

SUMMARY

[0007] Provided herein are anti- $\alpha\text{v}\beta 6$ antibodies and $\alpha\text{v}\beta 6$ -directed antibody-drug conjugates (ADCs). Also provided herein are methods of using anti- $\alpha\text{v}\beta 6$ -directed antibodies and ADCs to treat $\alpha\text{v}\beta 6$ -expressing disorders, including cancers. Preferred anti- $\alpha\text{v}\beta 6$ antibodies comprise heavy chain CDR sequences of SEQ ID NOs: 31, 32, and 33 and light chain CDR sequences of SEQ ID NOs: 37, 42, and 39, as determined by Kabat numbering.

[0007a] In one aspect, the present disclosure provides an isolated anti- $\alpha\text{v}\beta 6$ antibody, or antigen-binding fragment thereof, comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises:

- (i) a CDR-H1 comprising the amino acid sequence of SEQ ID NO:31;
- (ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO:32; and
- (iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO:33; and

wherein the light chain variable region comprises:

- (i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO:37;
- (ii) a CDR-L2 comprising the amino acid sequence of SEQ ID NO:42; and
- (iii) a CDR-L3 comprising the amino acid sequence of SEQ ID NO:39

wherein the CDRs are determined by Kabat.

[0007b] In another aspect, the present disclosure provides an isolated anti- $\alpha\text{v}\beta 6$ antibody, or antigen-binding fragment thereof, comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises:

- (i) a CDR-H1 comprising the amino acid sequence of SEQ ID NO:34;
- (ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO:35; and
- (iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO:36; and

wherein the light chain variable region comprises:

- (i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO:43;
- (ii) a CDR-L2 comprising the amino acid sequence of SEQ ID NO:44; and
- (iii) a CDR-L3 comprising the amino acid sequence of SEQ ID NO:45

wherein the CDRs are determined by IMGT.

[0007c] 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 another aspect, the present disclosure provides a nucleic acid encoding the heavy chain variable region and/or the light chain variable region as defined herein. In another aspect, the present disclosure provides a vector comprising the nucleic acid as described herein. In another aspect, the present disclosure provides a host cell comprising the nucleic acid as described herein.

[0007d] In a further aspect, the present disclosure provides a method of producing an anti- $\alpha v \beta 6$ antibody or antigen-binding fragment thereof comprising culturing the host cell as described herein under a condition suitable for production of the anti- $\alpha v \beta 6$ antibody or antigen-binding fragment thereof. In a further aspect, the present disclosure provides a method of producing an anti- $\alpha v \beta 6$ antibody-drug conjugate comprising culturing the host cell as described herein under a condition suitable for production of an anti- $\alpha v \beta 6$ antibody; isolating the anti- $\alpha v \beta 6$ antibody produced from the host cell; and conjugating the anti- $\alpha v \beta 6$ antibody to a cytotoxic or cytostatic agent.

[0007e] In still another aspect, the present disclosure provides a use of the antibody or antigen-binding fragment as described herein or the antibody-drug conjugate as described herein in the manufacture of a medicament for treating cancer in a subject. In still another aspect, the present disclosure provides a pharmaceutical composition comprising the antibody or antigen-binding fragment as described herein or the antibody-drug conjugate as described herein and one or more agents selected from the group consisting of a physiologically acceptable carrier, a diluent, an excipient and an auxiliary.

BRIEF DESCRIPTION OF THE DRAWINGS

[0008] FIGURE 1 shows the results of a LAP blockade ELISA assay with various anti- $\alpha v \beta 6$ antibodies.

[0009] FIGURE 2 shows the results of saturation binding studies on 293F cells expressing human and cyno $\alpha v \beta 6$ with the murine antibody clone 2A2 (referred to as m2A2).

[0010] FIGURE 3 shows an alignment of the amino acid sequences of the parental murine mAb (referred to as m2A2) heavy chain variable region with select human germline acceptor

(referred to as HIGHV1-46/HJ4) and humanized 2A2 heavy chain variants. Asterisks represent CDRs as determined by Kabat, and crosses represent CDRs as determined by IMGT.

