SUMMARY

[0006] Provided herein are anti-ALPP antibodies and ALPP-directed antibody-drug conjugates (ADCs), and anti-ALPPL2 antibodies and ALPPL2-directed ADCs. Also provided herein are antibodies that can bind both ALPP and ALPPL2 (anti-ALPP/ALPPL2 antibodies) and ADCs directed to both ALPP and ALPPL2 (ALPP/ALPPL2-directed ADCs). Also provided herein are methods of using anti-ALPP/ALPPL2-directed antibodies and ADCs to treat ALPP and/or ALPPL2-expressing disorders, including cancers. In one aspect, the present disclosure provides an antigen binding protein or fragment thereof that binds ALPP and/or ALPPL2, the antigen binding protein or fragment thereof comprising the following 6 CDRs: a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 56 or SEQ ID NO: 60; a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 57 or SEQ ID NO: 61; a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 58 or SEQ ID NO: 62; a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 63 or SEQ ID NO: 68; a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 64 or SEQ ID NO: 69; and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 65 or SEQ ID NO: 70; wherein the CDRs are determined by Kabat or IMGT.

[0006a] In another aspect, the present disclosure provides an antibody-drug conjugate comprising the antibody or antigen-binding fragment as described herein conjugated to a cytotoxic or cytostatic agent. In some ~~embodiment~~examples, the anti-ALPP/ALPPL2 antibodies comprise heavy chain CDR sequences of SEQ ID NOs: 56, 57, and 58, and light chain CDR sequences of SEQ ID NOs: 63, 64, and 65, as determined by Kabat numbering. In some ~~embodiment~~examples, the anti-ALPP/ALPPL2 antibodies comprise heavy chain CDR sequences of SEQ ID NOs: 60, 61, and 62, and light chain CDR sequences of SEQ ID NOs: 66, 67, and 68, as determined by IMGT numbering.

BRIEF DESCRIPTION OF THE DRAWINGS

[0007] FIGURE 1 shows a cytotoxicity evaluation of ALPP/ALPPL2-specific antibodies as ADCs using payloads with and without bystander activity on COV644 and NCI-H1651 tumor cells.

[0008] FIGURES 2A to 2C show the remaining viability of the cell lines CAOV3, COV644 and NCI-H1651 upon cytotoxicity evaluation of top ALPP/ALPPL2-specific antibodies as ADCs using payloads without bystander activity.

[0009] FIGURE 3 shows the binding affinities of ALPP/ALPPL2 specific antibodies.

[0010] FIGURE 4 shows the full binding profile for antibodies 1F7 and 12F3 to both ALPP and ALPPL2 expressing HEK293 cells by flow cytometry.

[0011] FIGURE 5 shows the sequence alignment of h12F3 variable heavy chain variants with human heavy acceptor sequence, IGHV3-49/HJ4.

[0012] FIGURE 6 shows the variable domain alignment of h12F3 variable heavy chain variants.

[0013] FIGURE 7 shows the sequence alignment of h12F3 variable light chain variants with human kappa acceptor sequence, IGKV1-33/KJ2.

WHAT IS CLAIMED IS:

1. An antigen binding protein or fragment thereof that binds ALPP and/or ALPPL2, the antigen binding protein or fragment thereof comprising the following 6 CDRs:

a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 56 or SEQ ID NO: 60;

a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 57 or SEQ ID NO: 61;

a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 58 or SEQ ID NO: 62;

a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 63 or SEQ ID NO: 68;

a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 64 or SEQ ID NO: 69; and

a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 65 or SEQ ID NO: 70;

wherein the CDRs are determined by Kabat or IMGT.

2. The antigen binding protein or fragment of claim 1 comprising a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 56; a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 57; a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 58; a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 63, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 64, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 65; wherein the CDRs are determined by Kabat.

3. The antigen binding protein or fragment of claim 1 comprising a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 60; a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 61; a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 62; a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 68, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 69, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 70; wherein the CDRs are determined by IMGT.

