ANTI-DLL3 ANTIBODY AND USE THEREOF

Cross-Reference To Related Applications

The present application is based on and claims priority to Chinese Patent Application No. 202410297394.5, filed on Mar. 15, 2024, which is incorporated herein by reference in its entirety.

The present invention provides an antibody or an antigen-binding fragment thereof that can specifically bind to a DLL3 protein, e.g., a human DLL3 protein. The present invention also provides a multispecific binding molecule, e.g., a multispecific antibody, that specifically binds to DLL3 and one or more tumor-associated antigens. The present invention further provides a nucleic acid molecule encoding the antibody, an expression vector for expressing the antibody, a host cell, and a preparation method therefor. The invention also provides methods of diagnosis and treatment using the antibodies of the invention.

Background of the Invention

DLL3 (i.e., Delta-like ligand 3 or Delta-like protein 3) is a type I single pass transmembrane protein and is one of the Notch ligands, comprising one DSL domain, six epidermal growth factor-like domains and one transmembrane domain. Under normal circumstances, DLL3 is limitedly expressed in normal tissues, localized inside the Golgi apparatus, with no detectable expression on the surface of the cell membrane. However, in cells of some neuroendocrine tumor types, including small cell lung cancer (SCLC) and large cell neuroendocrine carcinoma (LCNEC), DLL3 is aberrantly overexpressed at high levels (in approximately 70%-80% of SCLC cases). The abnormally expressed DLL3 is not only retained in the Golgi apparatus, but also detectable on the surface of the cell membrane.

Therefore, DLL3 is a promising target for neuroendocrine tumor therapy. Therapeutic agents against DLL3 have been developed in recent years for the treatment of DLL3-expressing tumors or cancers.

For DLL3, one approach is to develop an antibody-drug conjugate (ADC) that is formed by

conjugating an antibody with a cytotoxic payload. However, this strategy faces considerable clinical challenges, which may be attributable to the low expression abundance of DLL3 on the cell surface. Rova-T, which employs the highly potent pyrrolobenzodiazepine (PBD) dimer as its cytotoxic payload, is administered at a low clinical dose and has demonstrated only modest efficacy.

Attempts have also been made to combine DLL3 targeting with the targeting of other proteins. For example, WO2019234220A1, US10294300 or Susanne Hipp *et al.*, A Bispecific DLL3/CD3 IgG-Like T-Cell Engaging Antibody Induces Antitumor Responses in Small Cell Lung Cancer, Clin Cancer Res 2020;26:5258−68 or Giffin MJ *et al.*, AMG 757, a Half-Life Extended, DLL3-Targeted Bispecific T-Cell Engager, Shows High Potency and Sensitivity in Preclinical Models of Small-Cell Lung Cancer. Clin Cancer Res. 2021 Mar 1;27(5):1526-1537 each describe a bispecific antibody construct combining binding to human DLL3 on the surface of target cells with binding to human CD3 on the surface of T cells.

CD3 is a surface molecule specifically expressed on T cells, and as a co-receptor of the T cell receptor (TCR). Since the intracellular domain of the TCR lacks signaling domains, when the TCR receives a signal from MHC/HLA, the various subunits of CD3 cluster together to transmit a downstream signal for T cell activation. A functional CD3 molecule is formed by the dimeric association of two of the four different chains: ε, ζ, δ, and γ. The CD3 dimer arrangements include γ/ε, δ/ε, and ζ/ζ. Antibodies directed against CD3 have been shown to cluster CD3 on T cells, thereby inducing T cell activation in a manner similar to TCR engagement by peptide-loaded MHC molecules. Accordingly, anti-CD3 antibodies have been proposed for therapeutic purposes involving the activation of T cells. In addition, it has been proposed that a bispecific antibody capable of binding to CD3 and targeting a tumor surface antigen can engage tumor cells and T cells, thereby promoting the cross-linking of CD3 on the surface of the T cells and directly inducing T cells activation. The activated T cells release granzymes, perforins, and cytokines, which mediate tumor cell killing and ultimately exert a therapeutic effect against tumors.

However, there remains a need in the art to develop safe anti-DLL3 antibodies suitable for use as medicaments, as well as multispecific antigen-binding molecules constructed based thereon that specifically bind to DLL3 and other target antigens, particularly CD3, for use in the treatment of tumors.

Summary of the Invention

One aspect of the present invention relates to a binding molecule binding specifically to DLL3, e.g., an anti-DLL3 antibody or an antigen-binding fragment thereof, including a multispecific binding molecule binding specifically to DLL3 and other targets, e.g., a multispecific antibody such as a bispecific antibody.

One aspect of the present invention relates to an anti-DLL3 antibody or an antigen-binding fragment thereof.

In some embodiments, the anti-DLL3 antibody or antigen-binding fragment thereof of the present invention specifically binds to DLL3 (e.g., human DLL3). In some embodiments, the anti-DLL3 antibody or antigen-binding fragment thereof of the present invention binds to DLL3 (e.g., human DLL3) with a binding affinity (K_D value) of less than or equal to about 10 nM, 9 nM, 8 nM, 7 nM, 6 nM, 5 nM, 4 nM, 3 nM, 2 nM, 1 nM, 0.9 nM, 0.8 nM, 0.7 nM, or 0.6 nM, or a value therebetween. In some embodiments, the anti-DLL3 antibody or antigen-binding fragment thereof of the present invention for DLL3 (e.g., human DLL3) with a binding affinity (K_D value) of greater than or equal to about 0.5 nM.

In some embodiments, the anti-DLL3 antibody or antigen-binding fragment thereof of the present invention is capable of effectively binding to DLL3 and, via its Fc region, binding to FcR to activate downstream signaling pathways. For example, the Fc region of the anti-DLL3 antibody binds to macrophages and activates CD64-mediated downstream signaling pathways, thereby inducing macrophage phagocytosis.

Another aspect of the present invention relates to a multispecific binding molecule (e.g., a multispecific antibody, such as a bispecific antibody) constructed based on the DLL3 antibody of the present invention, comprising a target-binding region from the anti-DLL3 antibody of the present invention, and optionally one or more additional target-binding regions (antigen-binding regions).

In one embodiment, the present invention relates to a multispecific antibody, e.g., a bispecific antibody, that specifically binds to DLL3 and one or more additional antigens. In some embodiments, the additional antigen is CD3.

In one embodiment, the present invention relates to a bispecific antibody that specifically binds to DLL3 (e.g., human DLL3) and CD3 (e.g., human CD3).

In some embodiments, the bispecific antibody of the present invention is capable of activating the CD3 signaling pathway in a DLL3-dependent manner. In some embodiments, the bispecific antibody of the present invention is capable of activating T cells, such as CD4 or CD8 T cells, within a DLL3-positive tumor, for example, by inducing them to release cytokines, such as TNF, such as TNFα, or to release interleukins, such as IL2.

In some embodiments, the bispecific antibody of the present invention is capable of activating human T cells to kill tumor cells, e.g., activating human T cells to kill DLL3-positive tumor cells.

In some embodiments, the bispecific antibody of the present invention is capable of specifically binding to DLL3 (e.g., human DLL3) and CD3 (e.g., human CD3).

In some embodiments, the bispecific antibody of the present invention, such as a bispecific antibody specifically binding to DLL3 and CD3, has anti-tumor (e.g., DLL3-positive tumors) activity, such as in vivo anti-tumor activity.

In some embodiments, the bispecific antibody of the present invention may be used in combination with additional therapeutic agents for use in treating tumors, such as DLL3-positive tumors.

In some embodiments, the bispecific antibody of the present invention has a long half-life, greater than about 100 h, 110 h, 120 h, 130 h, 140 h, 145 h, 150 h, or 155 h, or a value therebetween, for example, between about 100 h and 200 h, e.g., between 140 h and 160 h, e.g., between about 148 h and 159 h.

In some embodiments, the bispecific antibody of the present invention exhibits favorable developability, for example, low developability risk; for example, the bispecific antibody has relatively good colloidal stability, relatively low non-specific binding, relatively low hydrophobicity, and relatively high thermal stability (e.g., Tagg above about 60°C, e.g., above about 61°C, 62°C, 63°C, or 64°C).

Description Of Drawings

FIG. 1 shows the binding of test antibodies to DLL3-overexpressing CHO cells ;

FIG. 2 shows dose-dependent activation of the CD64-mediated downstream signaling pathway by the test antibodies;

FIG. 3 shows the preparation route (A) and structure (B) of the bispecific antibody;

FIG. 4 shows the affinity of the test bispecific antibody DLL3/CD3 for human DLL3 (A) and human CD3 (B);

FIG. 5 shows the binding of the test bispecific antibody DLL3/CD3 to DLL3-overexpressing CHO cells ;

FIG. 6 shows the binding of the test bispecific antibody DLL3/CD3 to CD3 on the surfaces of human CD4 T cells (FIG. 6B) and CD8 T cells (FIG. 6A);

FIG. 7 shows the activity of the test bispecific antibody DLL3/CD3 in activating NFAT signaling in cells;

FIG. 8 shows dose-dependent killing effect of human CD8+ T cells induced by the test bispecific antibody DLL3/CD3 against SHP77 target cells (FIG. 8A) and non-target cells H460 (FIG. 8B);

FIG. 9 shows the binding activity to DLL3 and expression yield of the monoclonal antibody after removal of the hotspot mutation sites;

FIG. 10 shows the binding of the monoclonal antibody to DLL3-overexpressing CHO cells after removal of the hotspot mutation sites;

FIG. 11 shows the binding of the test bispecific antibody DLL3/CD3 to DLL3-overexpressing CHO cells;

FIG. 12 shows the activation of NFAT signaling in cells by the test bispecific antibody DLL3/CD3;

FIGs. 13 and 14 show dose-dependent killing effect of human CD8+ T cells against SHP77

target cells induced by the test bispecific antibody DLL3/CD3;

FIG. 15 shows the binding of test bispecific antibody to human DLL3;

FIG. 16 shows the binding of the test bispecific antibody to CHO-hDLL1 and 293T-hDLL4 cells;

FIG. 17 shows the binding of the test bispecific antibody to a DLL3-overexpressing cell line and T cells;

FIG. 18 shows activation of NFAT signaling in cells in vitro by the test bispecific antibody;

FIG. 19 shows the in vitro killing activity of the test bispecific antibody against tumor cells;

FIG. 20 shows the cytokine production by immune cells in PBMCs upon treatment with the tested bispecific antibody in the absence of DLL3 crosslinking;

FIG. 21 shows the in vivo tumor growth inhibitory activity of the test bispecific antibody;

FIG. 22 shows the infiltration of CD3-positive cells in tumors after administration of IgG1 at 0.1 mpk (FIG. 22A), BI-D3C1 analog at 0.01 mpk (FIG. 22B), and D3-107/CD3-C1 at 0.01 mpk (FIG. 22C);

FIG. 23 shows the number of CD3-positive cells per unit area in different administration groups;

FIG. 24 shows the in vivo tumor growth inhibitory activity of the test bispecific antibody;

FIG. 25 shows the in vivo tumor growth inhibitory activity of the test bispecific antibody;

FIG. 26 shows the in vivo tumor growth inhibitory activity of the test bispecific antibody in combination with standard chemotherapy;

FIG. 27 shows the in vivo tumor growth inhibitory activity of the test bispecific antibody in combination with an immune checkpoint inhibitor; and

FIG. 28 shows the in vivo PK assay results of the test bispecific antibody.

Detailed Description of the Invention

It is to be understood that the present invention is not limited to the particular method, protocols, examples, and reagents described herein, as these may vary. It is also to be understood that the terminology used herein are merely intended to describe particular embodiments rather than limiting the scope of the present invention, which will be limited only by the appended claims.

I. Definitons

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which the present invention belongs.

For the purpose of explaining this specification, the following definitions will be used, and wherever appropriate, terms used in the singular form may also include the plural form, and vice versa. It is to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

The term "about" used in combination with a numerical value is intended to encompass the numerical values in a range from a lower limit less than the specified numerical value by 5%, 4%, 3%, 2%, or 1% to an upper limit greater than the specified numerical value by 5%, 4%, 3%, 2%, or 1%.

As used herein, the term "and/or" refers to any one of the options, or any two or more of the options, or all of the options.

When referring to "first" and "second" herein, it is only to distinguish two domains or two chains, and does not indicate the location of the two domains in any way.

As used herein, the term "comprise" or "include" is intended to mean that the described elements, integers or steps are included, but not to the exclusion of any other elements, integers or steps. The term "comprise" or "include" used herein, unless otherwise indicated, also encompasses the instances consisting of the described elements, integers or steps. For example, when referring to an antibody variable region "comprising" a particular sequence, the term is also intended to encompass an antibody variable region consisting of the particular sequence.

The term "DLL3" as used herein refers to a type I transmembrane protein belonging to the

Notch ligand family. Human DLL3 comprises, for example, the amino acid sequence as set forth in UniProtKB/Swiss-Prot: Q9NYJ7. In some embodiments, the DLL3 is human DLL3.

As used herein, an "antibody binding to DLL3", "anti-DLL3 antibody", or "antibody specifically binding to DLL3" refers to an antibody that is capable of binding to DLL3 with an appropriate affinity.

In some aspects, the anti-DLL3 antibody described herein also encompasses multispecific antibodies such as bispecific antibodies that specifically bind to both DLL3 and other target antigens.

The term "CD3" as used herein refers to an antigen expressed on a T cell as part of a multimolecular T cell receptor (TCR), i.e., a T cell engaging antigen T cell surface glycoprotein CD3, which consists of a homodimer or heterodimer formed by two of the following four receptor chains: CD3-ε, CD3-δ, CD3-ζ, and CD3-γ. Human CD3-εn (hCD3ε) comprises an amino acid sequence as set forth in UniProtKB/Swiss-Prot: P07766. Human CD3-δ (hCD3δ) comprises an amino acid sequence as set forth in UniProtKB/Swiss-Prot: P04234. In some embodiments, the CD3 described herein refers to CD3 from a human or monkey (e.g., cynomolgus monkey).

The term "antibody binding to CD3" or "anti-CD3 antibody" as used herein includes antibodies that specifically recognize or bind to a single CD3 subunit (e.g., ε, δ, γ, or ζ), as well as antibodies that specifically recognize and bind to a dimeric complex of two CD3 subunits (e.g., γ/ε, δ/ε, and ζ/ζ CD3 dimers). The antibody and the antigen-binding fragment of the present invention can bind to soluble CD3, binding CD3, and/or CD3 expressed on the cell surface. Soluble CD3 includes native CD3 proteins as well as recombinant CD3 protein variants, e.g., monomeric and dimeric CD3 structures that lack a transmembrane region or are otherwise not associated with the cell membrane. The binding affinity of the antibody to CD3 can be detected by flow cytometry or bio-layer interferometry.

Effector cells include effector T cells (T lymphocytes), such as CD4+ T cells, CD8+ T cells, Th1, Th2, and regulatory T cells (Tregs). The effector cells may also include natural killer cells, macrophages, granulocytes, plasma cells, or B cells (lymphocytes).

General information on the amino acid sequences and nucleotide sequences of the human immunoglobulin light and heavy chains is given in Kabat, E.A. *et al.*, Sequences of Proteins of Immunological Interest, 5th Ed., Public Health Service, National Institutes of Health, Bethesda, MD

(1991).

The multispecific antibody of the present invention may comprise a linker. The term "linker" as used herein refers to any molecule that enables direct connection of different portions of a multispecific antibody. Examples of linkers to establish covalent linkages between different portions of a multispecific antibody include peptide linkers and non-proteinaceous polymers including, but not limited to, polyethylene glycol (PEG), polypropylene glycol, polyalkylene oxide, or copolymers of polyethylene glycol and polypropylene glycol. In some embodiments, the term "peptide linker" according to the present invention refers to an amino acid sequence, wherein the sequence links the amino acid sequences of various portions of a multispecific antibody together. Preferably, the peptide linker is of a length sufficient to link two entities in such a way that they maintain their conformation relative to each other without interfering with the desired activity. The peptide linker may or may not predominantly comprise the following amino acid residues: Gly, Ser, Ala or Thr.

The term "Fc domain" or "Fc region" is used herein to define a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. The term includes Fc regions of native sequences and variant Fc regions. A native immunoglobulin "Fc domain" comprises two or three constant domains, i.e., a CH2 domain, a CH3 domain, and an optional CH4 domain. For example, in native antibodies, an immunoglobulin Fc domain comprises the second and third constant domains (CH2 and CH3 domains) derived from two heavy chains of IgG, IgA, and IgD antibodies; or comprises the second, third, and fourth constant domains (CH2, CH3, and CH4 domains) derived from two heavy chains of IgM and IgE antibodies. Unless otherwise stated herein, amino acid residues in the Fc region or heavy chain constant region are numbered according to the EU numbering scheme (also known as the EU index) described in, for example, Kabat *et al.*, Sequences of Proteins of Immunological Interes, 5th Ed., Public Health Service, National Institutes of Health, Bethesda, MD, 1991. However, the C-terminal lysine (Lys447) of the Fc region may or may not be present. Two Fc regions can form a dimeric Fc by dimerization, and two different Fc form a heterodimeric Fc by heterodimerization. Herein, the terms "Fc region", "Fc portion", and "dimeric Fc (e.g., heterodimeric Fc)" do not comprise a heavy chain variable region VH and a light chain variable region VL as well as a heavy chain constant region

CH1 and a light chain constant region CL of an immunoglobulin, but in some cases, they may comprise a hinge region at the N-terminus of the heavy chain constant region. In one embodiment, a human IgG heavy chain Fc region extends from Asp221 or from Cys226 or from Asp231 to the carboxyl-terminus of the heavy chain.

In one embodiment, the human IgG1 Fc region polypeptide (comprising a portion of hinge region) comprises or consists of the following amino acid sequence:

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD GSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:26); or

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDS DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 27).

In one embodiment, the Fc region is derived from a human Fc region. In one embodiment, the Fc region comprises all or part of a human constant region. The antibody Fc region is directly involved in complement activation, C1q binding, C3 activation, and Fc receptor binding. In one embodiment, the Fc region is a human Fc region. In one embodiment, the Fc region belongs to a human IgG4 subclass. In one embodiment, the Fc region belongs to a human IgG1 subclass. In one embodiment, the Fc region is an Fc region of human IgG1, IgG2, IgG3, or IgG4.

Herein, a "heterodimeric Fc scaffold" refers to a scaffold comprising two different Fc regions or formed by the dimerization of two different Fc regions, which may be linked to an antigen-binding domain at its N-terminus or C-terminus (e.g., a heavy and/or light chain variable region of an antibody or an antigen-binding fragment of an antibody that can bind to a target molecule, or a soluble moiety of a ligand or receptor that can bind to a target molecule) to constitute a multispecific antibody, e.g., a bispecific antibody.

The term "CH1 region" refers to a portion of an antibody heavy chain polypeptide that extends from EU position 118 to EU position 220 (EU numbering scheme). In one embodiment, the CH1 domain comprises or consists of an amino acid sequence of

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC (SEQ ID NO: 28).

In some embodiments, the CH1 may comprise a portion of hinge region. In some embodiments, the Fc region may comprise a portion of hinge region.

Herein, specific amino acid residues of antibody IgG constant regions are described according to the EU numbering scheme. For example, "S364" refers to a serine at EU position 364.An amino acid mutation at a specific position in the constant region is indicated by "original amino acid, amino acid position, mutated amino acid". For example, "S364R" refers to a substitution of serine (S) at EU position 364 with arginine (R). When describing combinations of mutations, the combined mutations are connected by a plus (+) or "-" or "and". "S364R+D399K", "S364R-D399K", or "S364R and D399K" indicates that the Fc region comprises both mutations S364R and D399K. When there is a possibility of multiple mutations at a particular position, it is indicated herein by the symbol "/". For example, the mutation "K370T/S" indicates that residue K at position 370 may be substituted with T or S residue.

For polypeptide sequences, "conservative alterations" include substitutions, deletions, or additions to the polypeptide sequence that do not substantially alter the desired functional activity of the polypeptide sequence. For example, conservative substitutions often involve in the replacement of an amino acid with a chemically similar one. Tables of conservative substitution providing functionally similar amino acids are well known in the art. Listed below are eight groups of amino acids that are conservative substitutions for each other: 1) alanine (A) and glycine (G); 2) aspartic acid (D) and glutamic acid (E); 3) asparagine (N) and glutamine (Q); 4) arginine (R) and lysine (K); 5) isoleucine (I), leucine (L), methionine (M), and valine (V); 6) phenylalanine (F), tyrosine (Y), and tryptophan (W); 7) serine (S) and threonine (T); and 8) cysteine (C) and methionine (M). In some embodiments, the term "conservative sequence alteration" is used to refer to an amino acid modification that does not significantly affect or change the binding characteristics for an antigen of interest of the antibody molecule or binding protein molecule of the present invention comprising the amino acid sequence. For example, conservatively modified variants retain at least 80%, 85%, 90%, 95%, 98%, 99% or higher, e.g., 100%-110% or higher, binding affinity for an antigen of interest relative to the parent antibody or binding protein molecule.

The term "vector" as used herein refers to a nucleic acid molecule capable of proliferating another nucleic acid to which it is linked. The term includes vectors that serve as self-replicating nucleic acid structures as well as vectors that incorporate into the genome of a host cell into which they have been introduced. Some vectors are capable of directing the expression of a nucleic acid to which they are operably linked. Such vectors are referred to herein as "expression vectors."

The term "binding molecule" refers to any molecule capable of specifically binding to a target, e.g., an antibody or an antigen-binding fragment thereof or a fusion protein.

The term "target" refers to the bound substance against which the binding molecule is directed. The target may be an antigen, or may be a ligand or receptor. The term "antigen" refers to a molecule that elicits an immune response. This immune response may involve antibody production or activation of specific immune cells, or both. Those skilled in the art will appreciate that any macromolecule, including substantially all proteins or peptides, can be used as an antigen. Further, antigens may be derived from recombinant or genomic DNA.As used herein, the term "epitope" refers to a moiety of an antigen that specifically interacts with an antibody molecule. "Target" and "antigen" are used interchangeably when the binding molecule of the present invention relates to a target-binding region derived from an antibody. In some embodiments, the antigen is CD3.

The term "target-binding region" as used herein refers to a portion of a binding molecule, e.g., a multispecific binding molecule or a bispecific binding molecule, that binds to a particular target or antigen. The target-binding region may be, for example, an antibody or immunoglobulin per se or an antibody fragment. Such target-binding regions may or may not have a tertiary structure independent of the remainder of the binding molecule, and may or may not bind to their targets as separate entities. The target-binding region may also be a receptor or a ligand, or a domain of a receptor capable of binding to a ligand. In the case of multispecific antibodies or bispecific antibodies, the "target-binding region" is also referred to as the "antigen-binding region".

The term "antigen-binding region" as used herein refers to any portion of an antibody or an antigen-binding fragment thereof, e.g., a multispecific antibody or a bispecific antibody, that binds to a particular target or antigen. The antigen-binding region may be, for example, an antibody or immunoglobulin per se or an antibody fragment. Such antigen-binding region may or may not have

a tertiary structure independent of the remainder of the multispecific antibody or bispecific antibody, and may or may not bind to its antigen/epitope as a separate entity. When the binding molecule of the present invention relates to a target-binding region derived from an antibody, "target-binding region" and "antigen-binding region" are used interchangeably.

The term "multispecific binding molecule" refers to a multispecific binding molecule that is at least bispecific, e.g., a bispecific binding molecule, that is, the molecule comprises at least a first target-binding region and a second target-binding region, wherein the first target-binding region binds to one target or antigen and the second target-binding region binds to another antigen or target. The multispecific binding molecule according to the present invention also encompasses a multispecific binding molecule comprising a plurality of target-binding regions/sites. In some embodiments, the multispecific binding molecule of the present invention is a multispecific antibody. In some embodiments, the bispecific binding molecule of the present invention is a bispecific antibody.

As used herein, the term "monospecific" refers to a polypeptide/protein molecule having one or more target-binding regions, each of which binds to the same site or structure of the same target or the same epitope of the same antigen.

As used herein, the term "multispecific" binding molecule, e.g., an antibody, refers to a binding molecule having at least two target-binding regions, each of which binds to a different site/structure of the same target or a different target. A multispecific binding molecule is a binding molecule having binding specificities for at least two different targets or sites/structures. In one embodiment, provided herein is a bispecific binding molecule having binding specificity for a first target and a second target.

As used herein, the term "multispecific" antibody refers to an antibody having at least two antigen-binding regions, each of which binds to a different epitope of the same antigen or a different epitope of a different antigen. A multispecific antibody is an antibody having binding specificity for at least two different antigen epitopes. In one embodiment, provided herein is a bispecific antibody having binding specificity for a first antigen and a second antigen.

When referring to a "first antigen-binding region" in a multispecific antibody or a bispecific antibody, it refers to a binding region that binds to a first antigen and is not intended to limit the number of such antigen-binding regions comprised in the antibody. For example, the multispecific antibody or the bispecific antibody may comprise one or more first antigen-binding regions. For

example, the bispecific antibody comprises a first antigen-binding region and a second antigen-binding region, but may comprise one or more first antigen-binding regions and one or more second antigen-binding regions.

When referring to "first target-binding region" in a binding molecule, it refers to a binding region that binds to a first target, and is not intended to limit the number of such target-binding regions comprised in the binding molecule. For example, the multispecific binding molecule or bispecific binding molecule may comprise one or more first target-binding regions. For example, the bispecific binding molecule comprises a first target-binding region and a second target-binding region, and may comprise one or more first target-binding regions and one or more second target-binding regions.

When it is mentioned that "a target or an antigen-binding region is derived from an antibody", it is meant that the binding domain constituting the target/antigen-binding region is or is derived from a binding domain of the antibody that specifically binds to an antigen. For example, a fragment of the antigen-binding region that specifically binds to an antigen, such as an Fab, is or is derived from a corresponding fragment of the antibody, such as an Fab; or the heavy chain variable region and/or the light chain variable region of the antigen-binding region is or is derived from a heavy chain variable region and/or a light chain variable region of the antibody; or 1, 2, 3, 4, 5, or 6 complementarity-determining regions (CDRs) of the antigen-binding region are CDRs of the antibody.

The term "derived from" means that the fragment in the antigen-binding region is substantially identical to the fragment of the antibody from which it is derived, but has a mutation, such as a substitution, deletion, or addition, at one or more sites. In one particular embodiment, the mutation is not in a CDR of the antibody.

The terms "whole antibody" and "full-length antibody" are used interchangeably herein to refer to an antibody molecule having the structure of a native immunoglobulin molecule. In the case of a conventional four-chain IgG antibody, a full-length antibody comprises two heavy chains (H) and two light chains (L) interconnected by disulfide bonds. In the case of a heavy chain antibody having only heavy chains but lacking light chains, a full-length antibody comprises two heavy chains (H) interconnected by disulfide bonds. For a conventional four-chain IgG antibody, the full-length

antibody heavy chain generally consists of a heavy chain variable region (abbreviated herein as VH) and a heavy chain constant region, wherein the heavy chain constant region comprises at least 3 domains CH1, CH2, and CH3 (and optionally CH4). The full-length antibody light chain consists of a light chain variable region (abbreviated herein as VL) and a light chain constant region, wherein the light chain constant region consists of one domain CL. Each heavy chain variable region VH or each light chain variable region consists of three CDRs and 4 FRs arranged from amino-terminus to carboxyl-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4. The term "antibody fragment" includes a portion of an intact antibody. In a preferred embodiment, the antibody fragment is an antigen binding fragment.

The term "antigen-binding fragment" of an antibody refers to a molecule different from a full-length antibody, wherein the antigen-binding fragment comprises a portion of the full-length antibody, but is capable of binding to an antigen of the full-length antibody or competing with the full-length antibody (i.e., a full-length antibody from which the antigen-binding fragment is derived) for binding to an antigen. An antigen-binding fragment may be prepared by recombinant DNA techniques, or by enzymatic or chemical cleavage of an intact antibody. The antigen-binding fragment includes, but is not limited to, an Fab, an Fab', an $F(ab')_2$, an Fv, a single-chain Fv, a diabody, a single-domain antibody (sdAb), and a nanobody. For example, an Fab fragment can be obtained by papain digestion of a full-length antibody. In addition, the $F(ab')_2$, a dimer of the Fab', is a bivalent antibody fragment produced by pepsin digestion at a site below the disulfide bonds in a hinge region of an intact antibody. The $F(ab')_2$ can be reduced by disrupting the disulfide bonds in the hinge region under neutral conditions, thereby converting the $F(ab')_2$ dimer into an Fab' monomer. The Fab' monomer is essentially an Fab fragment with a hinge region. The Fv fragment consists of the VL and VH domains of a single arm of an antibody. The two domains VL and VH of the Fv fragment can be encoded by separate genes, but the two domains can also be linked, using recombinant methods, via a synthetic linker peptide so that they are produced as a single protein chain in which the VL and VH regions pair to form a single-chain Fv (scFv).

