

DLL3 ANTIBODY-DRUG CONJUGATES AND USES THEREOF**CROSS-REFERENCE TO RELATED APPLICATIONS**

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 63/454,009, filed March 22, 2023, the disclosure of which is incorporated by reference herein in its entirety.

SEQUENCE LISTING

[0002] This application contains a computer readable Sequence Listing which has been submitted in XML file format with this application, the entire content of which is incorporated by reference herein in its entirety. The Sequence Listing XML file submitted with this application is entitled “14529-133-228_SEQ_LISTING.xml”, was created on March 20, 2024, and is 49,227 bytes in size.

FIELD

[0003] The present disclosure relates generally to antibody-drug conjugates (ADCs) that comprise Delta-Like Canonical Notch Ligand (DLL3) antibodies and methods of their use.

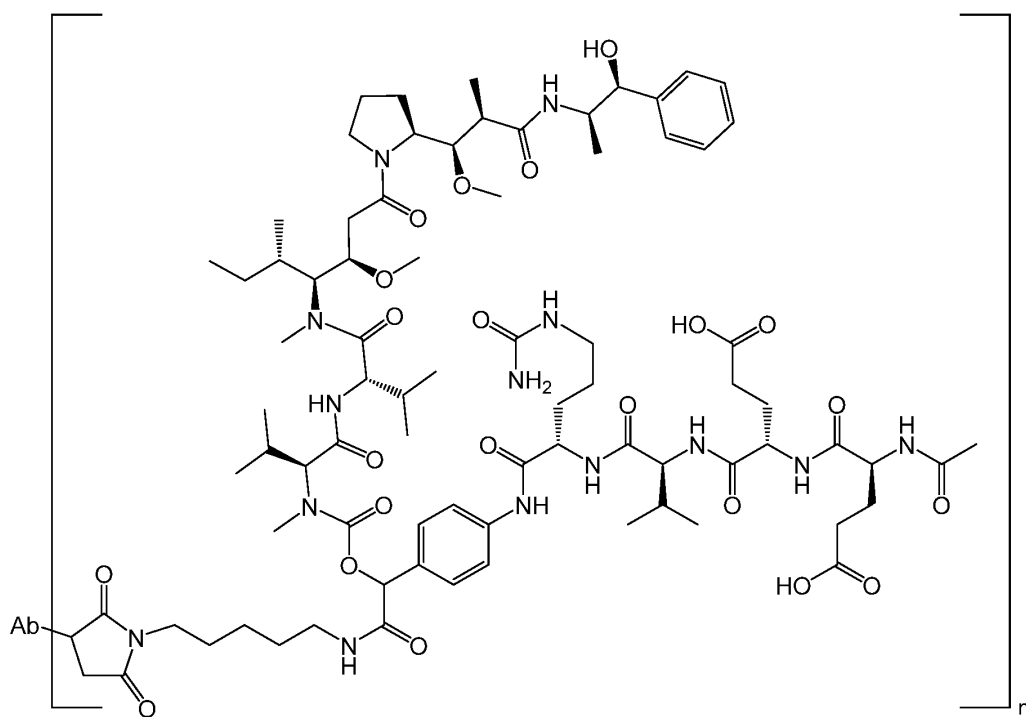
BACKGROUND

[0004] Delta Like Canonical Notch Ligand 3 (DLL3) is a type I transmembrane protein that belongs to the DSL family of Notch ligands. It is normally expressed exclusively on intracellular membranes, especially the Golgi apparatus. Additional Notch family ligands include Delta Like Canonical Notch Ligand 1 (D1), Delta Like Canonical Notch Ligand 4 (D4), Jagged Canonical Notch Ligand 1 (J1), and Jagged Canonical Notch Ligand 2 (J2). Except for DLL3, the other ligands can activate Notch signaling. DLL3 acts as an inhibitor of Notch signaling by interfering with the binding between Notch and its ligands. DLL3 is highly expressed on lung tumor cell surface, including small cell lung cancer (SCLC) and large cell neuroendocrine carcinoma (LCNEC). While it is normally expressed exclusively on the intracellular membranes, DLL3 is a potential therapeutic tumor target for any tumors that express DLL3, including SCLC and LCNEC. In recent years, the establishment of DLL3 as a unique target in SCLC has accelerated the development of therapeutic agents. However, therapeutic success with antibodies and ADCs targeting DLL3 has not yet been achieved.

[0005] There remains a need in the art for ADCs that can target DLL3 to treat, prevent, or alleviate DLL3-mediated diseases, disorders, or conditions, including lung cancer, such as small cell lung cancer (SCLC) or large cell neuroendocrine carcinoma (LCNEC).

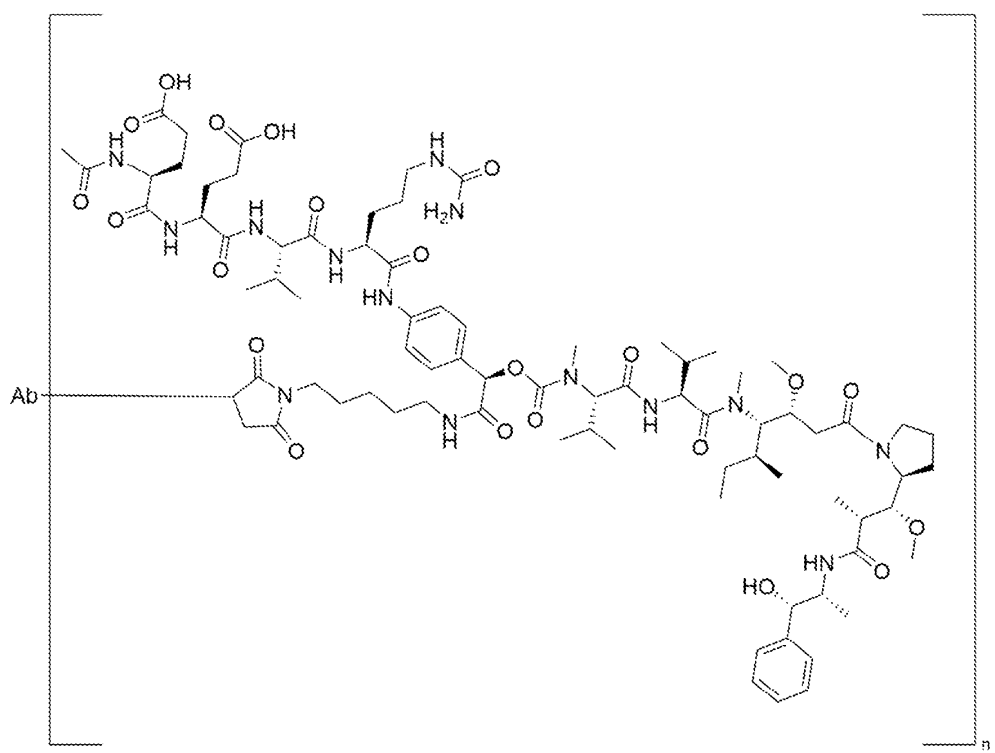
SUMMARY

[0006] In one aspect, provided herein is an antibody-drug conjugate (ADC) of Formula (I):



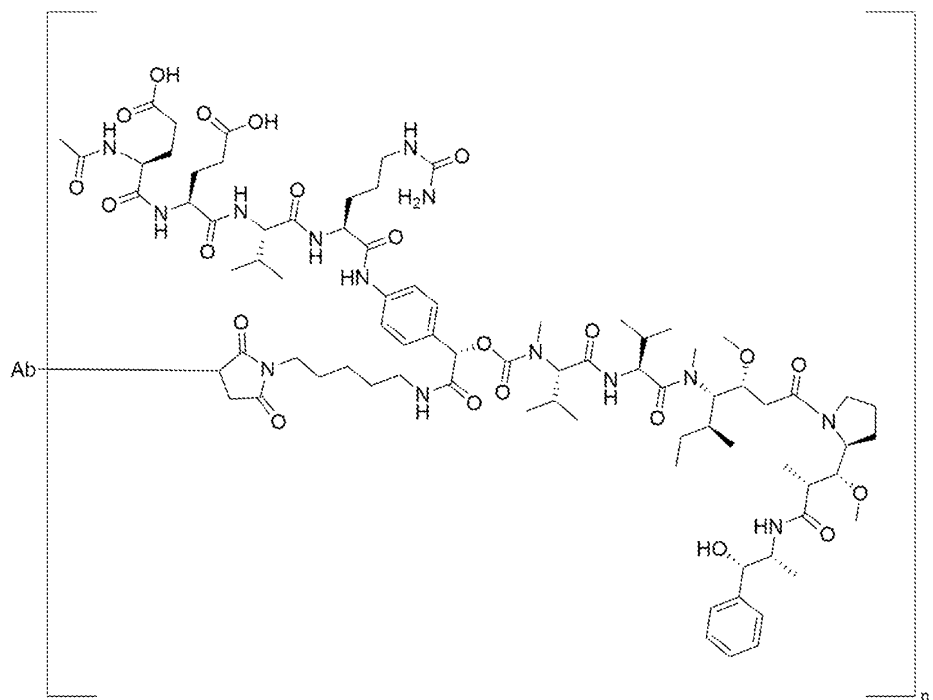
(I)

or a salt thereof, wherein n is an integer from 1 to 8, and Ab represents an antibody that binds to DLL3 (“DLL3 antibody”), wherein the point of attachment of the drug conjugate to the Ab is through a cysteine or a lysine residue of the DLL3 antibody. In certain embodiments, n is 2. In certain embodiments, n is 4. In some embodiments, the ADC is of Formula (I-A) or a salt thereof.



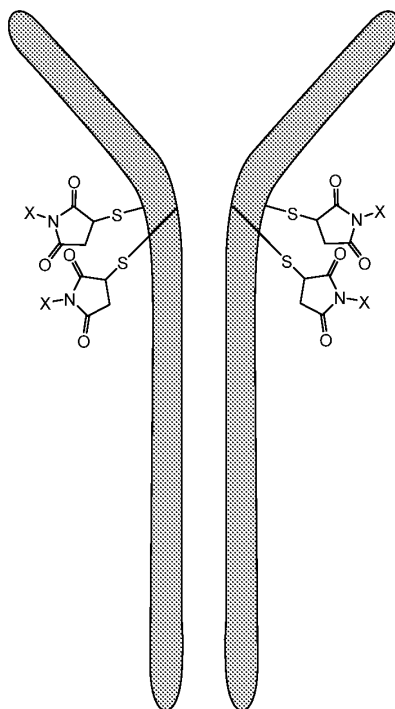
(I-A)

In other embodiments, the ADC is of Formula (I-B) or a salt thereof:



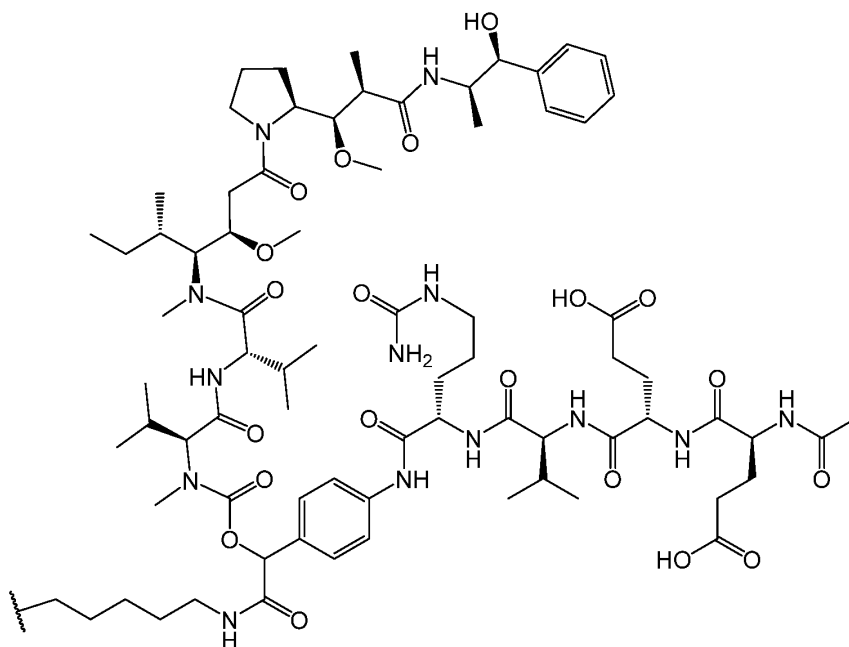
(I-B)

[0007] In another aspect, provided herein is an ADC of Formula (A):

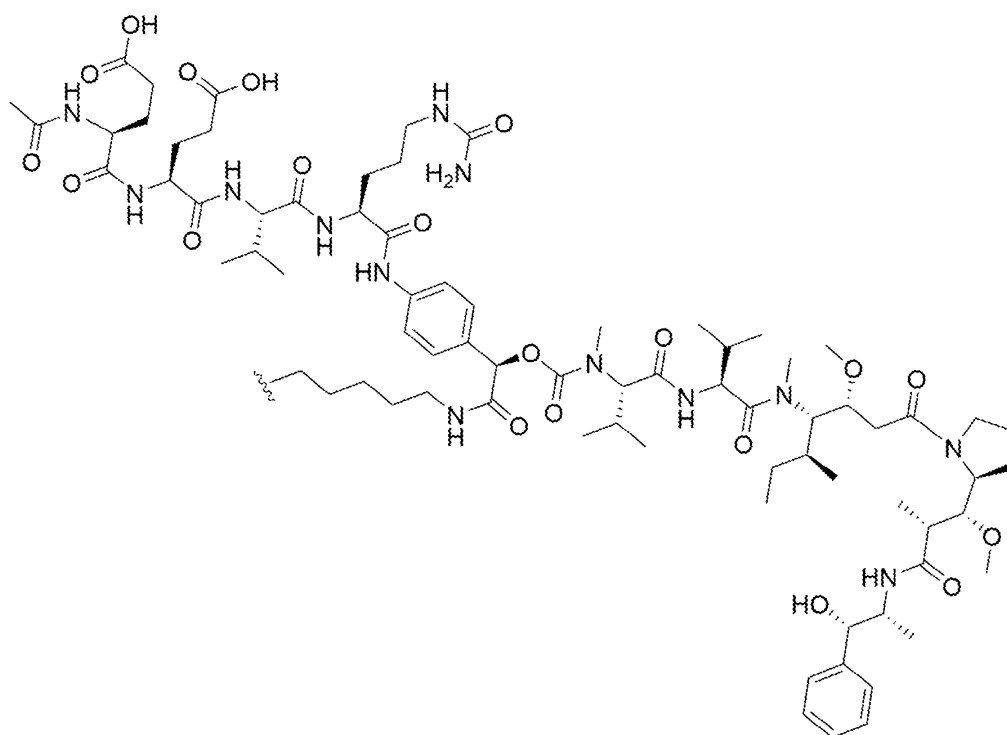


(A),

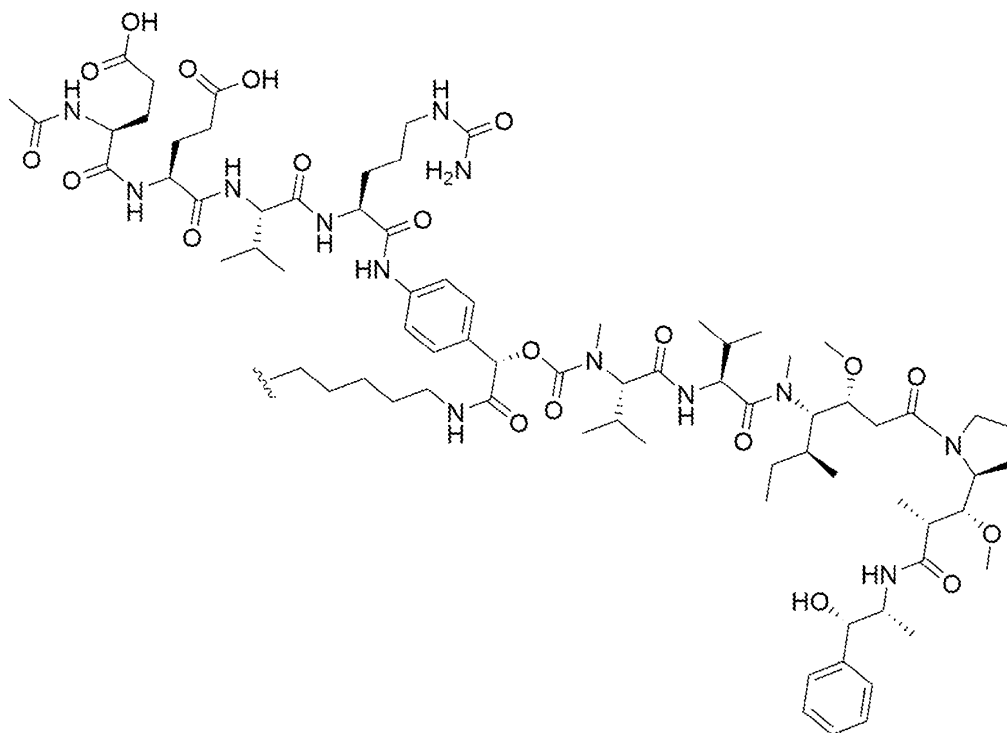
wherein each thick shaded line represents a chain of the DLL3 antibody, the depicted sulfurs are from cysteine residues of the DLL3 antibody, and X represents the structure below or a salt thereof:



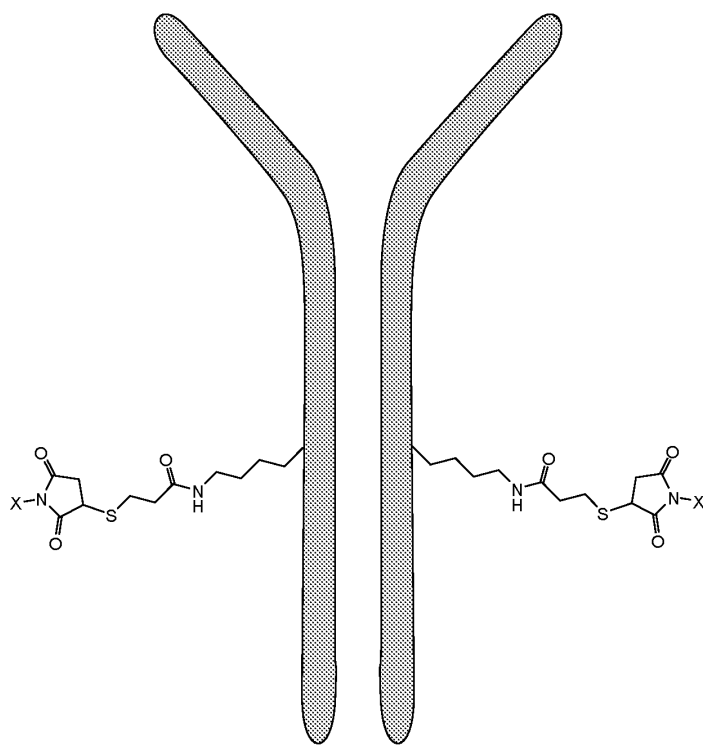
wherein the wavy line represents the point of attachment to the rest of the ADC of Formula (A). In some embodiments, the cysteine residues are in the hinge region of the DLL3 antibody. In further embodiments, the cysteine residues form an interchain disulfide bridge in a DLL3 antibody not conjugated directly or indirectly to X, for example, between two heavy chains, between a heavy chain and a light chain, between two VHH chains, or between two VHH-Fc chains. In yet further embodiments, the cysteine residue is at any one or more of positions 226 or 229 according to EU numbering (also referred to herein as C226 or C229, respectively). In some embodiments, X represents the structure below or a salt thereof:



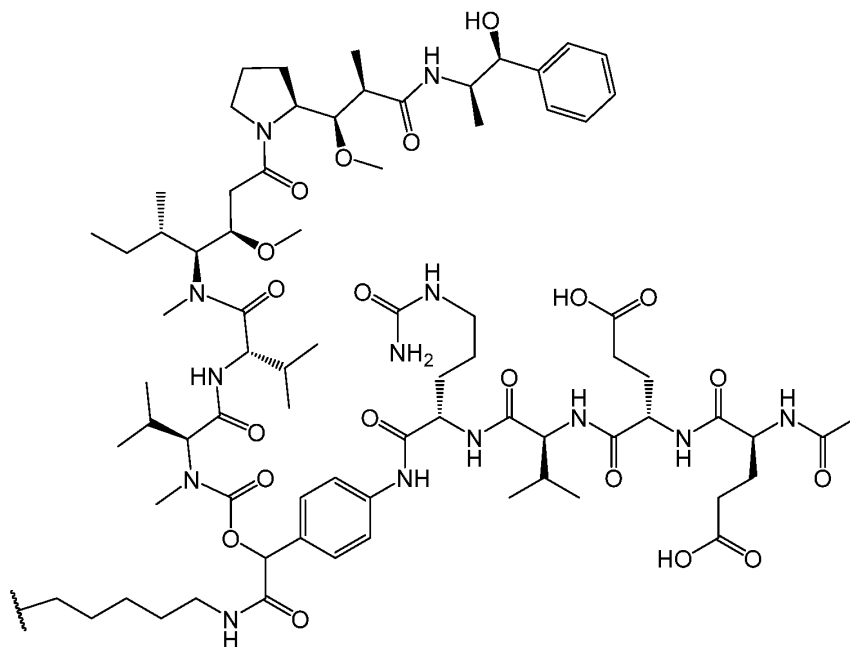
In other embodiments, X represents the structure below or a salt thereof:



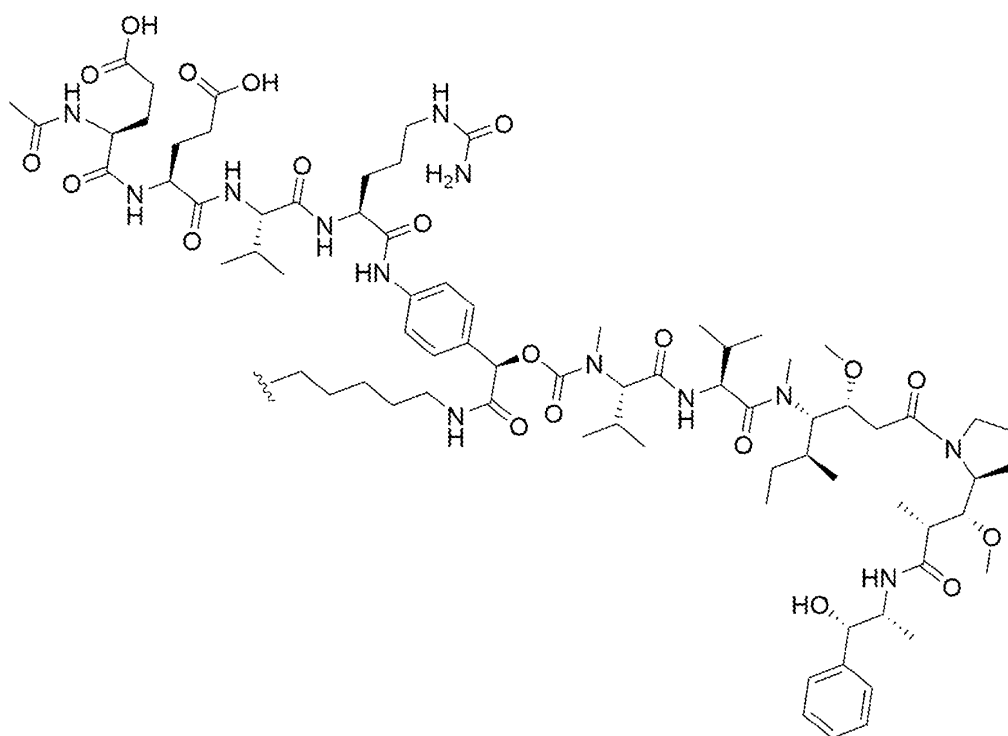
[0008] In another aspect, provided herein is an ADC of Formula (B):



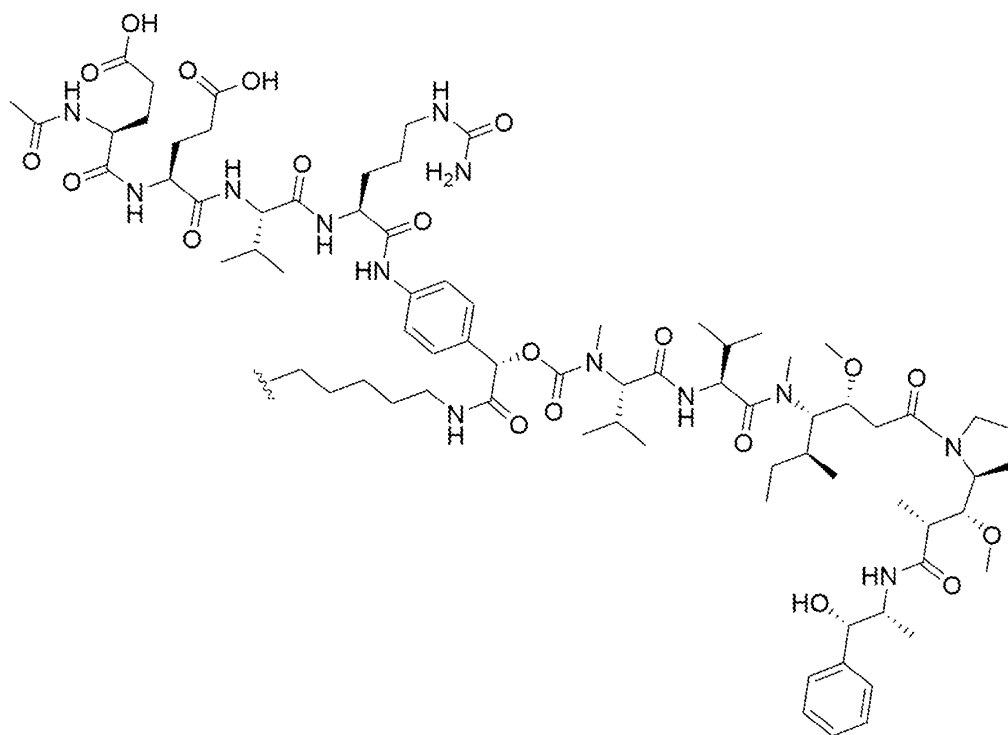
wherein each thick shaded line represents a chain of the DLL3 antibody, the depicted $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}$ moiety is from a lysine residue of the CH2 domain of the DLL3 antibody, and X represents the structure below:



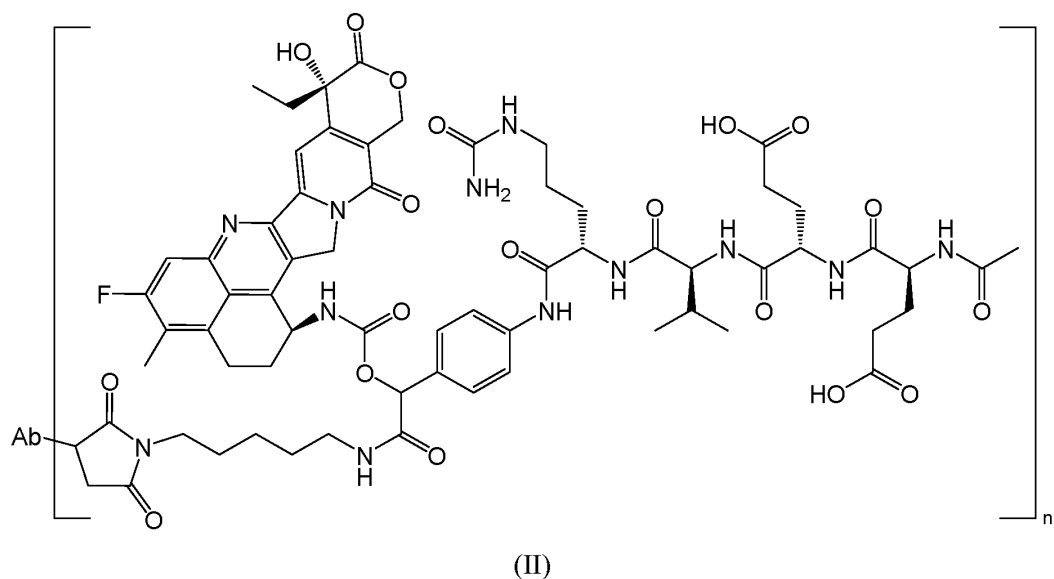
wherein the wavy line represents the point of attachment to the rest of the ADC of Formula (B). In some embodiments, the lysine residue is at any one or more of positions 246, 248, 288, 290, or 317 according to EU numbering (also referred to herein as K246, K248, K288, K290, or K317, respectively). In some embodiments, X represents the structure below or a salt thereof:



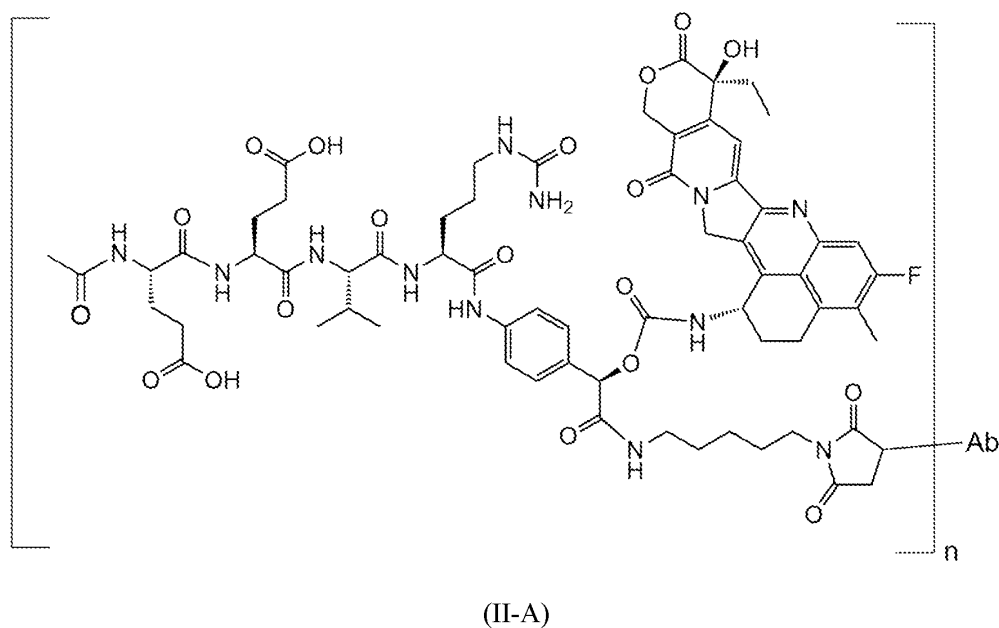
In other embodiments, X represents the structure below or a salt thereof:



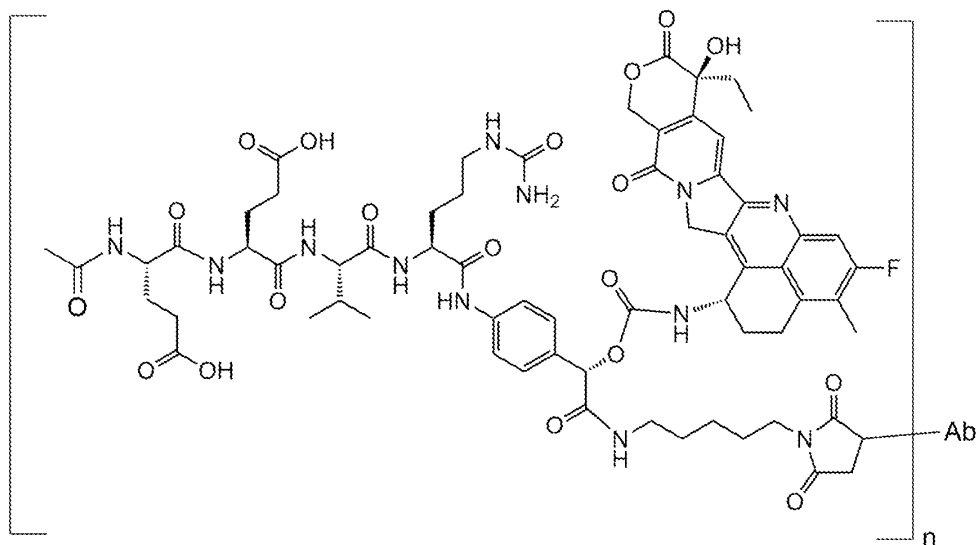
[0009] In another aspect, provided herein an ADC of Formula (II):



wherein n is an integer from 1 to 8, and Ab represents a DLL3 antibody. In some embodiments, the point of attachment of the drug conjugate to the Ab is through a cysteine or a lysine residue of the DLL3 antibody. In certain embodiments, n is 2. In certain embodiments, n is 4. In some embodiments, the ADC is of Formula (II-A) or a salt thereof:

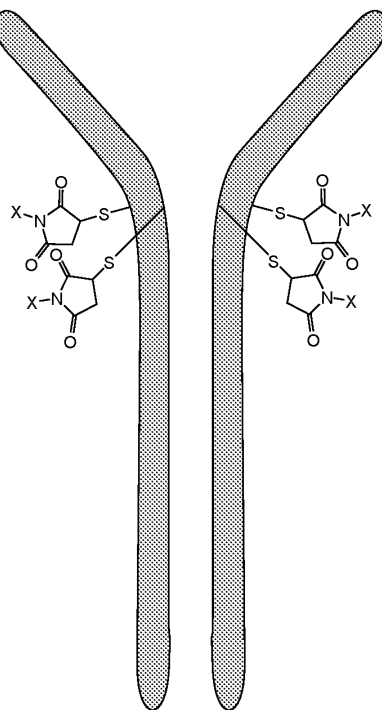


In other embodiments, the ADC is of Formula (II-B) or a salt thereof:



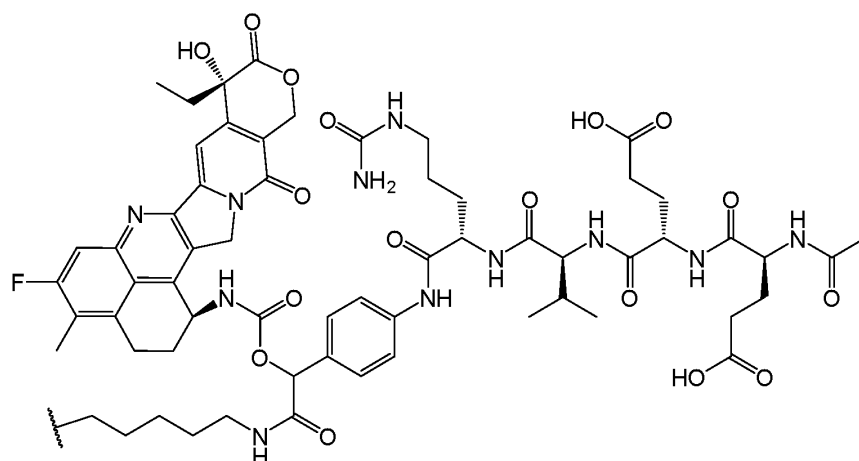
(II-B)

[0010] In another aspect, provided herein is an ADC of Formula (A):

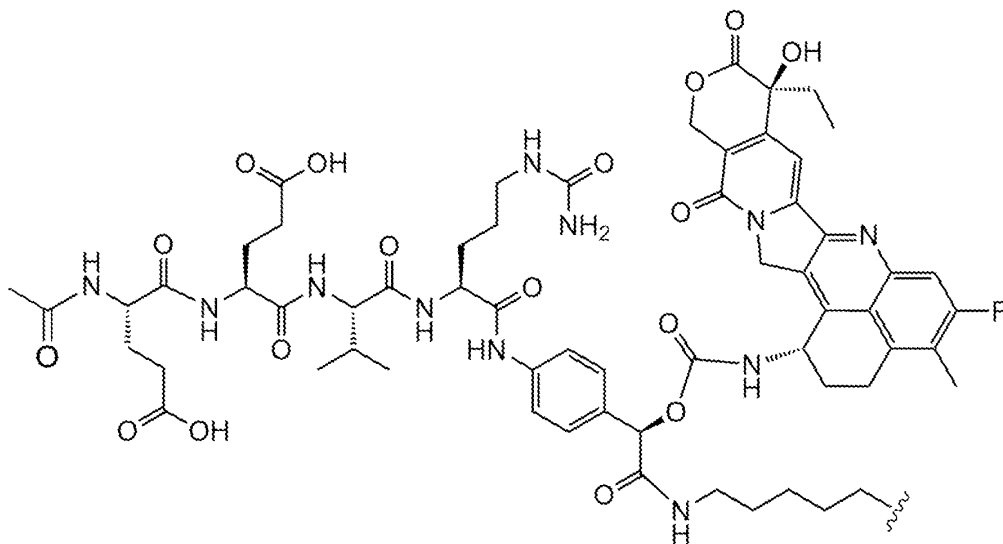


(A),

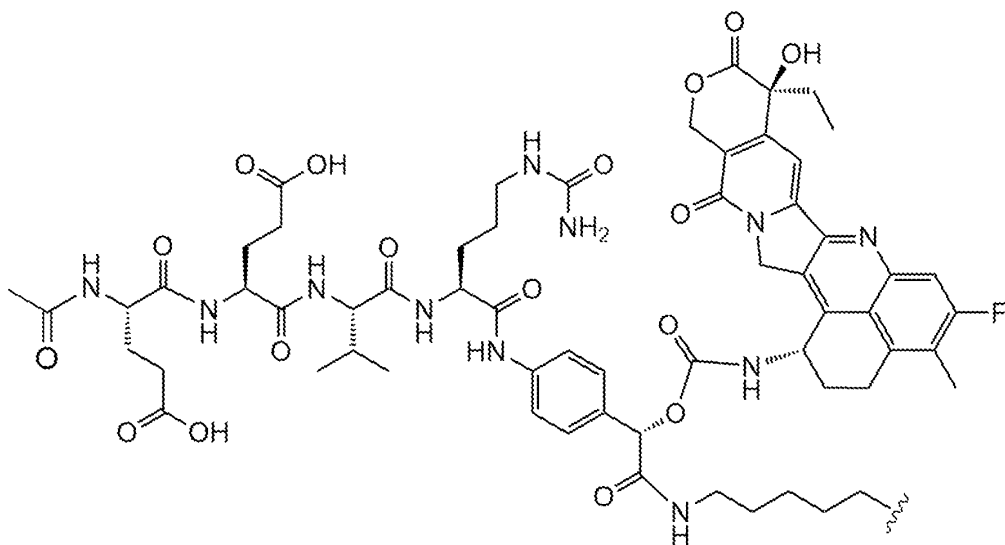
wherein each thick shaded line represents a chain of a DLL3 antibody, the depicted sulfurs are from cysteine residues of the DLL3 antibody, and X represents the structure below:



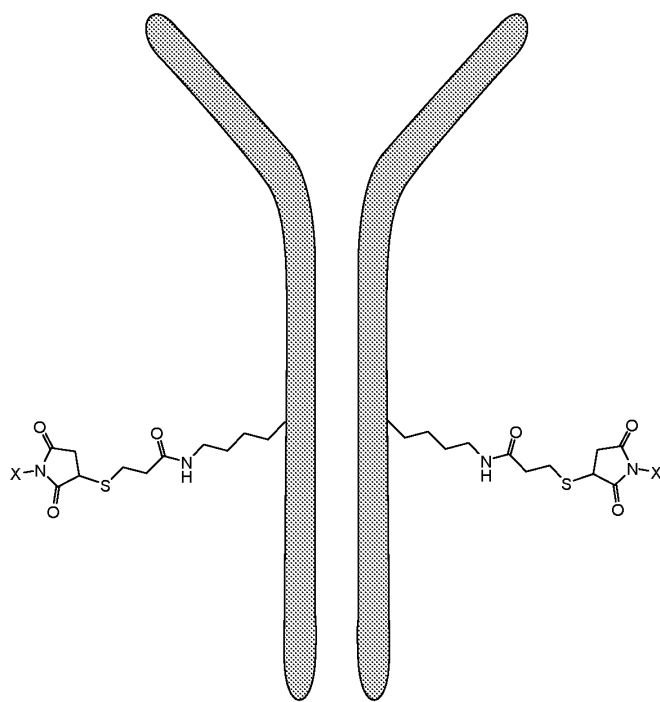
wherein the wavy line represents the point of attachment to the rest of the ADC of Formula (A). In some embodiments, the cysteine residues are in the hinge region of the DLL3 antibody. In further embodiments, the cysteine residues form an interchain disulfide bridge in a DLL3 antibody not conjugated directly or indirectly to X, for example, between two heavy chains, between a heavy chain and a light chain, between two VHH chains, or between two VHH-Fc chains. In yet further embodiments, the cysteine residue is at any one or more of positions 226 or 229 according to EU numbering (also referred to herein as C226 or C229, respectively). In some embodiments, X represents the structure below or a salt thereof:



In some embodiments, X represents the structure below or a salt thereof:

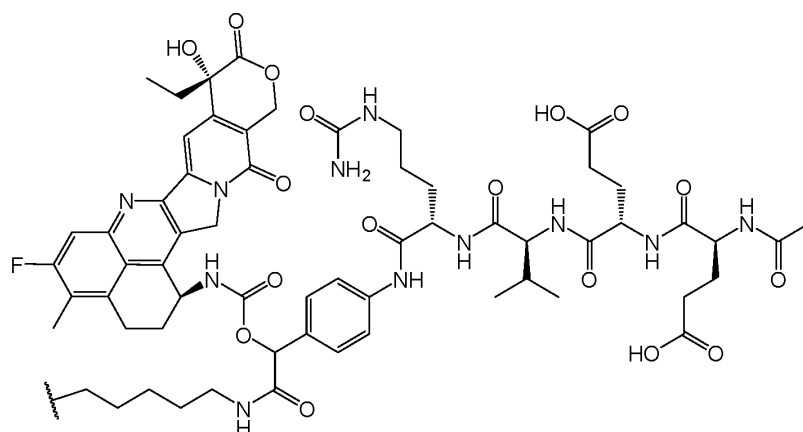


[0011] In another aspect, provided herein is an ADC of Formula (B):

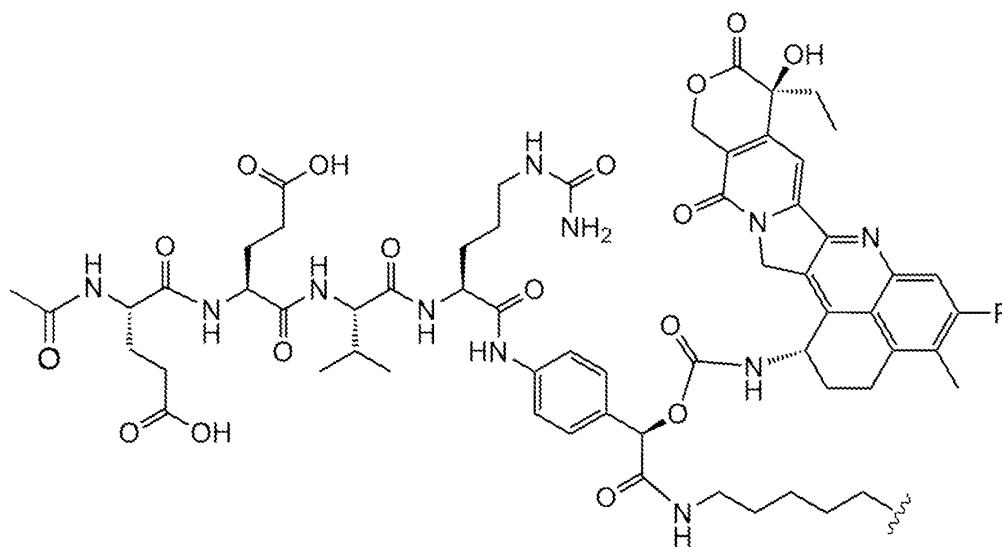


(B),

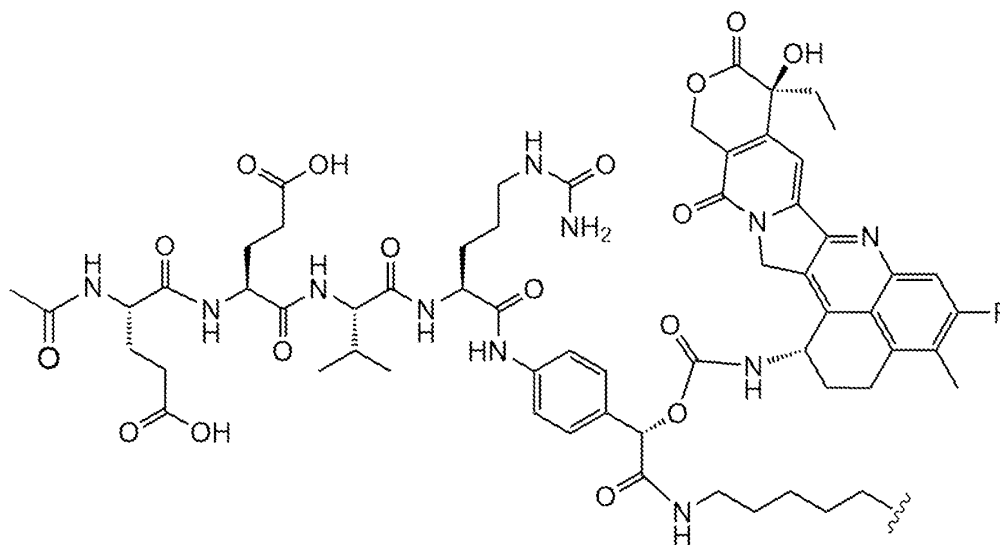
wherein each thick shaded line represents a chain of a DLL3 antibody, the depicted $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}$ moiety is from a lysine residue of the CH_2 domain of the DLL3 antibody, and X represents the structure below:



wherein the wavy line represents the point of attachment to the rest of the ADC of Formula (B). In some embodiments, the lysine residue is at any one or more of positions 246, 248, 288, 290, or 317 according to EU numbering (also referred to herein as K246, K248, K288, K290, or K317, respectively). In some embodiments, X represents the structure below or a salt thereof:



In some embodiments, X represents the structure below or a salt thereof:



[0012] In some embodiments, the DLL3 antibody as used herein comprises a VHH. In further embodiments, the DLL3 antibody as used herein comprises a VHH-Fc fusion, for example, as depicted in **Figure 1A**. Additionally or alternatively, the DLL3 antibody comprises a CDR1, CDR2, and CDR3 of a heavy chain variable domain (VH), wherein the VH comprises an amino acid sequence as set forth in SEQ ID NO:14, 15 or 16, for example, as those disclosed in **Table 1** and **Table 2**. In further embodiments, the DLL3 antibody comprises a VH as set forth in SEQ ID NO:14, 15 or 16. In yet further embodiments, the DLL3 antibody comprises a homodimer of a VHH-Fc chain as set forth in any one of SEQ ID NO:4, 5, or 10.

[0013] In another aspect, provided herein is a pharmaceutical composition comprising an ADC described herein, and a pharmaceutically acceptable carrier. In some embodiments, the pharmaceutical composition has a drug-to-antibody ratio (DAR) of about 1.5 to about 2.5. In further embodiments, the pharmaceutical composition has a DAR of about 1.9 to 2.1. In some embodiments, the pharmaceutical composition has a DAR of about 1 to about 4, for example, about 1 to about 3, about 1 to about 2, about 2 to about 4, or about 3 to about 4. In further embodiments, the pharmaceutical composition has a DAR of about 3.5 to about 4.0, such as about 3.6, about 3.7, about 3.8, about 3.9, or about 4.0.

[0014] In another aspect, provided herein is a method of modulating a DLL3 related immune response in a subject, comprising administering to the subject an ADC or a pharmaceutical composition described herein such that an immune response is modulated in the subject.

[0015] In another aspect, provided herein is a method for treating a cancer in a subject, comprising administering to the subject an ADC or a pharmaceutical composition described herein, wherein the cancer is DLL3-positive or overexpressed. In certain embodiments, the cancer is selected from lung cancer and neuroendocrine carcinoma. In certain embodiments, the cancer is small cell lung cancer (SCLC), large cell neuroendocrine carcinoma (LCNEC) or colorectal cancer.

[0016] In still further aspects, provided herein are methods of making an ADC described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] **Figure 1A** shows an antibody comprising VHH fused to an Fc. Two interchain disulfide bonds may be present between hinge regions of the two chains.

[0018] **Figure 1B** provides exemplary routes of synthesis for ADCs, wherein the antibody may be a conventional antibody comprising two heavy chains and two light chains, or a VHH-Fc fusion as illustrated in **Figure 1A**.

[0019] **Figure 2** provides an exemplary RP-HPLC analysis result of Linker-Drug (2-A), Linker-Drug (2-B), and Linker-Drug (2) (mixture of Linker-Drug (2-A) and Linker-Drug (2-B)), further described in Example 7.

[0020] **Figure 3** illustrates Linker-Drug (2-B).

[0021] **Figures 4A-4D** show exemplary results for mAb300, mAb301, mAb302, and relevant ADCs from developability assays, further described in **Example 15**. **Figure 4A** compares mAb302, ADC-013 and ADC-014 using size exclusion chromatography (SEC). **Figure 4B** compares mAb300 and mAb302 using hydrophobic interaction chromatography (HIC). **Figure 4C** compares mAb300, ADC-009, and ADC-010 using HIC. **Figure 4D** compares mAb302, ADC-013, and ADC-014 using HIC.

[0022] **Figure 5** shows exemplary results of assessing the binding characteristics of mAb300, mAb301 and mAb302 via a Biacore™ assay, further described in Example 16.

[0023] **Figure 6** shows exemplary results of assessing drug-to-antibody ratios (DARs) of ADC-015 (marked as mAb301-Linker-Drug (2) (Target DAR = 2.0)), ADC-016 (marked as mAb301-Linker-Drug (2) (Target DAR = 4.0)), and ADC-017 (marked as mAb301-Linker-Drug (1) (Target DAR = 4.0)), further described in Example 17.

[0024] **Figures 7A-7R** provide exemplary SEC-HPLC analysis results of the produced ADC-009-M (mAb300, Linker-Drug (2), DAR2, **Figure 7A**), ADC-009-A (mAb300, Linker-Drug (2-A), DAR2, **Figure 7B**), ADC-009-B (mAb300, Linker-Drug (2-B), DAR2, **Figure 7C**), ADC-015-M (mAb301, Linker-Drug (2), DAR2, **Figure 7D**), ADC-015-A (mAb301,

Linker-Drug (2-A), DAR2, **Figure 7E**), ADC-015-B (mAb301, Linker-Drug (2-B), DAR2, **Figure 7F**), ADC-013-M (mAb302, Linker-Drug (2), DAR2, **Figure 7G**), ADC-013-A (mAb302, Linker-Drug (2-A), DAR2, **Figure 7H**), ADC-013-B (mAb302, Linker-Drug (2-B), DAR2, **Figure 7I**), ADC-018-M (mAb300, Linker-Drug (2), DAR4, **Figure 7J**), ADC-018-A (mAb300, Linker-Drug (2-A), DAR4, **Figure 7K**), ADC-018-B (mAb300, Linker-Drug (2-B), DAR4, **Figure 7L**), ADC-016-M (mAb301, Linker-Drug (2), DAR4, **Figure 7M**), ADC-016-A (mAb301, Linker-Drug (2-A), DAR4, **Figure 7N**), ADC-016-B (mAb301, Linker-Drug (2-B), DAR4, **Figure 7O**), ADC-020-M (mAb302, Linker-Drug (2), DAR4, **Figure 7P**), ADC-020-A (mAb302, Linker-Drug (2-A), DAR4, **Figure 7Q**), ADC-020-B (mAb302, Linker-Drug (2-B), DAR4, **Figure 7R**), further described in **Example 18**.

[0025] **Figures 8A-8R** provide exemplary HIC-HPLC analysis results of the produced ADC-009-M (mAb300, Linker-Drug (2), DAR2, **Figure 8A**), ADC-009-A (mAb300, Linker-Drug (2-A), DAR2, **Figure 8B**), ADC-009-B (mAb300, Linker-Drug (2-B), DAR2, **Figure 8C**), ADC-015-M (mAb301, Linker-Drug (2), DAR2, **Figure 8D**), ADC-015-A (mAb301, Linker-Drug (2-A), DAR2, **Figure 8E**), ADC-015-B (mAb301, Linker-Drug (2-B), DAR2, **Figure 8F**), ADC-013-M (mAb302, Linker-Drug (2), DAR2, **Figure 8G**), ADC-013-A (mAb302, Linker-Drug (2-A), DAR2, **Figure 8H**), ADC-013-B (mAb302, Linker-Drug (2-B), DAR2, **Figure 8I**), ADC-018-M (mAb300, Linker-Drug (2), DAR4, **Figure 8J**), ADC-018-A (mAb300, Linker-Drug (2-A), DAR4, **Figure 8K**), ADC-018-B (mAb300, Linker-Drug (2-B), DAR4, **Figure 8L**), ADC-016-M (mAb301, Linker-Drug (2), DAR4, **Figure 8M**), ADC-016-A (mAb301, Linker-Drug (2-A), DAR4, **Figure 8N**), ADC-016-B (mAb301, Linker-Drug (2-B), DAR4, **Figure 8O**), ADC-020-M (mAb302, Linker-Drug (2), DAR4, **Figure 8P**), ADC-020-A (mAb302, Linker-Drug (2-A), DAR4, **Figure 8Q**), ADC-020-B (mAb302, Linker-Drug (2-B), DAR4, **Figure 8R**), further described in **Example 18**.

[0026] **Figures 9A-9E** provide exemplary results of cytotoxicity assays investigating effects of ADC-015, ADC-016, and ADC-017 on hDLL3 overexpressing B16F10 cells, further described in **Example 17**. **Figure 9A** compares various concentrations of MMAE, Exatecan and ADC-017 after 72 hours of incubation. **Figure 9B** compares various concentrations of MMAE, Exatecan and ADC-017 after 96 hours of incubation. Corresponding IC₅₀ and maximum inhibition percentages were calculated and are presented in **Figure 9C**. In addition, **Figure 9D** compares various concentrations of MMAE, Exatecan, ADC-015, ADC-016, ADC-017, IgG1 Fc conjugated to Exatecan, and IgG1 Fc conjugated to MMAE after 72 hours of incubation, while **Figure 9E** presents corresponding IC₅₀ and maximum inhibition percentages.

[0027] **Figures 10A-10E** provide exemplary results of *in vitro* cytotoxicity assays investigating the effects of ADC-015-M, ADC-015-A, ADC-015-B, ADC-016-M, ADC-016-A, and ADC-016-B on a DLL3 over-expressing 293F cell line, further described in **Example 20**. **Figures 10A** and **10B** plot the data from a first experiment, while **Figure 10A** plots the concentrations of the tested ADCs on the x-axis and **Figure 10B** plots the calibrated concentrations of the linker-payloads of the tested ADCs on the x-axis. **Figures 10C** and **10D** plot the data from a second experiment, while **Figure 10C** plots the concentrations of the tested ADCs on the x-axis and **Figure 10D** plots the calibrated concentrations of the linker-payloads of the tested ADCs on the x-axis. **Figure 10E** plots the data from a third experiment.

[0028] **Figures 11A-11F** provide exemplary results of cytotoxicity assays investigating the effect of ADC-015, ADC-016, and ADC-017 on H69 cells, further described in **Example 21**. **Figures 11A, 11C, and 11E** compare various concentrations of MMAE, Exatecan, ADC-015, ADC-016, and ADC-017 after 120 hours of incubation, while their IC₅₀ and maximum inhibition percentages are presented in **Figures 11B, 11D and 11F**, respectively.

[0029] **Figures 12A-12D** provide exemplary results of cytotoxicity assays investigating effects of ADC-015, ADC-016, and ADC-017 on CORL279 cells, further described in **Example 21**. **Figures 12A and 12C** compare various concentrations of MMAE, Exatecan, ADC-015, ADC-016, and ADC-017 after 120 hours of incubation, while their IC₅₀ and maximum inhibition percentages are presented in **Figures 12B and 12D**, respectively.

[0030] **Figures 13A-13B** provide exemplary results of cytotoxicity assays investigating effects of ADC-015, ADC-016, and ADC-017 on SHP77 cells, further described in **Example 21**. **Figure 13A** compares various concentrations of MMAE, Exatecan, ADC-015, ADC-016, and ADC-017 after 120 hours of incubation, while the corresponding IC₅₀ and maximum inhibition percentages are presented in **Figure 13B**.

[0031] **Figures 14A-14B** provide exemplary results of cytotoxicity assays investigating effects of ADC-015, ADC-016, and ADC-017 on H460 cells, further described in **Example 21**. **Figure 14A** compares various concentrations of MMAE, Exatecan, ADC-015, ADC-016, and ADC-017 after 120 hours of incubation, while the corresponding IC₅₀ and maximum inhibition percentages are presented in **Figure 14B**.

[0032] **Figures 15A-15E** provide exemplary results of cytotoxicity assays investigating effects of ADC-009 and ADC-010 on SHP77 cells (**Figure 15A**), DMS79 cells (**Figure 15B**), H69 cells (**Figure 15C**), and CORL279 cells (**Figure 15D**), further described in

Example 22. Corresponding IC₅₀ and maximum inhibition percentages were calculated and presented in **Figure 15E**.

[0033] **Figures 16A-16F** plot tumor volume (TV) measurements of mice implanted with SHP77 small cell lung cancer (SCLC) cells and treated with the indicated compounds, further described in **Example 23**. **Figure 16A**, **Figure 16C**, and **Figure 16E** plot the data over the observation period, while **Figure 16B** and **Figure 16D** compare the data on Day 28 (D28), and **Figure 16F** plots the data on Day 24 (D24).

[0034] **Figures 17A-17K** provide spider plots of mice implanted with SHP77 SCLC cells and treated with the indicated compounds (**Figure 17A**, Vehicle; **Figure 17B**, DAR 2 Isotype ADC 1 mg/kg; **Figure 17C**, DAR 4 Isotype ADC 1 mg/kg; **Figure 17D**, ADC-015-B 3 mg/kg; **Figure 17E**, ADC-015-B 1 mg/kg; **Figure 17F**, ADC-015-B 0.3 mg/kg x3; **Figure 17G**, ADC-015-M 1 mg/kg; **Figure 17H**, ADC-016-B 3 mg/kg; **Figure 17I**, ADC-016-B 1 mg/kg; **Figure 17J**, ADC-016-B 0.3 mg/kg x3; **Figure 17K**, ADC-016-M 1 mg/kg;), further described in **Example 23**.

[0035] **Figures 18A-18I** provide data obtained from the CXF 742 PDX model, further described in **Example 24**. **Figure 18A** provides an exemplary IHC staining image of the CXF 742 tumor sample. **Figure 18B** plots absolute tumor volumes of the tested groups over the observation period (the mean group TV was graphed until 2 animals were removed from the group due to the TV endpoint). **Figure 18C** plots tumor volumes on Day 45 (D45) (the mixed effect analysis was performed using Dunnett's post-test vs Vehicle group). **Figures 18D-18G** provide spider plots of mice treated with the indicated compounds (**Figure 18D**, Vehicle; **Figure 18E**, ADC-016-M, 5 mg/kg x 1; **Figure 18F**, ADC-016-M, 5 mg/kg x 2; **Figure 18G**, ADC-015-M, 5 mg/kg x 1). **Figure 18H** plots relative tumor volumes of the tested groups over the observation period. **Figure 18I** plots body weights of the tested groups over the observation period.