4. The antigen binding protein or fragment of any of claims 1-3 that comprises a VH and a VL, wherein the VH has at least 80%, 85%, 90%, 95% or 99% amino acid sequence identity to the amino acid sequence of SEQ ID NO: 15, and wherein the VL has at least 80%, 85%, 90%, 95% or 99% amino acid sequence identity to the amino acid sequence of SEQ ID NO: 30.

5. The antigen binding protein or fragment of any of claims 1-4 that comprises a VH and a VL, wherein the VH comprises the amino acid sequence of SEQ ID NO: 15.

6. The antigen binding protein or fragment of any of claims 1-4 that comprises a VH and a VL, wherein the VL comprises the amino acid sequence of SEQ ID NO: 30.

7. The antigen binding protein or fragment of any of claims 1-6 that comprises a VH and a VL, wherein the VH comprises the amino acid sequence of SEQ ID NO: 15, and the VL comprises the amino acid sequence of SEQ ID NO: 30.

8. The antigen binding protein or fragment of any of claims 1-7, comprising a heavy chain (HC) comprising the amino acid sequence of SEQ ID NO: 40.

9. The antigen binding protein or fragment of any of claims 1-7, comprising a light chain (LC) comprising the amino acid sequence of SEQ ID NO: 50.

10. The antigen binding protein or fragment of any of claims 1-9, comprising a HC comprising the amino acid sequence of SEQ ID NO: 40 and comprising an LC comprising the amino acid sequence of SEQ ID NO: 50.

11. The antigen binding protein or fragment of any one of claims 1-10, wherein the antigen binding protein is a monoclonal antibody or fragment thereof.

12. The antigen binding protein or fragment of any one of claims 1-11, wherein the antigen binding protein is a humanized antibody or fragment thereof.

13. The antigen binding protein or fragment of any one of claims 1-12, wherein the fragment is selected from a Fab, Fab', Fv, scFv or $(Fab')_2$ fragment.

14. An antibody-drug conjugate comprising the antibody or antigen-binding fragment of any one of claims 1-13 conjugated to a cytotoxic or cytostatic agent.

15. The antibody-drug conjugate of claim 14, wherein the antibody or antigen-binding fragment is conjugated to the cytotoxic or cytostatic agent via a linker.

16. The antibody-drug conjugate of any of claims 14-15, wherein the cytotoxic or cytostatic agent is a monomethyl auristatin.

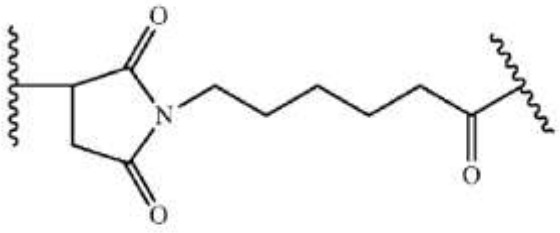
17. The antibody-drug conjugate of any of claims 14-16, wherein the monomethyl auristatin is monomethyl auristatin E (MMAE).

18. The antibody-drug conjugate of claim 17, wherein the antibody or antigen binding fragment thereof is conjugated to MMAE via an enzyme-cleavable linker unit.

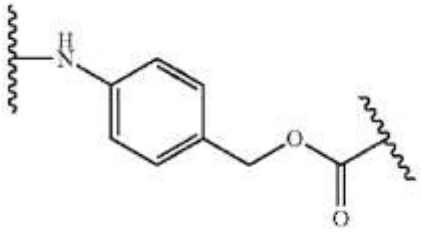
19. The antibody-drug conjugate of claim 18, wherein the enzyme-cleavable linker unit comprises a Val-Cit linker.

20. The antibody-drug conjugate of claim 19, wherein the antibody or antigen binding fragment thereof is conjugated to MMAE via a linker unit that has the formula: $–A_a–W_w–Y_y–$; wherein –A– is a stretcher unit, a is 0 or 1; –W– is an amino acid unit, w is an integer ranging from 0 to 12; and –Y– is a spacer unit, y is 0, 1, or 2.