"Fab fragment" and "Fab" are used interchangeably herein to refer to an immunoglobulin fragment consisting of two polypeptide chains and comprising an immunoglobulin heavy chain variable domain VH, a heavy chain constant domain CH1, a light chain variable domain VL, and a

light chain constant domain CL, wherein one polypeptide chain comprises, from N-terminus to C-terminus, a VH and one constant region selected from CH1 and CL, and the other polypeptide chain comprises, from N-terminus to C-terminus, a VL and the other constant region selected from CL and CH1, wherein the VH and VL domains are paired to form an antigen-binding site. Herein, an Fab polypeptide chain comprising a heavy chain constant region CH1 is also referred to as a "Fab heavy chain"; correspondingly, an Fab polypeptide chain comprising a light chain constant region CL is also referred to as a "Fab light chain".

"Complementarity-determining region" or "CDR region" or "CDR" is a region in an antibody variable domain that is highly variable in sequence and forms a structurally defined loop ("hypervariable loop") and/or comprises antigen-contacting residues ("antigen contact site"). The CDRs are primarily responsible for binding to antigenic epitopes. The CDRs of heavy and light chains are generally referred to as CDR1, CDR2, and CDR3, and are numbered sequentially from the N-terminus. The CDRs located in a heavy chain variable domain of an antibody are referred to as HCDR1, HCDR2, and HCDR3, whereas the CDRs located in a light chain variable domain of an antibody are referred to as LCDR1, LCDR2, and LCDR3. In a given amino acid sequence of a light chain variable region or a heavy chain variable region, the exact amino acid sequence boundary of each CDR can be determined using any one or a combination of many well-known antibody CDR assignment schemes including, e.g., Chothia based on the three-dimensional structure of antibodies and the topology of the CDR loops (Chothia *et al.* (1989) Nature 342: 877-883; Al-Lazikani *et al.*, "Standard conformations for the canonical structures of immunoglobulins", Journal of Molecular Biology, 273: 927-948 (1997)), Kabat based on antibody sequence variability (Kabat *et al.*, Sequences of Proteins of Immunological Interest, 4th Ed., U.S. Department of Health and Human Services, National Institutes of Health (1987)), AbM (University of Bath), Contact (University College London), International ImMunoGeneTics database (IMGT) (available on www.imgt.cines.fr/), and North CDR definition based on the affinity propagation clustering using a large number of crystal structures. Unless otherwise stated, the term "CDR" or "CDR sequence" used herein encompasses CDR sequences determined by any one of the schemes described above or a combination thereof. CDRs can also be determined based on having the same Kabat numbering positions as a reference CDR sequence (e.g., any of the exemplary CDRs of the present invention).

In some embodiments, the CDRs of the heavy chain variable region of the antibody of the present invention are each defined according to Kabat or Chothia, or a combination of Kabat and Chothia (hereinafter referred to as "Kabat & Chothia"; corresponding to H26-H32 under the Kabat numbering scheme). In some embodiments, the CDRs of the light chain variable region of the antibody of the present invention are determined according to Kabat.

In one embodiment, in the anti-DLL3 antibody of the present invention, HCDR1 is defined according to the Kabat & Chothia scheme, HCDR2 and HCDR3 are each defined according to the Kabat scheme, and LCDRs are each defined according to the Kabat scheme.

CDR	Kabat scheme	Chothia scheme	Kabat& Chothia
LCDR1 (Kabat numbering system)	L24-L34		
LCDR2 (Kabat numbering system)	L50-L56		
LCDR3 (Kabat numbering system)	L89-L97		
HCDR1 (Kabat and Chothia numbering systems)	H31-H35	H26-H32	H26-H35
HCDR2 (Kabat numbering system)	H50-H65		
HCDR3 (Kabat numbering system)	H95-H102		

The percent identity between sequences is calculated as follows.

To determine the percent identity of two amino acid sequences or two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced into one or both of the first and second amino acid sequences or nucleic acid sequences for optimal alignment, or non-homologous sequences can be disregarded for comparison). In a preferred embodiment, for comparison purposes, the length of the aligned reference sequence is at least 30%, preferably at

least 40%, more preferably at least 50% or 60%, and even more preferably at least 70%, 80%, 90%, or 100% of the length of the reference sequence. The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide at the corresponding position in the second sequence, the molecules are identical at this position.

"Humanized antibody" is an antibody that retains the antigen-specific reactivity of a non-human antibody and has lower immunogenicity when administered to humans as a therapeutic agent. This can be achieved, for example, by retaining non-human antigen-binding sites and substituting the remainder of the antibodies with their human counterparts (i.e., the portions of the variable regions not involved in binding are substituted with the corresponding parts of human antibodies).

As used herein, the term "anti", "binding", or "specific binding" means that the binding effect is selective for targets or antigens and can be distinguished from unwanted or non-specific interactions. The ability of a binding site to bind to a particular target or antigen may be determined by flow cytometry or enzyme-linked immunosorbent assay (ELISA) or conventional binding assays known in the art, such as radioimmunoassay (RIA), bio-layer interferometry, MSD assay, or surface plasmon resonance (SPR).

"Affinity" or "binding affinity" refers to the inherent binding affinity that reflects the interaction between members of a binding pair. The affinity of a molecule X for its partner Y can be represented generally by the dissociation constant (K_D), which is the ratio of the dissociation rate constant to the association rate constant (K_{dis} and K_{on}, respectively). Affinity can be measured by common methods known in the art. One specific method for measuring affinity is the ForteBio kinetic binding assay described herein.

The term "host cell" refers to a cell into which an exogenous polynucleotide has been introduced, including progeny of such cells. Host cells include "transformants" and "transformed cells", which include primary transformed cells and progeny derived therefrom.

The terms "individual" and "subject" are used interchangeably and refer to a mammal. The mammals include, but are not limited to, domesticated animals (e.g., cows, sheep, cats, dogs, and

horses), primates (e.g., human and non-human primates such as monkeys), rabbits, and rodents (e.g., mice and rats). In particular, the subject is a human.

The term "treatment" (or "treat" or "treating") refers to slowing, interrupting, arresting, alleviating, stopping, lowering, or reversing the onset of symptoms, complications, or biochemical indications of a disease, or alleviating symptoms, or arresting or inhibiting the further progression of the disease, condition, or disorder.

The term "prevention" (or "prevent" or "preventing") includes the inhibition of the development or progression of symptoms of a disease or disorder, or a specific disease or disorder.

The term "therapeutic agent" described herein encompasses any substance effective in preventing or treating a tumor, e.g., cancer, including a chemotherapeutic agent, a cytokine, a cytotoxic agent, an additional antibody (e.g., an immune checkpoint molecule antibody), a small molecule drug, or an immunomodulatory agent (e.g., an immunosuppressant or an agonist).

The term "combination therapy" refers to the administration of two or more therapeutic agents or modalities to treat the diseases described herein. Such administration includes co-administration of these therapeutic agents in a substantially simultaneous manner, e.g., in a single capsule having a fixed ratio of active ingredients. Alternatively, such administration includes co-administration of the active ingredients in a variety of or separate containers (e.g., tablets, capsules, powder and liquid). The powder and/or liquid may be reconstituted or diluted to a desired dose prior to administration. In addition, such administration also includes using each type of therapeutic agent in a sequential manner at approximately the same time or at different times. In either case, the therapeutic regimen will provide the beneficial effect of the drug combination in treating the disorders or conditions described herein.

The term "pharmaceutical combination or combination product" refers to a non-fixed combination product or a fixed combination product, including, but not limited to, a kit and a pharmaceutical composition. The term "non-fixed combination" means that the active ingredients (e.g., (i) the antibody of the present invention, and (ii) an additional therapeutic agent) are administered to a patient as separate entities, either simultaneously or sequentially (without specific time constraint or at the same or different time intervals), wherein such administration provides two

or more prophylactically or therapeutically effective active agents in the patient. The term "fixed combination" means that two or more active agents are administered to a patient simultaneously as a single entity. The dosage and/or time interval of the two or more active agents is preferably selected such that the combined use of the components can result in a therapeutic effect on the disease or disorder greater than that achieved by the use of either component alone. The ingredients may each be in a separate formulation, which may be the same or different.

The term "pharmaceutical composition" refers to a composition that exists in a form allowing the biological activity of active ingredients contained therein to be effective and does not contain additional ingredients having unacceptable toxicity to a subject to which the composition is administered.

The term "pharmaceutical supplementary material" refers to diluents, adjuvants (e.g., Freund's adjuvants (complete and incomplete)), excipients, carriers, stabilizers, or the like, which are administered with the active substance.

"Subject/patient/individual sample" refers to a collection of cells or fluids obtained from a patient or a subject. The source of the tissue or cell sample may be solid tissue as from a fresh, frozen and/or preserved organ or tissue sample or biopsy or puncture sample; bodily fluids such as tears, vitreous humor, cerebrospinal fluid, amniotic fluid, peritoneal fluid, or interstitial fluid.

Examples of tumor samples herein include, but are not limited to, tumor biopsies, fine needle aspirates, bronchial lavage fluids, pleural fluids, sputum, urine, surgical specimens, circulating tumor cells, serum, plasma, circulating plasma proteins, ascites, primary cell cultures or cell lines derived from tumors or exhibiting tumor-like properties, and archived tumor samples such as formalin-fixed, paraffin-embedded tumor samples or frozen tumor samples.

II. Anti-DLL3 Antibody

In some embodiments, the anti-DLL3 antibody or antigen-binding fragment thereof of the present invention comprises three complementarity-determining regions from a heavy chain variable region (HCDRs): HCDR1, HCDR2, and HCDR3. In some embodiments, the anti-DLL3 antibody or antigen-binding fragment thereof of the present invention comprises three complementarity-determining regions from a light chain variable region (LCDRs): LCDR1, LCDR2,

and LCDR3. In some embodiments, the anti-DLL3 antibody or antigen-binding fragment thereof of the present invention comprises three complementarity-determining regions from a heavy chain variable region (HCDRs) and three complementarity-determining regions from a light chain variable region (LCDRs).

In some aspects, the anti-DLL3 antibody or antigen-binding fragment thereof of the present invention comprises a heavy chain variable region (VH). In some aspects, the anti-DLL3 antibody or antigen-binding fragment thereof of the present invention comprises a light chain variable region (VL). In some aspects, the anti-DLL3 antibody or antigen-binding fragment thereof of the present invention comprises a heavy chain variable region (VH) and a light chain variable region (VL). In some embodiments, the heavy chain variable region comprises three complementarity-determining regions from the heavy chain variable region (HCDRs): HCDR1, HCDR2, and HCDR3. In some embodiments, the light chain variable region comprises three complementarity-determining regions from the light chain variable region (LCDRs): LCDR1, LCDR2, and LCDR3.

In some embodiments, the heavy chain variable region VH of the anti-DLL3 antibody of the present invention

(i) comprises or consists of an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence as set forth in SEQ ID NO: 12, 13, 14, or 15; or

(ii) comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 12, 13, 14, or 15; or

(iii) comprises or consists of an amino acid sequence having one or more (preferably no more than 10, and more preferably no more than 5, 4, 3, 2, or 1) amino acid alterations (preferably amino acid substitutions, and more preferably conservative amino acid substitutions) compared with the amino acid sequence as set forth in SEQ ID NO: 12, 13, 14, or 15, wherein preferably, the amino acid alterations do not occur in the CDRs.

In some embodiments, the light chain variable region VL of the anti-DLL3 antibody of the present invention

(i) comprises or consists of an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence as set forth in SEQ ID NO: 16, 17, or 18; or

(ii) comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 16, 17, or 18; or

(iii) comprises or consists of an amino acid sequence having one or more (preferably no more than 10, and more preferably no more than 5, 4, 3, 2, or 1) amino acid alterations (preferably amino acid substitutions, and more preferably conservative amino acid substitutions) compared with the amino acid sequence as set forth in SEQ ID NO: 16, 17, or 18, wherein preferably, the amino acid alterations do not occur in the CDRs.

In some embodiments, the three complementarity-determining regions from the heavy chain variable region (HCDRs) of the anti-DLL3 antibody of the present invention, HCDR1, HCDR2, and HCDR3, are

(i) three complementarity-determining regions HCDR1, HCDR2 and HCDR3 comprised in the VH as set forth in SEQ ID NO: 12, 13, 14 or 15, or

(ii) a sequence comprising, collectively across the three HCDR regions, at least one and no more than 5, 4, 3, 2, or 1 amino acid alterations (preferably amino acid substitutions, and more preferably conservative substitutions) relative to the sequence of (i);

For example, the HCDR1, the HCDR2, and the HCDR3 may each be defined according to any scheme for determining CDRs, e.g., according to the Kabat, Chothia, IMGT, AbM, or North schemes, or a combination thereof. Preferably, the HCDR1 is defined according to Kabat & Chothia schemes, and the HCDR2 and the HCDR3 are each defined according to Kabat schemes.

In some embodiments, the three complementarity-determining regions from the light chain variable region (LCDRs) of the anti-DLL3 antibody of the present invention, LCDR1, LCDR2, and LCDR3, are

(i) three complementarity-determining regions LCDR1, LCDR2 and LCDR3 comprised in the VL as set forth in SEQ ID NO: 16, 17 or 18, or

(ii), a sequence comprising, collectively across the three LCDR regions, at least one and no more than 5, 4, 3, 2, or 1 amino acid alterations (preferably amino acid substitutions, and more preferably conservative substitutions) in the three LCDRs relative to the sequence of (i);

For example, the LCDR1, LCDR2, and LCDR3 may each be defined according to any for determining CDRs, e.g., according to the Kabat, Chothia, IMGT, AbM, or North scheme, or a combination thereof, and preferably, the LCDR1, LCDR2, and LCDR3 are each defined according to Kabat scheme.

In some embodiments, the HCDR1 described herein comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 1, or an amino acid sequence having 1, 2, or 3 amino acid alterations (preferably amino acid substitutions, and more preferably conservative substitutions) compared thereto.

In some embodiments, the HCDR2 described herein comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 2, 3, 4 or 5, or an amino acid sequence having 1, 2 or 3 amino acid alterations (preferably amino acid substitutions, and more preferably conservative substitutions) compared thereto.

In some embodiments, the HCDR3 described herein comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 6, or an amino acid sequence having 1, 2, or 3 amino acid alterations (preferably amino acid substitutions, and more preferably conservative substitutions) compared thereto.

In some embodiments, the LCDR1 described herein comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 7, or an amino acid sequence having 1, 2, or 3 amino acid alterations (preferably amino acid substitutions, and more preferably conservative substitutions) compared thereto.

In some embodiments, the LCDR2 described herein comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 8, or an amino acid sequence having 1, 2, or 3 amino acid alterations (preferably amino acid substitutions, and more preferably conservative substitutions) compared thereto.

In some embodiments, the LCDR3 described herein comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 9, or an amino acid sequence having 1, 2, or 3 amino acid

alterations (preferably amino acid substitutions, and more preferably conservative substitutions) compared thereto.

In some embodiments, in the anti-DLL3 antibody or antigen-binding fragment thereof of the present invention,

the HCDR1 comprises or consists of the amino acid sequence of SEQ ID NO: 1; the HCDR2 comprises or consists of the amino acid sequence of SEQ ID NO2, 3, 4, or 5; the HCDR3 comprises or consists of the amino acid sequence of SEQ ID NO: 6; the LCDR1 comprises or consists of the amino acid sequence of SEQ ID NO: 7; the LCDR2 comprises or consists of the amino acid sequence of SEQ ID NO: 8; and/or the LCDR3 comprises or consists of the amino acid sequence of SEQ ID NO: 9.

In some particular embodiments of the present invention, the anti-DLL3 antibody or antigen-binding fragment thereof of the present invention comprises a VH and a VL, wherein

the VH comprises or consists of the amino acid sequenceas set forth in SEQ ID NO: 13, or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereto, and the VL comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 18, or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereto;

the VH comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 12, or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereto, and the VL comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 16, or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereto;

the VH comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 13, or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereto, and the VL comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 16, or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereto;

the VH comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 14, or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereto, and the VL comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 16, or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereto;

the VH comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 15, or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereto, and the VL comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 16, or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereto;

the VH comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 12, or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereto, and the VL comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 17, or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereto;

the VH comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 12, or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereto, and the VL comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 18, or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereto; or

the VH comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 14, or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereto, and the VL comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 18, or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereto.

In some particular embodiments of the present invention, the anti-DLL3 antibody or antigen-binding fragment thereof of the present invention comprises:

(i). three complementarity-determining regions HCDR1, HCDR2, and HCDR3 comprised in

the VH as set forth in SEQ ID NO: 13, andthree complementarity-determining regions LCDR1, LCDR2 and LCDR3 comprised in the VL as set forth in SEQ ID NO: 18;

(ii). three complementarity-determining regions HCDR1, HCDR2, and HCDR3 comprised in the VH as set forth in SEQ ID NO: 12, andthree complementarity-determining regions LCDR1, LCDR2 and LCDR3 comprised in the VL as set forth in SEQ ID NO: 16;

(iii). three complementarity-determining regions HCDR1, HCDR2, and HCDR3 comprised in the VH as set forth in SEQ ID NO: 13, and three complementarity-determining regions LCDR1, LCDR2 and LCDR3 comprised in the VL as set forth in SEQ ID NO: 16;

(iv). three complementarity-determining regions HCDR1, HCDR2, and HCDR3 comprised in the VH as set forth in SEQ ID NO: 14, and three complementarity-determining regions LCDR1, LCDR2 and LCDR3 comprised in the VL as set forth in SEQ ID NO: 16;

(v). three complementarity-determining regions HCDR1, HCDR2, and HCDR3 comprised in the VH as set forth in SEQ ID NO: 15, and three complementarity-determining regions LCDR1, LCDR2 and LCDR3 comprised in the VL as set forth in SEQ ID NO:16;

(vi). three complementarity-determining regions HCDR1, HCDR2, and HCDR3 comprised in the VH as set forth in SEQ ID NO: 12, and three complementarity-determining regions LCDR1, LCDR2 and LCDR3 comprised in the VL as set forth in SEQ ID NO: 17;

(vii). three complementarity-determining regions HCDR1, HCDR2, and HCDR3 comprised in the VH as set forth in SEQ ID NO: 12, and three complementarity-determining regions LCDR1, LCDR2 and LCDR3 comprised in the VL as set forth in SEQ ID NO: 18; or

(viii). three complementarity-determining regions HCDR1, HCDR2 and HCDR3 comprised in the VH as set forth in SEQ ID NO: 14, and three complementarity-determining regions LCDR1, LCDR2 and LCDR3 comprised in the VL as set forth in SEQ ID NO: 18;

For example, the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 may be defined according to any scheme for determining CDRs, e.g., according to the Kabat, Chothia, IMGT, AbM, or North schemes, or a combination thereof; preferably, the HCDR1 is determined according to the Kabat & Chothia scheme, and the HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 are each

defined according to the Kabat scheme.

In some particular embodiments of the present invention, the anti-DLL3 antibody or antigen-binding fragment thereof of the present invention comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, wherein

the HCDR1 consists of the amino acid sequence of SEQ ID NO: 1; the HCDR2 consists of the amino acid sequence of SEQ ID NO: 2, 3, 4, or 5; the HCDR3 consists of the amino acid sequence of SEQ ID NO: 6; the LCDR1 consists of the amino acid sequence of SEQ ID NO: 7; the LCDR2 consists of the amino acid sequence of SEQ ID NO8; and the LCDR3 consists of the amino acid sequence of SEQ ID NO: 9.

In some particular embodiments of the present invention, the anti-DLL3 antibody or antigen-binding fragment thereof of the present invention comprises a VH and a VL, wherein

the VH comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 13, and the VL comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 18;

the VH comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 12, and the VL comprises or consists of the amino acid sequence as set forth in SEQ ID NO:16;

the VH comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 13, and the VL comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 16;

the VH comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 14, and the VL comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 16;

the VH comprises or consists of the amino acid sequence as set forth in SEQ ID NO:15, and the VL comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 16;

the VH comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 12, and the VL comprises or consists of the amino acid sequence as set forth in SEQ ID NO:17;

the VH comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 12, and the VL comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 18; or

the VH comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 14, and

the VL comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 18.

In one embodiment of the present invention, the amino acid alterations described herein include amino acid substitutions, insertions, or deletions. In a preferred embodiment, the amino acid alterations described herein occur in regions outside the CDRs (e.g., in FRs). More preferably, the amino acid alterations described herein occur in a region outside the heavy chain variable region and/or outside the light chain variable region. Preferably, the amino acid alterations described herein are amino acid substitutions. In some embodiments, the amino acid substitution is a conservative substitution.

In a preferred embodiment of the present invention, the anti-DLL3 antibody or antigen-binding fragment thereof of the present invention comprises a mutation that removes a post-translational modification (PTM), such as an amino acid substitution. In one embodiment, a hotspot site in the anti-DLL3 antibody or antigen-binding fragment thereof of the present invention is mutated; for example, any one or two of the consecutive amino acids DG in a heavy chain variable region CDR, such as HCDR2, are mutated, e.g., D is mutated to E or Q, and/or G is mutated to V. In one embodiment, a glycosylation site in the anti-DLL3 antibody or antigen-binding fragment thereof of the present invention is mutated to eliminate glycosylation; for example, for example, any one or more of the consecutive amino acids NFS in the framework region of the light chain variable region (e.g., FR3) are mutated, for example, N of NFS is mutated into Q, or S of NFS is mutated into A.

In some embodiments, the anti-DLL3 antibody or antigen-binding fragment thereof of the present invention is an antibody in the form of IgG1, IgG2, IgG3, or IgG4, for example, an antibody in the form of IgG1 or an antigen-binding fragment thereof.

In some embodiments, the anti-DLL3 antibody or antigen-binding fragment thereof of the present invention further comprises an antibody heavy chain constant region HC. In some embodiments, the anti-DLL3 antibody or antigen-binding fragment thereof of the present invention further comprises an antibody light chain constant region LC. In some embodiments, the anti-DLL3 antibody or antigen-binding fragment thereof of the present invention further comprises a heavy chain constant region HC and a light chain constant region LC. In some embodiments, the heavy chain constant region of the present invention comprises an Fc region.

In some embodiments, the heavy chain constant region of the anti-DLL3 antibody of the present invention is derived from IgG, e.g., the heavy chain constant region of human IgG, such as the heavy chain constant region of (human) IgG1, IgG2, IgG3, or IgG4, e.g., tthe heavy chain constant region of human IgG1, IgG2, IgG3, or IgG4, such as a heavy chain constant region of human IgG1.

In some embodiments, the IgG1 heavy chain constant region suitable for use in the antibody molecule of the present invention

(i) comprises or consists of an amino acid sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence as set forth in SEQ ID NO: 23;

(ii) comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 23; or

(iii) comprises or consists of an amino acid sequence having one or more (preferably no more than 10 or 10, more preferably no more than 5, 4, 3, 2, or 1) amino acid alterations (preferably amino acid substitutions, more preferably conservative amino acid substitutions) as compared to the amino acid sequence as set forth in SEQ ID NO: 23.

In some embodiments, the heavy chain constant region comprises a mutation that reduces binding to the Fcγ receptor, such as a LALA mutation.

In some embodiments, the heavy chain constant region described herein comprises an Fc region, e.g., a wild-type Fc region or a mutated Fc region. For example, the heavy chain constant region described herein may comprise an Fc region having modifications in the effector functions of the Fc region (e.g., complement activation functions of the Fc region) or an Fc region having modifications that alter the binding affinity to one or more Fc receptors.

In some embodiments, the light chain constant region of the anti-DLL3 antibody of the present invention is a light chain constant region from a Lambda or Kappa light chain constant region, e.g., a Lambda or Kappa light chain constant region, such as a human Lambda or Kappa light chain constant region.

In some embodiments, the light chain constant region

(i) comprises or consists of an amino acid sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence as set forth in SEQ ID NO: 24 or 25;

(ii) comprises or consists of the amino acid sequence as set forth in SEQ ID NO:24 or 25; or

(iii) comprises or consists of an amino acid sequence having one or more (preferably no more than 10 or 10, and more preferably no more than 5, 4, 3, 2, or 1) amino acid alterations (preferably amino acid substitutions, more preferably conservative amino acid substitutions) as compared to the amino acid sequence as set forth in SEQ ID NO: 24 or 25.

In some embodiments, the anti-DLL3 antibody or antigen-binding fragment thereof of the present invention comprises an antibody heavy chain. In some embodiments, the anti-DLL3 antibody or antigen-binding fragment thereof of the present invention comprises an antibody light chain. In some embodiments, the anti-DLL3 antibody or antigen-binding fragment thereof of the present invention comprises a heavy chain and a light chain. In some embodiments, the anti-DLL3 antibody or antigen-binding fragment thereof of the present invention comprises two heavy chains and two light chains. In some embodiments, the anti-DLL3 antibody or antigen-binding fragment thereof of the present invention comprises two identical heavy chains and two identical light chains. In some embodiments, the heavy chain comprises or consists of a heavy chain variable region and a heavy chain constant region. In some embodiments, the light chain comprises or consists of a light chain variable region and a light chain constant region.

In some embodiments, the anti-DLL3 antibody or antigen-binding fragment thereof has one or more of the following properties:

(i) showing the same or similar binding affinity and/or specificity for DLL3 as the antibody of the present invention;

(ii) inhibiting (e.g., competitively inhibiting) the binding of the antibody of the present invention to DLL3;

(iii) binding to the same or overlapping epitope as the antibody of the present invention;

(iv) competing with the antibody of the present invention for binding to DLL3;

(v) having one or more biological properties of the antibody of the present invention.

In some embodiments, the anti-DLL3 antibody is a monoclonal antibody.

In some embodiments, the anti-DLL3 antibody is a full-length antibody.

In some embodiments, the anti-DLL3 antibody is humanized. Humanization can be achieved by replacing one or more amino acid residues, particularly framework region sequences, in the heavy and light chain variable regions of a non-human natural antibody with residues from the corresponding positions in the variable regions of a human conventional antibody. Methods of humanizing antibodies are well known in the art. Typically, the humanization substitutions are made in a manner that preserves the favorable binding properties of the antibody. Assays for determining biological properties of the humanized antibody, such as binding affinity, are well known in the art to determine and select suitable mutations or combinations of mutations of humanized residues.

In one embodiment, the anti-DLL3 antibody of the present invention also encompasses an antibody fragment (e.g., an antigen-binding fragment) thereof, preferably an antibody fragment selected from: an Fab, an Fab', an Fab'-SH, an Fv, a single-chain antibody (e.g., scFv), an $(Fab')_2$, a single-domain antibody (e.g., VHH), a diabody, a domain antibody (dAb), a heavy-chain antibody, or a linear antibody. In one embodiment, the anti-DLL3 antibody fragment of the present invention is an Fab comprising the VH and VL described herein, as well as a CH1 and a CL.

In one embodiment, the anti-DLL3 antibody of the present invention may also be a bispecific antibody or a multispecific antibody that specifically binds to DLL3 and one or more additional antigens (e.g., CD3).

III. Multispecific Binding Molecule

In one aspect of the present invention, the present invention relates to a multispecific binding molecule capable of specifically binding to DLL3 and specifically binding to one or more additional targets or antigens. In some embodiments, the additional antigen is CD3. In some embodiments, the multispecific binding molecule is a multispecific antibody, e.g., a bispecific antibody.

In some embodiments, the antibody of the present invention is a bispecific antibody. The term "bispecific antibody" refers to an antibody comprising a first antigen-binding region and a second antigen-binding region, wherein the first antigen-binding region binds to one antigen or epitope and the second antigen-binding region binds to another antigen or another epitope. Therefore, the bispecific antibody according to the present invention comprises specificity for two different

antigens, or for two different epitopes of one antigen. Bispecific antibody formats comprise IgG-like antibodies (Fan *et al.* (2015) Journal of Hematology & Oncology. 8: 130). The most common type of IgG-like antibodies comprises two Fab regions and two Fc regions, and the heavy and light chains of each Fab may be from a separate monoclonal antibody. The bispecific antibody of the present invention can be prepared using bispecific antibody formats or techniques known in the art. Specific exemplary bispecific formats that can be used in the context of the present invention can be found, for example, in Labrijn, *et al.*, Bispecific antibodies: a mechanistic review of the pipeline, Nature Reviews Drug Discovery, 2019, 18(8):1-24.