[0036] **Figures 19A-19I** provide data obtained from the LXFS 2156 PDX model, further described in **Example 24**. **Figure 19A** provides an exemplary IHC staining image of the LXFS 2156 tumor sample. **Figure 19B** plots absolute tumor volumes of the tested groups over the observation period (the mean group TV was graphed until 2 animals were removed from group due to TV endpoint). **Figure 19C** plots tumor volumes on Day 31 (D31) (mixed effect analysis, Dunnett's post-test vs Vehicle group). **Figures 19D-19G** provide spider plots of mice treated with the indicated compounds (**Figure 19D**, Vehicle; **Figure 19E**, ADC-016-M, 5 mg/kg x 1; **Figure 19F**, ADC-016-M, 5 mg/kg x 2; **Figure 19G**, ADC-015-M, 5 mg/kg

x 1). **Figure 19H** plots relative tumor volumes of the tested groups over the observation period. **Figure 19I** plots body weights of the tested groups over the observation period.

[0037] **Figures 20A-20I** provide data obtained from the LXFS 573 PDX model, further described in **Example 24**. **Figure 20A** provides an exemplary IHC staining image of the LXFS 573 tumor sample. **Figure 20B** plots absolute tumor volumes of the tested groups over the observation period. **Figure 20C** plots tumor volumes on Day 46 (D46) (Mixed effect analysis, Dunnett's post-test vs Vehicle group). **Figures 20D-20G** provide spider plots of mice treated with the indicated compounds (**Figure 20D**, Vehicle; **Figure 20E**, ADC-016-M, 5 mg/kg x 1; **Figure 20F**, ADC-016-M, 5 mg/kg x 2; **Figure 20G**, ADC-015-M, 5 mg/kg x 1). **Figure 20H** plots relative tumor volumes of the tested groups over the observation period. **Figure 20I** plots body weights of the tested groups over the observation period.

[0038] **Figures 21A-21I** provide data obtained from the CXF 94 PDX model, further described in **Example 24**. **Figure 21A** provides an exemplary IHC staining image of the CXF 94 tumor sample. **Figure 21B** plots absolute tumor volumes of the tested groups over the observation period (the mean group TV was graphed until 2 animals were removed from group due to TV endpoint). **Figure 21C** plots tumor volumes on Day 31 (D31) (Mixed effect analysis, Dunnett's post-test vs Vehicle group). **Figures 21D-21G** provide spider plots of mice treated with the indicated compounds (**Figure 21D**, Vehicle; **Figure 21E**, ADC-016-M, 5 mg/kg x 1; **Figure 21F**, ADC-016-M, 5 mg/kg x 2; **Figure 21G**, ADC-015-M, 5 mg/kg x 1). **Figure 21H** plots relative tumor volumes of the tested groups over the observation period. **Figure 21I** plots body weights of the tested groups over the observation period.

[0039] **Figures 22A-22J** provide data obtained from the LXFS 538 PDX model, further described in **Example 24**. **Figure 22A** provides an exemplary IHC staining image of the LXFS 538 tumor sample. **Figure 22B** plots absolute tumor volumes of the tested groups over the observation period (the mean group TV was graphed until 2 animals were removed from group due to TV endpoint). **Figure 22C** plots tumor volumes on Day 35 (D35), while **Figure 22D** plots tumor volumes on Day 42 (D42) (Mixed effect analysis, Dunnett's post-test vs Vehicle group). **Figures 22E-22H** provide spider plots of mice treated with the indicated compounds (**Figure 22E**, Vehicle; **Figure 22F**, ADC-016-M, 5 mg/kg x 1; **Figure 22G**, ADC-016-M, 5 mg/kg x 2; **Figure 22H**, ADC-015-M, 5 mg/kg x 1). **Figure 22I** plots relative tumor volumes of the tested groups over the observation period. **Figure 22J** plots body weights of the tested groups over the observation period.

DETAILED DESCRIPTION

[0040] The present disclosure provides an antibody-drug conjugate (ADC) comprising an antibody that binds to DLL3 and a drug conjugated (directly or indirectly) thereto. Such DLL3-ADCs are useful in compositions and in methods of treating, preventing, or alleviating a DLL3-mediated disease, disorder, or condition, including one or more symptoms of the disease, disorder, or condition. DLL3-mediated diseases, disorders, and conditions include cancer, for example, lung cancer (such as small cell lung cancer (SCLC)), large cell neuroendocrine carcinoma (LCNEC), or colorectal cancer. The DLL3-ADCs described herein comprise a DLL3 antibody conjugated to one or more linker-drug conjugates.

[0041] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

[0042] The terms “about” and “approximately” mean within 20%, within 15%, within 10%, within 9%, within 8%, within 7%, within 6%, within 5%, within 4%, within 3%, within 2%, within 1%, or less variation of a given value or range.

[0043] As used herein, comparative terms as used herein, such as reduce, decrease, increase, or any grammatical variation thereof, can refer to certain variation from the reference. In some embodiments, such variation can refer to about 10%, or about 20%, or about 30%, or about 40%, or about 50%, or about 60%, or about 70%, or about 80%, or about 90%, or about 1 fold, or about 2 fold, or about 3 fold, or about 4 fold, or about 5 fold, or about 10 fold, or about 20 fold, or about 30 fold, or about 40 fold, or about 100 fold or higher than the reference. In some embodiments, such variation can refer to about 1%, or about 2%, or about 3%, or about 4%, or about 5%, or about 6%, or about 7%, or about 8%, or about 9%, or about 10%, or about 20%, or about 30%, or about 40%, or about 50%, or about 60%, or about 70%, or about 80%, or about 90%, or about 95%, or about 96%, or about 97%, or about 98%, or about 99% of the reference.

[0044] As used in the present disclosure and claims, the singular forms “a,” “an” and “the” include plural forms unless the context clearly dictates otherwise.

[0045] In some embodiments, the terms “first,” “second,” “third,” “fourth” and similar in a component name are used to distinguish and identify more than one component sharing

certain identity in their names. For example, “first antibody” and “second antibody” are used to distinguish two antibodies.

[0046] It is understood that wherever embodiments are described herein with the term “comprising” otherwise analogous embodiments described in terms of “consisting of” and/or “consisting essentially of” are also provided. It is also understood that wherever embodiments are described herein with the phrase “consisting essentially of” otherwise analogous embodiments described in terms of “consisting of” are also provided.

[0047] The term “between” as used in a phrase as such “between A and B” or “between A-B” refers to a range including both A and B.

[0048] The term “and/or” as used in a phrase such as “A and/or B” herein is intended to include both A and B; A or B; A (alone); and B (alone). Likewise, the term “and/or” as used in a phrase such as “A, B, and/or C” is intended to encompass each of the following embodiments: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

[0049] The term “optional” or “optionally” means that the subsequently described circumstance may or may not occur, so that the description includes instances wherein the circumstance occurs, and the instances wherein the circumstance does not occur.

DLL3 ANTIBODIES

[0050] In order to better understand the disclosure, the definitions and explanations of the relevant terms are provided as follows.

[0051] The term “Delta Like Canonical Notch Ligand 3,” “DLL3,” “SCDO1,” “Drosophila Delta Homolog 3,” “Delta-Like Protein 3,” “Delta3,” “D3,” “Delta (Drosophila)-Like 3,” “Delta-Like 3 (Drosophila),” “Delta-Like 3” or similar terms refers to a polypeptide (“polypeptide” and “protein” are used interchangeably herein) or any native DLL3 from any vertebrate source, including mammals such as primates (*e.g.*, humans, cynomolgus monkey (*cyno*)), dogs, and rodents (*e.g.*, mice and rats), unless otherwise indicated. The term DLL3 encompasses “full-length” DLL3, as well as any form of DLL3 or any fragment thereof that results from processing in the cell. The term DLL3 also encompasses naturally occurring variants of DLL3, such as SNP variants, splice variants and allelic variants. Other related DLL3 polypeptides that are also encompassed by the term DLL3 include fragments, derivatives (*e.g.*, substitution, deletion, truncations, and insertion variants), fusion polypeptides, and interspecies homologs that retain DLL3 activity. Orthologs to the DLL3 polypeptide are also well known in the art. Exemplary DLL3 sequences and additional information can be found at GeneCards: GC19P039498; HGNC: 2909; NCBI Entrez Gene:

10683; Ensembl: ENSG00000090932; OMIM®: 602768; and UniProtKB/Swiss-Prot: Q9NYJ7, each of which is incorporated herein by reference in its entirety. In further embodiments, an exemplary human DLL3 sequence is provided herein as SEQ ID NO:11, an exemplary cyno DLL3 sequence is provided herein as SEQ ID NO:12, and an exemplary murine DLL3 sequence is provided herein as SEQ ID NO:13.

[0052] The term “antibody” (*e.g.*, DLL3 antibody) is used in the broadest sense and encompass any form of antibody that exhibits the desired biological or binding activity. It covers, but is not limited to, humanized antibodies, fully human antibodies, chimeric antibodies, and single-domain antibodies (sdAbs, comprising just one chain, which is typically similar to a heavy chain, such as VHH), as well as fragments of any of the foregoing as long as they exhibit the desired antigen-binding activity, including, for example, an antibody comprising at least one VHH domain. A conventional antibody comprises a heavy chain(s) and a light chain(s). Heavy chains may be classified into μ , δ , γ , α and ϵ , which define isotypes of an antibody as IgM, IgD, IgG, IgA and IgE, respectively. A heavy chain can comprise a heavy chain variable region (VH) and a heavy chain constant region (CH). A heavy chain can comprise one or more constant regions, for example, 3 constant regions (CH1, CH2 and CH3). A light chain can comprise a light chain variable region (VL) and a light chain constant region (CL). A VH and a VL region can further be divided into hypervariable regions (called complementary determining regions (CDRs)), which are interspaced by relatively conservative regions (called framework regions (FRW)). A VH and a VL can comprise 3 CDRs (Complementarity determining regions) and 4 FRs (Framework regions) in the following order: FRW1, CDR1, FRW2, CDR2, FRW3, CDR3, FRW4 from N-terminal to C-terminal. Antibodies can be of different antibody isotypes, for example, IgG (*e.g.*, IgG1, IgG2, IgG3 or IgG4 subtype), IgA1, IgA2, IgD, IgE or IgM antibody.

[0053] A universal numbering system for hypervariable regions has been developed and widely adopted, ImMunoGeneTics (IMGT®) Information System (Lefranc et al., Dev. Comp. Immunol. 27(1):55-77 (2003)). IMGT® is an integrated information system specializing in immunoglobulins (IG), T cell receptors (TR) and major histocompatibility complex (MHC) of human and other vertebrates. Herein, the CDRs are referred to in terms of both the amino acid sequence and the location within the light or heavy chain. As the “location” of the CDRs within the structure of the immunoglobulin variable region is conserved between species and present in structures called loops, by using numbering systems that align variable region sequences of structural features, CDR and framework residues and are readily identified. This information can be used in grafting and replacement of CDR residues from immunoglobulins

of one species into an acceptor framework from, typically, a human antibody. An additional numbering system (AHon) has been developed by Honegger and Plückthun, *J. Mol. Biol.* 309: 657-670 (2001). Correspondence between the numbering system, including, for example, the Kabat numbering and the IMGT[®] unique numbering system, is well known to one skilled in the art (see, *e.g.*, Kabat, *supra*; Chothia and Lesk, *supra*; Martin, *supra*; Lefranc et al., *supra*) and is also illustrated below. Various systems known in the art or described herein represent different ways of delineating CDRs, and when they are used to define the same antibody, they are often considered equivalent. An exemplary system, shown herein, combines Kabat and Chothia.

	Exemplary	IMGT[®]	Kabat	AbM	Chothia	Contact
V_H CDR1	26-35	27-38	31-35	26-35	26-32	30-35
V_H CDR2	50-65	56-65	50-65	50-58	53-55	47-58
V_H CDR3	95-102	105-117	95-102	95-102	96-101	93-101
V_L CDR1	24-34	27-38	24-34	24-34	26-32	30-36
V_L CDR2	50-56	56-65	50-56	50-56	50-52	46-55
V_L CDR3	89-97	105-117	89-97	89-97	91-96	89-96

[0054] The term “Fc region” is used to define a C-terminal region of an immunoglobulin heavy chain, including, for example, native sequence Fc regions, recombinant Fc regions, and variant Fc regions. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy chain Fc region is often defined to stretch from an amino acid residue at position Cys226 (according to the EU numbering system), or from Pro230 (according to the EU numbering system) to the carboxyl-terminus thereof. The C-terminal lysine (residue 447 according to the EU numbering system) of the Fc region may be removed, for example, during production or purification of the antibody, or by recombinantly engineering the nucleic acid encoding a heavy chain of the antibody.

[0055] A “functional Fc region” possesses an “effector function” of a native sequence Fc region. Exemplary “effector functions” include C1q binding; complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (*e.g.*, B cell receptor; BCR), etc. Such effector functions generally require the Fc region to be combined with a binding region or binding domain (*e.g.*, an antibody variable region or domain, including a VHH domain) and can be assessed using various assays as disclosed.

[0056] A “native sequence Fc region” comprises an amino acid sequence identical to the amino acid sequence of an Fc region found in nature, and not manipulated, modified, and/or changed (*e.g.*, isolated, purified, selected, including, or combining with other sequences such

as variable region sequences) by a human. Native sequence human Fc regions include a native sequence human IgG1 Fc region (non-A and A allotypes); native sequence human IgG2 Fc region; native sequence human IgG3 Fc region; and native sequence human IgG4 Fc region as well as naturally occurring variants thereof.

[0057] A “variant Fc region” comprises an amino acid sequence which differs from that of a native sequence Fc region by virtue of at least one amino acid modification, (*e.g.*, substituting, addition, or deletion) preferably one or more amino acid substitution(s). In some embodiments, the variant Fc region has at least one amino acid substitution compared to a native sequence Fc region or to the Fc region of a parent polypeptide, for example, from about one to about ten amino acid substitutions, and preferably from about one to about five amino acid substitutions in a native sequence Fc region or in the Fc region of the parent polypeptide. A variant Fc region can possess at least about 80% homology with a native sequence Fc region and/or with an Fc region of a parent polypeptide, or at least about 90% homology therewith, for example, at least about 95% homology therewith. The variant Fc region herein described herein may have a loss of effector function (*e.g.*, silent Fc).

[0058] DLL3 antibodies described herein include, but are not limited to, synthetic antibodies, monoclonal antibodies, recombinantly produced antibodies, multispecific antibodies (*e.g.*, including bispecific antibodies), human antibodies, humanized antibodies, chimeric antibodies, intrabodies, single-chain Fvs (scFv) (*e.g.*, including monospecific, bispecific, etc.), camelized antibodies, Fab fragments, F(ab') fragments, disulfide-linked Fvs (sdFv), anti-idiotypic (anti-Id) antibodies, and epitope-binding fragments of any of the above.

[0059] The term “immunoglobulin single variable domain” or “single variable domain” or “VHH domain” or “VHH” or “heavy chain only antibody variable domain” may be used interchangeably herein and refers to a single chain antigen binding domain that is capable of binding to an antigen or epitope, independently of a different variable domain. A VHH domain (*e.g.*, variable domain of a heavy chain antibody) represents the smallest known antigen-binding unit generated by adaptive immune responses (Koch-Nolte F. et al., FASEB J. Nov; 21(13):3490-8. Epub 2007 Jun 15 (2007)). A VHH domain may be a human domain, but also includes a single domain from other species such as rodent, nurse shark and camelid VHH domains. Camelid VHH are immunoglobulin single variable domain polypeptides that are derived from species including camel, llama, alpaca, dromedary, and guanaco, which produce heavy chain antibodies naturally devoid of light chains. Such VHH domains may be humanized according to standard techniques available in the art and are considered as “single

domain antibodies.” As used herein, VHH includes camelid VHH domains and humanized VHH domains.

[0060] The term “humanized antibody” is intended to refer to antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, llama, or alpaca, have been grafted onto human framework sequences. Additional framework region modifications may be made within the human framework sequences.

[0061] The term “ K_a ,” as used herein, is intended to refer to the association rate of a particular antibody-antigen interaction, whereas the term “ K_d ” as used herein, is intended to refer to the dissociation rate of a particular antibody-antigen interaction. K_d values for antibodies can be determined using methods well established in the art. The term “ K_D ” as used herein, is intended to refer to the dissociation constant of a particular antibody-antigen interaction, which is obtained from the ratio of K_d to K_a (*e.g.*, K_d/K_a) and is expressed as a molar concentration (M). A preferred method for determining the K_D of an antibody is by using surface plasmon resonance, preferably using a biosensor system such as a Biacore® system.

[0062] The term “specific binding” or “specifically binds” as used herein refers to a non-random binding reaction between two molecules, such as for example between an antibody and an antigen.

[0063] The term “high affinity,” as used herein, refers to a DLL3 antibody having a K_D of 1×10^{-7} M or less, more preferably 5×10^{-8} M or less, even more preferably 1×10^{-8} M or less, even more preferably 5×10^{-9} M or less and even more preferably 1×10^{-9} M or less for a target antigen.

[0064] The term “epitope,” as used herein, refers to a portion of an antigen that an immunoglobulin or antibody specifically binds to. “Epitope” is also known as “antigenic determinant.” Epitope or antigenic determinant generally comprises chemically active surface groups of a molecule such as amino acids, carbohydrates, or sugar side chains, and generally has a specific three-dimensional structure and a specific charge characteristic. For example, an epitope generally comprises at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 consecutive or non-consecutive amino acids in a unique steric conformation, which may be “linear” or “conformational”. See, for example, Epitope Mapping Protocols in Methods in Molecular Biology, Vol. 66, G. E. Morris, Ed. (1996). In a linear epitope, all the interaction sites between a protein and an interaction molecule (*e.g.*, an antibody) are present linearly along the primary amino acid sequence of the protein. In a conformational epitope, the interaction sites span over amino acid residues that are separate from each other in a protein. Antibodies

may be screened depending on competitiveness of binding to the same epitope by conventional techniques known by a person skilled in the art. For example, studies on competition or cross-competition may be conducted to obtain antibodies that compete or cross-compete with each other for binding to antigens. High-throughput methods for obtaining antibodies binding to the same epitope, which are based on their cross-competition, are described in an international patent application WO 03/48731.

[0065] The term “isolated antibody,” as used herein, is intended to refer to an antibody that is substantially free of other antibodies that have different antigenic specificities (*e.g.*, an isolated antibody that specifically binds a DLL3 protein is substantially free of antibodies that specifically bind antigens other than DLL3 proteins). An isolated antibody that specifically binds a human DLL3 protein may, however, have cross-reactivity to other antigens, such as DLL3 proteins from other species. Moreover, an isolated antibody can be substantially free of other cellular material and/or chemicals.

[0066] Examples of DLL3 antibodies for use in the DLL3-ADCs described herein include, but are not limited to, chimeric antibodies, humanized antibodies, human antibodies, and single-domain antibodies. In some embodiments, a DLL3-ADC as disclosed herein comprise a DLL3 antibody comprising at least one VHH that specifically binds to DLL3. Further, a DLL3 antibody may be a single-domain antibody and comprising one VHH. For example, a single-domain antibody is able to bind selectively to a specific antigen (*e.g.*, DLL3). In some embodiments, a DLL3-ADC as disclosed herein comprise a DLL3 antibody comprising a VHH fused to an immunoglobulin Fc region, for example, an Fc region of IgG (*e.g.*, IgG4 or IgG1). In some embodiments, the Fc region is an Fc region of human IgG1. By fusing a VHH to an Fc region, it may be more efficient to recruit effector functions. Also, fusion of a VHH to an Fc region may help a DLL3 antibody to form a dimer and may also help the extension of the half-life of the DLL3-ADC *in vivo*. In some embodiments, a DLL3 antibody for use in the DLL3-ADC as disclosed herein comprises two VHHs and an immunoglobulin Fc region, wherein each of the VHH directly or indirectly conjugated to a chain of the Fc. See, for example, **Figure 1A**. In some embodiments, the VHH is conjugated directly or indirectly to the N-terminus of the Fc.

[0067] In some embodiments, a DLL3 antibody of a DLL3-ADC as disclosed herein is a VHH-Fc fusion protein. As used herein, a VHH-Fc fusion refers to one or more VHH directly or indirectly conjugated to a Fc. In further embodiments, the VHH is conjugated directly or indirectly to the N-terminus of the Fc. In some embodiments, a VHH-Fc fusion comprises two VHHs and an immunoglobulin Fc region, wherein each of the VHH directly or indirectly

conjugated to a chain of the Fc. In further embodiments, each of the VHHs is conjugated directly or indirectly to the N-terminus of the Fc chain. See, for example, **Figure 1A**. Additionally or alternatively, a VHH-Fc fusion comprise two chains, wherein each of the two chains comprises, from the N terminus to the C terminus, a VHH, a hinge region, a CH2, and a CH3. In further embodiments, one or more linkers may present between any two of the VHH, the hinge region, the CH2, and the CH3. In some embodiments, the two chains of the VHH-Fc fusion are identical to each other. In other embodiments, the two chains of the VHH-Fc fusion are different from each other. For example, the first chain of the VHH-Fc fusion comprises a first VHH which is different from the VHH of the second chain. Additionally, or alternatively, the CH3 domains of the two chains are modified to facilitate production of the VHH-Fc fusion, such as by introducing knob-into-hole mutations. As it would be understood by one of skill in the art that, in some embodiments, a chain of a VHH-Fc fusion may not comprise a CH1 between the VHH and the hinge region, and thus is different from a conventionally defined heavy chain of an antibody. Yet, when describing a DLL3-ADC or embodiments thereof and when appropriate, the term “heavy chain” as used herein may also be interpreted as referring to a chain of a VHH-Fc fusion protein.

[0068] As known in the art, VHH molecules derived from Camelidae antibodies are among the smallest intact antigen-binding domains known (approximately 15 kDa, or 10 times smaller than a conventional IgG) and hence are well suited towards delivery to dense tissues and for accessing the limited space between macromolecules.

[0069] VHHs as disclosed herein may be made by the skilled artisan according to methods known in the art or any future method. For example, VHHs may be obtained using methods known in the art such as by immunizing a camel and obtaining hybridoma's therefrom, or by cloning a library of VHHs of the disclosure using molecular biology techniques known in the art and subsequent selection by using phage display.

[0070] For example, a VHH can be obtained by immunization of llamas or alpacas with the desired antigen and subsequent isolation of the mRNA coding for single-domain heavy-chain antibodies. By reverse transcription and polymerase chain reaction, a gene library of single-domain antibodies containing several million clones is produced. Screening techniques like phage display and ribosome display help to identify the clones binding the antigen. One technique is phage display in which a library of (*e.g.*, human) antibodies is synthesized on phages, the library is screened with the antigen of interest or an antibody-binding portion thereof, and the phage that binds the antigen is isolated, from which one may obtain the immunoreactive fragments. Methods for preparing and screening such libraries are well

known in the art and kits for generating phage display libraries are commercially available (*e.g.*, the Pharmacia Recombinant Phage Antibody System, catalog no. 27-9400-01; and the Stratagene SurfZAPTM phage display kit, catalog no. 240612). There also are other methods and reagents that can be used in generating and screening antibody display libraries (see, *e.g.*, Barbas *et al.*, *Proc. Natl. Acad. Sci. USA* 88:7978-7982 (1991)).

[0071] When potent clones have been identified, their sequence is optimized, for example, by affinity maturation or humanization. Humanization may prevent immunological reactions of the human organism against the antibody.

[0072] Accordingly, the VHHs can be obtained (1) by isolating the VHH domain of a naturally occurring heavy chain antibody; (2) by expression of a nucleotide sequence encoding a naturally occurring VHH domain; (3) by “humanization” (as described below) of a naturally occurring VHH domain or by expression of a nucleic acid encoding a such humanized VHH domain; (4) by “camelization” of a naturally occurring VH domain from any animal species, in particular a species of mammal, such as from a human being, or by expression of a nucleic acid encoding such a camelized VH domain; (5) by “camelization” of a “domain antibody” or “Dab” as described by Ward *et al* (*supra*), or by expression of a nucleic acid encoding such a camelized VH domain; (6) using synthetic or semi-synthetic techniques for preparing proteins, polypeptides or other amino acid sequences; (7) by preparing a nucleic acid encoding a VHH using techniques for nucleic acid synthesis, followed by expression of the nucleic acid thus obtained; (8) subjection heavy chain antibodies or VHHs to affinity maturation, to mutagenesis (*e.g.*, random mutagenesis or site directed mutagenesis,) and/or any other technique(s) in order to increase the affinity and/or specificity of the VHH; and/or (9) by any combination of the foregoing. Suitable methods and techniques for performing the foregoing will be clear to the skilled person based on the disclosure herein and, for example, include methods and techniques described in more detail herein.

[0073] Single-domain antibodies are usually generated by PCR cloning of variable domain repertoire from blood, lymph node, or spleen cDNA obtained from immunized animals into a phage display vector. Antigen-specific single-domain antibodies are commonly selected by panning phage libraries on immobilized antigen, for example, antigen coated onto the plastic surface of a test tube, biotinylated antigens immobilized on Streptavidin beads, or membrane proteins expressed on the surface of cells. The affinity of sdAbs can often be improved by mimicking this strategy *in vitro*, for example, by site directed mutagenesis of the CDR regions and further rounds of panning on immobilized antigen under conditions of increased

stringency (higher temperature, high or low salt concentration, high or low pH, and low antigen concentrations) (Wesolowski et al., Single domain antibodies: promising experimental and therapeutic tools in infection and immunity. *Med Microbiol Immunol* (2009) 198: 157-174).

[0074] Methods for preparing a VHH specifically binding to an antigen or epitope was described in references, for example: R. van der Linden et al., *Journal of Immunological Methods*, 240(2000) 185-195; Li et al., *J Biol Chem.*, 287(2012)13713-13721; Deffar et al., *African Journal of Biotechnology* Vol. 8(12), pp.2645, 17 June, 2009 and WO 94/04678.

[0075] In some embodiments, a VHH may be truncated at the N-terminus or C-terminus such that it comprises only a partial FRW1 and/or FRW4, or lacks one or both of those framework regions, so long as the VHH substantially maintains antigen binding and specificity (*e.g.*, substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%).

[0076] DLL3 antibodies of the DLL3-ADCs described herein may be monospecific, bispecific, trispecific, or of greater multispecificity. Such agents may include antibodies. Multispecific antibodies, such as bispecific antibodies, are monoclonal antibodies that have binding specificities for at least two different targets (*e.g.*, antigens) or two different epitopes on the same target (*e.g.*, a bispecific antibody directed to DLL3 with a first binding domain for a first epitope of DLL3, and a second binding domain for a second epitope of DLL3). In some embodiments, the multispecific (*e.g.*, bispecific) antibodies can be constructed based on the sequences of the antibodies described herein. In some embodiments, the multispecific antibodies described herein are bispecific antibodies. In some embodiments, bispecific antibodies are mouse, chimeric, human, or humanized antibodies. In some embodiments, one of the binding specificities of the multispecific antibody is for DLL3 and the other is for any other target (*e.g.*, antigen). In some embodiments, a multispecific (*e.g.*, bispecific) antibody can comprise more than one target (*e.g.*, antigen) binding domain, in which different binding domains are specific for different targets (*e.g.*, a first binding domain that binds to DLL3 and a second binding domain that binds another target (*e.g.*, antigen), such as an immune checkpoint regulator (*e.g.*, a negative checkpoint regulator)). In some embodiments, multispecific (*e.g.*, bispecific) antibody molecules can bind more than one (*e.g.*, two or more) epitopes on the same target (*e.g.*, antigen). In some embodiments, one of the binding specificities is DLL3 and the other is for one or more of Cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD80, CD86, Programmed cell death 1 (PD-1), Programmed cell death ligand 1 (PD-L1), Programmed cell death ligand 2 (PD-L2), Lymphocyte activation gene-3 (LAG-3;

also known as CD223), Galectin-3, B and T lymphocyte attenuator (BTLA), T-cell membrane protein 3 (TIM3), Galectin-9 (GAL9), B7-H1, B7-H3, B7-H4, T-Cell immunoreceptor with Ig and ITIM domains (TIGIT/Vstm3/WUCAM/VSIG9), V-domain Ig suppressor of T-Cell activation (VISTA), Glucocorticoid-induced tumor necrosis factor receptor-related (GITR) protein, Herpes Virus Entry Mediator (HVEM), OX40, CD27, CD28, CD137. CGEN-15001T, CGEN-15022, CGEN-15027, CGEN-15049, CGEN-15052, and CGEN-15092.

[0077] Methods for making multispecific antibodies are known in the art, for example, by co-expression of two immunoglobulin heavy chain-light chain pairs, where the two heavy chains have different specificities (see, *e.g.*, Milstein and Cuello, 1983, *Nature* 305:537-40). For further details of generating multispecific antibodies (*e.g.*, bispecific antibodies), see, for example, *Bispecific Antibodies* (Kontermann ed., 2011).

[0078] The present disclosure provides humanized antibodies that bind DLL3. Various methods for humanizing non-human antibodies are known in the art. For example, a humanized antibody can have one or more amino acid residues introduced into it from a source that is non-human. These non-human amino acid residues are often referred to as “import” residues, which are typically taken from an “import” variable domain. Humanized antibodies that bind DLL3 may be produced using techniques known to those skilled in the art (*e.g.*, Zhang et al., *Molecular Immunology*, 42(12): 1445-1451, 2005; Hwang et al., *Methods*, 36(1): 35-42, 2005; Dall’Acqua et al., *Methods*, 36(1): 43-60, 2005; Clark, *Immunology Today*, 21(8): 397-402, 2000, and U.S. Patent Nos. 6,180,370; 6,054,927; 5,869,619; 5,861,155; 5,712,120; and 4,816,567).

[0079] The term “identity,” as used herein, refers to a relationship between the sequences of two or more polypeptide molecules or two or more nucleic acid molecules, as determined by aligning and comparing the sequences. “Percent identity” means the percent of identical residues between the amino acids or nucleotides in the compared molecules and is calculated based on the size of the smallest of the molecules being compared. For these calculations, gaps in alignments (if any) are preferably addressed by a particular mathematical model or computer program (*e.g.*, an “algorithm”). Methods that can be used to calculate the identity of the aligned nucleic acids or polypeptides include those described in *Computational Molecular Biology*, (Lesk, A. M., ed.), 1988, New York: Oxford University Press; *Biocomputing Informatics and Genome Projects*, (Smith, D. W., ed.), 1993, New York: Academic Press; *Computer Analysis of Sequence Data, Part I*, (Griffin, A. M., and Griffin, H. G., eds.), 1994, New Jersey: Humana Press; von Heinje, G., 1987, *Sequence Analysis in Molecular Biology*,

New York: Academic Press; Sequence Analysis Primer, (Gribskov, M. and Devereux, J., eds.), 1991, New York: M. Stockton Press; and Carillo et al, 1988, SIAMJ. Applied Math. 48:1073. Alignment methods are available to one of skilled in the art, such as BLAST as disclosed herein and/or Clustal Omega.

[0080] “Polynucleotide” or “nucleic acid,” as used interchangeably herein, refers to polymers of nucleotides of any length and includes DNA and RNA. The nucleotides can be deoxyribonucleotides, ribonucleotides, modified nucleotides or bases, and/or their analogs, or any substrate that can be incorporated into a polymer by DNA or RNA polymerase or by a synthetic reaction. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and their analogs. A cell that produces a binding molecule of the present disclosure may include a parent hybridoma cell, as well as bacterial and eukaryotic host cells into which nucleic acids encoding the antibodies have been introduced. Unless specified otherwise, the left-hand end of any single-stranded polynucleotide sequence disclosed herein is the 5' end; the left-hand direction of double-stranded polynucleotide sequences is referred to as the 5' direction. The direction of 5' to 3' addition of nascent RNA transcripts is referred to as the transcription direction; sequence regions on the DNA strand having the same sequence as the RNA transcript that are 5' to the 5' end of the RNA transcript are referred to as “upstream sequences”; sequence regions on the DNA strand having the same sequence as the RNA transcript that are 3' to the 3' end of the RNA transcript are referred to as “downstream sequences.”

[0081] The term “vector,” as used herein, refers to a nucleic acid vehicle which can have a polynucleotide inserted therein. When the vector allows for the expression of the protein encoded by the polynucleotide inserted therein, the vector is called an expression vector. The vector can have carried genetic material elements expressed in a host cell by transformation, transduction, or transfection into the host cell. Vectors are well known by a person skilled in the art, including, but not limited to plasmids, phages, cosmids, artificial chromosome such as yeast artificial chromosome (YAC), bacterial artificial chromosome (BAC) or P1-derived artificial chromosome (PAC); phage such as λ phage or M13 phage and animal virus. The animal viruses that can be used as vectors, include, but are not limited to, retrovirus (including lentivirus), adenovirus, adeno-associated virus, herpes virus (such as herpes simplex virus), pox virus, baculovirus, papillomavirus, papova virus (such as SV40). A vector may comprise multiple elements for controlling expression, including, but not limited to, a promoter sequence, a transcription initiation sequence, an enhancer sequence, a selection element, and a reporter gene. In addition, a vector may comprise an origin of replication.

[0082] The term “host cell,” as used herein, refers to a cell into which a vector can be introduced, including, but not limited to, a prokaryotic cell such as *E. coli* or *Bacillus subtilis*, a fungal cell such as yeast cell or *Aspergillus*, an insect cell such as S2 *Drosophila* cell or Sf9, and an animal cell such as fibroblast, CHO cell, COS cell, NSO cell, HeLa cell, BHK cell, HEK 293 cell or human cell.

[0083] The term “transfection” or “transfect,” as used herein, refers to a process by which nucleic acids are introduced into eukaryotic cells, particularly mammalian cells. Protocols and techniques for transfection include but not limited to lipid transfection and chemical and physical methods such as electroporation. A number of transfection techniques are well known in the art and are disclosed herein. See, *e.g.*, Graham et al., 1973, *Virology* 52:456; Sambrook et al., 2001, *Molecular Cloning: A Laboratory Manual*, supra; Davis et al., 1986, *Basic Methods in Molecular Biology*, Elsevier; Chu et al, 1981, *Gene* 13:197.

[0084] The DLL3-ADCs disclosed herein comprise a DLL3 antibody. DLL3 antibodies includes, but not limited to, a chimeric antibody, a humanized antibody, a human antibody, or a single-domain antibody.

[0085] DLL3 antibodies of the disclosure including, for example, antibodies comprising at least one VHH domain, are characterized by particular functional features or properties of the antibodies. In some embodiments, the antibodies have one or more of the following properties:

- (a) bind to human DLL3, cyno DLL3 and mouse DLL3 with EC₅₀ at nM grade, as measured by ELISA or FACS;
- (b) show dose-dependent internalization potency in human cells engineered to express DLL3 expressing; and
- (c) bind to human DLL3 extracellular domain (ECD) with a K_D no more than 0.1 nM, as measured by SPR.

[0086] A DLL3-ADC disclosed herein comprises a DLL3 antibody that binds to cell surface DLL3 with high affinity. The binding of an antibody, and therefore of the DLL3-ADC of which the antibody is part, can be assessed using one or more techniques well established in the art, for example, ELISA. The binding specificity of a DLL3-ADC can also be determined by monitoring binding of the DLL3-ADC to cells expressing a DLL3 protein, *e.g.*, by flow cytometry. For example, an antibody can be tested by a flow cytometry assay (*e.g.*, FACS) in which the DLL3-ADC is reacted with a cell line that expresses human DLL3, such as CHO cells and 293 cells that have been transfected to express DLL3 on their cell surface. Additionally, or alternatively, the binding of the DLL3-ADC, including the binding

kinetics (*e.g.*, K_D value) can be tested in Biacore™ binding assays. Still other suitable binding assays include ELISA assays, for example using a recombinant DLL3 protein. For example, a DLL3-ADC as disclosed herein may bind to a cell surface DLL3 (*e.g.*, human DLL3 ECD) protein with a K_D of 1×10^{-7} M or less, 5×10^{-8} M or less, 2×10^{-8} M or less, 5×10^{-9} M or less, 4×10^{-9} M or less, 3×10^{-9} M or less, 2×10^{-9} M or less, 1×10^{-9} M or less, 5×10^{-10} M or less, or 1×10^{-10} M or less.

[0087] The term “fluorescence-activated cell sorting” or “FACS,” as used herein, refers to a specialized type of flow cytometry. It provides a method for sorting a heterogeneous mixture of biological cells into two or more containers, one cell at a time, based upon the specific light scattering and fluorescent characteristics of each cell (FlowMetric. “Sorting Out Fluorescence Activated Cell Sorting.” Retrieved 2017-11-09.). Instruments for carrying out FACS are known to those of skill in the art and are commercially available to the public. Examples of such instruments include FACS Star Plus, FACScan and FACSsort instruments from Becton Dickinson (Foster City, Calif.) Epics C from Coulter Epics Division (Hialeah, Fla.) and MoFlo from Cytomation (Colorado Springs, Colo.).

[0088] The term “SPR” or “surface plasmon resonance,” as used herein, refers to and includes an optical phenomenon that allows for an analysis of real-time biospecific interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIAcore system (Pharmacia Biosensor AB, Uppsala, Sweden and Piscataway, N.J.). For further descriptions, see Jönsson, U., et al. (1993) *Ann. Biol. Clin.* 51:19-26; Jönsson, U., et al. (1991) *Biotechniques* 11:620-627; Johnsson, B., et al. (1995) *J. Mol. Recognit.* 8:125-131; and Johnsson, B., et al. (1991) *Anal. Biochem.* 198:268-277.

[0089] The term “ EC_{50} ”, as used herein, which is also termed as “half maximal effective concentration” refers to the concentration of a drug, antibody or toxicant which induces a response halfway between the baseline and maximum after a specified exposure time. In the context of the present disclosure, EC_{50} is expressed in the unit of “nM”. In some embodiments, the antibodies of the disclosure bind to cynomolgus monkey or mouse DLL3 at an EC_{50} of no more than or 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, 0.6 nM, 0.5 nM, 0.4 nM, 0.3 nM, 0.2 nM, 0.1 nM, 0.09 nM, 0.08 nM, 0.07 nM, 0.06 nM, 0.05 nM, 0.04 nM, 0.03 nM, 0.02 nM, or 0.01 nM, as measured by FACS.

DLL3 Antibodies Comprising VHH CDRs

[0090] In some embodiments, a DLL3-ADC as disclosed herein comprises a DLL3 antibody comprising a heavy chain variable domain (VH, such as a VHH), wherein the VH

comprises CDR1, CDR2 and CDR3, such as a VHH or a VHH conjugated to an Fc. In further embodiments, CDR1 comprises an amino acid sequence as set forth in SEQ ID NO:1 or 7, CDR2 comprises an amino acid sequence as set forth in SEQ ID NO:2 or 8, and CDR3 comprises an amino acid sequence as set forth in SEQ ID NO:3 or 9. In some embodiments, the CDR numbering are according to a combination of Kabat and AbM numbering. The extent of the framework region and CDRs can be precisely identified using methodology known in the art, for example, by the Kabat definition, the Chothia definition, the AbM definition, the contact definition, the IMGT definition (all of which are well known in the art) and any combinations thereof. See, *e.g.*, Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest* (Fifth Edition), U.S. Department of Health and Human Services, NIH Publication No. 91-3242; Chothia *et al.*, (1989) *Nature* 342:877; Chothia, C. *et al.* (1987) *J. Mol. Biol.* 196:901-917, Al-lazikani *et al.* (1997) *J. Molec. Biol.* 273:927-948; Edelman *et al.*, *Proc Natl Acad Sci U S A.* 1969 May, 63(1):78-85; and Martin and Allen, in “*Handbook of Therapeutic Antibodies*”, chapter 5, 2007. See also hgmp.mrc.ac.uk and bioinf.org.uk/abs. Correspondence or alignments between numberings according to different definitions can for example be found at www.imgt.org/ (see also Giudicelli V *et al.* IMGT, the international ImMunoGeneTics database. *Nucleic Acids Res.* (1997) 25:206–11; and Lefranc MP *et al.*, IMGT unique numbering for immunoglobulin and T cell receptor variable domains and Ig superfamily V-like domains. *Dev Comp Immunol.* (2003) 27:55–77).

[0091] As will be appreciated by those in the art, the exact numbering and placement of the CDRs can be different among different numbering systems. However, it should be understood that the disclosure of a variable heavy sequence, a variable light sequence and/or a VHH sequence includes the disclosure of the associated (inherent) CDRs. Accordingly, the disclosure of each variable region is a disclosure of the CDRs (*e.g.*, CDR1, CDR2 and CDR3). Two antibodies having the same VH, VL or VHH CDRs means that their CDRs are identical when determined by the same approach (*e.g.*, the Kabat, AbM, Chothia, Contact, and IMGT numbering approaches as known in the art).

[0092] Variable regions and CDRs in an antibody sequence can be identified according to general rules that have been developed in the art (for example, the Kabat, AbM, Chothia, Contact, and IMGT numbering system) or by aligning the sequences against a database of known variable regions. Methods for identifying these regions are described in Kontermann and Dubel, eds., *Antibody Engineering*, Springer, New York, NY, 2001 and Dinarello *et al.*, *Current Protocols in Immunology*, John Wiley and Sons Inc., Hoboken, NJ, 2000. Exemplary databases of antibody sequences are described in, and can be accessed through, the “Abysis”

website at www.bioinf.org.uk/abs (maintained by A.C. Martin in the Department of Biochemistry & Molecular Biology University College London, London, England) and the VBASE2 website at www.vbase2.org, as described in Retter *et al.*, Nucl. Acids Res., 33 (Database issue): D671 -D674 (2005). Preferably sequences are analyzed using the Abysis database, which integrates sequence data from Kabat, IMGT and the Protein Data Bank (PDB) with structural data from the PDB. See Dr. Andrew C. R. Martin's book chapter *Protein Sequence and Structure Analysis of Antibody Variable Domains*. In: *Antibody Engineering Lab Manual* (Ed.: Duebel, S. and Kontermann, R., Springer-Verlag, Heidelberg, ISBN-13: 978-3540413547, also available on the website bioinf.org.uk/abs). The Abysis database website further includes general rules that have been developed for identifying CDRs which can be used in accordance with the teachings herein. Unless otherwise indicated, all CDRs set forth herein are derived according to the Abysis database website as per Kabat, except for Chothia CDRs (see, *e.g.*, Chothia & Lesk (1987) J. Mol. Biol. 196:901-917).

[0093] In some embodiments, the DLL3 antibody (such as a VHH or a VHH conjugated to an Fc, *e.g.*, a VHH-Fc fusion) comprises at least one VH comprising one, two, and/or three heavy chain CDRs as described in **Table 1** or **Table 2**.

[0094] Table 1: Antibody mAb300 and mAb301

	VHH CDR1	VHH CDR2	VHH CDR3	Exemplary	IMGT	Kabat	Chothia	Contact	AbM
VHH CDR Seq.				GLTFSTATVG (SEQ ID NO:1)	GLTFSTAT (SEQ ID NO:20)	TATVG (SEQ ID NO:23)	GLTFSTA (SEQ ID NO:24)	STATVG (SEQ ID NO:27)	GLTFSTATVG (SEQ ID NO:1)
				AIPAYYSTYYA SSVKG (SEQ ID NO:2)	IPAYYST (SEQ ID NO:21)	AIPAYYSTYYA SSVKG (SEQ ID NO:2)	AYY (SEQ ID NO:25)	LIAAIPAYYSTY (SEQ ID NO:28); or LVAAIPAYYSTY (SEQ ID NO:56)	AIPAYYSTY (SEQ ID NO:30)
				DDTPSPSRSPFY KH (SEQ ID NO:3)	AADDTPSPSRSPFYKH (SEQ ID NO:22)	DDTPSPSRSPFY KH (SEQ ID NO:3)	DDTPSPSRSPFYKH (SEQ ID NO:26)	AADDTPSPSRSPFYKH (SEQ ID NO:29)	DDTPSPSRSPFYKH (SEQ ID NO:3)
VHH Sequence mAb300									
EVQLVESGGGLVQPGGSLRLSCAASGLTFSTATVGFWRQAPGKGRELIAAIPAYYSTYYASSVKGRFTISRDNAKNSLYLQMNSLRPDTAVYYCAADDTPSPSRSPFYKHRGQGTMTVSS (SEQ ID NO:14)									
VHH-Fc Fusion Sequence mAb300									
EVQLVESGGGLVQPGGSLRLSCAASGLTFSTATVGFWRQAPGKGRELIAAIPAYYSTYYASSVKGRFTISRDNAKNSLYLQMNSLRPDTAVYYCAADDTPSPSRSPFYKHRGQGTMTVSSDKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGK (SEQ ID NO:4)									
VHH Sequence mAb301									
EVQLVESGGGLVQPGGSLRLSCAASGLTFSTATVGFWRQAPGKGRELVA AIPAYYSTYYASSVKGRFTISRDNAKNSLYLQMNSLRPDTAVYYCAADDTPSPSRSPFYKHRGQGTMTVSS (SEQ ID NO:15)									
VHH-Fc Fusion sequence mAb301									
EVQLVESGGGLVQPGGSLRLSCAASGLTFSTATVGFWRQAPGKGRELVA AIPAYYSTYYASSVKGRFTISRDNAKNSLYLQMNSLRPDTAVYYCAADDTPSPSRSPFYKHRGQGTMTVSSDKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGK (SEQ ID NO:5)									

[0095] Table 2: Antibody mAb302

	VHH	Exemplary	IMGT	Kabat	Chothia	Contact	AbM
VHH CDR Seq.	CDR1	GRTRSYAMG (SEQ ID NO:7)	GRTRSYA (SEQ ID NO:42)	SYAMG (SEQ ID NO:45)	GRTRSY (SEQ ID NO:46)	RSYAMG (SEQ ID NO:49)	GRTRSYAMG (SEQ ID NO:7)
	VHH CDR2	AISWIGGGTY ADSVKG (SEQ ID NO:8)	ISWIGGGT (SEQ ID NO:43)	AISWIGGGTY ADSVKG (SEQ ID NO:8)	WIGG (SEQ ID NO:47)	FVA AISWIGGGT Y (SEQ ID NO:50)	AISWIGGGTY (SEQ ID NO:52)
	VHH CDR3	SSLRRHGHMFE ESDY (SEQ ID NO:9)	AASSLLRHGHM FEESDY (SEQ ID NO:44)	SSLRRHGHMFE ESDY (SEQ ID NO:9)	SLLRHGHMFE SD (SEQ ID NO:48)	AASSLLRHGHM FEESD (SEQ ID NO:51)	SLLRRHGHMFE ESDY (SEQ ID NO:9)
	VHH Sequence mAb302 QVQLVESGGGVVQPGGSLRLSCAASGRTRSYAMGWFRQAPGKEREFVAAISWIGGGTYADSVKGRFTISGDNSKNTLYLQMNSLRAEDTAV YYCAASSLLRHGHMFEESDYWGQGTMTVTVSS (SEQ ID NO:16) VHH-Fc Fusion Sequence mAb302 QVQLVESGGGVVQPGGSLRLSCAASGRTRSYAMGWFRQAPGKEREFVAAISWIGGGTYADSVKGRFTISGDNSKNTLYLQMNSLRAEDTAV YYCAASSLLRHGHMFEESDYWGQGTMTVTVSSDKTHTCPPCPAPELLGGPSVFLPPKPKDTLMISRTPETCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:10)						

[0096] In some embodiments, a DLL3-ADC as disclosed herein comprises a DLL3 antibody comprising a heavy chain variable domain (VH), such as a VHH or a VHH conjugated to an Fc, wherein the VH comprises FRW1-CDR1-FRW2-CDR2-FRW3-CDR3-FRW4, and wherein CDR1 comprises an amino acid sequence as set forth in SEQ ID NO:1 or 7, CDR2 comprises an amino acid sequence as set forth in SEQ ID NO:2 or 8, and CDR3 comprises an amino acid sequence as set forth in SEQ ID NO:3 or 9. In some embodiments, the FRW1 and FRW4 at the N and C terminal of the VHH comprised in a DLL3 antibody may be truncated such that it comprise only a partial FRW1 and/or FRW4, or the VHH lacks one or both of these framework regions, so long as the VHH substantially maintains antigen binding and specificity.

[0097] In some embodiments, a DLL3-ADC as disclosed herein comprises a DLL3 antibody (such as an anti-DLL3 single domain antibody, a VHH or a VHH conjugated to an Fc) comprising one, two, or all three CDRs of the amino acid sequence as set forth in SEQ ID NO:14. In some embodiments, a DLL3-ADC comprises a DLL3 antibody (such as an anti-DLL3 single domain antibody, a VHH or a VHH conjugated to an Fc) comprising one, two, or all three CDRs of the amino acid sequence as set forth in SEQ ID NO:15. In some embodiments, a DLL3-ADC comprises a DLL3 antibody (such as an anti-DLL3 single domain antibody, a VHH or a VHH conjugated to an Fc) comprising one, two, or all three CDRs of the amino acid sequence as set forth in SEQ ID NO:16. In some embodiments, a DLL3-ADC as disclosed herein comprises a DLL3 antibody which comprises a camelid VHH. In some embodiments, a DLL3-ADC as disclosed herein comprises a DLL3 antibody which is humanized. In some embodiments, a DLL3-ADC as disclosed herein comprises a DLL3 antibody comprising an acceptor human framework, *e.g.*, a human immunoglobulin framework or a human consensus framework.

[0098] In some embodiments, a DLL3-ADC as disclosed herein comprises a DLL3 antibody comprising a CDR1 having an amino acid sequence of the CDR1 as set forth in SEQ ID NO:1. In some embodiments, a DLL3-ADC comprises a DLL3 antibody comprising a CDR2 having an amino acid sequence of the CDR2 as set forth in SEQ ID NO:2. In some embodiments, a DLL3-ADC comprises a DLL3 antibody comprising a CDR3 having an amino acid sequence of the CDR3 as set forth in SEQ ID NO:3. In some embodiments, a DLL3-ADC comprises a DLL3 antibody comprising a CDR1 and a CDR2 having amino acid sequences of the CDR1 and the CDR2 as set forth in SEQ ID NO:1 and 2, respectively. In some embodiments, a DLL3-ADC comprises a DLL3 antibody comprising a CDR1 and a CDR3 having amino acid sequences of the CDR1 and the CDR3 as set forth in SEQ ID NO:1

and 3, respectively. In some embodiments, a DLL3-ADC comprises a DLL3 antibody comprising a CDR2 and a CDR3 having amino acid sequences of the CDR2 and the CDR3 as set forth in SEQ ID NO:2 and 3, respectively. In some embodiments, a DLL3-ADC comprises a DLL3 antibody comprising a CDR1, a CDR2, and a CDR3 having amino acid sequences of the CDR1, the CDR2, and the CDR3 as set forth in SEQ ID NO:1, 2, and 3, respectively. In some embodiments, a DLL3-ADC comprises a DLL3 antibody (such as an anti-DLL3 single domain antibody, a VHH or a VHH conjugated to an Fc) comprising one, two, or all three CDRs of the amino acid sequence as set forth in SEQ ID NO:14. In some embodiments, a DLL3-ADC comprises a DLL3 antibody (such as an anti-DLL3 single domain antibody, a VHH or a VHH conjugated to an Fc) comprising one, two, or all three CDRs of the amino acid sequence as set forth in SEQ ID NO:15. CDR sequences can be determined according to well-known numbering systems. In some embodiments, the CDRs are according to IMGT numbering. In some embodiments, the CDRs are according to Kabat numbering. In other embodiments, the CDRs are according to Chothia numbering. In other embodiments, the CDRs are according to Contact numbering. In some embodiments, the CDRs are according to AbM numbering. In some embodiments, a DLL3-ADC comprises a DLL3 antibody which comprises a camelid VHH. In some embodiments, a DLL3-ADC comprises a DLL3 antibody which is humanized. In some embodiments, a DLL3-ADC comprises a DLL3 antibody which comprises an acceptor human framework, *e.g.*, a human immunoglobulin framework or a human consensus framework.

[0099] In some embodiments, a DLL3-ADC comprises a DLL3 antibody comprising a CDR1 having an amino acid sequence of the CDR1 as set forth in SEQ ID NO:7. In some embodiments, a DLL3-ADC comprises a DLL3 antibody comprising a CDR2 having an amino acid sequence of the CDR2 as set forth in SEQ ID NO:8. In some embodiments, a DLL3-ADC comprises a DLL3 antibody comprising a CDR3 having an amino acid sequence of the CDR3 as set forth in SEQ ID NO:9. In some embodiments, a DLL3-ADC comprises a DLL3 antibody comprising a CDR1 and a CDR2 having amino acid sequences of the CDR1 and the CDR2 as set forth in SEQ ID NO:7 and 8, respectively. In some embodiments, a DLL3-ADC comprises a DLL3 antibody comprising a CDR1 and a CDR3 having amino acid sequences of the CDR1 and the CDR3 as set forth in SEQ ID NO:7 and 9, respectively. In some embodiments, a DLL3-ADC comprises a DLL3 antibody comprising a CDR2 and a CDR3 having amino acid sequences of the CDR2 and the CDR3 as set forth in SEQ ID NO:8 and 9, respectively. In some embodiments, a DLL3-ADC comprises a DLL3 antibody comprising a CDR1, a CDR2, and a CDR3 having amino acid sequences of the CDR1, the

CDR2, and the CDR3 as set forth in SEQ ID NO:7, 8, and 9, respectively. In some embodiments, a DLL3-ADC comprises a DLL3 antibody (such as an anti-DLL3 single domain antibody, a VHH or a VHH conjugated to an Fc) comprising one, two, or all three CDRs of the amino acid sequence as set forth in SEQ ID NO:16. CDR sequences can be determined according to well-known numbering systems. In some embodiments, the CDRs are according to IMGT numbering. In some embodiments, the CDRs are according to Kabat numbering. In other embodiments, the CDRs are according to Chothia numbering. In other embodiments, the CDRs are according to Contact numbering. In some embodiments, the CDRs are according to AbM numbering. In some embodiments, a DLL3-ADC comprises a DLL3 antibody which comprises a camelid VHH. In some embodiments, a DLL3-ADC comprises a DLL3 antibody which is humanized. In some embodiments, a DLL3-ADC comprises a DLL3 antibody which comprises an acceptor human framework, *e.g.*, a human immunoglobulin framework or a human consensus framework.

[00100] In some embodiments, provided herein is a DLL3-ADC comprising a DLL3 antibody comprising the following structure: FRW1-CDR1-FRW2-CDR2-FRW3-CDR3-FRW4, wherein (i) the CDR1 comprises an amino acid sequence as set forth in any one of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:20, SEQ ID NO:23, SEQ ID NO:24, or SEQ ID NO:27; (ii) the CDR2 comprises an amino acid sequence as set forth in any one of SEQ ID NO:2, SEQ ID NO:21, SEQ ID NO:25, SEQ ID NO:28, SEQ ID NO:56, or SEQ ID NO:30; and/or (iii) the CDR3 comprises an amino acid sequence as set forth in any one of SEQ ID NO:3, SEQ ID NO:22, SEQ ID NO:26, or SEQ ID NO:29. In some embodiments, a DLL3-ADC as disclosed herein comprises a DLL3 antibody which comprises a camelid VHH. In some embodiments, a DLL3-ADC as disclosed herein comprises a DLL3 antibody which is humanized. In some embodiments, a DLL3-ADC as disclosed herein comprises a DLL3 antibody which comprises an acceptor human framework, *e.g.*, a human immunoglobulin framework or a human consensus framework.

[00101] In some embodiments, the CDR1 of the DLL3 antibody of the DLL3-ADC comprises the exemplary amino acid sequence as set forth in SEQ ID NO:1; the CDR2 comprises the exemplary amino acid sequence as set forth in SEQ ID NO:2; and the CDR3 comprises the exemplary amino acid sequence as set forth in SEQ ID NO:3. In some embodiments, the CDR1 of the DLL3 antibody of the DLL3-ADC is according to IMGT numbering, comprising the amino acid sequence as set forth in SEQ ID NO:20; the CDR2 is according to IMGT numbering, comprising the amino acid sequence as set forth in SEQ ID NO:21; and the CDR3 is according to IMGT numbering, comprising the amino acid sequence

as set forth in SEQ ID NO:22. In some embodiments, the CDR1 of the DLL3 antibody of the DLL3-ADC is according to Kabat numbering, comprising the amino acid sequence as set forth in SEQ ID NO:23; the CDR2 is according to Kabat numbering, comprising the amino acid sequence as set forth in SEQ ID NO:2; and the CDR3 is according to Kabat numbering, comprising the amino acid sequence as set forth in SEQ ID NO:3. In some embodiments, the CDR1 of the DLL3 antibody of the DLL3-ADC is according to Chothia numbering, comprising the amino acid sequence as set forth in SEQ ID NO:24; the CDR2 is according to Chothia numbering, comprising the amino acid sequence as set forth in SEQ ID NO:25; and the CDR3 is according to Chothia numbering, comprising the amino acid sequence as set forth in SEQ ID NO:26. In some embodiments, the CDR1 of the DLL3 antibody of the DLL3-ADC is according to Contact numbering, comprising the amino acid sequence as set forth in SEQ ID NO:27; the CDR2 is according to Contact numbering, comprising the amino acid sequence as set forth in SEQ ID NO:28 or 56; and the CDR3 is according to Contact numbering, comprising the amino acid sequence as set forth in SEQ ID NO:29. In some embodiments, the CDR1 of the DLL3 antibody of the DLL3-ADC is according to AbM numbering, comprising the amino acid sequence as set forth in SEQ ID NO:1; the CDR2 is according to AbM numbering, comprising the amino acid sequence as set forth in SEQ ID NO:30; and the CDR3 is according to AbM numbering, comprising the amino acid sequence as set forth in SEQ ID NO:3. In some embodiments, a DLL3-ADC as disclosed herein comprises a DLL3 antibody which comprises a camelid VHH. In some embodiments, DLL3-ADC comprising a DLL3 antibody which is humanized. In some embodiments, a DLL3-ADC as disclosed herein comprises a DLL3 antibody which comprises an acceptor human framework, *e.g.*, a human immunoglobulin framework or a human consensus framework.