21. The antibody-drug conjugate of claim 20, wherein the stretcher unit has the structure of Formula I below; wherein the amino acid unit is Val-Cit; and wherein the spacer unit is a p-aminobenzyl alcohol (PABC) group having the structure of Formula II below;

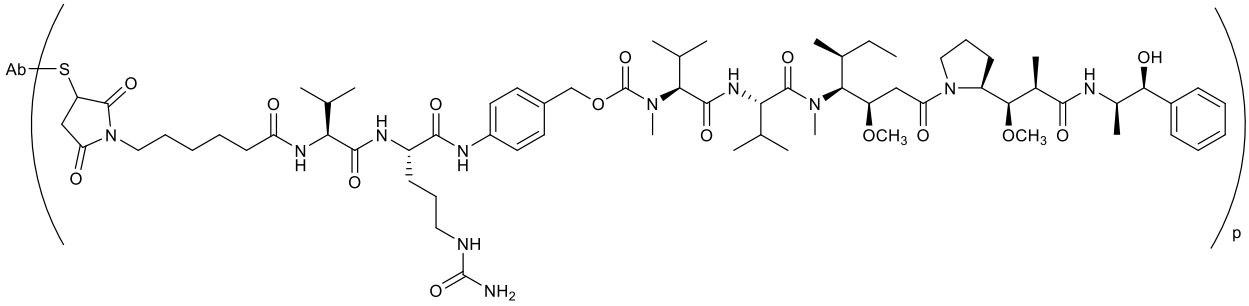


Formula I;



Formula II.

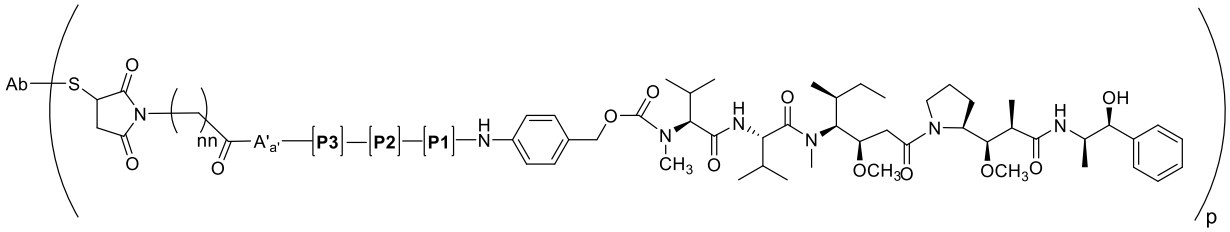
22. The antibody-drug conjugate of any of claims 14-21, wherein the linker is attached to monomethyl auristatin E forming an antibody-drug conjugate having the structure:



wherein Ab is the antibody h12F3 and p denotes a number from 1 to 16.

23. The antibody-drug conjugate of claim 22, wherein the average value of p in a population of the antibody-drug conjugate is about 4.

24. The antibody-drug conjugate of any of claims 14-18, wherein the antibody-drug conjugate is represented by the structure:



or a pharmaceutically acceptable salt thereof, wherein:

Ab is the antibody h12F3 and p denotes a number from 1 to 12;

subscript nn is a number from 1 to 5;

subscript a' is 0, and A' is absent;

P1, P2, and P3 are each an amino acid, wherein:

a first one of the amino acids P1, P2, or P3 is negatively charged;

a second one of the amino acids P1, P2, or P3 has an aliphatic side chain with hydrophobicity no greater than that of leucine; and

a third one of the amino acids P1, P2, or P3 has hydrophobicity lower than that of leucine,

wherein the first one of the amino acids P1, P2, or P3 corresponds to any one of P1, P2, or P3, the second one of the amino acids P1, P2, or P3 corresponds to one of the two remaining amino acids P1, P2, or P3, and the third one of the amino acids P1, P2, or P3 corresponds to the last remaining amino acids P1, P2, or P3,

provided that -P3-P2-P1- is not -Glu-Val-Cit- or -Asp-Val-Cit-.