Therefore, one aspect of the present invention relates to a bispecific antibody, which comprises

a first antigen-binding region and a second antigen-binding region, wherein the first antigen-binding region specifically binds to DLL3, and the second antigen-binding region specifically binds to CD3.

The first antigen-binding region suitable for use in the bispecific antibody of the present invention may comprise or consist of the anti-DLL3 full-length antibody or antigen-binding fragment thereof of the present invention, as long as it is capable of specifically binding to DLL3, including, but not limited to, for example, a full-length antibody, a half-antibody, an Fab, an Fab', an Fab'-SH, an Fv, a single-chain antibody (e.g., scFv), or an $(Fab')_2$, a single-domain antibody (e.g., VHH), a domain antibody (dAb), a diabody, a heavy-chain antibody, or a linear antibody specifically binding to DLL3, or the like.

In some embodiments, the first antigen-binding region is derived from the anti-DLL3 antibody or antigen-binding fragment thereof described herein, e.g., the Fab of the anti-DLL3 antibody described herein. In some embodiments, the first antigen-binding region specifically binding to DLL3 comprises 1, 2, 3, 4, 5, or 6 CDRs of the anti-DLL3 antibody described herein. In some embodiments, the first antigen-binding region comprises 1, 2, or 3 heavy chain variable region CDRs, i.e., HCDR1, HCDR2, and HCDR3, of the anti-DLL3 antibody described herein. In some embodiments, the first antigen-binding region comprises 1, 2, or 3 light chain variable region CDRs, i.e., LCDR1, LCDR2, and LCDR3, of the anti-DLL3 antibody described herein. In some embodiments, the first antigen-binding region comprises 3 heavy chain variable region CDRs and 3

light chain variable region CDRs of the anti-DLL3 antibody described herein. In some embodiments, the first antigen-binding region comprises the heavy chain variable region of the anti-DLL3 antibody described herein. In some embodiments, the first antigen-binding region comprises the light chain variable region of the anti-DLL3 antibody described herein. In some embodiments, the first antigen-binding region comprises the heavy chain variable region and the light chain variable region of the anti-DLL3 antibody described herein. In some embodiments, the first antigen-binding region comprises or consists of the Fab of the anti-DLL3 antibody described herein.

The second antigen-binding region suitable for use in the bispecific antibody of the present invention may comprise or consist of the anti-CD3 antibody or an antigen-binding fragment thereof, as long as it is capable of specifically binding to CD3, including, but not limited to, for example, a full-length antibody, a half-antibody, an Fab, an Fab', an Fab'-SH, an Fv, a single-chain antibody (e.g., scFv), and an $(Fab')_2$., a single-domain antibody (e.g., VHH), a domain antibody (dAb), a diabody, a heavy-chain antibody, or a linear antibody specifically binding to CD3, or the like.

In some embodiments, the second antigen-binding region is derived from the anti-CD3 antibody or antigen-binding fragment thereof, e.g., the Fab of anti-CD3. In some embodiments, the second antigen-binding region suitable for use in the bispecific antibody of the present invention may comprise or consist of the anti-CD3 antibody or antigen-binding fragment thereof (e.g., the anti-CD3 antibody or antigen-binding fragment thereof disclosed in WO2022068809A1, e.g., the HzSP34.24 antibody or antigen-binding fragment thereof), as long as it is capable of specifically binding to CD3, including, but not limited to, for example, a full-length antibody, a half-antibody, an Fab, an Fab', an Fab'-SH, an Fv, a single-chain antibody (e.g., scFv), and an $(Fab')_2$, a single-domain antibody (e.g., VHH), a diabody, a heavy-chain antibody, or a linear antibody specifically binding to CD3, or the like.

In some embodiments, the bispecific antibody of the present invention is an IgG-like bispecific antibody. In some embodiments, the IgG-like bispecific antibody of the present invention refers to a bispecific antibody comprising an Fc dimer (e.g., a heterodimeric Fc scaffold). Thus, in some embodiments, the bispecific antibody of the present invention comprises an Fc dimer, e.g., a heterodimeric Fc scaffold.

In one embodiment, the bispecific antibody may comprise one or more first antigen-binding regions. In one embodiment, the bispecific antibody may comprise one or more second antigen-binding regions. In some embodiments, the bispecific antibody comprises 2 first antigen-binding regions and 2 second antigen-binding regions.

The present invention has been described in detail with respect to components of multispecific binding molecules, such as bispecific antibodies e.g., antigen-binding regions or domains comprised therein, such as VH, VL, CL, CH1, or Fc regions. It will be understood by those skilled in the art that any combination of any of the technical features of these components is within the scope of the invention, unless otherwise expressly stated to the contrary. Furthermore, it will be understood by those skilled in the art that the antibody of the present invention (including any form of antibody) may comprise any such combination, unless otherwise expressly stated to the contrary.

III-1 Second Antigen-binding Region

In some embodiments, the second antigen-binding region specifically binds to CD3.

In some embodiments, the second antigen-binding region is an antigen-binding region specifically binding to CD3. In some embodiments, the second antigen-binding region specifically binding to CD3 is derived from an antibody specifically binding to CD3, e.g., the antibody specifically binding to CD3 disclosed in WO2022068809A1 (incorporated herein in its entirety), e.g., HzSP34.24 disclosed therein.

In some embodiments, the second antigen-binding region specifically binding to CD3 comprises 1, 2, 3, 4, 5, or 6 CDRs of an antibody known to specifically bind to CD3 (e.g., the CD3 antibody disclosed in WO2022068809A1, e.g., HzSP34.24 disclosed therein). In some embodiments, the second antigen-binding region comprises 1, 2, or 3 heavy chain variable region CDRs, i.e., HCDR1, HCDR2, and HCDR3, of an antibody known to specifically bind to CD3 (e.g., the CD3 antibody disclosed in WO2022068809A1, e.g., HzSP34.24 disclosed therein). In some embodiments, the second antigen-binding region comprises 1, 2, or 3 light chain variable region CDRs, i.e., LCDR1, LCDR2, and LCDR3, of an antibody known to specifically bind to CD3 (e.g., the CD3 antibody disclosed in WO2022068809A1, such as HzSP34.24 disclosed therein). In some embodiments, the second antigen-binding region comprises 3 heavy chain variable region CDRs

and 3 light chain variable region CDRs of an antibody known to specifically bind to CD3 (e.g., the CD3 antibody disclosed in WO2022068809A1, such as HzSP34.24 disclosed therein). In some embodiments, the second antigen-binding region comprises a heavy chain variable region of an antibody known to specifically bind to CD3 (e.g., the CD3 antibody disclosed in WO2022068809A1, such as HzSP34.24 disclosed therein). In some embodiments, the second antigen-binding region comprises a light chain variable region of an antibody known to specifically bind to CD3 (e.g., the CD3 antibody disclosed in WO2022068809A1, such as HzSP34.24 disclosed therein). In some embodiments, the second antigen-binding region comprises a heavy chain variable region and a light chain variable region of an antibody known to specifically bind to CD3 (e.g., the CD3 antibody disclosed in WO2022068809A1, such as HzSP34.24 disclosed therein). In some embodiments, the second antigen-binding region comprises an Fab of an antibody known to specifically bind to CD3 (e.g., the antibody specifically binding to CD3 disclosed in WO2022068809A1, e.g., HzSP34.24 disclosed therein).

In some embodiments, the second antigen-binding region specifically binding to CD3 comprises three complementarity-determining regions from a heavy chain variable region (HCDRs): HCDR1, HCDR2, and HCDR3, wherein the HCDR1, HCDR2, and HCDR3 are the HCDR1, HCDR2, and HCDR3 of the heavy chain variable region as set forth in SEQ ID NO: 31. In some embodiments, the second antigen-binding region specifically binding to CD3 comprises three complementarity-determining regions from a light chain variable region (LCDRs): LCDR1, LCDR2, and LCDR3, wherein the LCDR1, LCDR2, and LCDR3 are the LCDR1, LCDR2, and LCDR3 of the light chain variable region as set forth in SEQ ID NO: 35. In some embodiments, the HCDR1 of the second antigen-binding region specifically binding to CD3 comprises or consists of the amino acid sequence of SEQ ID NO: 32; hCDR2 comprises or consists ofSEQ ID NO: 33, HCDR3 comprises or consists of the amino acid sequence of SEQ ID NO: 34, LCDR1 comprises or consists of the amino acid sequence of SEQ ID NO: 36, LCDR2 comprises or consists of the amino acid sequence of SEQ ID NO: 37 and/or LCDR3 comprises or consists of the amino acid sequence of SEQ ID NO: 38.

In some embodiments, the second antigen-binding region specifically binding to CD3 comprises a VH, wherein the VH

(a) comprises three complementarity-determining regions from a heavy chain variable region (HCDRs): HCDR1, HCDR2, and HCDR3, wherein the HCDR1 comprises or consists of the amino acid sequence of SEQ ID NO: 32; the hCDR2 comprises or consists of the amino acid sequence of SEQ ID NO: 33, and the HCDR3 comprises or consists of the amino acid sequence of SEQ ID NO: 34; or

(b) comprises or consists of the amino acid sequence of SEQ ID NO: 31.

In some embodiments, the second antigen-binding region specifically binding to CD3 comprises a VL, wherein the VL

(a) comprises three complementarity-determining regions from a light chain variable region (HCDRs): HCDR1, HCDR2, and HCDR3, wherein the LCDR1 comprises or consists of the amino acid sequence of SEQ ID NO: 36, the LCDR2 comprises or consists of the amino acid sequence of SEQ ID NO: 37, and the LCDR3 comprises or consists of the amino acid sequence of SEQ ID NO: 38; or

(b) comprises or consists of the amino acid sequence of SEQ ID NO: 35.

In some embodiments, the second antigen-binding region specifically binding to CD3 comprises three complementarity-determining regions from a heavy chain variable region (HCDRs): HCDR1, HCDR2, and HCDR3, and three complementarity-determining regions from a light chain variable region (LCDRs): LCDR1, LCDR2, and LCDR3, wherein the HCDR1 comprises or consists of the amino acid sequence of SEQ ID NO: 32; the hCDR2 comprises or consists of the amino acid sequence of SEQ ID NO: 33, the HCDR3 comprises or consists of the amino acid sequence of SEQ ID NO: 34, the LCDR1 comprises or consists of the amino acid sequence of SEQ ID NO: 36, the LCDR2 comprises or consists of the amino acid sequence of SEQ ID NO: 37, and the LCDR3 comprises or consists of the amino acid sequence of SEQ ID NO: 38.

In some embodiments, the second antigen-binding region specifically binding to CD3 comprises a VH and a VL, wherein the VH comprises or consists of the sequence as set forth in SEQ ID NO: 31; and/or the VL comprises or consists of the sequence as set forth in SEQ ID NO: 35.

In some embodiments, the second antigen-binding region specifically binding to CD3 is an Fab fragment.

III-2 Fab Fragment

In some embodiments, the first antigen-binding region and/or the second antigen-binding region of the present invention are Fab fragments. The Fab fragments suitable for use as the antigen-binding regions of the multispecific antibodies (such as bispecific antibodies) described herein a are composed of two polypeptide chains comprising VH, CH1, VL, and CL domains of the antibody, wherein the VH is paired with the VL, and the CH1 is paired with the CL to form the antigen-binding region. In some embodiments, in the Fab, one chain comprises or consists of, from N-terminus to C-terminus, a VH and a CH1 (i.e., VH-CH1), and the other chain comprises or consists of, from N-terminus to C-terminus, a VL and a CL (i.e., VL-CL). In some embodiments, In the the multispecific antibodies (such as bispecific antibodies) of the present invention, the Fab may be linked to the N-terminus of the Fc domain of the antibody via the C-terminus of the chain comprising the VH. Preferably, the Fab comprises a VH-CH1 chain and a VL-CL chain and may be linked to the Fc domain of the antibody via the C-terminus of the CH1 of the VH-CH1 chain. In some embodiments, the linkage is direct or via a linker. Herein, an Fab chain comprising or consisting of VH-CH1 is also referred to as a nFab heavy chain, and an Fab chain comprising or consisting of VL-CL is also referred to as an Fab light chain.

In some embodiments, the CH1 is a CH1 derived from IgG1, IgG2, IgG3, or IgG4, preferably a CH1 derived from IgG1, preferably a CH1 derived from human IgG1, IgG2, IgG3, or IgG4, such as a CH1 of human IgG1, IgG2, IgG3, or IgG4. In some embodiments, the CH1

(i) comprises or consists of an amino acid sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence as set forth in SEQ ID NO: 28;

(ii) comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 28; or

(iii) comprises or consists of an amino acid sequence having one or more (preferably no more than 10, and more preferably no more than 5, 4, 3, 2, or 1) amino acid alterations (preferably amino acid substitutions, and more preferably conservative amino acid substitutions) as compared to the amino acid of SEQ ID NO: 28.

In some embodiments, the CL is a light chain constant region from a Lambda or Kappa light chain constant region, e.g., a Lambda or Kappa light chain constant region, such as a human Lambda or Kappa light chain constant region. In some embodiments, the CL is a human Kappa light chain constant region or a human Lambda light chain constant region. In some embodiments, the CL

(i) comprises or consists of an amino acid sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence of SEQ ID NO: 24 or 25;

(ii) comprises or consists of the amino acid sequence of SEQ ID NO: 24 or 25; or

(iii) comprises or consists of an amino acid sequence having one or more (preferably no more than 10, and more preferably no more than 5, 4, 3, 2, or 1) amino acid alterations (preferably amino acid substitutions, and more preferably conservative amino acid substitutions) as compared to the amino acid sequence of SEQ ID NO: 24 or 25.

In some embodiments, the first antigen-binding region is an Fab specifically binding to DLL3, wherein the Fab fragment is from the anti-DLL3 antibody of the present invention and comprises the heavy chain variable region VH and the light chain variable region VL of the anti-DLL3 antibody of the present invention. In some embodiments, the Fab heavy chain of the Fab specifically binding to DLL3 as the first antigen-binding region comprises or consists of a VH and a CH1, wherein the VH is the VH of the anti-DLL3 antibody of the present invention. In some embodiments, the Fab light chain of the Fab specifically binding to DLL3 as the first antigen-binding region comprises or consists of a VL and a CL, wherein the VL is the VL of the anti-DLL3 antibody of the present invention. In some embodiments, the first antigen-binding region comprises a CH1 from IgG1 and/or a Kappa light chain constant region.

In some embodiments, the Fab heavy chain specifically binding to DLL3

(i) comprising and or consists of an amino acid sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence as set forth in SEQ ID NOs: 19, 20, 21, or 22;

(ii) comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 19, 20, 21 or 22; or

(iii) comprises or consists ofan amino acid sequence having one or more (preferably no more

than 10, and more preferably no more than 5, 4, 3, 2, or 1) amino acid alterations (preferably amino acid substitutions, and more preferably conservative amino acid substitutions) as compared to an amino acid as set forth in SEQ ID NOs: 19, 20, 21, and 22.

In some embodiments, the Fab light chain specifically binding to DLL3

(i) comprises or consists of an amino acid sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence as set forth in SEQ ID NOs: 45, 46, or 47;

(ii) comprises or consists of the amino acid sequence as set forth in SEQ ID NOs: 45, 46, or 47; or

(iii) comprises or consists of an amino acid sequence having one or more (preferably no more than 10, and more preferably no more than 5, 4, 3, 2, or 1) amino acid alterations (preferably amino acid substitutions, and more preferably conservative amino acid substitutions) as compared to an amino acid as set forth in SEQ ID NOs: 45, 46, and 47.

In some embodiments, the Fab specifically binding to DLL3 comprises or consists of an Fab heavy chain and an Fab light chain, wherein

the Fab heavy chain comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 19, and the Fab light chain comprises or consists of or consists of the amino acid sequence as set forth in SEQ ID NO: 45;

Fab heavy chain comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 20, and the Fab light chain comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 45;

Fab heavy chain comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 21, and the Fab light chain comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 45;

Fab heavy chain comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 22, and the Fab light chain comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 45;

Fab heavy chain comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 19, and the Fab light chain comprises or consists of the amino acid sequence as shown in SEQ ID NO: 46;

Fab heavy chain comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 19, and the Fab light chain comprises or consists of the amino acid sequence as shown in SEQ ID NO: 47;

Fab heavy chain comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 20, and the Fab light chain comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 47; or

Fab heavy chain comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 21, and the Fab light chain comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 47.

In some embodiments, the second antigen-binding region is an Fab specifically binding to CD3, wherein the Fab fragment is derived from the anti-CD3 antibody and comprises the heavy chain variable region VH and the light chain variable region VL of the anti-CD3 antibody.

In some embodiments, the Fab as the second antigen-binding region comprises a VH or a VL of the second antigen-binding region described herein, or comprises a VH and a VL of the second antigen-binding region described herein.

In some embodiments, the Fab heavy chain of the antibody specifically binding to CD3 as the second antigen-binding region comprises or consists of a VH and a CH1, wherein the VH is the VH of the second antigen-binding region described herein. In some embodiments, the Fab light chain specifically binding to CD3 as the second antigen-binding region comprises or consists of a VL and a CL, wherein the VL is the VL of the second antigen-binding region described herein. In some embodiments, the second antigen-binding region comprises CH1 from IgG1 and/or a Lambda light chain constant region.

In some embodiments, the Fab heavy chain specifically binding to CD3

(i) comprises or consists of an amino acid sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence of SEQ ID NO: 39;

(ii) comprises or consists of the amino acid sequence of SEQ ID NO: 39; or

(iii) comprises or consists of an amino acid sequence having one or more (preferably no more than 10 or 10, and more preferably no more than 5, 4, 3, 2, or 1) amino acid alterations (preferably amino acid substitutions, and more preferably conservative amino acid substitutions) as compared

to the amino acid sequence of SEQ ID NO: 39.

In some embodiments, the Fab light chain specifically binding to CD3

(i) comprises or consists of an amino acid sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence of SEQ ID NO: 40;

(ii) comprises or consists of the amino acid sequence of SEQ ID NO: 40; or

(iii) comprises or consists of an amino acid sequence having one or more (preferably no more than 10 or 10, and more preferably no more than 5, 4, 3, 2, or 1) amino acid alterations (preferably amino acid substitutions, and more preferably conservative amino acid substitutions) as compared to the amino acid sequence of SEQ ID NO: 40.

In some embodiments, the Fab heavy chain specifically binding to CD3 comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 39, and the Fab light chain comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 40.

In some embodiments, the light chain constant region of the Fab light chain of the first antigen-binding region is the same as or different from the light chain constant region of the Fab light chain of the second antigen-binding region. In some embodiments, the light chain constant regions of the Fab light chains of the first antigen-binding region and the second antigen-binding region are both a Kappa light chain constant region or a Lambda light chain constant region. In some embodiments, the light chain constant region of the Fab light chain of the first antigen-binding region is a Kappa light chain constant region, and the light chain constant region of the Fab light chain of the second antigen-binding region is a Lambda light chain constant region; or the light chain constant region of the Fab light chain of the first antigen-binding region is a Lambda light chain constant region, and the light chain constant region of the Fab light chain of the second antigen-binding region is a Kappa light chain constant region.

III-3 Fc Region

In some embodiments, one or more target-binding regions (antigen-binding regions) of the multispecific antibody (e.g., a bispecific antibody) of the present invention further comprise Fc regions, wherein the Fc regions contained may be the same or different.

In some embodiments, the first Fc region and the second Fc region are different and are capable of dimerizing to form a heterodimeric Fc scaffold.

Herein, the Fc region refers to a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region, and may include Fc regions of native sequences and variant Fc regions. The F regions of native sequences encompass naturally occurring Fc sequences of various immunoglobulins, such as Fc regions of various Ig subclasses or allotypes thereof (Gestur Vidarsson *et al.*, IgG subclasses and allotypes: from structure to effector functions, 20 October 2014, doi: 10.3389/fimmu.2014.00520). In some embodiments, the Fc region of the present invention comprises a CH2 and a CH3 of the antibody. In some embodiments, the antibody Fc region may also bear an IgG hinge region or a part of the IgG hinge region at the N-terminus, e.g., an IgG1 hinge region or a part of the IgG1 hinge region, e.g., according to EU numbering, a sequence of D221 to P230. The hinge region may comprise a mutation.

Unless otherwise indicated herein, amino acid residues in the Fc region are numbered according to the EU numbering system, also known as the EU index, as described in Kabat, E.A. *et al.*, Sequences of Proteins of Immunological Interest, 5th Ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991), NIH Publication 91-3242.

In some embodiments, the Fc region is human IgG Fc, e.g., human IgG1 Fc, human IgG2 Fc, human IgG3 Fc, or human IgG4 Fc. In one embodiment, the Fc region comprises or consists of the amino acid sequence of SEQ ID NO: 26 or 27, oran amino acid sequence having at least 90% identity, e.g., 95%, 96%, 97%, 98%, 99% or higher identity thereto.

As understood by those skilled in the art, to facilitate the formation of the multispecific antibody of the present invention as a heterodimer, the Fc region comprised in the multispecific antibody of the present invention may comprise mutations that favor the heterodimerization of the first Fc region and the second Fc region. In one embodiment, mutations are introduced into the CH3 regions of the two Fc regions.

Methods for promoting the heterodimerization of the Fc regions are known in the art.For example, the CH3 region of the first Fc region and the CH3 region of the second Fc region are engineered in a complementary manner, such that each CH3 region (or the heavy chain comprising the same) can no longer homodimerize with itself but is forced to heterodimerize with other CH3 regions that are complementarily engineered (such that heterodimerization occurs between the CH3

regions of the first and second Fc regions and no homodimers are formed between the two first CH3 regions or the two second CH3 regions). For example, corresponding Knob and Hole mutations are introduced into the first Fc region and the second Fc region based on the Knob-into-Hole technique. See, for example, US 5,731,168; US 7,695,936; Ridgway *et al.*, Prot Eng 9, 617-621 (1996) and Carter, J Immunol Meth 248, 7-15 (2001).

Corresponding mutations can also be introduced into the first CH3 region of the first Fc region and the second CH3 region of the second Fc region based on the Innobody technology. See, for example, PCT/CN2021/143141 (said patent incorporated herein in its entirety).

In one particular embodiment, the first CH3 region comprises an S364R/K mutation (preferably S364R), and optionally one or more additional mutations. In some embodiments, the second CH3 region comprises a K370S/T/A/V mutation (preferably K370S), and optionally one or more additional mutations. In some embodiments, the first CH3 region comprises an S364R/K mutation, and the second CH3 region comprises a K370S/T/A/V mutation. In some embodiments, the first CH3 region comprises an S364R mutation, and the second CH3 region comprises a K370S mutation.

In some embodiments, the first CH3 region comprises an S364R/K (preferably S364R) mutation and a D399K/R (preferably D399K) mutation. In some embodiments, the second CH3 region comprises a K370S/T/A/V (preferably K370S) mutation and a K409D/E (preferably K409D) mutation and/or Y349 T/S/A/V mutations. In some embodiments, the first CH3 region comprises S364R/K + D399K/R, and the second CH3 region comprises K370S/T/A/V + Y349T/S/A/V. In some embodiments, the first CH3 region comprises S364R + D399K, and the second CH3 region comprises K370S + Y349T. In some embodiments, the first CH3 region further comprises E375N/Q (preferably E375N) and/or T350V/A (preferably T350V). In some embodiments, the second CH3 region further comprises K409D/E (preferably K409D), Q347D/E (preferably Q347D), and/or T350V/A (preferably T350V).

In some embodiments, the first CH3 region comprises S364R + D399K, and the second CH3 region comprises K370S + Y349T + K409D. In some embodiments, the first CH3 region further comprises E357N. In some embodiments, the second CH3 region further comprises Q347D. In

some embodiments, the first CH3 region further comprises E357N, and the second CH3 region further comprises Q347D. In some embodiments, the first CH3 region and the second CH3 region each further comprise T350V, or both comprise T350V.

Thus, in some embodiments, the first CH3 region comprises S364R + D399K, and the second CH3 region comprises K370S + Y349T + K409D + Q347D. In some embodiments, the first CH3 region comprises S364R + D399K + E357N, and the second CH3 region comprises K370S + Y349T + K409D + Q347D. In some embodiments, the first CH3 region comprises S364R + D399K + E357N + T350V, and the second CH3 region comprises K370S + Y349T + K409D + Q347D + T350V.

In some embodiments, the first CH3 region comprises K409E/D (preferably K409E). In some embodiments, the second CH3 region comprises D399K/R (preferably D399K) or K370T/S/A/V (preferably K370T). In some embodiments, the first CH3 region comprises K409E/D (preferably K409E), and the second CH3 region comprises D399K/R (preferably D399K). In some embodiments, the first CH3 region further comprises T411R/K (preferably T411R). In some embodiments, the second CH3 region further comprises K370T/S/A/V (preferably K370T). In some embodiments, the CH3 region comprises K409E/D + T411R/K, and the second CH3 region comprises D399K/R + K370T/S/A/V. In some embodiments, the CH3 region comprises K409E + T411R, and the second CH3 region comprises D399K + K370T.

In some particular embodiments, the first CH3 region and the second CH3 region have the following 5 mutation combinations:

First CH3 region	Second CH3 region
S364R, D399K, E357N, T350V	K370S, Y349T, Q347D, K409D, T350V
K409E, T411R	D399K, K370T
S364R, D399K, E357N	K370S, Y349T, Q347D, K409D
S364R, D399K	K370S, Y349T, Q347D, K409D
S364R, D399K	K370S, Y349T, K409D

In one embodiment, the CH3 of one Fc region comprises S364R and D399K mutations, and the CH3 mutation of the other Fc region comprises Y349T, K370S, and K409D mutations.

In some embodiments, the Fc region further comprises other mutations that facilitate heterodimer purification.

The Fc region in the binding molecule (such as an antibody molecule) of the present invention can also be mutated to obtain desired properties. Mutations to the Fc region are known in the art.

In one embodiment, the Fc region is modified in properties of effector functions of the Fc region (e.g., complement activation functions of the Fc region). In one embodiment, the effector functions have been reduced or eliminated relative to a wild-type isotype Fc region. In one embodiment, the effector function is reduced or eliminated by using a method selected from: using an Fc isotype that naturally has a reduced or an eliminated effector function, and modifying the Fc region.

In one preferred embodiment, the Fc region has reduced effector functions mediated by the Fc region, such as reduced or eliminated ADCC or ADCP or CDC effector functions, for example, comprising mutations for achieving the above functions.

As understood by those skilled in the art, according to the intended use of the binding molecule (e.g., an antibody molecule) of the present invention, the binding molecule (e.g., an antibody molecule) of the present invention may also comprise modifications to the Fc domain that alter the binding affinity for one or more Fc receptors. In one embodiment, the Fc receptor is an Fcγ receptor, particularly a human Fcγ receptor. In some embodiments, the Fc region comprises a mutation that reduces the binding to Fcγ receptors. For example, in some embodiments, the Fc region used in the present invention has a L234A/L235A mutation that reduces the binding to Fcγ receptors. In yet another preferred embodiment, the Fc fragment may have a mutation that results in an increased serum half-life, e.g., a mutation that improves the binding of the Fc fragment to FcRn.

Thus, in one particular embodiment, the two Fc regions comprised in the antibodyof the present invention, such as a multispecific antibody, are heterodimerized, wherein

one Fc-region polypeptide comprises mutations S364R and D399K, and the other Fc-region polypeptide comprises mutations Y349T, K370S, and K409D.

In some embodiments, the Fc region further comprises a L234A/L235A mutation, and optionally a deletion of proline at position P329 (P329delP).

Thus, in one particular embodiment, the two Fc regions comprised in the antibody of the present invention, such as a multispecific antibody, are heterodimerized, wherein one Fc region comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 29, and the other Fc region polypeptide comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 30.

In one particular embodiment, the two Fc regions comprised in the antibody of the present invention, such as a multispecific antibody, are heterodimerized, wherein one Fc region comprises an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence as set forth in SEQ ID NO: 29, and the other Fc region polypeptide comprises an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence as set forth in SEQ ID NO: 30.

Thus, in one particular embodiment, the two Fc regions comprised in the binding molecule of the present invention, e.g., in an antibody such as a multispecific antibody, are heterodimerized, wherein

a) one Fc region comprises an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence as set forth in SEQ ID NO: 29, and comprises mutations S364R and D399K, and the other Fc region polypeptide comprises an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence as set forth in SEQ ID NO: 30, and comprises mutations Y349T, K370S, and K409D; or

b) one Fc region comprises an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence as set forth in SEQ ID NO: 29, and comprises mutations S364R and D399K and a L234A/L235A mutation, and the other Fc region polypeptide comprises an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence as set forth inSEQ ID NO: 30, and comprises mutations Y349T, K370S, and K409D, and a L234A/L235A mutation.