[00102] In some embodiments, the CDR1 of the DLL3 antibody of the DLL3-ADC comprises the exemplary amino acid sequence as set forth in SEQ ID NO:7; the CDR2 comprises the exemplary amino acid sequence as set forth in SEQ ID NO:8; and the CDR3 comprises the exemplary amino acid sequence as set forth in SEQ ID NO:9. In some embodiments, the CDR1 of the DLL3 antibody of the DLL3-ADC is according to IMGT numbering, comprising the amino acid sequence as set forth in SEQ ID NO:42; the CDR2 is according to IMGT numbering, comprising the amino acid sequence as set forth in SEQ ID NO:43; and the CDR3 is according to IMGT numbering, comprising the amino acid sequence as set forth in SEQ ID NO:44. In some embodiments, the CDR1 of the DLL3 antibody of the DLL3-ADC is according to Kabat numbering, comprising the amino acid sequence as set forth in SEQ ID NO:45; the CDR2 is according to Kabat numbering, comprising the amino

acid sequence as set forth in SEQ ID NO:8; and the CDR3 is according to Kabat numbering, comprising the amino acid sequence as set forth in SEQ ID NO:9. In some embodiments, the CDR1 of the DLL3 antibody of the DLL3-ADC is according to Chothia numbering, comprising the amino acid sequence as set forth in SEQ ID NO:46; the CDR2 is according to Chothia numbering, comprising the amino acid sequence as set forth in SEQ ID NO:47; and the CDR3 is according to Chothia numbering, comprising the amino acid sequence as set forth in SEQ ID NO:48. In some embodiments, the CDR1 of the DLL3 antibody of the DLL3-ADC is according to Contact numbering, comprising the amino acid sequence as set forth in SEQ ID NO:49; the CDR2 is according to Contact numbering, comprising the amino acid sequence as set forth in SEQ ID NO:50; and the CDR3 is according to Contact numbering, comprising the amino acid sequence as set forth in SEQ ID NO:51. In some embodiments, the CDR1 of the DLL3 antibody of the DLL3-ADC is according to AbM numbering, comprising the amino acid sequence as set forth in SEQ ID NO:7; the CDR2 is according to AbM numbering, comprising the amino acid sequence as set forth in SEQ ID NO:52; and the CDR3 is according to AbM numbering, comprising the amino acid sequence as set forth in SEQ ID NO:9. In some embodiments, a DLL3-ADC as disclosed herein comprises a DLL3 antibody which comprises a camelid VHH. In some embodiments, a DLL3-ADC as disclosed herein comprises a DLL3 antibody which is humanized. In some embodiments, a DLL3-ADC as disclosed herein comprises a DLL3 antibody which comprises an acceptor human framework, *e.g.*, a human immunoglobulin framework or a human consensus framework.

[00103] In some embodiments, the DLL3-ADC comprises a DLL3 comprising a single domain antibody comprising one or more framework(s) derived from a VHH domain comprising the sequence as set forth in SEQ ID NO:14. In some embodiments, the DLL3-ADC comprises a DLL3 antibody comprising a single domain antibody comprising one or more framework(s) derived from a VHH domain comprising the sequence as set forth in SEQ ID NO:15. In some embodiments, the DLL3-ADC comprises a DLL3 antibody comprising a single domain antibody comprising one or more framework(s) derived from a VHH domain comprising the sequence as set forth in SEQ ID NO:16.

[00104] In some embodiments, the DLL3-ADC provided herein comprises a single domain antibody which is a humanized single domain antibody. Framework regions described herein are determined based upon the boundaries of the CDR numbering system. In other words, if the CDRs are determined by, *e.g.*, IMGT, Kabat, Chothia, Contact, or AbM, then the framework regions are the amino acid residues surrounding the CDRs in the variable region

in the format, from the N-terminus to C-terminus: FRW1-CDR1-FRW2-CDR2- FRW3-CDR3-FRW4. For example, FRW1 is defined as the amino acid residues N-terminal to the CDR1 amino acid residues as defined by, *e.g.*, the IMGT numbering system, the Kabat numbering system, the Chothia numbering system, the Contact numbering system, or the AbM numbering system; FRW2 is defined as the amino acid residues between CDR1 and CDR2 amino acid residues as defined by, *e.g.*, the IMGT numbering system, the Kabat numbering system, the Chothia numbering system, the Contact numbering system, or the AbM numbering system; FRW3 is defined as the amino acid residues between CDR2 and CDR3 amino acid residues as defined by, *e.g.*, the IMGT numbering system, the Kabat numbering system, the Chothia numbering system, the Contact numbering system, or the AbM numbering system; and FRW4 is defined as the amino acid residues C-terminal to the CDR3 amino acid residues as defined by, *e.g.*, the IMGT numbering system, the Kabat numbering system, the Chothia numbering system, the Contact numbering system, or the AbM numbering system.

[00105] In some embodiments, the present disclosure provides a DLL3-ADC comprising an isolated DLL3 antibody comprising a VHH domain having the amino acid sequence as set forth in SEQ ID NO:14. In some embodiments, the present disclosure provides a DLL3-ADC comprising a polypeptide comprising the amino acid sequence as set forth in SEQ ID NO:14. In some embodiments, the present disclosure provides a DLL3-ADC comprising an isolated DLL3 antibody comprising a VHH domain having the amino acid sequence as set forth in SEQ ID NO:15. In some embodiments, the present disclosure provides a DLL3-ADC comprising a polypeptide comprising the amino acid sequence as set forth in SEQ ID NO:15. In some embodiments, the present disclosure provides a DLL3-ADC comprising an isolated DLL3 antibody comprising a VHH domain having the amino acid sequence as set forth in SEQ ID NO:16. In some embodiments, the present disclosure provides a DLL3-ADC comprising a polypeptide comprising the amino acid sequence as set forth in SEQ ID NO:16.

DLL3 Antibodies Comprising VHH Sequences

[00106] In some embodiments, a DLL3-ADC as disclosed herein comprises a DLL3 antibody comprising a heavy chain variable domain (VH) (such as a VHH), wherein the VH comprises or consists of:

- (a) an amino acid sequence as set forth in any one of SEQ ID NOs: 14-16;
- (b) an amino acid sequence which is at least 85%, at least 90%, or at least 95% identical to any one of SEQ ID NOs: 14-16; or

(c) an amino acid sequence with addition, deletion and/or substitution of one or more (for example, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10) amino acids compared with any one of SEQ ID NOs: 14-16.

[00107] The percent identity between two amino acid sequences can be determined using the algorithm of E. Meyers and W. Miller (Comput. Appl. Biosci., 4:11-17 (1988)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. In addition, the percent identity between two amino acid sequences can be determined by the algorithm of Needleman and Wunsch (J. Mol. Biol. 48:444-453 (1970)) which has been incorporated into the GAP program in the GCG software package (available at www.gcg.com), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6.

[00108] Additionally, or alternatively, protein (*e.g.*, antibody) sequences of the present disclosure can further be used as a “query sequence” to perform a search against public databases to, for example, identify related sequences. Such searches can be performed using the XBLAST program (version 2.0) of Altschul, et al. (1990) J. Mol. Biol. 215:403-10. BLAST protein searches can be performed with the XBLAST program, score = 50, word length = 3 to obtain amino acid sequences homologous to the antibody molecules of the disclosure. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al, (1997) Nucleic Acids Res. 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used. See www.ncbi.nlm.nih.gov.

[00109] In some embodiments, the amino acid sequence of a VH (such as a VHH) can be at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to any one of SEQ ID NOs: 14-16.

[00110] In some further embodiments, DLL3 antibodies may contain conservative substitution or modification of amino acids in the variable regions of the heavy chain and/or light chain, such as VH or VHH. It is understood in the art that certain conservative sequence modification can be made which do not remove antigen binding. See, *e.g.*, Brummell et al. (1993) Biochem 32:1180-8; de Wildt et al. (1997) Prot. Eng. 10:835-41; Komissarov et al. (1997) J. Biol. Chem. 272:26864- 26870; Hall et al. (1992) J. Immunol. 149:1605-12; Kelley and O’Connell (1993) Biochem. 32:6862-35; Adib-Conquy et al. (1998) Int. Immunol. 10:341-6 and Beers et al. (2000) Clin. Can. Res. 6:2835-43.

[00111] As described above, the term “conservative substitution,” as used herein, refers to an amino acid substitution which would not disadvantageously affect or change the essential properties of a protein/polypeptide comprising the amino acid sequence. For example, a conservative substitution may be introduced by standard techniques known in the art such as site-directed mutagenesis and PCR-mediated mutagenesis. Conservative amino acid substitutions include substitutions wherein an amino acid residue is substituted with another amino acid residue having a similar side chain, for example, a residue physically or functionally similar (such as, having similar size, shape, charge, chemical property including the capability of forming covalent bond or hydrogen bond, etc.) to the corresponding amino acid residue. The families of amino acid residues that have similar side chains have been defined in the art. These families include amino acids having alkaline side chains (for example, lysine, arginine and histidine), amino acids having acidic side chains (for example, aspartic acid and glutamic acid), amino acids having uncharged polar side chains (for example, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), amino acids having nonpolar side chains (for example, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), amino acids having β -branched side chains (such as threonine, valine, isoleucine) and amino acids having aromatic side chains (for example, tyrosine, phenylalanine, tryptophan, histidine). Therefore, a corresponding amino acid residue is preferably substituted with another amino acid residue from the same side-chain family. Methods for identifying amino acid conservative substitutions are well known in the art (see, for example, Brummell et al., *Biochem.* 32: 1180-1187 (1993); Kobayashi et al., *Protein Eng.* 12(10): 879-884 (1999); and Burks et al., *Proc. Natl. Acad. Sci. USA* 94: 412-417 (1997), which are incorporated herein by reference).

[00112] In some embodiments, a DLL3-ADC comprises a DLL3 antibody comprising at least one VHH, and the VHH comprises an amino acid sequence as set forth in any one of SEQ ID NOs: 14-16. In some embodiments, a DLL3-ADC comprises a DLL3 antibody comprising a VHH which has the amino acid sequence as set forth in any one of SEQ ID NOs: 14-16.

[00113] In some embodiments, a DLL3-ADC comprises a DLL3 antibody comprising a VHH fused to an Fc region of human IgG1 or IgG4 (referred to herein as a VHH-Fc fusion), wherein the VHH comprises an amino acid sequence as set forth in any one of SEQ ID NOs: 14-16. In some embodiments, a DLL3-ADC comprises a DLL3 antibody comprising a VHH and an Fc region of human IgG1. In some further embodiments, a DLL3-ADC comprises a DLL3 antibody which is a humanized antibody comprising a VHH and an Fc region of

human IgG1. In some embodiments, an antibody or antigen-binding portion thereof comprises a VHH domain comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 14-16, and a Fc region comprising an amino acid sequence as set forth in SEQ ID NO:17. In some embodiments, a DLL3-ADC comprises a DLL3 antibody comprising a polypeptide having an amino acid sequence as set forth in any one of SEQ ID NO:4, 5, or 10. In further embodiments, a DLL3-ADC comprises a DLL3 antibody comprising a homodimer of a polypeptide having an amino acid sequence as set forth in any one of SEQ ID NO:4, 5, or 10.

[00114] In some embodiments, the addition, deletion and/or substitution of at least one of the amino acids in the VHH region is not in any of the CDR sequences, but in the framework (FRW) sequences. For example, an antibody or antigen-binding portion thereof as described above may comprise one or more substitutions of the amino acids in the framework sequences, *e.g.*, FRW1, FRW2, FRW3, and/or FRW4 of the VHH region.

[00115] In some embodiments, an antibody or antigen-binding portion thereof as provided herein comprises any suitable framework region (FRW) sequences, as long as the antigen-binding domains can specifically bind to DLL3.

[00116] As described above, an antibody or antigen-binding portion thereof may contain modification of one or more amino acids in the variable regions and/or constant regions, including wherein the modification is a conservative substitution. It is understood in the art that certain conservative sequence modifications can be made which do not remove antigen binding. See, *e.g.*, Brummell et al. (1993) *Biochem* 32:1180-8; de Wildt et al. (1997) *Prot. Eng.* 10:835-41; Komissarov et al. (1997) *J. Biol. Chem.* 272:26864- 26870; Hall et al. (1992) *J. Immunol.* 149:1605-12; Kelley and O' Connell (1993) *Biochem.* 32:6862-35; Adib-Conquy et al. (1998) *Int. Immunol.* 10:341-6 and Beers et al. (2000) *Clin. Can. Res.* 6:2835-43.

[00117] An antigen-binding domain of a DLL3 antibody of a DLL3-ADC is not limited to the VHH form and may adopt a variety of other formats, such as but not limited to, a Fab, a Fab', a F(ab')₂, an Fv fragment, a single-chain antibody molecule (scFv). In some embodiments, an antigen-binding domain is a Fv fragment with a VH region and a VL region in separate chains held together by tight, non-covalent interactions.

Fc Region Comprising IgG Constant Domains

[00118] A DLL3-ADC as disclosed herein comprises a DLL3 antibody that further comprises an Fc region comprising one or more human IgG constant domains. A human IgG constant domain may be a human IgG1, IgG2, IgG3 or IgG4 constant domain, preferably a

human IgG1 constant domain. An example of the amino acid sequence of an Fc region comprising human IgG1 constant regions is set forth in SEQ ID NO:17. In some embodiments, the DLL3-ADC comprises a DLL3 antibody comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 4, 5, and 10. In some embodiments, the Fc region is a human IgG1 Fc region, such as a wild-type Fc region or a Fc variant comprising one or more amino acid modifications (Leu234Ala/Leu235Ala or LALA) that alters the antibody-dependent cellular cytotoxicity (ADCC) or other effector functions.

[00119] In some embodiments, the Fc modification comprises a LALA mutation, *e.g.*, mutations of L234A and L235A, according to EU numbering as in Kabat et al. The Kabat numbering system is generally used when referring to a residue in the variable domain (approximately residues 1-107 of the light chain and residues 1-113 of the heavy chain) (*e.g.*, Kabat et al., Sequences of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)). The “EU numbering system” or “EU index” is generally used when referring to a residue in an immunoglobulin heavy chain constant region (*e.g.*, the EU index reported in Kabat et al., *supra*). *See*, www.imgt.org/IMGTScientificChart/Numbering/Hu_IGHGnber.html. The “EU numbering as in Kabat” or “EU index as in Kabat” refers to the residue numbering of the human IgG1 EU antibody. Unless stated otherwise herein, references to residue numbers in the constant domain of antibodies means residue numbering by the EU numbering system.

[00120] Modifications of antibodies are also included within the scope of the present disclosure. Covalent modifications include reacting targeted amino acid residues of an antibody with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C- terminal residues of the antibody, such as those disclosed herein. Other modifications include deamidation of glutamyl and asparaginy residues to the corresponding glutamyl and aspartyl residues, respectively, hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the α -amino groups of lysine, arginine, and histidine side chains (*see, e.g.*, Creighton, Proteins: Structure and Molecular Properties 79-86 (1983)), glycosylation (such as N-linked glycosylation), isomerization of Asp, oxidation, acetylation of the N-terminal amine, and amidation of any C-terminal carboxyl group.

Nucleic Acid Molecules Encoding DLL3 Antibodies

[00121] A nucleic acid molecule encoding a DLL3 antibody as disclosed herein can be readily selected by one of skill in the art. In one aspect, the DLL3 antibody of the DLL3-ADC provided herein may be generated using a nucleic acid molecule comprises a nucleic

acid sequence encoding the amino acid sequence as set forth in SEQ ID NO:14. In one aspect, the DLL3 antibody of the DLL3-ADC provided herein may be generated using a nucleic acid molecule comprises a nucleic acid sequence encoding the amino acid sequence as set forth in SEQ ID NO:15. In one aspect, the DLL3 antibody of the DLL3-ADC provided herein may be generated using a nucleic acid molecule comprises a nucleic acid sequence encoding the amino acid sequence as set forth in SEQ ID NO:16.

[00122] In some embodiments, the percent identity is derived from the degeneracy of the genetic code, and the encoded protein sequences remain unchanged.

Host Cells

[00123] Host cells as disclosed in the present disclosure may be any cell which is suitable for expressing the antibodies of the present disclosure, for example, yeast, bacterial, plant, and mammalian cells. Mammalian host cells for expressing the antibodies of the present disclosure include Chinese Hamster Ovary (CHO cells) (including dhfr CHO cells, described in Urlaub and Chasin, (1980) Proc. Natl. Acad. Sci. USA 77:4216-4220, used with a DHFR selectable marker, *e.g.*, as described in R. J. Kaufman and P. A. Sharp (1982) J. Mol. Biol. 159:601-621), 293F cells, NSO myeloma cells, COS cells and SP2 cells. In particular, for use with NSO myeloma cells, another expression system is the GS gene expression system disclosed in WO 87/04462, WO 89/01036, and EP 338,841. Also included are monkey kidney CV1 line transformed by SV4010 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham et al., J. Gen. Virol. 36:59 (1977)); baby hamster kidney cells (BHK, ATCC CCL); Chinese hamster ovary cells/-DHFR (CHO, Urlaub et al., 1980, Proc. Natl. Acad. Sci. USA 77:4216); mouse Sertoli cells (TM4, Mather, 1980, Biol. Reprod. 23:243-251); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather et al., 1982, Annals N.Y. Acad. Sci. 383:44-68); MRC-5 cells; FS4 cells; mouse myeloma cells, such as NSO (*e.g.*, RCB0213, 1992, Bio/Technology 10:169) and SP2/0 cells (*e.g.*, SP2/0-Ag14 cells, ATCC CRL 1581); rat myeloma cells, such as YB2/0 cells (*e.g.*, YB2/3HL.P2.G11.16Ag.20 cells, ATCC CRL 1662); PER.C6 cells; and a human hepatoma line (Hep G2). CHO cells are one of the cell lines that can be used herein, with CHO-K1, DUK-B11, CHO-DP12, CHO-DG44 (Somatic Cell and Molecular Genetics 12:555 (1986)), and Lec13 being exemplary host cell lines. In the case of CHO-K1, DUK-

B11, DG44 or CHO-DP12 host cells, these may be altered such that they are deficient in their ability to fucosylate proteins expressed therein. In some embodiments, the host cells herein are selected from CHO, CHO-S, HEK, HEK293, HEK-293F, Expi293F, PER.C6 or NSO cells or lymphocytic cells.

[00124] Suitable prokaryotes for this purpose include eubacteria, such as Gram-negative or Gram-positive organisms, for example, Enterobacteriaceae such as *Escherichia*, e.g., *E. coli*, Enterobacter, Erwinia, Klebsiella, Proteus, Salmonella, e.g., Salmonella typhimurium, Serratia, e.g., *Serratia marcescens*, and Shigella, as well as Bacilli such as *B. subtilis* and *B. licheniformis*, Pseudomonas such as *P. aeruginosa*, and Streptomyces.

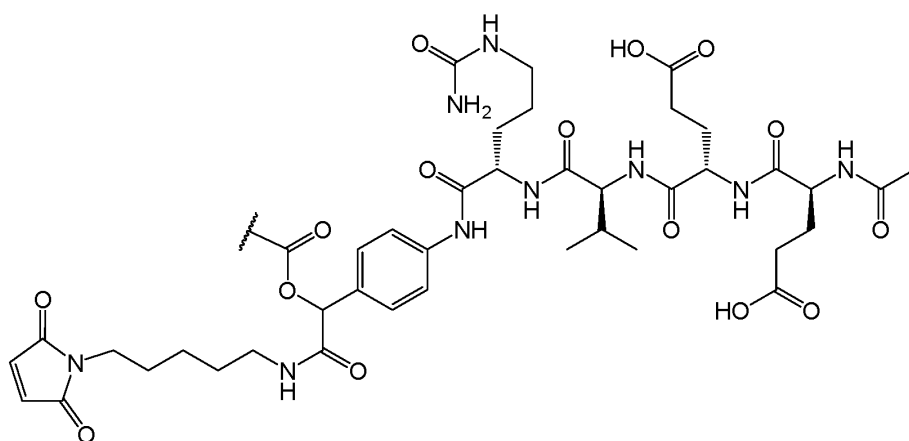
[00125] In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are also suitable cloning or expression hosts for antibody-encoding vectors. *Saccharomyces cerevisiae*, or common baker's yeast, is the most commonly used among lower eukaryotic host microorganisms. However, a number of other genera, species, and strains are commonly available and useful herein, such as *Schizosaccharomyces pombe*; Kluyveromyces hosts such as, e.g., *K. lactis*, *K. fragilis* (ATCC 12,424), *K. bulgaricus* (ATCC 16,045), *K. wickerhamii* (ATCC 24,178), *K. waltii* (ATCC 56,500), *K. drosophilae* (ATCC 36,906), *K. thermotolerans*, and *K. marxianus*; *Yarrowia* (EP 5 402,226); *Pichia pastoris* (EP 183,070); *Candida*, *Trichoderma reesei* (EP 244,234); *Neurospora crassa*; Schwanniomycetes such as *Schwanniomycetes occidentalis*; and filamentous fungi such as, e.g., Neurospora, Penicillium, Tolypocladium, and Aspergillus hosts such as *A. nidulans* and *A. niger*.

[00126] When recombinant expression vectors encoding an antibody are introduced into mammalian host cells, the antibody is produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or, secretion of the antibody into the culture medium in which the host cells are grown. Antibodies can be recovered from the culture medium using standard protein purification methods.

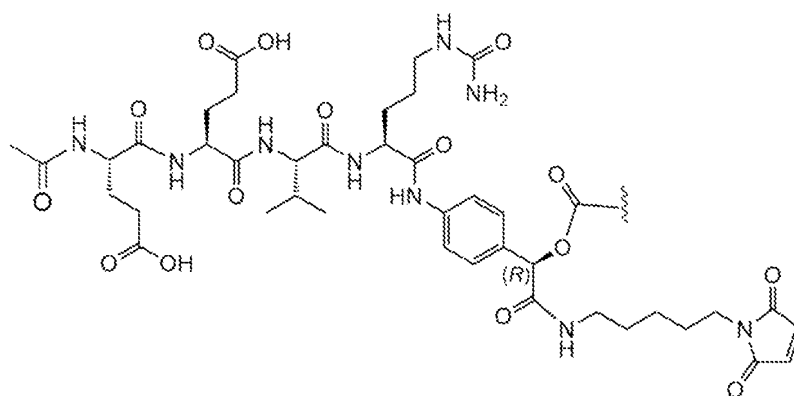
LINKER-DRUG CONJUGATES

[00127] The DLL3-ADCs comprises a DLL3 antibody conjugated to a drug via a linker. The linker and drug may be collectively referred to as a "linker-drug" herein.

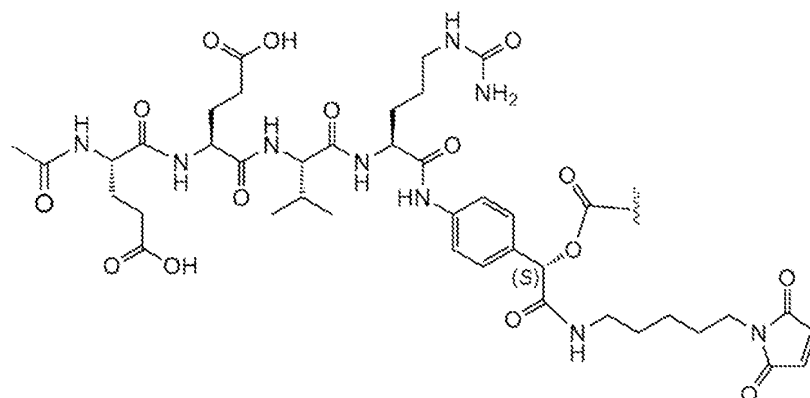
[00128] In one embodiment, the linker structure is:



or a salt thereof, wherein the wavy line represents the point of attachment to the drug. In further embodiments, the linker structure is an (*R*)-epimer of the linker structure above or a salt thereof. Accordingly, the linker structure is

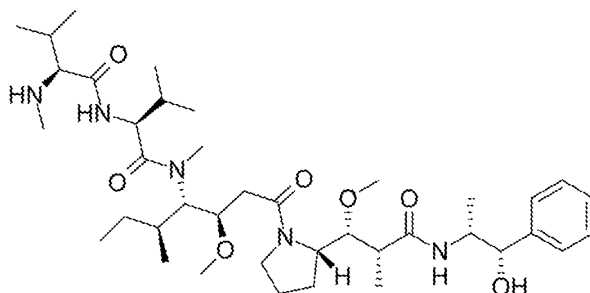


or a salt thereof. In other embodiments, the linker structure is an (*S*)-epimer of the linker structure above or a salt thereof. Accordingly, the linker structure is



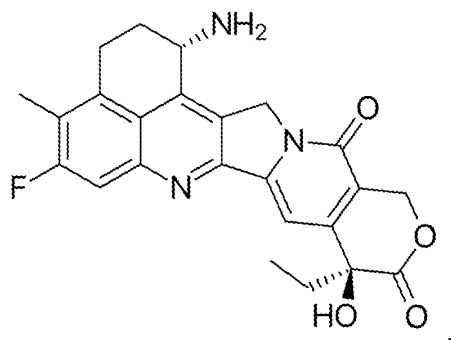
or a salt thereof.

[00129] In one embodiment, the drug is an auristatin, for example, MMAE. The structure of MMAE is provided below:



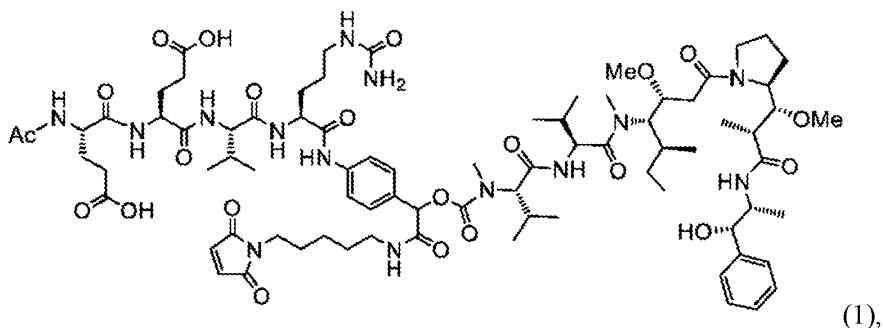
MMAE is also known as (2S)-N-[(2S)-1-[(3R,4S,5S)-1-[(2S)-2-[(1R,2R)-3-[(1S,2R)-1-hydroxy-1-phenylpropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-methylamino]-3-methyl-1-oxobutan-2-yl]-3-methyl-2-(methylamino)butanamide.

[00130] In one embodiment, the drug is a topoisomerase inhibitor, for example, camptothecin or a derivative thereof, such as Exatecan. The structure of Exatecan is provided below:

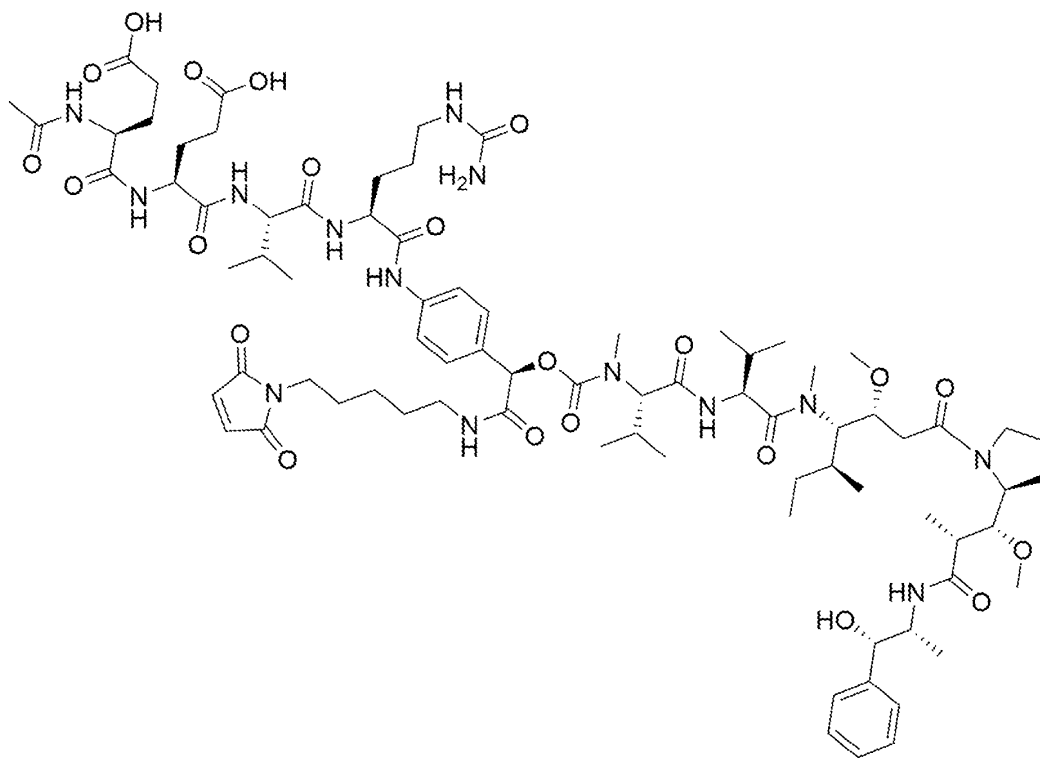


which is also known as (1S,9S)-1-amino-9-ethyl-5-fluoro-9-hydroxy-4-methyl-1,2,3,9,12,15-hexahydro-10H,13H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinoline-10,13-dione.

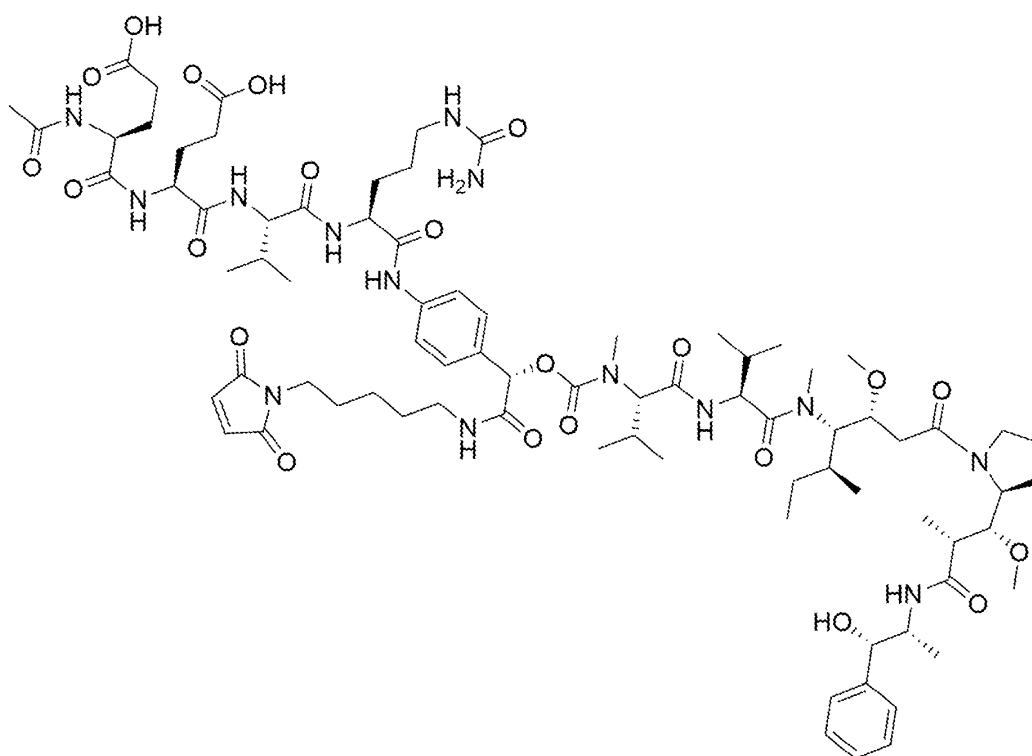
[00131] In one embodiment, the linker-drug is Linker-Drug (1):



or a salt thereof, wherein “Ac” is an acetyl group. Linker-Drug (1) (or a salt thereof) comprises a linker conjugated to monomethyl auristatin E (MMAE). In further embodiments, the linker-drug is an (*R*)-epimer of Linker-Drug (1) or a salt thereof. Accordingly, the linker-drug is

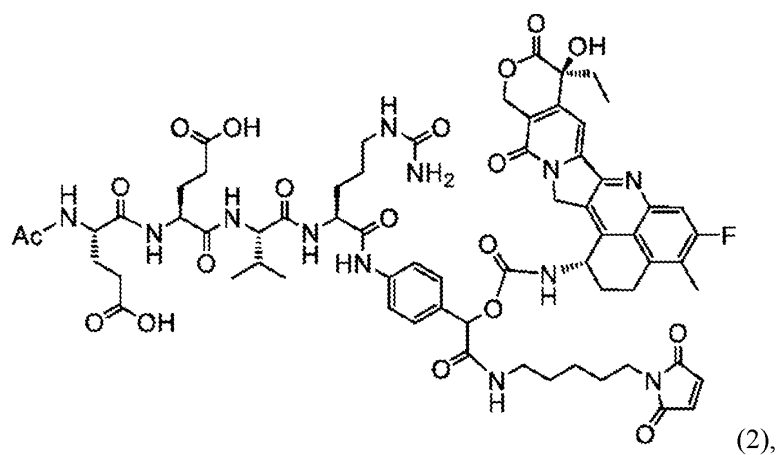


(which is also referred to herein as Linker-Drug (1) Form A or Linker-Drug (1-A)) or a salt thereof. In other embodiments, the linker-drug is an (*S*)-epimer of Linker-Drug (1) or a salt thereof. Accordingly, the linker-drug is

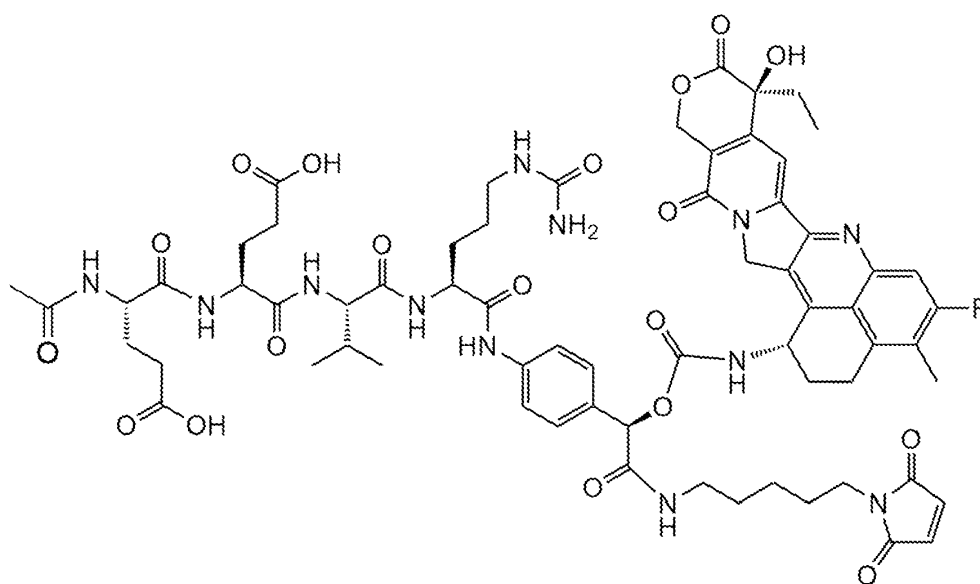


(which is also referred to herein as Linker-Drug (1) Form B or Linker-Drug (1-B)) or a salt thereof.

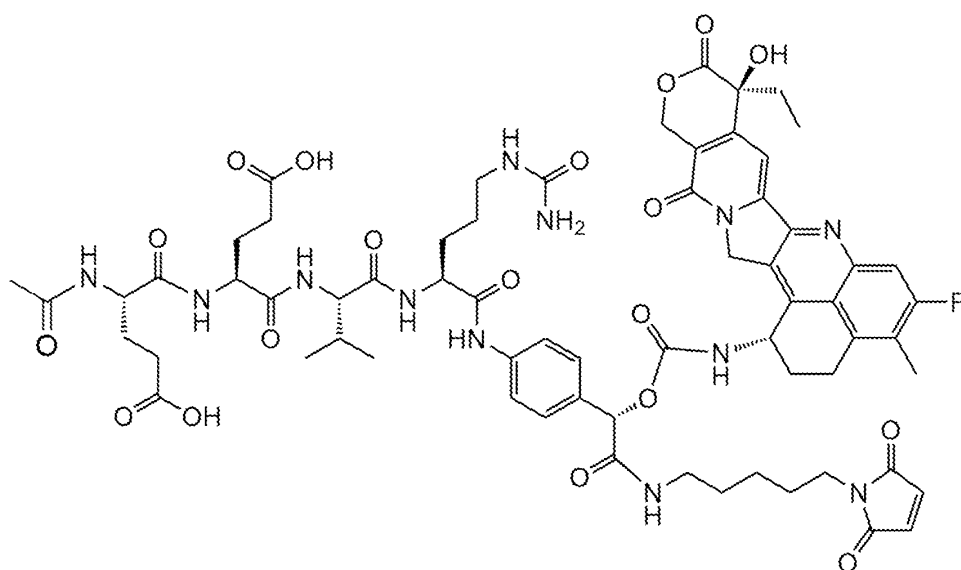
[00132] In one embodiment, the linker-drug is Linker-Drug (2):



or a salt thereof, wherein “Ac” is an acetyl group. Linker-Drug (2) (or a salt thereof) comprises a linker conjugated to Exatecan. In further embodiments, the linker-drug is an (*R*)-epimer of Linker-Drug (2) or a salt thereof. Accordingly, the linker-drug is



(which is also referred to herein as Linker-Drug (2) Form A or Linker-Drug (2-A)) or a salt thereof. In other embodiments, the linker-drug is an (*S*)-epimer of Linker-Drug (2) or a salt thereof. Accordingly, the linker-drug is



(which is also referred to herein as Linker-Drug (2) Form B or Linker-Drug (2-B)) or a salt thereof.

[00133] Other suitable linker-drug can be found in the international PCT patent application nos. PCT/JP2021/162299, PCT/JP2022/036852 (international patent application publication no. WO2023054714 published April 6, 2023) and PCT/JP2022/036835 (international patent

application publication no. WO2023054706 published April 6, 2023), each of which is incorporated herein by reference in its entirety.

[00134] As used herein, the term “salt” includes, for example, salts of inorganic acids, salts of organic acids, salts of inorganic bases, salts of organic bases, and salts of amino acids. Examples of salts of inorganic acids include salts of hydrogen chloride, hydrogen bromide, phosphoric acid, sulfuric acid, and nitric acid. Examples of salts of organic acids include salts of with formic acid, acetic acid, trifluoroacetic acid, lactic acid, tartaric acid, fumaric acid, oxalic acid, maleic acid, citric acid, succinic acid, malic acid, benzenesulfonic acid, and p-toluenesulfonic acid. Examples of salts of inorganic bases include salts of alkali metals (*e.g.*, sodium, potassium), alkaline earth metals (*e.g.*, calcium, magnesium), other metals such as zinc and aluminum, and ammonium. Examples of salts of organic bases include salts of trimethylamine, triethylamine, propylenediamine, ethylenediamine, pyridine, ethanolamine, monoalkyl ethanolamines, dialkyl ethanolamines, diethanolamine, and triethanolamine. Examples of salts of amino acids include salts of basic amino acids (*e.g.*, arginine, histidine, lysine, ornithine) and acidic amino acids (*e.g.*, aspartic acid, glutamic acid). The salt is preferably a salt of an inorganic acid (*e.g.*, hydrogen chloride) or a salt of an organic acid (*e.g.*, trifluoroacetic acid).

Antibody-Linker-Drug Conjugation

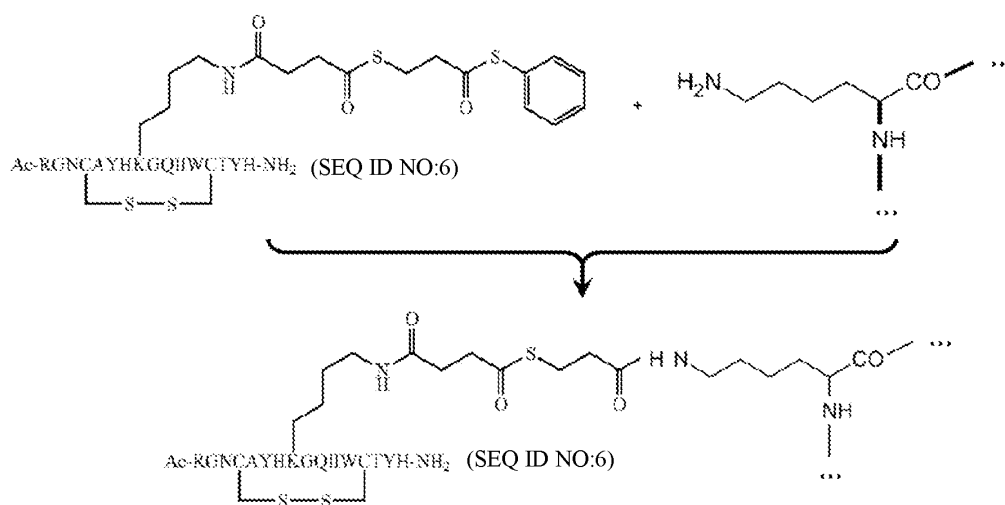
[00135] In some embodiments, the antibody can be modified before conjugation to the linker-drug. Modification of the antibody can produce a modified antibody that contains one or more reactive groups suitable for conjugation to the linker-drug.

[00136] In some embodiments, the antibody can be modified at one or more amino acid residues to provide one or more reactive groups suitable for conjugation to the linker-drug. For example, amino acid residues and side chains thereof in the antibody can be chemically modified, as described below, and conjugated to a linker-drug. In certain embodiments, the point of attachment of the linker-drug (*e.g.*, drug conjugate moiety) to the Ab is through a cysteine or a lysine residue of the DLL3 antibody, as described below.

[00137] Chemical Conjugation at Lysine Residue

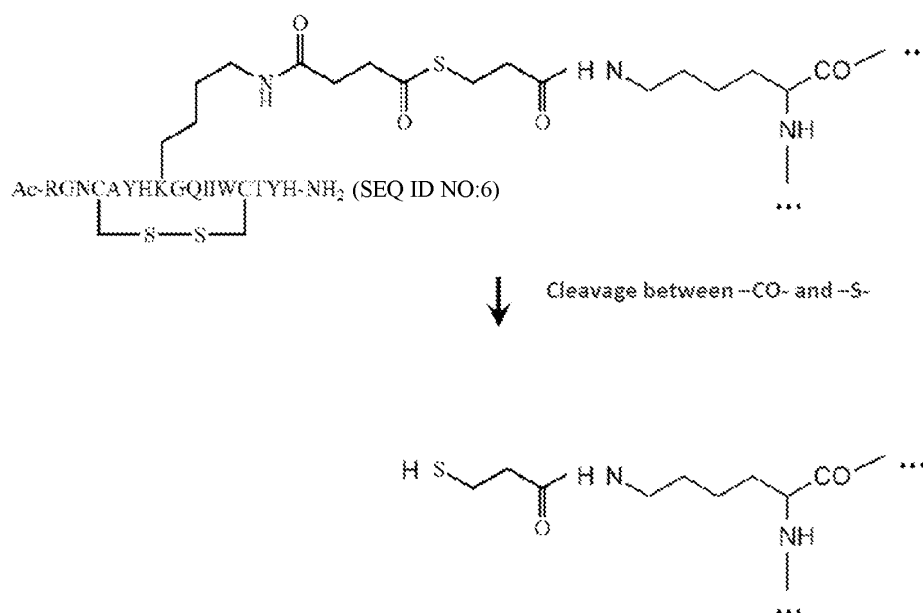
[00138] In some embodiments, the antibody can be conjugated to a linker-drug using chemical conjugation by affinity peptide (CCAP) methods disclosed in US Patent Application Publication No. 20210139549A1 (Ajinomoto Co. Inc.), which is incorporated herein by reference in its entirety. In particular, a lysine residue of a DLL3 antibody as disclosed herein may be chemically modified by contacting the DLL3 antibody with an affinity peptide conjugated to a thiophenol-activation moiety to react the thiophenol with the

lysine residue on the antibody to conjugate the affinity peptide to the lysine via a peptide bond as shown below in **SCHEMES 1 and 2**:



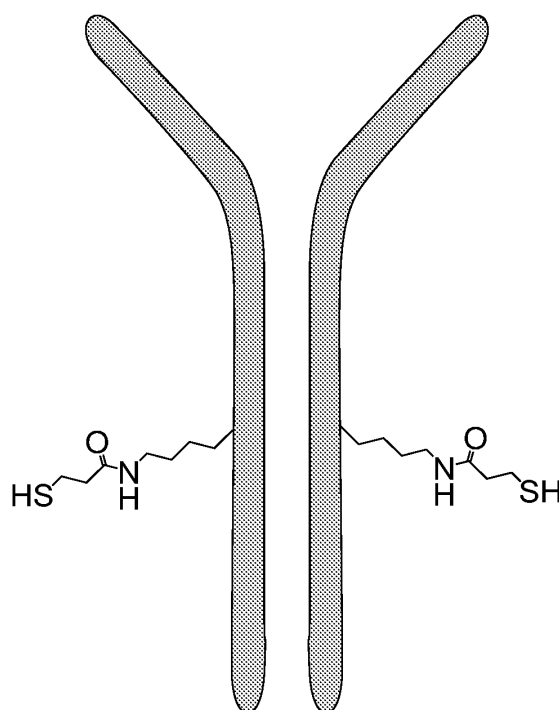
SCHEME 1

[00139] The bond between the carboxyl (C=O) and sulfur may then be cleaved to yield a modified lysine residue with a terminal sulfhydryl group as shown below in **SCHEME 2**:



SCHEME 2

[00140] The modification may occur at a lysine on each chain of the antibody (*e.g.*, K246 or K248 according to the EU numbering), thereby resulting in two conjugation sites as shown in **SCHEME 3** below:



SCHEME 3

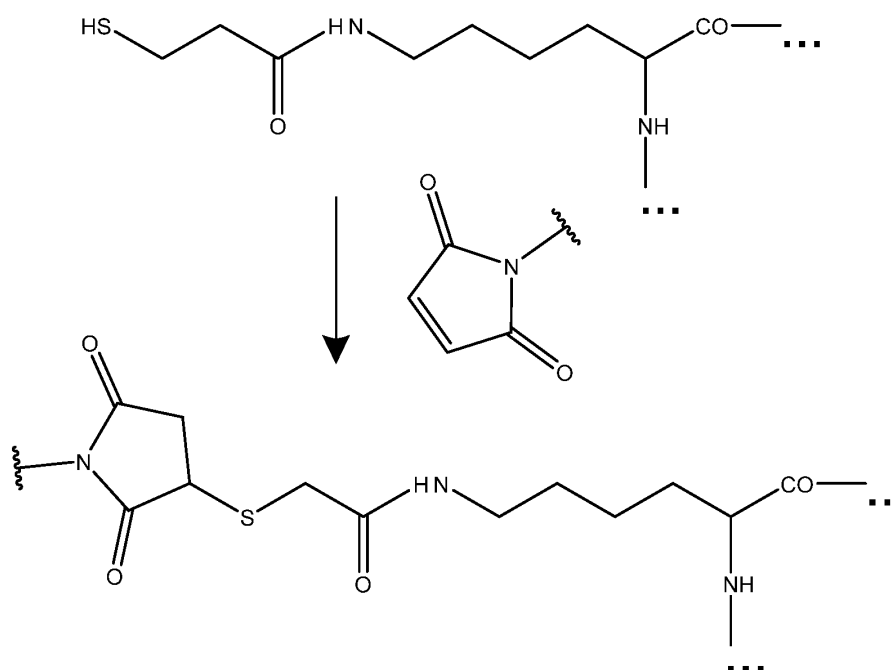
[00141] In some embodiments, the lysine residue being modified is any one or more of positions 246, 248, 288, 290, or 317 according to EU numbering (also referred to herein as K246, K248, K288, K290, or K317, respectively). In further embodiments, the lysine residue is K246 and/or K248 according to EU numbering. In yet further embodiments, the lysine residue is K248 accordingly to EU numbering.

[00142] As used herein, K246, K248, K288, K290, or K317 refer to amino acid residues (lysine, Lys, K) of an immunoglobulin identified according to the EU numbering. As it would be understood by one of skill in the art, such numberings accordingly represent amino acid residues of a polypeptide aligned to those identified in an immunoglobulin, such as the one shown in www.imgt.org/IMGTScientificChart/Numbering/Hu_IGHGnber.html. For example, in some embodiments, K246 refers to the 150th amino acid residue of SEQ ID NO:10 which is lysine, K248 refers to the 152nd amino acid residue of SEQ ID NO:10 which is also lysine, K288 refers to the 192nd amino acid residue of SEQ ID NO:10, K290 refers to the 194th amino acid residue of SEQ ID NO:10, and K317 refers to the 221st amino acid residue of SEQ ID NO:10. In another embodiment, K246 refers to the 148th amino acid residue of SEQ ID NO:4 or 5 which is lysine, K248 refers to the 150th amino acid residue of SEQ ID NO:4 or 5 which is also lysine, K288 refers to the 190th amino acid residue of SEQ ID NO:4 or 5,

K290 refers to the 192nd amino acid residue of SEQ ID NO:4 or 5, and K317 refers to the 219th amino acid residue of SEQ ID NO:4 or 5.

[00143] Accordingly, provided herein is a DLL3 antibody having a side chain lysyl ($-(CH_2)_4NH_2$) of one or more of its lysine residues modified to $-(CH_2)_4NHCO(CH_2)_2SH$, for example, as depicted in SCHEME 3. In some embodiments, the DLL3 antibody comprises a VHH comprising a CDR1, CDR2, and CDR3 as disclosed herein. In further embodiments, the DLL3 antibody comprises a VHH as disclosed herein. In yet further embodiments, the DLL3 antibody comprises a VHH-Fc fusion as disclosed herein, for example, comprising an amino acid sequence as set forth in any one of SEQ ID NO:4, 5, or 10. Additionally or alternatively, the modified lysine residue is selected from one or more of the following: K246, K248, K288, K290, or K317. In further embodiments, the modified lysine residue is K246 and/or K248. In yet further embodiments, the modified lysine residue is K248. These modified DLL3 antibodies of the present invention are useful, for example, as synthetic intermediates in the production of a DLL3 ADC as disclosed herein.

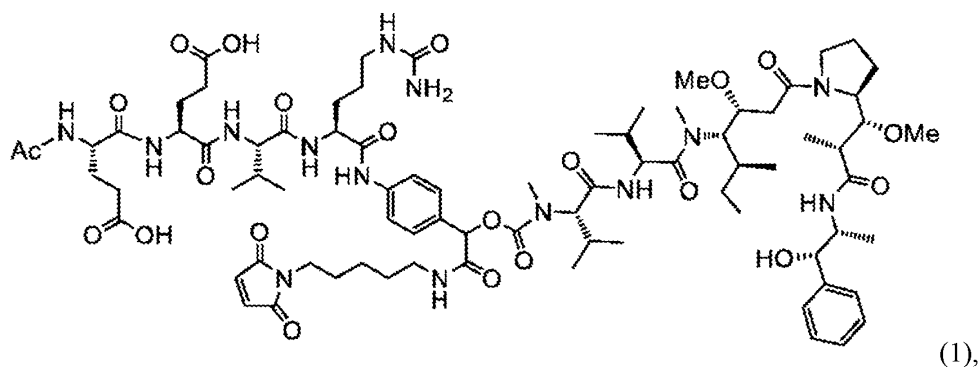
[00144] The terminal sulfhydryl group may be reacted with a maleimide moiety on a linker-drug, such as the maleimide moiety on Linker-Drug (1) (or a salt thereof), Linker-Drug (1-A) (or a salt thereof), Linker-Drug (1-B) (or a salt thereof), Linker-Drug (2) (or a salt thereof), Linker-Drug (2-A) (or a salt thereof), or Linker-Drug (2-B) (or a salt thereof) to conjugate the linker-drug thereto as follows according to **SCHEME 4**:



SCHEME 4

[00145] In one embodiment, the present disclosure provides a method of making a DLL3-ADC of Formula (I) or a salt thereof, as disclosed herein, comprising the steps of:

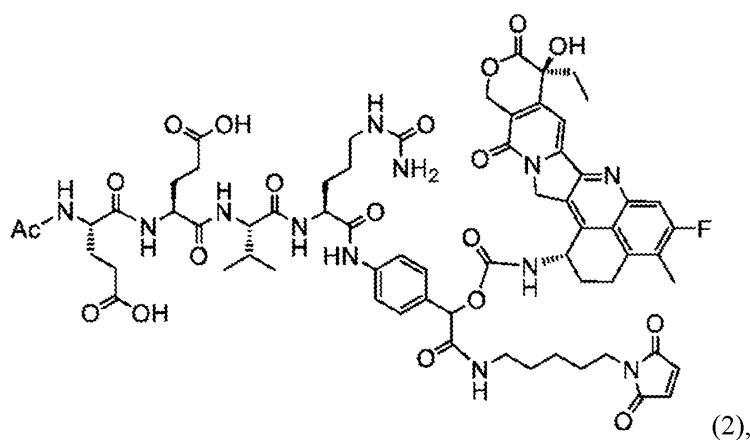
- a) providing a solution comprising the DLL3 antibody;
- b) contacting the solution of a) with an affinity peptide conjugated to a thiophenol activation moiety;
- c) contacting the solution of b) with a solution comprising Linker-Drug (1) or a salt thereof:



wherein the ADC is made. In some embodiments, the Linker-Drug (1) is Linker-Drug (1-A). In some embodiments, the Linker-Drug (1) is Linker-Drug (1-B). In some embodiments, the Linker-Drug (1) is a mixture of Linker-Drug (1-A) and Linker-Drug (1-B).

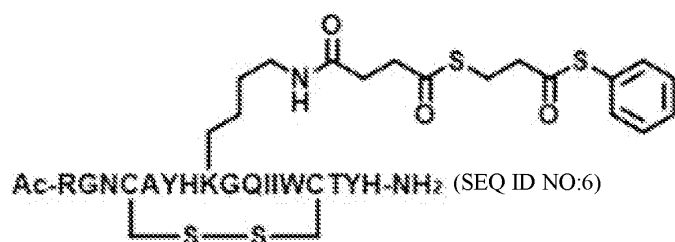
[00146] In another embodiment, the present disclosure provides a method of making a DLL3-ADC of Formula (II) or a salt thereof, as disclosed herein, comprising the steps of:

- a) providing a solution comprising the DLL3 antibody;
- b) contacting the solution of a) with an affinity peptide conjugated to a thiophenol activation moiety;
- c) contacting the solution of b) with a solution comprising Linker-Drug (2) or a salt thereof:



wherein the ADC is made. In some embodiments, the Linker-Drug (2) is Linker-Drug (2-A). In some embodiments, the Linker-Drug (2) is Linker-Drug (2-B). In some embodiments, the Linker-Drug (2) is a mixture of Linker-Drug (2-A) and Linker-Drug (2-B).

[00147] In one embodiment, the affinity peptide is a peptide of SEQ ID NO:6. In one embodiment, the affinity peptide conjugated to a thiophenol activation moiety is:



, wherein Ac is an acetyl moiety

and the lines linking the sulfurs of the cysteine residues represent a disulfide bridge. Other suitable affinity peptides and thiophenol activation moieties can be found, for example, in US Patent Application Publication No. 20210139549A1.

[00148] Stochastic Conjugation of Reduced Interchain Disulfide Bonds

[00149] In some embodiments, the DLL3 antibody is conjugated to a linker-drug via stochastic conjugation to reduced interchain disulfide bonds in the antibody. For example, an IgG1 antibody consists of four polypeptide chains, two heavy chains comprising VH, CH1 and Fc (*e.g.*, hinge, CH2 and CH3) domains, and two light chains comprising VL and CL domains, connected by interchain cysteine disulfide (-S-S-) bonds (*e.g.*, two heavy chain-light chain interchain disulfide bonds and two hinge heavy chain-heavy chain interchain disulfide bonds). In certain embodiments, when these disulfide bonds are broken under reducing conditions, eight (8) reactive cysteine sulfhydryl moieties are produced. In certain embodiments, each of the eight reactive cysteine sulfhydryl moieties is a point of attachment for a linker-drug, such that a maximum of eight ($n = 8$) linker-drugs may be attached to the reduced antibody. For a VHH-Fc fusion antibody, the antibody may comprise two chains comprising VHH domains fused to an Fc (*e.g.*, hinge, CH2 and CH3), where the two chains are linked through two hinge interchain disulfide bonds (*see, e.g.*, **Figure 1A**). In certain embodiments, when these disulfide bonds are broken under reducing conditions, four (4) reactive cysteine sulfhydryl moieties are produced. In certain embodiments, each of the four reactive cysteine sulfhydryl moieties is a point of attachment for a linker-drug, such that a maximum of four ($n = 4$) linker-drugs may be attached to the reduced antibody.

[00150] In some embodiments, the interchain disulfide bond is between two cysteine residues, and broken under reducing conditions, resulting in two reactive cysteine sulfhydryl

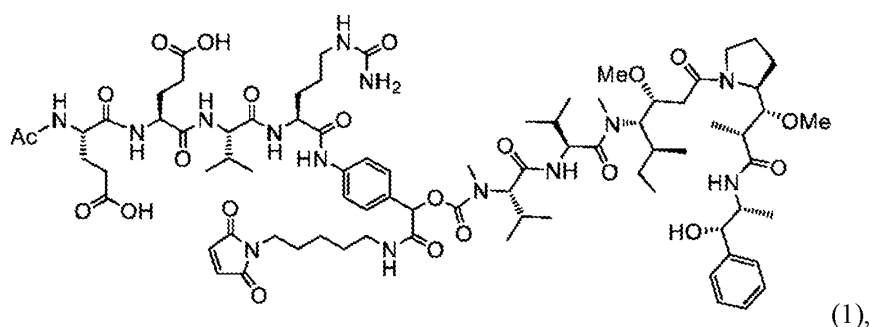
moieties. In further embodiments, the interchain disulfide bridge in a DLL3 antibody is between a heavy chain and a light chain. Additionally, or alternatively, the interchain disulfide bridge in a DLL3 antibody is between two heavy chains. In some embodiments, the interchain disulfide bridge in a DLL3 antibody is between two VHH chains. In some embodiments, the interchain disulfide bridge in a DLL3 antibody is between two VHH-Fc chains. In some embodiments, the cysteine residues are in the hinge region of the DLL3 antibody. In some embodiments, the cysteine residue is at any one or more of positions 226 or 229 according to EU numbering (also referred to herein as C226 or C229, respectively).

[00151] As used herein, C226 and C229 refer to amino acid residues (cysteine, Cys, C) of an immunoglobulin identified according to the EU numbering. As it would be understood by one of skill in the art, such numberings accordingly represent amino acid residues of a polypeptide aligned to those identified in an immunoglobulin, such as the one shown in www.imgt.org/IMGTScientificChart/Numbering/Hu_IGHGnber.html. For example, in some embodiments, C226 refers to the 130th amino acid residue of SEQ ID NO:10, which is cysteine, and C229 refers to the 133rd amino acid residue of SEQ ID NO:10 which is also cysteine. In another embodiment, C226 refers to the 128th amino acid residue of SEQ ID NO:4 or 5 which is cysteine, and C229 refers to the 131st amino acid residue of SEQ ID NO:4 or 5 which is also cysteine.

[00152] In certain embodiments, the DLL3 antibodies disclosed herein comprise two interchain disulfide bonds in the hinge region which may be reduced, thereby breaking the bond, and revealing a reactive sulfhydryl moiety that may be conjugated with a maleimide moiety on a linker-drug, such as the maleimide moiety on Linker-Drug (1) (or a salt thereof) or Linker-Drug (2) (or a salt thereof).