[0011] FIGURE 4 shows an alignment of the amino acid sequences of the parental murine mAb (referred to as m2A2) light chain variable region with select human germline acceptor (referred to as hIGKV1D-33/KJ2) and humanized 2A2 light chain variants. Asterisks represent CDRs as determined by Kabat, and crosses represent CDRs as determined by IMGT.

[0012] FIGURE 5 shows the results of competition binding studies on 293F cells expressing human $\alpha v \beta 6$ with humanized antibodies having the LA and LB light chains and the parental murine and chimeric antibodies (referred to as m2A2 and c2A2, respectively).

[0013] FIGURE 6 shows the results of competition binding studies on 293F cells expressing human $\alpha v \beta 6$ with humanized antibodies having the HA and HC heavy chains and the parental murine and chimeric antibodies (referred to as m2A2 and c2A2, respectively).

CLAIMS

1. An isolated anti- $\alpha v \beta 6$ antibody, or antigen-binding fragment thereof, comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises:

- (i) a CDR-H1 comprising the amino acid sequence of SEQ ID NO:31;
- (ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO:32; and
- (iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO:33; and

wherein the light chain variable region comprises:

- (i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO:37;
- (ii) a CDR-L2 comprising the amino acid sequence of SEQ ID NO:42; and
- (iii) a CDR-L3 comprising the amino acid sequence of SEQ ID NO:39

wherein the CDRs are determined by Kabat.

2. An isolated anti- $\alpha v \beta 6$ antibody, or antigen-binding fragment thereof, comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises:

- (i) a CDR-H1 comprising the amino acid sequence of SEQ ID NO:34;
- (ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO:35; and
- (iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO:36; and

wherein the light chain variable region comprises:

- (i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO:43;
- (ii) a CDR-L2 comprising the amino acid sequence of SEQ ID NO:44; and
- (iii) a CDR-L3 comprising the amino acid sequence of SEQ ID NO:45

wherein the CDRs are determined by IMGT.

3. The antibody or antigen-binding fragment of claim 1 or claim 2, wherein the antibody is humanized.

4. The antibody or antigen-binding fragment of any one of claims 1-3, wherein the heavy chain variable region comprises an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 6 and the light chain variable region comprises an amino

acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 17.

5. The antibody or antigen-binding fragment of any one of claims 1-4, wherein the heavy chain variable region comprises an amino acid sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 6 and the light chain variable region comprises an amino acid sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 17.

6. The antibody or antigen-binding fragment of any one of claims 1-5, wherein the heavy chain variable region comprises an amino acid sequence having at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 6 and the light chain variable region comprises an amino acid sequence having at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 17.

7. The antibody or antigen-binding fragment of any one of claims 1-6, wherein the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 6 and the light chain variable region comprises the amino acid sequence of SEQ ID NO:17.

8. The antibody or antigen-binding fragment of any of claims 4-7, wherein H2 is occupied by F, H28 is occupied by S, H48 is occupied by I, H67 is occupied by A, H69 is occupied by L, H71 is occupied by V, H73 is occupied by K, H78 is occupied by A, H93 is occupied by T, L69 is occupied by R, and L71 is occupied by Y, and wherein the numbering is via the Kabat numbering system.

9. The antibody or antigen-binding fragment of any of claims 1-8, wherein the heavy chain has an amino acid sequence comprising SEQ ID NO:21 and the light chain has an amino acid sequence comprising SEQ ID NO:29.

10. The antibody or antigen-binding fragment of any of claims 1-9, wherein the antibody or antigen-binding fragment is an antigen-binding fragment, and wherein the antigen-binding

fragment is selected from the group consisting of Fab, Fab', F(ab')₂, Fab'-SH, Fv, diabody, linear antibody, and single-chain antibody fragment.

11. The antibody or antigen-binding fragment of any of claims 1-10, wherein a heavy chain variable region of the antibody is fused to a heavy chain constant region and a light chain variable region is fused to a light chain constant region.

12. The antibody or antigen-binding fragment of claim 11, wherein the heavy chain constant region is of the IgG1 isotype.