III-4. Exemplary Bispecific Antibody

In some embodiments, the bispecific antibody of the present invention comprises the first antigen-binding region specifically binding to the first antigen described herein and the second antigen-binding region specifically binding to the second antigen described herein, wherein the first antigen-binding region comprises a first Fab and the second antigen-binding region comprises a second Fab, wherein the first Fab is linked at the C-terminus of its CH1 to the N-terminus of the first Fc region (directly or via a linker such as a hinge region), and the second Fab is linked at the C-terminus of its CH1 to the N-terminus of the second Fc region (directly or via a linker such as a hinge region). In one particular embodiment, the first Fab and the first Fc region constitute a first half antibody binding to a first antigen, the second Fab and the second Fc region constitute a second half antibody binding to a second antigen, and the first half antibody and the second half antibody constitute the bispecific antibody of the present invention.

In one particular embodiment, the bispecific antibody is an IgG-like antibody having a configuration shown in FIG. 3. In one particular embodiment, the first Fc region and the second Fc region comprise Innobody mutations, and optionally a mutation that reduces effector function mediated by the Fc region or a mutation that reduces binding to Fcγ receptors. In one embodiment, the bispecific antibody comprises or consists of:

heavy chain 1: comprising or consisting of, from N-terminus to C-terminus, afirst Fab heavy chain variable region-a heavy chain constant region CH1-a first Fc region, wherein the heavy chain constant region CH1 is linked at its C-terminus to the N-terminus of the first Fc region directlyor via a linker (e.g., a hinge region);

light chain 1: comprising or consisting of, from N-terminus to C-terminus, a first Fab light chain variable region–a light chain constant region;

heavy chain 2: comprising or consisting of, from N-terminus to C-terminus, a second Fab heavy chain variable region-a heavy chain constant region CH1-a second Fc region, wherein the heavy chain constant region CH1 is linked at its C-terminus to the N-terminus of the second Fc region via or not via a linker (e.g., a hinge region);

light chain 2: comprising or consisting of, from the N-terminus to the C-terminus: a second

Fab light chain variable region –a light chain constant region.

In some embodiments, the Fab heavy chain variable region and the heavy chain constant region CH1 constitute an Fab heavy chain. In some embodiments, the Fab light chain variable region and the light chain constant region constitute the Fab light chain.

In some embodiments, the heavy chain variable region-heavy chain constant region CH1 of the first Fab and the light chain variable region-light chain constant region of the first Fab constitute a first Fab; the heavy chain variable region-heavy chain constant region CH1 of the second Fab and the light chain variable region-light chain constant region of the second Fab constitute a second Fab.

In some embodiments, the first Fc region comprises S364R and D399K mutations and the second Fc region comprises Y349T, K370S, and K409D mutations.

In one particular embodiment, the first Fab is an anti-DLL3 Fab of the present invention, and the second Fab is the anti-CD3 Fab, or vice versa.

In one particular embodiment, the bispecific antibody of the present invention specifically binds to DLL3 and CD3 and comprises or consists of the following:

heavy chain 1: comprising or consisting of, from N-terminus to C-terminus: an Fab heavy chain variable region specifically binding to DLL3–a heavy chain constant region CH1–a first Fc region, wherein the heavy chain constant region CH1 is linked at its C-terminus to the N-terminus of the first Fc region directly or via a linker (e.g., a hinge region);

light chain 1: comprising or consisting of, from N-terminus to C-terminus, an Fab light chain variable region specifically binding to DLL3–a light chain constant region;

heavy chain 2: comprising or consisting of, from N-terminus to C-terminus: an Fab heavy chain variable region specifically binding to CD3–a heavy chain constant region CH1–a second Fc region, wherein the heavy chain constant region CH1 is linked at its C-terminus to the N-terminus of the second Fc region directly or via a linker (e.g., a hinge region); and/or

light chain 2: comprising or consisting of, from N-terminus to C-terminus: an Fab light chain variable region specifically binding to CD3–a light chain constant region.

In some embodiments, the first Fc region comprises S364R and D399K mutations and the second Fc region comprises Y349T, K370S, and K409D mutations, or vice versa; optionally, the first Fc region and the second Fc region each further comprise a L234A/L235A mutation,. In some embodiments, the light chain constant region in the light chain 1 is the same as or different from the light chain constant region in the light chain 2. For example, the light chain constant region in the light chain 1 is a Kappa light chain constant region and the light chain constant region in the light chain 2 is a Lambda light chain constant region.

The domains of the bispecific antibody of the present invention are as defined herein.

In some particular embodiments, the bispecific antibody that specifically binds to DLL3 and CD3 comprises or consists of a heavy chain 1, a heavy chain 2, a light chain 1, and a light chain 2, wherein

the heavy chain 1 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 41, 42, 43, or 44, or an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto;

the light chain 1 comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 45, 46 or 47, or an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98% or 99% identity thereto;

the heavy chain 2 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 48, or an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto; and/or

the light chain 2 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 40, or an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

In some particular embodiments, the bispecific antibody that specifically binds to DLL3 and CD3 comprises or consists of a heavy chain 1, a heavy chain 2, a light chain 1, and a light chain 2, wherein

the heavy chain 1 comprises or consists of the amino acid sequence as set forth in SEQ ID NO:

41, or an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto; the light chain 1 comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 45, or an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto; the heavy chain 2 comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 48, or an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto; and the light chain 2 comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 40, or an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto;

the heavy chain 1 comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 42, or an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto; the light chain 1 comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 47, or an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto; the heavy chain 2 comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 48, or an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto; and the light chain 2 comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 40, or an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto; or

the heavy chain 1 comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 43, or an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto; the light chain 1 comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 47, or an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto; the heavy chain 2 comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 48, or an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto; and the light chain 2 comprises the amino acid sequence as set forth in SEQ ID NO: 40, or an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

In some particular embodiments, the bispecific antibody that specifically binds to DLL3 and CD3 comprises or consists of a heavy chain 1, a heavy chain 2, a light chain 1, and a light chain 2, wherein

the heavy chain 1 comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 41; the light chain 1 comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 45; the heavy chain 2 comprises or consists of the amino acid sequence the as set forth in SEQ ID NO: 48; and the light chain 2 comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 40;

the heavy chain 1 comprises or consists of the amino acid sequence as set forth in SEQ ID NO:42; the light chain 1 comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 47; the heavy chain 2 comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 48; and the light chain 2 comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 40; or

the heavy chain 1 comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 43; the light chain 1 comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 47; the heavy chain 2 comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 48; and the light chain 2 comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 40.

IV. Polynucleotide, Vector and Host Cell

The present invention provides a nucleic acid encoding any of the above antibody molecules of the present invention (e.g., an anti-DLL3 antibody or antigen-binding fragment thereof or a multispecific antibody such as a bispecific antibody of the present invention).

In one aspect, the present invention provides a nucleic acid encoding any of the aforementioned anti-DLL3 antibodies or antigen-binding fragments thereof, or multispecific antibodies such as bispecific antibodies.

For ease of production and purification, the anti-DLL3 antibody or antigen-binding fragment thereof or the multispecific antibody such as a bispecific antibody may be fused at the N-terminus or C-terminus (e.g., C-terminus) to a secretory signal peptide, and/or a tag peptide that facilitates purification, such as a hexahistidine tag or a biotin tag.

As will be appreciated by those skilled in the art, each antibody or polypeptide amino acid sequence can be encoded by a variety of nucleic acid sequences because of codon degeneracy.

In some embodiments, the nucleic acid of the present invention comprises a nucleic acid selected from any one of SEQ ID NOs: 10 and 11, or a nucleic acid having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the nucleic acid selected from any one of SEQ ID NOs: 10 and 11.

In some embodiments, the nucleic acid of the present invention comprises a nucleic acid encoding an amino acid sequence selected from any one of SEQ ID NOs: 12-22 and 41-47, or a nucleic acid encoding an amino acid sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence selected from any one of SEQ ID NOs: 12-22 and 41-47.

Nucleic acid sequences encoding the molecules of the present invention may be produced using methods well known in the art, for example by de novo solid phase DNA synthesis, or by PCR amplification.

In one aspect, the present invention also provides a vector comprising a nucleic acid of the present invention. In one embodiment, the vector is an expression vector, e.g., a prokaryotic expression vector or a eukaryotic expression vector. The vector includes, but is not limited to, a virus, a plasmid, a cosmid, a Lambda phage, or a yeast artificial chromosome (YAC). In a preferred embodiment, the expression vector is pCDNA, such as pCDNA3.1.

In one aspect, the present invention also provides a host cell comprising the nucleic acid or the vector. In one embodiment, the host cell is eukaryotic. In another embodiment, the host cell is selected from a yeast cell and a mammalian cell (e.g., a CHO cell or a 293 cell, such as a HEK 293 or a 293F cell or a 293FT cell or an Expi293 cell). In another embodiment, the host cell is prokaryotic.

Suitable host cells include prokaryotic microorganisms such as E. coli, eukaryotic microorganisms such as filamentous fungi or yeast, or various eukaryotic cells such as Chinese hamster ovary cells (CHO) and insect cells. Mammalian cell lines suitable for suspension culture can be used.Examples of useful mammalian host cell lines include monkey kidney CV1 line (COS-7) transformed by SV40; human embryonic kidney line (a HEK 293 or 293F cell or a 293FT cells or an Expi293 cell), baby hamster kidney cells (BHK), monkey kidney cells (CV1), African

green monkey kidney cells (VERO-76), human cervical cancer cells (HELA), canine kidney cells (MDCK), buffalo rat liver cells (BRL 3A), human lung cells (W138), human liver cells (Hep G2), CHO cells, NSO cells, and myeloma cell lines such as YO, NS0, P3X63, and Sp2/0.Mammalian host cell lines suitable for antibody production are known in the art. In a preferred embodiment, the host cell is a CHO or HEK293 cell or a 293FT cell or an Expi293 cell.

V. Production and Purification of Molecules of the Present Invention

In yet another aspect, the present invention provides a method for producing the antibody molecule (e.g., an anti-DLL3 antibody or antigen-binding fragment thereof, or a multispecific antibody such as a bispecific antibody) of the present invention, the method comprising: culturing a host cell comprising a polynucleotide encoding the polypeptide chains of the molecule under conditions suitable for the expression of the polypeptide chains; and optionally further comprising assembling the polypeptide chains to produce the molecule under conditions suitable for the assembly of the polypeptide chains into the molecule.

For recombinant production, the polynucleotides encoding the polypeptide chains of the molecule of the present invention may be inserted into one or more vectors for further cloning and/or expression in host cells. Methods which are well known to those skilled in the art can be used to construct expression vectors. The expression vector includes, but is not limited to, a virus, a plasmid, a cosmid, a Lambda phage, or a yeast artificial chromosome (YAC). Once an expression vector comprising one or more polynucleotides of the present invention has been prepared for expression, the expression vector can be transfected or introduced into a suitable host cell. Various techniques can be used for this purpose, for example, protoplast fusion, calcium phosphate precipitation, electroporation, retroviral transduction, viral transfection, biolistics, liposome-based transfection, or other conventional techniques.

The molecule prepared as described herein can be purified by known prior art such as high-performance liquid chromatography, ion exchange chromatography, gel electrophoresis, affinity chromatography (e.g., Protein A affinity chromatography), and size exclusion chromatography. The actual conditions used to purify a particular protein also depend on factors such as net charge, hydrophobicity, and hydrophilicity, and these will be apparent to those skilled in the art.

The purity of the molecules of the present invention can be determined by any of a variety of well-known analytical methods including size exclusion chromatography, gel electrophoresis, high-performance liquid chromatography, and the like. The physical/chemical properties and/or biological activities of the antibody molecule provided herein can be identified, screened, or characterized through a variety of assays known in the art.

VI. Assays

The molecule provided herein (e.g., an anti-DLL3 antibody or antigen-binding fragment thereof, or a multispecific antibody such as a bispecific antibody) may be identified, screened, or characterized for their physical/chemical properties and/or biological activities using various assays known in the art. Assays for determining the properties of the anti-DLL3 antibody or antigen-binding fragment thereof or the bispecific antibody of the present invention, are exemplified in the Examples, including bio-layer interferometry (BLI), a T cell reporter gene assay method, a T cell activation assay method, a mediated T cell killing experiment, or an anti-tumor activity assay, and the like.

VII. Immunoconjugate

In one aspect, the present invention provides an immunoconjugate comprising the molecule of the present invention (e.g., an anti-DLL3 antibody or an antigen-binding fragment thereof, or a multispecific antibody such as a bispecific antibody) and one or more additional active ingredients (e.g., an active ingredient from a drug or therapeutic agent for treating a disease of the present invention, such as a small molecule that enhances the therapeutic effect of the molecule of the present invention).

In some embodiments, the immunoconjugate is an antibody-drug-conjugate (ADC).

VIII. Medicament and Use

In some embodiments, the present invention also relates to a composition (e.g., a pharmaceutical composition or a pharmaceutical formulation) comprising the molecule of the present invention (e.g., an anti-DLL3 antibody or antigen-binding fragment thereof, a multispecific antibody such as a bispecific antibody, or the immunoconjugate).

In one embodiment, the composition, e.g., the pharmaceutical composition, comprises the

molecule of the present invention, and a combination of one or more additional therapeutic agents.

The composition of the present invention may further comprise a suitable pharmaceutical auxiliary material, such as a pharmaceutical carrier and a pharmaceutical excipient, including buffer, as known in the art. As used herein, "pharmaceutical carrier" includes any and all solvents, dispersion media, isotonic or absorption delaying agents, and the like that are physiologically compatible. For use and application of the pharmaceutical excipients, see Handbook of Pharmaceutical Excipients, 8th Ed. R. C. Rowe, P. J. Seskey and S. C. Owen, Pharmaceutical Press, London, Chicago. The composition of the present invention may be in a variety of forms. These forms include, for example, liquid, semi-solid, and solid dosage forms, such as liquid solutions (e.g., injectable solutions and infusible solutions), powders or suspensions, liposomes, and suppositories. The preferred form depends on the intended mode of administration and therapeutic use.

The medicament comprising the antibody molecule or the immunoconjugate described herein can be prepared, e.g., in the form of a lyophilized formulation or an aqueous solution, by mixing the antibody molecule (e.g., an anti-DLL3 antibody or antigen-binding fragment thereof, or a multispecific antibody such as a bispecific antibody) or the immunoconjugate of the present invention, having desired purity, with one or more optional pharmaceutical supplementary materials.

In some embodiments, the present invention further provides a pharmaceutical combination or a pharmaceutical combination product comprising a molecule of the present invention (e.g., an anti-DLL3 antibody or antigen-binding fragment thereof, or a multispecific antibody such as a bispecific antibody, or an immunoconjugate), and one or more additional therapeutic agents, and the like.

Another object of the present invention is to provide a kit of parts comprising the pharmaceutical combination of the present invention, preferably, the kit is in the form of a pharmaceutical dose unit., whereby dose units may be provided according to a dosing regimen or an administration interval.

In one embodiment, the kit of parts of the present invention comprises in the same package:

- a first container containing a pharmaceutical composition comprising the molecule (e.g., an

anti-DLL3 antibody or antigen-binding fragment thereof, or a multispecific antibody such as a bispecific antibody, or an immunoconjugate) of the present invention; and

- one or more containers containing a pharmaceutical composition comprising one or more additional therapeutic agents.

In some embodiments, when the molecule of the present invention (e.g., an anti-DLL3 antibody or antigen-binding fragment thereof, the bispecific antibody, or the immunoconjugate thereof of the present invention) is used for treating a tumor, the additional therapeutic agent is, e.g., various therapeutic agents used for treating the tumor, such as a chemotherapeutic agent, an angiogenesis inhibitor, a cytokine, a cytotoxic agent, an additional antibody, a small molecule drug, or an immunomodulatory agent (e.g., an immune checkpoint inhibitor or an agonist).

In some particular embodiments, the additional therapeutic agent can be an additional antibody, for example, an antibody that specifically binds to an immune checkpoint molecule, e.g., an antibody that inhibits the PD-1/PD-L1 signaling pathway, such as an anti-PD-1 antibody, or an anti-PD-L1 antibody or an anti-PD-L2 antibody.

In some particular embodiments, the pharmaceutical combination of the present invention comprises the molecule of the present invention (e.g., an anti-DLL3 antibody or antigen-binding fragment thereof, a bispecific antibody, or an immunoconjugate thereof of the present invention) and an antibody that inhibits the PD-1/PD-L1 signaling pathway, such as an anti-PD-1 antibody, or an anti-PD-L1 antibody or an anti-PD-L2 antibody.

In some embodiments, the anti-PD-1 antibody is selected from the group consisting of nivolumab, pembrolizumab, cemiplimab, toripalimab, sintilimab, tislelizumab, camrelizumab, penpulimab, zimberelimab, putlimab, and adebrelimab.

In some particular embodiments, the additional therapeutic agent is a chemotherapeutic agent, e.g., a chemotherapeutic agent used in standard chemotherapy methods for treating tumors. In some particular embodiments, the additional chemotherapeutic agent is selected from a platinum-based chemotherapeutic drug, such as carboplatin; or a topoisomerase inhibitor, e.g., a topoisomerase II inhibitor, such as etoposide.

In some particular embodiments, the pharmaceutical combination of the present invention comprises the molecule of the present invention (e.g., an anti-DLL3 antibody or antigen-binding fragment thereof, a bispecific antibody, or an immunoconjugate thereof of the present invention) and one, two, or more chemotherapeutic agents, e.g., two chemotherapeutic agents, wherein one of the chemotherapeutic agents is a platinum-based chemotherapeutic drug such as carboplatin, and the other chemotherapeutic agent is a topoisomerase inhibitor, e.g., a topoisomerase II inhibitor such as etoposide.

In some particular embodiments, the pharmaceutical combination of the present invention comprises the molecule of the present invention (e.g., an anti-DLL3 antibody or antigen-binding fragment thereof, or a bispecific antibody or an immunoconjugate thereof of the present invention), and a combination of carboplatin and etoposide.

Another aspect of the present invention provides a method for preventing or treating a disease in a subject, comprising administering to the subject an effective amount of the molecule (e.g., an anti-DLL3 antibody or antigen-binding fragment thereof, or a multispecific antibody such as a bispecific antibody or an immunoconjugate thereof), the pharmaceutical composition, the pharmaceutical combination, or the kit of the present invention. In some embodiments, the disease is, for example, a tumor such as cancer.

In some embodiments, the present invention relates to the molecule (e.g., an anti-DLL3 antibody or antigen-binding fragment thereof, or a multispecific antibody such as a bispecific antibody or an immunoconjugate thereof), the pharmaceutical composition, the pharmaceutical combination, or the kit of the present invention for use in therapy, e.g., in the treatment of a tumor such as cancer.

In some embodiments, the present invention relates to a method for treating a disease, e.g., a tumor such as cancer, with the molecule (e.g., an anti-DLL3 antibody or antigen-binding fragment thereof, or a multispecific antibody such as a bispecific antibody or an immunoconjugate thereof), the pharmaceutical composition, the pharmaceutical combination, or the kit of the present invention, or to use thereof in the treatment, or to use thereof in the preparation of a medicament for the treatment.

In some embodiments, the tumor is a solid tumor or a hematological tumor and a metastatic lesion. In one embodiment, examples of the solid tumor include a malignant tumor. The cancer may be at an early, intermediate or advanced stage or be a metastatic cancer. In some embodiments, the tumor has tumor immune escape.

In some embodiments, the tumor is a DLL3-positive tumor or cancer. In some embodiments, the tumor is associated with abnormal expression or abnormal activity of DLL3.

In some embodiments, the DLL3-positive tumor or cancer refers to abnormal expression or activity of DLL3 in a subject having the tumor, e.g., cancer. In some embodiments, the subject (particularly an adult subject) has DLL3 expression. In some embodiments, the subject has DLL3, e.g., at an elevated level, e.g., at a nucleic acid or protein level or activity, e.g., as compared to a healthy subject. In some embodiments, the subject has DLL3 in a biological sample (e.g., a tumor cell or tumor tissue) thereof, e.g., at an elevated level, such as at a nucleic acid or protein level or activity, e.g., compared to DLL3 in a biological sample from a healthy subject (e.g., the corresponding tissue or cell in a healthy subject), or compared to DLL3 in an adjacent healthy tissue or cell of the subject.

In some embodiments, the DLL3-positive tumor refers to a tumor containing tumor cells abnormally expressing DLL3. In some embodiments, the abnormal expression of DLL3 refers to the expression of DLL3 on the cell membrane of the tumor cell. In some embodiments, the abnormal expression of DLL3 refers to DLL3 expression on tumor cells that is higher than that on control cells (e.g., healthy cells in the corresponding tissue from a healthy individual, or healthy cells adjacent to the tumor cells).

In some embodiments, the tumor is a neuroendocrine tumor (e.g., a DLL3-positive neuroendocrine tumor), such as lung cancer (e.g., DLL3-positive lung cancer), e.g., small cell lung cancer (SCLC).

Depending on its therapeutic use, the molecule (e.g., an anti-DLL3 antibody or antigen-binding fragment thereof, or a multispecific antibody such as a bispecific antibody or an immunoconjugate thereof) or the pharmaceutical composition of the present invention may also be administered in combination with one or more additional therapies, e.g., therapeutic modalities and/or additional therapeutic agents, for the method or use described herein, e.g., for use in

preventing and/or treating the related disease or disorder mentioned herein.

In some embodiments, when the molecule of the present invention (e.g., an anti-DLL3 antibody or antigen-binding fragment thereof, a bispecific antibody, or an immunoconjugate thereof of the present invention) is used for treating a tumor, the additional therapeutic agent is, e.g., various therapeutic agents used for treating the tumor, such as a chemotherapeutic agent, an angiogenesis inhibitor, a cytokine, a cytotoxic agent, an additional antibody, a small molecule drug, or an immunomodulatory agent (e.g., an immune checkpoint inhibitor or an agonist). In some embodiments, the therapeutic agent is a PD-1 antibody or an antigen-binding fragment thereof. In some embodiments, when the molecule of the present invention (e.g., an anti-DLL3 antibody or antigen-binding fragment thereof, a bispecific antibody, or an immunoconjugate thereof of the present invention) is used for treating a tumor, the therapeutic modality includes surgery, radiotherapy, partial irradiation, focused irradiation, or the like.

In some particular embodiments, the additional therapeutic agent can be an additional antibody, e.g., an antibody that specifically binds to an immune checkpoint molecule, e.g., an antibody that inhibits the PD-1/PD-L1 signaling pathway, such as an anti-PD-1 antibody, or an anti-PD-L1 antibody or an anti-PD-L2 antibody.

In some particular embodiments, the molecule of the present invention (e.g., an anti-DLL3 antibody or antigen-binding fragment thereof, a bispecific antibody, or an immunoconjugate thereof of the present invention) is administered in combination with an antibody that inhibits the PD-1/PD-L1 signaling pathway, such as an anti-PD-1 antibody, or an anti-PD-L1 antibody or an anti-PD-L2 antibody, for the method or use described herein.

In some embodiments, the anti-PD-1 antibody is selected from the group consisting of nivolumab, pembrolizumab, cemiplimab, toripalimab, sintilimab, tislelizumab, camrelizumab, penpulimab, zimberelimab, putlimab, and adebrelimab.

In some particular embodiments, the additional therapeutic agent is a chemotherapeutic agent, e.g., a chemotherapeutic agent used in standard chemotherapy methods for treating tumors. In some particular embodiments, the additional chemotherapeutic agent is selected from a platinum-based chemotherapeutic drug, such as carboplatin; or a topoisomerase inhibitor, e.g., a topoisomerase II

inhibitor, such as etoposide.

In some particular embodiments, the molecule of the present invention (e.g., an anti-DLL3 antibody or antigen-binding fragment thereof, a bispecific antibody, or an immunoconjugate thereof of the present invention) is administered in combination with a standard chemotherapy for the method or use described herein.

As described herein, standard chemotherapy refers to a chemotherapy method for a specific cancer type that has been clinically studied and verified in practice, is widely accepted and used as a conventional treatment regimen. In some particular embodiments, the standard chemotherapy includes standard chemotherapy methods suitable for treating the tumors described herein.

In some embodiments, the standard chemotherapy method for treating the tumor described herein comprises combined administration of one, two or more chemotherapeutic agents, e.g., two chemotherapeutic agents, e.g., one of the chemotherapeutic agents is a platinum-based chemotherapeutic drug such as carboplatin, and the other chemotherapeutic agent is a topoisomerase inhibitor, e.g., a topoisomerase II inhibitor such as etoposide. In some embodiments, standard chemotherapy methods for treating the tumors described herein comprise combined administration of carboplatin and etoposide.

In some particular embodiments, the molecule of the present invention (e.g., an anti-DLL3 antibody or antigen-binding fragment thereof, a bispecific antibody, or an immunoconjugate thereof of the present invention) is administered in combination with one, two, or more chemotherapeutic agents, e.g., two chemotherapeutic agents, for the method or use described herein, preferably, one of the chemotherapeutic agents is a platinum-based chemotherapeutic drug, such as carboplatin, and the other chemotherapeutic agent is a topoisomerase inhibitor, e.g., a topoisomerase II inhibitor, such as etoposide.

In some particular embodiments, the molecule of the present invention (e.g., an anti-DLL3 antibody or antigen-binding fragment thereof, a bispecific antibody, or an immunoconjugate thereof of the present invention) is administered in combination with carboplatin and etoposide for the method or use described herein.

In other aspects, the present invention provides use of the molecule (e.g., an anti-DLL3

antibody or antigen-binding fragment thereof, a bispecific antibody, or an immunoconjugate thereof) of the present invention, or the composition or combination product comprising the same in the manufacture or preparation of a medicament for the use described herein, e.g., for use in preventing or treating the related disease or disorder mentioned herein.

In other aspects, the present invention also provides the molecule of the present invention (e.g., an anti-DLL3 antibody or antigen-binding fragment thereof, a bispecific antibody, or an immunoconjugate thereof), or the composition or the medicament or the formulation or the combination product comprising the same, for use in therapy, e.g., in the treatment of the related disease or disorder mentioned herein.

IX. Diagnostics and Detection

In certain embodiments, the molecule provided herein (e.g., an anti-DLL3 antibody or antigen-binding fragment thereof, a bispecific antibody, or an immunoconjugate thereof) may be used to detect the presence of DLL3 in a biological sample.

The term "detection" used herein includes quantitative and qualitative detections, and exemplary detections may involve immunohistochemistry, immunocytochemistry, flow cytometry (e.g., FACS), magnetic beads complexed with antibody molecules, ELISA, and PCR techniques (e.g., RT-PCR). In certain embodiments, the biological sample is a bodily fluid.

In certain embodiments, the method comprises contacting the biological sample with the molecule (e.g., a bispecific antibody or an immunoconjugate thereof) described herein under a condition that allows the molecule to bind to DLL3, and detecting whether a complex is formed between the molecule and DLL3, wherein the formation of the complex indicates the presence of DLL3. The method may be an in vitro or an in vivo method. In one embodiment, the molecule of the present invention is used to select a subject suitable for treatment with the anti-DLL3 antibody or antigen-binding fragment thereof, or the bispecific antibody, or the immunoconjugate thereof of the present invention, e.g., wherein DLL3 is a biomarker for selecting the subject.

In certain embodiments, provided is a labeled molecule of the present invention (e.g., an anti-DLL3 antibody or antigen-binding fragment thereof, a bispecific antibody, or an immunoconjugate thereof of the present invention). Labels include, but are not limited to, labels or

moieties that are detected directly (such as fluorescent labels, chromophoric labels, electron-dense labels, chemiluminescent labels, and radioactive labels), as well as moieties that are detected indirectly, such as an enzyme or a ligand, e.g., through an enzymatic reaction or molecular interaction.

In some embodiments provided herein, the sample is obtained prior to treatment with the molecule, or the composition, the medicament, the formulation, or the combination product comprising same of the present invention. In some embodiments, the sample is obtained prior to treatment with other therapies. In some embodiments, the sample is obtained during or after treatment with other therapies.

In some embodiments, DLL3 is detected prior to treatment, e.g., prior to an initial treatment or prior to a certain treatment following a treatment interval.

In some embodiments, provided is a method for treating a disease of the present invention, which comprises: detecting the presence of DLL3 in a subject (e.g., a sample) (such as a subject sample), thereby determining a DLL3 value; comparing the DLL3 value to a control value (e.g., a value in a normal individual); and if the DLL3 value is greater than the control value, administering to the subject a therapeutically effective amount of the bispecific antibody of the present invention or the composition, medicament, or formulation comprising the same, optionally in combination with one or more additional therapies, thereby treating the disease.