[00153] In one embodiment, the present disclosure provides a method of making a DLL3-ADC of Formula (I) or a salt thereof, comprising the steps of:

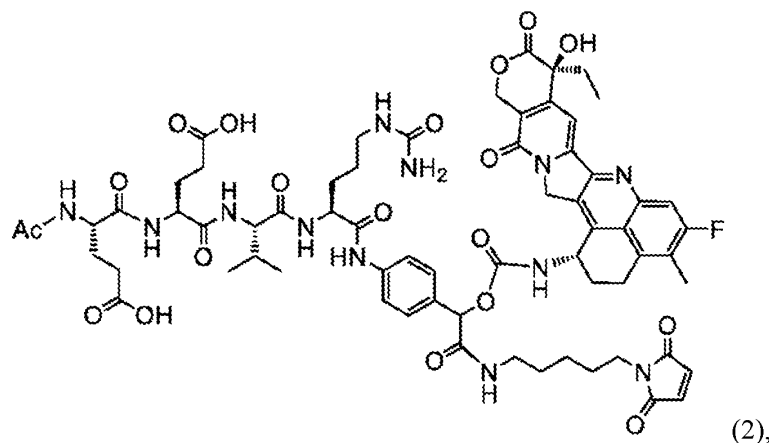
- a) providing a solution comprising the DLL3 antibody;
- b) contacting the solution of a) with a reducing agent;
- c) contacting the solution of b) with a solution comprising Linker-Drug (1) or a salt thereof:



wherein the ADC is made. In some embodiments, the Linker-Drug (1) is Linker-Drug (1-A). In some embodiments, the Linker-Drug (1) is Linker-Drug (1-B). In some embodiments, the Linker-Drug (1) is a mixture of Linker-Drug (1-A) and Linker-Drug (1-B).

[00154] In one embodiment, the present disclosure provides a method of making a DLL3-ADC of Formula (II) or a salt thereof, comprising the steps of:

- providing a solution comprising the DLL3 antibody;
- contacting the solution of a) with a reducing agent;
- contacting the solution of b) with a solution comprising Linker-Drug (2) or a salt thereof:



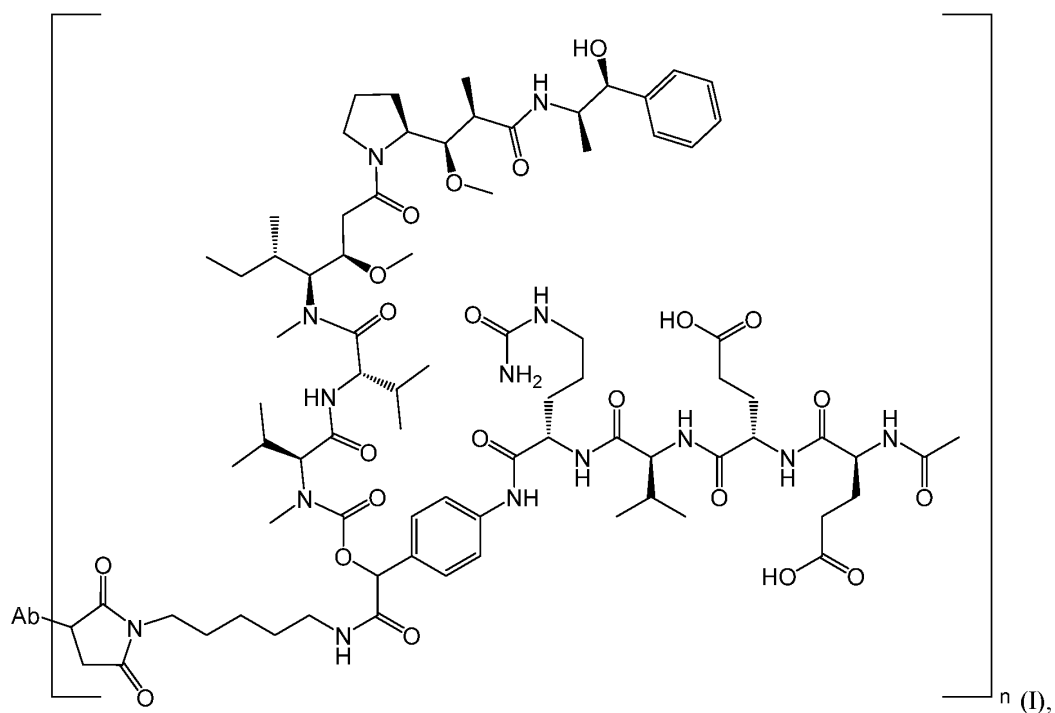
wherein the ADC is made. In some embodiments, the Linker-Drug (2) is Linker-Drug (2-A). In some embodiments, the Linker-Drug (2) is Linker-Drug (2-B). In some embodiments, the Linker-Drug (2) is a mixture of Linker-Drug (2-A) and Linker-Drug (2-B).

[00155] In one embodiment, the reducing agent is tris(2-carboxyethyl) phosphine (TCEP).

DLL3-ADCS

[00156] Any of the DLL3 antibodies described herein and any of the linker-drugs or salts thereof as described herein may be conjugated to generate a DLL3-ADC.

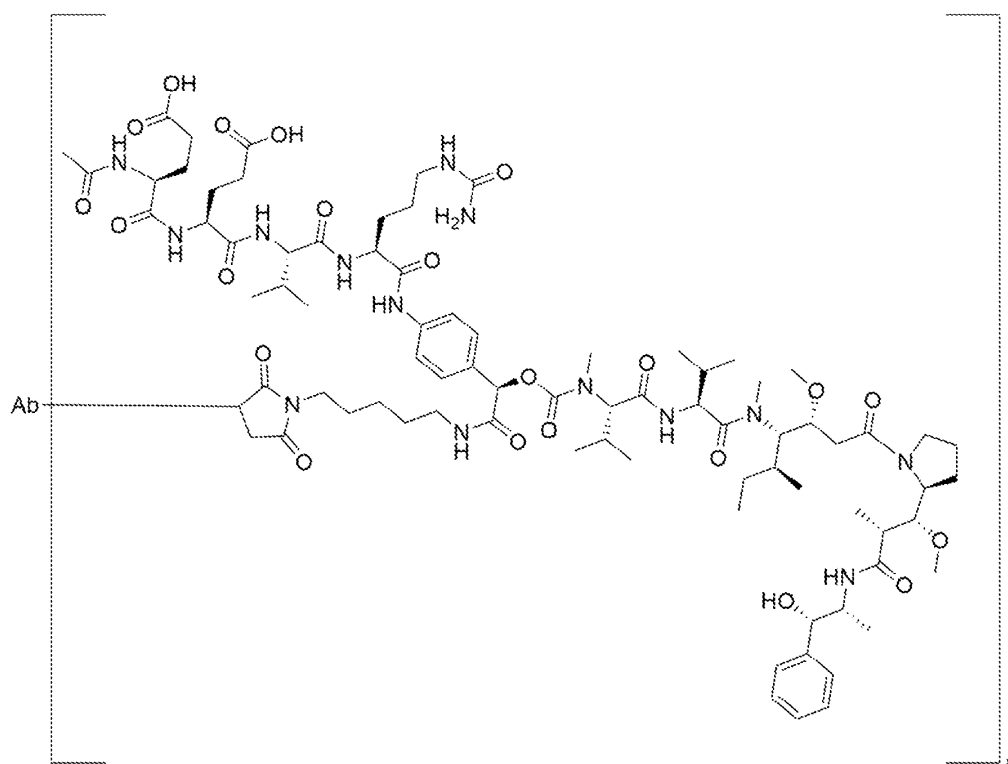
[00157] In one embodiment, the present disclosure provides an ADC of Formula (I):



or a salt thereof, wherein:

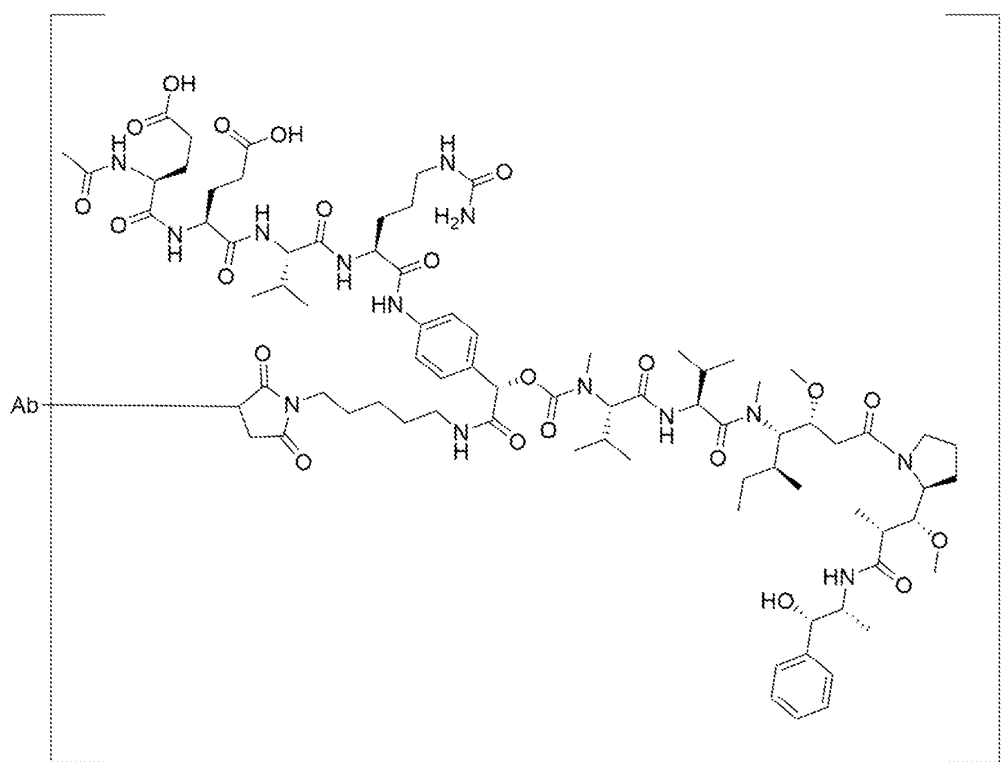
n is an integer from 1 to 8, and

Ab represents an antibody that binds to DLL3 (“DLL3 antibody”). In some embodiments, the ADC is an (*R*)-epimer of Formula (I) or a salt thereof. Accordingly, the ADC is of Formula (I-A):



(I-A)

or a salt thereof. In some embodiments, the ADC is an (*S*)-epimer of Formula (I) or a salt thereof. Accordingly, the ADC is of Formula (I-B):



(I-B)

or a salt thereof.

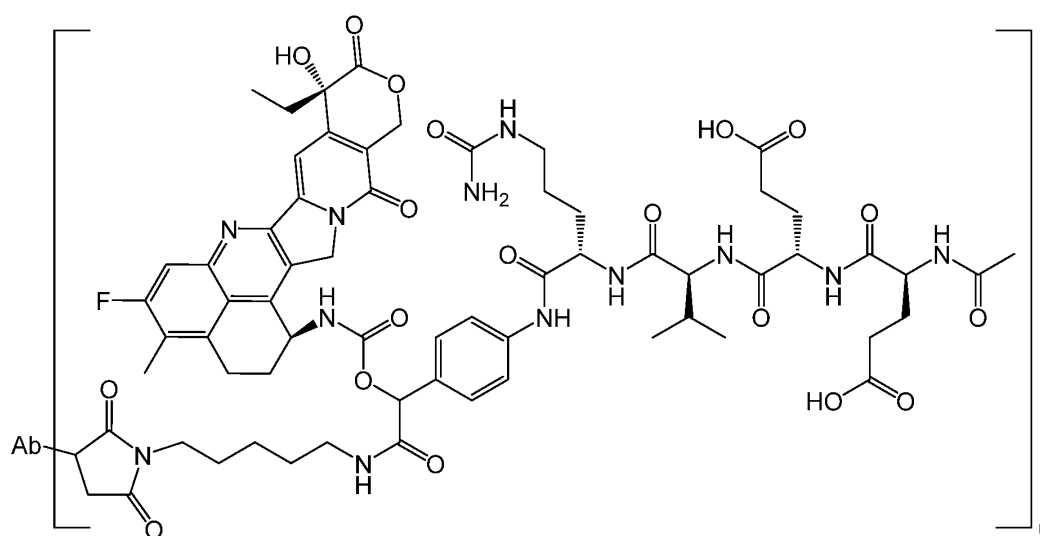
[00158] In one embodiment, the DLL3-ADC of Formula (I), (I-A), (I-B), or salt of any thereof comprises a DLL3 antibody comprising: (1) a CDR1, CDR2, and CDR3 of a VHH, wherein the VHH comprises an amino acid sequence as set forth in SEQ ID NO:14, 15 or 16; or (2) at least one VHH, wherein the at least one VHH comprises CDR1, CDR2 and CDR3, and wherein: (i) the CDR1 comprises an amino acid sequence as set forth in SEQ ID NO:1 or 7; (ii) the CDR2 comprises an amino acid sequence as set forth in SEQ ID NO:2 or 8; and (iii) the CDR3 comprises an amino acid sequence as set forth in SEQ ID NO:3 or 9.

[00159] In one embodiment, the DLL3-ADC of Formula (I), (I-A), (I-B), or salt of any thereof comprises a DLL3 antibody comprising: (a) a CDR1 as set forth in SEQ ID NO:1; a CDR2 as set forth in SEQ ID NO:2; and a CDR3 as set forth in SEQ ID NO:3; or (b) a CDR1 as set forth in SEQ ID NO:7; a CDR2 as set forth in SEQ ID NO:8; and a CDR3 as set forth in SEQ ID NO:9. In one embodiment, the DLL3-ADC of Formula (I), (I-A), (I-B), or a salt of any thereof comprises a DLL3 antibody comprising a VHH comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 14-16. In one embodiment, the DLL3-ADC of Formula (I), (I-A), (I-B), or salt of any thereof comprises a DLL3 antibody further comprising a human IgG constant domain. In one embodiment, the human IgG is a human

IgG1. In one embodiment, the DLL3-ADC of Formula (I), (I-A), (I-B), or salt of any thereof comprises a DLL3 antibody further comprising an Fc fragment. In one embodiment, the Fc fragment comprises a homodimer of an amino acid sequence as set forth in SEQ ID NO:17. In one embodiment, the DLL3-ADC of Formula (I), (I-A), (I-B), or salt of any thereof comprises a DLL3 antibody comprising at least one VHH domain. In one embodiment, the DLL3-ADC of Formula (I), (I-A), (I-B), or salt of any thereof comprises a DLL3 antibody comprising two VHH domains, each of which is conjugated to a chain of an Fc fragment. In one embodiment, the DLL3-ADC of Formula (I), (I-A), (I-B), or salt of any thereof comprises a homodimer of an amino acid sequence as set forth in any one of SEQ ID NOs:4, 5, or 10.

[00160] In one embodiment, n is 1 in Formula (I), (I-A), or (I-B) (or salt thereof). In one embodiment, n is 2. In one embodiment, n is 3. In one embodiment, n is 4. In one embodiment, n is 5. In one embodiment, n is 6. In one embodiment, n is 7. In one embodiment, n is 8.

[00161] In one embodiment, the present disclosure provides an ADC of Formula (II):

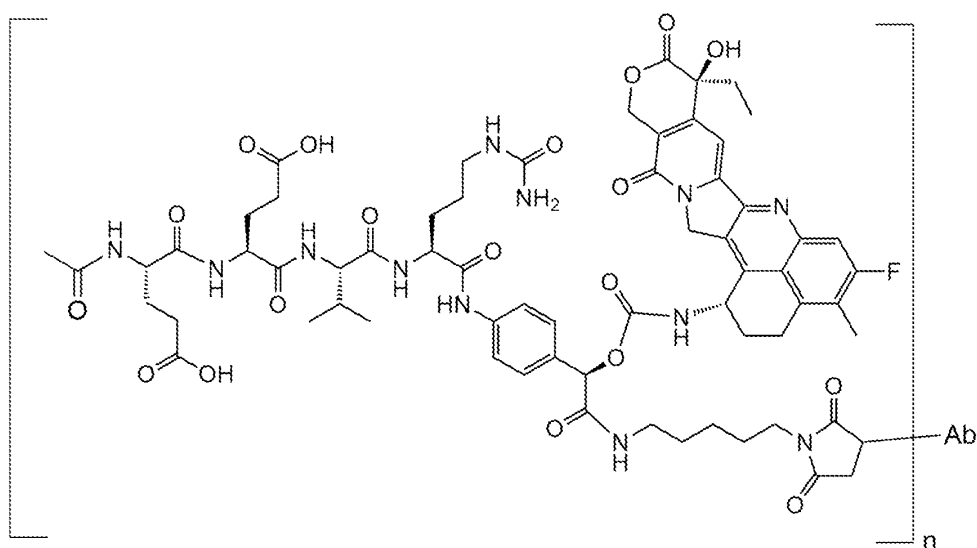


(II),

or a salt thereof, wherein:

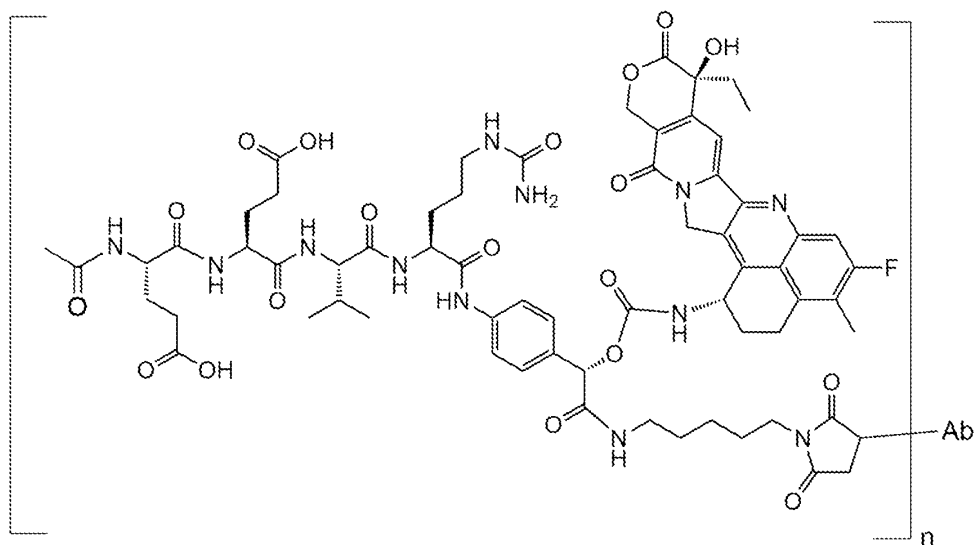
n is an integer from 1 to 8, and

Ab represents an antibody that binds to DLL3 (“DLL3 antibody”). In some embodiments, the ADC is an (*R*)-epimer of Formula (II) or a salt thereof. Accordingly, the ADC is of Formula (II-A):



(II-A)

or a salt thereof. In some embodiments, the ADC is an (*S*)-epimer of Formula (II) or a salt thereof. Accordingly, the ADC is of Formula (II-B):



(II-B)

or a salt thereof.

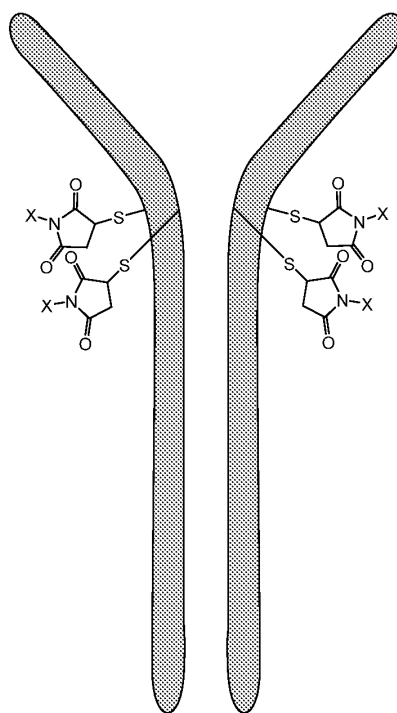
[00162] In one embodiment, the DLL3-ADC of Formula (II), (II-A), (II-B), or salt of any thereof comprises a DLL3 antibody comprising: (1) a CDR1, CDR2, and CDR3 of a VHH, wherein the VHH comprises an amino acid sequence as set forth in SEQ ID NO:14, 15 or 16; or (2) at least one VHH, wherein the at least one VHH comprises CDR1, CDR2 and CDR3, and wherein: (i) the CDR1 comprises an amino acid sequence as set forth in SEQ ID NO:1 or

7; (ii) the CDR2 comprises an amino acid sequence as set forth in SEQ ID NO:2 or 8; and (iii) the CDR3 comprises an amino acid sequence as set forth in SEQ ID NO:3 or 9.

[00163] In one embodiment, the DLL3-ADC of Formula (II), (II-A), (II-B), or salt of any thereof comprises a DLL3 antibody comprising: (a) a CDR1 as set forth in SEQ ID NO:1; a CDR2 as set forth in SEQ ID NO:2; and a CDR3 as set forth in SEQ ID NO:3; or (b) a CDR1 as set forth in SEQ ID NO:7; a CDR2 as set forth in SEQ ID NO:8; and a CDR3 as set forth in SEQ ID NO:9. In one embodiment, the DLL3-ADC of Formula (II), (II-A), (II-B), or a salt of any thereof comprises a DLL3 antibody comprising a VHH as set forth in any one of SEQ ID NOs: 14-16. In one embodiment, the DLL3-ADC of Formula (II), (II-A), (II-B), or a salt of any thereof comprises a DLL3 antibody further comprising a human IgG constant domain. In one embodiment, the human IgG is a human IgG1. In one embodiment, the DLL3-ADC of Formula (II), (II-A), (II-B), or a salt of any thereof comprises a DLL3 antibody further comprising an Fc fragment. In one embodiment, the Fc fragment comprises a homodimer of an amino acid sequence as set forth in SEQ ID NO:17. In one embodiment, the DLL3-ADC of Formula (II), (II-A), (II-B), or a salt of any thereof comprises a DLL3 antibody comprising at least one VHH domain. In one embodiment, the DLL3-ADC of Formula (II), (II-A), (II-B), or a salt of any thereof comprises a DLL3 antibody comprising two VHH domains, each of which is conjugated to a chain of an Fc fragment. In one embodiment, the DLL3-ADC of Formula (II), (II-A), (II-B), or a salt of any thereof comprises a homodimer of an amino acid sequence as set forth in any one of SEQ ID NOs:4, 5, or 10.

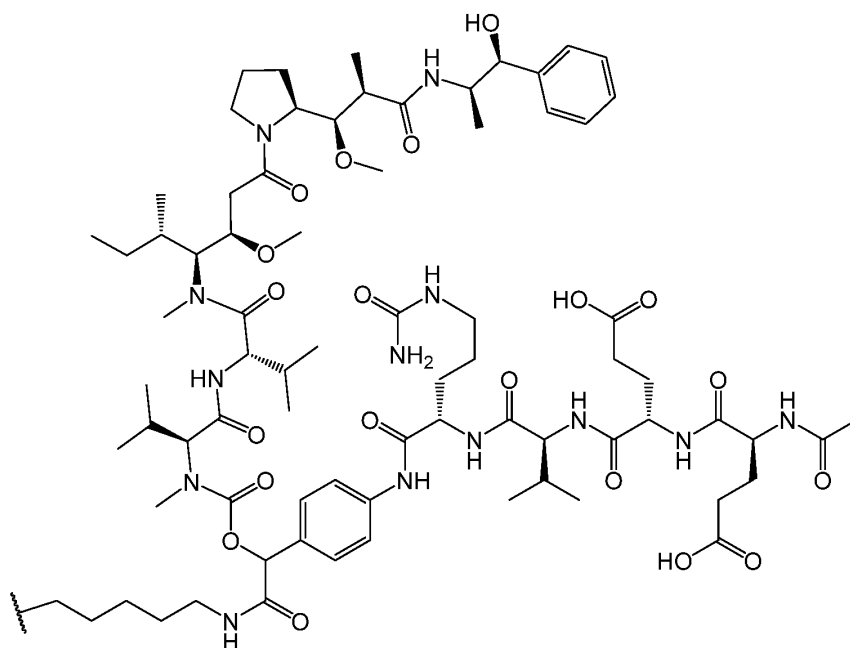
[00164] In one embodiment, n is 1 in Formula (II), (II-A), or (II-B) (or salt thereof). In one embodiment, n is 2. In one embodiment, n is 3. In one embodiment, n is 4. In one embodiment, n is 5. In one embodiment, n is 6. In one embodiment, n is 7. In one embodiment, n is 8.

[00165] In one embodiment, the DLL3-ADC is of the following Formula (A):



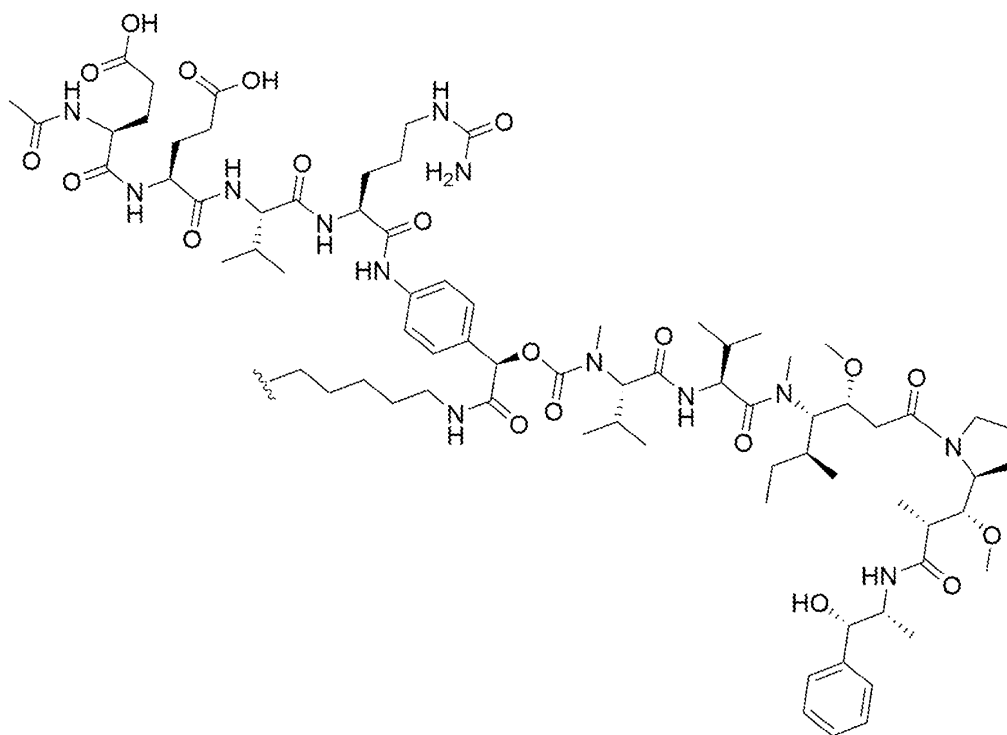
(A),

wherein each thick shaded line represents a chain of an antibody that binds to DLL3 (“DLL3 antibody”), the depicted sulfurs are from cysteine residues of the DLL3 antibody, and X represents the structure below or a salt thereof:

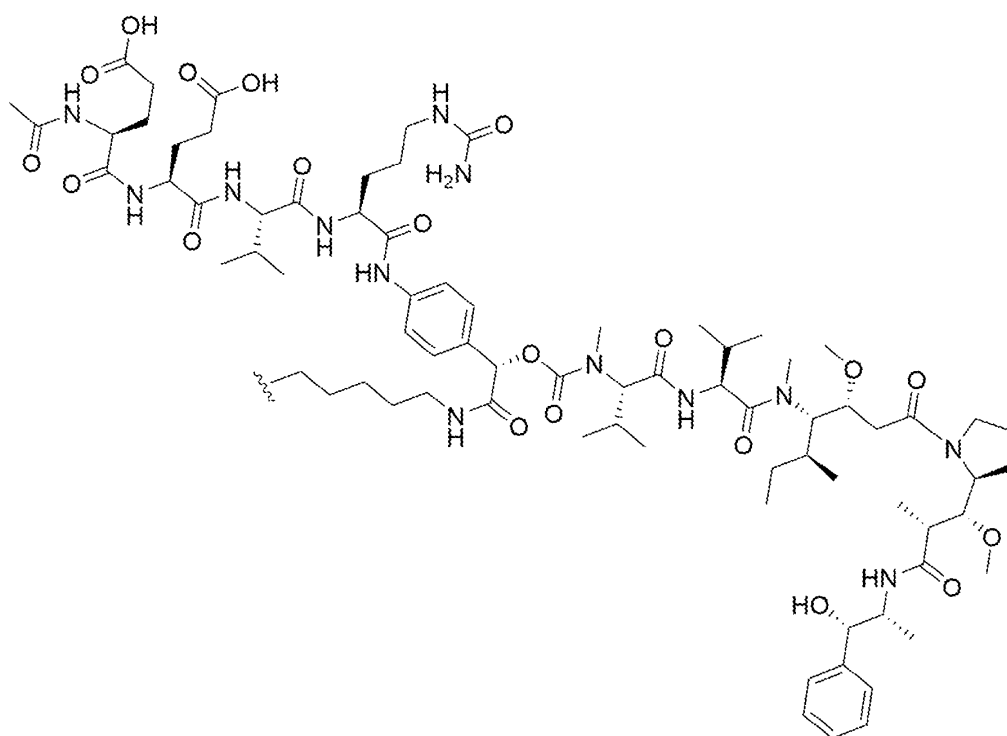


wherein the wavy line represents the point of attachment to the rest of the ADC of Formula (A). In some embodiments, the cysteine residues are in the hinge region of the

DLL3 antibody. In further embodiments, the cysteine residues form an interchain disulfide bridge in a DLL3 antibody not conjugated directly or indirectly to X, for example, between two heavy chains, between a heavy chain and a light chain, between two VHH chains, or between two VHH-Fc chains. In yet further embodiments, the cysteine residue is at any one or more of positions 226 or 229 according to EU numbering (also referred to herein as C226 or C229, respectively). In some embodiments, X is an (*R*)-epimer of the structure above or a salt thereof. Accordingly, X represents:

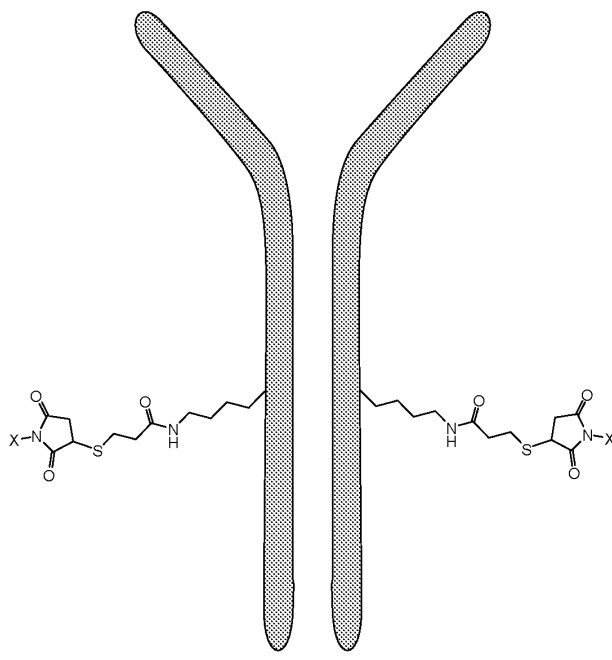


or a salt thereof, wherein the wavy line represents the point of attachment to the rest of the ADC of Formula (A). In some embodiments, X is an (*S*)-epimer of the structure above or a salt thereof. Accordingly, X represents:



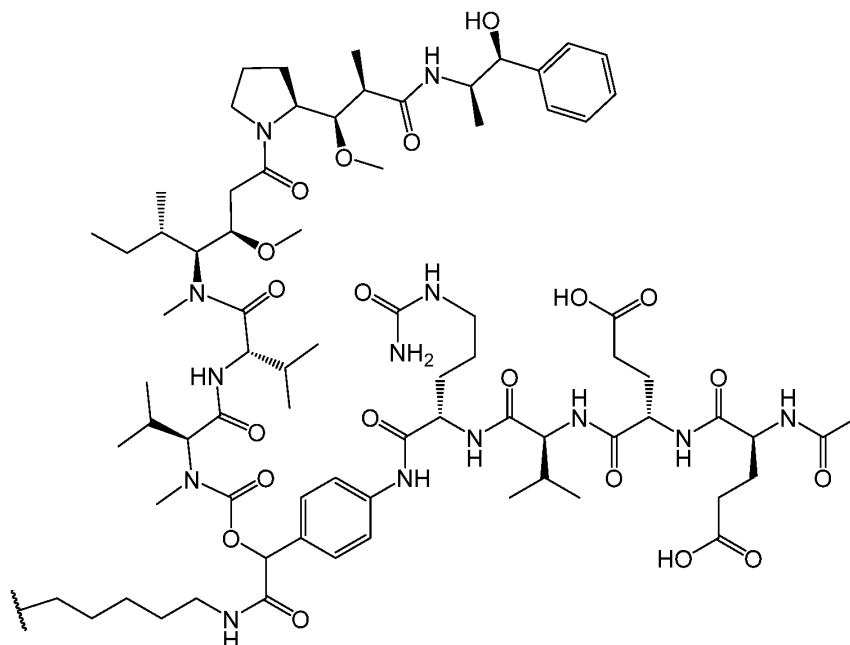
or a salt thereof, wherein the wavy line represents the point of attachment to the rest of the ADC of Formula (A).

[00166] In one embodiment, the DLL3-ADC is of the following Formula (B):

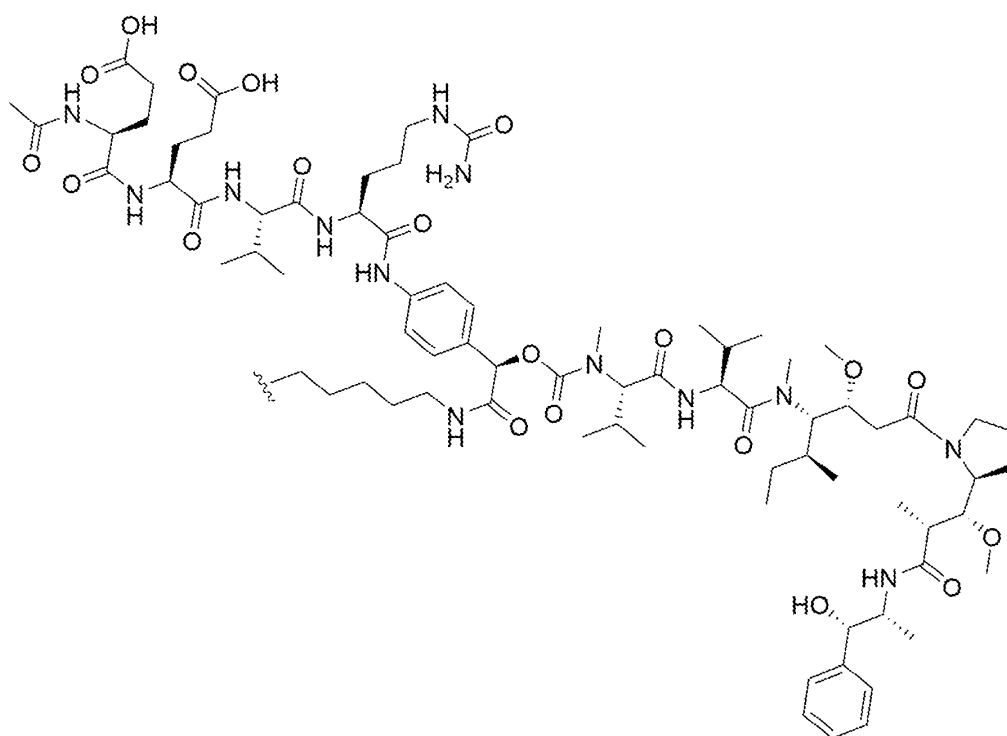


(B),

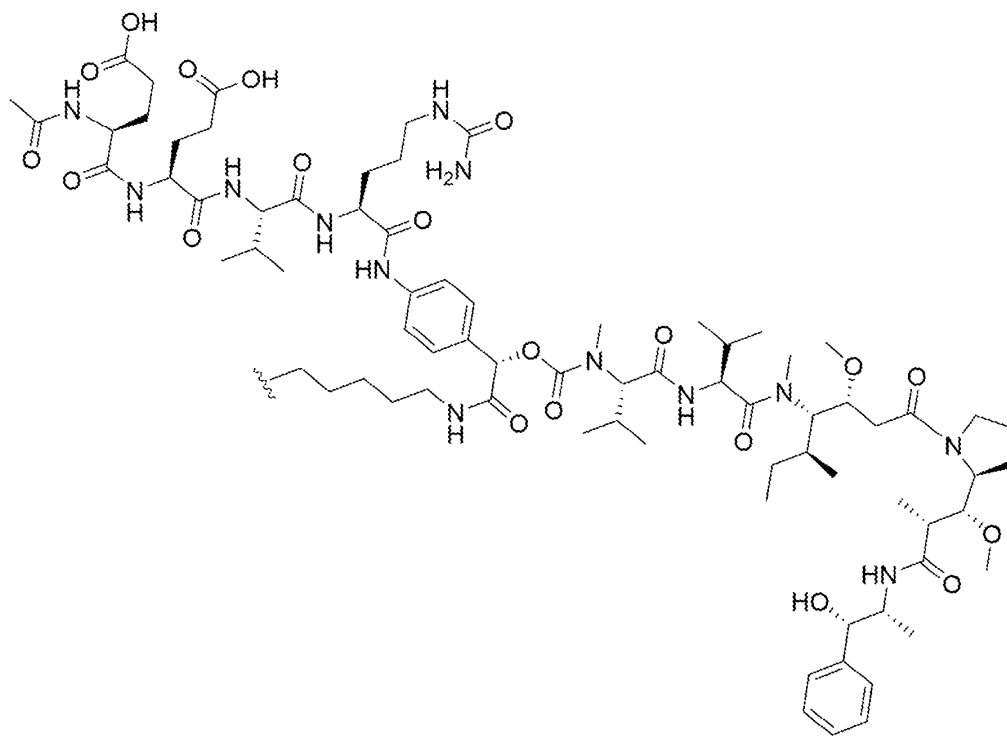
wherein each thick shaded line represents a chain of an antibody that binds to DLL3 (“DLL3 antibody”), the depicted $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}$ moiety is from a lysine residue of the CH2 domain of the DLL3 antibody, and X represents the structure below or a salt thereof:



wherein the wavy line represents the point of attachment to the rest of the ADC of Formula (B). In some embodiments, the lysine residue is at any one or more of positions 246, 248, 288, 290, or 317 according to EU numbering (also referred to herein as K246, K248, K288, K290, or K317, respectively). In some embodiments, X is an (*R*)-epimer of the structure above or a salt thereof. Accordingly, X represents:

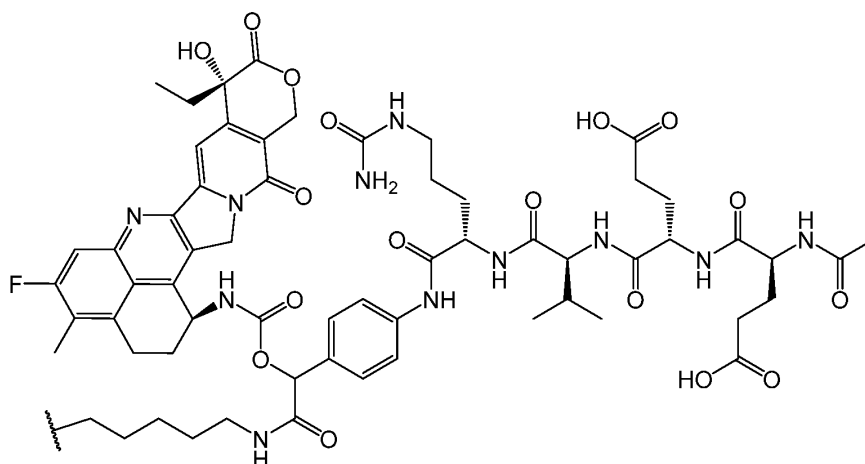


or a salt thereof, wherein the wavy line represents the point of attachment to the rest of the ADC of Formula (B). In some embodiments, X is an (*S*)-epimer of the structure above or a salt thereof. Accordingly, X represents:

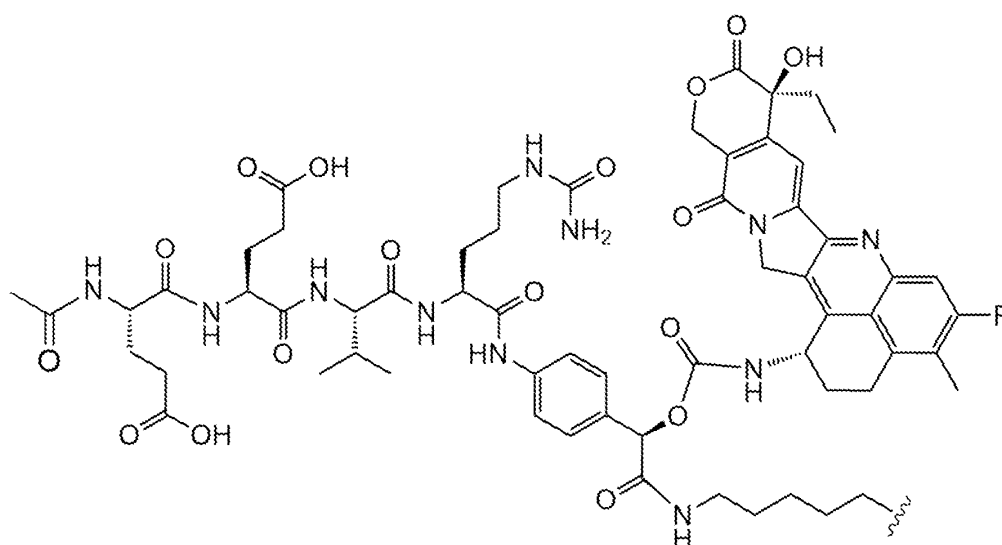


or a salt thereof, wherein the wavy line represents the point of attachment to the rest of the ADC of Formula (B).

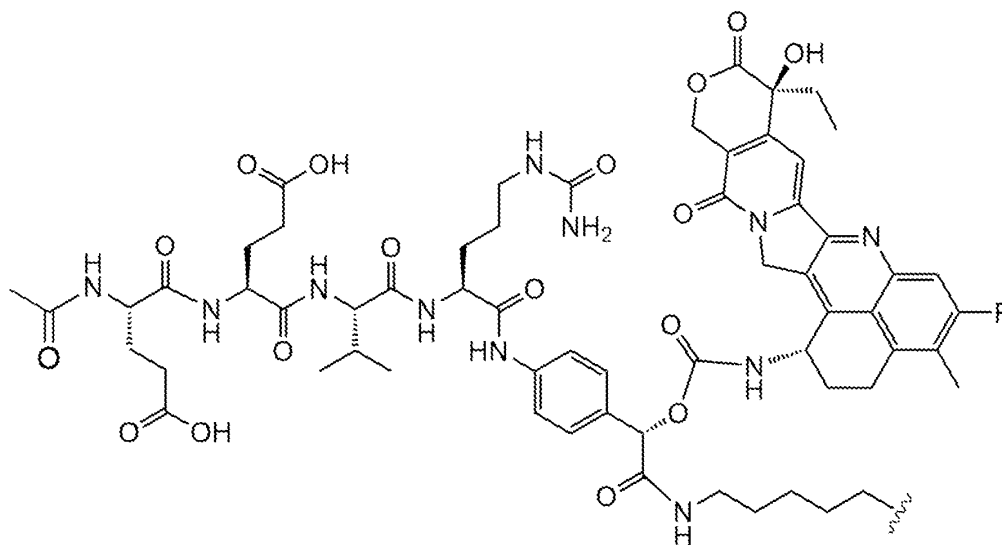
[00167] In one embodiment, the DLL3-ADC is of Formula (A), or a salt thereof as depicted above, wherein each thick shaded line represents a chain of an antibody that binds to DLL3 (“DLL3 antibody”), the depicted sulfurs are from cysteine residues of the DLL3 antibody, and X represents the structure below or a salt thereof:



wherein the wavy line represents the point of attachment to each depicted maleimide moiety of Formula (A). In some embodiments, the cysteine residues are in the hinge region of the DLL3 antibody. In further embodiments, the cysteine residues form an interchain disulfide bridge in a DLL3 antibody not conjugated directly or indirectly to X, for example, between two heavy chains, between a heavy chain and a light chain, between two VHH chains, or between two VHH-Fc chains. In yet further embodiments, the cysteine residue is at any one or more of positions 226 or 229 according to EU numbering (also referred to herein as C226 or C229, respectively). In some embodiments, X is an (*R*)-epimer of the structure above or a salt thereof. Accordingly, X represents:

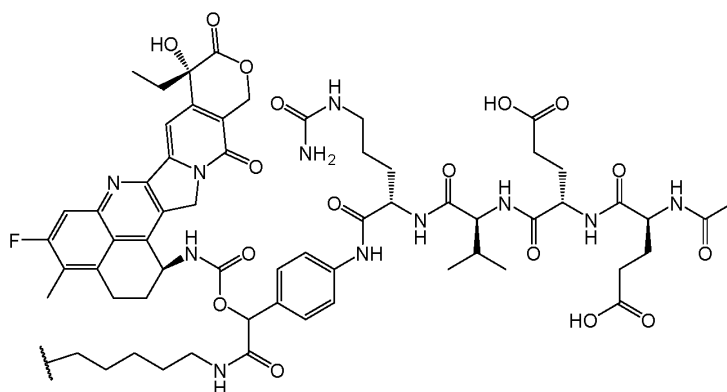


or a salt thereof, wherein the wavy line represents the point of attachment to the rest of the ADC of Formula (A). In some embodiments, X is an (*S*)-epimer of the structure above or a salt thereof. Accordingly, X represents:

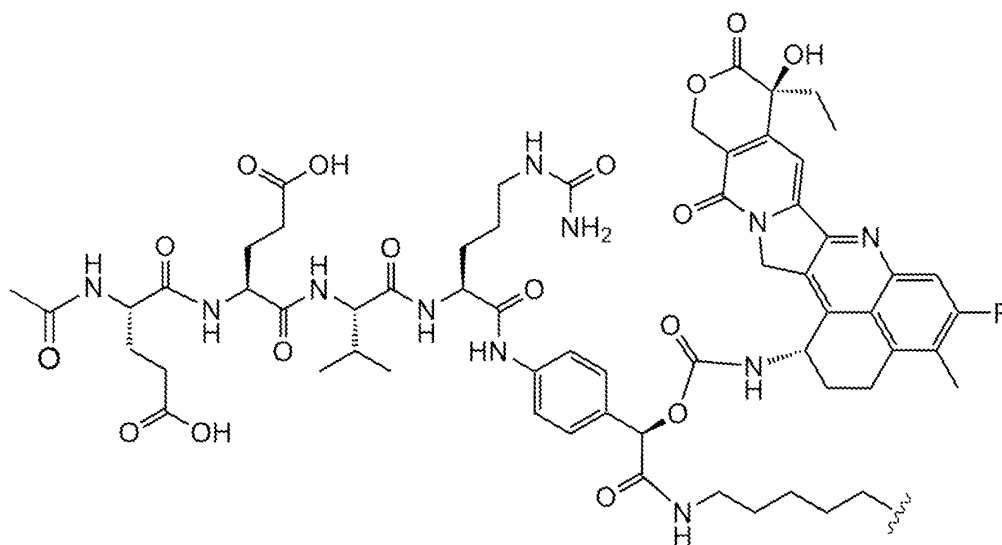


or a salt thereof, wherein the wavy line represents the point of attachment to the rest of the ADC of Formula (A).

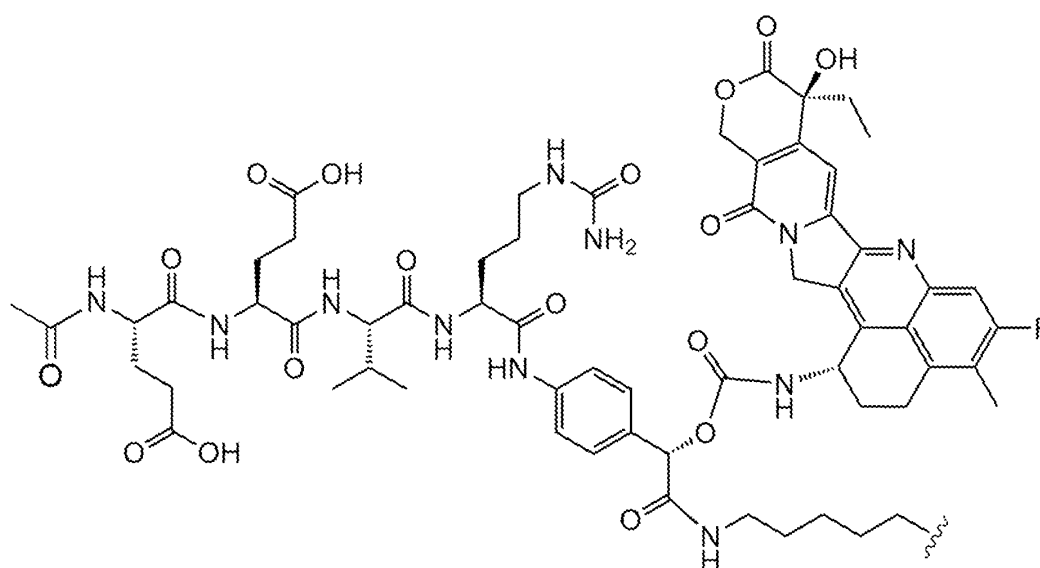
[00168] In one embodiment, the DLL3-ADC is of Formula (B) as depicted above, wherein each thick shaded line represents a chain of an antibody that binds to DLL3 (“DLL3 antibody”), the depicted $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}$ moiety is from a lysine residue of the CH2 domain of the DLL3 antibody, and X represents the structure below or a salt thereof:



wherein the wavy line represents the point of attachment to each depicted maleimide moiety of Formula (B). In some embodiments, the lysine residue is at any one or more of positions 246, 248, 288, 290, or 317 according to EU numbering (also referred to herein as K246, K248, K288, K290, or K317, respectively). In some embodiments, X is an (*R*)-epimer of the structure above or a salt thereof. Accordingly, X represents:



or a salt thereof, wherein the wavy line represents the point of attachment to the rest of the ADC of Formula (B). In some embodiments, X is an (*S*)-epimer of the structure above or a salt thereof. Accordingly, X represents:

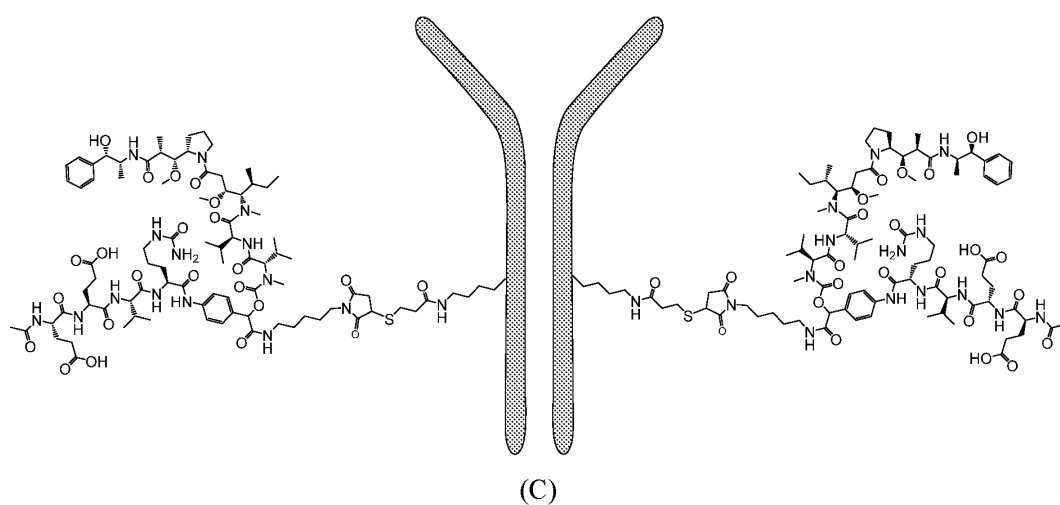


or a salt thereof, wherein the wavy line represents the point of attachment to the rest of the ADC of Formula (B).

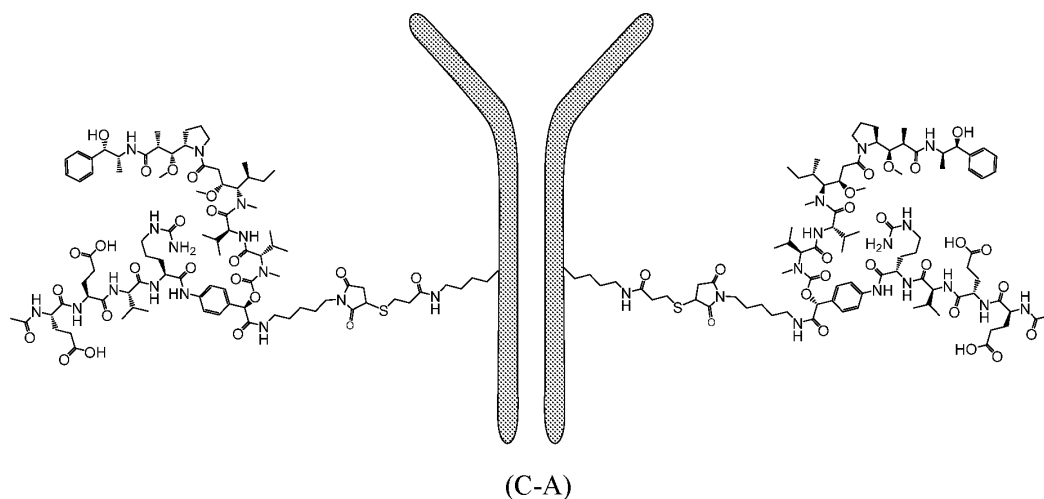
[00169] In some embodiments, the DLL3 antibody of Formula (A) or Formula (B) comprises an amino acid sequence as set forth in SEQ ID NO:4, wherein the lysine residue is K248 of the DLL3 antibody, *e.g.*, the 150th amino acid residue of SEQ ID NO:4. In some embodiments, the DLL3 antibody of Formula (A) or Formula (B) or a salt of any thereof comprises an amino acid sequence as set forth in SEQ ID NO:5 wherein the lysine residue is K248 of the DLL3 antibody, *e.g.*, the 150th amino acid residue of SEQ ID NO:5. In some embodiments, the DLL3 antibody of Formula (A) or Formula (B) or a salt of any thereof comprises an amino acid sequence as set forth in SEQ ID NO:10 wherein the lysine residue is K248 of the DLL3 antibody, *e.g.*, the 152nd amino acid residue of SEQ ID NO:10.

[00170] Additionally or alternatively, the DLL3 antibody of Formula (A) or Formula (B) comprises an amino acid sequence as set forth in SEQ ID NO:4, wherein the lysine residue is K246 of the DLL3 antibody, *e.g.*, the 148th amino acid residue of SEQ ID NO:4. In some embodiments, the DLL3 antibody of Formula (A) or Formula (B) or a salt of any thereof comprises an amino acid sequence as set forth in SEQ ID NO:5 wherein the lysine residue is K246 of the DLL3 antibody, *e.g.*, the 148th amino acid residue of SEQ ID NO:5. In some embodiments, the DLL3 antibody of Formula (A) or Formula (B) or a salt of any thereof comprises an amino acid sequence as set forth in SEQ ID NO:10 wherein the lysine residue is K246 of the DLL3 antibody, *e.g.*, the 150th amino acid residue of SEQ ID NO:10.

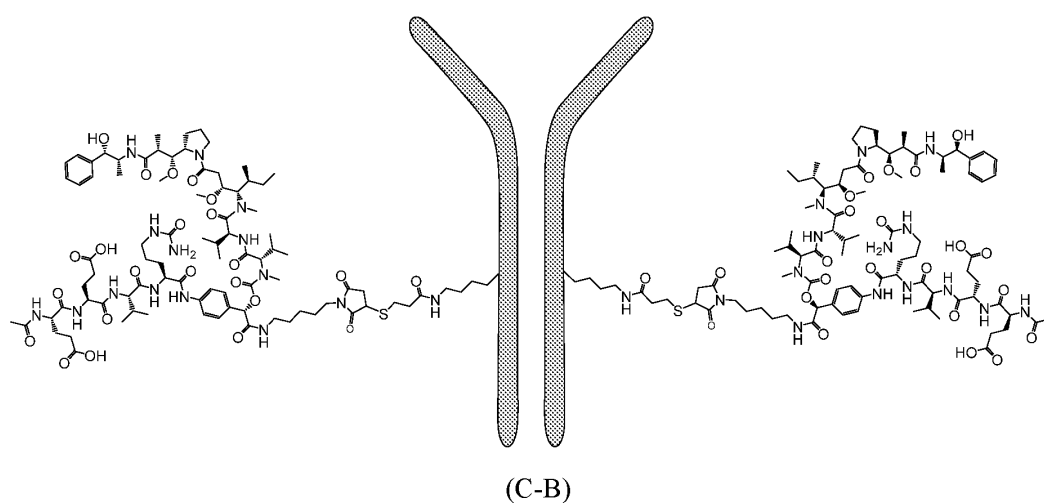
[00171] In one embodiment, the DLL3-ADC is of the following Formula (C):



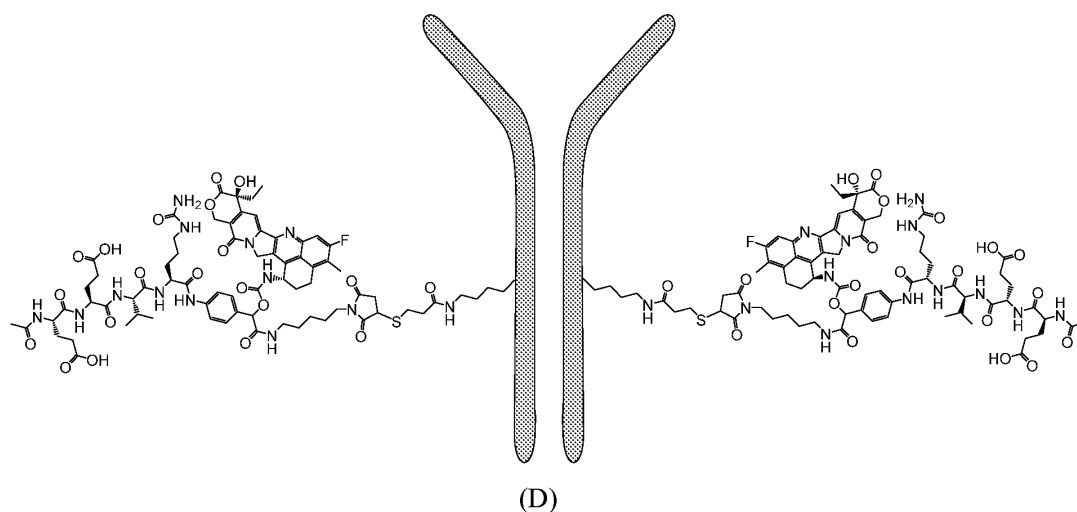
or a salt thereof, wherein each thick shaded line represents a chain of an antibody that binds to DLL3 (“DLL3 antibody”), the depicted $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}$ moiety is from a lysine residue of the CH_2 domain of the DLL3 antibody. In some embodiments, the DLL3-ADC is an (*R*)-epimer of Formula (C) or a salt thereof:



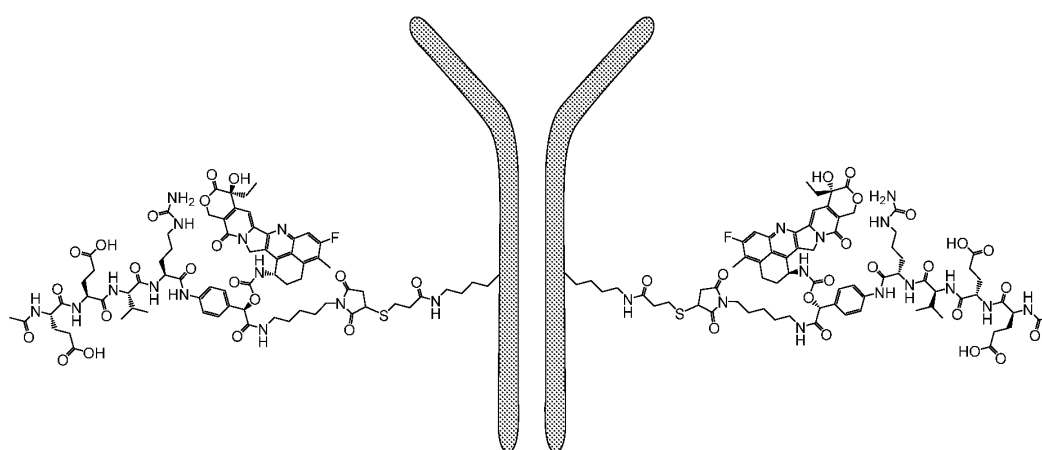
In some embodiments, the DLL3-ADC is an (*S*)-epimer of Formula (C), *e.g.*, Formula (C-B), or a salt thereof:



[00172] In one embodiment, the DLL3-ADC is of the following Formula (D):

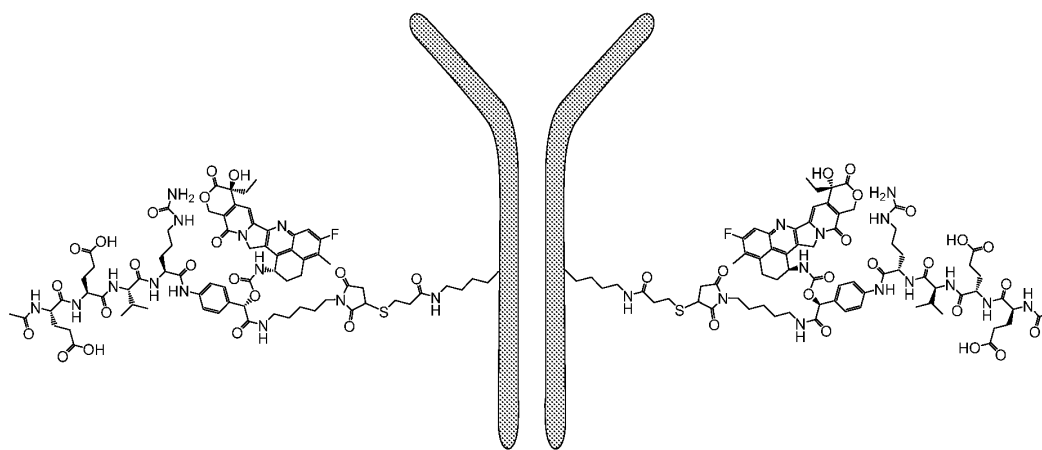


or a salt thereof, wherein each thick shaded line represents a chain of an antibody that binds to DLL3 (“DLL3 antibody”), the depicted $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}$ moiety is from a lysine residue of the CH_2 domain of the DLL3 antibody. In some embodiments, the DLL3-ADC is an (*R*)-epimer of Formula (D), *e.g.*, Formula (D-A), or a salt thereof:



(D-A)

In some embodiments, the DLL3-ADC is an (*S*)-epimer of Formula (D), *e.g.*, Formula (D-B), or a salt thereof:



(D-B)

[00173] In some embodiments, the DLL3 antibody of Formula (C) or Formula (D) or a salt of any thereof comprises an amino acid sequence as set forth in SEQ ID NO:4, wherein the lysine residue is K248 of the DLL3 antibody, *e.g.*, the 150th amino acid residue of SEQ ID NO:4. In some embodiments, the DLL3 antibody of Formula (C) or Formula (D) or a salt of any thereof comprises an amino acid sequence as set forth in SEQ ID NO:5 wherein the lysine residue is K248 of the DLL3 antibody, *e.g.*, the 150th amino acid residue of SEQ ID NO:5. In some embodiments, the DLL3 antibody of Formula (C) or Formula (D) or a salt of any thereof comprises an amino acid sequence as set forth in SEQ ID NO:10 wherein the lysine residue is K152 of the DLL3 antibody, *e.g.*, the 152nd amino acid residue of SEQ ID NO:10.