25. The antibody-drug conjugate of claim 24, wherein subscript nn is 2.

26. The antibody-drug conjugate of claim 24 or 25, wherein:

the P3 amino acid of the tripeptide is in the D-amino acid configuration;

one of the P2 and P1 amino acids has an aliphatic side chain with hydrophobicity lower than that of leucine; and

the other of the P2 and P1 amino acids is negatively charged.

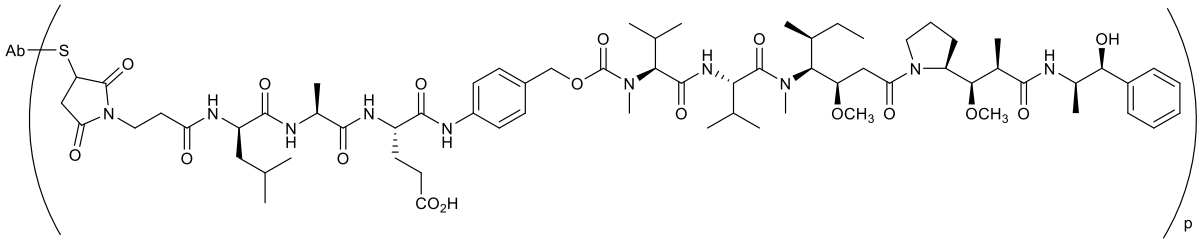
27. The antibody-drug conjugate of any one of claims 24-26, wherein the P3 amino acid is D-Leu or D-Ala.

28. The antibody-drug conjugate of any one of claims 24-27, wherein the P3 amino acid is D-Leu or D-Ala, the P2 amino acid is Ala, Glu, or Asp, and the P1 amino acid is Ala, Glu, or Asp.

29. The antibody-drug conjugate of any one of claims 24-28, wherein -P3-P2-P1- is -D-Leu-Ala-Asp-, -D-Leu-Ala-Glu-, -D-Ala-Ala-Asp-, or -D-Ala-Ala-Glu-.

30. The antibody-drug conjugate of any one of claims 24-29, wherein -P3-P2-P1- is -D-Leu-Ala-Glu-.

31. The antibody-drug conjugate of any one of claims 24-30, wherein the antibody-drug conjugate is represented by the structure:



or a pharmaceutically acceptable salt thereof,
wherein Ab is the antibody h12F3 and p denotes a number from 1 to 12.

~~32. An isolated nucleic acid encoding the antigen binding protein or fragment of any one of claims 1-13.~~

~~33. A vector comprising the nucleic acid of claim 32.~~

~~34. A host cell comprising the vector of claim 33.~~

~~35. The host cell of claim 34, wherein the host cell is a CHO cell.~~

~~36. A host cell that produces the antigen binding protein or fragment of any one of claims 1-13.~~

~~37. A method for making an antigen binding protein or fragment thereof, the method comprising culturing the host cell of claim 36 under conditions suitable for production of the antigen binding protein.~~

~~38. The method of claim 37, further comprising recovering the antigen binding protein or fragment produced by the host cell.~~

~~39. The method of claim 37 or claim 38 wherein the host cell is a CHO cell.~~

~~40. An antigen binding protein or fragment thereof produced by the method of any one of claims 37-39.~~

~~41. A pharmaceutical composition comprising the antigen binding protein or fragment of any one of claims 1-31 or 40 and a pharmaceutically acceptable carrier.~~