X. Particular Embodiments

1. An anti-DLL3 antibody or an antigen-binding fragment thereof, comprising three CDRs of a heavy chain variable region VH: HCDR1, HCDR2, and HCDR3, and three CDRs of a light chain variable region VL: LCDR1, LCDR2, and LCDR3, wherein the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 are selected from

(i). the three complementarity-determining regions HCDR1, HCDR2 and HCDR3 comprised in the VH as set forth in SEQ ID NO: 13, and the three complementarity-determining regions LCDR1, LCDR2 and LCDR3 comprised in the VL as set forth in SEQ ID NO: 18;

(ii). the three complementarity-determining regions HCDR1, HCDR2 and HCDR3 comprised in the VH as set forth in SEQ ID NO: 12, and the three complementarity-determining regions LCDR1,

LCDR2 and LCDR3 comprised in the VL as set forth in SEQ ID NO: 16;

(iii). the three complementarity-determining regions HCDR1, HCDR2 and HCDR3 comprised in the VH as set forth in SEQ ID NO: 13, and the three complementarity-determining regions LCDR1, LCDR2 and LCDR3 comprised in the VL as set forth in SEQ ID NO: 16;

(iv). the three complementarity-determining regions HCDR1, HCDR2 and HCDR3 comprised in the VH as set forth in SEQ ID NO: 14, and the three complementarity-determining regions LCDR1, LCDR2 and LCDR3 comprised in the VL as set forth in SEQ ID NO: 16;

(v). the three complementarity-determining regions HCDR1, HCDR2 and HCDR3 comprised in the VH as set forth in SEQ ID NO: 15, and the three complementarity-determining regions LCDR1, LCDR2 and LCDR3 comprised in the VL as set forth in SEQ ID NO: 16;

(vi). the three complementarity-determining regions HCDR1, HCDR2 and HCDR3 comprised in the VH as set forth in SEQ ID NO: 12, and the three complementarity-determining regions LCDR1, LCDR2 and LCDR3 comprised in the VL as set forth in SEQ ID NO: 17;

(vii). the three complementarity-determining regions HCDR1, HCDR2 and HCDR3 comprised in the VH as set forth in SEQ ID NO: 12, and the three complementarity-determining regions LCDR1, LCDR2 and LCDR3 comprised in the VL as set forth in SEQ ID NO: 18; or

(viii). the three complementarity-determining regions HCDR1, HCDR2, and HCDR3 comprised in the VH as set forth in SEQ ID NO: 14, and the three complementarity-determining regions LCDR1, LCDR2 and LCDR3 comprised in the VL as set forth in SEQ ID NO: 18.

2. An anti-DLL3 antibody or an antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, wherein the HCDR1 consists of SEQ ID NO: 1; the HCDR2 consists of SEQ ID NO: 2; the HCDR3 consists of SEQ ID NO: 6; the LCDR1 consists of SEQ ID NO: 7; the LCDR2 consists of SEQ ID NO: 8; and the LCDR3 consists of SEQ ID NO: 9;

optionally, any one of the amino acids D or G in the contiguous amino acids DG in the HCDR2 may be substituted, e.g., wherein D may be substituted with E or Q, and/or G may be substituted with V, e.g., HCDR2 consists of the amino acid sequence as set forth in SEQ ID NO: 3, 4, or 5.

3. The antibody or antigen-binding fragment thereof according to embodiment 1 or 2, comprising a heavy chain variable region VH, wherein the heavy chain variable region

(i) comprises or consists of an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence as set forth in SEQ ID NO: 12, 13, 14, or 15; or

(ii) comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 12, 13, 14, or 15.

4. The antibody or antigen-binding fragment thereof according to any one of embodiments 1-3, comprising a light chain variable region VL, wherein the light chain variable region

(i) comprises or consists of an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence as set forth in SEQ ID NO: 16; or

(ii) comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 16;

optionally, any one of the amino acids in the consecutive amino acids NFS within a framework region FR3 of the VL may be mutated, for example, wherein N may be substituted with Q and/or wherein S may be substituted with A; for example,

the light chain variable region

(i) comprises or consists of an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence as set forth in SEQ ID NO: 17 or 18; or

(ii) comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 17 or 18.

5. The antibody or antigen-binding fragment thereof according to any one of embodiments 1-4, comprising a heavy chain variable region VH and a light chain variable region VL, wherein

(i). the VH comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 13 or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereto, and the VL comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 18 or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereto;

(ii). the VH comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 12 or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereto, and the VL comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 16 or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereto;

(iii). the VH comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 13 or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereto, and the VL comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 16 or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereto;

(iv). the VH comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 14 or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereto, and the VL comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 16 or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereto;

(v). the VH comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 15 or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereto, and the VL comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 16 or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereto;

(vi). the VH comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 12 or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereto, and the VL comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 17 or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereto;

(vii). the VH comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 12 or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereto, and the VL comprises or consists of the amino acid sequence as set forth in

SEQ ID NO: 18 or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereto; or

(viii). the VH comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 14 or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereto, and the VL comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 18 or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereto.

6. The antibody or antigen-binding fragment thereof according to any one of embodiments 1-5, comprising a heavy chain variable region VH and a light chain variable region VL, wherein

(i). the VH comprises or consists of the amino acid sequence as set forth in SEQ ID NO:13, and the VL comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 18;

(ii). the VH comprises or consists of the amino acid sequence as set forth in SEQ ID NO:12, and the VL comprises or consists of the amino acid sequence as set forth in SEQ ID NO:16;

(iii). the VH comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 13, and the VL comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 16;

(iv). the VH comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 14, and the VL comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 16;

(v). the VH comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 15, and the VL comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 16;

(vi). the VH comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 12, and the VL comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 17;

(vii). the VH comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 12, and the VL comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 18; or

(viii). the VH comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 14, and the VL comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 18.

7. The antibody or antigen-binding fragment thereof according to any one of embodiments 1-6, further comprising a heavy chain constant region HC, for example, the antibody heavy chain

constant region HC is a heavy chain constant region from an IgG, such as human IgG, e.g., a heavy chain constant region of IgG1, IgG2, IgG3, or IgG4, preferably a heavy chain constant region of human IgG1, IgG2, IgG3, or IgG4.

8. The antibody or antigen-binding fragment thereof according to embodiment 7, wherein the heavy chain constant region

(i) comprises or consists of an amino acid sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence as set forth in SEQ ID NO: 23; or

(ii) comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 23.

9. The antibody or antigen-binding fragment thereof according to embodiment 7 or 8, wherein the heavy chain constant region comprises an Fc region, wherein the Fc region is an Fc region from IgG, such as human IgG, e.g., an Fc region of IgG1, IgG2, IgG3, or IgG4, preferably, a human IgG1, IgG2, IgG3 or IgG4 Fc, optionally the Fc region

(i) comprises or consists of an amino acid sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence as set forth in SEQ ID NO: 26 or 27; or

(ii) comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 26 or 27.

10. The antibody or antigen-binding fragment thereof according to any one of embodiments 1-9, further comprising a light chain constant region, for example, the light chain constant region is a light chain constant region from a Lambda or Kappa light chain constant region, such as a Lambda or Kappa light chain constant region, e.g., a human Lambda or Kappa light chain constant region.

11. The antibody or antigen-binding fragment thereof according to embodiment 10, wherein the light chain constant region

(i) comprises or consists of an amino acid sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence as set forth in SEQ ID NO: 24 or 25; or

(ii) comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 24 or 25.

12. The antibody or antigen-binding fragment thereof according to any one of embodiments 1-11, wherein the antibody is a humanized antibody or a chimeric antibody.

13. The antibody or antigen-binding fragment thereof according to any one of embodiments 1-12, wherein the antibody is a monoclonal antibody.

14. The antibody or antigen-binding fragment thereof according to any one of embodiments 1-13, wherein the antigen-binding fragment is an antibody fragment selected from the group consisting of an Fab, anFab', an Fab'-SH, an Fv, a single-chain antibody (e.g., scFv), and an $(Fab')_2$, a diabody, or a linear antibody.

15. The antibody or antigen-binding fragment thereof according to any one of embodiments 1-11, wherein the antibody is a bispecific antibody or a multispecific antibody comprising a first binding specificity for DLL3 and additional binding specificities for one or more additional antigens.

16. The antibody or antigen-binding fragment thereof according to embodiment 15, wherein the additional antigen is CD3.

17. A bispecific antibody, comprising a first antigen-binding region and a second antigen-binding region,

wherein the first antigen-binding region specifically binds to DLL3 and comprises a VH and a VL, wherein

the VH comprises HCDR1, HCDR2, and HCDR3 as defined in embodiment 1 or 2, and the VL comprises LCDR1, LCDR2, and LCDR3 as defined in embodiment 1 or 2; or

the VH and VL are the VH and VL as defined in any one of embodiments 3-6; and

the second antigen-binding region specifically binds to CD3.

18. The bispecific antibody according to embodiment 17, wherein the first antigen-binding region is the Fab of the anti-DLL3 antibody as defined in any one of embodiments 1-14.

19. The bispecific antibody according to embodiment 17 or 18, wherein the second antigen-binding region is the Fab specifically binding to CD3.

20. The bispecific antibody according to Embodiment 18 or 19, wherein the Fab as the first antigen-binding region or the second antigen-binding region comprises CH1, wherein the CH1 is a CH1 from IgG1, IgG2, IgG3, or IgG4, preferably a CH1 from human IgG1, IgG2, IgG3, or IgG4, e.g., a CH1 of human IgG1, IgG2, IgG3, or IgG4.

21. The bispecific antibody according to embodiment 20, wherein the CH1

(i) comprises or consists of an amino acid sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence as set forth in SEQ ID NO: 28; or

(ii) comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 28.

22. The bispecific antibody according to any one of embodiments 18-21, wherein the Fab serving as the first antigen-binding region or the second antigen-binding region comprises a light chain constant region, wherein the light chain constant region is a light chain constant region from a Lambda or Kappa light chain constant region, e.g., a Lambda or Kappa light chain constant region, such as a human Lambda or Kappa light chain constant region; optionally, the first antigen-binding region and the second antigen-binding region comprise the same or different light chain constant regions; for example, the first antigen-binding region comprises a Kappa light chain constant region and the second antigen-binding region comprises a Lambda light chain constant region.

23. The bispecific antibody according to embodiment 22, wherein the Kappa light chain constant region

(i) comprises or consists of an amino acid sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence as set forth in SEQ ID NO: 25; or

(ii) comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 25;

and/or

the Lambda light chain constant region

(i) comprises or consists of an amino acid sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence as set forth in SEQ ID NO: 24; or

(ii) comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 24.

24. The bispecific antibody according to any one of embodiments 17-23, wherein the bispecific antibody is an IgG-like bispecific antibody comprising an Fc dimer, wherein two Fc regions constituting the Fc dimer, the first Fc region and second Fc region are the same or different, and optionally, the Fc region is an Fc region from IgG, such as human IgG, e.g., an Fc region of IgG1, IgG2, IgG3, or IgG4, preferably, a human IgG1, IgG2, IgG3 or IgG4 Fc, optionally, the Fc region

(i) comprises or consists of an amino acid sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence as set forth in SEQ ID NO: 26 or 27; or

(ii) comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 26 or 27.

25. The bispecific antibody according to embodiment 24, wherein the two Fc regions are different, wherein mutations are introduced into the first Fc region and the second Fc region based on the Innobody technology to facilitate heterodimerization of the first Fc region and the second Fc region.

26. The antibody or antigen-binding fragment thereof according to embodiment 25, wherein the CH3 of one Fc region comprises S364R and D399K mutations, and the CH3 mutation of the other Fc region comprises Y349T, K370S, and K409D mutations.

27. The bispecific antibody according to any one of embodiments 24-26, wherein the first and/or second Fc region comprises a L234A/L235A mutation.

28. The bispecific antibody according to embodiment 26 or 27, wherein

a) one Fc region comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 29, and the other Fc region comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 30;

b) one Fc region comprises an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence as set forth in SEQ ID NO: 29 and comprises the mutations S364R and D399K, while the other Fc region comprises an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence as set forth in SEQ ID NO: 30 and comprises the mutations Y349T, K370S, and K409D; or

c) one Fc region comprises an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence as set forth in SEQ ID NO: 29 and comprises the mutations S364R, D399K, and L234A/L235A, while the other Fc region comprises an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence as set forth in SEQ ID NO: 30 and comprises the mutations Y349T, K370S, K409D, and L234A/L235A.

29. The bispecific antibody according to any one of embodiments 17-28, wherein the second antigen-binding region comprises three complementarity-determining regions from a heavy chain

variable region (HCDRs): HCDR1, HCDR2 and HCDR3 and three complementarity-determining regions from a light chain variable region (LCDRs): LCDR1, LCDR2 and LCDR3, wherein

HCDR1 comprises or consists of the amino acid sequence of SEQ ID NO: 32; HCDR2 comprises or consists of the amino acid sequence of SEQ ID NO: 33, HCDR3 comprises or consists of the amino acid sequence of SEQ ID NO: 34, LCDR1 comprises or consists of the amino acid sequence of SEQ ID NO: 36, LCDR2 comprises or consists of the amino acid sequence of SEQ ID NO: 37, and LCDR3 comprises or consists of the amino acid sequence of SEQ ID NO: 38.

30. The bispecific antibody according to embodiment 29, wherein the second antigen-binding region comprises a VH and a VL, wherein the VH comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 31; and the VL comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 35.

31. The bispecific antibody according to any one of embodiments 17-30, wherein the bispecific antibody comprises a first antigen-binding region specifically binding to DLL3 and a second antigen-binding region specifically binding to CD3, wherein the first antigen-binding region comprises a first Fab and the second antigen-binding region comprises a second Fab, wherein the first Fab is linked at the C-terminus of its CH1 to the N-terminus of the first Fc region (directly or via a linker such as a hinge region), and the second Fab is linked at the C-terminus of its CH1 to the N-terminus of the second Fc region (directly or via a linker such as a hinge region).

32. The bispecific antibody according to embodiment 31, wherein the bispecific antibody is an IgG-like antibody having a configuration shown in FIG. 3.

33. The bispecific antibody according to Embodiment 31 or 32, comprising a first Fab as the first antigen-binding region specifically binding to DLL3 and a second Fab as the second antigen-binding region specifically binding to CD3, wherein the bispecific antibody comprises

heavy chain 1: comprising or consisting of, from N-terminus to C-terminus, a first Fab heavy chain variable region-a heavy chain constant region CH1-a first Fc region, wherein the heavy chain constant region CH1 is linked at its C-terminus to the N-terminus of the first Fc region directly or via a linker (e.g., a hinge region);

light chain 1: comprising or consisting of, from N-terminus to C-terminus, a first Fab light chain variable region–a light chain constant region;

heavy chain 2: comprising or consisting of, from N-terminus to C-terminus, a second Fab heavy chain variable region-a heavy chain constant region CH1-a second Fc region, wherein the

heavy chain constant region CH1 is linked at its C-terminus to the N-terminus of the second Fc region directly or via a linker (e.g., a hinge region);

light chain 2: comprising or consisting of, from N-terminus to C-terminus: a second Fab light chain variable region–a light chain constant region;

wherein the first Fab heavy chain variable region-the heavy chain constant region CH1 and the first Fab light chain variable region-the light chain constant region constitute a first Fab, and the second Fab heavy chain variable region-the heavy chain constant region CH1 and the second Fab light chain variable region-the light chain constant region constitute a second Fab;

optionally, the light chain constant region in the light chain 1 is different from the light chain constant region in the light chain 2, for example, the light chain constant region in the light chain 1 is a Kappa light chain constant region and the light chain constant region in the light chain 2 is a Lambda light chain constant region.

34. The bispecific antibody according to embodiment 33, wherein

the heavy chain 1 comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 41, or an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto; the light chain 1 comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 45, or an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto; the heavy chain 2 comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 48, or an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto; and the light chain 2 comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 40, or an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto;

the heavy chain 1 comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 42, or an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto; the light chain 1 comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 47, or an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto; the heavy chain 2 comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 48, or an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto; and the light chain 2 comprises or consists of the amino acid sequence as set forth

in SEQ ID NO: 40, or an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto; or

the heavy chain 1 comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 43, or an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto; the light chain 1 comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 47, or an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto; the heavy chain 2 comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 48, or an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto; and the light chain 2 comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 40, or an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

35. A nucleic acid molecule, comprising or consisting of a polynucleotide encoding any one of the chains of the antibody or antigen-binding fragment thereof according to any one of embodiments 1-16 or any one of the chains of the bispecific antibody according to any one of embodiments 17-34.

36、An expression vector, comprising the nucleic acid molecule according to embodiment 35, preferably, the expression vector is pCDNA, such as pCDNA3.1.

37. A host cell, comprising the nucleic acid molecule according to embodiment 35 or the expression vector according to embodiment 36, preferably, the host cell is prokaryotic or eukaryotic, e.g., a 293 cell or a CHO cell, such as an Expi293 cell.

38. A method of producing the antibody or antigen-binding fragment thereof according to any one of claims 1-10, or the bispecific antibody according to any one of claims 11-19, the method comprising: culturing a host cell comprising the nucleic acid molecule according to claim 20 or the expression vector according to claim 21 under conditions suitable for expression of the polypeptide chain(s) of the antibody or antigen-binding fragment thereof or the bispecific antibody; and optionally, recovering the antibody or antigen-binding fragment thereof or the bispecific antibody from the host cell or from the host cell culture medium.

39. An immunoconjugate, comprising the antibody or antigen-binding fragment thereof according to any one of embodiments 1 to 16 or the bispecific antibody according to any one of embodiments 17 to 34.

40. A pharmaceutical composition or a medicament or a formulation, comprising the antibody

or antigen-binding fragment thereof according to any one of embodiments 1-16 or the bispecific antibody according to any one of embodiments 17-34, or the immunoconjugate according to embodiment 39, and optionally a pharmaceutical supplementary material.

41. A pharmaceutical combination, comprising the antibody or antigen-binding fragment thereof according to any one of Embodiments 1-16 or the bispecific antibody according to any one of Embodiments 17-34, or the immunoconjugate according to embodiment 39, and one or more additional therapeutic agents, preferably, the therapeutic agents are various therapeutic agents for treating a tumor, such as a chemotherapeutic agent, an angiogenesis inhibitor, a cytokine, a cytotoxic agent, an additional antibody, a small molecule drug, or an immunomodulatory agent (e.g., an immune checkpoint inhibitor or an agonist).

42. The pharmaceutical combination according to embodiment 41, comprising the antibody or antigen-binding fragment thereof according to any one of embodiments 1-16 or the bispecific antibody according to any one of embodiments 17-34, or the immunoconjugate according to embodiment 39 and an additional antibody, preferably, the additional antibody is an antibody that inhibits the PD-1/PD-L1 signaling pathway, such as an anti-PD-1 antibody; or an anti-PD-L1 antibody or an anti-PD-L2 antibody; or

The antibody or antigen-binding fragment thereof according to any one of embodiments 1 to 16 or the bispecific antibody according to any one of embodiments 17 to 34, or the immunoconjugate according to embodiment 39 and one or more chemotherapeutic agents, for example, a platinum-based chemotherapeutic drug such as carboplatin; or a topoisomerase inhibitor, such as a topoisomerase II inhibitor, e.g., etoposide, or a combination thereof;

preferably, the pharmaceutical combination comprises the antibody or antigen-binding fragment thereof according to any one of embodiments 1-16 or the bispecific antibody according to any one of embodiments 17-34., or the immunoconjugate according to embodiment 39, and a combination of carboplatin and etoposide.

43. A method for preventing or treating a tumor in a subject, comprising administering to the subject an effective amount of the antibody or antigen-binding fragment thereof according to any one of embodiments 1-16 or the bispecific antibody according to any one of embodiments 17-34, or the immunoconjugate according to embodiment 39, or the pharmaceutical composition or formulation according to embodiment 40, or the pharmaceutical combination according to embodiment 41 or 42.

44. The method according to embodiment 43, wherein the tumor is a solid tumor or a

hematological tumor, e.g., a DLL3-positive tumor or cancer; optionally the tumor is a neuroendocrine tumor (e.g., a DLL3 positive neuroendocrine tumor), for example lung cancer (e.g., a DLL3 positive lung cancer), for example small cell lung cancer (SCLC).

45. The method according to embodiment 43 or 44, wherein the method further comprises administering in combination with an additional therapy such as a therapeutic modality (e.g., surgical therapy or radiotherapy) and/or an additional therapeutic agent, preferably, the therapeutic agent is various therapeutic agents for treating a tumor, e.g., a chemotherapeutic agent, an angiogenesis inhibitor, a cytokine, a cytotoxic agent, an additional antibody, a small molecule drug, or an immunomodulatory agent (e.g., an immune checkpoint inhibitor or an agonist).

46. The method according to embodiment 45, wherein the method further comprises administering in combination with an additional antibody, preferably, the additional antibody is an antibody that inhibits the PD-1/PD-L1 signaling pathway, such as an anti-PD-1 antibody; or an anti-PD-L1 antibody or an anti-PD-L2 antibody; or

the method further comprises administering in combination with one or more chemotherapeutic agents, wherein the chemotherapeutic agent is selected from a platinum-based chemotherapeutic drug, such as carboplatin; or a topoisomerase inhibitor, such as a topoisomerase II inhibitor, such as etoposide, or a combination thereof; or

the method further comprises administering in combination with carboplatin and etoposide.

Example.

Example 1. Generation and Screening of DLL3 Monoclonal Antibodyy

Immunization

RenMab mice (purchased from Biocytogen) were immunized with human DLL3 extracellular domain protein (Kactus, DLL-HM103) emulsified in Freund's complete adjuvant (Sigma, F5881); two weeks later, they received three additional immunizations with the protein emulsified in Freund's incomplete adjuvant (Sigma, F5506), administered via subcutaneous injection every two weeks (50 μg of protein per mouse).

Single B Cell Fluorescence Staining and Sorting

When the serum titer met the requirement, the spleen was harvested from the mouse to obtain

suspended B cells. The cell density was then adjusted to 1×10^8 cells/vial in a volume of 0.25-2 mL with EasySep Buffer (Stemcell, 20144). 50 μL/mL Rat Serum and 50 μL/mL Isolation Cocktails were added, and the mixture was incubated at room temperature for 5 min. Subsequently, 50 μL/mL RapidSpheres were added, and the mixture was incubated at room temperature for 2.5 min. EasySep Buffer was added to bring the volume to 2.5 mL. The flow cytometry tube was placed on a magnetic rack at room temperature for 2.5 min, and the cell suspension was collected. The cells were washed with 10 mL of EasySep Buffer and t adjusted to a cell density of 1×10^8 cells/mL. PE-Cy 7 Rat Anti-Mouse CD45R/B220 (BD, 552772) and FITC (Invitrogen, A20181)-labeled hu-DLL3 were added, and the mixture was incubated in the dark for 1 h. The cells were washed once with 10 mL of pre-cooled EasySep Buffer, and adjusted to a cell density of 1×10^8 cells/vial. APC-Cyanine 7 anti-mouse IgM Antibody (Biolegend, 406516), Brilliant Violet 510 anti-mouse IgD Antibody (Biolegend, 405723), and R-Phycoerythrin AffiniPure $F(ab')_2$ Fragment Goat Anti-Mouse IgG (Jackson Immunoresearch Inc, 115-116-071) were added to the, and the mixture was incubated in the dark for 20 min. The cells were washed once with 10 mL of pre-cooled EasySep Buffer, and adjusted to a cell density of 10^7 cells/mL.Single B cells were sorted using an Aria III flow cytometer. The gating strategy for flow cytometry sorting was as follows: PE-Cy 7^{pos}/ FITC ^{pos} /APC ^{pos} / APC-Cyanine 7^{neg} / Brilliant Violet 510^{neg} / R-Phycoerythrin ^{pos}. The cells were sorted into a 96-well PCR plate containing a lysis buffer.

Antibody Variable Region Amplification, Vector Construction, Expression and Detection

Amplification products of the antibody variable regions were obtained by one round of RT-PCR and nested PCR. The paired amplification products were sequenced, and the sequencing results were analyzed and aligned using MEGA7 software. Meanwhile, the paired amplification products were introduced into the pcDNA3.1 vector digested with BsmBI (NEB; R0739L) via homologous recombination, wherein the IgG1 wild type (SEQ ID NO: 23) was selected for the heavy chain constant region, and Kappa (SEQ ID NO: 25) was selected for the light chain constant region. 5 μL of the homologous recombination product was taken and transformed into 30 μL of DH5a competence, and the resulting bacterial solution was inoculated into 2 mL of Amp-resistant LB medium and cultured overnight on a shaker at 220 rpm at 37°C. The bacterial solution cultured overnight was extracted with 96 Plus Miniprep Kit (QIAGENE, 27291), and the plasmid

concentration was determined by nanodrop. The light chain plasmid and the heavy chain plasmid of the same antibody were mixed in a molar ratio of 1:1, and 293F cells were transfected with polyethylenimine (PEI) (Polysciences, 23966). After 5-7 days of culture, the supernatant was collected for subsequent detection.

Anti-DLL3 antibody supernatants were screened for anti-DLL3 activity by enzyme-linked immunosorbent assay (ELISA) and flow cytometry (FACS). In the ELISA assay, each well was coated with 100 μL of Human-DLL3 (1 μg/mL) and blocked with 200 μL of 5% BSA. The primary antibody, consisting of transient-transfection supernatant and 5A4 (Benchmark, in-house), was added at 50 μL/well, and then 100 μL of anti-human IgG Fc secondary antibody (Thermo, A24476), diluted at a 1:10000 ratio in antibody diluent, was added to each well. The processes of coating, blocking, the primary antibody incubation and the secondary antibody incubation were all performed at 37°C for 1 h. Color development was performed with TMB (Solarbio, PR1200) at 100 μL/well for 3 min in the dark, and finally, ELISA stop solution (Solarbio, C1058) was added at 50 μL/well. The absorbance at OD450 nm was measured with a microplate reader. For the FACS assay, the cells to be tested (huDLL3/GS-CHO, in-houseconstructed) were diluted to 2×10^6 cells/mL, and the diluent was added to a U-bottom 96-well plateL at 50 μL/well. 50 μL/well of the supernatant was added to the U-shaped plate to resuspend the cells, followed by incubation 4°C for 30 min.The supernatant was removed by centrifugation at 500 × g for 5 min, and the cells were washed once with PBS. Then, 100 μL/well of PE-labeled anti-human Fab secondary antibody (BioLegend, 409304), diluted 1:500 in PBS, was added to each well, and the mixture was incubated at 4°C in the dark for 30 min. After another centrifugation at 500 × g for 5 min, the supernatant was removed, and the cells were washed once with PBS. The cells were then resuspended in 50 μL of 1× PBS and subjected to FACS analysis. 89 positive clone supernatants were selected, and monoclonal antibodies were purified using a Protein A affinity column. The binding regions of the antibodies were determined by FACS analysis of the binding of the purified antibodies to cells expressing different DLL3 truncations (in-house constructed). Meanwhile, these purified antibodies were subjected to affinity assay by Fortebio. According to the detection results and the sequence diversity, 19 paired sequences were obtained for subsequent construction of bispecific antibodies. The amino acid sequences and DNA sequences of the VH and VL of D3-107 obtained therein are shown in the

sequence listing.

Table 1: Primary screening results for DLL3 clones

ID	ELISA	FACS (MFI ratio)											domain
	human DLL3	human DLL3	DSL	EGF-1	EGF-2	EGF-3	EGF-4	EGF-5	EGF-6	Cyno DLL3	DLL1	DLL4	
D3-107	1.7391	42.55	54.74	106.88	63.88	44.71	86.76	21.82	147.61	1.70	1.59	2.01	EGF-6

Table 2: Affinity results for DLL3 clones

Sample ID	KD (M) antibodies on AHC sensor tip, Human DLL3 (100nM) in solution				KD (M) antibodies on AHC sensor tip, Rhesus DLL3 (100nM) in solution			
	Response	KD (M)	kon(1/Ms)	kdis(1/s)	Response	KD (M)	kon(1/Ms)	kdis(1/s)
D3-107	0.6325	8.84E-10	2.26E+05	2.00E-04	0.6631	9.65E-10	2.07E+05	2.00E-04

Example 2. Affinity Assay of DLL3 Monoclonal Antibody

The binding affinity (KD) of antibody D3-107 to DLL3 was determined by bio-layer interferometry (BLI). Half an hour before the experiment, an appropriate number of AHC sensors (18-5060, Sartorius) were taken according to the number of samples and soaked in SD buffer (1× PBS, 0.1% BSA, 0.05% Tween-20). The antibodies and human DLL3 (DL3-H52H4, Acro biosystems) were each diluted to 100 nM.