[00174] Additionally or alternatively, the DLL3 antibody of Formula (C), (C-A), (C-B), (D), (D-A), (D-B), or a salt of any thereof comprises an amino acid sequence as set forth in SEQ ID NO:4, wherein the lysine residue is K246 of the DLL3 antibody, *e.g.*, the 148th amino acid residue of SEQ ID NO:4. In some embodiments, the DLL3 antibody of Formula (C), (C-A), (C-B), (D), (D-A), (D-B), or a salt of any thereof comprises an amino acid sequence as set forth in SEQ ID NO:5 wherein the lysine residue is K246 of the DLL3 antibody, *e.g.*, the 148th amino acid residue of SEQ ID NO:5. In some embodiments, the DLL3 antibody of Formula (C), (C-A), (C-B), (D), (D-A), (D-B), or a salt of any thereof comprises an amino acid sequence as set forth in SEQ ID NO:10 wherein the lysine residue is K246 of the DLL3 antibody, *e.g.*, the 150th amino acid residue of SEQ ID NO:10.

[00175] In some embodiments, the DLL3-ADC is represented by Formula (I), (I-A), (I-B), (II), (II-A), (II-B), or a salt of any thereof, wherein Ab is DLL3 antibody comprising a VHH comprising one or more CDRs comprising an amino acid sequence identical or substantially identical to one or more of SEQ ID NOs:1-3 and 7-9.

[00176] In some embodiments, the DLL3-ADC is represented by Formula (I), (I-A), (I-B), (II), (II-A), (II-B), or a salt of any thereof, wherein Ab is DLL3 antibody comprising a VHH comprising an amino acid sequence identical or substantially identical to one or more of SEQ ID NOs:14-16.

[00177] In one embodiment, the DLL3-ADC of Formula (I), (I-A), (I-B), or a salt of any thereof comprises an antibody comprising a VHH comprising an amino acid sequence identical or substantially identical to SEQ ID NO:14 and n is 2. In one embodiment, the DLL3-ADC of Formula (I), (I-A), (I-B), or a salt of any thereof comprises an antibody comprising a VHH comprising an amino acid sequence identical or substantially identical to SEQ ID NO:14 and n is 4. In one embodiment, the DLL3-ADC of Formula (I), (I-A), (I-B), or a salt of any thereof comprises Linker-Drug (1), Linker-Drug (1-A), Linker-Drug (1-B), or a salt of any thereof, which is conjugated to the DLL3 antibody of Formula (I), (I-A), (I-B), or a salt of any thereof via the K248 residue of each chain of the DLL3 antibody, wherein the DLL3 antibody comprises a VHH comprising an amino acid sequence identical or substantially identical to SEQ ID NO:14. Accordingly, n is 2. In one embodiment, the DLL3-ADC of Formula (I), (I-A), (I-B), or a salt of any thereof comprises Linker-Drug (1), Linker-Drug (1-A), Linker-Drug (1-B), or a salt of any thereof, which is conjugated to the DLL3 antibody of Formula (I), (I-A), (I-B), or a salt of any thereof via one or more reduced disulfide bridges of each chain of the DLL3 antibody, wherein the antibody comprises a VHH comprising an amino acid sequence identical or substantially identical to SEQ ID

NO:14. In some embodiments, the one or more disulfide bridges prior to the reduction are selected from those formed among C226 and C229 of two antibody chains. Accordingly, n is 2 or 4. In some embodiments, both C226 and C229 of both antibody chains are modified to conjugated directly or indirectly to a linker-drug as disclosed herein, and accordingly, n is 4.

[00178] In one embodiment, the DLL3-ADC of Formula (I), (I-A), (I-B), or a salt of any thereof comprises an antibody comprising a VHH comprising an amino acid sequence identical or substantially identical to SEQ ID NO:15 and n is 2. In one embodiment, the DLL3-ADC of Formula (I), (I-A), (I-B), or a salt of any thereof comprises an antibody comprising a VHH comprising an amino acid sequence identical or substantially identical to SEQ ID NO:15 and n is 4. In one embodiment, the DLL3-ADC of Formula (I), (I-A), (I-B), or a salt of any thereof comprises Linker-Drug (1), Linker-Drug (1-A), Linker-Drug (1-B), or a salt of any thereof, which is conjugated to the DLL3 antibody of Formula (I), (I-A), (I-B), or a salt of any thereof via the K248 residue of each chain of the DLL3 antibody, wherein the DLL3 antibody comprises a VHH comprising an amino acid sequence identical or substantially identical to SEQ ID NO:15. Accordingly, n is 2. In one embodiment, the DLL3-ADC of Formula (I), (I-A), (I-B), or a salt of any thereof comprises Linker-Drug (1), Linker-Drug (1-A), Linker-Drug (1-B), or a salt of any thereof, which is conjugated to the DLL3 antibody of Formula (I), (I-A), (I-B), or a salt of any thereof via one or more reduced disulfide bridges of each chain of the DLL3 antibody, wherein the antibody comprises a VHH comprising an amino acid sequence identical or substantially identical to SEQ ID NO:15. In some embodiments, the one or more disulfide bridges prior to the reduction are selected from those formed among C226 and C229 of two antibody chains. Accordingly, n is 2 or 4. In some embodiments, both C226 and C229 of both antibody chains are modified to conjugated directly or indirectly to a linker-drug as disclosed herein, and accordingly, n is 4.

[00179] In one embodiment, the DLL3-ADC of Formula (I), (I-A), (I-B), or a salt of any thereof comprises an antibody comprising a VHH comprising an amino acid sequence identical or substantially identical to SEQ ID NO:16 and n is 2. In one embodiment, the DLL3-ADC of Formula (I), (I-A), (I-B), or a salt of any thereof comprises an antibody comprising a VHH comprising an amino acid sequence identical or substantially identical to SEQ ID NO:16 and n is 4. In one embodiment, the DLL3-ADC of Formula (I), (I-A), (I-B), or a salt of any thereof comprises Linker-Drug (1), Linker-Drug (1-A), Linker-Drug (1-B), or a salt of any thereof, which is conjugated to the DLL3 antibody of Formula (I), (I-A), (I-B), or a salt of any thereof via the K248 residue of each chain of the DLL3 antibody, wherein the DLL3 antibody comprises a VHH comprising an amino acid sequence identical or

substantially identical to SEQ ID NO:16. Accordingly, n is 2. In one embodiment, the DLL3-ADC of Formula (I), (I-A), (I-B), or a salt of any thereof comprises Linker-Drug (1), Linker-Drug (1-A), Linker-Drug (1-B), or a salt of any thereof, which is conjugated to the DLL3 antibody of Formula (I), (I-A), (I-B), or a salt of any thereof via one or more reduced disulfide bridges of each chain of the DLL3 antibody, wherein the antibody comprises a VHH comprising an amino acid sequence identical or substantially identical to SEQ ID NO:16. In some embodiments, the one or more disulfide bridges prior to the reduction are selected from those formed among C226 and C229 of two antibody chains. Accordingly, n is 2 or 4. In some embodiments, both C226 and C229 of both antibody chains are modified to conjugated directly or indirectly to a linker-drug as disclosed herein, and accordingly, n is 4.

[00180] In one embodiment, the DLL3-ADC of Formula (II), (II-A), (II-B), or a salt of any thereof comprises an antibody comprising a VHH comprising an amino acid sequence identical or substantially identical to SEQ ID NO:14 and n is 2. In one embodiment, the DLL3-ADC of Formula (II), (II-A), (II-B), or a salt of any thereof comprises an antibody comprising a VHH comprising an amino acid sequence identical or substantially identical to SEQ ID NO:14 and n is 4. In one embodiment, the DLL3-ADC of Formula (II), (II-A), (II-B), or a salt of any thereof comprises Linker-Drug (2), Linker-Drug (2-A), Linker-Drug (2-B), or a salt of any thereof, which is conjugated to the DLL3 antibody of Formula (II), (II-A), (II-B), or a salt of any thereof via the K248 residue of each chain of the DLL3 antibody, wherein the DLL3 antibody comprises a VHH comprising an amino acid sequence identical or substantially identical to SEQ ID NO:14. Accordingly, n is 2. In one embodiment, the DLL3-ADC of Formula (II), (II-A), (II-B), or a salt of any thereof comprises Linker-Drug (2), Linker-Drug (2-A), Linker-Drug (2-B), or a salt of any thereof, which is conjugated to the DLL3 antibody of Formula (II), (II-A), (II-B), or a salt of any thereof via one or more reduced disulfide bridges of each chain of the DLL3 antibody, wherein the antibody comprises a VHH comprising an amino acid sequence identical or substantially identical to SEQ ID NO:14. In some embodiments, the one or more disulfide bridges prior to the reduction are selected from those formed among C226 and C229 of two antibody chains. Accordingly, n is 2 or 4. In some embodiments, both C226 and C229 of both antibody chains are modified to conjugated directly or indirectly to a linker-drug as disclosed herein, and accordingly, n is 4.

[00181] In one embodiment, the DLL3-ADC of Formula (II), (II-A), (II-B), or a salt of any thereof comprises an antibody comprising a VHH comprising an amino acid sequence identical or substantially identical to SEQ ID NO:15 and n is 2. In one embodiment, the

DLL3-ADC of Formula (II), (II-A), (II-B), or a salt of any thereof comprises an antibody comprising a VHH comprising an amino acid sequence identical or substantially identical to SEQ ID NO:15 and n is 4. In one embodiment, the DLL3-ADC of Formula (II), (II-A), (II-B), or a salt of any thereof comprises Linker-Drug (2), Linker-Drug (2-A), Linker-Drug (2-B), or a salt of any thereof, which is conjugated to the DLL3 antibody of Formula (II), (II-A), (II-B), or a salt of any thereof via the K248 residue of each chain of the DLL3 antibody, wherein the DLL3 antibody comprises a VHH comprising an amino acid sequence identical or substantially identical to SEQ ID NO:15. Accordingly, n is 2. In one embodiment, the DLL3-ADC of Formula (II), (II-A), (II-B), or a salt of any thereof comprises Linker-Drug (2), Linker-Drug (2-A), Linker-Drug (2-B), or a salt of any thereof, which is conjugated to the DLL3 antibody of Formula (II), (II-A), (II-B), or a salt of any thereof via one or more reduced disulfide bridges of each chain of the DLL3 antibody, wherein the antibody comprises a VHH comprising an amino acid sequence identical or substantially identical to SEQ ID NO:15. In some embodiments, the one or more disulfide bridges prior to the reduction are selected from those formed among C226 and C229 of two antibody chains. Accordingly, n is 2 or 4. In some embodiments, both C226 and C229 of both antibody chains are modified to conjugated directly or indirectly to a linker-drug as disclosed herein, and accordingly, n is 4.

[00182] In one embodiment, the DLL3-ADC of Formula (II), (II-A), (II-B), or a salt of any thereof comprises an antibody comprising a VHH comprising an amino acid sequence identical or substantially identical to SEQ ID NO:16 and n is 2. In one embodiment, the DLL3-ADC of Formula (II), (II-A), (II-B), or a salt of any thereof comprises an antibody comprising a VHH comprising an amino acid sequence identical or substantially identical to SEQ ID NO:16 and n is 4. In one embodiment, the DLL3-ADC of Formula (II), (II-A), (II-B), or a salt of any thereof comprises Linker-Drug (2), Linker-Drug (2-A), Linker-Drug (2-B), or a salt of any thereof, which is conjugated to the DLL3 antibody of Formula (II), (II-A), (II-B), or a salt of any thereof via the K248 residue of each chain of the DLL3 antibody, wherein the DLL3 antibody comprises a VHH comprising an amino acid sequence identical or substantially identical to SEQ ID NO:16. Accordingly, n is 2. In one embodiment, the DLL3-ADC of Formula (II), (II-A), (II-B), or a salt of any thereof comprises Linker-Drug (2), Linker-Drug (2-A), Linker-Drug (2-B), or a salt of any thereof, which is conjugated to the DLL3 antibody of Formula (II), (II-A), (II-B), or a salt of any thereof via one or more reduced disulfide bridges of each chain of the DLL3 antibody, wherein the antibody comprises a VHH comprising an amino acid sequence identical or substantially identical to

SEQ ID NO:16. In some embodiments, the one or more disulfide bridges prior to the reduction are selected from those formed among C226 and C229 of two antibody chains. Accordingly, n is 2 or 4. In some embodiments, both C226 and C229 of both antibody chains are modified to conjugated directly or indirectly to a linker-drug as disclosed herein, and accordingly, n is 4.

[00183] In some embodiments, the DLL3-ADC is represented by Formula (I), (I-A), (I-B), (II), (II-A), (II-B), or a salt of any thereof, wherein Ab is DLL3 antibody comprising an amino acid sequence identical or substantially identical to one of SEQ ID NOs:4, 5, and 10. In some embodiments, a cysteine or lysine residue of the DLL3 antibody is modified as disclosed herein and conjugated to a linker-drug as disclosed herein.

[00184] In one embodiment, the DLL3-ADC comprises a DLL3 antibody and two or more of Linker-Drug (1), or a salt thereof, conjugated thereto. In one embodiment, the DLL3-ADC comprises a DLL3 antibody and two or more of Linker-Drug (1-A), or a salt thereof, conjugated thereto. In one embodiment, the DLL3-ADC comprises a DLL3 antibody and two or more of Linker-Drug (1-B), or a salt thereof, conjugated thereto.

[00185] In one embodiment, the DLL3-ADC of Formula (I), (I-A), (I-B), or a salt of any thereof comprises a DLL3 antibody comprising an amino acid sequence identical or substantially identical to SEQ ID NO:4, wherein n is 2. In one embodiment, the DLL3-ADC of Formula (I), (I-A), (I-B), or a salt of any thereof comprises a DLL3 antibody comprising an amino acid sequence identical or substantially identical to SEQ ID NO:4, wherein n is 4. In one embodiment, the DLL3-ADC of Formula (I), (I-A), (I-B), or a salt of any thereof comprises Linker-Drug (1), Linker-Drug (1-A), Linker-Drug (1-B), or a salt of any thereof, which is conjugated to the DLL3 antibody of Formula (I), (I-A), (I-B), or a salt of any thereof via the K248 residue of each chain of the DLL3 antibody, wherein the antibody comprises an amino acid sequence identical or substantially identical to SEQ ID NO:4. Accordingly, n is 2. In one embodiment, the DLL3-ADC of Formula (I), (I-A), (I-B), or a salt of any thereof comprises Linker-Drug (1), Linker-Drug (1-A), Linker-Drug (1-B), or a salt of any thereof, which is conjugated to the DLL3 antibody of Formula (I), (I-A), (I-B), or a salt of any thereof via one or more reduced disulfide bridges of each chain of the DLL3 antibody, wherein the antibody comprises an amino acid sequence identical or substantially identical to SEQ ID NO:4. In some embodiments, the one or more disulfide bridges prior to the reduction are selected from those formed among C226 and C229 of two antibody chains. Accordingly, n is 2 or 4. In some embodiments, both C226 and C229 of both antibody chains are modified to conjugated directly or indirectly to a linker-drug as disclosed herein, and accordingly, n is 4.

[00186] In one embodiment, the DLL3-ADC of Formula (I), (I-A), (I-B), or a salt of any thereof comprises a DLL3 antibody comprising an amino acid sequence identical or substantially identical to SEQ ID NO:5, wherein n is 2. In one embodiment, the DLL3-ADC of Formula (I), (I-A), (I-B), or a salt of any thereof comprises a DLL3 antibody comprising an amino acid sequence identical or substantially identical to SEQ ID NO:5, wherein n is 4. In one embodiment, the DLL3-ADC of Formula (I), (I-A), (I-B), or a salt of any thereof comprises Linker-Drug (1), Linker-Drug (1-A), Linker-Drug (1-B), or a salt of any thereof, which is conjugated to the DLL3 antibody of Formula (I), (I-A), (I-B), or a salt of any thereof via the K248 residue of each chain of the DLL3 antibody, wherein the antibody comprises an amino acid sequence identical or substantially identical to SEQ ID NO:5. Accordingly, n is 2. In one embodiment, the DLL3-ADC of Formula (I), (I-A), (I-B), or a salt of any thereof comprises Linker-Drug (1), Linker-Drug (1-A), Linker-Drug (1-B), or a salt of any thereof, which is conjugated to the DLL3 antibody of Formula (I), (I-A), (I-B), or a salt of any thereof via one or more reduced disulfide bridges of each chain of the DLL3 antibody, wherein the antibody comprises an amino acid sequence identical or substantially identical to SEQ ID NO:5. In some embodiments, the one or more disulfide bridges prior to the reduction are selected from those formed among C226 and C229 of two antibody chains. Accordingly, n is 2 or 4. In some embodiments, both C226 and C229 of both antibody chains are modified to conjugated directly or indirectly to a linker-drug as disclosed herein, and accordingly, n is 4.

[00187] In one embodiment, the DLL3-ADC of Formula (I), (I-A), (I-B), or a salt of any thereof comprises a DLL3 antibody comprising an amino acid sequence identical or substantially identical to SEQ ID NO:10, wherein n is 2. In one embodiment, the DLL3-ADC of Formula (I), (I-A), (I-B), or a salt of any thereof comprises a DLL3 antibody comprising an amino acid sequence identical or substantially identical to SEQ ID NO:10, wherein n is 4. In one embodiment, the DLL3-ADC of Formula (I), (I-A), (I-B), or a salt of any thereof comprises Linker-Drug (1), Linker-Drug (1-A), Linker-Drug (1-B), or a salt of any thereof, which is conjugated to the DLL3 antibody of Formula (I), (I-A), (I-B), or a salt of any thereof via the K248 residue of each chain of the DLL3 antibody, wherein the antibody comprises an amino acid sequence identical or substantially identical to SEQ ID NO:10. Accordingly, n is 2. In one embodiment, the DLL3-ADC of Formula (I), (I-A), (I-B), or a salt of any thereof comprises Linker-Drug (1), Linker-Drug (1-A), Linker-Drug (1-B), or a salt of any thereof, which is conjugated to the DLL3 antibody of Formula (I), (I-A), (I-B), or a salt of any thereof via one or more reduced disulfide bridges of each chain of the DLL3 antibody, wherein the antibody comprises an amino acid sequence identical or substantially identical to SEQ ID

NO:10. In some embodiments, the one or more disulfide bridges prior to the reduction are selected from those formed among C226 and C229 of two antibody chains. Accordingly, n is 2 or 4. In some embodiments, both C226 and C229 of both antibody chains are modified to conjugated directly or indirectly to a linker-drug as disclosed herein, and accordingly, n is 4.

[00188] In one embodiment, the DLL3-ADC comprises a DLL3 antibody and two or more of Linker-Drug (2), or a salt thereof, conjugated thereto.

[00189] In one embodiment, the DLL3-ADC of Formula (II), (II-A), (II-B), or a salt of any thereof comprises a DLL3 antibody comprising an amino acid sequence identical or substantially identical to SEQ ID NO:4, wherein n is 2. In one embodiment, the DLL3-ADC of Formula (II), (II-A), (II-B), or a salt of any thereof comprises a DLL3 antibody comprising an amino acid sequence identical or substantially identical to SEQ ID NO:4, wherein n is 4. In one embodiment, the DLL3-ADC of Formula (II), (II-A), (II-B), or a salt of any thereof comprises Linker-Drug (2), Linker-Drug (2-A), Linker-Drug (2-B), or a salt of any thereof, which is conjugated to the DLL3 antibody of Formula (II), (II-A), (II-B), or a salt of any thereof via the K248 residue of each chain of the DLL3 antibody, wherein the antibody comprises an amino acid sequence identical or substantially identical to SEQ ID NO:4. Accordingly, n is 2. In one embodiment, the DLL3-ADC of Formula (II), (II-A), (II-B), or a salt of any thereof comprises Linker-Drug (2), Linker-Drug (2-A), Linker-Drug (2-B), or a salt of any thereof, which is conjugated to the DLL3 antibody of Formula (II), (II-A), (II-B), or a salt of any thereof via one or more reduced disulfide bridges of each chain of the DLL3 antibody, wherein the antibody comprises an amino acid sequence identical or substantially identical to SEQ ID NO:4. In some embodiments, the one or more disulfide bridges prior to the reduction are selected from those formed among C226 and C229 of two antibody chains. Accordingly, n is 2 or 4. In some embodiments, both C226 and C229 of both antibody chains are modified to conjugated directly or indirectly to a linker-drug as disclosed herein, and accordingly, n is 4.

[00190] In one embodiment, the DLL3-ADC of Formula (II), (II-A), (II-B), or a salt of any thereof comprises a DLL3 antibody comprising an amino acid sequence identical or substantially identical to SEQ ID NO:5, wherein n is 2. In one embodiment, the DLL3-ADC of Formula (II), (II-A), (II-B), or a salt of any thereof comprises a DLL3 antibody comprising an amino acid sequence identical or substantially identical to SEQ ID NO:5, wherein n is 4. In one embodiment, the DLL3-ADC of Formula (II), (II-A), (II-B), or a salt of any thereof comprises Linker-Drug (2), Linker-Drug (2-A), Linker-Drug (2-B), or a salt of any thereof, which is conjugated to the DLL3 antibody of Formula (II), (II-A), (II-B), or a salt of any

thereof via the K248 residue of each chain of the DLL3 antibody, wherein the antibody comprises an amino acid sequence identical or substantially identical to SEQ ID NO:5. Accordingly, n is 2. In one embodiment, the DLL3-ADC of Formula (II), (II-A), (II-B), or a salt of any thereof comprises Linker-Drug (2), Linker-Drug (2-A), Linker-Drug (2-B), or a salt of any thereof, which is conjugated to the DLL3 antibody of Formula (II), (II-A), (II-B), or a salt of any thereof via one or more reduced disulfide bridges of each chain of the DLL3 antibody, wherein the antibody comprises an amino acid sequence identical or substantially identical to SEQ ID NO:5. In some embodiments, the one or more disulfide bridges prior to the reduction are selected from those formed among C226 and C229 of two antibody chains. Accordingly, n is 2 or 4. In some embodiments, both C226 and C229 of both antibody chains are modified to conjugated directly or indirectly to a linker-drug as disclosed herein, and accordingly, n is 4.

[00191] In one embodiment, the DLL3-ADC of Formula (II), (II-A), (II-B), or a salt of any thereof comprises a DLL3 antibody comprising an amino acid sequence identical or substantially identical to SEQ ID NO:10, wherein n is 2. In one embodiment, the DLL3-ADC of Formula (II), (II-A), (II-B), or a salt of any thereof comprises a DLL3 antibody comprising an amino acid sequence identical or substantially identical to SEQ ID NO:10, wherein n is 4. In one embodiment, the DLL3-ADC of Formula (II), (II-A), (II-B), or a salt of any thereof comprises Linker-Drug (2), Linker-Drug (2-A), Linker-Drug (2-B), or a salt of any thereof, which is conjugated to the DLL3 antibody of Formula (II), (II-A), (II-B), or a salt of any thereof via the K248 residue of each chain of the DLL3 antibody, wherein the antibody comprises an amino acid sequence identical or substantially identical to SEQ ID NO:10. Accordingly, n is 2. In one embodiment, the DLL3-ADC of Formula (II), (II-A), (II-B), or a salt of any thereof comprises Linker-Drug (2), Linker-Drug (2-A), Linker-Drug (2-B), or a salt of any thereof, which is conjugated to the DLL3 antibody of Formula (II), (II-A), (II-B), or a salt of any thereof via one or more reduced disulfide bridges of each chain of the DLL3 antibody, wherein the antibody comprises an amino acid sequence identical or substantially identical to SEQ ID NO:10. In some embodiments, the one or more disulfide bridges prior to the reduction are selected from those formed among C226 and C229 of two antibody chains. Accordingly, n is 2 or 4. In some embodiments, both C226 and C229 of both antibody chains are modified to conjugated directly or indirectly to a linker-drug as disclosed herein, and accordingly, n is 4.

[00192] The DLL3-ADC of the present invention can have superior properties such as long retention time within the body, a high monomer ratio (low aggregation rate), high functional

substance releasability in human cells, high stability (for example in mouse plasma), and desirable cytotoxicity to target cells (such as DLL3 expressing or overexpressing cancer cells).

COMPOSITIONS

[00193] In another embodiment, the present disclosure provides a composition comprising DLL3-ADCs as disclosed herein. A composition may comprise a plurality of DLL3-ADCs as disclosed herein, wherein each DLL3-ADC in the composition independently comprises a linker-drug as shown in Formula (I), (I-A), (I-B), (II), (II-A), (II-B), or a salt of any thereof, wherein n is independently 1, 2, 3, or 4. In other words, each antibody molecule in the composition may be conjugated to 1, 2, 3, or 4 linker-drugs. Therefore, a composition may be characterized by a “drug-to-antibody” ratio (DAR) ranging from about 1 to about 4. Methods to determine DAR are well known to the skilled person and include methods using Reverse Phase Chromatography, or HPLC-MS.

[00194] For example, in any embodiment, a composition comprising a plurality of DLL3-ADCs may exhibit a DAR of about 1 to about 4, about 1 to about 3, about 1 to about 2, about 2 to about 4, about 2 to about 3, about 3 to about 4, about 1.5 to about 2, about 1.8 to about 2, about 1.9 to about 2, about 3.5 to about 4, about 3.6 to about 4, about 3.7 to about 4, about 3.8 to about 4, about 3.9 to about 4, about 3.5 to about 3.9, about 3.6 to about 3.9, about 3.7 to about 3.9, about 3.8 to about 3.9, about 3.5 to about 3.8, about 3.6 to about 3.8, about 3.7 to about 3.8, about 3.5 to about 3.7, about 3.6 to about 3.7, or about 3.5 to about 3.6. In one embodiment, the DAR is about 1 to about 2. In one embodiment, the DAR is about 1.1, about 1.2, about 1.3, about 1.4, about 1.5, about 1.6, about 1.7, about 1.8, about 1.9, or about 2. In one embodiment, the DAR is about 2 to about 4. In one embodiment, the DAR is about 2, about 2.1, about 2.2, about 2.4, about 2.4, about 2.5, about 2.6, about 2.7, about 2.8, about 2.9, about 3, about 3.1, about 3.2, about 3.3, about 3.4, about 3.5, about 3.6, about 3.7, about 3.8, about 3.9 or about 4.

[00195] In some embodiments, the DLL3-ADC comprises a DLL3 antibody as disclosed herein conjugated to a linker-drug as disclosed herein through a lysine residue of the antibody as disclosed herein, such as K246, K248, K288, K290, or K317. Accordingly, provided herein is a composition comprising the DLL3-ADC. In some embodiments, a composition comprising the DLL3-ADC exhibits a DAR of about 1.5 to about 2.5. In some embodiments, the composition exhibits a DAR of 1.5 or greater, 1.6 or greater, 1.7 or greater, 1.8 or greater, or 1.9 or greater. Additionally, or alternatively, the composition exhibits a DAR of 2.5 or less, 2.4 or less, 2.3 or less, 2.2 or less, or 2.1 or less. In some embodiments, the composition

exhibits a DAR of 1.6 to 2.4, 1.7 to 2.3, 1.8 to 2.2, or 1.9 to 2.1. In some embodiments, a composition comprising the DLL3-ADC exhibits a DAR of about 1.9 to about 2.1.

[00196] In one embodiment, a composition comprising DLL3-ADCs as disclosed herein may exhibit a DAR of about 2, wherein the linker-drugs are conjugated via site specific conjugation, as described herein, to a lysine of the DLL3 antibody of the DLL3-ADC (*e.g.*, K246, K248, K288, K290, or K317 in the CH2 domain). In one embodiment, a composition comprising DLL3-ADCs as disclosed herein may exhibit a DAR of about 4, wherein the linker-drugs are conjugated to reduced cysteine moieties of an interchain disulfide bridge, *e.g.*, in the hinge region, of the DLL3 antibody of the DLL3-ADC.

ADMINISTRATION, FORMULATION, AND DOSAGE

[00197] In another aspect, the present disclosure provides a pharmaceutical composition comprising a DLL3-ADC as disclosed herein and a pharmaceutically acceptable carrier. In another aspect, the present disclosure provides a pharmaceutical composition comprising a composition of DLL3-ADCs as disclosed herein and a pharmaceutically acceptable carrier (“DLL3-ADC pharmaceutical composition”). A DLL3-ADC pharmaceutical composition may comprise a plurality of DLL3-ADCs as disclosed herein, wherein each DLL3-ADC in the pharmaceutical composition independently comprises a linker-drug as shown in Formula (I), (I-A), (I-B), (II), (II-A), (II-B), or a salt of any thereof, wherein *n* is independently 1, 2, 3, or 4. In other words, each antibody molecule in the DLL3-ADC pharmaceutical composition may be conjugated to 1, 2, 3, or 4 linker-drugs. Therefore, a DLL3-ADC pharmaceutical composition may be characterized by a DAR ranging from about 1 to about 4.

[00198] In one embodiment, a DLL3-ADC pharmaceutical composition may exhibit a DAR of about 2, wherein the linker-drugs are conjugated via site specific conjugation, as described herein, to a lysine of a DLL3 antibody (*e.g.*, K246, K248, K288, K290, or K317 of the CH2 domain) of the DLL3-ADC. In one embodiment, a DLL3-ADC pharmaceutical composition may exhibit a DAR of about 4, wherein the linker-drugs are conjugated to reduced cysteine moieties of an interchain disulfide bridge, *e.g.*, in the hinge region, of a DLL3 antibody.

[00199] In further embodiments, a DLL3-ADC pharmaceutical composition comprises 20 mM Histidine. Additionally or alternatively, a DLL3-ADC pharmaceutical composition comprises 5% trehalose. Additionally or alternatively, a DLL3-ADC pharmaceutical composition has a pH of about 5 to about 6, such as 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, or 6.0. In further embodiments, a DLL3-ADC pharmaceutical composition comprises 20 mM histidine and 5% trehalose and has a pH of about 5 to about 6, such as 5.1, 5.2, 5.3, 5.4, 5.5,

5.6, 5.7, 5.8, 5.9, or 6.0. In further embodiments, a DLL3-ADC pharmaceutical composition comprises 20 mM histidine and 5% trehalose and has a pH of 6.0.

[00200] A pharmaceutical composition of the disclosure may be administered to a subject in need thereof, by various routes, including, but not limited to, oral, intravenous, intra-arterial, subcutaneous, parenteral, intranasal, intramuscular, intracranial, intracardiac, intraventricular, intratracheal, buccal, rectal, intraperitoneal, intradermal, topical, transdermal, and intrathecal, or otherwise by implantation or inhalation. The subject compositions may be formulated into preparations in solid, semi-solid, liquid, or gaseous forms; including, but not limited to, tablets, capsules, powders, granules, ointments, solutions, suppositories, enemas, injections, inhalants, and aerosols. The appropriate formulation and route of administration may be selected according to the intended application and therapeutic regimen.

[00201] Suitable formulations for enteral administration include hard or soft gelatin capsules, pills, tablets, including coated tablets, elixirs, suspensions, syrups or inhalations and controlled release forms thereof.

[00202] Formulations suitable for parenteral administration (*e.g.*, by injection), include aqueous or non-aqueous, isotonic, pyrogen-free, sterile liquids (*e.g.*, solutions, suspensions), in which the active ingredient is dissolved, suspended, or otherwise provided (*e.g.*, in a liposome or other microparticulate). Such liquids may additionally contain other pharmaceutically acceptable ingredients, such as anti-oxidants, buffers, preservatives, stabilizers, bacteriostats, suspending agents, thickening agents, and solutes which render the formulation isotonic with the blood (or other relevant bodily fluid) of the intended recipient. Examples of excipients include, for example, water, alcohols, polyols, glycerol, vegetable oils, and the like. Examples of suitable isotonic carriers for use in such formulations include Sodium Chloride Injection, Ringer's Solution, or Lactated Ringer's Injection. Similarly, the particular dosage regimen, including dose, timing, and repetition, will depend on the particular individual and that individual's medical history, as well as empirical considerations such as pharmacokinetics (*e.g.*, half-life, clearance rate, etc.).

[00203] Frequency of administration may be determined and adjusted over the course of therapy and is based on reducing the number of proliferative or tumorigenic cells, maintaining the reduction of such neoplastic cells, reducing the proliferation of neoplastic cells, or delaying the development of metastasis. In some embodiments, the dosage administered may be adjusted or attenuated to manage potential side effects and/or toxicity. Alternatively, sustained continuous release formulations of a subject therapeutic composition may be appropriate.

[00204] It will be appreciated by one of skill in the art that appropriate dosages can vary from patient to patient. Determining the optimal dosage will generally involve the balancing of the level of therapeutic benefit against any risk or deleterious side effects. The selected dosage level will depend on a variety of factors including, but not limited to, the activity of the particular compound, the route of administration, the time of administration, the rate of excretion of the compound, the duration of the treatment, other drugs, compounds, and/or materials used in combination, the severity of the condition, and the species, sex, age, weight, condition, general health, and prior medical history of the patient. The amount of compound and route of administration will ultimately be at the discretion of the physician, veterinarian, or clinician, although generally the dosage will be selected to achieve local concentrations at the site of action that achieve the desired effect without causing substantial harmful or deleterious side-effects.

[00205] In general, a DLL3-ADC of the disclosure may be administered in various ranges. These include about 5 µg/kg body weight to about 100 mg/kg body weight per dose; about 50 µg/kg body weight to about 5 mg/kg body weight per dose; about 100 µg/kg body weight to about 10 mg/kg body weight per dose; and any values within the aforementioned ranges. Other ranges include about 100 µg/kg body weight to about 20 mg/kg body weight per dose and about 0.5 mg/kg body weight to about 20 mg/kg body weight per dose. In some embodiments, the dosage is at least about 100 µg/kg body weight, at least about 250 µg/kg body weight, at least about 750 µg/kg body weight, at least about 3 mg/kg body weight, at least about 5 mg/kg body weight, at least about 10 mg/kg body weight.

[00206] In any event, an antibody or antigen binding portion thereof of the disclosure is preferably administered as needed to subjects (or a subject) in need thereof. Determination of the frequency of administration may be made by persons skilled in the art, such as an attending physician based on considerations of the condition being treated, age of the subject being treated, severity of the condition being treated, general state of health of the subject being treated and the like.

[00207] In some embodiments, the course of treatment involving a DLL3-ADC of the present disclosure will comprise multiple doses of the selected drug product over a period of weeks or months. For example, a DLL3-ADC of the present disclosure may be administered once every day, every two days, every four days, every week, every ten days, every two weeks, every three weeks, every month, every six weeks, every two months, every ten weeks, or every three months. In this regard, it will be appreciated that the dosages may be altered or the interval may be adjusted based on patient response and clinical practices.

[00208] Dosages and regimens may also be determined empirically for the disclosed therapeutic compositions in individuals who have been given one or more administration(s). For example, individuals may be given incremental dosages of a therapeutic composition produced as described herein. In some embodiments, the dosage may be gradually increased, reduced, or attenuated based respectively on empirically determined or observed side effects or toxicity. To assess efficacy of the selected composition, a marker of the specific disease, disorder or condition can be followed as described previously. For cancer, these include direct measurements of tumor size via palpation or visual observation, indirect measurement of tumor size by x-ray or other imaging techniques; an improvement as assessed by direct tumor biopsy and microscopic examination of the tumor sample; the measurement of an indirect tumor marker (*e.g.*, PSA for prostate cancer) or a tumorigenic antigen, a decrease in pain or paralysis; improved speech, vision, breathing or other disability associated with the tumor; increased appetite; or an increase in quality of life as measured by accepted tests or prolongation of survival. It will be apparent to one of skill in the art that the dosage will vary depending on the individual, the type of neoplastic condition, the stage of neoplastic condition, whether the neoplastic condition has begun to metastasize to other location in the individual, and the past and concurrent treatments being used.

[00209] Compatible formulations for parenteral administration (*e.g.*, intravenous injection) may comprise a DLL3-ADC as disclosed herein in concentrations of from about 10 µg/mL to about 100 mg/mL. In some embodiments, the concentrations of the DLL3-ADC (*e.g.*, antibody or the antigen binding portion thereof) will comprise 20 µg/mL, 40 µg/mL, 60 µg/mL, 80 µg/mL, 100 µg/mL, 200 µg/mL, 300 µg/mL, 400 µg/mL, 500 µg/mL, 600 µg/mL, 700 µg/mL, 800 µg/mL, 900 µg/mL, or 1 mg/mL. In some embodiments, the concentrations of the DLL3-ADC will comprise 2 mg/mL, 3 mg/mL, 4 mg/mL, 5 mg/mL, 6 mg/mL, 8 mg/mL, 10 mg/mL, 12 mg/mL, 14 mg/mL, 16 mg/mL, 18 mg/mL, 20 mg/mL, 25 mg/mL, 30 mg/mL, 35 mg/mL, 40 mg/mL, 45 mg/mL, 50 mg/mL, 60 mg/mL, 70 mg/mL, 80 mg/mL, 90 mg/mL, or 100 mg/mL.

[00210] The term “pharmaceutically acceptable,” as used herein, means that the vehicle, diluent, excipient and/or salts thereof, are chemically and/or physically compatible with other ingredients in the formulation, and physiologically compatible with the recipient.

[00211] As used herein, the term “a pharmaceutically acceptable carrier and/or excipient” refers to a carrier, stabilizer, and/or excipient pharmacologically and/or physiologically compatible with a subject and an active agent, which is well known in the art (see, *e.g.*, Remington's Pharmaceutical Sciences. Edited by Gennaro AR, 19th ed. Pennsylvania: Mack

Publishing Company, 1995), and includes, but is not limited to a pH adjuster, surfactant, adjuvant, or an ionic strength enhancer. For example, a pH adjuster includes, but is not limited to, phosphate buffer; a surfactant includes, but is not limited to, cationic, anionic, or non-ionic surfactant, *e.g.*, Tween-80; an ionic strength enhancer includes, but is not limited to, sodium chloride. Carriers, excipients, or stabilizers are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the carrier is an aqueous pH buffered solution. Examples of carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (*e.g.*, less than about 10 amino acid residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN™, polyethylene glycol (PEG), and PLURONICS™. The term “carrier” can also refer to a diluent, adjuvant (*e.g.*, Freund’s adjuvant (complete or incomplete)), excipient, or vehicle with which the therapeutic is administered. Such carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable, or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is an exemplary carrier when a composition (*e.g.*, a pharmaceutical composition) is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable excipients (*e.g.*, pharmaceutical excipients) include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. Compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. Oral compositions, including formulations, can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable carriers are described in Remington’s Pharmaceutical Sciences (1990) Mack Publishing Co., Easton, PA. Compositions, including pharmaceutical compounds, may contain a prophylactically or therapeutically effective amount of a D3-binding agent (*e.g.*, an anti-D3 antibody), for example, in isolated or purified form, together with a suitable amount of carrier so as to

provide the form for proper administration to the subject (*e.g.*, patient). The formulation should suit the mode of administration.

[00212] As used herein, the term “adjuvant” refers to a non-specific immunopotentiator, which can enhance immune response to an antigen or change the type of immune response in an organism when it is delivered together with the antigen to the organism or is delivered to the organism in advance. There are a variety of adjuvants, including, but not limited to, aluminum adjuvants (for example, aluminum hydroxide), Freund’s adjuvants (for example, Freund’s complete adjuvant and Freund’s incomplete adjuvant), *Corynebacterium parvum*, lipopolysaccharide, cytokines, and the like. Freund’s adjuvant is the most commonly used adjuvant in animal experiments. Aluminum hydroxide adjuvant is more commonly used in clinical trials.

METHODS OF TREATMENT

[00213] Also provided herein are methods of treating, preventing, or alleviating a DLL3-mediated disease, disorder, or condition, including one or more symptoms of the DLL3-mediated disease, disorder, or condition with a DLL3-ADC as described herein. In some embodiments, the present disclosure provides a method for treating, preventing, or alleviating a DLL3 positive or DLL3 overexpressed cancer with a DLL3-ADC as described herein. In some embodiments, the cancer is lung cancer, including, for example, small cell lung cancer (SCLC), large cell neuroendocrine carcinoma (LCNEC), or colorectal cancer. Also provided herein are methods of killing tumor cells with a DLL3-ADC as described herein. In some embodiments, a method of killing a tumor cell comprises contacting the tumor cell with a DLL3-ADC of Formula (I), (I-A) (I-B), (II), (II-A), (II-B), or a salt of any thereof. In some embodiments, the contacting is *in vivo* or *in vitro*.

[00214] The term “subject” includes any human or nonhuman animal, preferably humans.

[00215] The term “condition associated with DLL3” or “condition related to DLL3”, as used herein, refers to any condition that is caused by, exacerbated by, or otherwise linked to increased or decreased (generally increased) expression or activities of DLL3 (*e.g.*, a human DLL3).

[00216] The term “cancer,” as used herein, refers to any tumor or any malignant cell growth, proliferation, primary or metastasis-mediated, including solid tumors and non-solid tumors such as leukemia.

[00217] The term “treatment,” “treating” or “treated,” as used herein in the context of treating a condition, pertains generally to treatment or therapy, whether of a human or an animal, in which some desired therapeutic effect is achieved, for example, inhibition of the

progress of a condition, and includes a reduction in the rate of progress, a halt in the rate of progress, regression of the condition, amelioration of the condition, and cure of the condition. Treatment as a prophylactic measure (*e.g.*, prophylaxis, prevention) is also included. For cancer, “treating” may refer to a dampening or slowing of a tumor or malignant cell growth, proliferation, or metastasis, or some combination thereof. For tumors, “treatment” includes removal of all or part of a tumor, inhibiting or slowing tumor growth and metastasis, preventing, or delaying the development of a tumor, or some combination thereof.

[00218] The term “therapeutically-effective amount,” as used herein, pertains to that amount of an active compound, or a material, composition, or dosage from comprising an active compound, which is effective for producing some desired therapeutic effect, commensurate with a reasonable benefit/risk ratio, when administered in accordance with a desired treatment regimen. For example, a “therapeutically-effective amount,” of a DLL3-ADC refers to an amount or concentration effective to treat a human DLL3-related disease or condition.

[00219] Treatment of Disorders Including Cancer

[00220] In one aspect, the present disclosure provides a method of treating a disorder or a disease in a mammal, which comprises administering to the subject (for example, a human) in need of treatment a therapeutically effective amount of DLL3-ADC, such as in the form of a DLL3-ADC pharmaceutical composition, as disclosed herein. In one aspect, the present disclosure provides a DLL3-ADC as disclosed herein for use in treating a disease or disorder. In another aspect, provided herein is a use of a DLL3-ADC as disclosed herein for the manufacture of a medicament for the treatment of a disease or disorder. The disorder or disease may be a cancer.

[00221] A variety of cancers where DLL3 is implicated, whether malignant or benign and whether primary or secondary, may be treated or prevented with a method provided by the disclosure. The cancers may include, but not limited to, lung (including various subtypes, *e.g.*, small cell and non-small cell lung cancer), adrenal, liver, kidney, bladder, breast, gastric, ovarian, cervical, uterine, esophageal, colorectal, prostate pancreatic, thyroid, carcinomas, sarcomas, glioblastomas and various head and neck tumors. Exemplary cancers include, for example, small cell lung cancer, large cell neuroendocrine carcinoma, glioblastoma, Ewing's sarcoma, and cancers with neuroendocrine phenotype.

[00222] DLL3-ADCs and compositions and pharmaceutical compositions comprising the same as disclosed herein can be used for treating lung cancers such as bronchogenic carcinoma, non-small cell lung cancer, squamous cell carcinoma, small cell carcinoma, large

cell carcinoma, and adenocarcinoma, *e.g.*, lung adenocarcinoma. The lung cancers may be refractory, relapsed, or resistant to a platinum based agent (*e.g.*, carboplatin, cisplatin, oxaliplatin, topotecan) and/or a taxane (*e.g.*, docetaxel, paclitaxel, larotaxel, or cabazitaxel).

[00223] Cancers to be treated by DLL3-ADCs as disclosed herein may also be large cell neuroendocrine carcinoma (LCNEC), medullary thyroid cancer, glioblastoma, neuroendocrine prostate cancer (NEPC), high-grade gastroenteropancreatic cancer (GEP) and malignant melanoma. DLL3-ADCs and compositions and pharmaceutical compositions comprising the same, as disclosed herein, may be used to treat neuroendocrine tumors (both NET and pNET) arising in the kidney, genitourinary tract (bladder, prostate, ovary, cervix, and endometrium), gastrointestinal tract (colon, stomach), thyroid (medullary thyroid cancer), and lung (small cell lung carcinoma and large cell neuroendocrine carcinoma).

[00224] As described above, DLL3-ADCs and compositions and pharmaceutical compositions comprising the same are especially effective at treating lung cancer, including the following subtypes: small cell lung cancer and non-small cell lung cancer (*e.g.*, squamous cell non-small cell lung cancer or squamous cell small cell lung cancer) and large cell neuroendocrine carcinoma.

Stimulation of an Immune Response

[00225] The disclosure also provides a method of enhancing (for example, stimulating) an immune response in a subject comprising administering to the subject a DLL3-ADC of the disclosure such that an immune response in the subject is enhanced. In one aspect, the present disclosure provides a DLL3-ADC for use in enhancing (*e.g.*, stimulating) an immune response in a subject. In another aspect, provided herein is use of a DLL3-ADC for the manufacture of a medicament for enhancing (*e.g.*, stimulating) an immune response in a subject. In some embodiments, the subject is a mammal. In some embodiments, the subject is a human.

[00226] The term “enhancing an immune response” or its grammatical variations, means stimulating, evoking, increasing, improving, or augmenting any response of a mammal’s immune system. The immune response may be a cellular response (*e.g.*, cell-mediated, such as cytotoxic T lymphocyte mediated) or a humoral response (*e.g.*, antibody mediated response), and may be a primary or secondary immune response. Examples of enhancement of immune response include increased CD4⁺ helper T cell activity and generation of cytolytic T cells. The enhancement of immune response can be assessed using a number of *in vitro* or *in vivo* measurements known to those skilled in the art, including, but not limited to, cytotoxic T lymphocyte assays, release of cytokines (for example IL-2 production or IFN-γ

production), regression of tumors, survival of tumor bearing animals, antibody production, immune cell proliferation, expression of cell surface markers, and cytotoxicity. For example, methods of the disclosure are useful to enhance the immune response by a mammal when compared to the immune response by an untreated mammal or a mammal not treated using the methods as disclosed herein.

[00227] A DLL3-ADC may be used alone as a monotherapy, or may be used in combination with chemical therapies, radiotherapies, targeted therapies, or cell immunotherapies etc.

[00228] Combined Use with Chemotherapies

[00229] A DLL3-ADC may be used in combination with chemotherapies, including, for example, an anti-cancer agent, a cytotoxic agent or chemotherapeutic agent.

[00230] The term “anti-cancer agent” or “anti-proliferative agent” means any agent that can be used to treat a cell proliferative disorder such as cancer, and includes, but is not limited to, cytotoxic agents, cytostatic agents, anti-angiogenic agents, debulking agents, chemotherapeutic agents, radiotherapy and radiotherapeutic agents, targeted anti-cancer agents, BRMs, therapeutic antibodies, cancer vaccines, cytokines, hormone therapies, radiation therapy and anti-metastatic agents and immunotherapeutic agents. It will be appreciated that, in some embodiments as discussed above, such anti-cancer agents may comprise conjugates and may be associated with a DLL3-ADC as disclosed herein prior to administration. For example, in some embodiments selected anti-cancer agents will be linked to the unpaired cysteines of the engineered antibodies to provide engineered conjugates (*e.g.*, antibody-drug conjugates) as set forth herein. Accordingly, such engineered conjugates are expressly contemplated as being within the scope of the present disclosure. In some embodiments, the disclosed anti-cancer agents will be given in combination with a DLL3-ADC comprising a different therapeutic agent as set forth above.

[00231] As used herein the term “cytotoxic agent” means a substance that is toxic to the cells and decreases or inhibits the function of cells and/or causes destruction of cells. In some embodiments, the substance is a naturally occurring molecule derived from a living organism. Examples of cytotoxic agents include, but are not limited to, small molecule toxins or enzymatically active toxins of bacteria (*e.g.*, Diphtheria toxin, Pseudomonas endotoxin and exotoxin, Staphylococcal enterotoxin A), fungal (*e.g.*, α -sarcin, restrictocin), plants (*e.g.*, abrin, ricin, modeccin, viscumin, pokeweed anti-viral protein, saporin, gelonin, momoridin, trichosanthin, barley toxin, Aleurites fordii proteins, dianthin proteins, Phytolacca mericana proteins (PAPI, PAPII, and PAP-S), Momordica charantia inhibitor, curcin, crotin, saponaria officinalis inhibitor, gelonin, mitegellin, restrictocin, phenomycin, neomycin, and the

trichothecenes) or animals, (*e.g.*, cytotoxic RNases, such as extracellular pancreatic RNases; DNase I, including fragments and/or variants thereof).

[00232] For the purposes of the present disclosure a “chemotherapeutic agent” comprises a chemical compound that non-specifically decreases or inhibits the growth, proliferation, and/or survival of cancer cells (*e.g.*, cytotoxic, or cytostatic agents). Such chemical agents are often directed to intracellular processes necessary for cell growth or division, and are thus particularly effective against cancerous cells, which generally grow and divide rapidly. For example, vincristine depolymerizes microtubules, and thus inhibits cells from entering mitosis. In general, chemotherapeutic agents can include any chemical agent that inhibits, or is designed to inhibit, a cancerous cell or a cell likely to become cancerous or generate tumorigenic progeny (*e.g.*, TIC). Such agents are often administered, and are often most effective, in combination, *e.g.*, in regimens such as CHOP or FOLFIRI.

[00233] Examples of anti-cancer agents that may be used in combination with DLL3-ADCs of the present disclosure (either as a component of a site specific conjugate or in an unconjugated state) include, but are not limited to, alkylating agents, alkyl sulfonates, aziridines, ethylenimines and methylamelamines, acetogenins, a camptothecin, bryostatin, callistatin, CC-1065, cryptophycins, dolastatin, duocarmycin, eleutherobin, pancratistatin, a sarcodictyin, spongistatin, nitrogen mustards, antibiotics, enediyne antibiotics, dynemicin, bisphosphonates, esperamicin, chromoprotein enediyne antibiotic chromophores, aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabycin, carminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, ADRIAMYCIN[®] doxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites, erlotinib, vemurafenib, crizotinib, sorafenib, ibrutinib, enzalutamide, folic acid analogues, purine analogs, androgens, anti-adrenals, folic acid replenisher such as frolic acid, aceglatone, aldophosphamide glycoside, aminolevulinic acid, eniluracil, amsacrine, bestabucil, bisantrene, edatraxate, defofamine, demecolcine, diaziquone, elfornithine, elliptinium acetate, an epothilone, etoglucid, gallium nitrate, hydroxyurea, lentinan, lonidainine, maytansinoids, mitoguazone, mitoxantrone, mopidanmol, nitraerine, pentostatin, phenamet, pirarubicin, losoxantrone, podophyllinic acid, 2-ethylhydrazide, procarbazine, PSK[®] polysaccharide complex (JHS Natural Products, Eugene, OR), razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and

anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, chloranbucil; GEMZAR[®] gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs, vinblastine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; NAVELBINE[®] vinorelbine; novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; irinotecan (Camptosar, CPT-11), topoisomerase inhibitor RFS 2000; difluoromethylornithine; retinoids; capecitabine; combretastatin; leucovorin; oxaliplatin; inhibitors of PKC-alpha, Raf, H-Ras, EGFR and VEGF-A that reduce cell proliferation and pharmaceutically acceptable salts, acids or derivatives of any of the above. Also included in this definition are anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens and selective estrogen receptor modulators, aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the adrenal glands, and anti-androgens; as well as troxacitabine (a 1,3- dioxolane nucleoside cytosine analog); antisense oligonucleotides, ribozymes such as a VEGF expression inhibitor and a HER2 expression inhibitor; vaccines, PROLEUKIN[®] rIL-2; LURTOTECAN[®] topoisomerase 1 inhibitor; ABARELIX[®] rmRH; Vinorelbine and Esperamicins and pharmaceutically acceptable salts, acids or derivatives of any of the above.

Combined Use with Radiotherapies

[00234] The present disclosure also provides for the use of a combination of a DLL3-ADC with a radiotherapy (*e.g.*, any mechanism for inducing DNA damage locally within tumor cells such as gamma-irradiation, X-rays, UV-irradiation, microwaves, electronic emissions and the like). Combination therapy using the directed delivery of radioisotopes to tumor cells is also contemplated, and the disclosed DLL3-ADCs may be used in connection with a targeted anti-cancer agent or other targeting means. Typically, radiation therapy is administered in pulses over a period of time from about 1 to about 2 weeks. The radiation therapy may be administered to subjects having head and neck cancer for about 6 to 7 weeks. Optionally, the radiation therapy may be administered as a single dose or as multiple, sequential doses.

[00235] It is understood that modifications that do not substantially affect the activity of the various embodiments described herein are also provided within the definition of the subject matter described herein. Accordingly, the following examples are intended to illustrate but not limit the present disclosure.

SEQUENCE TABLE SUMMARY

[00236] Appended to the instant application is a sequence table comprising a number of amino acid sequences. The following **Table 3** provides a summary of the included sequences, where the underlined sequences represent the CDRs and the bold underlined lysine (K) residue is the site of modification for conjugating the linker-drug via site specific modification/conjugation.