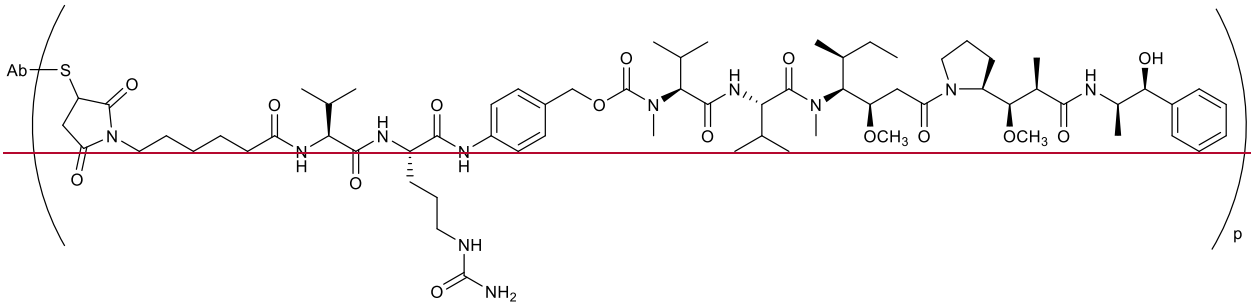
~~42. A method of treating an ALPP and/or ALPPL2-expressing cancer in an individual comprising administering to an individual in need thereof an effective amount of an antigen binding protein or fragment of any one of claims 1-31 or 40, or the pharmaceutical composition of claim 41.~~

~~43. The method of claim 42, wherein the cancer is ovarian cancer, lung cancer, endometrial cancer, bladder cancer, gastric cancer, or testicular cancer.~~

~~44. The method of claim 43, wherein the cancer is ovarian cancer.~~

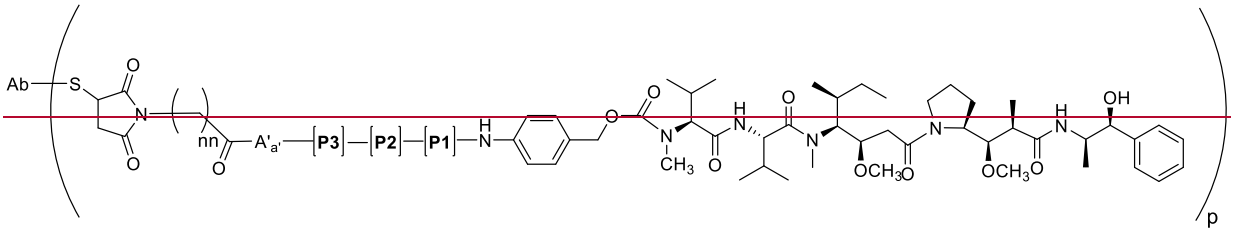
~~45. An antibody-drug conjugate comprising an isolated anti-ALPP/ALPPL2 antibody conjugated to mc-vc-PABC-MMAE, wherein the antibody has a heavy chain variable region comprising the~~

amino acid sequence of SEQ ID NO: 15 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 30, and wherein the antibody-drug conjugate has the structure:



wherein Ab is the antibody and p denotes a number from 1 to 16.

46. An antibody-drug conjugate comprising an antigen binding protein or fragment thereof that binds ALPP and ALPPL2, wherein the antibody-drug conjugate is represented by the structure:



or a pharmaceutically acceptable salt thereof, wherein:

Ab is an anti-ALPP/ALPPL2 antibody and p denotes a number from 1 to 12;

subscript nn is a number from 1 to 5;

subscript a' is 0, and A' is absent;

P1, P2, and P3 are each an amino acid, wherein:

a first one of the amino acids P1, P2, or P3 is negatively charged;

a second one of the amino acids P1, P2, or P3 has an aliphatic side chain with hydrophobicity no greater than that of leucine; and

a third one of the amino acids P1, P2, or P3 has hydrophobicity lower than that of leucine,

wherein the first one of the amino acids P1, P2, or P3 corresponds to any one of P1, P2, or P3, the second one of the amino acids P1, P2, or P3 corresponds to one of the two remaining

~~amino acids P1, P2, or P3, and the third one of the amino acids P1, P2, or P3 corresponds to the last remaining amino acids P1, P2, or P3,~~