The SD buffer, the antibody solution, and human DLL3 were separately added to 96-well black polystyrene microplates (Greiner, 655209). The assay was performed using a Fortebio Octet Red96e instrument. The plate was arranged according to sample positions, and the sensor positions were selected accordingly.

The instrument parameters were set as follows: the assay was run with the following steps: baseline equilibration for 120 s, antibody loading for 100 s, baseline equilibration for 120 s, antigen association for 100 s, and dissociation for 120 s, at an agitation speed of 1000 rpm and a temperature of 30°C. After the experiment was completed, KD values were analyzed using ForteBio Octet analysis software. The results are shown in Table 3 below.

Table 3. Affinity assay of the exemplary antibody to human DLL3

Name	Antigen	Ka(1/Ms)	kd(1/s)	KD (M)
D3-107	Human DLL3	3.59E+05	2E-04	5.57E-10

Example 3. In Vitro Binding Assay of DLL3 Monoclonal Antibody

CHO-hDLL3 (obtained by electroporating CHO cells with a pXC17.4 vector constructed with a human DLL3 gene, NCBI ID: 10683, followed by sorting positive monoclones) was cultured and passaged according to conventional procedures.

After centrifugation and resuspension, the cells were counted and adjusted to a cell density of 4×10^6 cells/mL. The cell suspension was then transferred to a reagent reservoir and dispensed into a 96-well plate at 50 μL/well using a multi-channel pipette. Then, 50 μL of serially diluted antibody samples (starting at 100 nM, with 3-fold dilutions across 11 concentrations) were added to each well containing 50 μL of cells, and the plate was incubated in a refrigerator at 4°C for 30 min. The plate was centrifuged at 400 g for 5 min, and the supernatant was discarded. The cells were then resuspended in 200 μL of PBS, centrifuged again, and resuspended. This wash procedure was repeated twice. PE anti-human IgG Fc recombinant antibody (Biolegend, 409304, 1:200) was added, and the mixture was incubated at 4°C in the dark for 30 min. The cells were then washed twice with 200 μL of PBS, centrifuged, andresuspended in 100 μL of PBS, and analyzed by flow cytometry. The data were fitted to a dose-response curve, and the EC50 values were calculated.

The results are shown in FIG. 1. The candidate monoclonal antibodies and the positive control were comparable in their binding ability to DLL3-overexpressing CHO cells.

Example 4. Verification of ADCP Activity of DLL3 Monoclonal Antibody

The exemplary DLL3 antibody binds, via its Fab region, to DLL3 on the surface of small cell lung cancer cells, and simultaneously binds, via its Fc region, to CD64 on the surface of FcγRI ADCP Bioassay Effector Cells, thereby activating downstream signaling pathways through DLL3-dependent CD64 crosslinking.

In this study, a luciferase reporter assay was employed to assess the antibody activation activity by measuring luciferase expression in FcγRI ADCP bioassay effector cells co-cultured with DLL3-positive cells following overnight incubation with the test antibody. CHO-hDLL3 and FcγRI ADCP Bioassay Effector Cells (Promega G9871) cells (Effector cells) were adjusted to a density of 6.0×10^5. The overexpression cells CHO-hDLL3 and Effortor cells were mixed at a ratio of 1:1 for later use. 100 μL of cell suspension and serially diluted test antibodies (D3-107, DLL3 BMK antibody (Rova-T), DLL3 BMK (Amgen)) (starting at 30 nM, with 3× dilutions across 10 concentrations) were added to each well, and the plate was incubated overnight. The next day, 80 μL of Bio-Glo Luciferase Assay System (Promega, G7940) assay solution was added to each well. The plate was read on a microplate reader (Molecular Device, SpectraMax I3).

In experiments performed as described in the above assay, the exemplary antibody was able to dose-dependently activate CD64-mediated downstream signaling (see FIG. 2), exhibiting a slightly stronger activation activity than the control antibody.

Example 5. Expression and Purification of DLL3 Bispecific Antibody Molecule

All bispecific antibodies of the present invention were generated by first expressing and purifying the two parental proteins individually, and then subjecting them to in vitro redox recombination. The preparation route of the bispecific antibodies is shown in FIG. 3A, and the structures are shown in FIG. 3B.

Plasmid construction: The gene sequences were synthesized by Genewiz and cloned onto the pcDNA3.1 vector. The coding sequences are shown in Table 4 below:

DLL3 arm specifically binding to DLL3	
Anti-DLL3 antibody D3-107 VH	SEQ ID NO: 12
Anti-DLL3 antibody D3-107 VL	SEQ ID NO: 16
Fc region (LALA + S364R/D399K)	SEQ ID NO: 29
Light chain constant region	SEQ ID NO: 25
Heavy chain 1	SEQ ID NO: 41
Light chain 1	SEQ ID NO: 45
CD3 arm specifically binding to CD3	
Anti-CD3 antibody VH	SEQ ID NO:31
Anti-CD3 antibody VL	SEQ ID NO:35

Fc region (LALA+ K370S, K409D, Y349T)	SEQ ID NO: 30
Light chain constant region	SEQ ID NO: 24
Heavy chain 2	SEQ ID NO: 48
Light chain 2	SEQ ID NO: 40

Expi293F cells (purchased from Gibco) were cultured in an Expi293F medium (Gibco, REF# A14351-01). The cell density was measured the day before transfection (with viability required to be greater than 95%), and the cell was adjusted to a density of 3×10^6 cells/mL with fresh Expi293F medium for continued culture. On the day of transfection, the cell was adjusted to a density of 3×10^6 cells/ml.

1/10 of the final transfection volume of Opti-MEM medium (Gibco, REF# 31985-070) was used as a transfection buffer. The DNA to be transfected was added at a concentration of 1 mg/L, with the light and heavy chain plasmids at a ratio of 1:1. The mixture was mixed well, and PEIMax (Polysciences Inc. Cat# 24765-1), and the mixture was mixed well. PEIMax (Polysciences Inc. Cat# 24765-1) was added at a DNA:PEI mass ratio of 1:3, and the mixture was mixed well and incubated at room temperature for 20 min. Then the mixture was gently poured into the Expi293F cell suspension with swirling. The cells were cultured in a shaker under the conditions of 8% CO2, 36.5°C and 120 rpm.

After culturing for 16-18 h, the cell suspension was supplemented with 2% (v/v) of Feed (100 g/L Phytone Peptone + 100 g/L Difco Select Phytone) at a concentration of 200 g/L, a glucose solution at a final concentration of 5 g/L, and Valproic acid sodium salt (Merk, Cat# P4543-100G) at a final concentration of 2.2 mM, mixed well gently. The cells were continuously cultured at 8% CO_2, 36.5°C, and 120 rpm for 7 days before sample collection. The cell culture liquid was mixed well with celite (diatomaceous earth, Sartorius, Cat. No. 1000037025) (1 L of the cell culture liquid was mixed with 40 g of celite), and the mixture was filtered using a 0.22 μm disposable vacuum filtration apparatus.

Purification of target protein by affinity chromatography: A HiTrap MabSelect PrismA (GE Healthcare, Cat# 17549853) affinity chromatography column was selected for affinity capture. Before purification, 10-20 column volumes of 0.1 M NaOH were passed through the pipeline and the affinity chromatography column, and then the pipeline and the column were washed with 10-20 column volumes of distilled water. The packing column was equilibrated with 5 column volumes of

1× PBS (Gibco). The filtered cell culture liquid was passed through the column, and then the packing column was washed with 10 column volumes of 1× PBS to remove non-specific binding proteins. The packing was washed with 5 column volumes of an elution buffer (100 mM sodium citrate, pH 3.5), and the eluent was collected, and adjusted to pH6.0 with 2 M Tris. The eluent was filtered and sterilized for further ion exchange chromatography.

In vitro reduction oxidation: The protein collections from the affinity chromatography were mixed in a molar ratio of 1:1. A proper amount of GSH was added, and the reaction was adjusted to pH 8.0 with 2 M Tris. The reaction system was incubated at room temperature overnight. The reactant was buffer-exchanged into PBS and left to stand at 4°C for later use.

Purification of bispecific antibodies by ion exchange chromatography: a Mono S 5/50 GL (obtained from GE Healthcare) ion exchange chromatography column was selected and placed in an AKTApure system (available from GE healthcare). The AKTApure system equipped with a Mono S 5/50 GL ion exchange chromatography column was treated with 0.5 M NaOH for 2 h to remove endotoxins, and then the system and the column were washed with distilled water. The column was equilibrated with 5-10 column volumes of loading buffer (20 mM NaPO4, pH 6.6) until the conductivity and pH were stable. The protein obtained by affinity chromatography was diluted 10-fold with the loading buffer before loading. The column was reequilibrated with 5 column volumes of the loading buffer. Linear elution was performed with a gradient of 0-40% elution buffer (20 mM NaPO4, 1 M NaCl, pH 6.6) for a total of 30 column volumes, and samples were collected based on the UV absorption value.

Collected samples in all fraction tubes were analyzed for purity by size exclusion chromatography (SEC). According to the SEC results, the samples in the fraction tubes with a purity greater than 95% were combined.

The purified bispecific antibody solution was centrifuged in a 15 mL ultrafiltration centrifuge tube at 4500 rpm for 30 min. The protein was diluted with PBS and then centrifuged again at 4500 rpm for 30 min, and this operation was repeated several times to exchange the buffer. The antibodies after buffer exchange were combined, and the antibody concentration was measured. The composition and content of the bispecific antibody DLL3/CD3 were further qualitatively and

quantitatively determined by capillary electrophoresis (CE-SDS) in combination with liquid chromatography-mass spectrometry (LC-MS).

In a similar manner, the positive control antibody AMGEN757 (AMG757, INN Prop. List 123 tarlatamab, referred to herein as AMG757 or AMG757 analog) and the DLL3/CD3 bispecific antibody BI-D3C1 from Boehringer Ingelheim (WO 2019/234220 A1; EQ ID NO: 75 DLL3#3 chain; sEQ ID NO: 79 CD3#1 chain, referred to herein as BI-D3C1 or BI-D3C1 analog) were expressed and purified. The negative control IgG1 was from BioXCell BP0297.

The antibody Anti-mPD-1 (heavy chain: SEQ ID NO: 49, light chain: SEQ ID NO: 50) was similarly expressed and purified.

Example 6. Affinity of DLL3/CD3 Bispecific Antibody

The binding affinity (KD) of the bispecific antibody to DLL3 was determined by bio-layer interferometry (BLI). Half an hour prior to the assay, an appropriate number of AHC sensors (18-5060, Sartorius) and HIS1K sensors (18-5120, Sartorius) were taken according to the number of samples and soaked in SD buffer (1× PBS, 0.1% BSA, 0.05% Tween-20). The bispecific antibodies, human DLL3 (DL3-H52H4, Acro biosystems) and human CD3 (CDD-H52W1, Acro biosystems) were diluted to 100 nM.

The SD buffer, the antibody solution, human DLL3, and human CD3 were separately added to 96-well black polystyrene microplates (Greiner, 655209). The assay was performed using a Fortebio Octet Red96e instrument. The plate was arranged according to sample positions, and the sensor positions were selected accordingly. The instrument parameters were set as follows: the assay was run with the following steps: baseline equilibration for 120 s, antibody loading for 100 s, baseline equilibration for 120 s, antigen association for 100 s, and dissociation for 120 s, at an agitation speed of 1000 rpm and a temperature of 30°C. After the experiment was completed, KD values were analyzed using ForteBio Octet analysis software. The results are shown in the table below.

In experiments performed as described in the above assay, the affinity of DLL3/CD3 to human DLL3-His is shown in Table 5.

Sample ID	KD (M) antibodies on AHC sensor tip, Human DLL3 His (100 nM) in solution	Response	KD (M)	kon(1/Ms)	kdis(1/s)
D3-107/CD3	FIG.4A	0.3671	4.91E-10	4.07E+05	2.00E-04

The affinity of DLL3/CD3 to human CD3 is shown in Table 6.

Sample ID	KD (M) Human CD3 on HIS1K sensor tip, antibody (100nM) in solution	Response	KD (M)	kon(1/Ms)	kdis(1/s)
D3-107/CD3	FIG.4B	1.2595	9.92E-09	2.93E+05	2.90E-03

The binding affinities obtained are shown in Table 7.

Name	Antigen	Ka(1/Ms)	kd(1/s)	KD (M)
D3-107/CD3	Human DLL3	4.07E+05	2.00E-04	4.91E-10
D3-107/CD3	Human CD3	2.93E+05	2.90E-03	9.92E-09

Example 7. Cell Binding Assay of DLL3/CD3 Bispecific Antibody

The binding ability of the DLL3/CD3 bispecific antibody to DLL3-overexpressing cell lines was detected by flow cytometry.

CHO-hDLL3 cells were cultured and passaged according to standard procedures. After centrifugation and resuspension, the cells were counted and adjusted to a cell density of 4×10^6 cells/mL. The cells were transferred to a reagent reservoir and dispensed into a 96-well plate at 50 μL/well using a multi-channel pipette. Then, 50 μL of serially diluted antibody samples (starting at 300 nM, with 3-fold dilutions across 12 concentrations) were added to each well containing 50 μL of cells, and then the plate was incubated in a refrigerator at 4°C for 30 min. The plate was centrifuged at 400 g for 5 min, and the supernatant was discarded. The cells were then in 200 μL of PBS, centrifuged again, and resuspended. This wash procedure was repeated twice. PE anti-human IgG Fc recombinant antibody (1:200) was added, and the mixture was incubated at 4°C in the dark

for 30 min. The cells were then twice washed with 200 μL of PBS, centrifuged, resuspended in 100 μL of PBS, and analyzed by flow cytometry. The data were fitted to a dose-response curve, and the EC50 values were calculated.

In experiments performed as described in the above assay, the binding of the exemplary antibody to DLL3 on the surface of overexpressed cells is shown in FIG. 5. As can be seen from FIG. 5, the binding plateau of the exemplary antibody to the DLL3-overexpressing cell line was slightly stronger than that of the control antibody.

PBMCs (Oricells, Cat#: FPB005-C) were thawed according to standard procedures. After thawing, the cells were centrifuged and resuspended, and counted, and adjusted to a cell density of 4×10^6 cells/mL. Human TruStain FcXTM (Biolegend, 422302) was added, and the cells were mixed well and blocked at 4°C for 10 min. After blocking, an FITC anti-human CD4 antibody (Biolegend, 300506) and APC anti-human CD8a antibody (Biolegend, 301014) were added and mixed well. The cell suspension was transferred to a reagent reservoir and dispensed into a 96-well plate at 50 μL per well using a multi-channel pipette. Then, 50 μL of serially diluted antibody samples (starting at 300 nM, with 3-fold dilutions across 12 concentrations) were added to each well containing 50 μL of cells, and then the plate was incubated in a refrigerator at 4 C for 30 min. The plate was centrifuged at 400 g for 5 min, and the supernatant was discarded. After adding 200 μL of PBS, the mixture was centrifuged at 400 g for 5 min, and the supernatant was discarded. This wash procedure was repeated 3 times. The mixture was centrifuged at 400 g for 5 min, and the supernatant was discarded. The mixture was resuspened in 200 μL of PBS, centrifuged again, and resuspended. This wash procedure was repeated twice. PE anti-human IgG Fc recombinant antibody (1:200) was added, and the mixture was incubated at 4°C in the dark for 30 min. The cells were then washed twice with 200 μL of PBS, centrifuged, resuspended in 100 μL of PBS, and analyzed by flow cytometry. The data were fitted to a dose-response curve, and the EC50 values were calculated.

In experiments performed as described in the above assay, the binding of exemplary antibody to CD3 on the surface of human CD4 T cells (FIG. 6B) and CD8 T cells (FIG. 6A) is shown in FIG. 6. As can be seen from FIG. 6, the binding ability of the exemplary antibody to CD3 on the surface of T cells was comparable to that of the control antibody.

Example 8. In Vitro Jurkat-NFAT-Luc Activation Assay of DLL3/CD3 Bispecific Antibody

The DLL3/CD3 bispecific antibody simultaneously binds to DLL3 on the surface of small cell lung cancer cells and CD3 on the surface of Jurkat-NFAT-Luc cells, and activates the NFAT-Luc downstream signaling pathway through DLL3-dependent CD3 cross-linking. In this study, a luciferase reporter assay was used to assess the activation activity of a test antibody by measuring luciferase expression in Jurkat-NFAT-Luc cells co-cultured with DLL3-positive cells following overnight incubation with the antibody.

SHP77, H82 (ATCC, HTB-175), DMS53 (Nanjing Cobioer, CBP60172), H460 (ATCC, HTB-177, DLL3 negative cells) and Jurkat-NFAT-Luc cells were resuspended using the Assay medium, and was adjusted to a cell density of 6.0 × 10 cells^5 cells/mL. The tumor cells and Jurkat-NFAT-Luc cells were mixed at a ratio of 1:1 for later use. The cell suspension and the serially diluted test antibody (starting at 50 nM, with 3-fold dilutions across 12 concentrations) were added at 100 μL/well, and the plate was incubated overnight. The next day, 80 μL of Bio-Glo Luciferase Assay System (Promega, G7940) assay solution was added to each well. The plate was read on a microplate reader (Molecular Device, SpectraMax I3).

In experiments performed as described in the above assay, in the DLL3-positive cell experimental systems (SHP77, H82, DMS53), the exemplary antibody was able to dose-dependently activate NFAT signaling (see FIGs. 7A, 7B and 7C), with an activation activity comparable to that of the control antibody. The activation activity was strongest in SHP77 with the highest expression of DLL3, and was slightly lower in H982 and DMS53 with slightly lower expression; while the exemplary antibody showed no activation in the DLL3 negative cell H460 experimental system (see FIG. 7D). Therefore, the activation activity of the DLL3/CD3 bispecific antibody for NFAT signaling was correlated with the expression level.

Example 9. In Vitro T Cell-Mediated Killing of Small Cell Lung Cancer by DLL3/CD3 Bispecific Antibody

The DLL3/CD3 bispecific antibody simultaneously binds to DLL3 on the surface of small cell

lung cancer cells (SCLC) and CD3 on the surface of primary T cells. Upon DLL3-dependent cross-linking of CD3, the antibody activates the T cells and mediates the killing against DLL3-positive tumor cells. In this study, Propidium Iodide (PI) staining was used to assess the cytotoxicity of human $CD8^+$ T cells against DLL3-positive tumor cells by measuring the percentage of PI-positive tumor cells 24 hours after the addition of the test antibody to a co-culture of human PBMCs and DLL3-positive cells.

PBMCs were taken out from a liquid nitrogen tank, rapidly thawed at 37°C, and added dropwise to a preheated 1640 medium containing 10% FBS (containing 0.1% DNase) to obtain 10 mL of a mixed solution. The mixture was centrifuged at 400 g for 5 min and resuspended in 10 mL of 1640 medium containing 10% FBS, and 10 μL of DNase was added, and the plate was placed in an incubator for adherence culture overnight at 37°C with 5% CO_2.

DLL3-positive cells SHP77 and DLL3-negative cells H460 (negative control) were used as target cells and centrifuged at 400 g for 5 min, and the supernatant was removed. The cells were washed twice with 30 mL of PBS and centrifuged, and the supernatant was removed. CellTrace Far Red Cell staining solution (THERMO FISHER, C34564) was added to label the cells, and the cells were incubated in an incubator at 37°C in the dark for 20 min. After the incubation, a 1640 complete medium was added, and the mixture was centrifuged at 400 g for 5 min, and the supernatant was removed. The cells were resuspended in the 1640 complete medium and transferred to a CO_2 incubator for culture overnight.

The target cells were adjusted to a density of 2 × 10E5 cells/mL, and PBMC s were adjusted to a density of 4 × 10E6 cells/mL. 50 μL of the serially diluted antibody (starting at 10 nM with 3-fold dilutions across 11 concentrations), 50 μL of the target cells and 50 μL of PBMC cells were added, and the mixture was mixed well and then placed in a CO_2 incubator for culture. After 24 h of killing, the 96-well plate was taken out, 100 μL of PI assay solution was added to each well. After mixing with the cells, the samples were assayed by flow cytometry.

In experiments performed as described in the above assay, the exemplary antibody was able to dose-dependently induce killing of human CD8+ T cells against SHP77 target cells (see FIG. 8A), and showed no killing against non-target cells H460 (see FIG. 8B).

Example 10. PTM Removal of DLL3/CD3 bispecific antibody

The D3-107/CD3 bispecific antibody molecule has a DG as a hotspot in the heavy chain variable region (HCDR2) of the DLL3 arm (D3-107) and an NFS glycosylation site in the light chain framework region (FR3). To reduce the analytical difficulties in CMC and the potential risk of developability, corresponding mutations were designed for the above hotspot (Table 8). The mutated molecules were verified for binding activity and expression yield based on the methods of Examples 2 and 3 (starting at 300 nM, with 3-fold dilutions across 12 concentrations), demonstrating that the mutant molecules can maintain good DLL3 binding activity and expression yield (Tables 8 and 10; FIG. 9).

According to the results of the expression level and the cell-based binding ability, the heavy chain D3-107-VH-P1 and D3-107-VH-P2 were each combined with the light chain D3-107-VL-P2 to generate D3-107-C1 and D3-107-C2, respectively. The binding activity of the resulting novel monoclonal antibody sequence was re-verified by flow cytometry (see Example 2). The PTM-removed molecules all exhibited favorable binding activity than that of the parent molecule (Tables 9 and 10, and FIG. 10).

Next, D3-107-C1 and D3-107-C2 were assembled with a CD3 arm to generate the bispecific antibodies D3-107/CD3-C1 and D3-107/CD3-C2, respectively. The two bispecific antibodies were evaluated for cell-based binding ability(see Examples 3 and 7 for the method; and results shown in FIG. 11), reporter activation (see Example 8 for the method; and results shown in FIG. 12), and in vitro killing function (see Example 9 for the method; and results shown in FIGs. 13 and 14). The two candidate molecules were found to be comparable in in vitro activity to each other and consistent with that of their parent molecules. Therefore, D3-107/CD3-C1 was selected.

Table 8: PTM removal mutations of DLL3 candidate molecule D3-107

NO.	New plasmid name	Sequences	SEQ ID NO
1	D3-107-VH	D at position 6 and G at position 7 of CDR2	SEQ ID NO:12
2	D3-107-P1-VH	D at position 6 of CDR2 mutated to E	SEQ ID NO:13
3	D3-107-P2-VH	D at position 6 of CDR2 mutated to Q	SEQ ID NO:14

4	D3-107-P3-VH	G at position 6 of CDR2 mutated to V	SEQ ID NO:15
5	D3-107-VL	NFS comprised in FR3	SEQ ID NO:16
6	D3-107-VL-P1	N of NFS in FR3 mutated to Q	SEQ ID NO:17
7	D3-107-VL-P2	S of NFS in FR3 mutated to A	SEQ ID NO:18

The light chain variable regions and the heavy chain variable regions described above were combined to prepare corresponding monoclonal antibodies as described in Example 1. The obtained antibodies are shown in Table 9 below.

Table 9: Names and variable regions of monoclonal antibodies

Protein name	Heavy chain variable region	Light chain variable region
D3-107	D3-107-VH SEQ ID NO:12	D3-107-VL SEQ ID NO:16
D3-107-P1	D3-107-P1-VH SEQ ID NO:13	D3-107-VL SEQ ID NO:16
D3-107-P2	D3-107-P2-VH SEQIDNO:14	D3-107-VL SEQ ID NO:16
D3-107-P3	D3-107-P3-VH SEQ ID NO:15	D3-107-VL SEQ ID NO:16
D3-107-P4	D3-107-VH SEQ ID NO:12	D3-107-P1-VL SEQID NO:17
D3-107-P5	D3-107-VH SEQ ID NO:12	D3-107-P2-VL SEQ ID NO:18
D3-107-C1	D3-107-P1-VH SEQ ID NO:13	D3-107-P2-VL SEQ ID NO:18
D3-107-C2	D3-107-P2-VH SEQIDNO:14	D3-107-P2-VL SEQ ID NO:18

Table 10. DLL3 binding activity and expression level of DLL3-S4-107 after removal of single hotspot sites

Protein name	Expression level (mg/L)	Purity（SEC）	KD	EC50
D3-107	69.3	99.871%	9.24E-10	1.575
D3-107-P1	72.9	99.819%	9.34E-10	1.541
D3-107-P2	75.4	100.000%	1.22E-09	1.252
D3-107-P3	80.3	99.791%	9.98E-10	1.664
D3-107-P4	52.8	99.809%	9.71E-10	1.01
D3-107-P5	73.8	99.866%	9.97E-10	0.9556

D3-107	69.3	99.871%	9.24E-10	0.6575
D3-107-C1	108.9	99.924%	6.68E-10	0.7164
D3-107-C2	121.3	99.964%	7.75E-09	0.5609

The specific sequences of the resulting D3-107/CD3-C1 bispecific antibodies are as follows:

DLL3 arm specifically binding to DLL3	
Anti-DLL3 antibody D3-107 VH (D3-107-P1-VH)	SEQ ID NO: 13
Anti-DLL3 antibody D3-107 VL (D3-107-P2-VL)	SEQ ID NO: 18
Fc region (LALA + S364R/D399K)	SEQ ID NO: 29
Light chain constant region	SEQ ID NO: 25
Heavy chain 1	SEQ ID NO: 42
Light chain 1	SEQ ID NO: 47
CD3 arm specifically binding to CD3	
Anti-CD3 antibody VH	SEQ ID NO: 31
Anti-CD3 antibody VL	SEQ ID NO: 35
Fc region (LALA+ K370S, K409D, Y349T)	SEQ ID NO: 30
Light chain constant region	SEQ ID NO: 24
Heavy chain 2	SEQ ID NO: 48
Light chain 2	SEQ ID NO: 40

The specific sequences of the resulting D3-107/CD3-C2 bispecific antibodies are as follows:

DLL3 arm specifically binding to DLL3	
Anti-DLL3 antibody D3-107 VH (D3-107-P2-VH)	SEQIDNO: 14
Anti-DLL3 antibody D3-107 VL (D3-107-P2-VL)	SEQ ID NO: 18
Fc region (LALA + S364R/D399K)	SEQ ID NO: 29
Light chain constant region	SEQ ID NO: 25
Heavy chain 1	SEQ ID NO: 43
Light chain 1	SEQ ID NO: 47
CD3 arm specifically binding to CD3	
Anti-CD3 antibody VH	SEQ ID NO: 31
Anti-CD3 antibody VL	SEQ ID NO: 35
Fc region (LALA+ K370S, K409D, Y349T)	SEQ ID NO: 30
Light chain constant region	SEQ ID NO: 24
Heavy chain 2	SEQ ID NO: 48
Light chain 2	SEQ ID NO: 40

Example 11. Affinity of PTM-removed DLL3/CD3 Bispecific Antibody

The binding affinity (KD) of the bispecific antibody to DLL3 was determined by bio-layer interferometry (BLI). Half an hour prior to the assay, an appropriate number of AHC sensors (18-5060, Sartorius) were taken according to the number of samples and soaked in SD buffer (1 × PBS, 0.1% BSA, 0.05% Tween-20). The bispecific antibodies and human DLL3 (DL3-H52H4, Acro biosystems) were diluted to 100 nM.

The SD buffer, the antibody solution, and human DLL3 were separately added to 96-well black polystyrene microplates (Greiner, 655209). The assay was performed using a Fortebio Octet Red96e instrument. The plate was arranged according to sample positions, and the sensor positions were selected accordingly. The instrument parameters were set as follows: the assay was run with the following steps: baseline equilibration for 120 s, antibody loading for 100 s, baseline equilibration for 120 s, antigen binding for 100 s, and dissociation for 120 s, at an agitation speed of 1000 rpm and a temperature of 30°C. After the experiment was completed, KD values were analyzed using ForteBio Octet analysis software. The results are shown in Table 11 below and FIG. 15.

Sample ID	KD (M) antibodies on AHC sensor tip, Human DLL3 His (100 nM) in solution	Response	KD (M)	kon(1/Ms)	kdis(1/s)
D3-107/CD3-C1	FIG. 15A	0.7832	6.68E-10	2.99E+05	2.00E-04
D3-107/CD3-C2	FIG. 15B	0.8162	7.75E-10	2.58E+05	2.00E-04

Example 12. Verification of binding of PTM-removed DLL3/CD3 bispecific antibody to DLL1 and DLL4

In vitro affinity determination of the exemplary antibody to DLL1 and DLL4

The binding affinity (KD) of the bispecific antibody to DLL3 was determined by bio-layer interferometry (BLI). Half an hour prior to the assay, an appropriate number of AHC sensors (18-5120, Sartorius) were taken according to the number of samples and soaked in SD buffer (1× PBS, 0.1% BSA, 0.05% Tween-20). The bispecific antibodies, human DLL1 (DL1-H52H8, Acro biosystems) and human DLL4 (DL4-H5227, Acro biosystems) were diluted to 100 nM.