[00237] Table 3: Sequence Table

SEQ ID NO:	DESCRIPTION	AMINO ACID SEQUENCE
1	CDR1 (of mAbs 300 and 301)	GLTFSTATVG
2	CDR2 (of mAbs 300 and 301)	AIPAYYSTYYASSVKG
3	CDR3 (of mAbs 300 and 301)	DDTPSPSRSPFYKH
4	mAb300	EVQLVESGGG LVQPGGSLRL SCAASGLTFS TATVGWFRQA PGKGRELIAA IPAYYSTYYA SSVKGRFTIS RDNAKNSLYL QMNSLRPEDT AVYYCAADD T PPSRSPFYK HRGQGTMTVT SSDKTHTCPP CPAPELLGGP SVFLFPPKP K DTLMISRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK TKPREEQYNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL PAPIEKTISK AKGQPREPQV YTLPPSREEM TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTTPVL DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQK SLSLSPGK
5	mAb301	EVQLVESGGG LVQPGGSLRL SCAASGLTFS TATVGWFRQA PGKGRELIAA IPAYYSTYYA SSVKGRFTIS RDNAKNSLYL QMNSLRPEDT AVYYCAADD T PPSRSPFYK HRGQGTMTVT SSDKTHTCPP CPAPELLGGP SVFLFPPKP K DTLMISRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK TKPREEQYNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL PAPIEKTISK AKGQPREPQV YTLPPSREEM TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTTPVL DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ KSLSLSPGK
6	Affinity Peptide	Ac-RGN C AYHKQI I W C TYH
7	CDR1 (of mAb302)	GRTFRSYAMG
8	CDR2 (of mAb302)	AISWIGGGTYADSVKG
9	CDR3 (of mAb302)	SLLRHGHMFEEVDY
10	mAb302	QVQLVESGGG VVQPGGSLRL SCAASGRFTS SYAMGWFRQA PGKEREFVAA ISWIGGGTYA ADSVKGRFTI SGDNSKNTLY LQMNSLRAED TAVYYCAASS LLRHGHMFEE SDYWGQGTMTV TVSSDKTHTC PPCAPELLG GPSVFLFPPK P K DTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTISKAKGQPREP QVYTLPPSRE EMTKNQVSLT CLVKGFYPSDIAVEWESNGQ PENNYKTTTP VLDSGSFFL YSKLTVDKSRWQWQGNVFSCS VMHEALHNHY TQKSLSLSPG K

SEQ ID NO:	DESCRIPTION	AMINO ACID SEQUENCE
11	Delta-like protein 3 (Homo sapiens (Human))	MVSPRMSGLLSQTVILALIFLPQTRPAGVFELQIHSFGPGPGPG APRSPCSARLPCLRFVRVCLKPGLSEEAESPICALGAALSARGP VYTEQPGAPAPDLPLPDGLLQVPFRDAWPGTFSFIIETWREELG DQIGGPAWSLLARVAGRRRLAAGGPWARDIQRAGAWELRFSYRA RCEPPAVGTACTRLCRPRSAPSRCPGLRPCAPLEDECEAPLVC RAGCSPEHGFC EQPGECRCLEGWTGPLCTVPVSTSSCLSPRGPS SATTGCLVPGPGPCDGNPCANGGSCSETPRSFECTCPRGFYGLR CEVSGVTCADGPCFNGLCVGGADPDSAYICHCPGFGQGSNCEK RVDRCSLQPCRNGGLCLDLGHALRCRCRAGFAGPRCEHDLDDCA GRACANGGTCVEGGGAHRCSCALGFGGRDCRERADPCAARPCAH GGRCYAHFSGLVACAPGYMGARCEFPVHPDGASALPAAPGLR PGDPQRYLLPPALGLLVAAGVAGAALLLVHVRRRGHSGDAGSRL LAGTPEPSVHALPDALNNLRTQEGSGDGPSSSVVDWNRPEDVDPQ GIYVISAPSIYAREVATPLFPPLHTGRAGQRQHLLFPYPSSILS VK
12	Delta like canonical Notch ligand 3 (Macaca fascicularis (Crab-eating macaque) (Cynomolgus monkey))	MVSPRMSRLLSQTVILALIFIPQARPAGVFELQIHSFGPGPGGA PRSPYSARGPCLRFVRVCLKPGLSEEAESPICALGAALSARGPV YTEQPEAPAPDLPLPNGLLQVPFRDAWPGTFSLIETWREELGD QIGGPAWSLLARVTRRRRLAAGGPWAGDIQRAGAWELRFSYRAR CELPAVGTACTRLCRPRSASRCGPGLRPCAPLEDECEAPPVCRA GCSLEHGFC EQPGECRCLEGWTGPLCMVPVSTSSCLGLRGPSST TTGCLVPGPGPCDGNPCANGGSCSETPGSFECTCPRGFYGLRCE VSGVTCADGPCFNGLCVGGADPDSAYICHCPGFGQGSNCEKRV DRCSLQPCRNGGLCLDLGHALRCRCRAGFAGPRCEHDLNDCAGR ACANGGTCVEGGGAHRCSCALGFGGRDCRERADPCAARPCAHGG RCYAHFSGLVACAPGYMGARCEFPVHPDGVSALPAAPGLRPG DPQRYPLPPALGLLVAAGVAGAALLLVHVRRRGHAQDAGSRLLA GTPEPSVHALPDALNNLRTQEGPGDVPSSSVVDWNRPEDVDSRGI YVISAPSIYAREA
13	Delta-like protein 3 (Mus musculus (Mouse))	MVSLQVSPLSQTLILAFLLPQALPAGVFELQIHSFGPGPGLGTP RSPCNARGPCLRFVRVCLKPGVSQEATESLICALGAALSTSVPVY TEHPGESAAALPLPDGLVVRVPFRDAWPGTFSLVIETWREQLGEH AGGPAWNLLARVVGRRLAAGGPWARDVQRTGTWELHFSYRARC BPPAVGAACARLCRSRSAPSRCPGLRPCPTFPDECEAPSVC RP GCSPHEGYCEEPDECRCLEGWTGPLCTVPVSTSSCLNSRVPGPA STGCLLPGPGPCDGNPCANGGSCSETSGSFECACPRGFYGLRCE VSGVTCADGPCFNGLCVGGEDPDSAYVCHCPGFGQGSNCEKRV DRCSLQPCQNGGLCLDLGHALRCRCRAGFAGPRCEHDLDDCAGR ACANGGTCVEGGGSRRCSCALGFGGRDCRERADPCASRPCAHGG RCYAHFSGLVACAPGYMGVRCEFAVRPDGADAVPAAPRGLRQA DPQRFLLPPALGLLVAAGLAGAALLVIHVRRRGPGQDTGTRLLS GTREPSVHTLPDALNNLRLQDGAGDGPSSSADWNHPEDGDSRSI YVIPAPSIYAREDWLIQVLF
14	mAb300 VHH region	EVQLVESGGG LVQPGGSLRL SCAASGLTFS TATVGWFRQA PGKGRELIAA IPAYYSTYYA SSVKGRFTIS RDNAKNSLYL QMNSLRPEDT AVYYCAADD T PSPSRSPFYK HRGQGTMTVTV SS
15	mAb301 VHH Region	EVQLVESGGG LVQPGGSLRL SCAASGLTFS TATVGWFRQA PGKGRELVAA IPAYYSTYYA SSVKGRFTIS RDNAKNSLYL QMNSLRPEDT AVYYCAADD T PSPSRSPFYK HRGQGTMTVTV SS
16	mAb302 VHH region	QVQLVESGGG VVQPGGSLRL SCAASGRFTFR SYAMGWFRQA PGKEREFVAA ISWIGGGTY Y ADSVKGRFTI SGDNSKNTLY

SEQ ID NO:	DESCRIPTION	AMINO ACID SEQUENCE
		LQMNSLRAED TAVYYCAASS LLRHGHMFEE SDYWGQGTMTV TVSS
17	Amino acid sequence of human IgG1 Fc region	DKTHTCPPCP APELLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTTPVLD S DGSFFLYSKL TVDKSRWQQG NVFSCSV MHE ALHNHYTQKSLSLSPGK

EXAMPLES

Example 1. Preparation of DLL3 Antibodies

Antigen generation

[00238] DNA sequences encoding the extracellular domain (ECD) sequence of cynomolgus DLL3 (Uniprot No. A0A2K5WSR4) and mouse DLL3 (Uniprot No. O88516), were synthesized in Sangon Biotech (Shanghai, China), and then subcloned into modified pcDNA3.3 expression vectors with MBP tag in N-terminal and AVI-His tag or human Fc tag in C-terminal. Human DLL3 (Uniprot No. Q9NYJ7) was purchased from AcroBiosystems (Cat. DL3-H52H4).

[00239] DNA sequences (as disclosed in WO 2017/021349, Bispecific Antibody Constructs Binding D3 and CD3) encoding the truncated isoforms of human DLL3 were synthesized in Sangon Biotech (Shanghai, China), and then subcloned into modified pcDNA3.3 expression vectors with MBP tag and AVI-His tag in C-terminal.

[00240] Expi293 cells (Invitrogen-A14527) were transfected with the purified expression vectors. Cells were cultured for 5 days, and the supernatant was collected for protein purification using Ni-NTA column (GE Healthcare, Cat. 175248) or Protein A column (GE Healthcare, Cat. 175438). The obtained mouse DLL3, cynomolgus DLL3 and truncated human DLL3 were analyzed by SDS-PAGE and SEC, and then stored at -80°C.

[00241] Human DLL3 (ACRO DL3-H52H4) was named as WT115-hPro1.ECD.His. The obtained mouse DLL3 was named as WT115-MBP-mPro1.ECD.hFc. Truncated isoforms of human DLL3 were named WT115-hPro1.V1.ECD.MBP.AVI.His (DSL domain + EGF1-6 domain + membrane proximal), WT115-hPro1.V2.ECD.MBP.AVI.His (EGF1-6 domain + membrane proximal), WT115-hPro1.V3.ECD.MBP.AVI.His (EGF2-6 domain + membrane proximal), WT115-hPro1.V4.ECD.MBP.AVI.His (EGF3-6 domain + membrane proximal), WT115-hPro1.V5.ECD.MBP.AVI.His (EGF4-6 domain + membrane proximal), WT115-

hPro1.V6.ECD.MBP.AVI.His (EGF5-6 domain + membrane proximal), WT115-hPro1.V7.ECD.MBP.AVI.His (EGF6 domain + membrane proximal).

Construction of expression vector of BMK antibodies

[00242] Two DLL3 antibodies were used as benchmark antibodies and referred to as WT115-BMK1 and WT115-BMK2 herein. DNA sequences encoding the variable region of WT115-BMK1 (SEQ ID NO:212 and SEQ ID NO:213 in US 2019/0046656, which is incorporated herein by reference in its entirety) and WT115-BMK2 (SEQ ID NO:37 and SEQ ID NO:38 in WO 2017/021349, which is incorporated herein by reference in its entirety) were synthesized in Sangon Biotech (Shanghai, China), and then subcloned into modified pcDNA3.3 expression vectors encoding an Fc region of human IgG1.

[00243] The plasmid containing VH and VL genes was co-transfected into Expi293 cells. Cells were cultured for 5 days, and the supernatant was collected for protein purification using Protein A column (GE Healthcare, 175438). The obtained antibodies were analyzed by SDS-PAGE and SEC, and then stored at -80°C.

Establishment of Stable Cell Lines/Cell Pool

[00244] Using Lipofectamine 2000, 293F cells were transfected with the expression vector containing a gene encoding full-length human DLL3 (UniProt, Q9NYJ7-1). Flpin293 cells were transfected with the expression vector containing a gene encoding full-length cynomolgus DLL3. Cells were cultured in a medium containing a proper selection marker. Human DLL3 high expression stable cell line (WT115-293F.hPro1.2E5) was selected after limited dilution, and cynomolgus DLL3 high expression stable cell pool (WT115.Flpin293.cPro1.pool) with proper selection antibiotics.

Antibodies Biotinylation

[00245] For NHS-PEO4-Biotinylation, 1-10 mg/mL antibodies (IgG) were incubated with 20-fold molar excess of NHS-PEO4-Biotin reagent at 25 °C for 75 minutes in metal bath or on ice for two hours. Excess biotin was then removed using a desalting spin column, and the purified protein sample was collected from the flow-through solution. The level of biotin incorporation in the protein was determined by HABA assay: dilute the biotinylated sample 10-fold with HABA/Avidin solution and measure the absorbance of the mixed solution at A500. The moles of biotin per mole of protein were calculated based on the A500 value.

Example 2. Production of VHH-comprising DLL3 antibodies

Generation of anti-DLL3 VHHs

[00246] DLL3 antibody VHHs were generated by immunization of Camelidae animals and phage display technology. Briefly, Alpacas (*Vicugna pacos*) were subcutaneously immunized with hFc tagged human DLL3 ECD protein (ACRO, DL3-H5255). After immunization, peripheral blood was collected for construction of a phage library displaying VHH fragments. After bio-panning with corresponding target ECD proteins, the positive VHH clones binding to DLL3 were selected.

VHH sequencing

[00247] The positive *E. coli* clones selected by target specific binding ELISA and FACS with *E. coli* supernatants were sent to Biosune (Shanghai, China) for nucleotide sequencing of VHH gene. The sequencing results were analyzed using CLC Main Workbench (Qiagen, Hilden, Germany). The sequences of three (3) unique positive VHH clones were VHH300, VHH301, and VHH302, shown in Table 4 and Table 5.

[00248] Table 4. Amino acid sequences of the CDR region

VHH	CDR1	CDR2	CDR3
VHH300 and VHH301	SEQ ID NO:1 GLTFSTATVG	SEQ ID NO:2 AIPAYYSTYYASSVKG	SEQ ID NO:3 DDTPSPSRSPFYKH
VHH302	SEQ ID NO:7 GRFTRSYAMG	SEQ ID NO:8 AISWIGGGTYADSVKG	SEQ ID NO:9 SSLLRHGHMFEESD Y

[00249] Table 5. Amino acid sequences of the CDR region

	Amino acid sequence of VHH region	Amino acid sequence of human IgG1 Fc region
VHH300	SEQ ID NO:14 EVQLVESGGGLVQPGGSLRLS CAASGLTFSTATVGWFRQAP GKGRELIAAIPAYYSTYYASS VKGRFTISRDNANKNSLYLQM NSLRPEDTAVYYCAADDTSPSP SRSPFYKHRGQGTMVTVSS	SEQ ID NO:17 DKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYN STYRVVSVLTVLHQDWLNGKEYKC KVSNAKALPAIEKTISKAKGQPREPQ VYTLPPSREEMTKNQVSLTCLVKGF YPSDIAVEWESNGQPENNYKTTTPVL
VHH301	SEQ ID NO:15 EVQLVESGGGLVQPGGSLRLS CAASGLTFSTATVGWFRQAP GKGRELVAAPAYYSTYYASS VKGRFTISRDNANKNSLYLQM NSLRPEDTAVYYCAADDTSPSP SRSPFYKHRGQGTMVTVSS	DSDGSFFLYSKLTVDKSRWQQGNVF SCSVMHEALHNHYTQKSLSLSPGK

	Amino acid sequence of VHH region	Amino acid sequence of human IgG1 Fc region
VHH302	SEQ ID NO:16 QVQLVESGGGVVQPGGSLRL SCAASGRFTFRSYAMGWFRQA PGKEREFVAAISWIGGGTYA DSVKGRFTISGDNSKNTLYLQ MNSLRAEDTAVYYCAASSLL RHGHMFEESDYWGQGTMTV VSS	

Generation of human Fc fusion antibodies comprising the VHHs

[00250] The three unique positive VHH clones were converted to VHH-Fc (hIgG1) fusion antibodies. Briefly, the VHH genes were PCR amplified from the pET-bac vectors using VHH-specific cloning primers containing appropriate restriction sites then cloned by fusion into a modified human hIgG1 expression pcDNA3.3 vector to create corresponding clones of VHH-Fc (hIgG1) chimeric antibody. 293F or Expi293 cells were transiently transfected with the vectors for antibody expression. The cell culture supernatants containing antibodies were harvested and purified using Protein A chromatography. The generated antibodies were named mAb300, mAb301, and mAb302, respectively. The obtained antibodies were analyzed by SDS-PAGE and HPLC-SEC, and then stored at -80°C.

Humanization

[00251] VHH humanization was done by “Best Fit” approach. Briefly, amino acid sequences of VHH framework regions were blasted against human germline V-gene database, and humanized VHH sequences were generated by replacing human CDR sequences in the top hit with VHH CDR sequences using Kabat CDR definition. Then key residues in framework which play an important role in antibody affinity or developability were back mutated to parental residues alone or in combination. The variants were codon optimized for mammalian expression and then synthesized by GENEWIZ (SuZhou, China). The designed VHH variants and parental VHH proteins were cloned into human IgG1 expression vectors to generate human IgG1 constructs. Antibodies were produced in HEK293 cells and purified using Protein A chromatography. The variants with desired affinity were finally selected as the humanized leads.

Example 3: Characterization of DLL3-binding antibodies

[00252] Human DLL3 binding by FACS: WT115-293F.hPro1.2E5 cells (1×10^5 cells/well) were incubated with various concentrations of antibodies (5-fold serially diluted from 200 nM to 0.0128 nM) at 4 °C for 1 hour. After washing with 1×PBS/1%BSA, a secondary

antibody, R-PE-labeled goat anti-human IgG (1:150, Jackson ImmunoResearch, 109-115-098), was added and incubated with cells at 4 °C in dark for 1 hour. Anti-human DLL3 antibodies WT115-BMK1 and WT115-BMK2 were used as positive controls. Human IgG1 isotype antibody was used as a negative control. The cells were washed and resuspended in 4% paraformaldehyde. MFI of the cells was measured by a flow cytometer and analyzed by FlowJo.

[00253] Cynomolgus monkey DLL3 binding by FACS: WT115-Flpin293.cPro1.pool cells (1×10^5 cells/well) were incubated with various concentrations of antibodies (4-fold serially diluted from 10 nM to 0.00061 nM) at 4 °C for 1 hour. After washing with 1×PBS/1%BSA, a secondary antibody, Alexa Fluor 647-labeled goat anti-human IgG (1:150, Jackson ImmunoResearch, 109-605-098), was added and incubated with cells at 4 °C in dark for 1 hour. Anti-human DLL3 antibodies WT115-BMK1 and WT115-BMK2 were used as positive controls. Human IgG1 isotype antibody was used as a negative control. The cells were washed with 1×PBS/1%BSA, resuspended in 4% paraformaldehyde, and incubated at 4 °C in dark for 0.5 hour. Then the buffer was changed to 1×PBS/1%BSA and the cells were filtered. MFI of the cells was measured by a flow cytometer and analyzed by FlowJo.

[00254] Mouse DLL3 binding by ELISA: Plates were pre-coated with 1 µg/mL, 100 µL per well of WT115-MBP-mPro1.ECD.hFc at 4 °C overnight. The antigen was diluted in coating buffer from stock solution. Next day, the plates were washed using 1×PBST for one time, and blocking was done by adding 200 µL of 1×PBS/2%BSA. Antibodies were serially diluted (5-fold serially diluted from 20 nM to 0.000256 nM) in blocking buffer. After 1-hour blocking, the plates were washed using 1×PBST for 3 times, and then the antibody was added to the plates and incubated at ambient temperature for 1 hour. Anti-human DLL3 antibodies WT115-BMK1-Biotin and WT115-BMK2-Biotin were used as positive controls. WT114-BMK1-Biotin antibody was used as a negative control. Binding of antibodies to the immobilized mouse DLL3 was detected by HRP-labeled secondary antibody (Invitrogen, SNN1004), which was diluted in 1×PBS/2%BSA at a concentration of 1:30000. After incubation, the plates were washed using 1×PBST for 6 times. The color was developed by dispensing 100 µL of TMB substrate, and then reaction was stopped by adding 100 µL of 2M HCl. Absorbance was read at 450nm and 540nm using a microplate spectrophotometer. All samples were tested in duplicate.

[00255] Internalization: WT115-293F.hPro1.2E5 cells (4×10^4 cells/well) were plated in 96-well plate and the medium was removed from the plate after centrifuge. $1 \times$ final maximum concentration of primary antibodies (5-fold serially diluted from 40 nM to 0.00256 nM, or 5-

fold serially diluted from 200 nM to 0.0128 nM) and pHrodo (amine reactive, Thermo Fisher, P36011) labeled second antibodies (Affinipure F(ab')₂ fragment goat anti-human IgG, Jackson ImmunoResearch, 109-006-098, Ratio = 1:1 molecule) dilutions were prepared and added into plates with cell culture medium and the plates were incubated at 37°C for 5 hours. Anti-human DLL3 antibodies WT115-BMK1 and WT115-BMK2 were used as positive controls. Human IgG1 isotype antibody was used as a negative control. After incubation, cells were stained with reagent (cell nucleus-Hoechst33342, 1000 ng/mL; cytoplasm-Calcein AM, 1:2000 dilution in DPBS) and the plate was incubated at 37 °C for 15 mins. Finally, cells were photographed with Operatta CLS and the antibody endocytosis was analyzed by parameter of “Spots per cell.”

[00256] The data of mAb301 and two WT BMK antibodies is summarized in **Table 6**.

[00257] **Table 6. Characterization summary of mAb301.**

Antibody name	Human DLL3 binding FACS (EC ₅₀ , nM)	Cyno DLL3 Binding FACS (EC ₅₀ , nM)	Mouse DLL3 Binding ELISA (EC ₅₀ , nM)	Internalization (EC ₅₀ , nM)
mAb301	0.14	0.096	0.0075	12.2
WT115-BMK1	0.03	0.019	0.0039	3.47
WT115-BMK2	0.52	0.20	0.014	14.4

[00258] *Kinetic binding affinity of DLL3 antibodies:* The binding affinity of DLL3 antibodies to human DLL3 ECD protein was detected by SPR assay using Biacore T200. Each antibody tested was captured on an anti-human IgG Fc antibody immobilized CM5 sensor chip (GE). WT115-hPro1.ECD.His at different concentrations were injected over the sensor chip at a flow rate of 30 µL/min for an association phase of 180 seconds, followed by 3600 seconds dissociation. The chip was regenerated by 10 mM glycine (pH 1.5) after each binding cycle.

[00259] As shown in **Table 7**, the experimental data to human DLL3 was fitted by steady state affinity model. The experimental data of WT-115-BMK1 to human DLL3 was fitted by heterogeneous ligand model. The other experimental data was fitted by 1:1 model using Langmuir analysis. The sensorgrams of blank surface and buffer channel were subtracted from the test sensorgrams. Molecular weight of 34 KDa was used to calculate the molar concentration of analyte. The affinities of the tested antibodies to human DLL3 are shown in **Table 7**.

[00260] **Table 7. Binding kinetics of DLL3 antibodies.**

Antibody name	k _a (1/Ms)	k _d (1/s)	KD (M)
mAb301	1.85E+05	3.06E-05	1.65E-10

mAb302	8.02E+04	1.74E-05	2.17E-10
WT115-BMK1 (heterogeneous ligand)	2.10E+06	2.65E-03	1.26E-09
	1.73E+06	1.50E-04	8.67E-11
WT115-BMK2	4.04E+05	3.98E-05	9.87E-11

Human Serum Stability

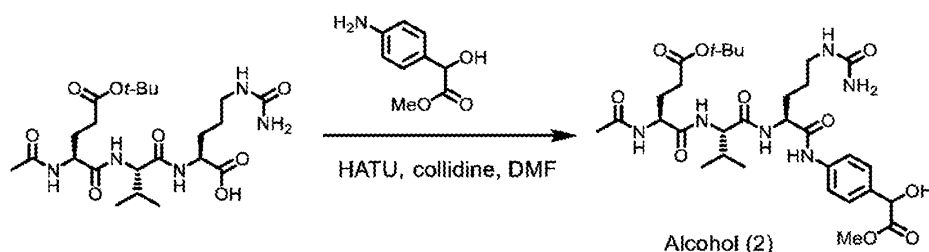
[00261] Human serum was freshly isolated from healthy donors by centrifugation. The samples were diluted in serum and serum volume accounted for more than 90% of total volume. Five aliquots of the sample were incubated at 37 °C. Samples were then collected at day 0, day 1, day 4, day 7 and day 14, respectively and quick-frozen until analysis together.

[00262] The stability of the samples was tested by binding to human DLL3 using ELISA. Briefly, plates were pre-coated with 100 μ L /well of 1 μ g/mL WT115-hPro1.ECD.his (in house) at 4°C overnight. Next day, the plates were washed using 1 $\times$ PBST (PBS containing 0.05% tween-20) for one time, and blocking was done by adding 200 μ L of 1 $\times$ PBS/2%BSA per well. During blocking, testing antibodies were added to the plates at various concentrations (4-fold serially diluted from 3 nM to 0.00018 nM). The plates were incubated at ambient temperature for 1 hour. Binding of antibodies to the immobilized human DLL3 was detected by Goat anti-human IgG-Fc Fragment Cross-absorbed Antibody HRP (Bethyl, A80-304P) and Mouse IgG-Fc Fragment cross-adsorbed Antibody HRP (Bethyl, A90-231P), which were diluted in 1 $\times$ PBS/2%BSA at 1:5000. After incubation, the plates were washed using 1 $\times$ PBST for 6 times. The color was developed by dispensing 100 μ L of TMB substrate, and then reaction was stopped by adding 100 μ L of 2M HCl. Absorbance was read at 450 nm and 540 nm using a microplate spectrophotometer. Human IgG1 isotype antibody was used as a negative control. All samples were tested in duplicate. After incubation in human serum at 37 °C for up to 14 days, the binding profile of mAb301 to human DLL3 protein did not change (data not shown).

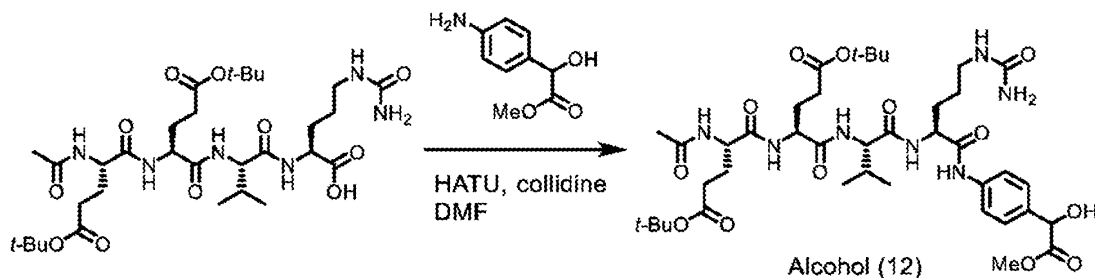
Example 4. Synthesis of Linker-Drug (1)

[00263] Linker-Drug (1) was prepared according to the following procedures:

Synthesis of Intermediate (2)



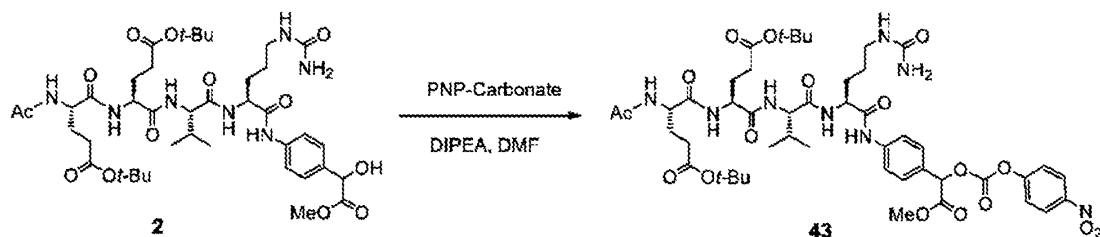
[00264] Ac-Glu(OtBu)-Val-Cit-OH (19.9 mg, 39.7 μ mol) was dissolved in N,N-dimethylformamide (400 μ L); 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (18.1 mg, 47.6 μ mol), and 2,4,6-trimethylpyridine (6.27 μ L, 47.6 μ mol) were added thereto; and the mixture was stirred at room temperature for 10 minutes. Next, methyl 4-aminomandelate (8.63 mg, 47.6 μ mol) was added, and the mixture was stirred at room temperature for 21.5 hours, then purified via reverse-phase fractionating chromatography. The fraction containing the product was recovered, vacuum-concentrated to remove acetonitrile, and lyophilized to obtain the alcohol (2) (28.5 mg, quant). ^1H NMR (400 MHz, DMSO- d_6) δ 9.95 (s, 1H), 8.07 (d, J = 7.4 Hz, 1H), 7.99 (d, J = 8.0 Hz, 1H), 7.66 (d, J = 8.4 Hz, 1H), 7.50 (d, J = 8.4 Hz, 2H), 7.25 (d, J = 8.4 Hz, 2H), 5.92 (brs, 1H), 5.36 (brs, 2H), 5.01 (s, 1H), 4.34-4.29 (m, 1H), 4.26-4.20 (m, 1H), 4.14-4.10 (m, 1H), 3.53 (s, 3H), 3.00-2.83 (m, 2H), 2.18-2.13 (m, 2H), 1.94-1.89 (m, 2H), 1.84-1.23 (m, 17H), 0.79 (d, J = 6.8 Hz, 3H), 0.75 (d, J = 6.8 Hz, 3H). MS (ESI) m/z : 665.30 $[\text{M}+\text{H}]^+$



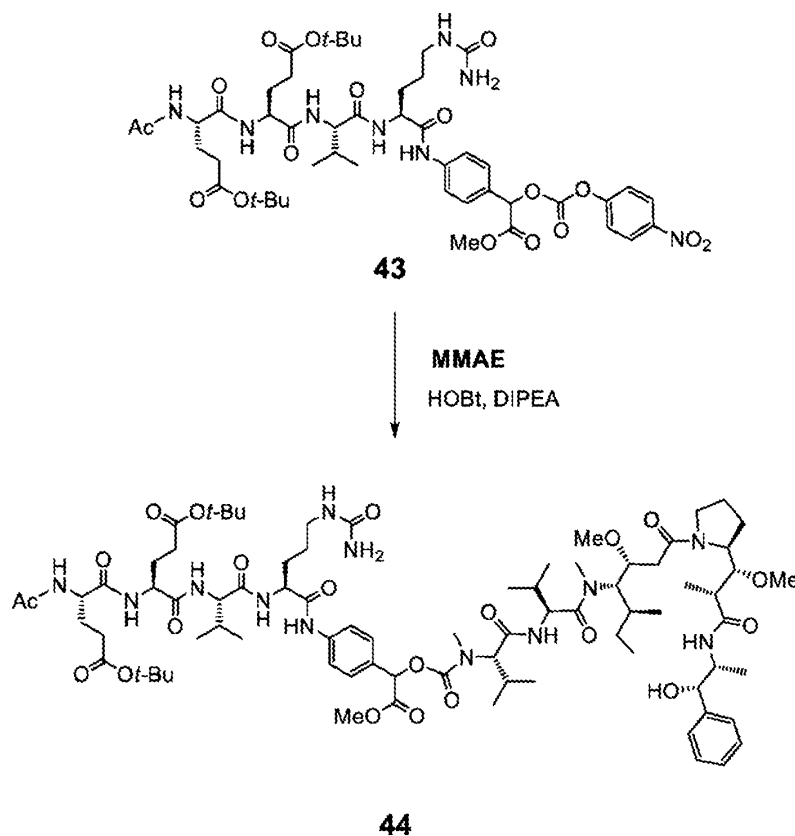
[00265] Ac-Glu(t-Bu)-Glu(t-Bu)-Val-Cit-OH (50.0 mg, 72.8 μ mol) was dissolved in N,N-dimethylformamide (800 μ L), 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (33.2 mg, 87.4 μ mol), and 2,4,6-trimethylpyridine (11.5 μ L, 87.4 μ mol) were added thereto, and the mixture was stirred at room temperature for 10 minutes. Next, methyl 4-aminomandelate (15.8 mg, 87.4 μ mol) was added, and the mixture was stirred at room temperature for 16 hours, then purified via reverse-phase fractionating chromatography. A product-containing fraction was recovered, vacuum-concentrated to remove acetonitrile, and lyophilized to obtain the alcohol (12) (54.0 mg, 63.5 μ mol).

[00266] ^1H NMR (400 MHz, DMSO- d_6) δ 10.00 (s, 1H), 8.26-7.88 (m, 3H), 7.68-7.60 (m, 1H), 7.57 (d, J = 8.4 Hz, 2H), 7.32 (d, J = 8.4 Hz, 2H), 6.00-5.97 (m, 1H), 5.43 (brs, 2H), 5.08 (s, 1H), 4.40-4.37 (m, 1H), 4.32-4.19 (m, 3H), 3.60 (s, 3H), 3.09-2.90 (m, 2H), 2.25-2.18 (m, 4H), 2.03-1.53 (m, 10H), 1.46-1.36 (m, 20H), 0.86 (d, J = 6.8 Hz, 3H), 0.82 (d, J = 6.8 Hz, 3H).

[00267] MS (ESI) m/z : 850.40 $[\text{M}+\text{H}]^+$

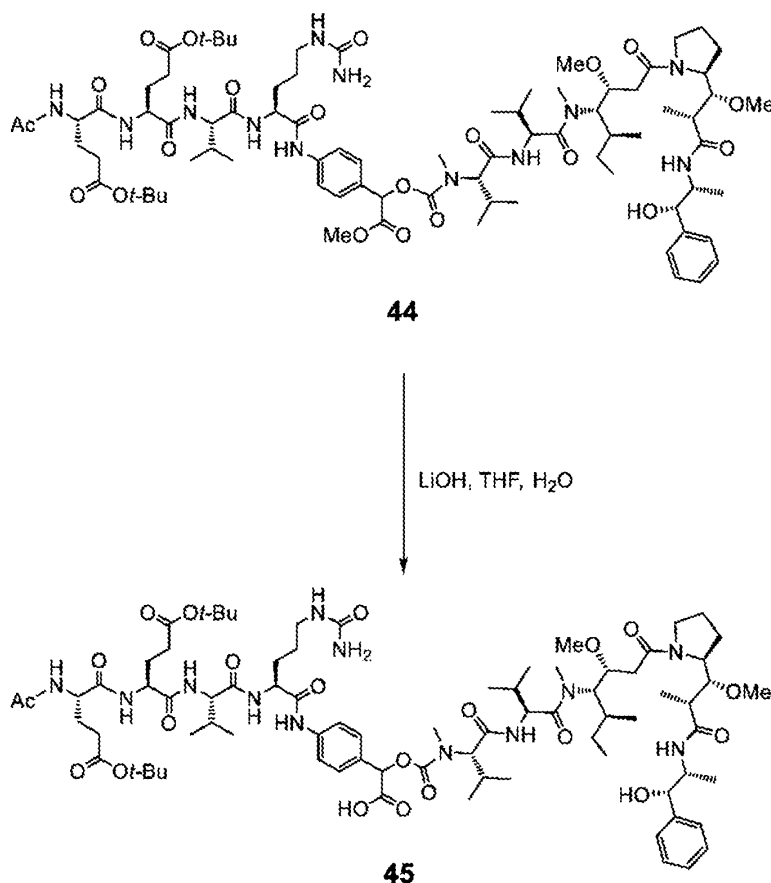
Synthesis of Intermediate (43)

[00268] Intermediate (**2**) (140 mg, 0.165 mmol) was dissolved in N,N-dimethylformamide (4 mL), and the mixture was stirred while being ice-cooled for five minutes, after which bis(4-nitrophenyl) carbonate (108 mg, 0.355 mmol) and N,N-diisopropylethylamine (100 μ L, 0.574 mmol) were added thereto, and the mixture was stirred for 18 hours in a nitrogen atmosphere at room temperature. The organic solvent was removed via evaporator, after which a 1:1 solution of acetonitrile and water was added thereto, and the mixture was purified via reverse-phase fractionating chromatography. A product-containing fraction was recovered, vacuum-concentrated to remove acetonitrile, and lyophilized to obtain Intermediate (**43**) (130 mg, 0.128 mmol). MS (ESI) m/z : 1015.6 $[M+H]^+$

Synthesis of Intermediate (44)

[00269] Intermediate (43) (85 mg, 0.084 mmol) was dissolved in N,N-dimethylformamide (2 mL), and 1-hydroxybenzotriazole (20 mg, 0.13 mmol) and commercially available monomethyl auristatin E (MMAE, 63 mg, 0.088 mmol) were added thereto at room temperature. Next, diisopropylethylamine (75 μ L, 0.43 mmol) was added thereto, after which the mixture was stirred for 23 hours in a nitrogen atmosphere at room temperature. The organic solvent was removed via evaporator, after which a 1:1 solution of acetonitrile and water was added thereto, and the mixture was purified via reverse-phase fractionating chromatography. A product-containing fraction was recovered, vacuum-concentrated to remove acetonitrile, and lyophilized to obtain Intermediate (44) (94 mg, 0.059 mmol). MS (ESI) m/z : 1593.6 $[M+H]^+$

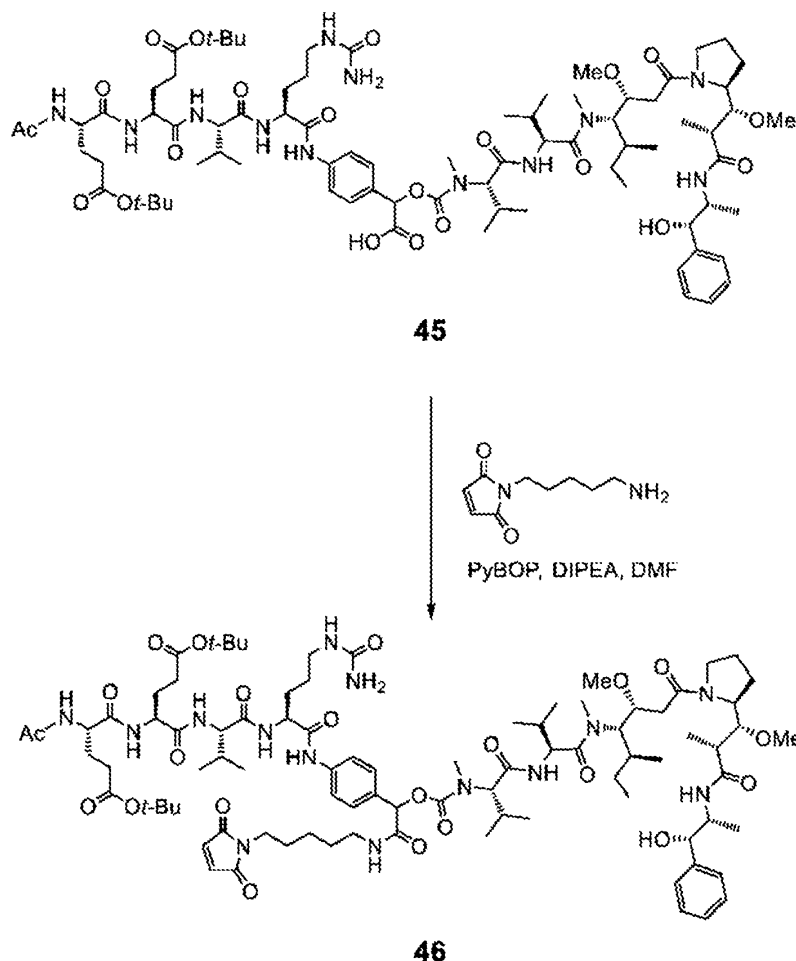
Synthesis of Intermediate (45)



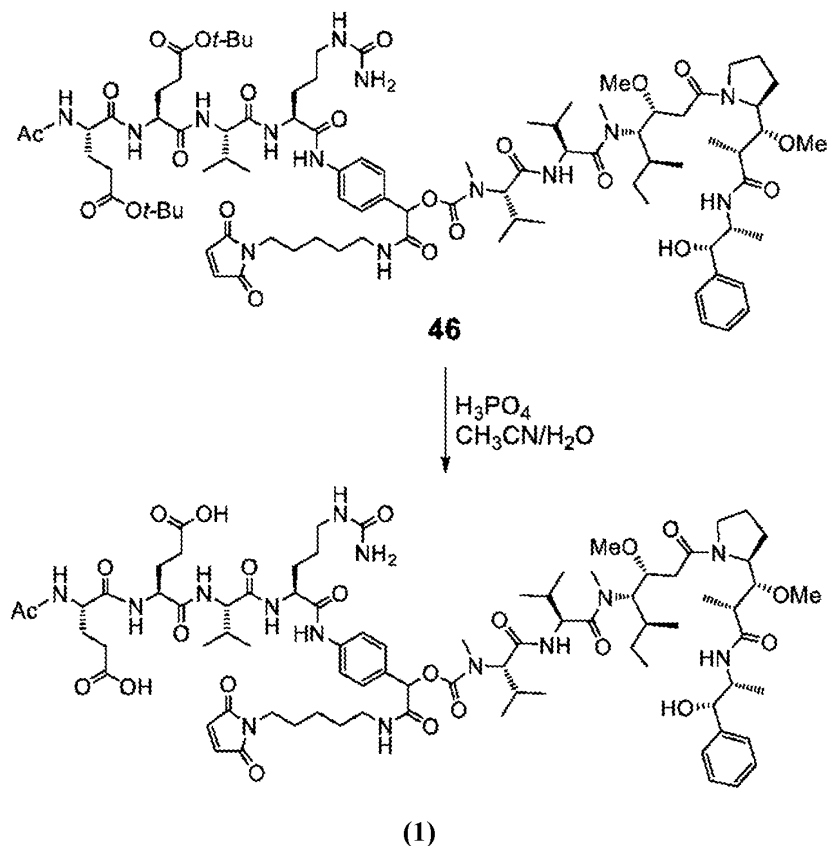
[00270] Intermediate (44) (94 mg, 0.059 mmol) was dissolved in tetrahydrofuran (5 mL) and water (2 mL), lithium hydroxide (1.0 M, 0.6 mL, 0.6 mmol) was added thereto while the mixture was being ice-cooled, and the mixture was stirred in that state for 1 hour. Hydrochloric acid was added to the reaction solution to adjust the pH to 5, after which a 1:1

mixture of acetonitrile and water was added thereto, and the mixture was purified via reverse-phase fractionating chromatography. A product-containing fraction was recovered, vacuum-concentrated to remove acetonitrile, and lyophilized to obtain Intermediate (45) (77 mg, 0.049 mmol). MS (ESI) m/z : 1579.7 $[M+H]^+$

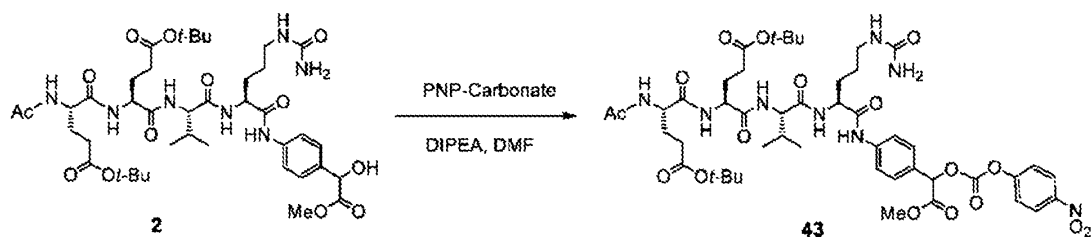
Synthesis of Intermediate (46)



[00271] Intermediate (45) (77 mg, 0.049 mmol) was dissolved in N,N-dimethylformamide (3 mL), then ice-cooled, and N,N-diisopropylethylamine (50 μ L, 0.29 mmol) and 1H-benzotriazole-1-yloxytripyrrolidinophosphonium hexafluorophosphate (87 mg, 0.17 mmol) were added thereto. Next, N-(5-aminopentyl) maleimide hydrochloride (35 mg, 0.16 mmol) was added thereto, and the mixture was returned to room temperature and stirred for 20 hours. Once the reaction was finished, purification was performed via reverse-phase fractionating chromatography. A product-containing fraction was recovered, vacuum-concentrated to remove acetonitrile, then lyophilized to obtain Intermediate (46) (65 mg, 0.037 mmol). MS (ESI) m/z : 1744.7 $[M+H]^+$

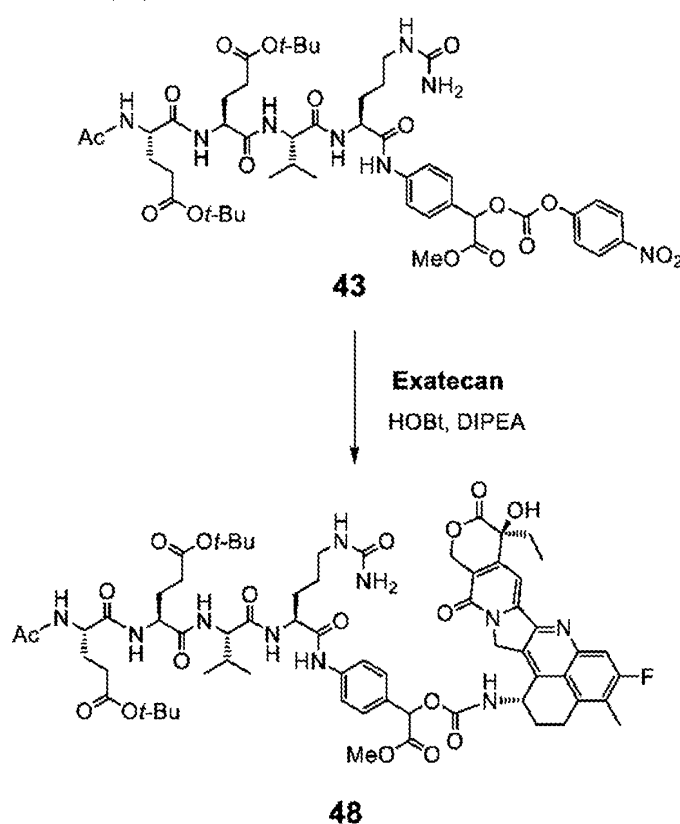
Conversion of Intermediate (46) to Linker-Drug (1)

[00272] Acetonitrile (2 mL) and an 85 wt. % aqueous solution of phosphoric acid (1.00 mL, 14.6 mmol) were sequentially added to compound (**46**) (65 mg, 0.037 mmol), and the mixture was stirred at room temperature for 6 hours. Once the reaction was complete, water (2 mL) was added thereto, the reaction solution was purified via reverse-phase fractionating chromatography, and a product-containing fraction was recovered and vacuum-concentrated to remove acetonitrile, then lyophilized to obtain **Linker-Drug (1)** (49 mg, 0.030 mmol). MS (ESI) m/z : 1631.6 $[\text{M}+\text{H}]^+$

Example 5. Synthesis of Linker-Drug (2)Synthesis of Intermediate (43)

[00273] Intermediate (2) (140 mg, 0.165 mmol) prepared as described in Example 1 was dissolved in N,N-dimethylformamide (4 mL), and the mixture was stirred while being ice-cooled for five minutes, after which bis(4-nitrophenyl) carbonate (108 mg, 0.355 mmol) and N,N-diisopropylethylamine (100 μ L, 0.574 mmol) were added thereto, and the mixture was stirred for 18 hours in a nitrogen atmosphere at room temperature. The organic solvent was removed via evaporator, after which a 1:1 solution of acetonitrile and water was added thereto, and the mixture was purified via reverse-phase fractionating chromatography. A product-containing fraction was recovered, vacuum-concentrated to remove acetonitrile, and lyophilized to obtain compound 43 (130 mg, 0.128 mmol). MS (ESI) m/z: 1015.6 $[M+H]^+$

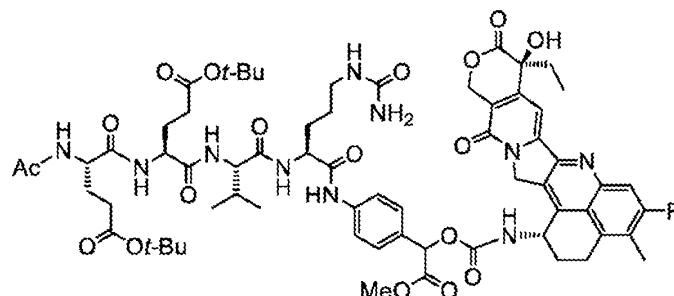
Synthesis of Intermediate (48)



[00274] Intermediate (43) (67 mg, 0.066 mmol) was dissolved in N,N-dimethylformamide (2 mL), and 1-hydroxybenzotriazole (17 mg, 0.11 mmol) and commercially available Exatecan mesylate (CAS: 169869-90-3, 35 mg, 0.066 mmol) were added thereto at room temperature. Next, diisopropylethylamine (50 μ L, 0.29 mmol) was added thereto, after which the mixture was stirred for 4 hours in a nitrogen atmosphere at room temperature. The organic solvent was removed via evaporator, after which a 1:1 solution of acetonitrile and water was added thereto, and the mixture was purified via reverse-phase fractionating

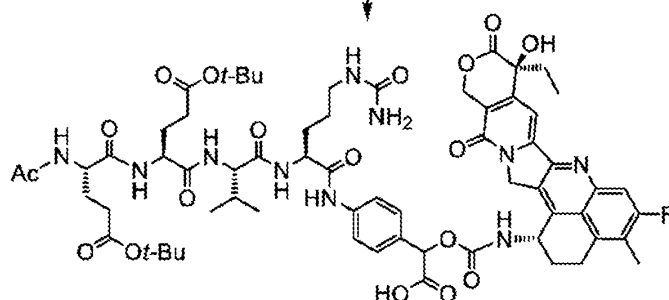
chromatography. A product-containing fraction was recovered, vacuum-concentrated to remove acetonitrile, and lyophilized to obtain compound 48 (57 mg, 0.043 mmol). MS (ESI) m/z : 1311.7 $[M+H]^+$

Synthesis of Intermediate (49)



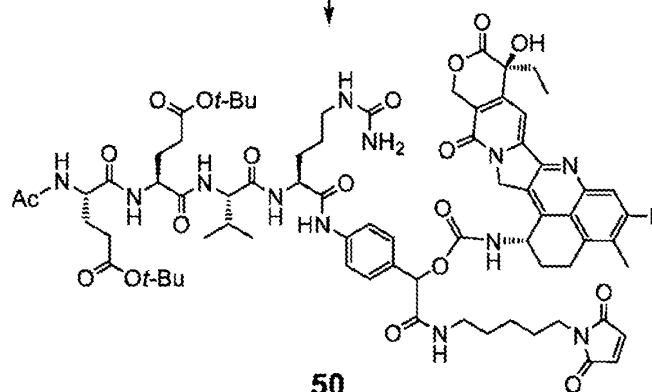
48

LiOH, THF, H₂O

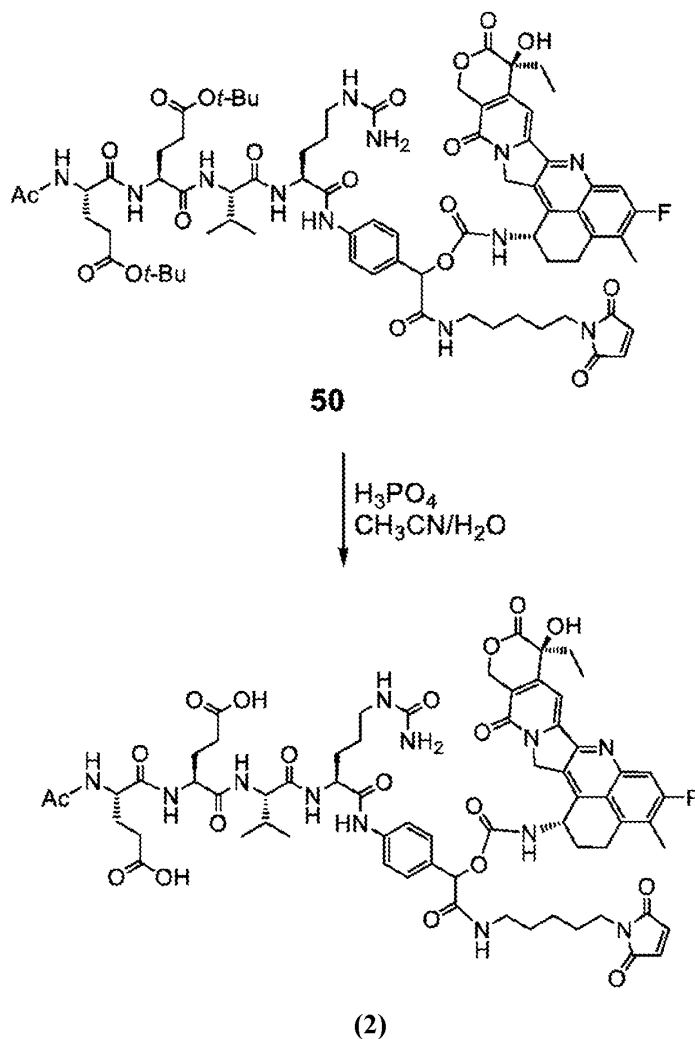


49

[00275] Intermediate (**48**) (57 mg, 0.043 mmol) was dissolved in tetrahydrofuran (3 mL) and water (1.5 mL), lithium hydroxide (1.0M, 0.5 mL, 0.5 mmol) was added thereto while the mixture was being ice-cooled, and the mixture was stirred in that state for 1 hour. Hydrochloric acid was added to the reaction solution to adjust pH to 5, after which a 1:1 mixture of acetonitrile and water was added thereto, and the mixture was purified via reverse-phase fractionating chromatography. A product-containing fraction was recovered, vacuum-concentrated to remove acetonitrile, and lyophilized to obtain compound 49 (45 mg, 0.035 mmol). MS (ESI) m/z : 1297.6 $[M+H]^+$

[illegible]NC(CCCCN)C1=CC=C(C=C1)C(=O)N2C=CC(=O)C=C2
PyBOP, DIPEA, DMF

117

Conversion of Intermediate (50) to Linker-Drug (2)

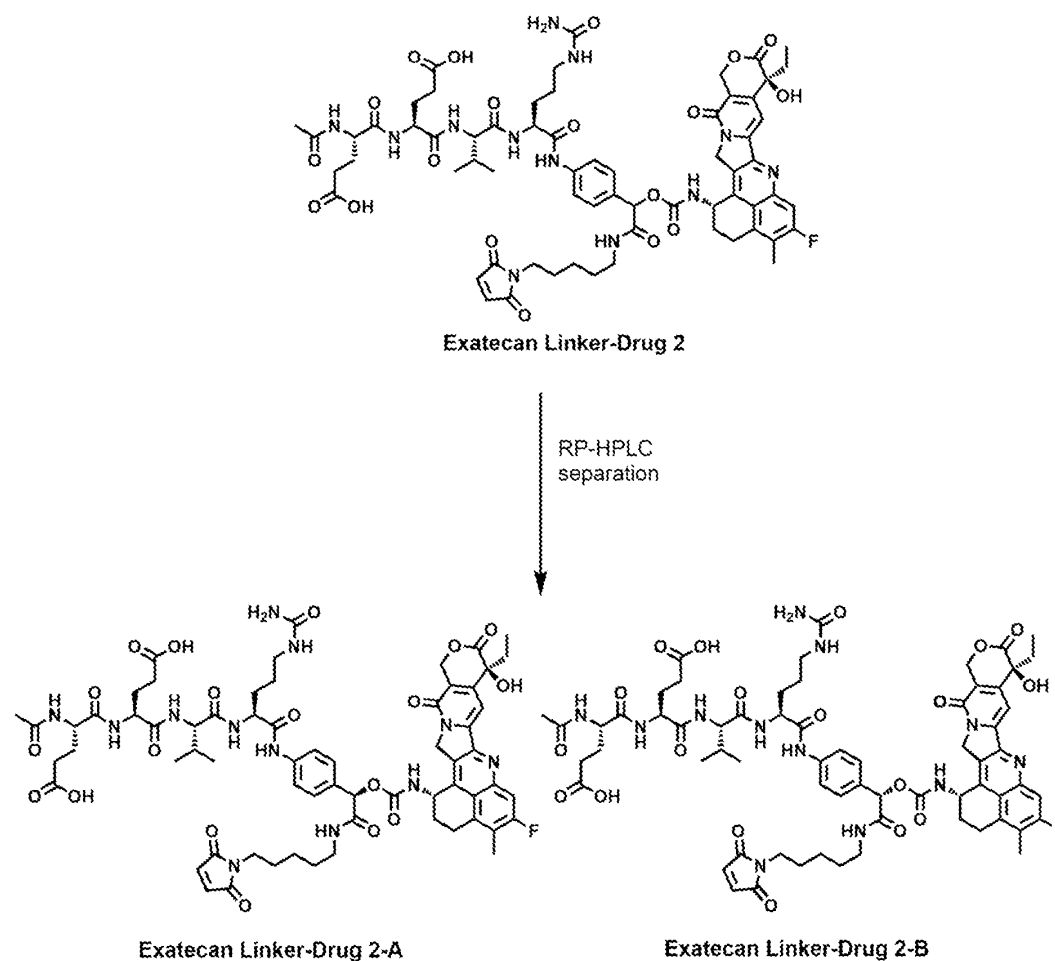
[00277] Acetonitrile (1 mL) and an 85 wt. % aqueous solution of phosphoric acid (1.0 mL, 14.6 mmol) were sequentially added to intermediate (**50**) (22 mg, 0.015 mmol), and the mixture was stirred at room temperature for 1.5 hours. Once the reaction was complete, water (1 mL) was added thereto, the reaction solution was purified via reverse-phase fractionating chromatography, and a product-containing fraction was recovered and vacuum-concentrated to remove acetonitrile, then lyophilized to obtain **Linker-Drug (2)** (18.8 mg, 0.0139 mmol). ^1H NMR (300 MHz; DMSO-d_6) δ 10.04 (s, 1H), 8.10-8.05 (m, 4H), 7.80-7.77 (m, 2H), 7.59-7.55 (m, 2H), 7.35-7.30 (m, 3H), 6.99 (d, $J = 8.8$ Hz, 2H), 6.54-6.53 (m, 1H), 6.01 (brs, 1H), 5.74 (d, $J = 9.0$ Hz, 1H), 5.43 (brs, 4H), 5.33-5.28 (m, 2H), 4.29-4.15 (m, 4H), 3.19-2.98 (m, 5H), 2.43-2.38 (m, 5H), 2.27-2.20 (m, 7H), 1.95-1.83 (m, 8H), 1.73-1.62 (m, 4H), 1.42-1.30 (m, 7H), 1.23-1.13 (m, 3H), 0.84 (m, 9H). MS (ESI) m/z : 1349.2 $[\text{M}+\text{H}]^+$

Example 6: Chromatographic Separation of Linker-Drug (1) Form A and Form B

[00278] Linker-Drug (1) is purified via reverse-phase fractionating chromatography on a C18 silica gel column (20X 150 mm, 5 μ m) by gradient conditions (mobile phase A: 0.1% TFA and 99.9% water, mobile phase B: 0.1% TFA and 99.9% MeCN). Each product-containing fractions (Linker-Drug (1) Form A, which is also referred to herein as Linker-Drug (1-A); and Linker-Drug (1) Form B, which is also referred to herein as Linker-Drug (1-B)) are recovered and vacuum-concentrated to remove acetonitrile, then are lyophilized to obtain Linker-Drug (1-A) and Linker-Drug (1-B) respectively.

[00279] RP-HPLC is further performed to analyze the produced Linker-Drugs. In brief, the analysis is conducted using an XBridge Shield RP 18, 4.6 x 150 mm, 3.5 μ m (Waters catalog# 186003045), on an Agilent 1260 HPLC system. This system is equipped with a binary gradient pump, a temperature-controlled column compartment, an autosampler, and a diode array detector. The operational conditions are set as follows: a flow rate of 1.0 mL/min at 50 °C, with mobile phase A (MPA) comprising 0.1% trifluoroacetic acid (TFA) in water, and mobile phase B (MPB) containing 0.1% TFA in acetonitrile. Other elution conditions can be found in **Table 8** below. UV at 215 nm, 252 nm, and 280 nm are used to detect the sample signals.

Example 7: Chromatographic Separation of Linker-Drug (2) (mixture) to Linker-Drug (2) Form A and Form B



[00280] Linker-Drug (2) (75 mg) was purified via reverse-phase fractionating chromatography on a C18 silica gel column (20X 150 mm, 5 μ m) by gradient conditions (mobile phase A: 0.1% TFA and 99.9% water, mobile phase B: 0.1% TFA and 99.9% MeCN). Each product-containing fractions (Linker-Drug (2) Form A, which is also referred to herein as Linker-Drug (2-A); and Linker-Drug (2) Form B, which is also referred to herein as Linker-Drug (2-B)) were recovered and vacuum-concentrated to remove acetonitrile, then lyophilized to obtain Linker-Drug (2-A) (40 mg) and Linker-Drug (2-B) (23 mg) respectively.

[00281] Linker-Drug (2-A): MS (ESI) m/Z: 1349.5 $[M=H]^+$

[00282] Linker-Drug (2-B): MS (ESI) m/Z: 1349.5 $[M=H]^+$

[00283] RP-HPLC was further performed to analyze the produced Linker-Drugs. In brief, the analysis was conducted using an XBridge Shield RP 18, 4.6 x 150 mm, 3.5 μ m (Waters

catalog# 186003045), on an Agilent 1260 HPLC system. This system was equipped with a binary gradient pump, a temperature-controlled column compartment, an autosampler, and a diode array detector. The operational conditions were set as follows: a flow rate of 1.0 mL/min at 50 °C, with mobile phase A (MPA) comprising 0.1% trifluoroacetic acid (TFA) in water, and mobile phase B (MPB) containing 0.1% TFA in acetonitrile. Other elution conditions can be found in **Table 8** below. UV at 215 nm, 252 nm, and 280 nm was used to detect the sample signals.