~~provided that -P3-P2-P1- is not -Glu-Val-Cit- or -Asp-Val-Cit-.~~

~~47. The antibody-drug conjugate of claim 46, wherein subscript nn is 2.~~

~~48. The antibody-drug conjugate of claim 46 or 47, wherein:~~

~~the P3 amino acid of the tripeptide is in the D-amino acid configuration;~~

~~one of the P2 and P1 amino acids has an aliphatic side chain with hydrophobicity lower than that of leucine; and~~

~~the other of the P2 and P1 amino acids is negatively charged.~~

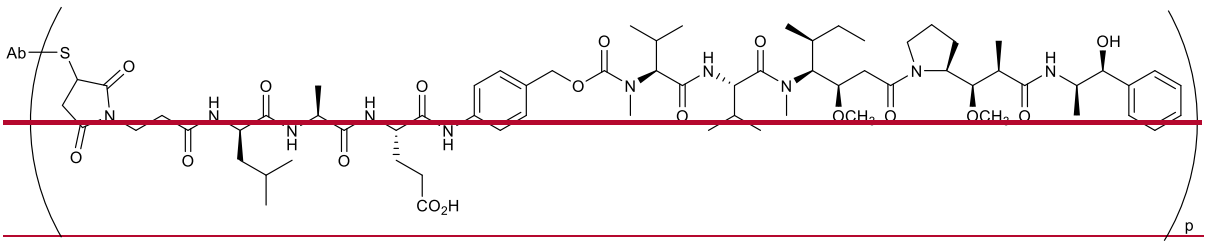
~~49. The antibody-drug conjugate of any one of claims 46-48, wherein the P3 amino acid is D-Leu or D-Ala.~~

~~50. The antibody-drug conjugate of any one of claims 46-49, wherein the P3 amino acid is D-Leu or D-Ala, the P2 amino acid is Ala, Glu, or Asp, and the P1 amino acid is Ala, Glu, or Asp.~~

~~51. The antibody-drug conjugate of any one of claims 46-50, wherein -P3-P2-P1- is -D-Leu-Ala-Asp-, -D-Leu-Ala-Glu-, -D-Ala-Ala-Asp-, or -D-Ala-Ala-Glu-.~~

~~52. The antibody-drug conjugate of any one of claims 46-51, wherein -P3-P2-P1- is -D-Leu-Ala-Glu-.~~

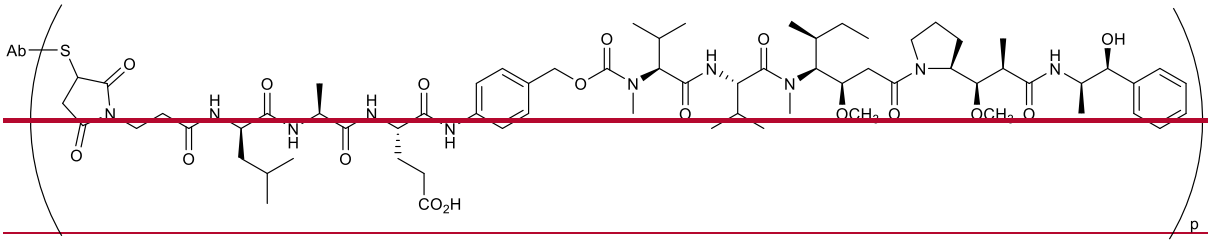
~~53. The antibody-drug conjugate of any one of claims 46-52, wherein the antibody-drug conjugate is represented by the structure:~~



~~or a pharmaceutically acceptable salt thereof,~~

~~wherein Ab is an anti-ALPP/ALPPL2 antibody and p denotes a number from 1 to 12.~~

~~54. An antibody-drug conjugate comprising an isolated anti-ALPP/ALPPL2 antibody conjugated to mp-dLAE-PABC-MMAE, wherein the antibody has a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 15 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 30, and wherein the antibody-drug conjugate has the structure:~~



~~wherein Ab is the antibody and p denotes a number from 1 to 12.~~

~~55. An ALPP and/or ALPPL2-binding antigen binding protein or fragment thereof that is capable of binding to one or more amino acids of a peptide comprising SEQ ID NO: 73 and/or SEQ ID NO: 74.~~