The SD buffer, the antibody solution, human DLL1, and human DLL4 were separately added to 96-well black polystyrene microplates (Greiner, 655209). The assay was performed using a

Fortebio Octet Red96e instrument. The plate was arranged according to sample positions, and the sensor positions were selected accordingly. The instrument parameters were set as follows: the assay was run with the following steps: baseline equilibration for 120 s, antibody loading for 100 s, baseline equilibration for 120 s, antigen association for 100 s, and dissociation for 120 s, at an agitation speed of 1000 rpm and a temperature of 30°C. After the experiment was completed, KD values were analyzed using ForteBio Octet analysis software. The results are shown in Table 12 below.

Table 12. In vitro cell-based binding of the exemplary antibody to DLL1 and DLL4

Name	Antigen	Response	Ka(1/Ms)	kd(1/s)	KD (M)
D3-107/CD3-C1	Human DLL1	-0.014	No binding		
D3-107/CD3-C1	Human DLL4	0.0405	No binding		

The binding ability of the DLL3/CD3 bispecific antibody to DLL1- and DLL4-overexpressing cell lines was detected by flow cytometry.

CHO-hDLL1 (obtained by electroporating CHO cells with a pXC17.4 vector constructed to contain the human DLL1 gene [NCBI ID: 28514], followed by sorting positive cell populations) and 293T-hDLL4 (obtained by packaging lentivirus in 293T cells using a pLenti-C-MYC-DDK-IRES-puro vector constructed to contain human DLL4 gene [NCBI Gene ID: 54567), followed by transduction of 293T cells with the resulting lentivirus and selection of positive cells).

The cells were cultured and passaged according to conventional procedures. After centrifugation and resuspension, the cells were counted andwas adjusted to a cell density of 4×10^6 cells/mL. The cell suspension was then transferred to a reagent reservoir and dispensed into a 96-well plate at 50 μL/well using a multi-channel pipette. Then, 50 μL of serially diluted antibody samples (starting at 100 nM, with 3-fold dilution across 10 concentrations) were added to each well containing 50 μL of cells, wherein the positive controls were a commercial DLL1 antibody (PE anti-human Delta-like protein 1 (DLL1); biolegend; 346404) and a commercial DLL4 antibody (PE anti-human Delta-like protein 4 (DLL4) Antibody; biolegend; 346506), and the plate was incubated in a refrigerator at 4°C for 30 min. The plate was centrifuged at 400 g for 5 min, and the supernatant

was discarded. The cells were then resuspended in 200 μL of PBS, centrifuged again, and resuspended. This wash procedure was repeated twice. PE anti-human IgG Fc recombinant antibody (1:200) was added, and the mixture was incubated at 4°C in the dark for 30 min. The cells were then washed twice with 200 μL of PBS, centrifuged, and resuspended in 100 μL of PBS, and analyzed by flow cytometry. analyzed by flow cytometry and the EC50 values were calculated.

In experiments performed as described in the above assay, the binding of the exemplary antibody to CHO-hDLL1 and 293T-hDLL4 cells is shown in FIG. 16. The exemplary antibody showed no binding to cell lines overexpressing DLL3 homologous proteins (DLL1/DLL4).

Example 13. Binding assay of PTM-removed DLL3/CD3 bispecific antibody to small cell lung cancer and T cells

The binding ability of the PTMremoved DLL3/CD3 bispecific antibody to DLL3-overexpressing cell lines and T cells was detected by flow cytometry. Detailed experimental procedures are described in Example 7.

The results are shown in FIG. 17. It can be seen from FIG. 17 that the binding ability of the PTM-removed DLL3/CD3 bispecific antibody is comparable to that of the positive control AMG757 analog and is superior to that of the BI-D3C1 analog.

Example 14. In Vitro Activation of NFAT-reporter cells by PTM-removed DLL3/CD3 Bispecific Antibody

The activation of NFAT reporter cells by the exemplary antibody was assessed in an in vitro experiment. Reference was made to Example 8 for the specific experimental procedure, with the difference that IgG1 was used as a negative control and BI-D3C1 analog was used as a positive control.

The experimental results are shown in FIG. 18. As can be seen from FIG. 18 that the D3-107/CD3-C1 antibody exhibited significant dose-dependent stimulatory activity on DLL3-positive cells, with the activation levels similar to those of the BI-positive drugs. However, in

the DLL3-negative cells of interest, the exemplary antibody showed no activation activity, indicating a favorable safety profile.

Example 15. In Vitro Killing of Tumor Cells by PTM-Removed DLL3/CD3 Bispecific Antibody

The in vitro killing activity of exemplary antibody against tumor cells was evaluated using a co-culture system of primary PBMCs and small cell lung cancer cells. Reference was made to Example 9 for the specific experimental procedure, except that the test antibodies were serially diluted 3-fold across 10 concentrations starting at 10 nM.

The specific results are shown in FIG. 19. The exemplary antibody D3-107/CD3-C1 was able to effectively kill DLL3-positive tumor cells (SHP-77 (ATCC CRL-2195), DMS-79 (ATCC CRL-2049), and H82 (ATCC HTB-175)) in a dose-dependent manner. However, for no non-specific killing was observed in DLL3-negative tumor cells (293T-hDLL4 (constructed as described above) and U87 (ATCC HTB-14)), even at a dose of up to 900 nM, suggesting that the exemplary antibody is safe and reliable.

Example 16. Cytokine Release Assay Of PTM-Removed DLL3/CD3 Bispecific Antibody with DLL3-Negative Cells

To further evaluate the safety profile of the exemplary antibody, the production of cytokines by immune cells in PBMCs was monitored in the absence of DLL3 crosslinking. Reference was made to Example 9 for the specific experimental method, with the difference that no target cells were added, only PBMCs and antibody dilutions were added, with the antibody serially diluted 3-fold across 12 concentrations starting at 10 nM. After incubation, the supernatant was collected, and the cytokine levels were determined using a Human Th1/Th2/Th17 CBA Kit and analyzed using FCAP.

The specific results, as shown in FIG. 20, demonstrated that the exemplary antibody D3-107/CD3-C1 exhibited little stimulatory effect on T cells or macrophages in the absence of DLL3 crosslinking, and the detected cytokines (TNFa, IL-6, etc.) were substantially comparable to the baseline, suggesting that the exemplary antibody has a favorable safety profile.

Example 17. Study On In-Vivo Anti-Tumor Effect Of PTM-Removed DLL3/CD3 Bispecific Antibody

In this study, the anti-tumor effect of the exemplary antibody was assessed in PBMC-humanized NOG mice models inoculated with the human small cell lung cancer (SCLC) cell lines DMS-79 and SHP-77.

17.1 In Vivo Anti-tumor Effect of the Exemplary Antibody in a DMS-79 Tumor-Bearing Humanized Mouse Model

Experimental Procedures

Female NOG mice (16-19 g) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. The mice were of SPF grade. Upon arrival, the mice were acclimated and quarantined for 3 days before the study was initiated.

Mice were injected intravenously with PBMC cells at 4×10^6 cells/mouse with an inoculation volume of 200 μL/mouse. DMS-79 cells were inoculated on the third day after PBMC injection. DMS-79 cells were outinely cultured and passaged, then harvested by centrifugation and dispersed in PBS. NOG mice were shaved on the right dorsal flank and inoculated with DMS79 cells at 5×10^6 cells/mouse with an inoculation volume of 200 μL/mouse.

Administration: On day 7 after the inoculation of DMS-79 cells, the mice were divided into groups (6 mice per group) based on tumor volume and PBMC proliferation for dosing. The initial tumor volume of the control group was about 129 mm^3.

The antibodies were administered once every 7 days for a total of 3 doses, atdoses of 0.1 mpk and 0.01 mpk, respectively. The administration groups were as follows:

hIgG: human IgG control (Equitech-Bio SLH56-0001);

D3-107/CD3-C1: exemplary antibody D3-107/CD3-C1

BI-D3C1 analog: positive control BI DLL3/CD3.

The tumor volume and body weight of the mice were monitored twice a week.

Tumor volume measurement: Tumor size was measured using a vernier caliper, with the

longest length (L) and widest width (W) recorded. The tumor volume was calculated using the following formula: $V = L \times W^2/2$. The mice were weighted using an electronic balance. Throughout the study, mice were euthanized when tumors reached an endpoint or when the mice had more than 20% of weight loss. The tumor size was counted, and the tumor growth inhibition (TGI%) was calculated.

The relative tumor growth inhibition (TGI%) was calculated on day 24 after inoculation, and the calculation formula is as follows:

TGI% = 100% × (hIgG control group tumor volume – treatment group tumor volume)/(hIgG control group tumor volume – hIgG control group initial tumor volume).

Experimental Results

Tumor growth curves are shown in FIG. 21. The exemplary antibody could significantly inhibit the growth of DMS79 cells. The tumor size was obtained on day 24, and the tumor growth inhibition rate and the proportion of complete tumor regression were calculated.

In contrast to hIgG, the exemplary antibody D3-107/CD3-C1 and BI positive control BI DLL3/CD3 at a high dose of 0.1 mg/kg exhibited tumor growth inhibition rates of 115% and 103%, respectively. In addition, complete tumor regression was observed in 5/6 mice in the exemplary antibody D3-107/CD3-C1-treated group and in 2/6 mice in the BI positive control BI DLL3/CD3-treated group. Thus, the exemplary antibody D3-107/CD3-C1 exhibited superior anti-tumor effects relative to the BI positive control BI DLL3/CD3, and no significant weight loss was observed in either group.

At the low dose of 0.01 mg/kg, the exemplary antibody D3-107/CD3-C1 and the BI positive control BI DLL3/CD3 exhibited tumor growth inhibition (TGI) rates of 75% and 53%, respectively, and with the exemplary antibody D3-107/CD3-C1 also exhibiting a superior anti-tumor effect.

DMS-79 Model Data

After the experiment was completed, tumor tissues from the low dose group were collected, fixed in formalin, and processed into paraffin-embedded blocks for immunohistochemical (IHC) staining of CD3, so as to evaluate the CD3 status in the tumor after the administration.

In FIG. 22, panels A, B, and C correspond to the groups IgG1 0.1 mpk, BI-D3C1 analog 0.01 mpk, and D3-107/CD3-C1 0.01 mpk, respectively. As can be seen from the figure, notable infiltration of CD3+ cells was observed in the D3-107/CD3-C1 0.01 mpk group, which was higher than that observed in the hIgG group

The number of CD3+ cells per unit area was counted by HALO software (Indica Labs), and the results are shown in FIG. 23. The results showed that the D3-107/CD3-C1 group had more positive cell infiltration in the tumor compared to the BI-D3C1 analog group, which was consistent with a stronger anti-tumor effect.

17.2 Anti-Tumor Effect of the Exemplary Antibody In SHP-77 Tumor-Bearing Humanized Mouse Models

Experimental Procedures

Female NOG mice (16-19 g) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. The mice were of SPF grade. Upon arrival, the mice were acclimated and quarantined for 3 days before the study was initiated.

PBMC cells were thawed in a pre-heated RPMI-1640 medium with 0.1% DNase, and then dispersed in PBS to prepare a cell suspension at a concentration of 20×10^6 cells/mL. Mice were intravenously injected with PBMC cell suspensions at 0.2 mL/mouse, corresponding to an inoculum size of 4×10^6 cells/mouse.

The SHP-77 cells were routinely cultured and passaged for subsequent in vivo study. Six days after PBMC cell inoculation, SHP-77 cells were dispersed in a 1:1 mixture of PBS and matrigelto prepare a cell suspension at a concentration of 25×10^6 cells/mL cells. NOG mice were shaved on the right dorsal flank and injected subcutaneously with the SHP-77 cell suspension at 0.2 mL/mouse, corresponding to an inoculum size of 5×10^6 cells/mouse.

4 days after the tumor cell inoculation, the mice were divided into groups (c) based on the tumor volume for dosing. The antibody was administered once every 7 days for 3 times via intraperitoneal injection, at a dose of 0.6 mpk and 0.06 mpk, respectively. The administration groups were as follows:

h-IgG: human IgG control (Equitech-Bio SLH56-0001);

D3-107/CD3-C1: exemplary antibody D3-107/CD3-C1

BI-D3C1 analog: positive control BI DLL3/CD3.

The tumor volume and body weight of the mice were monitored twice a week until the end of 24 days.

The relative tumor growth inhibition (TGI%) was calculated on day 24 after inoculation, and the calculation formula is as follows:

TGI% = 100% × (hIgG control group tumor volume – treatment group tumor volume)/(hIgG control group tumor volume – hIgG control group tumor volume before administration).

Tumor volume measurement: Tumor size was measured using a vernier caliper, with the longest length (L) and widest width (W) recorded. The tumor volume was calculated using the following formula: $V = L \times W^2/2$. The mice were weighted using an electronic balance.

Experimental Results

The tumor growth curves are shown in FIG. 24. The exemplary antibody D3-107/CD3-C1 could significantly inhibit the growth of SHP77. The tumor size was obtained on day 24, and the tumor growth inhibition was calculated. The antibody D3-107/CD3-C1 and BI positive control BI DLL3/CD3 at a high dose of 0.6 mg/kg exhibited tumor growth inhibitions of 108% and 111%, respectively. The Exemplary antibody D3-107/CD3-C1 and BI positive control BI DLL3/CD3 at a low dose of 0.06 mg/kg exhibited tumor inhibition rates of 75% and 34%, respectively. In addition, no significant body weight loss was observed in either the antibody D3-107/CD3-C1-treated group or the BI positive control BI DLL3/CD3-treated group.

17.3 Anti-Tumor Effect of the Exemplary Antibody In SHP-77 Tumor-Bearing Humanized Mouse Models

Experimental Procedures

Female NOG mice (16-19 g) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. The mice were of SPF grade. Upon arrival, the mice were acclimated and

quarantined for 3 days before the study was initiated.

PBMC cells were thawed in a pre-heated RPMI-1640 medium with 0.1% DNase, and then dispersed in PBS to prepare a cell suspension at a concentration of 20×10^6 cells/mL. Mice were intravenously injected with PBMC cell suspensions at 0.2 mL/mouse, corresponding to an inoculum size of 4×10^6 cells/mouse.

The SHP-77 cells were routinely cultured and passaged for subsequent in vivo study. 4 days after PBMC cell inoculation, SHP-77 cells were dispersed in a 1:1 mixture of PBS and matrigel to prepare a cell suspension with a cell concentration of 25×10^6 cells/mL. NOG mice were shaved on the right dorsal flank and injected subcutaneously with the SHP-77 cell suspension at 0.2 mL/mouse, corresponding to an inoculum size of 5×10^6 cells/mouse.

4 days after the tumor cell inoculation, the mice were divided into groups (6 mice per group) based on the tumor volume for dosing. The antibody was administered once every 7 days for 3 times via intraperitoneal injection. The doses administered are indicated in the legend of FIG. 25 (ensuring equivalent molar doses). The administration groups were as follows:

h-IgG: human IgG control (Equitech-Bio SLH56-0001);

D3-107/CD3-C1: exemplary antibody D3-107/CD3-C1

AMG757 analog: positive control AMG757.

The tumor volume and body weight of the mice were monitored twice a week until the end of 25 days. Dosing was performed based on equivalent molar doses.

The relative tumor growth inhibition (TGI%) rate was calculated on day 21 post-inoculation using the following formula:

TGI% = 100% × (hIgG control group tumor volume – treatment group tumor volume)/(hIgG control group tumor volume – hIgG control group tumor volume before administration).

Tumor volume measurement: Tumor size was measured using a vernier caliper, with the longest length (L) and widest width (W) recorded. The tumor volume was calculated using the following formula: $V = L \times W^2/2$. The mice were weighted using an electronic balance.

Experimental Results

The tumor growth curves are shown in FIG. 25. The exemplary antibody D3-107/CD3-C1 could significantly inhibit the growth of SHP77. The tumor size was obtained on day 20, and the tumor growth inhibition was calculated.

The antibody D3-107/CD3-C1 and Amgen positive control AMG757 analog at a high dose exhibited tumor growth inhibitions of 112% and 110%, respectively. The exemplary antibody D3-107/CD3-C1 and Amgen positive control AMG757 analog at a low dose exhibited tumor growth inhibitions of75% and 40%, respectively. In addition, no significant body weight loss was observed in either the antibody D3-107/CD3-C1-treated group or the Amgen positive control AMG757 analog-treated group.

17.4 Combination Antitumor Effect of the Exemplary Antibody with Standard Chemotherapy in H82 Tumor-Bearing Humanized Mouse Model

Experimental Procedures

Female NOG mice (16-19 g) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. The mice were of SPF grade. Upon arrival, the mice were acclimated and quarantined for 3 days before the study was initiated.

PBMC cells were thawed in a pre-heated RPMI-1640 medium with 0.1% DNase, and then dispersed in PBS to prepare a cell suspension at a concentration of 20×10^6 cells/mL. Mice were intravenously injected with PBMC cell suspensions at 0.2 mL/mouse, corresponding to an inoculum size of 4×10^6cells/mouse.

H82 cells were routinely cultured and passaged for subsequent in vivo study. Six days after PBMC cell inoculation, H82 cells were dispersed in a 1:1 mixture of PBS and matrigel to prepare a cell suspension at a concentration of 12.5×10^6 cells/mL. NOG mice were shaved on the right dorsal flank and injected subcutaneously with the H82 cell suspension at 0.2 mL/mouse, corresponding to an inoculum size of 2.5×10^6cells/mouse.

5 days after the tumor cell inoculation, the mice were divided into groups (6 mice per group) based on the tumor volume for dosing. The exemplary antibody was administered twice a week for

7 times, and the standard chemotherapeutic drug was administered once a week for 4 times, with theroute of administration of intraperitoneal injection, and the administration volume 10 mL/kg. The doses are shown in the figure legend to FIG. 26. The administration groups were as follows:

h-IgG: IgG control (Equitech-Bio SLH56-0001)

D3-107/CD3-C1: exemplary antibody D3-107/CD3-C1

Chemotherapy (Chemo): carboplatin+etoposide

Combination therapy: carboplatin+etoposide+ D3-107/CD3-C1

The tumor volume and body weight of the mice were monitored twice a week until day 31.

Tumor volume measurement: Tumor size was measured using a vernier caliper, with the longest length (L) and widest width (W) recorded. The tumor volume was calculated using the following formula: $V = L \times W^2/2$. The mice were weighted using an electronic balance.

Experimental Results

Tumor growth curves are shown in FIG. 26. The relative tumor growth inhibition (TGI%) on day 29 after inoculation was calculated by the following formula: TGI% = 100% × (hIgG control group tumor volume – treatment group tumor volume)/(hIgG control group tumor volume – hIgG control group tumor volume before administration).

The antibody D3-107/CD3-C1 and standard chemotherapeutics exhibited tumor growth inhibitions of23.0% and 65.9%, respectively. When administered in combination, the TGI rate reached to 83.5%, indicating markedly improved antitumor efficacy. Furthermore, no significant body weight loss was observed throughout the study.

17.5 Combination Antitumor Effect of Exemplary Antibody with Immune Checkpoint Inhibitor in MC38-hDLL3 Tumor-Bearing hCD3 KI Mouse Model

Experimental Procedures

Female hCD3EDG mice (6-8 w) were purchased from Shanghai Model Organisms Center, Inc. The mice were of SPF grade. Upon arrival, the mice were acclimated and quarantined for 3 days before the study was initiated.

MC38-hDLL3 cells were generated in-house as follows: the human DLL3 gene (NCBI Gene ID: 10683) was cloned into the pLenti-C-MYC-DDK-IRES-puro vector, packaged into lentivirus using 293T cells, used to infect MC38 cells (Shanghai Obio Technology (Group) Corp., Ltd., HYC0116), and positive cell populations were sorted. MC38-hDLL3 cells were routinely cultured and passaged for subsequent in vivo assays. MC38-hDLL3 cells were prepared into a cell suspension at a concentration of 15×10^6 cells/mL. The mice were shaved on the right dorsal flank and injected subcutaneously with the MC38-hDLL3 cell suspension at 0.2 mL/mouse, corresponding to an inoculum size of 3×10^6 cells/mouse.

Eight days after the tumor cell inoculation, the mice were divided into groups (6 mice per group) based on the tumor volume for dosinge. The exemplary antibody was administered twice a week for 5 times, and the immune checkpoint inhibitor was administered once, with the route of administration of intraperitoneal injection, and the administration volume of 10 mL/kg. The doses administered are indicated in the legend of FIG. 27. The administration groups were as follows:

h-IgG: IgG control (Equitech-Bio SLH56-0001), 5 mg/kg

D3-107/CD3-C1: exemplary antibody D3-107/CD3-C1, 3 mg/kg

Anti-mPD-1: mouse PD-1 inhibitor (prepared as described in Example 1), 2 mg/kg

Combination therapy: D3-107/CD3-C1, 3 mg/kg + Anti-mPD-1, 2 mg/kg

The tumor volume and body weight of the mice were monitored twice a week until day 29.

Tumor volume measurement: Tumor size was measured using a vernier caliper, with the longest length (L) and widest width (W) recorded. The tumor volume was calculated using the following formula: $V = L \times W^2/2$. The mice were weighted using an electronic balance.

Experimental Results

Tumor growth curves are shown in FIG. 27. On day 29 after inoculation, the relative tumor growth inhibition (TGI%) was calculated by the following formula: TGI% = 100% × (hIgG control group tumor volume – treatment group tumor volume)/(hIgG control group tumor volume – hIgG control group tumor volume before administration).

The antibody D3-107/CD3-C1 and the mouse PD-1 inhibitor exihibited tumor growth inhibition of 21.1% and 64.5%, respectively. When administered in combination, the TGI reached to 96.9%, indicating markedly improved antitumor efficacy. Furthermore, no significant body weight loss was observed throughout the study.

Example 18. PK study of PTM-removed DLL3/CD3 bispecific antibodies in mice

A mouse PK study was conducted to evaluate the in vivo stability of D3-107/CD3-C1. Nine BALB/c mice (Beijing Vital River) were administered with D3-107/CD3-C1 at 10 mg/kg via tail vein injection. Serum samples were collected at 5 min, 0.5 h, 2 h, 6 h, 24 h, 48 h, 72 h, 168 h, 336 h, and 504 h for analysis by ELISA.

ELISA was performed by two methods:

Method 1: A plate was coated with DLL3-his antigen (kactusbio, DLL-HM103), followed by addition of test serum. Then an anti-human Fc secondary antibody (BETHYL, A80-104P) was added, followed by color development with TMB.

Method 2: A plate was coated with DLL3-his antigen (kactusbio, DLL-HM103), followed by addition of test serum. Then, biotin-labeled CD3 (Acrobiosystem, CDD-H82W6) was added, followed by addition of a streptavidin-conjugated secondary antibody (BIOLEGEND, 405210), followed by color development with TMB. The PK detection results are shown in FIG. 28. The two arms of D3-107/CD3-C1 were relatively stable in mice, and the PK curves showed IgG-like PK profiles, with a half-life of 148-159 h and a clearance rate of 0.39-0.42 mL/kg/h. The detailed PK parameters are shown in Table 13.

Table 13. PK parameters of exemplary bispecific antibody molecules

D3-107 /CD3-C1	Cmax (ug/mL)	AUC0-t (ug*h/mL)	AUC0-inf _obs (ug*h/mL)	MRT0-inf _obs(h)	t1/2 (h)	CL(ml/kg/h)	Vss (ml/kg)
Method 1	249	23635	25613	198	148	0.39	77
Method 2	251	21700	23901	211	159	0.42	88

Example 19. Developability study of PTM-removed DLL3/CD3

1.1 Determination of colloidal stability of antibodies by SMAC column

1.1.1 Experimental procedure

The colloidal stability of the antibody was determined by recording its retention time on a Zenix-HPLC column. A 100 μL of the sample (D3-107/CD3-C1) was centrifuged at 13000 rpm for 5 min, and the supernatant was injected onto HPLC system. Chromatographic conditions: Zenix SEC-300 chromatographic column; mobile phase PBS; wavelength: 214 nm; column temperature: 25°C; flow rate: 0.35 mL/min; injection volume: 10 μL.

1.2 HIC Assay for Antibody Hydrophobicity

1.2.1 Experimental Procedure

The hydrophobicity of the antibody was detected by recording its retention time on an HIC-HPLC column. The sample (D3-107/CD3-C1) was diluted to 1 mg/mL, and 10 μL of the diluted sample was injected into a MAbPac HIC-10 chromatographic column. The chromatographic conditions were as follows: detection wavelength, 280 nm; column temperature, 25°C; flow rate, 1 mL/min. Mobile phase gradient: 0-20 min 100% A – 100% B; 20-25min 100% B； 25-30 min 100% A. (A: 1.8 M (NH4)2SO4, 100 mM NaH2PO4, pH 6.5; B: 100 mM NaH2PO4, 10% IPA, pH 6.5)

1.3 CIC Assay for Antibody Non-Specificity

Human serum-derived IgG was coated on an NHS-activated chromatography column, and the retention time of the antibody on this column was investigated by HPLC, with a significant negative correlation observed between retention time and antibody solubility. Therefore, antibodies with superio solubility can be screened by this method.

100 μL of the sample (D3-107/CD3-C1) diluted to 1 mg/mL was centrifuged at 13,000 rpm for 5 min. The supernatant was injected onto a chromatographic column pre-coupled with polyclonal human IgG, and the assay was performed under the following conditions: detection wavelength: 280 nm; flow rate: 0.35 mL/min; injection volume: 10 μL; temperature: room temperature.

1.4 DLS Assay for Antibody Thermal stability

Dynamic light scattering (DLS) utilizes a laser to irradiate small particles undergoing Brownian motion in a solution and detects changes in the intensity of the scattered light. DLS can be used to detect the particle size of proteins in solution, as well as stability at different temperatures or concentrations. The sample was centrifuged at 13000 G/min for 5 min and added to the sample plate, wwhich was then centrifuged for 1 min using a plate centrifuge to remove bubbles. For the detection of Tagg, a temperature ramp experiment was selected. After the read wells were selected, the DLS detection settings were as follows: acquisition time of 5 s, 5 acquisitions, and an experimental temperature range of 25–85°C. After the experiment, the changes in particle size as a function of temperature were analyzed.

1.5 DSC Assay for Antibody thermostability

Scanning calorimetry (DSC) is a method of thermal analysis. Under programmed temperature ramp conditions, it measures the energy difference between a sample and a reference as a function of temperature. As the temperature increased, the protein structure changes occurred, and the associated heat changes were recorded as a DSC curve by a differential scanning calorimeter. The Tm values of the proteins were obtained by analyzing the DSC curve.

The sample (D3-107/CD3-C1) was diluted with PBS to 0.5-1 mg/mL. After the diluted sample and the PBS buffer were degassed, the sample was added to the left sample plate, and the PBS buffer was added to the corresponding position in the right reference plate. The initial temperature was 30°C, with an equilibration time of 10 min, followed by heating at a rate of 1°C/min, to a termination temperature of 90°C.

The titer of transient transfection of the bispecific antibody molecule anti-DLL3 half molecule 107-C1-innoA.LALA (heavy chain SEQ ID NO: 42; light chain: SEQ ID NO: 47) in CHO cells was 109 mg/L, and the titer of the anti-CD3 half molecule hzsp34.24-innoB.LALA (heavy chain SEQ ID NO: 48; light chain: SEQ ID NO: 40) was 244 mg/L.

The specific developability parameters obtained are shown in Table14. The recovery rate of the assembled bispecific antibody molecules D3-107/CD3-C1 was 69%, and the purity after CEX purification was 97% as determined by SEC; the early developability HPLC chromatography retention time assay showed colloidal stability (SMAC 8.3 min), non-specific binding (CIC 9.8

min), and hydrophobicity (HIC 13 min), indicating that the risk of developability indexes was low.