13. An antibody-drug conjugate comprising the antibody or antigen-binding fragment of any one of claims 1-12 conjugated to a cytotoxic or cytostatic agent.

14. The antibody-drug conjugate of claim 13, wherein the antibody or antigen-binding fragment is conjugated to the cytotoxic or cytostatic agent via a linker.

15. The antibody-drug conjugate of any of claims 13-14, wherein the cytotoxic or cytostatic agent is a monomethyl auristatin.

16. The antibody-drug conjugate of any of claims 13-15, wherein the monomethyl auristatin is monomethyl auristatin E (MMAE).

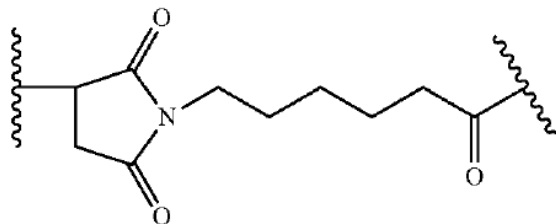
17. The antibody-drug conjugate of claim 16, wherein the antibody or antigen binding fragment thereof is conjugated to MMAE via an enzyme-cleavable linker unit.

18. The antibody-drug conjugate of claim 17, wherein the enzyme-cleavable linker unit comprises a Val-Cit linker.

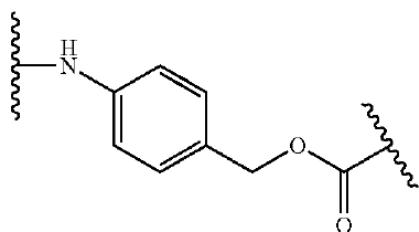
19. The antibody-drug conjugate of claim 18, wherein the antibody or antigen binding fragment thereof is conjugated to MMAE via a linker unit that has the formula: $-A_a-W_w-Y_y-$; wherein $-$

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.

20. The antibody-drug conjugate of claim 19, 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 (PAB) group having the structure of Formula II below;

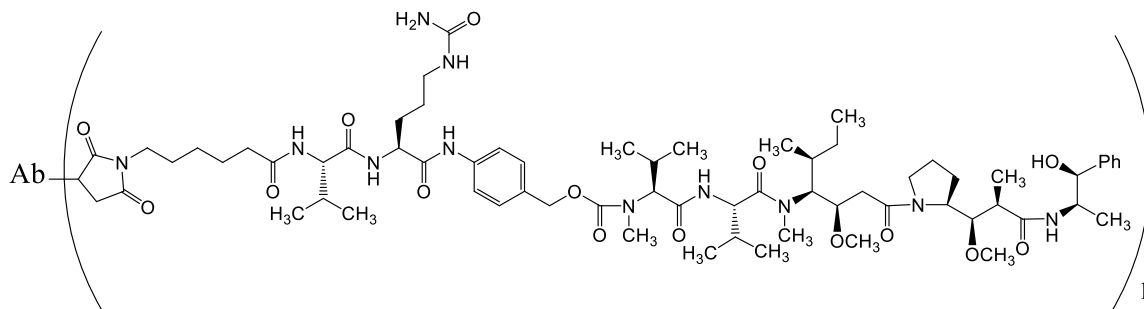


Formula I;



Formula II.

21. The antibody-drug conjugate of any of claims 14-20, wherein the linker is attached to monomethyl auristatin E forming an antibody-drug conjugate having the structure:



wherein Ab is the antibody h2A2 and p denotes a number from 1 to 16.