[00284] **Table 8. RP-HPLC Elution Conditions**

Time (min)	A (%)	B (%)
0.0	95	5
5.0	60	40
15.0	60	40
20.0	10	90
25.0	10	90
25.1	95	5
31.0	95	5

[00285] An exemplary result is presented in **Figure 2**, showing the separation of the two linker-drug diastereomers overlaid on the diastereomeric mixture chromatogram.

Example 8: Synthesis of Linker-Drug (2) Form B

[00286] Linker-Drug (2-B) (the (S) epimer) as illustrated in **Figure 3** was also produced. Linker-Drug (2-B) is a synthetic derivative of Exatecan, a cytotoxic agent of the camptothecin family, containing a cleavable peptide linker (EEVC) and antibody conjugation site Maleimide-C₅-amine which are attached to *p*-amino mandelic acid fragment. Linker-Drug (2-B) was synthesized (for example, as described herein in **Example 5**), using stereospecific intermediates. Linker-Drug (2-B) (0.54 g, 86 % yield) was obtained. MS (ESI, pos.): Calculated for C₆₅H₇₇FN₁₂O₁₉ [M+H]:1350.37, Found: 1350.30. NMR was further performed using the obtained Linker-Drug (2-B) (in DMSO) (data not shown).

Example 9: Stochastic Conjugation through Reduction of Disulfides to make DLL3-ADCs (*see, e.g., Jackson, David, Organic Process Research & Development* 2016, 20, 852-866, incorporated herein by reference in its entirety, and Tsuchikama, K. et al., *Protein & Cell* 2018, 9(1), 33-46, incorporated herein by reference in its entirety)

[00287] This example provides an exemplary protocol for reduction of the disulfides of the antibodies disclosed herein, and conjugation of the reduced antibodies to the linker-drug conjugates, *e.g.*, Linker-Drug (1), (1-A), (1-B), (2), (2-A), or (2-B), described herein.

Step 1: Antibody Disulfide Reduction

- a) Dilute antibody to 15 mg/mL (0.1 mM IgG) in PBS (pH 7.4);
- b) Prepare a fresh 20 mM (5.7 mg/mL) stock solution of tris(2 carboxyethyl)phosphine (TCEP) in H₂O;
- c) Add 25 μ L of TCEP stock solution to 1 mL of antibody from 1a (0.5 mM final concentration TCEP);
- d) Incubate at 37 °C for 2 hours (check for free thiols using 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) test); and
- e) Aliquot the reduced antibody into 4 tubes (250 μ L each).

Step 2: Linker-Drug Conjugation to Antibody

- a) Prepare a 10 mM stock solution of linker-cytotoxin conjugate in DMSO (DMA, DMF or CH₃CN are also acceptable);
- b) Add 5 equivalents of 12.5 μ L stock solution to each tube of reduced antibody (0.5 mM final concentration linker-cytotoxin conjugate stock solution);
- c) Incubate overnight at 4 °C for 4 hours at room temperature; check for free thiols using DTNB test; and
- d) Run analytical HIC to determine DAR and homogeneity.

Example 10: Stochastic Conjugation through Reduction of Disulfides to make DLL3-ADCs (*see, e.g.,* Jackson, David, *Organic Process Research & Development* 2016, 20, 852-866, incorporated herein by reference in its entirety, and Tsuchikama, K. et al., *Protein & Cell* 2018, 9(1), 33-46, incorporated herein by reference in its entirety)

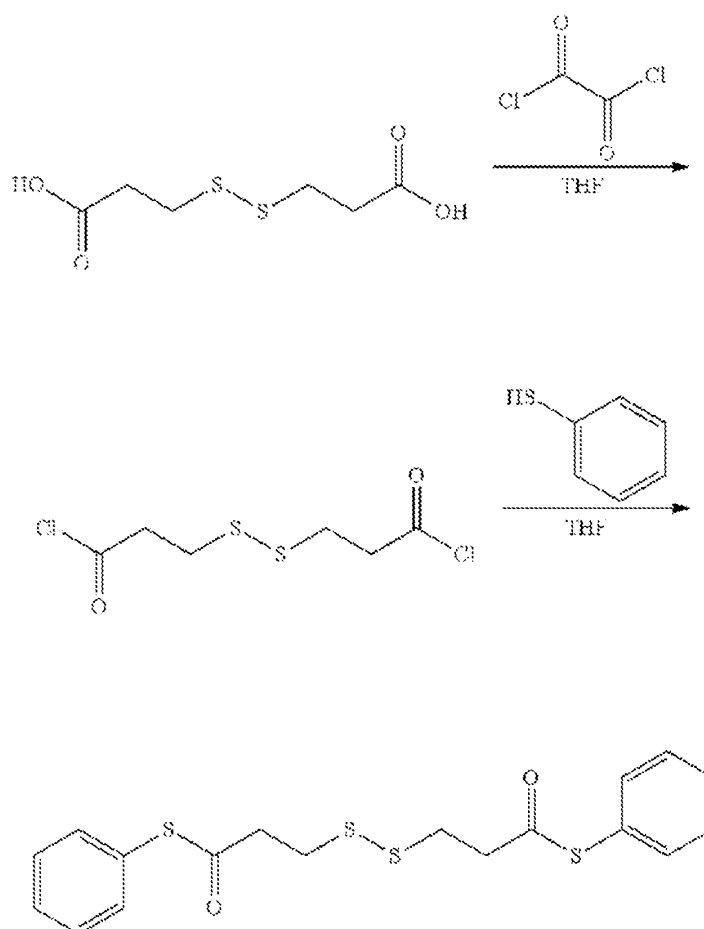
[00288] This example provides an additional exemplary protocol for reduction and purification of a DLL3 antibody for conjugation to the linker-cytotoxin conjugates *e.g.*, Linker-Drug (1), (1-A), (1-B), (2), (2-A), or (2-B), described herein. All buffers and DMSO stock solutions are purged with argon for 1 hour prior to use.

- 1) Aliquot 1 mL of antibody from 10 mg/mL stock into a 2 mL Eppendorf tube.
- 2) Dilute with 1 mL 100 mM borate (pH 8.4) to afford a 10 mg/mL stock solution (67 μ M).
- 3) Prepare a 50 mM stock solution of TCEP in water.
- 4) Add 20 mL of TCEP to 2 mL of antibody and incubate at 37 °C for 3 hours.
- 5) Aliquot into 4 x 0.5 mL Eppendorf tubes and place 3 tubes in storage at 20 °C.
- 6) Purify one 0.5 mL aliquot (approx. 5 mg) via SEC on Bio-Rad using degassed PBS.
- 7) Collect monomeric antibody peak in a sealed tube (approx. 4 mL total volume) at 4 °C.

- 8) Aliquot into 4 equal 1 mL Eppendorf tubes (1 mg/mL).
- 9) Add 6 equivalents of the linker-drug from 2 mM stock solutions in DMSO to each tube.
- 10) Incubate at 4 °C for 48 hours.
- 11) Analyze by HIC, SDS-PAGE and LC/MS, and compare against control.

Example 11: Site Specific Conjugation at Lysine to make DLL3-ADC

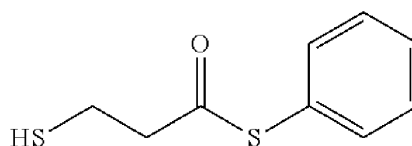
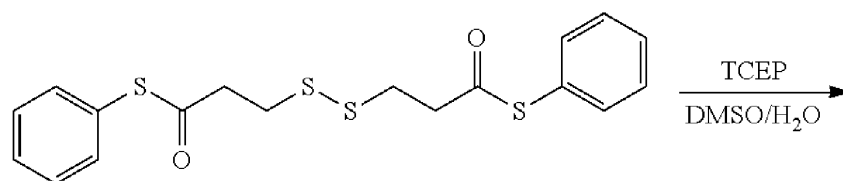
Synthesis of Thioester Linker



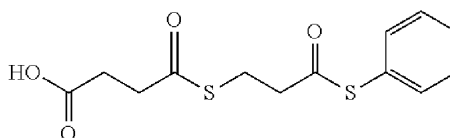
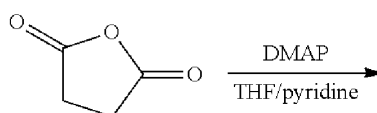
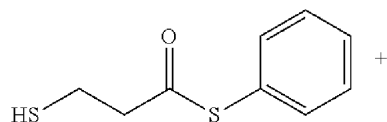
[00289] 3,3'-Dithiopropionic acid (1.0 g, 5.0 mmol) was dissolved in 10 mL of THF and 100 μ L of DMF, pyridine (4.0 mL, 50 mmol) and oxalyl chloride (1.5 mL, 15.0 mmol) were added dropwise thereto under ice cooling, and the resulting mixture was stirred at room temperature for two hours. After two hours, thiophenol (1.53 mL, 15.0 mmol) was added dropwise thereto, and the resulting mixture was further stirred at room temperature for two hours. The reaction solution was concentrated under reduced pressure and purified by normal phase chromatography with hexane/ethyl acetate to obtain S-phenyl 3-[(3-oxo-3-

phenylsulfanyl-propyl)disulfanyl]propanethioate (1.77 g, 4.5 mmol) as a target product.

MS(ESI)m/z:395.1[M+H]⁺



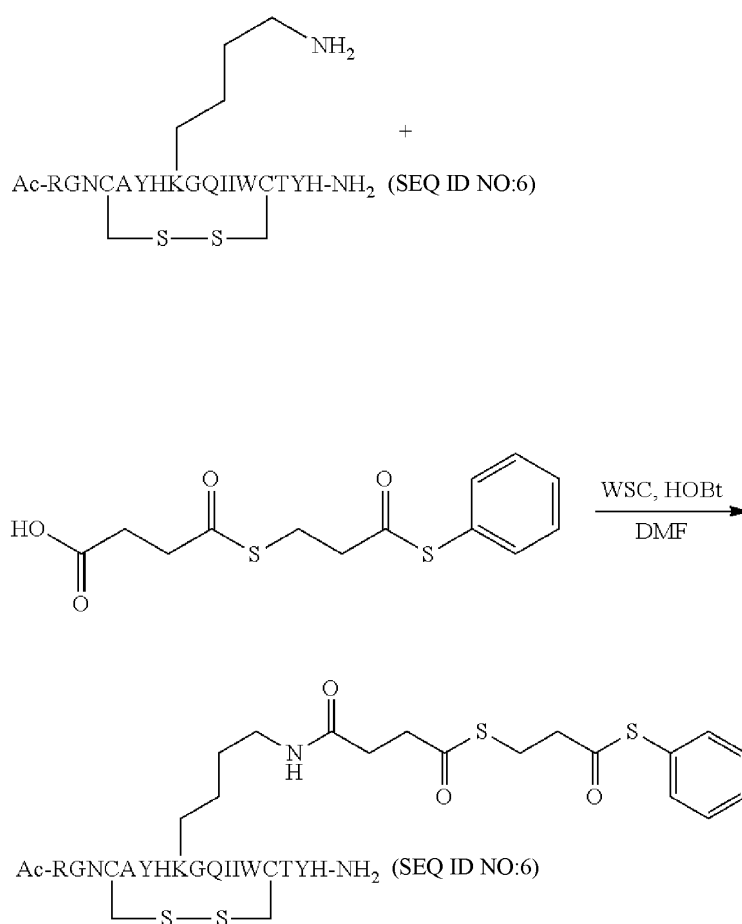
[00290] S-Phenyl 3-[(3-oxo-3-phenylsulfanyl-propyl)disulfanyl]propanethioate (1.77 g, 4.5 mmol) was dissolved in 5.0 mL of H₂O and 1.0 mL of DMSO, tris(2-carboxyethyl) phosphine hydrochloride (1.93 g, 6.75 mmol) was added thereto, and the resulting mixture was stirred at room temperature for two hours. After 2 hours, the solution was extracted with ethyl acetate to obtain S-phenyl 3-sulfanylpropanethioate (1.29 g, 6.5 mmol) as a target product. MS(ESI)m/z:199.2[M+H]⁺



[00291] 2.7 mL of acetonitrile and 0.3 mL of pyridine were added to and dissolved in S-phenyl 3-sulfanyl propanethioate (1.29 g, 6.5 mmol). Thereafter, succinic anhydride (0.65 g, 6.5 mmol) and 4-dimethylaminopyridine (12.2 mg, 0.1 mmol) were added thereto, and the

resulting mixture was stirred for one hour. 20 mL of 1 M aqueous hydrochloric acid solution was added thereto, and the resulting mixture was extracted with ethyl acetate. The solution was concentrated under reduced pressure to obtain (4-oxo-4-(3-oxo-3-phenylsulfanyl-propyl) sulfanyl-butanoic acid (1.25 g, 4.2 mmol). MS(ESI)m/z:299.0[M+H]⁺

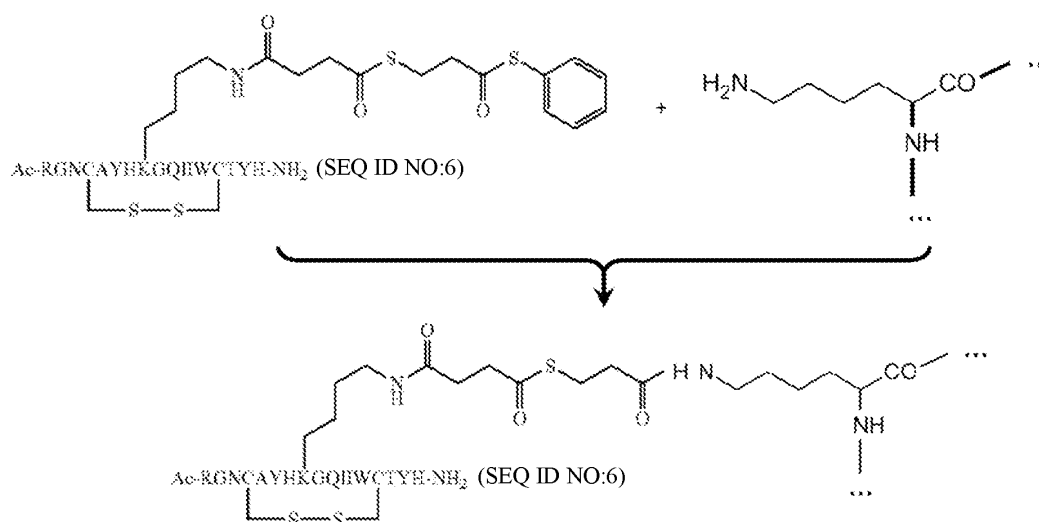
Thioester Linker and Peptide Coupling



[00292] Ac-RGNCA YHKGQIIWCTYH-NH₂ (SEQ ID NO:6) (30 mg, 14.4 μmol, the 4th and 14th of two cysteines form a disulfide bond in the molecule) was dissolved in 1 mL of N,N'-dimethylformamide, and ((4-oxo-4-(3-oxo-3-phenylsulfanyl-propyl) sulfanyl-butanoic acid (64 mg, 0.22 mmol) and WSC·HCl (41 mg, 0.22 mmol) were added thereto. The solution was stirred at room temperature for 12 hours, a 0.1% trifluoroacetic acid solution was added thereto, and fractions were eluted by reversed phase preparative chromatography. A fraction comprising a product was collected, was concentrated under reduced pressure to remove only acetonitrile, and was then freeze-dried to obtain the peptide- and linker-coupled

thiophenol-activation compound (22.3 mg, 9.4 μmol). MS (ESI) m/z : $z=3$ 790 $[\text{M}+3\text{H}]^{3+}$, $z=4$ 593 $[\text{M}+4\text{H}]^{4+}$.

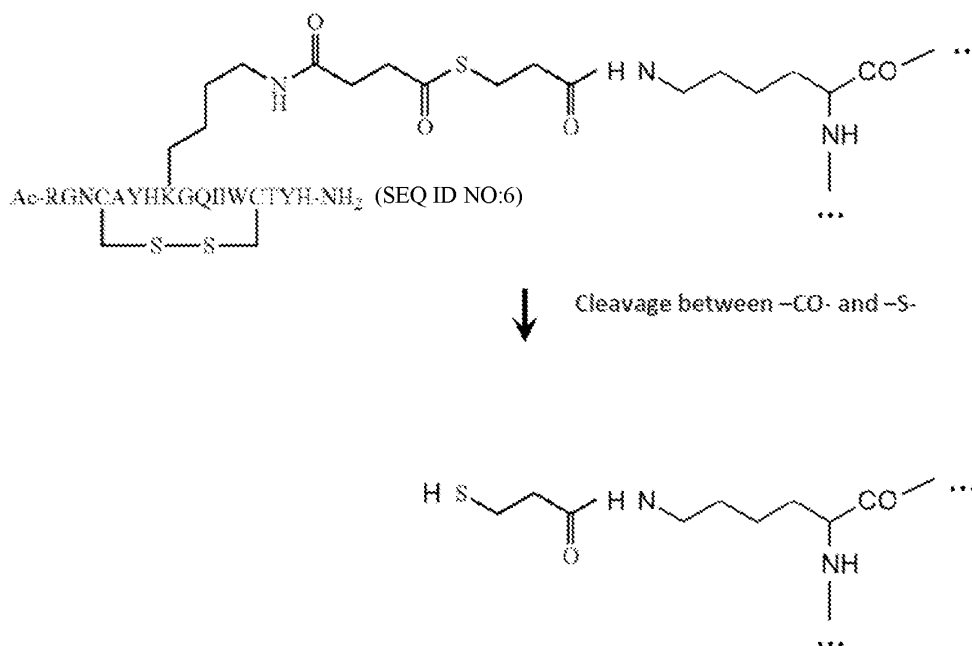
Site Specific Modification of DLL3 Antibody



[00293] The peptide- and linker-coupled thiophenol-activation compound is dissolved in $\text{N,N'$ -dimethylformamide. DLL3 antibody is dissolved in sodium acetate buffer (pH 4.7), peptide reagent (30 equivalents with respect to the antibody) is added thereto, and the solution is stirred at room temperature for one hour. The reaction solution is substituted with a 100 mM sodium citrate buffer (pH 2.9) to stop the reaction and is further substituted with a 20 mM PBS buffer to generate an DLL3-antibody-peptide conjugate.

[00294] The mass of the DLL3-antibody-peptide conjugate is measured by ESI-TOFMS by adding tris(2-carboxyethyl) phosphine hydrochloride solution (an equivalent with respect to the antibody) to the DLL3-antibody-peptide conjugate and the solution is stirred at room temperature. Samples are then analyzed by ESI-TOFMS.

[00295] The DLL3-antibody-peptide conjugate formed above and the DLL3 unconjugated antibody are also analyzed by HIC using detector with a wavelength of 280 nm.

Linker Cleavage of DLL3-antibody-peptide conjugate

[00296] The solution of the DLL3-antibody-peptide conjugate (20 mM PBS buffer) obtained above is substituted with a 0.5 M hydroxylamine 10 mM EDTA (pH 5.5) solution (Amicon 3K) and allowed to stand at room temperature for two hours. After two hours, the solution is substituted with 20 mM PBS buffer, 10 mM EDTA (pH 7.4) to obtain a thiol-introduced DLL3 antibody.

[00297] The mass of the DLL3-antibody-peptide conjugate is measured by ESI-TOFMS by adding tris(2-carboxyethyl) phosphine hydrochloride solution (an equivalent with respect to the antibody), and the solution is stirred at room temperature. Samples are then analyzed by ESI-TOFMS.

Linker-Drug Conjugation to Modified (Thiol-Introduced) DLL3-Antibody-Peptide Conjugate

[00298] This example provides two exemplary protocols for conjugation of the modified DLL3 antibody to the linker-drug conjugates, *e.g.*, Linker-Drug (1), (1-A), (1-B), (2), (2-A), or (2-B), described herein:

[00299] Protocol 1: (*see, e.g.*, Jackson, David, *Organic Process Research & Development* 2016, 20, 852-866, incorporated herein by reference in its entirety, and Tsuchikama, K. et al., *Protein & Cell* 2018, 9(1), 33-46, incorporated herein by reference in its entirety)

- a) Prepare a 10 mM stock solution of linker-cytotoxin conjugate in DMSO (DMA, DMF or CH₃CN are also acceptable);

- b) Add 5 equivalents of 12.5 μ L stock solution to each tube of DLL3-antibody-peptide conjugate (0.5 mM final concentration linker-cytotoxin conjugate stock solution);
- c) Incubate overnight at 4 °C for 4 hours at room temperature under reducing conditions; and
- d) Run analytical HIC to determine DAR and homogeneity.

[00300] Protocol 2: (see, e.g., Matsuda et al., *ACS Omega* 2019, 4, 24, 20564–20570, incorporated herein by reference in its entirety)

- e) To a solution of DLL3-antibody-peptide conjugate in conjugation buffer is added dimethylacetamide (DMA) and a 10 mM DMA solution of linker-cytotoxin conjugate (10 equiv), and the mixture incubated at 20 °C.
- f) After 2 h, a small amount of the reaction mixture (0.5 mL) is sampled for IPC analysis.
- g) After checking the IPC analysis, the reaction mixture is quenched by an excess amount of a 50 mM aqueous solution of N-acetyl cysteine and incubated at 25 °C for 15 min.
- h) This reaction mixture is purified by a TFF system using a Sartocore Slice 200 ECO Hydrosart membrane (30 kDa; Sartorius) and conjugation buffer as DF buffer at an antibody concentration of 20 mg/mL.
- i) Next, the buffer exchange of this solution is conducted by a TFF system using a Sartocore Slice 200 ECO Hydrosart membrane (30 kDa; Sartorius) and formulation buffer as DF buffer to afford ADC in formulation buffer.

Example 12: Alternative Synthesis of Thiol-Introduced DLL3 Antibody by One-Pot Method

[00301] The peptide- and linker-coupled thiophenol-activation compound are dissolved in N,N'-dimethylformamide. DLL3 antibody (e.g., 500 μ g) is dissolved in sodium acetate buffer (pH 5.5) and peptide reagent (30 equivalents with respect to the antibody) is added thereto. The solution is stirred at room temperature for one hour. Thereafter, a 0.5 M hydroxylamine 10 mM EDTA (pH 5.5) solution is added in the same amount as sodium acetate, and the resulting mixture is allowed to stand at room temperature for two hours. After two hours, the solution is substituted with 20 mM PBS buffer, 10 mM EDTA (pH 7.4) to obtain thiol-introduced DLL3 antibody.

Example 13: Alternative Conjugation Method (see, e.g., Matsuda et al., *ACS Omega* 2019, 4, 24, 20564–20570, incorporated herein by reference in its entirety)

[00302] Step 1: Conjugation of Affinity Peptide to Antibody To a solution of antibody in AcONa buffer (pH 5.5) is added a dimethylformamide (DMF) solution of peptide reagent 1 (9 equiv.) and stirred at 25 °C using the Chemglass system. After 1 h, a small amount of reaction mixture is sampled for IPC analysis. After checking the IPC analysis, the reaction mixture is purified by a TFF system using a Sartocan® Slice 200 ECO Hydrosart® membrane (30 kDa; Sartorius) and AcONa buffer (pH 5.5) as diafiltration (DF) buffer. Next, the buffer exchange of this solution is conducted by a TFF system using a Sartocan® Slice 200 ECO Hydrosart® membrane (30 kDa; Sartorius) and formulation buffer (20 mM histidine containing 5% trehalose, pH 5.2) as DF buffer at an antibody concentration of 6.9 mg/mL to afford antibody-peptide conjugate 2 in formulation buffer.

[00303] Step 2: Linker Cleavage To a solution of antibody-peptide conjugate in formulation buffer is added 0.25 M aqueous solution of ethylenediaminetetraacetic acid (EDTA) (12 equiv, pH 7.4), polysorbate 20 (0.0265 mL), and 0.5 M aqueous solution of TCEP (20 equiv) at 37 °C. The resulting mixture is measured by a pH meter, showing that the final pH is 5.2. After 1 h at 37 °C, a small amount of reaction mixture (*e.g.*, 0.5 mL) is sampled for IPC analysis. After checking the IPC analysis, the reaction mixture is purified by a TFF system using a Sartocan® Slice 200 ECO Hydrosart® membrane (30 kDa; Sartorius) and 10 mM AcONa buffer (pH 5.5) as DF buffer. Next, the buffer exchange of this solution is conducted by a TFF system using a Sartocan® Slice 200 ECO Hydrosart® membrane (30 kDa; Sartorius) and conjugation buffer (50 mM PBS, 10 mM EDTA, pH 7.4) as DF buffer to afford a linker cleavage product in conjugation buffer.

[00304] Step 3: Reoxidation To a solution of linker cleavage product in conjugation buffer is added a 50 mM dimethyl sulfoxide solution of DHAA (40 equiv), and the mixture is incubated at room temperature. After 3 h, a small amount of reaction mixture (*e.g.*, 0.5 mL) is sampled for IPC analysis. After checking the IPC analysis, the reaction mixture is purified by a TFF system using a Sartocan® Slice 200 ECO Hydrosart® membrane (30 kDa; Sartorius) and conjugation buffer as DF buffer to afford linker reoxidation product in conjugation buffer.

[00305] Step 4: Drug Conjugation To a solution of reoxidation product in conjugation buffer is added dimethylacetamide (DMA) and a 10 mM DMA solution of linker-drug (10 equiv), and the mixture is incubated at 20 °C. After 2 h, a small amount of the reaction mixture (*e.g.*, 0.5 mL) is sampled for IPC analysis. After checking the IPC analysis, the reaction mixture is quenched by an excess amount of a 50 mM aqueous solution of N-acetyl cysteine and incubated at 25 °C for 15 min. This reaction mixture is purified by a TFF system using a

Sartocon Slice 200 ECO Hydrosart® membrane (30 kDa; Sartorius) and conjugation buffer as DF buffer. Next, the buffer exchange of this solution is conducted by a TFF system using a Sartocon Slice 200 ECO Hydrosart® membrane (30 kDa; Sartorius) and formulation buffer as DF buffer to afford the ADC in formulation buffer.

Example 14: ADC Production

[00306] The ADCs as described in **Table 9** below were produced using a method as described in the Examples above. All produced ADCs have < 3 % high-molecular weight species (% HMW) aggregates. Each of the ADCs was then prepared in a formulation buffer (20 mM histidine, 5% (w/v) trehalose, pH 5 to 6, such as 5.2, 5.5, or 6.0).

[00307] Table 9. Produced ADCs

ADC Ref No.	DLL3 Antibody	Linker-Drug (Drug)	Conjugation Site	DAR
ADC-009 or ADC-009-M (used interchangeably herein)	mAb300	Linker-Drug (2) (Exatecan)	K248	2
ADC-009-A	mAb300	Linker-Drug (2) (Exatecan) Form A	K248	2
ADC-009-B	mAb300	Linker-Drug (2) (Exatecan) Form B	K248	2
ADC-013 or ADC-013-M (used interchangeably herein)	mAb302	Linker-Drug (2) (Exatecan)	K248	2
ADC-013-A	mAb302	Linker-Drug (2) (Exatecan) Form A	K248	2
ADC-013-B	mAb302	Linker-Drug (2) (Exatecan) Form B	K248	2
ADC-015 or ADC-015-M (used interchangeably herein)	mAb301	Linker-Drug (2) (Exatecan)	K248	2
ADC-015-A	mAb301	Linker-Drug (2) (Exatecan) Form A	K248	2
ADC-015-B	mAb301	Linker-Drug (2) (Exatecan) Form B	K248	2
ADC-010	mAb300	Linker-Drug (1) (MMAE)	K248	2
ADC-014	mAb302	Linker-Drug (1) (MMAE)	K248	2
ADC-018 or ADC-018-M (used interchangeably herein)	mAb300	Linker-Drug (2) (Exatecan)	Interchain	4
ADC-018-A	mAb300	Linker-Drug (2) (Exatecan) Form A	Interchain	4
ADC-018-B	mAb300	Linker-Drug (2) (Exatecan) Form B	Interchain	4
ADC-020 or ADC-020-M (used interchangeably herein)	mAb302	Linker-Drug (2) (Exatecan)	Interchain	4

ADC Ref No.	DLL3 Antibody	Linker-Drug (Drug)	Conjugation Site	DAR
ADC-020-A	mAb302	Linker-Drug (2) (Exatecan) Form A	Interchain	4
ADC-020-B	mAb302	Linker-Drug (2) (Exatecan) Form B	Interchain	4
ADC-016 or ADC-016-M (used interchangeably herein)	mAb301	Linker-Drug (2) (Exatecan)	Interchain	4
ADC-016-A	mAb301	Linker-Drug (2) (Exatecan) Form A	Interchain	4
ADC-016-B	mAb301	Linker-Drug (2) (Exatecan) Form B	Interchain	4
ADC-019 or ADC-019-M (used interchangeably herein)	mAb300	Linker-Drug (1) (MMAE)	Interchain	4
ADC-017	mAb301	Linker-Drug (1) (MMAE)	Interchain	4

Example 15: Developability

[00308] Size exclusion chromatography (SEC) analysis was performed using a 4.6 mm ID x 300 mm AdvanceBio column (SEC 300Å, 2.7 µm, Agilent, catalog # PL1580-5301) on an HPLC. The maximum column pressure was 400 bar. The running buffer was 1 x PBS (HyClone) + 10% IPA. Sample was loaded and the flow rate was 0.2 mL/minute. The length of isocratic gradient was 27 min. Absorbance was monitored at 280 nm. Chromatographic peaks were integrated to determine percentage of homogeneity and retention time. The column stationary phase along with choice of mobile phase supports hydrophobic and electrostatic interactions in addition to molecular sizing (secondary interactions much milder compared to SMAC).

[00309] Hydrophobic interaction chromatography (HIC) analysis was performed using a 4.6 mm ID x 3.5 cm TSKgel Butyl-NPR column (2.5 µm particle size, Tosoh Bioscience LLC, PN 14947) on an HPLC. Maximum column pressure was 200 bar. The column was equilibrated with 100% mobile phase Buffer A (50 mM sodium phosphate, pH 7 with 1.5 M ammonium sulfate) at a flow rate of 0.5 mL/minute at ambient temperature. Sample was loaded and eluted using a gradient from 100% mobile phase buffer A to 100% mobile phase buffer B (50 mM sodium phosphate, pH 7 with 20 % (v/v) isopropanol) at 0.5 mL/minute. The elution time and buffer are specified in **Table 10** below, and the length of linear gradient was 20 min. Absorbance was monitored at 280 nm. Sample retention time was calculated and compared to a set of standard controls to identify antibodies with increased retention time (increased hydrophobicity) and the presence of multiple species.

[00310] **Table 10. HIC Elution Conditions**

Time (min)	A (%)	B (%)
0	100	0
2	100	0
22	0	100
24	0	100
24.1	100	0
27	100	0

[00311] Reverse phase liquid chromatography (RP-HPLC) was also performed to analyze the DLL3 antibodies and the produced ADCs. Briefly, samples were analyzed on an Agilent Instrument equipped with a 4.6 mm X 100 mm column (Agilent AdvanceBio RP-mAb Diphenyl, 3.5 μ m particle size, Agilent catalog # 795975-944). The column Temperature was 70 °C and the maximum column pressure was 600 bar. Buffer A as used was water + 0.1% TFA, while Buffer B as used was acetonitrile + 0.1% TFA. The flow rate was set to 0.4 mL/minute and the length of linear gradient was 27 minutes. Other elution conditions can be found in **Table 11** below. UV at 215 nm, 252 nm, and 280 nm was used to detect the sample signals.

[00312] Table 11. RP-HPLC Elution Conditions

Time (min)	A (%)	B (%)
0	70	30
2	70	30
24	52	48
24.1	5	95
27	5	95
27.1	70	30
35	70	30

[00313] Representative results are provided herein as **Figure 4A-4D**. **Figure 4A** showed SEC results indicating that mAb302 had <3% HMW aggregates, which was higher than mAb300, and conjugation had very little effect on aggregation. **Figure 4B** compared mAb300 and mAb302 using HIC and showed that mAb302 was more hydrophobic than mAb300. mAb300 was further compared with mAb 301 (data not shown), indicating that mAb300 and mAb301 had similar hydrophobicity profiles. SEC was also performed to compare mAb300 and mAb301 (data not shown), and mAb300 and mAb301 were shown to have similar elution times.

[00314] **Figure 4C** showed that mAb300 conjugates (ADC-009 and ADC-010) were more hydrophobic than the antibody, and MMAE conjugate was more hydrophobic than the Exatecan conjugate. Similarly, as shown in **Figure 4D**, mAb302 conjugates (ADC-013 and

ADC-014) were more hydrophobic than the antibody, and MMAE conjugate was more hydrophobic than the Exatecan conjugate.

Example 16: Binding Characterization (Biacore™)

[00315] Kinetic measurements were performed using a Cytiva Biacore T200 instrument in HBS-EP+ buffer. An anti-human Fc antibody was covalently coupled with a Series S CM5 chip (Cytiva) using EDC/NHS chemistry and deactivated with ethanolamine before use. After equilibration in running buffer, the anti-DLL3 antibodies were captured in Channels 2, 3, and 4, while Channel 1 was used as the reference. The human, cyno, mouse, and rat DLL3 antigens (Acro) were tested in a 4-fold serial dilution from 100 nM to 0.02 with a buffer blank included. After each antigen cycle, the chip surface was regenerated using 10 mM glycine which facilitated the removal of both the antibody and antigen. The data were processed using a 1:1 binding model in the Biacore T200 Evaluation software.

[00316] **Figure 5** provides exemplary Biacore sensorgrams of the tested antibodies (mAb300, mAb301, mAb302, and a DLL3 IgG1 α (having a heavy chain disclosed as SEQ ID NO:71 in WO2022153195A1 and a light chain disclosed as SEQ ID NO:72 in WO2022153195A1) as a positive control) binding to cyno, human, mouse, and rat DLL3. The calculated affinity data is provided in **Table 12** below, indicating that mAb300 and mAb301 have similar binding characteristics compared to mAb302.

[00317] Table 12. Biacore™ Affinity Analysis

	K _D (nM)				k _a (M ⁻¹ s ⁻¹)				k _d (s ⁻¹)			
	mAb300	mAb301	mAb302	DLL3 IgG1a	mAb300	mAb301	mAb302	DLL3 IgG1a	mAb300	mAb301	mAb302	DLL3 IgG1a
Human	0.012	0.022**	< 0.01	0.044	1.8x 10 ⁶	2.0 x 10 ⁶	5.4 x 10 ⁵	2.7x10 ⁷	2.1x10 ⁻⁵	4.5x10 ⁻⁵	< 10 ⁻⁵	1.2x10 ⁻³
Cyno	2.3	2.2	1.6	0.32*	4.7x10 ⁵	3.2x10 ⁵	1.2x10 ⁵	3.8x10 ⁶	1.1x10 ⁻³	7.0x10 ⁻⁴	1.9x10 ⁻⁴	1.2x10 ⁻³
Mouse	< 0.05	< 0.25	37*	0.86	1.6x10 ⁵	1.7x10 ⁵	2.2x10 ⁴	7.1x10 ⁵	< 10 ⁻⁵	< 4x10 ⁻⁵	8.1x10 ⁻⁴	6.1x10 ⁻⁴
Rat	< 0.01	< 0.08	3.2*	0.56*	5.5x10 ⁵	4.8x10 ⁵	1.4x10 ⁵	2.1x10 ⁶	< 10 ⁻⁵	< 4x10 ⁻⁵	4.5x10 ⁻⁴	1.2x10 ⁻³

*High bulk contributions

**50 nM excluded from analysis due to two-phase decay

Example 17: DAR Determination (HIC and RP-HPLC)

[00318] Experiments were performed to assess DARs of the produced ADC compositions, using HIC and RP-HPLC, both of which were described above in **Example 15**.

[00319] Representative results are provided in **Figure 6**. Briefly, good conjugation efficiency was observed for DAR 2 and DAR 4 ADCs, and the HIC data aligned with the RP-HPLC results: the ADC-016 composition exhibited a DAR of 3.9 using HIC and 3.6 using RP-HPLC; the ADC-017 composition exhibited a DAR of 4.0 using HIC and 3.9 using RP-HPLC; and the ADC-015 composition exhibited a DAR of 1.8 using HIC and 1.7 using RP-HPLC.

Example 18: Characterization of ADC-015-M, ADC-015-A, ADC-015-B, ADC-016-M, ADC-016-A, and ADC-016-B.

[00320] The ADCs, including ADC-015-M, ADC-015-A, ADC-015-B, ADC-016-M, ADC-016-A, and ADC-016-B, were produced as described in the preceding Examples, formulated in 20 mM Histidine, 5% Trehalose, pH 6.0, and the subject to further analysis concerning their aggregation percentages, DARs, endotoxins, and yield percentages by following the methods below.

[00321] Aggregation Determination: ADCs (25 µg) were injected onto an AdvanceBio SEC 300Å size exclusion chromatography column (2.7 µm, 4.6 x 300 mm, Agilent PN PL1580-5301) at room temperature via isocratic elution in 1 x PBS (Cytiva Life Sciences PN SH30258.01) with 10 % (v/v) isopropanol at a flow rate of 0.2 mL/minute. Samples were monitored at 280-nm and 254-nm on an Agilent 1260 Infinity II Bio-Inert HPLC system equipped with a multi-wavelength detector. ChemStation software was utilized to quantify each sample chromatography to determine aggregation.

[00322] DAR Determination: ADCs (50 µg) were injected onto a TSKgel Butyl-NPR hydrophobic interaction chromatography column (2.5 µm, 4.6 x 35 mm, Tosoh Bioscience PN 14947) at room temperature and eluted with a 20-minute linear gradient from 0 to 100% B at a flow rate of 0.5 mL/minute, with Mobile Phase A: 50 mM sodium phosphate, 1.5 M ammonium sulfate, pH 7, and Mobile Phase B: 50 mM sodium phosphate, 20% (v/v) isopropanol, pH 7. Samples were monitored at 280-nm and 254-nm on an Agilent 1260 Infinity II Bio-Inert HPLC system equipped with a multi-wavelength detector. ChemStation software was utilized to quantify each sample chromatography, allowing for DAR determination.

[00323] Endotoxin Determination: ADCs diluted (1:10) with LAL Reagent Water (Associates of Cape Cod Inc. P/N W020P) were loaded onto Endosafe endotoxin LAL test cartridges (FDA-licensed), 0.01 EU/mL (Charles River P/N PTS2001F) per the User's Guide and analyzed for endotoxin on the Endosafe nexgen MCS (Charles River).

[00324] Yield Determination: Absorbances of unconjugated and conjugate antibodies were quantified at 280-nm on a NanoDrop One microvolume UV-Vis spectrophotometer (Thermo Scientific). The resulting measurements were utilized to calculate concentrations using the extinction coefficient of the unconjugated antibody. Total amounts were determined by multiplying the volume and calculated concentration, with the overall yield calculated by subtracting the final amount from the starting amount.

[00325] HIC analysis was performed using a column of Tosoh TSKgel Butyl-NPR, 4.6 x 35 mm, 2.5 µm on an HPLC. The column was equilibrated with 100% mobile phase Buffer A (1.5 M (NH₄)₂SO₄, 50 mM Na₂HPO₄/NaH₂PO₄, pH 7.0) at a flow rate of 0.5 mL/minute at ambient temperature. Sample (20 µL, 1 mg/mL) was loaded and eluted using a gradient from 100% mobile phase buffer A to 100% mobile phase buffer B (50 mM Na₂HPO₄/NaH₂PO₄ 25 v/v% IPA, pH 7.0) at 0.5 mL/minute. The elution time and buffer are specified in **Table 13** below. Absorbance was monitored at 280 nm.

[00326] **Table 13.** HIC Elution Conditions

Time (min)	A (%)	B (%)
0.00	100.0	0.0
2.00	100.0	0.0
22.00	0.0	100.0
24.00	0.0	100.0
24.20	100.0	0.0
27.00	100.0	0.0

[00327] Size exclusion chromatography (SEC) analysis was performed using a 4.6 mm ID x 300 mm AdvanceBio column (SEC 300Å, 2.7 µm, Agilent, catalog # PL1580-5301) on an HPLC. The maximum column pressure was 400 bar. The running buffer was 1 x PBS (HyClone) + 10% IPA. Sample was loaded and the flow rate was 0.2 mL/minute. The length of isocratic gradient was 27 min. Absorbance was monitored at 280 nm. Chromatographic peaks were integrated to determine percentage of homogeneity and retention time. The column stationary phase along with choice of mobile phase supports hydrophobic and electrostatic interactions in addition to molecular sizing (secondary interactions much milder compared to SMAC).

[00328] Exemplary results are provided in **Table 14** below as well as in **Figures 7A-7R**, and **8A-8R**. The SEC data showed less than 3% aggregates observed for all the tested ADCs. The HIC chromatogram showed that the ADC having a Form B exhibited an earlier retention time, suggesting that Form B is less hydrophobic than Form A.

[00329] **Table 14.** Analytical Results.

ADC Ref. No.	% Aggregation	DAR	Endotoxin (EU/mg)	% yield
ADC-015-A	< 1%	1.1	0.02	86%
ADC-015-B	< 1%	1.9	< 0.03	60%
ADC-015-M	< 1%	1.9	< 0.02	81%
ADC-016-A	1.2	3.8	—	—
ADC-016-B	< 1%	4	< 0.05	50%
ADC-016-M	1.6%	3.6	< 0.03	31%

Example 19: Cytotoxicity Assays (hDLL3 B16F10 cell line)

[00330] The produced ADCs were evaluated for their cytotoxicity effects on mouse melanoma cell line B16F10 engineered to express human DLL3 (referred to herein as hDLL3 B16F10). Briefly, hDLL3 B16F10 cells were seeded per well on Day -1 (referred to herein as D-1) and cultured under 5% CO₂ at 37°C. On the following day (*e.g.*, Day 0 or D0), the tested compounds were added to each well at a series of dilutions. At the end of the observation time period, the cells were harvested, and the cell viability was assessed using CellTiter-Glo® Luminescent Cell Viability (CTG) assay following the manufacture protocols.

[00331] One representative result is provided in **Figures 9A-9C**. 1,000 cells were seeded per well on Day -1. The tested compound was ADC-017 comprising mAb301 and MMAE and having a target DAR of 4. MMAE (NJ Bio stock- lot# NJBP-75-184-002) and Exatecan (NJ Bio stock- lot# NJBP-75-184-001) were also evaluated in parallel, serving as controls. The dilution series in DPBS were 500 – 0.008 nM, (9 concentrations, 4x dilution). The endpoints were selected as 72 hours of incubation and 96 hours of incubation, and the corresponding cell survival curves were plotted in **Figure 9A** and **Figure 9B**, respectively. IC₅₀ and maximum inhibition percentages were then calculated using Prism and the result is shown in **Figure 9C**.

[00332] A further experiment was performed to test additional mAb301 ADCs. 1,500 cells were seeded per well on Day -1. The tested compounds included ADC-015 (comprising

mAb301 and Exatecan, and having a target DAR of 2), ADC-016 (comprising mAb301 and Exatecan, and having a target DAR of 4), and ADC-017 (comprising mAb301 and MMAE, and having a target DAR of 4). MMAE (NJ Bio stock- lot# NJBP-75-184-002) and Exatecan (NJ Bio stock- lot# NJBP-75-184-001) were also evaluated in parallel, serving as controls. Additional controls were used, including isotype control conjugated to Linker-Drug (1) (MMAE) and having a target DAR of 4 as well as isotype control conjugated to Linker-Drug (2) (Exatecan) and having a target DAR of 4. The dilution series in DPBS were 500 – 0.008 nM, (9 concentrations, 4x dilution). Each compound at each concentration was tested in triplicates. The endpoint was selected as 72 hours of incubation, and the corresponding cell survival curve was plotted in **Figure 9D**. IC₅₀ and maximum inhibition percentages were then calculated using Prism and the result is shown in **Figure 9E**. These results suggest that MMAE conjugates showed better activity than Exatecan conjugates. Additional endpoints, such as 96 hours and 120 hours of incubation, are under investigation. Additionally or alternatively, an initial cell seeding of 1,000 cells is also assessed.

Example 20: Cytotoxicity Assays (Human DLL3 high expression stable cell line (WT115-293F.hPro1.2E5))

[00333] The produced ADCs (ADC-015-A, ADC-015-B, ADC-015-M, ADC-016-A, ADC-016-B, and ADC-016-M) were evaluated for their cytotoxicity effects on the HEK293F tumor cell line engineered to overexpressing hDLL3 as described above (WT115-293F.hPro1.2E5). Exatecan and an isotype ADC (anti-HEWL VHH-Fc isotype conjugated to Linker-Drug (2), mixture of Form A and Form B, having a target DAR of 4) were tested in parallel and served as controls.

[00334] Briefly, on day -1, cells were seeded in a 96 well plate at a density of 3,000 cells/well by diluting 1 million cells in 30 mL of pre-warmed FreeStyle 293 Expression Medium (ThermoFisher 12338018) supplemented with 6 µg/mL Blasticidin (Thermo Scientific J67216.XF). Cells used for these assays were between passages 5 and 13 and their viability verified to be > 90%. Test compounds (ADC-015-A, ADC-015-B, ADC-015-M, ADC-016-A, ADC-016-B, ADC-016-M, Exatecan and isotype ADC) were added on day 0 as 10x concentrated working stocks serially diluted in PBS from a top concentration of 500 nM following a 3 or 4 fold dilution series. Each concentration was tested in triplicates. Test plates were incubated in a humidified incubator at 37°C with 5% CO₂ for 96 hours. Endpoint was measured using the Cell Titer Glow reagent and protocol and luminescence was recorded on

SpectraMax i3x. The cell survival rate in each well was determined using the following calculation:

$$\text{surviving rate (\%)} = (\text{LumTest article-LumMedium control}) / (\text{LumVehicle control-LumMedium control}) \times 100\%$$

[00335] A dose-response curve using nonlinear regression model with a log(inhibitor) vs response- Variable slope response was fitted to the data using GraphPad Prism 9 to calculate relative IC₅₀. Maximum response was defined as “100 – minimal survival rate”.

[00336] Exemplary results are plotted in **Figures 10A-10E** and **Tables 15-19** below. **Figure 10A** and **Figure 10B** provide data from one exemplary experiment, and the obtained signal fold change vs day 0 in PBS control was 6.9. **Figure 10C** and **Figure 10D** provide data from another exemplary experiment, and the obtained signal fold change vs day 0 in PBS control was 13. Concerning the data of **Figure 10E**, the obtained signal fold change vs day 0 in PBS control was also 13. Further, **Figure 10A**, **Figure 10C**, and **Figure 10E** plot the concentration of the ADCs (or Exatecan for the Exatecan treated group) on the x-axis, while **Figure 10B** and **Figure 10D** plot the concentration of Linker-Drug (2) (Form A, or Form B, or a mixture thereof) (or Exatecan for the Exatecan treated group) on the x-axis.

[00337] As shown in **Figures 10A-10D** and **Tables 15- 18**, similar cytotoxicity was observed for ADC-015-B and ADC-015-M. As shown in **Figure 10E** and **Table 19**, similar cytotoxicity was observed for ADC-016-M, ADC-016-A, and ADC-016-B.

[00338] **Table 15.** IC₅₀ and Other Calculations Corresponding to **Figure 10A**.

Compound	DAR	IC ₅₀ (nM)*	95% CI	50% inhibition (nM) [#]	Max inhibition
Exatecan		2.7		2.8	96%
Isotype ADC	4	201		157	75%
ADC-015-A	1.1	10.3	8.4 to 12.8	14.8	85%
ADC-015-B	1.9	5.5	4.9 to 6.2	5.9	91%
ADC-015-M	1.9	5.8	4.8 to 7.1	6.9	92%
ADC-016-M	3.6	4.9	4.5 to 5.4	5.0	92%

* Prism, log (inhibitor) vs response – Variable slope

[#] Prism, interpolated mean values

[00339] Table 16. IC₅₀ Calculation Corresponding to Figure 10B.

Compound	IC ₅₀ (nM)*
Exatecan	2.7
ADC-015-A	11.4
ADC-015-B	10.5
ADC-015-M	11
ADC-016-M	17.4

* Prism, log (inhibitor) vs response – Variable slope

[00340] Table 17. IC₅₀ and Other Calculations Corresponding to Figure 10C.

Compound	DAR	IC ₅₀ (nM)*	95% CI	50% inhibition (nM)#	Max inhibition
Exatecan		3.5		2.8	95%
Isotype ADC	4	191		199	76%
ADC-015-A	1.1	18	14.2 to 23.6	32	81%
ADC-015-B	1.9	5.7	4.9 to 6.5	7.0	91%
ADC-015-M	1.9	5.1	4.2 to 6.3	6.1	91%
ADC-016-M	3.6	4.8	3.6 to 6.2	5.3	90%

* Prism, log (inhibitor) vs response – Variable slope

Prism, interpolated mean values

[00341] Table 18. IC₅₀ Calculation Corresponding to Figure 10D.

Compound	IC ₅₀ (nM)*
Exatecan	3.5
ADC-015-A	19.8
ADC-015-B	10.8
ADC-015-M	9.8
ADC-016-M	17.2

* Prism, log (inhibitor) vs response – Variable slope

[00342] Table 19. IC₅₀ and Other Calculations Corresponding to Figure 10E.

Compound	DAR	IC ₅₀ (nM)*	50% inhibition (nM)#	Max inhibition
ADC-016-M	3.6	4.8	5.3	90%
ADC-016-A	3.8	4.1		92%
ADC-016-B	3.8	5.2		93%

* Prism, log (inhibitor) vs response – Variable slope

Prism, interpolated mean values

Example 21: Cytotoxicity Assays (ADC-015, ADC-016, and ADC-017 on H69, CORL279, SHP77, and H460 cells)

[00343] The produced ADCs were further evaluated for their cytotoxicity effects on human SCLC cell lines endogenously expressing DLL3, such as H69, CORL279 and SHP77. H460,

which is a DLL-negative non-small cell lung cancer cell line, was also tested in parallel, serving as a control.

[00344] Briefly, the cells were seeded per well on Day -1 (referred to herein as D-1) and cultured under 5% CO₂ at 37 °C. For CORL279 and SHP77, the seeding density was 3,000 cells per well. For H69, the seeding density was 9,000 cells per well. And, for H460, the seeding density was 1,000 cells per well. On the following day (*e.g.*, Day 0 or D0), the tested compounds were added to each well at a series of dilutions, including ADC-015 (comprising mAb301 and Exatecan, and having a target DAR of 2), ADC-016 (comprising mAb301 and Exatecan, and having a target DAR of 4), and ADC-017 (comprising mAb301 and MMAE, and having a target DAR of 4). MMAE (NJ Bio stock- lot# NJBP-75-184-002) and Exatecan (NJ Bio stock- lot# NJBP-75-184-001) were also evaluated in parallel, serving as controls. Additional controls were used, including isotype control conjugated to Linker-Drug (1) (MMAE) and having a target DAR of 4 as well as isotype control conjugated to Linker-Drug (2) (Exatecan) and having a target DAR of 4. The dilution series in DPBS were 500 – 0.008 nM, (9 concentrations, 4x dilution). At the end of the observation time period (120 hours of incubation), the cells were harvested, and the cell viability was assessed using CellTiter-Glo® Luminescent Cell Viability (CTG) assay following the manufacture protocols.

[00345] H69 relevant data is provided in **Figures 11A-11F**. Three representative cell survival curves were plotted in **Figures 11A, 11C and 11E**. Their IC₅₀ and maximum inhibition percentages were then calculated using Prism and the results are shown in **Figures 11B, 11D and 11F**, respectively.

[00346] CORL279 relevant data is provided in **Figures 12A-12D**. Two representative cell survival curves were plotted in **Figures 12A and 12C**. Their IC₅₀ and maximum inhibition percentages were then calculated using Prism and the results are shown in **Figures 12B and 12D**, respectively.

[00347] SHP77 relevant data is provided in **Figures 13A-13B**. One representative cell survival curve was plotted in **Figure 13A**. Their IC₅₀ and maximum inhibition percentages were then calculated using Prism and the results are shown in **Figure 13B**.

[00348] H460 relevant data is provided in **Figures 14A-14B**. One representative cell survival curve was plotted in **Figure 14A**. Their IC₅₀ and maximum inhibition percentages were then calculated using Prism and the results are shown in **Figure 14B**. Lower sensitivity to MMAE (and MMAE conjugate) was observed. In some embodiments, such data may suggest that IC₅₀>150nM for Exatecan conjugate may reflect non-DLL3 specific activity.

Example 22: Cytotoxicity Assays (ADC-009 and ADC-010 on H69, CORL279, SHP77, and DMS79)

[00349] The produced ADCs were further evaluated for their cytotoxicity effects on human SCLC cell lines endogenously expressing DLL3, such as H69, CORL279, SHP77, and DMS79.

[00350] Briefly, the cells were seeded per well on Day -1 (referred to herein as D-1) at a density of 9,000 cells per well and cultured under 5% CO₂ at 37 °C. On the following day (e.g., Day 0 or D0), the tested compounds were added to each well at a series of dilutions, including ADC-009 (comprising Exatecan) and ADC-010 (comprising MMAE). MMAE (NJ Bio stock- lot# NJBP-75-184-002) was also evaluated in parallel, serving as a control. The dilution series in DPBS were 500 – 0.008 nM, (9 concentrations, 4x dilution). At the end of the observation time period (96 hours of incubation), the cells were harvested, and the cell viability was assessed using CellTiter-Glo® Luminescent Cell Viability (CTG) assay following the manufacture protocols.

[00351] Relevant data is provided in **Figures 15A-15E**. Representative cell survival curves of SHP77, DMS79, H69, and CORL279 were plotted in **Figures 15A, 15B, 15C and 15D**, respectively. Their IC₅₀ and maximum inhibition percentages were then calculated using Prism and the results are shown in **Figure 15E**.

Example 23: Anti-Tumor Effects in Cell Line-Derived Xenograft (CDX) Models

[00352] Briefly, female CB17.SCID mice, between 5 and 8 weeks of age, were implanted subcutaneously with 5x10⁶ SHP77 (~5,000 DLL3 surface expression) or 7x10⁶ H69 cells (~2,000 DLL3 surface expression) in a 1:1 cell: Matrigel ratio. Upon reaching a mean tumor volume of 200 mm³, animals were randomized into treatment groups. The tested compounds were administered by the intravenous (*i.v.*) route. Animals were removed from study when they reached humane endpoints including tumor volume exceeding 2000 mm³ and body weight loss endpoint. Tumor Growth Inhibition (TGI) was calculated by following $TGI = (1 - (\Delta TV [\text{treatment group}] / \Delta TV [\text{Vehicle group}])) * 100$

[00353] In an initial study, ADC-015-B and ADC-016-B were tested using the SHP77 CDX model as described herein. The tested compounds at various dosages (1 mg/kg or 3 mg/kg) were administered by the intravenous (*i.v.*) route on Day 1. Vehicle, DAR 2 Isotype ADC (anti-HEWL VHH-Fc isotype conjugated to Linker-Drug (2), mixture of Form A and Form B, having a target DAR of 2), and DAR 4 Isotype ADC (anti-HEWL VHH-Fc isotype conjugated to Linker-Drug (2), mixture of Form A and Form B, having a target DAR of 4)

were also tested in parallel as controls. Tumor volumes and body weights were monitored during the study.

[00354] The corresponding results can be found in **Figure 16A** (the mean group tumor volume (TV) graphing stops when 1 animal reached TV endpoint and was removed from study or on Day 31), **Figure 16B**, and **Table 20**. The result indicated that mAb301 Exatecan ADCs (DAR 2 and DAR 4) showed tumor growth inhibition with single dose; both DAR 2 and DAR 4 ADCs show delayed tumor growth vs non-DLL3 binding ADCs (isotype controls) at 3 mg/kg and 1 mg/kg (and vehicle); and no significant effect changes was observed in body weight (<10%).

[00355] **Table 20.** TGI of the Treatment Groups.

Treatment Group	TGI (%)
1 mg/kg DAR 2 Isotype ADC	49
1 mg/kg DAR 4 Isotype ADC	43
3 mg/kg ADC-015-B	94
1 mg/kg ADC-015-B	87
3 mg/kg ADC-016-B	96
1 mg/kg ADC-016-B	99

[00356] In a further study, the anti-tumor activity of ADC-015-M, ADC-015-B, ADC-016-M, and ADC-016-B were investigated in two CDX models of small cell lung (SCL) cancer SHP77 and H69, implanted subcutaneously in female CB17 SCID mice. DLL3 expression on these cell lines was confirmed by flow cytometry using a control anti-DLL3 antibody (having a heavy chain as set forth in CAS Registry Number: 2897724-56-8 and a light chain as set forth in CAS Registry Number: 2897724-55-7).

[00357] The tested compounds (including ADC-015-B, ADC-015-M, ADC-016-B, and ADC-016-M) were administered by the intravenous (*i.v.*) route, either as a single dose or on a repeat dose schedule depending on the studies. Vehicle, mAb301, DAR 2 Isotype ADC (anti-HEWL VHH-Fc isotype conjugated to Linker-Drug (2), mixture of Form A and Form B, having a target DAR of 2), and DAR 4 Isotype ADC (anti-HEWL VHH-Fc isotype conjugated to Linker-Drug (2), mixture of Form A and Form B, having a target DAR of 4) were also included as controls. Tumor volumes and body weights were monitored twice a week up to 56 days post initial treatment. The dose levels and the dosing schedules are outlined in **Table 21** below.

[00358] Table 21. Dosing Levels and Schedules

Group No.	Compound	Target DAR	Dose level (mg/kg)	Dosing day(s)	Route	Vehicle	n=
1	Vehicle	NA	NA	D1	i.v.	PBS	9
2	ADC-015-B	2	3	D1	i.v.	PBS	9
3	ADC-015-B	2	1	D1	i.v.	PBS	9
4	ADC-015-B	2	0.3	D1, D8, D15	i.v.	PBS	9
5	ADC-015-M	2	1	D1	i.v.	PBS	9
6	ADC-016-B	4	3	D1	i.v.	PBS	9
7	ADC-016-B	4	1	D1	i.v.	PBS	9
8	ADC-016-B	4	0.3	D1, D8, D15	i.v.	PBS	7*
9	ADC-016-M	4	1	D1	i.v.	PBS	9
10	DAR 4 Isotype ADC	4	1	D1	i.v.	PBS	9
11	DAR 2 Isotype ADC	2	1	D1	i.v.	PBS	9

* 2 animals removed from study-independent from dosing or disease

[00359] The SHP77 CDX results were obtained and are provided in **Figures 16A-17K** and **Table 22** below. In **Figure 16A**, the mean group tumor volume (TV) graphing was stopped when the first animal reached TV endpoint and was removed from study.

[00360] Table 22. Data Analysis of the SHP77 CDX Results.