Table 14: Developability parameters of exemplary bispecific antibody molecules

	Colloidal stability	Polyspecificity	Hydrophobicity	Thermostability	
	SMAC (min)	CIC (min)	HIC (min)	Tm1(°C)	Tagg(°C)
D3-107/CD3-C1	8.3	9.8	13.0	65.6	64.3

Summary of sequences

SEQ ID NO	Sequence name	Specific sequence
1	D3-107-VH-CDR1	GYTFTSYYMH
2	D3-107-VH-CDR2	IINPSDGRKSYAQKFLG
3	D3-107-P1-VH-CDR2	IINPSEGRKSYAQKFLG
4	D3-107-P2-VH-CDR2	IINPSQGRKSYAQKFLG
5	D3-107-P3-VH-CDR2	IINPSDVRKSYAQKFLG
6	D3-107-VH-CDR3	GEKVAGPYYYYYSMDV
7	D3-107-VL-CDR1	RASQGISNYLA
8	D3-107-VL-CDR2	AASSLQS
9	D3-107-VL-CDR3	QQYDSYPLT
10	D3-107-VH-DNA	GAAGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTTTCCTGCAAGGCATCTGGATACACCTTCACCAGCTACTATATGCACTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGAATAATCAACCCTAGTGATGGTAGGAAAAGCTACGCACAGAAGTTCCTGGGCAGAGTCACCATGACCAGGGACACGTCCACGAGCACAGTCTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGTGCGAGGGGGGAGAAAGTGGCTGGTCCCTACTACTATTACTACAGTATGGACGTCTGGGGCCAAGGGACCACGGTCACCGTCTCCTCA
11	D3-107-VL-DNA	GAGATTGTGATGACCCAGTCTCCATCCTCACTGTCTGCATCTATAGGAGACAGAGTCACCATCACTTGTCGGGCGAGTCAGGGCATTAGCAATTATTTAGCCTGGTTTCAGCAGAAACCAGGGAAAGCCCCTAAGTCCCTGATCTATGCTGCATCCAGTTTGCAAAGTGGGGTCCCATCAAACTTCAGCGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGTAGCCTACAGCCTGAAGATTTTGCAACTTATTACTGCCAACAGTATGATAGTTACCCGCTCACTTTCGGCGGAGGGACCAAGGTGGAGATCAAA
12	D3-107-VH	EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINPSDGRKSYAQKFLGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARGEKVAGPYYYYYSMDVWGQGTTVTVSS
13	D3-107- P1-VH	EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINPSEGRKSYAQKFLGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARGEKVAGPYYYYYSMDVWGQGTTVTVSS
14	D3-107 -P2-VH	EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINPSQGRKSYAQKFLGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARGEKVAGPYYYYYSMDVWGQGTTVTVSS
15	D3-107 -P3-VH	EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINPSDVRKSYAQKFLGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARGEKVAGPYYYYYSMDVWGQGTTVTVSS
16	D3-107-VL-amino acid	EIVMTQSPSSLSASIGDRVTITCRASQGISNYLAWFQQKPGKAPKSLIYAASSLQSGVPSNFSGSGSGTDFTLTISSLQPEDFATYYCQQYDSYPLTFGGGTKVEIK
17	D3-107-P1-VL	EIVMTQSPSSLSASIGDRVTITCRASQGISNYLAWFQQKPGKAPKSLIYAASSLQSGVPSQFSGSGSGTDFTLTISSLQPEDFATYYCQQYDSYPLTFGGGTKVEIK
18	D3-107-P2-VL	EIVMTQSPSSLSASIGDRVTITCRASQGISNYLAWFQQKPGKAPKSLIYAASSLQSGVPSNFAGSGSGTDFTLTISSLQPEDFATYYCQQYDSYPLTFGGGTKVEIK

19	D3-107-Fab heavy chain (VH+CH1)	EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINPSDGRKSYAQK FLGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARGEKVAGPYYYYYSMDVWGQGTTVTVSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC
20	D3-107-P1-Fab heavy chain (VH+CH1)	EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINPSEGRKSYAQK FLGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARGEKVAGPYYYYYSMDVWGQGTTVTVSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC
21	D3-107-P2-Fab heavy chain (VH+CH1)	EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINPSQGRKSYAQK FLGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARGEKVAGPYYYYYSMDVWGQGTTVTVSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC
22	D3-107-P3-Fab heavy chain (VH+CH1)	EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINPSDVRKSYAQK FLGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARGEKVAGPYYYYYSMDVWGQGTTVTVSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC
23	Heavy chain constant region (IgG1 wild type)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDT LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS PGK
24	Light chain constant region Lambda	GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKY AASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
25	Light chain constant region Kappa	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST YSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
26	IgG1 Fc (wild-type)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
27	IgG1 Fc (wild-type)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
28	CH1	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC
29	IgG1 Fc (LALA+S364R/ D399K)	DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSRDELTKNQVRLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLKSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
30	IgG1 Fc (LALA+ K370S, K409D, Y349T)	DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVT TLPPSRDELTKNQVSLTCLVSGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSDLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
31	Anti-CD3 antibody VH	EVQLVESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQASGKGLEWVGRIRSKYNNYATYYA DSVKDRFTISRDDSKSTLYLQMNSLKTEDTAVYYCARHGNFGQSYVSWFAYWGQGTTVTVSS
32	Anti-CD3 antibody VH-HCDR1	GFTFNTYAMN
33	Anti-CD3 antibody VH-HCDR2	RIRSKYNNYATYYADSVKD
34	Anti-CD3 antibody VH-HCDR3	HGNFGQSYVSWFAY
35	Anti-CD3 antibody VL	QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFS GSLLGDKAALTLLGAQPEDEAEYYCALWYSNLWVFGQGTKLTVL

36	Anti-CD3 antibody VL-LCDR1	RSSTGAVTTSNYAN
37	Anti-CD3 antibody VL-LCDR2	GTNKRAP
38	Anti-CD3 antibody VL-LCDR3	ALWYSNLWV
39	Anti-CD3 antibody Fab heavy chain	EVQLVESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQASGKGLEWVGRIRSKYNNYATYYA DSVKDRFTISRDDSKSTLYLQMNSLKTEDTAVYYCARHGNFGQSYVSWFAYWGQGTTVTVSSAS TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC
40	Anti-CD3 antibody Fab light chain	QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFS GSLLGDKAALTLLGAQPEDEAEYYCALWYSNLWVFGQGTKLTVLGQPKAAPSVTLFPPSSEELQ ANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSY SCQVTHEGSTVEKTVAPTECS
41	D3-107-Fab heavy chain-Fc	EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINPSDGRKSYAQK FLGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARGEKVAGPYYYYYSMDVWGQGTTVTVSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVRLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLKSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS PGK
42	D3-107-P1-Fab heavy chain-Fc	EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINPSEGRKSYAQK FLGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARGEKVAGPYYYYYSMDVWGQGTTVTVSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVRLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLKSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS PGK
43	D3-107-P2-Fab heavy chain-Fc	EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINPSQGRKSYAQK FLGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARGEKVAGPYYYYYSMDVWGQGTTVTVSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVRLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLKSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS PGK
44	D3-107-P3-Fab heavy chain-Fc	EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINPSDVRKSYAQK FLGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARGEKVAGPYYYYYSMDVWGQGTTVTVSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVRLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLKSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS PGK
45	D3-107-VL-CL	EIVMTQSPSSLSASIGDRVTITCRASQGISNYLAWFQQKPGKAPKSLIYAASSLQSGVPSNFSGSGS GTDFTLTISSLQPEDFATYYCQQYDSYPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCL LNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ GLSSPVTKSFNRGEC
46	D3-107-P1-VL-CL	EIVMTQSPSSLSASIGDRVTITCRASQGISNYLAWFQQKPGKAPKSLIYAASSLQSGVPSQFSGSGS GTDFTLTISSLQPEDFATYYCQQYDSYPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCL LNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ GLSSPVTKSFNRGEC

47	D3-107-P2-VL-CL	EIVMTQSPSSLSASIGDRVTITCRASQGISNYLAWFQQKPGKAPKSLIYAASSLQSGVPSNFAGSGS GTDFTLTISSLQPEDFATYYCQQYDSYPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCL LNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ GLSSPVTKSFNRGEC
48	Anti-CD3 antibody Fab heavy chain-Fc	EVQLVESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQASGKGLEWVGRIRSKYNNYATYYA DSVKDRFTISRDDSKSTLYLQMNSLKTEDTAVYYCARHGNFGQSYVSWFAYWGQGTTVTVSSAS TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVTTLPPSRDELTKNQVSLTCLVSGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSDLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS PGK
49	Anti-mPD-1 heavy chain	EVQLQESGPGLVKPSQSLSLTCSVTGYSITSSYRWNWIRKFPGNRLEWMGYINSAGISNYNPSLKR RISITRDTSKNQFFLQVNSVTTEDAATYYCARSDNMGTTPFTYWGQGTLVTVSSASTKGPSVFPLA PSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
50	Anti-mPD-1 light chain	DIVMTQGTLPNPVPSGESVSITCRSSKSLLYSDGKTYLNWYLQRPGQSPQLLIYWMSTRASGVSD RFSGSGSGTDFTLKISGVEAEDVGIYYCQQGLEFPTFGGGTKLELKRTVAAPSVFIFPPSDEQLKSG TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY ACEVTHQGLSSPVTKSFNRGEC

Claims

1. An anti-DLL3 antibody or an antigen-binding fragment thereof, comprising three CDRs of a heavy chain variable region VH: HCDR1, HCDR2, and HCDR3, and three CDRs of a light chain variable region VL: LCDR1, LCDR2, and LCDR3, wherein the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 are selected from

(i). the three complementarity-determining regions HCDR1, HCDR2, and HCDR3 comprised in the VH as set forth in SEQ ID NO: 13, and the three complementarity-determining regions LCDR1, LCDR2 and LCDR3 comprised in the VL as set forth in SEQ ID NO: 18;

(ii). the three complementarity-determining regions HCDR1, HCDR2 and HCDR3 comprised in the VH as set forth in SEQ ID NO: 12, and the three complementarity-determining regions LCDR1, LCDR2 and LCDR3 comprised in the VL as set forth in SEQ ID NO: 16;

(iii). the three complementarity-determining regions HCDR1, HCDR2, and HCDR3 comprised in the VH as set forth in SEQ ID NO: 13, and the three complementarity-determining regions LCDR1, LCDR2 and LCDR3 comprised in the VL as set forth in SEQ ID NO: 16;

(iv). the three complementarity-determining regions HCDR1, HCDR2, and HCDR3 comprised in the VH as set forth in SEQ ID NO: 14, and the three complementarity-determining regions LCDR1, LCDR2 and LCDR3 comprised in the VL as set forth in SEQ ID NO: 16;

(v). the three complementarity-determining regions HCDR1, HCDR2 and HCDR3 comprised in the VH as set forth in SEQ ID NO: 15, and the three complementarity-determining regions LCDR1, LCDR2 and LCDR3 comprised in the VL as set forth in SEQ ID NO: 16;

(vi). the three complementarity-determining regions HCDR1, HCDR2 and HCDR3 comprised in the VH as set forth in SEQ ID NO: 12, and the three complementarity-determining regions LCDR1, LCDR2 and LCDR3 comprised in the VL as set forth in SEQ ID NO: 17;

(vii). the three complementarity-determining regions HCDR1, HCDR2 and HCDR3 comprised in the VH as set forth in SEQ ID NO: 12, and the three complementarity-determining regions LCDR1, LCDR2 and LCDR3 comprised in the VL as set forth in SEQ ID NO: 18; or

(viii). the three complementarity-determining regions HCDR1, HCDR2, and HCDR3 comprised in

the VH as set forth in SEQ ID NO: 14, and the three complementarity-determining regions LCDR1, LCDR2 and LCDR3 comprised in the VL as set forth in SEQ ID NO: 18.

2. An anti-DLL3 antibody or an antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, wherein the HCDR1 consists of SEQ ID NO: 1; the HCDR2 consists of SEQ ID NO: 2; the HCDR3 consists of SEQ ID NO: 6; the LCDR1 consists of SEQ ID NO: 7; the LCDR2 consists of SEQ ID NO: 8; and the LCDR3 consists of SEQ ID NO: 9;

optionally, any one of the amino acids D or G in the contiguous amino acids DG in the HCDR2 may be substituted, e.g., wherein D may be substituted with E or Q, and/or G may be substituted with V, e.g., HCDR2 consists of the amino acid sequence as set forth in SEQ ID NO: 3, 4, or 5.

3. The antibody or antigen-binding fragment thereof according to claim 1 or 2, comprising a heavy chain variable region VH, wherein the heavy chain variable region

(i) comprises or consists of an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence as set forth in SEQ ID NO: 12, 13, 14, or 15; or

(ii) comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 12, 13, 14, or 15;

and/or, comprising a light chain variable region VL, wherein the light chain variable region

(i) comprises or consists of an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence as set forth in SEQ ID NO: 16; or

(ii) comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 16;

optionally, any one of the amino acids in the consecutive amino acids NFS within a framework region FR3 of the VL may be substituted, for example, wherein N may be substituted with Q and/or S may be substituted with A; for example,

the light chain variable region

(i) comprises or consists of an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence as set forth in SEQ ID NO: 17 or

18; or

(ii) comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 17 or 18.

4. The antibody or antigen-binding fragment thereof according to any one of claims 1-3, comprising a heavy chain variable region VH and a light chain variable region VL, wherein

(i). the VH comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 13 or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereto, and the VL comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 18 or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereto;

(ii). the VH comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 12 or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereto, and the VL comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 16 or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereto;

(iii). the VH comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 13 or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereto, and the VL comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 16 or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereto;

(iv). the VH comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 14 or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereto, and the VL comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 16 or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereto;

(v). the VH comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 15 or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereto, and the VL comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 16 or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%,

96%, 97%, 98%, or 99% identity thereto;

(vi). the VH comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 12 or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereto, and the VL comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 17 or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereto;

(vii). the VH comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 12 or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereto, and the VL comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 18 or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereto; or

(viii). the VH comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 14 or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereto, and the VL comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 18 or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereto.

5. The antibody or antigen-binding fragment thereof according to any one of claims 1-4, comprising a heavy chain variable region VH and a light chain variable region VL, wherein

(i). the VH comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 13, and the VL comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 18;

(ii). the VH comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 12, and the VL comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 16;

(iii). the VH comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 13, and the VL comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 16;

(iv). the VH comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 14, and the VL comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 16;

(v). the VH comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 15, and

the VL comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 16;

(vi). the VH comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 12, and the VL comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 17;

(vii). the VH comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 12, and the VL comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 18; or

(viii). the VH comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 14, and the VL comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 18.

6. The antibody or antigen-binding fragment thereof according to any one of claims 1-5, further comprising a heavy chain constant region HC, for example, the antibody heavy chain constant region HC is a heavy chain constant region from an IgG, such as human IgG, e.g., a heavy chain constant region of IgG1, IgG2, IgG3, or IgG4, preferably a heavy chain constant region of human IgG1, IgG2, IgG3, or IgG4;

optionally, the heavy chain constant region

(i) comprises or consists of an amino acid sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence of SEQ ID NO: 23; or

(ii) comprises or consists of the amino acid sequence of SEQ ID NO: 23;

optionally, the heavy chain constant region comprises an Fc region from IgG, such as human IgG, e.g., an Fc region of IgG1, IgG2, IgG3, or IgG4, preferably, a human IgG1, IgG2, IgG3 or IgG4 Fc, optionally the Fc region

(i) comprises or consists of an amino acid sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence as set forth in SEQ ID NO: 26 or 27; or

(ii) comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 26 or 27.

7. The antibody or antigen-binding fragment thereof according to any one of claims 1-6, further comprising a light chain constant region, for example, the light chain constant region is a light chain constant region from a Lambda or Kappa light chain constant region, such as a Lambda or Kappa light chain constant region, e.g., a human Lambda or Kappa light chain constant region;

optionally, the light chain constant region

(i) comprises or consists of an amino acid sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence of SEQ ID NO: 24 or 25; or

(ii) comprises or consists of the amino acid sequence of SEQ ID NO: 24 or 25.

8. The antibody or antigen-binding fragment thereof according to any one of claims 1-7, wherein the antibody is a humanized antibody or a chimeric antibody, or the antibody is a monoclonal antibody.

9. The antibody or antigen-binding fragment thereof according to any one of claims 1-8, wherein the antigen-binding fragment is an antibody fragment selected from the group consisting of an Fab, an Fab', an Fab'-SH, an Fv, a single-chain antibody (e.g., scFv), and an $(Fab')_2$, a diabody or a linear antibody.

10. The antibody or antigen-binding fragment thereof according to any one of claims 1-9, wherein the antibody is a bispecific antibody or a multispecific antibody comprising a first binding specificity for DLL3 and binding specificities for one or more additional antigens; optionally, the additional antigen is CD3.

11. A bispecific antibody, comprising a first antigen-binding region and a second antigen-binding region,

wherein the first antigen-binding region specifically binds to DLL3 and comprises a VH and a VL, wherein

the VH comprises HCDR1, HCDR2 and HCDR3 as defined in claim 1 or 2, and the VL comprises LCDR1, LCDR2 and LCDR3 as defined in claim 1 or 2; or

the VH and the VL are the VH and the VL as defined in any one of claims 3-6; and

the second antigen-binding region specifically binds to CD3;

optionally, the first antigen-binding region is the Fab of the anti-DLL3 antibody as defined in any one of claims 1-14; and/or the second antigen-binding region is the Fab specifically binding to CD3.

12. The bispecific antibody according to claim 11, wherein the Fab as the first antigen-binding region or the second antigen-binding region comprises CH1, wherein the CH1 is a CH1 from IgG1, IgG2, IgG3, or IgG4, preferably a CH1 from human IgG1, IgG2, IgG3, or IgG4, e.g., a CH1 of human IgG1, IgG2, IgG3, or IgG4;

optionally, the CH1

(i) comprises or consists of an amino acid sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence as set forth in SEQ ID NO: 28; or

(ii) comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 28.

13. The bispecific antibody according to claim 11 or 12, wherein the Fab serving as the first antigen-binding region or the second antigen-binding region comprises a light chain constant region, wherein the light chain constant region is a light chain constant region from a Lambda or Kappa light chain constant region, e.g., a Lambda or Kappa light chain constant region, such as a human Lambda or Kappa light chain constant region; optionally, the first antigen-binding region and the second antigen-binding region comprise the same or different light chain constant regions; for example, the first antigen-binding region comprises a Kappa light chain constant region and the second antigen-binding region comprises a Lambda light chain constant region;

optionally, the Kappa light chain constant region

(i) comprises or consists of an amino acid sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence as set forth in SEQ ID NO: 25; or

(ii) comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 25;

and/or

the Lambda light chain constant region

(i) comprises or consists of an amino acid sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence as set forth in SEQ ID NO: 24; or

(ii) comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 24.

14. The bispecific antibody according to any one of claims 11-13, wherein the bispecific antibody is an IgG-like bispecific antibody comprising an Fc dimer, wherein two Fc regions constituting the Fc dimer, the first Fc region and second Fc region are the same or different, and optionally, the Fc region is an Fc region from IgG, such as human IgG, e.g., an Fc region of IgG1, IgG2, IgG3, or IgG4, preferably a human IgG1, IgG2, IgG3 or IgG4 Fc, optionally, the Fc region

(i) comprises or consists of an amino acid sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence as set forth in SEQ ID NO: 26 or 27; or

(ii) comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 26 or 27;

optionally, the two Fc regions are different, wherein mutations are introduced into the first Fc region and the second Fc region based on the Innobody technology to facilitate heterodimerization of the first Fc region and the second Fc region; for example, the CH3 of one Fc region comprises S364R and D399K mutations, and the CH3 mutation of the other Fc region comprises Y349T, K370S, and K409D mutations;

optionally, the first and/or second Fc region comprises a L234A/L235A mutation;

for example,

a) one Fc region comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 29, and the other Fc region comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 30;

b) one Fc region comprises an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence as set forth in SEQ ID NO: 29 and comprises the mutations S364R and D399K, while the other Fc region comprises an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence as set forth in SEQ ID NO: 30 and comprises the mutations Y349T, K370S, and K409D; or

c) one Fc region comprises an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence as set forth in SEQ ID NO: 29 and comprises the mutations S364R, D399K, and L234A/L235A, while the other Fc region comprises an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence as set forth in SEQ ID NO: 30 and comprises the mutations Y349T, K370S, K409D, and L234A/L235A.

15. The bispecific antibody according to any one of claims 11-14, wherein the second antigen-binding region comprises: three complementarity-determining regions from a heavy chain variable region (HCDRs): HCDR1, HCDR2 and HCDR3, and three complementarity-determining regions from a light chain variable region (LCDRs): LCDR1, LCDR2 and LCDR3, wherein

HCDR1 comprises or consists of the amino acid sequence of SEQ ID NO: 32; HCDR2

comprises or consists of the amino acid sequence of SEQ ID NO: 33, HCDR3 comprises or consists of the amino acid sequence of SEQ ID NO: 34, LCDR1 comprises or consists of the amino acid sequence of SEQ ID NO: 36, LCDR2 comprises or consists of the amino acid sequence of SEQ ID NO: 37, and LCDR3 comprises or consists of the amino acid sequence of SEQ ID NO: 38.

16. The bispecific antibody according to claim 15, wherein the second antigen-binding region comprises a VH and a VL, wherein the VH comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 31; and the VL comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 35.

17. The bispecific antibody according to any one of claims 11-16, wherein the bispecific antibody comprises a first antigen-binding region specifically binding to DLL3 and a second antigen-binding region specifically binding to CD3, wherein the first antigen-binding region comprises a first Fab and the second antigen-binding region comprises a second Fab, wherein the first Fab is linked at the C-terminus of its CH1 to the N-terminus of the first Fc region (directly or via a linker such as a hinge region), and the second Fab is linked at the C-terminus of its CH1 to the N-terminus of the second Fc region (directly or via a linker such as a hinge region);

optionally, the bispecific antibody is an IgG-like antibody having a configuration shown in FIG. 3.

18. The bispecific antibody according to claim 16 or 17, comprising a first Fab as the first antigen-binding region specifically binding to DLL3 and a second Fab as the second antigen-binding region specifically binding to CD3, wherein the bispecific antibody comprises

heavy chain 1: comprising or consisting of, from N-terminus to C-terminus, a first Fab heavy chain variable region-a heavy chain constant region CH1-a first Fc region, wherein the heavy chain constant region CH1 is linked at its C-terminus to the N-terminus of the first Fc region directly or via a linker (e.g., a hinge region);

light chain 1: comprising or consisting of, from N-terminus to C-terminus, a first Fab light chain variable region–a light chain constant region;

heavy chain 2: comprising or consisting of, from N-terminus to C-terminus, a second Fab heavy chain variable region-a heavy chain constant region CH1-a second Fc region, wherein the heavy chain constant region CH1 is linked at its C-terminus to the N-terminus of the second Fc region directly or via a linker (e.g., a hinge region);

light chain 2: comprising or consisting of, from N-terminus to C-terminus: a second Fab light

chain variable region–a light chain constant region;

wherein the first Fab heavy chain variable region-the heavy chain constant region CH1 and the first Fab light chain variable region-the light chain constant region constitute a first Fab, and the second Fab heavy chain variable region-the heavy chain constant region CH1 and the second Fab light chain variable region-the light chain constant region constitute a second Fab;

optionally, the light chain constant region in the light chain 1 is different from the light chain constant region in the light chain 2, for example, the light chain constant region in the light chain 1 is a Kappa light chain constant region and the light chain constant region in the light chain 2 is a Lambda light chain constant region.

19. The bispecific antibody according to claim 18, wherein

(i) the heavy chain 1 comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 41, or an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto; the light chain 1 comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 45, or an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto; the heavy chain 2 comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 48, or an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto; and the light chain 2 comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 40, or an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto;

(ii) the heavy chain 1 comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 42, or an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto; the light chain 1 comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 47, or an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto; the heavy chain 2 comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 48, or an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto; and the light chain 2 comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 40, or an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto; or

(iii) the heavy chain 1 comprises or consists of the amino acid sequence as set forth in SEQ ID

NO: 43, or an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto; the light chain 1 comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 47, or an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto; the heavy chain 2 comprises or consists of the amino acid sequence as set forth in SEQ ID NO: 48, or an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto; and the light chain 2 comprises of consists of the amino acid sequence as set forth in SEQ ID NO: 40, or an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

20. A nucleic acid molecule, comprising or consisting of a polynucleotide encoding any one of the chains of the antibody or antigen-binding fragment thereof according to any one of claims 1-10 or any one of the chains of the bispecific antibody according to any one of claims 11-19.

21、An expression vector, comprising the nucleic acid molecule according to claim 20, preferably, the expression vector is pCDNA, such as pCDNA3.1.

22. A host cell, comprising the nucleic acid molecule according to claim 20 or the expression vector according to claim 21, preferably, the host cell is prokaryotic or eukaryotic, e.g., a 293 cell or a CHO cell, such as an Expi293 cell.

23. A method of producing the antibody or antigen-binding fragment thereof according to any one of claims 1-10, or the bispecific antibody according to any one of claims 11-19, the method comprising:

culturing a host cell comprising the nucleic acid molecule according to claim 20 or the expression vector according to claim 21 under conditions suitable for expression of the polypeptide chain(s) of the antibody or antigen-binding fragment thereof or the bispecific antibody; and

optionally, recovering the antibody or antigen-binding fragment thereof or the bispecific antibody from the host cell or from the host cell culture medium.

24. An immunoconjugate, comprising the antibody or antigen-binding fragment thereof according to any one of claims 1-10 or the bispecific antibody according to any one of claims 11-19.

25. A pharmaceutical composition or a medicament or a formulation, comprising the antibody or antigen-binding fragment thereof according to any one of claims 1-10 or the bispecific antibody according to any one of claims 11-19, or the immunoconjugate according to claim 24, and optionally a pharmaceutical supplementary material.

26. A pharmaceutical combination, comprising the antibody or antigen-binding fragment thereof according to any one of claims 1-10 or the bispecific antibody according to any one of claims 11-19, or the immunoconjugate according to claim 24, and one or more additional therapeutic agents, preferably, the therapeutic agents are various therapeutic agents for treating a tumor, such as a chemotherapeutic agent, an angiogenesis inhibitor, a cytokine, a cytotoxic agent, an additional antibody, a small molecule drug, or an immunomodulatory agent (e.g., an immune checkpoint inhibitor or an agonist);

optionally, the pharmaceutical combination

comprises the antibody or antigen-binding fragment thereof according to any one of claims 1-10 or the bispecific antibody according to any one of claims 11-19, or the immunoconjugate according to claim 39 and an additional antibody, preferably, the additional antibody is an antibody that inhibits the PD-1/PD-L1 signaling pathway, such as an anti-PD-1 antibody; or an anti-PD-L1 antibody or an anti-PD-L2 antibody; or

comprises the antibody or antigen-binding fragment thereof according to any one of claims 1-10 or the bispecific antibody according to any one of claims 1-19, or the immunoconjugate according to claim 39 and one or more chemotherapeutic agents, e.g.,a platinum-based chemotherapeutic drug such as carboplatin; or a topoisomerase inhibitor, such as a topoisomerase II inhibitor, e.g., etoposide, or a combination thereof;

preferably, the pharmaceutical combination comprises the antibody or antigen-binding fragment thereof according to any one of claims 1-10 or the bispecific antibody according to any one of claims 11-19, or the immunoconjugate according to claim 39, and a combination of carboplatin and etoposide.

27. A method for preventing or treating a tumor in a subject, comprising administering to the subject an effective amount of the antibody or antigen-binding fragment thereof according to any one of claims 1-10 or the bispecific antibody according to any one of claims 11-19, or the immunoconjugate according to claim 24, or the pharmaceutical composition or formulation according to claim 25, or the pharmaceutical combination according to claim 26;

optionally, the tumor is a solid tumor or a hematological tumor, e.g., a DLL3-positive tumor or cancer; optionally the tumor is a neuroendocrine tumor (e.g. a DLL3-positive neuroendocrine tumor), for example lung cancer (e.g. a DLL3-positive lung cancer), for example small cell lung cancer (SCLC).

28. The method according to claim 27, wherein the method further comprises administering in combination with an additional therapy such as a therapeutic modality (e.g., surgical therapy or radiotherapy) and/or an additional therapeutic agent, preferably, the therapeutic agent is various therapeutic agents for treating a tumor, e.g., a chemotherapeutic agent, an angiogenesis inhibitor, a cytokine, a cytotoxic agent, an additional antibody, a small molecule drug, or an immunomodulatory agent (e.g., an immune checkpoint inhibitor or an agonist);

optionally, the method further comprises administering in combination with an additional antibody; preferably, the additional antibody is an antibody that inhibits the PD-1/PD-L1 signaling pathway, such as an anti-PD-1 antibody; or an anti-PD-L1 antibody or an anti-PD-L2 antibody; or

the method further comprises administering in combination with one or more chemotherapeutic agents, wherein the chemotherapeutic agent is selected from a platinum-based chemotherapeutic drug, such as carboplatin; or a topoisomerase inhibitor, such as a topoisomerase II inhibitor, such as etoposide, or a combination thereof; or

the method further comprises administering in combination with carboplatin and etoposide.