22. The antibody-drug conjugate of claim 21, wherein the average value of p in a population of the antibody-drug conjugate is about 4.
23. A nucleic acid encoding the heavy chain variable region and/or the light chain variable region as defined by any one of claims 1-12.
24. A vector comprising the nucleic acid of claim 23.
25. The vector of claim 24, wherein the vector is an expression vector.
26. A host cell comprising the nucleic acid of claim 25.
27. The host cell of claim 26, wherein the host cell is a Chinese hamster ovary (CHO) cell.
28. A method of producing an anti- $\alpha v \beta 6$ antibody or antigen-binding fragment thereof comprising culturing the host cell of claim 26 or 27 under a condition suitable for production of the anti- $\alpha v \beta 6$ antibody or antigen-binding fragment thereof.
29. The method of claim 28, further comprising isolating the anti- $\alpha v \beta 6$ antibody or antigen-binding fragment thereof produced by the host cell.
30. A method of producing an anti- $\alpha v \beta 6$ antibody-drug conjugate comprising culturing the host cell of claim 26 or 27 under a condition suitable for production of an anti- $\alpha v \beta 6$ antibody; isolating the anti- $\alpha v \beta 6$ antibody produced from the host cell; and conjugating the anti- $\alpha v \beta 6$ antibody to a cytotoxic or cytostatic agent.
31. The method of claim 30, wherein the anti- $\alpha v \beta 6$ antibody is conjugated to the cytotoxic or cytostatic agent via a linker.
32. The method of claim 31, wherein the linker is a Val-Cit linker.

33. The method of any of claims 30-32, wherein the cytotoxic or cytostatic agent is monomethyl auristatin E (MMAE).

34. ~~Use of A method of treating cancer in a subject, the method comprising administering to the~~ subject the antibody or antigen-binding fragment of any one of claims 1-12 or the antibody-drug conjugate of any one of claims 13-22 in the manufacture of a medicament for treating cancer in a subject.

35. The ~~use method~~ of claim 34, wherein the cancer is selected from the group consisting of non-small cell lung cancer (NSCLC), head and neck cancer, esophageal cancer, breast cancer, ovarian cancer, bladder cancer, skin cancer (SCC), ovarian cancer, cervical cancer, gastric cancer, and pancreatic cancer.

36. The ~~use method~~ of claim 34, wherein the lung cancer is non-small cell lung cancer.

37. The ~~use method~~ of claim 34, wherein the cancer is head and neck cancer.

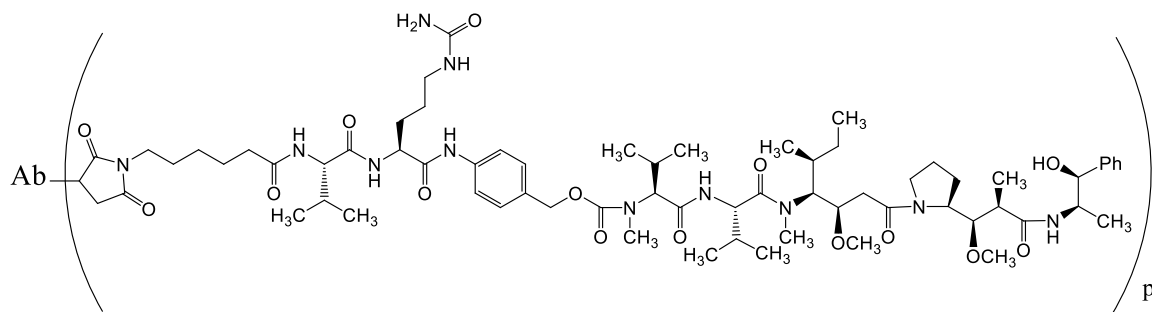
38. The ~~use method~~ of any one of claims 34-37, wherein the antibody or antigen-binding fragment or antibody-drug conjugate is in a pharmaceutical composition comprising the antibody or antigen-binding fragment or antibody-drug conjugate and a pharmaceutically acceptable carrier.

39. The ~~use method~~ of any one of claims 34-38, wherein the subject is a human.

40. A pharmaceutical composition comprising the antibody or antigen-binding fragment of any one of claims 1-12 or the antibody-drug conjugate of any one of claims 13-22 and one or more agents selected from the group consisting of a physiologically acceptable carrier, a diluent, an excipient and an auxiliary.

41. The ~~pharmaceutical composition~~ use of any one of claims 34-39 ~~40~~, wherein the composition is to be administered in combination with radiation or a chemotherapeutic agent.

42. An antibody-drug conjugate comprising isolated anti- $\alpha v \beta 6$ antibody conjugated to vcMMAE, wherein the antibody has a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 6 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 17, and wherein the antibody-drug conjugate has the structure:



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