Treatment Group	Day 28 TV (Mean $\pm$ SEM, mm ³)	One Way ANOVA (Dunnett's post-test vs Vehicle)	Day 28 Percentage of Tumor Growth Inhibition (%TGI) (vs Vehicle)
Vehicle	1277 $\pm$ 125		
DAR 2 Isotype ADC, 1 mg/kg	815 $\pm$ 88	<0.0001	49%
DAR 4 Isotype ADC, 1 mg/kg	757 $\pm$ 103	<0.0001	43%
ADC-015-B, 3 mg/kg	267 $\pm$ 19	<0.0001	94%
ADC-015-B, 1 mg/kg	340 $\pm$ 23	<0.0001	87%
ADC-015-B, 0.3 mg/kg x 3	378 $\pm$ 83	<0.0001	84%
ADC-015-M, 1 mg/kg	272 $\pm$ 31	<0.0001	93%
ADC-016-B, 3 mg/kg	244 $\pm$ 19	<0.0001	96%
ADC-016-B, 1 mg/kg	221 $\pm$ 18	<0.0001	99%
ADC-016-B, 0.3 mg/kg x 3	239 $\pm$ 14	<0.0001	97%
ADC-016-M, 1 mg/kg	257 $\pm$ 9	<0.0001	96%

[00361] The results indicate that all ADC treated groups showed delayed tumor growth vs vehicle control; all mAb301 ADCs showed delayed tumor growth vs isotype control ADCs; a dose dependent efficacy was apparent in the ADC-015-B treatment groups; and ADC-015-B and ADC-016-B showed similar efficacy to ADC-015-M and ADC-016-M, respectively (1 mg/kg).

[00362] In a third study, mAb300 and the ADCs comprising thereof were tested using the SHP77 CDX model as described herein. The tested compounds at various dosages (including 5 mg/kg ADC-009-M, 10 mg/kg ADC-009-M, 5 mg/kg ADC-018-M, 2.5 mg/kg ADC-018-M, 5 mg/kg ADC-019-M, 2.5 mg/kg ADC-019-M) were administered by the intravenous (*i.v.*) route on Day 1, Day 4, and Day 8. Vehicle and 5 mg/kg mAb300 were also tested in parallel as controls. Tumor volumes and body weights were monitored during the study.

[00363] The corresponding results can be found in **Figure 16E** (the mean group tumor volume (TV) graphing stops when 1 animal reached TV endpoint and was removed from study or on Day 31), **Figure 16F**, and **Table 23**. The result indicated that all tested mAb300 Exatecan ADC dose groups showed tumor regression; tumor growth inhibition for the mAb300 MMAE ADC was similar to the unconjugated antibody group; and no significant effect was seen on body weight with any treatment.

[00364] **Table 23**. Tumor Growth Inhibition (TGI) of the Treatment Groups.

Treatment Group	TGI (%)
5 mg/kg mAb300	57
5 mg/kg ADC-009-M (mAb300 Exatecan ADC DAR 2)	113
10 mg/kg ADC-009-M (mAb300 Exatecan ADC DAR 2)	114
5 mg/kg ADC-018-M (mAb300 Exatecan ADC DAR 4)	110
2.5 mg/kg ADC-018-M (mAb300 Exatecan ADC DAR 4)	109
5 mg/kg ADC-019-M (mAb300 MMAE ADC DAR 4)	45
2.5 mg/kg ADC-019-M (mAb300 MMAE ADC DAR 4)	77

Example 24: Anti-Tumor Effects in Patient-Derived Xenograft (PDX) Models

[00365] To investigate the anti-tumor activity of the ADCs as described herein in monotherapy, the small cell lung (SCL) cancer (SCLC) patient derived xenograft models LXFS 538, LXFS573 and LXFS 2156 as well as in the colon cancer models CXF 94 and CXF 742 implanted subcutaneously in female NMRI nude mice were established.

[00366] These PDX models were selected based on the expression data of DLL3, confirmed by immunohistochemistry (IHC) on formalin-fixed paraffin-embedded (FFPE) tumor samples (monoclonal rabbit anti-human DLL3 antibody clone E3J5R; Cell Signaling Technology cat # 71804/3) and the related metadata of the model.

[00367] Female NMRI nu/nu mice (from Charles River Laboratories), between 5 and 8 weeks of age, were implanted subcutaneously with tumor pieces of LXFS 538 (SCLC, IHC scores: 152 (membranous); 212 (cytoplasmic)), LXFS573 (SCLC, IHC scores: 42 (membranous); 51 (cytoplasmic)), LXFS 2156 (SCLC, IHC scores: 8 (membranous); 16 (cytoplasmic)), CXF 94 (SCLC, IHC scores: 5 (membranous); 9 (cytoplasmic)), or CXF 742 (colorectal, IHC scores: 142 (membranous); 168 (cytoplasmic)). Upon reaching individual tumor volumes of 50-250 mm³, mice were assigned to treatment groups based on tumor volumes aiming at comparable group mean/median tumor volumes and were subsequently treated according to **Table 24** below. Dosing started within 24 hours of the randomization. The observation period was max. 5 weeks post last treatment.

[00368] **Table 24.** Group Assignment and Therapy Schedule

Group No.	Compound	Single Dose* (mg/kg)	Dosing Volume* (mL/kg)	Dosing days	Route	Vehicle	n
1	Vehicle	NA	5	D1, D8	<i>i.v.</i>	PBS	8
2	ADC-016-M	5	5	D1	<i>i.v.</i>	PBS	8
3	ADC-016-M	5	5	D1, D8	<i>i.v.</i>	PBS	8
4	ADC-015-M	5	5	D1	<i>i.v.</i>	PBS	8
Total number of mice:							32

*based on last body weight measurement

[00369] Briefly, the efficacy experiments comprised 4 groups with 8 animals/group. All treatments were administered by the intravenous (*i.v.*) route, at a dose level of 5 mg/kg. ADC-015-M was tested as a single dose and ADC-016-M as both a single and repeat dose, on day 1 (D1) and day 8 (D8). As a reference, a vehicle control was included and dosed on a repeat schedule on day 1 and day 8. Tumor volume and body weight were monitored twice a week for 6 weeks post initial treatment. The tumor volumes were determined by two-dimensional measurement with a digital caliper and calculated according to the formula Tumor volume = $(l \times w^2) \times 0.5$, where *l* = tumor length and *w* = tumor width. Animals were removed from study when they reach humane endpoints including tumor volume exceeding 2000mm³ and body weight loss endpoint.

[00370] The results obtained from the CXF 742 PDX model are provided in **Figures 18A-18I** and **Table 25** below, indicating that TGI was observed with both ADC-016-M and ADC-015-M. Animals removed from the study because of TV endpoint ($TV > 2000 \text{ mm}^3$) are listed below: Vehicle: 1 on Day 17, 1 on Day 45, and 1 on Day 49; ADC-016-M, 5 mg/kg $\times$ 1: 1 on Day 38; and ADC-015-M, 5 mg/kg $\times$ 1: 2 on Day 49.

[00371] **Table 25.** TV and TGI of the Treatment Groups in the CXF 742 PDX model.

Group/ADC	TV Mean (mm^3)	TGI (%)
Vehicle	1,655	
ADC-016-M; 5 mg/kg $\times$ 1	476	76
ADC-016-M; 5 mg/kg $\times$ 2	457	78
ADC-015-M; 5 mg/kg $\times$ 1	806	56

[00372] The results obtained from the LXFS 2156 PDX model are provided in **Figures 19A-19I** and **Table 26** below, indicating that TGI was observed with ADC-016-M, with 2-dose treatment regimen. Animals removed from the study because of TV endpoint ($TV > 2000 \text{ mm}^3$) are listed below: Vehicle: 1 on Day 24, 1 on Day 27, 2 on Day 38, 2 on Day 42; ADC-016-M, 5 mg/kg $\times$ 1: 2 on Day 31, 1 on Day 35, 1 on Day 38, 2 on Day 42; ADC-016-M, 5 mg/kg $\times$ 2: 1 on Day 24; and ADC-015-M, 5 mg/kg $\times$ 1: 1 on Day 27, 3 on Day 35, and 4 on Day 38.

[00373] **Table 26.** TV and TGI of the Treatment Groups in the LXFS 2156 PDX model.

Group/ADC	TV Mean (mm^3)	TGI (%)
Vehicle	1,344	
ADC-016-M; 5 mg/kg $\times$ 1	1,127	20
ADC-016-M; 5 mg/kg $\times$ 2	318	85
ADC-015-M; 5 mg/kg $\times$ 1	1,350	1

[00374] The results obtained from the LXFS 573 PDX model are provided in **Figures 20A-20I** and **Table 27** below, showing that tumor growth delay was observed with ADC-016-M.

[00375] **Table 27.** TV and TGI of the Treatment Groups in the LXFS 573 PDX model.

Group/ADC	TV Mean (mm^3)	TGI (%)
Vehicle	1,505	
ADC-016-M; 5 mg/kg $\times$ 1	711	57
ADC-016-M; 5 mg/kg $\times$ 2	788	51
ADC-015-M; 5 mg/kg $\times$ 1	1,318	13

[00376] The results obtained from the CXF 94 PDX model are provided in **Figures 21A-21I** and **Table 28** below, indicating that no TGI was observed with ADC-016-M and ADC-015-M. Animals removed from the study because of TV endpoint ($TV > 2000 \text{ mm}^3$) are listed below: Vehicle: 2 on Day 31, and 1 on Day 38; ADC-016-M, 5 mg/kg $\times$ 1: 1 on Day 38; ADC-016-M, 5 mg/kg $\times$ 2: 1 on Day 31 (due to size of accessory tumor); and ADC-015-M, 5 mg/kg $\times$ 1: 2 on Day 31.

[00377] **Table 28.** TV and TGI of the Treatment Groups in the CXF 94 PDX model.

Group/ADC	TV Mean (mm^3)	TGI (%)
Vehicle	1,280	
ADC-016-M; 5 mg/kg $\times$ 1	825	38
ADC-016-M; 5 mg/kg $\times$ 2	748	46
ADC-015-M; 5 mg/kg $\times$ 1	1,214	1

[00378] The results obtained from the LXFS 538 PDX model are provided in **Figures 22A-22J** and **Tables 29- 30** below, indicating that TGI was observed with ADC-015-M and ADC-016-M and ADC-016-M with 2-dose regimen showed prolonged tumor regression. The results showed that tumor regression was observed in all dosed groups starting Day 7 post 1st dose; regrowth was observed in single treatment groups (with ADC-015-M and ADC-016-M) starting Day 18 post dose; and marginal tumor regrowth was seen in the group treated with ADC-016-M twice (Day 1 and Day 8) up to Day 46 post dose, and 3 complete responders (> 3 consecutive $TV = 0 \text{ mm}^3$ measurements) were observed. Animals removed from the study because of TV endpoint ($TV > 2000 \text{ mm}^3$) are listed below: Vehicle: 1 on Day 35; and ADC-015-M, 5 mg/kg $\times$ 1: 1 found dead on Day 25.

[00379] **Table 29.** TV and TGI of the Treatment Groups on Day 35 (D35) in the LXFS 538 PDX model.

Group/ADC	TV Mean (mm^3)	TGI (%)
Vehicle	1,108	
ADC-016-M; 5 mg/kg $\times$ 1	208	92
ADC-016-M; 5 mg/kg $\times$ 2	5	114
ADC-015-M; 5 mg/kg $\times$ 1	500	69

[00380] **Table 30.** TV and TGI of the Treatment Groups on Day 42 (D42) in the LXFS 538 PDX model.

Group/ADC	TV Mean (mm ³)	TGI (%)
Vehicle	1,422	
ADC-016-M; 5 mg/kg × 1	376	79
ADC-016-M; 5 mg/kg × 2	9	110
ADC-015-M; 5 mg/kg × 1	897	49

[00381] Other ADCs as disclosed herein are also studied using the established PDX models.

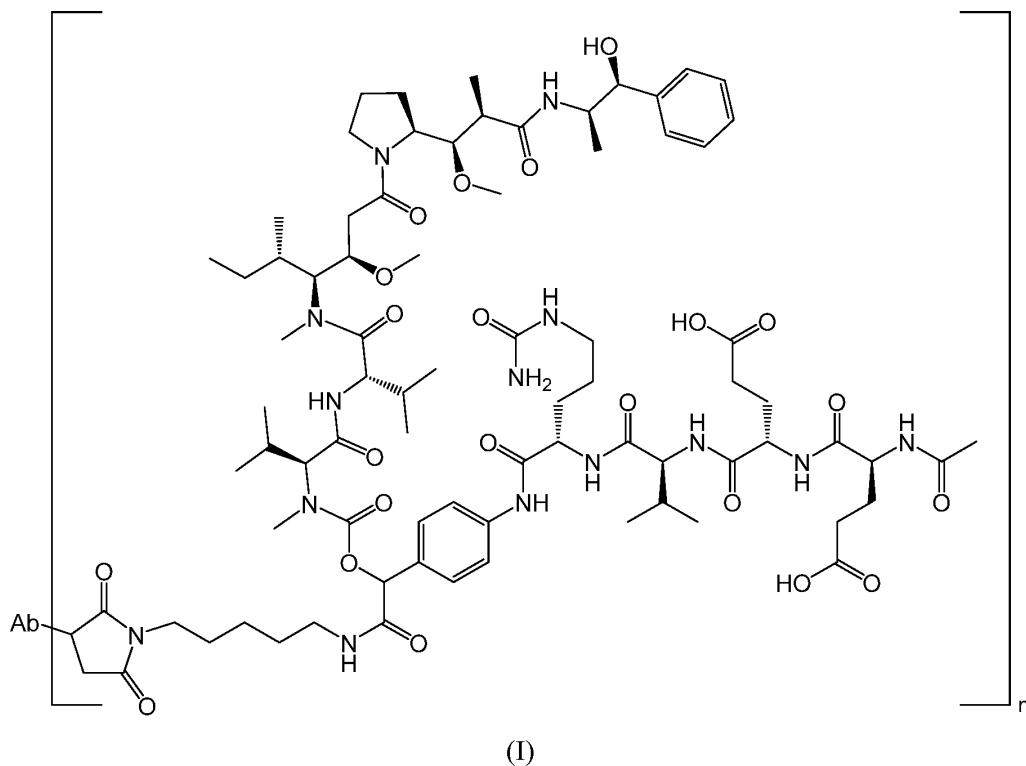
* * * * *

[00382] Throughout this application, various publications, patents, patent applications and other documents have been referenced. The disclosures of these publications, patents, patent applications and other documents in their entireties are hereby incorporated by reference in this application for all purposes, including in order to more fully describe the state of the art to which this the subject matter disclosed herein pertains. Although the disclosed subject matter has been described with reference to the examples provided above, it should be understood that various modifications could be made without departing from the spirit of the disclosed subject matter. Many variations will become apparent to those skilled in the art upon review of this specification.

CLAIMS

What is claimed is:

1. An antibody-drug conjugate (ADC) of Formula (I):

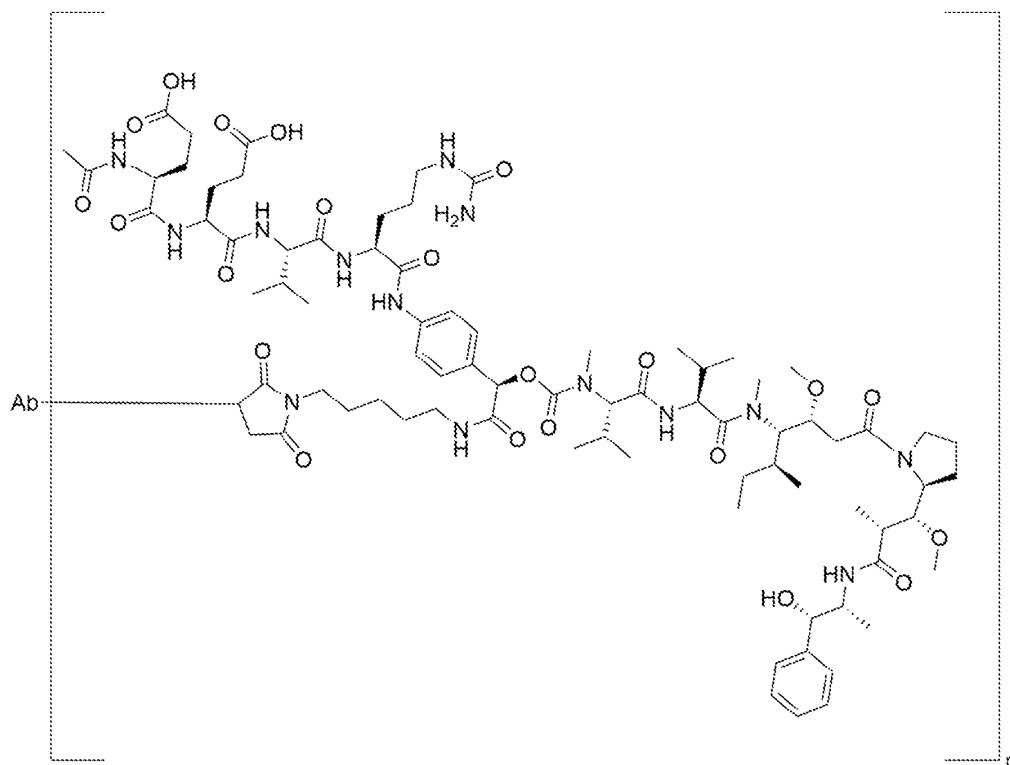


or a salt thereof, wherein:

n is an integer from 1 to 8, and

Ab represents an antibody that binds to DLL3 ("DLL3 antibody").

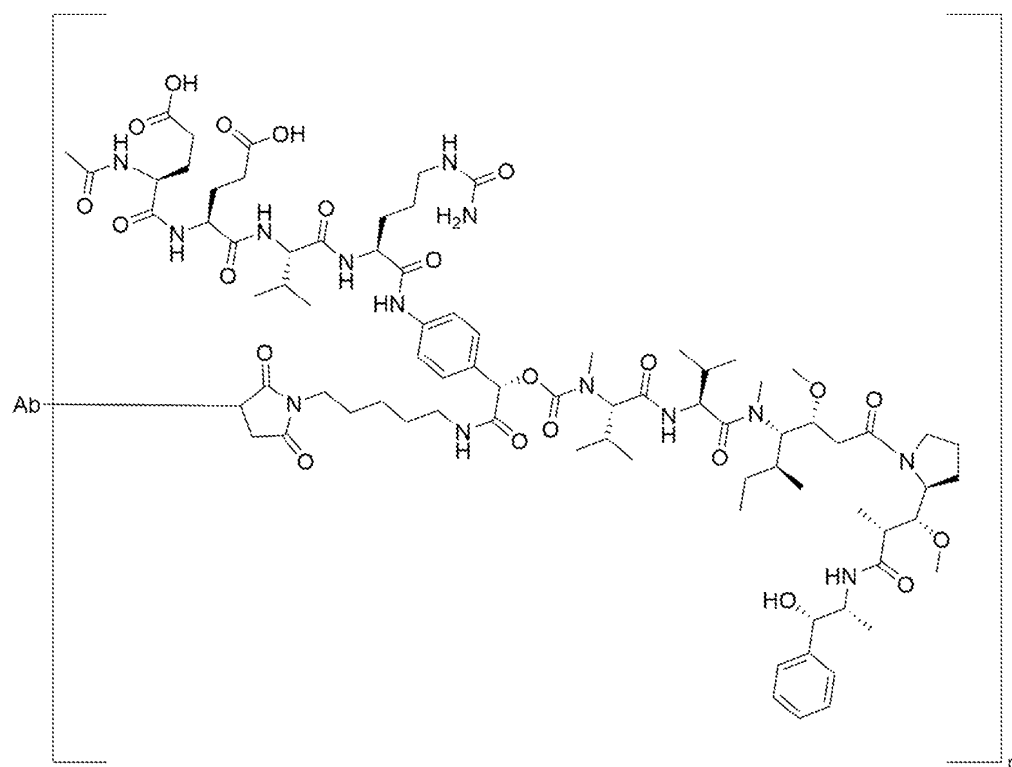
2. The ADC of claim 1, wherein the ADC is of Formula (I-A):



(I-A)

or a salt thereof.

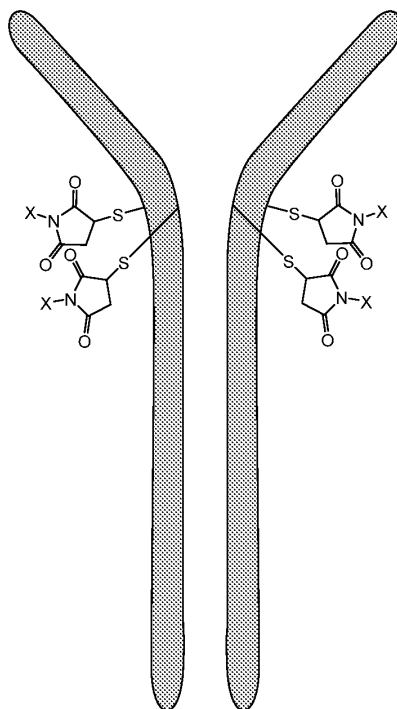
3. The ADC of claim 1, wherein the ADC is of Formula (I-B):



(I-B)

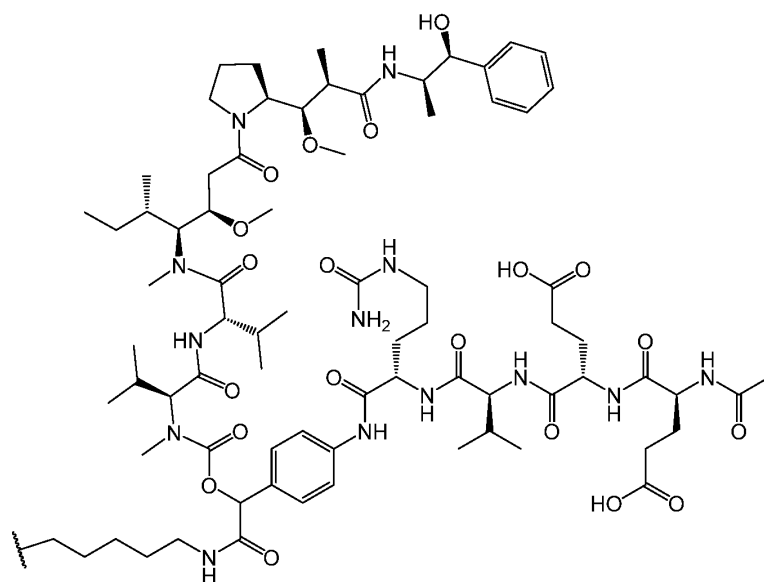
or a salt thereof.

4. An antibody-drug conjugate (ADC) of Formula (A):



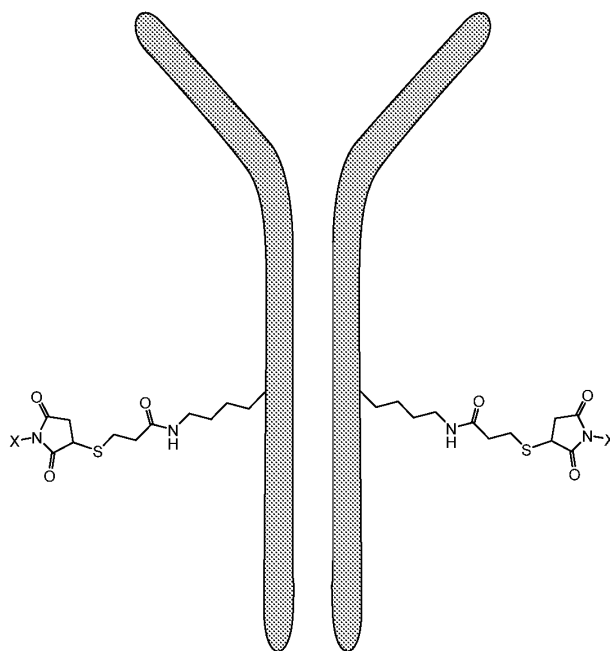
(A),

wherein each thick shaded line represents a chain of an antibody that binds to DLL3 (“DLL3 antibody”), the depicted sulfurs are from cysteine residues (optionally C226 and/or C229) of the DLL3 antibody, and X represents the structure below or a salt thereof:



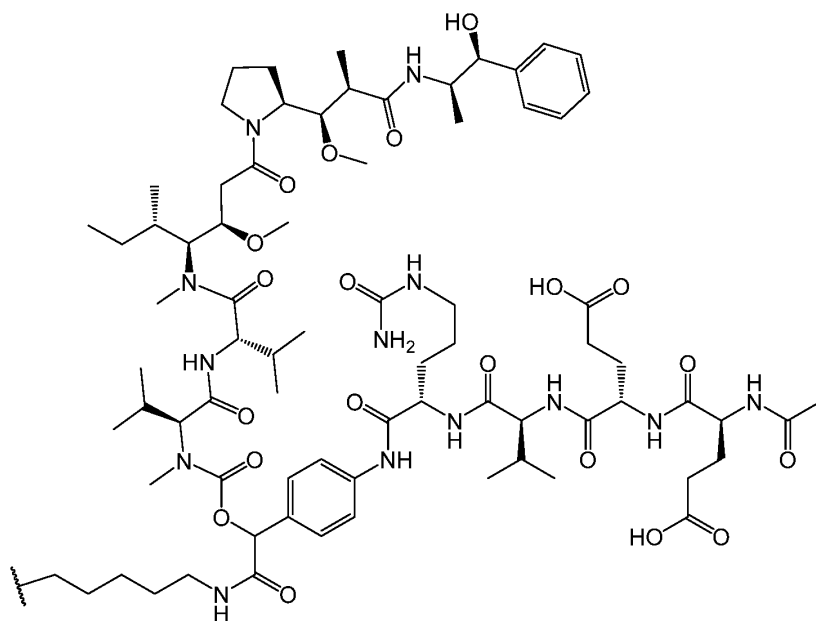
wherein the wavy line represents the point of attachment to the rest of the ADC of Formula (A).

5. An antibody-drug conjugate (ADC) of Formula (B):



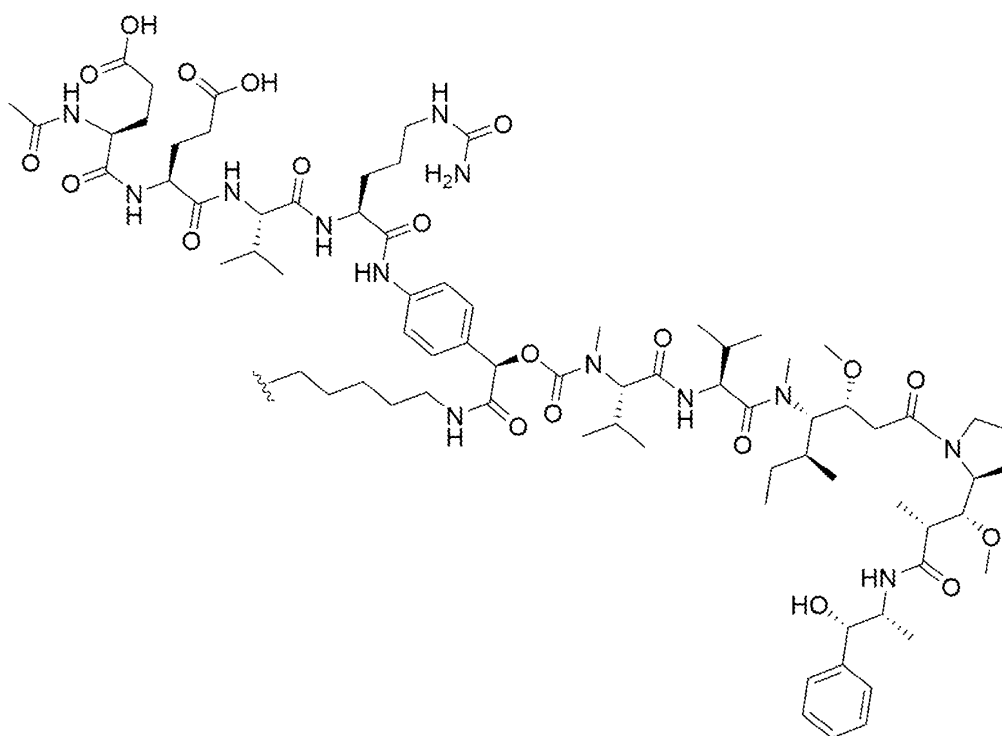
(B),

wherein each thick shaded line represents a chain of an antibody that binds to DLL3 (“DLL3 antibody”), the depicted $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}$ moiety is from a lysine residue (optionally any one or more of K246, K248, K288, K290, or K317 according to the EU numbering) of the DLL3 antibody, and X represents the structure below or a salt thereof:



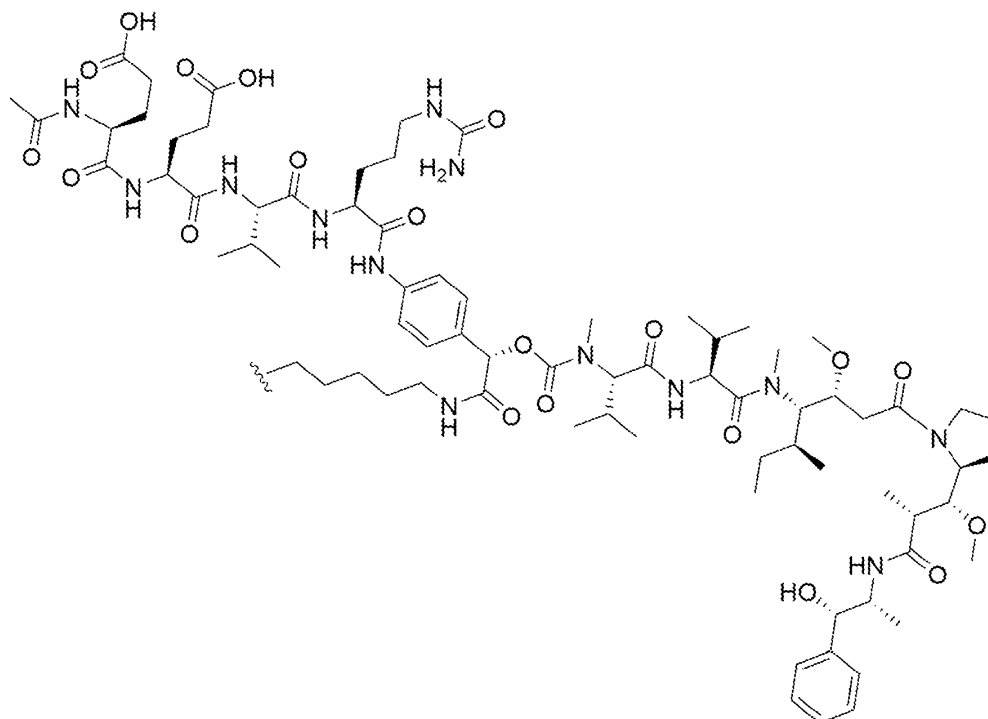
wherein the wavy line represents the point of attachment to the rest of the ADC of Formula (B).

6. The ADC of claim 5, wherein the DLL3 antibody comprises an amino acid sequence as set forth in SEQ ID NO:4 and wherein the lysine residue is the 150th amino acid residue of SEQ ID NO:4 (*e.g.*, K248 according to the EU numbering).
7. The ADC of claim 5, wherein the DLL3 antibody comprises an amino acid sequence as set forth in SEQ ID NO:5 and wherein the lysine residue is the 150th amino acid residue of SEQ ID NO:5 (*e.g.*, K248 according to the EU numbering).
8. The ADC of claim 5, wherein the DLL3 antibody comprises an amino acid sequence as set forth in SEQ ID NO:10 and wherein the lysine residue is the 152nd amino acid residue of SEQ ID NO:10 (*e.g.*, K248 according to the EU numbering).
9. The ADC of any one of claims 4-8, wherein the X represents the structure below or a salt thereof:



wherein the wavy line represents the point of attachment to the rest of the ADC of Formula (A) or (B).

10. The ADC of any one of claims 4-8, wherein the X represents the structure below or a salt thereof:



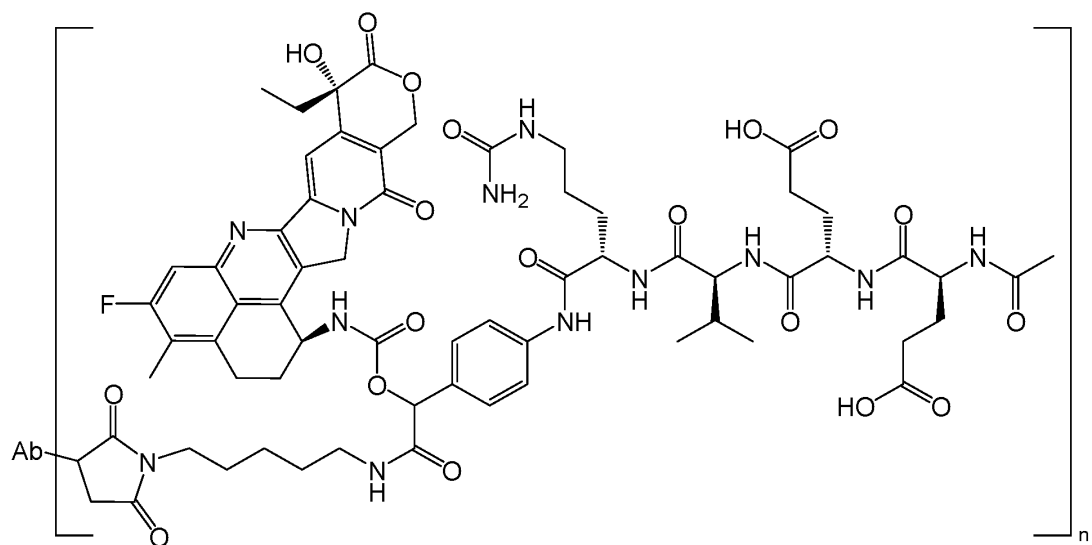
wherein the wavy line represents the point of attachment to the rest of the ADC of Formula (A) or (B).

11. The ADC of any of claims 1-10, wherein the DLL3 antibody comprises: (1) a CDR1, CDR2, and CDR3 of a heavy chain only antibody variable domain (VHH), wherein the VHH comprises an amino acid sequence as set forth in SEQ ID NO:14, 15 or 16; or (2) at least one VH, wherein the VH comprises CDR1, CDR2 and CDR3, and wherein: (i) the CDR1 comprises an amino acid sequence as set forth in SEQ ID NO:1 or 7; (ii) the CDR2 comprises an amino acid sequence as set forth in SEQ ID NO:2 or 8; and (iii) the CDR3 comprises an amino acid sequence as set forth in SEQ ID NO:3 or 9.
12. The ADC of any one of claims 1-11, wherein the DLL3 antibody comprises:
- (a) a CDR1 as set forth in SEQ ID NO:1; a CDR2 as set forth in SEQ ID NO:2; and a CDR3 as set forth in SEQ ID NO:3; or

(b) a CDR1 as set forth in SEQ ID NO:7; a CDR2 as set forth in SEQ ID NO:8; and a CDR3 as set forth in SEQ ID NO:9.

13. The ADC of any one of claims 1-12, wherein the DLL3 antibody comprises a VHH that comprises the amino acid sequence as set forth in any one of SEQ ID NOs: 14-16.
14. The ADC of any one of claims 1-13, wherein the DLL3 antibody further comprises a human IgG constant domain.
15. The ADC of any one of claims 1-14, wherein the DLL3 antibody further comprises an Fc fragment.
16. The ADC of claim 14, wherein the human IgG is a human IgG1.
17. The ADC of claim 15, wherein the Fc fragment comprises a homodimer of an amino acid sequence as set forth in SEQ ID NO:17.
18. The ADC of any one of claims 1-17, wherein the DLL3 antibody comprises at least one VHH single domain.
19. The ADC of any one of claims 1-18, wherein the DLL3 antibody comprises two VHH single domains, each of which is conjugated to a chain of an Fc fragment.
20. The ADC of any one of claims 1-19, wherein the DLL3 antibody comprises a homodimer of an amino acid sequence as set forth in any one of SEQ ID NO:4, 5, or 10.
21. The ADC of any one of claims 1-3 and 11-20, wherein n is 1.
22. The ADC of any one of claims 1-20, wherein n is 2.
23. The ADC of any one of claims 1-3 and 11-20, wherein n is 3.
24. The ADC of any one of claims 1-4 and 9-20, wherein n is 4.

25. An antibody-drug conjugate (ADC) of Formula (II):



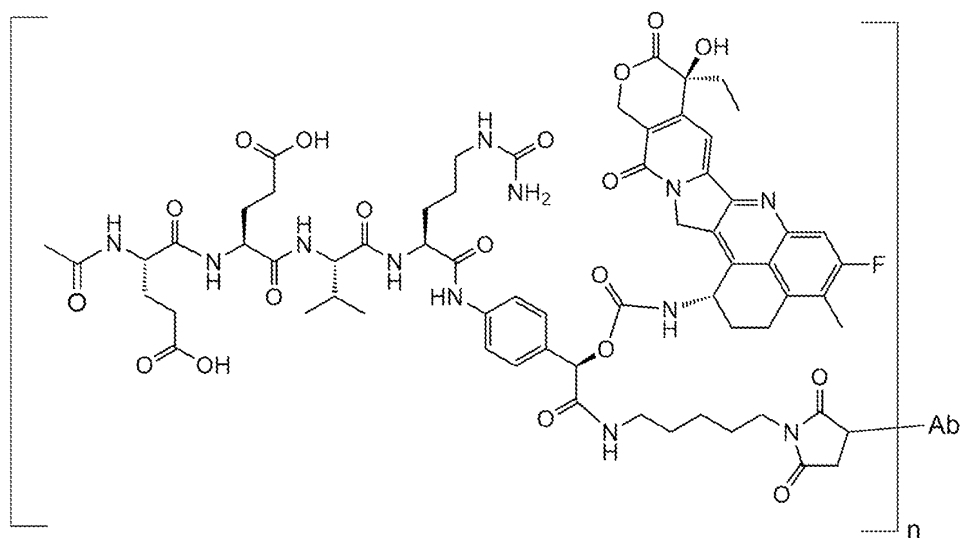
(II)

or a salt thereof, wherein:

n is an integer from 1 to 8, and

Ab represents an antibody that binds to DLL3 ("DLL3 antibody").

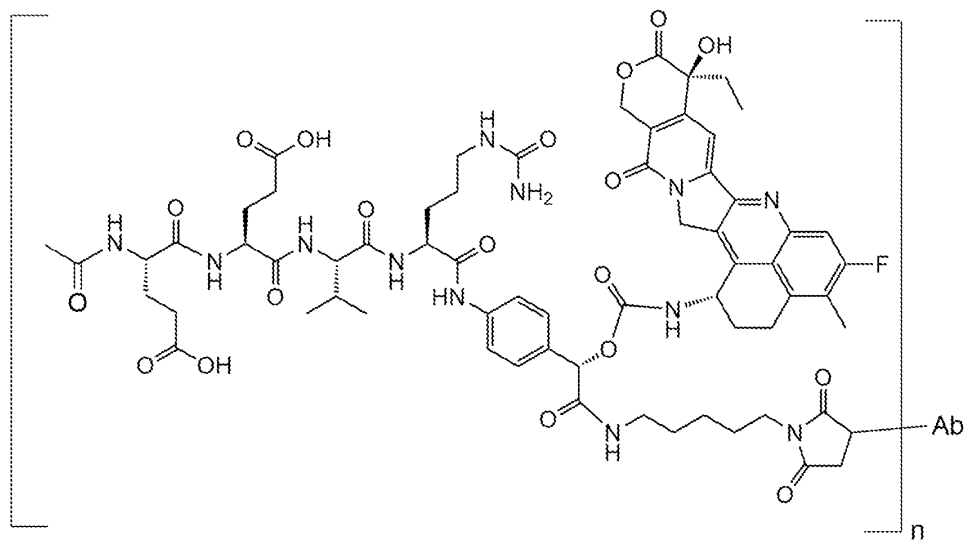
26. The ADC of claim 25, wherein the ADC is Formula (II-A)



(II-A)

or a salt thereof.

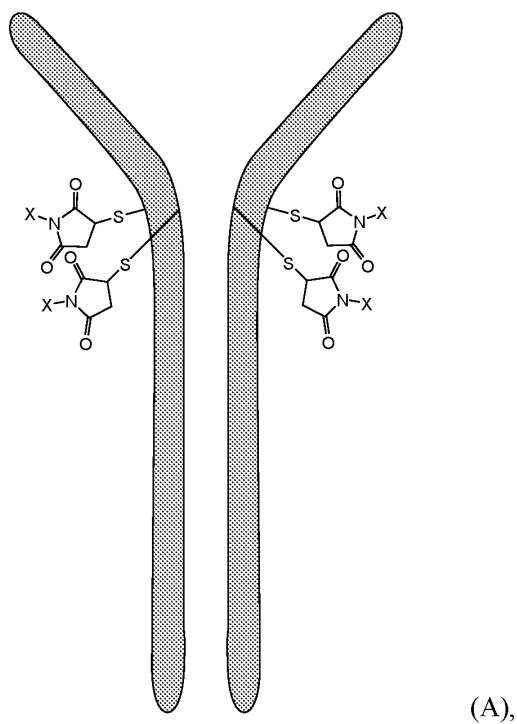
27. The ADC of claim 25, wherein the ADC is Formula (II-B)



(II-B)

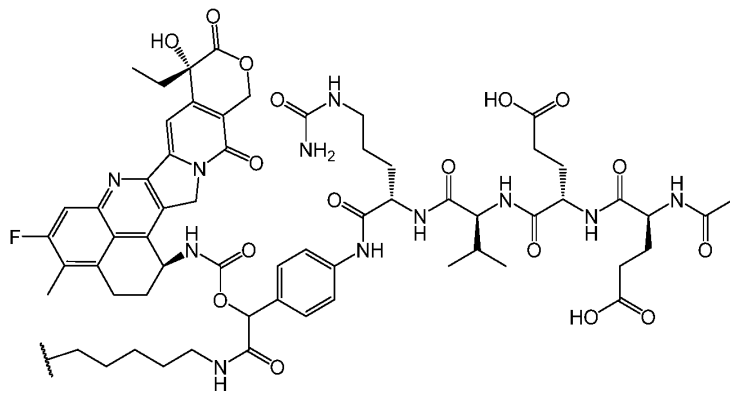
or a salt thereof.

28. An antibody-drug conjugate (ADC) of Formula (A):



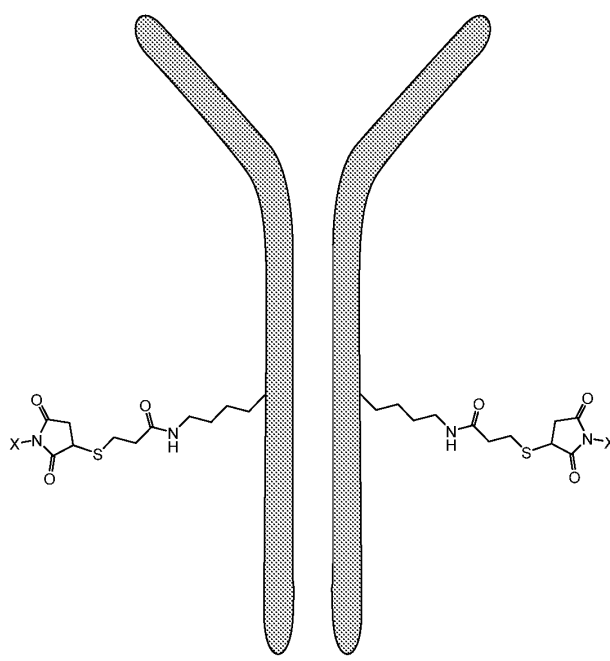
(A),

wherein each thick shaded line represents a chain of an antibody that binds to DLL3 (“DLL3 antibody”), the depicted sulfurs are from cysteine residues (optionally C226 and/or C229) of the DLL3 antibody, and X represents the structure below or a salt thereof:



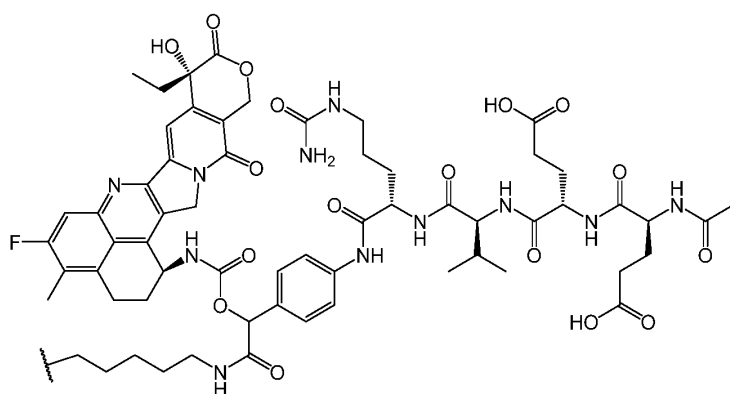
wherein the wavy line represents the point of attachment to the rest of the ADC of Formula (A).

29. An antibody-drug conjugate (ADC) of Formula (B):



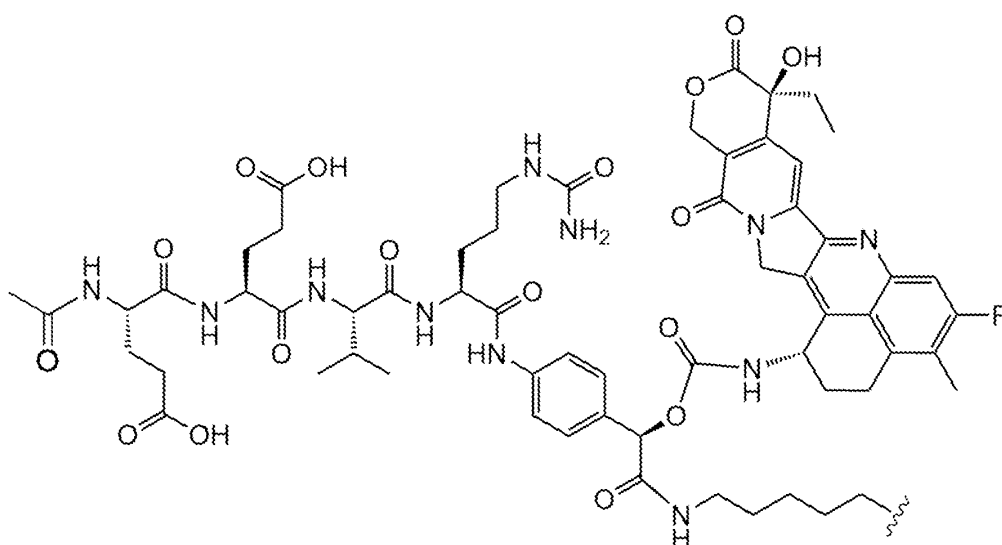
(B),

wherein each thick shaded line represents a chain of an antibody that binds to DLL3 (“DLL3 antibody”), the depicted $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}$ moiety is from a lysine residue (optionally any one or more of K246, K248, K288, K290, or K317 according to the EU numbering) of the CH2 domain of the DLL3 antibody, and X represents the structure below or a salt thereof:



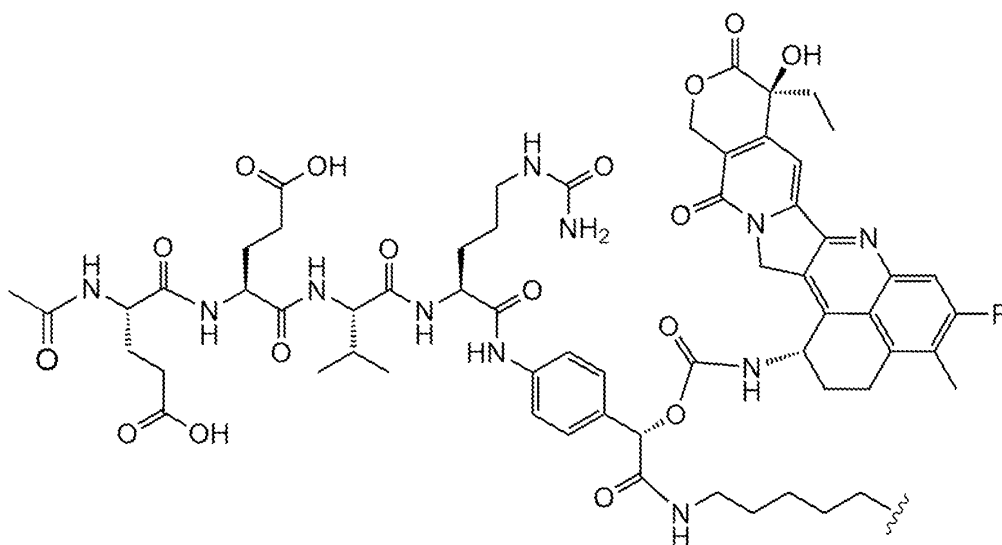
wherein the wavy line represents the point of attachment to the rest of the ADC of Formula (B).

30. The ADC of claim 29, wherein the DLL3 antibody comprises an amino acid sequence as set forth in SEQ ID NO:4 and wherein the lysine residue is the 150th amino acid residue of SEQ ID NO:4 (*e.g.*, K248 according to the EU numbering).
31. The ADC of claim 29, wherein the DLL3 antibody comprises an amino acid sequence as set forth in SEQ ID NO:5 and wherein the lysine residue is the 150th amino acid residue of SEQ ID NO:5 (*e.g.*, K248 according to the EU numbering).
32. The ADC of claim 29, wherein the DLL3 antibody comprises an amino acid sequence as set forth in SEQ ID NO:10 and wherein the lysine residue is the 152nd amino acid residue of SEQ ID NO:10 (*e.g.*, K248 according to the EU numbering).
33. The ADC of any one of claims 28-32, wherein X represents the structure below or a salt thereof:



wherein the wavy line represents the point of attachment to the rest of the ADC of Formula (A) or (B).

34. The ADC of any one of claims 28-32, wherein X represents the structure below or a salt thereof:



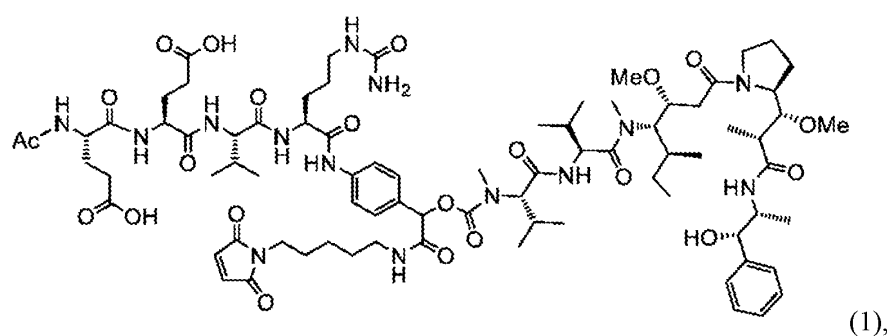
wherein the wavy line represents the point of attachment to the rest of the ADC of Formula (A) or (B).

35. The ADC of any one of claims 25-34, wherein the DLL3 antibody comprises: (1) a CDR1, CDR2, and CDR3 of a heavy chain only antibody variable domain (VHH), wherein the VHH comprises an amino acid sequence as set forth in SEQ ID NO:14,

- 15 or 16; or (2) at least one VH, wherein the VH comprises CDR1, CDR2 and CDR3, and wherein: (i) the CDR1 comprises an amino acid sequence as set forth in SEQ ID NO:1 or 7; (ii) the CDR2 comprises an amino acid sequence as set forth in SEQ ID NO:2 or 8; and (iii) the CDR3 comprises an amino acid sequence as set forth in SEQ ID NO:3 or 9.
- 36.** The ADC of any one of claims 25-35, wherein the DLL3 antibody comprises:
- (a) a CDR1 as set forth in SEQ ID NO:1; a CDR2 as set forth in SEQ ID NO:2; and a CDR3 as set forth in SEQ ID NO:3; or
- (b) a CDR1 as set forth in SEQ ID NO:7; a CDR2 as set forth in SEQ ID NO:8; and a CDR3 as set forth in SEQ ID NO:9.
- 37.** The ADC of any one of claims 25-36, wherein the DLL3 antibody comprises a VH that comprises the amino acid sequence as set forth in any one of SEQ ID NOs: 14-16.
- 38.** The ADC of any one of any of claims 25-37, wherein the DLL3 antibody further comprises a human IgG constant domain.
- 39.** The ADC of any one of claims 25-38, wherein the DLL3 antibody further comprises an Fc fragment.
- 40.** The ADC of claim 38, wherein the human IgG is a human IgG1.
- 41.** The ADC of claim 39, wherein the Fc fragment comprises a homodimer of an amino acid sequence as set forth in SEQ ID NO:17.
- 42.** The ADC of any one of claims 25-41, wherein the DLL3 antibody comprises at least one VHH single domain.
- 43.** The ADC of any one of claims 25-42, wherein the DLL3 antibody comprises two VHH single domains, each of which is conjugated to a chain of an Fc fragment.
- 44.** The ADC of any one of claims 25-43, wherein the DLL3 antibody comprises a homodimer of an amino acid sequence as set forth in any one of SEQ ID NO:4, 5, or 10.
- 45.** The ADC of any one of claims 25-27 and 35-44, wherein n is 1.

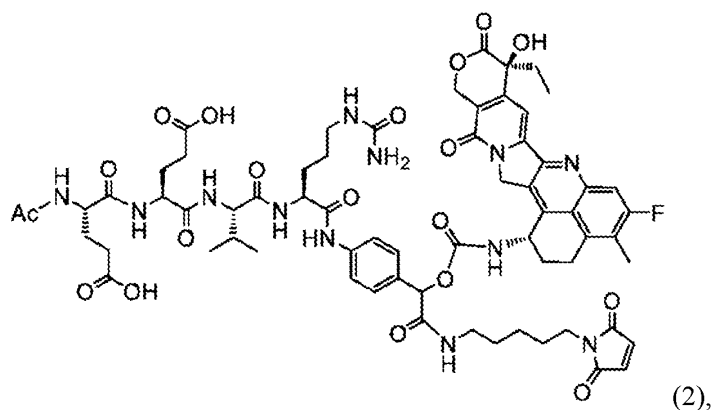
46. The ADC of any one of claims 25-44, wherein n is 2.
47. The ADC of any one of claims 25-27 and 35-44, wherein n is 3.
48. The ADC of any one of claims 25-28 and 35-44, wherein n is 4.
49. A pharmaceutical composition comprising one or more of the ADCs of any one of claims 1-48 and a pharmaceutically acceptable carrier.
50. The pharmaceutical composition of claim 49, wherein the composition is characterized by a drug-to-antibody ratio ("DAR") of about 1 to about 8.
51. The pharmaceutical composition of claim 50, wherein the DAR is about 1 to about 2.
52. The pharmaceutical composition of claim 50 or 51, wherein the DAR is about 1.1, about 1.2, about 1.3, about 1.4, about 1.5, about 1.6, about 1.7, about 1.8, about 1.9, or about 2.
53. The pharmaceutical composition of claim 50, wherein the DAR is about 2 to about 4.
54. The pharmaceutical composition of claim 50 or 53, wherein the DAR is about 2, about 2.1, about 2.2, about 2.4, about 2.4, about 2.5, about 2.6, about 2.7, about 2.8, about 2.9, about 3, about 3.1, about 3.2, about 3.3, about 3.4, about 3.5, about 3.6, about 3.7, about 3.8, about 3.9 or about 4.
55. A method of modulating a DLL3 related immune response in a subject, comprising administering to the subject the ADC of any one of claims 1-48 or the pharmaceutical composition of any one of claims 49-54 such that an immune response is modulated in the subject.
56. A method for treating a cancer in a subject, comprising administering to the subject the ADC of any one of claims 1-48 or the pharmaceutical composition of any one of claims 49-54, wherein the cancer is DLL3-positive or overexpressed.
57. The method of claim 56, wherein the cancer is selected from lung cancer, neuroendocrine carcinoma, and colorectal cancer.
58. The method of claim 56 or 57, wherein the cancer is SCLC or LCNEC.

59. Use of the ADC of any one of claims 1-48 or the pharmaceutical composition of any one of claims 49-54, in the manufacture of a medicament for diagnosing or treating a DLL3 positive cancer.
60. The ADC of any one of claims 1-48 or the pharmaceutical composition of any one of claims 49-54 for use in treating a DLL3 positive cancer.
61. A method of making the ADC of any one of claims 1-24, comprising the steps of:
- providing a solution comprising the DLL3 antibody;
 - contacting the solution of a) with a reducing agent;
 - contacting the solution of b) with a solution comprising Linker-Drug (1):



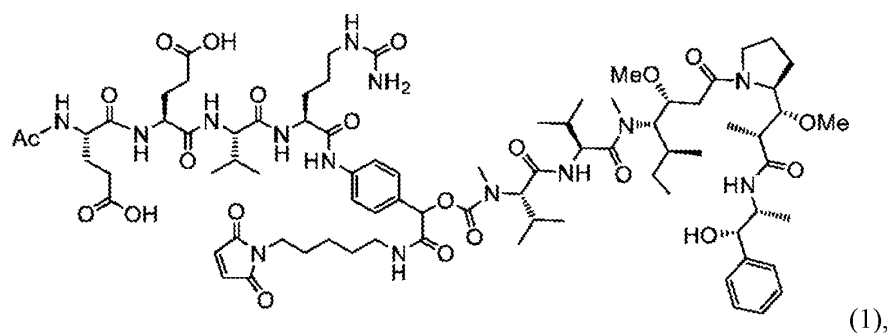
wherein the ADC is made.

62. A method of making the ADC of any one of claims 25-48, comprising the steps of:
- providing a solution comprising the DLL3 antibody;
 - contacting the solution of a) with a reducing agent;
 - contacting the solution of b) with a solution comprising Linker-Drug (2):



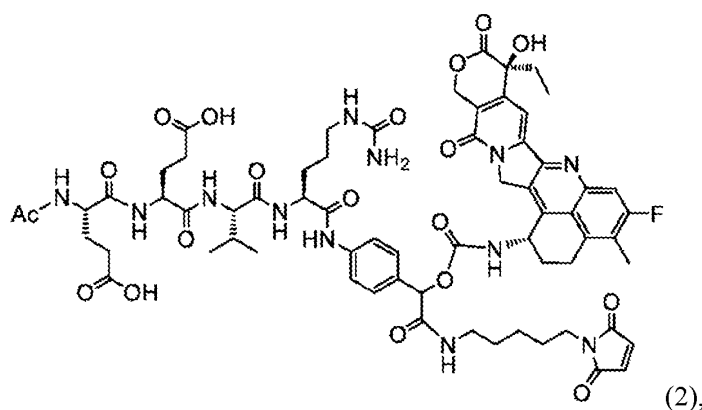
wherein the ADC is made.

63. The method of claim 61 or 62, wherein the reducing agent is tris(2 carboxyethyl)phosphine (TCEP).
64. A method of making the ADC of any one of claims 1-24, comprising the steps of:
- providing a solution comprising the DLL3 antibody;
 - contacting the solution of a) with an affinity peptide conjugated to a thiophenol activation moiety;
 - contacting the solution of b) with a solution comprising Linker-Drug (1):



wherein the ADC is made.

65. A method of making the ADC of any one of claims 25-48, comprising the steps of:
- providing a solution comprising the DLL3 antibody;
 - contacting the solution of a) with an affinity peptide conjugated to a thiophenol activation moiety;
 - contacting the solution of b) with a solution comprising Linker-Drug (2):



wherein the ADC is made.

66. The method of claim 64 or 65, wherein the affinity peptide is a peptide of SEQ ID NO:6.

67. The method of any one of claims 64-66, wherein the affinity peptide conjugated to a thiophenol activation moiety is:

