

bispecific molecules that do not require lymphocyte preconditioning or co-stimulation in order to elicit efficient lysis of target cells. Several bispecific antibody formats have been developed and their suitability for T cell mediated immunotherapy investigated. These include BiTE (bispecific T cell engager) molecules (Nagorsen and B  uerle, Exp Cell Res 317, 1255-1260 (2011)),
5 diabodies (Holliger et al., Prot Eng 9, 299-305 (1996)) and derivatives thereof, such as tandem diabodies (Kipriyanov et al., J Mol Biol 293, 41-66 (1999)), DART (dual affinity retargeting) molecules, (Moore et al., Blood 117, 4542-51 (2011)), and triomabs (Seimetz et al., Cancer Treat Rev 36, 458-467 (2010)).

The task of generating bispecific molecules suitable for treatment provides several technical
10 challenges related to efficacy, toxicity, applicability and produceability that have to be met. In instances where the bispecific molecule targets an antigen on a target cell, e.g., a cancer cell, that is also expressed in non-target tissue, toxicity can occur. There is thus a need for efficacious T cell activating bispecific molecules that unleash full T cell activation in the presence of target cells but not in the presence of normal cells or tissue.

SUMMARY

The disclosure generally relates to T cell activating bispecific molecules that are activated selectively in the presence of a target cell.

The disclosure relates to a protease-activatable T cell activating bispecific molecule comprising

- (a) a first antigen binding moiety capable of specific binding to CD3;
- 20 (b) a second antigen binding moiety capable of specific binding to a target cell antigen; and
- (c) a masking moiety covalently attached to the T cell bispecific binding molecule through a protease-cleavable linker, wherein the masking moiety is capable of specific binding to the idiotype of the first or the second antigen binding moiety thereby reversibly concealing the first or second antigen binding moiety.

25 In one aspect, the disclosure relates to a protease-activatable T cell activating bispecific molecule comprising

- (a) a first antigen binding moiety capable of specific binding to CD3, wherein the first antigen binding moiety is an antibody or fragment thereof;
- (b) a second antigen binding moiety capable of specific binding to a target cell antigen
- 30 wherein the second antigen binding moiety is an antibody or fragment thereof; and

(c) a masking moiety covalently attached to the protease-activatable T cell activating bispecific molecule through a protease-cleavable linker, wherein the masking moiety is capable of specific binding to the idiotype of the first or the second antigen binding moiety thereby reversibly concealing the first or the second antigen binding moiety, wherein the masking moiety is an anti-idiotypic scFv.

In one embodiment, the masking moiety of the protease-activatable T cell activating bispecific molecule is covalently attached to the first antigen binding moiety. In one embodiment the masking moiety is covalently attached to the heavy chain variable region of the first antigen binding moiety. In one embodiment the masking moiety is covalently attached to the light chain variable region of the first antigen binding moiety. In one embodiment the masking moiety is an anti-idiotypic scFv.

In one particular embodiment the protease-activatable T cell activating bispecific molecule described herein comprises

- (a) a first heavy chain comprising the amino acid sequence of SEQ ID NO:132;
- (b) a second heavy chain comprising the amino acid sequence of SEQ ID NO:136;
- (c) a first light chain comprising an amino acid sequence of SEQ ID NO:81; and
- (d) a second light chain comprising an amino acid sequence of SEQ ID NO:133.

In one particular embodiment the protease-activatable T cell activating bispecific molecule described herein comprises

- (a) a first heavy chain comprising the amino acid sequence of SEQ ID NO:137;
- (b) a second heavy chain comprising the amino acid sequence of SEQ ID NO:139;
- (c) a first light chain comprising an amino acid sequence of SEQ ID NO:81; and
- (d) a second light chain comprising an amino acid sequence of SEQ ID NO:138.

The disclosure relates to an idiotypic-specific polypeptide for reversibly concealing an anti-CD3 antigen binding site of a molecule. In one aspect, the disclosure relates to an idiotypic-specific polypeptide for reversibly concealing an anti-CD3 antigen binding site of a molecule, wherein the idiotypic-specific polypeptide is an anti-idiotypic scFv wherein the idiotypic-specific polypeptide is covalently attached to the molecule through a linker. In one embodiment the idiotypic-specific polypeptide is an anti-idiotypic scFv. In one embodiment the idiotypic-specific polypeptide is covalently attached to the molecule through a linker. In one embodiment the linker is a peptide linker. In one embodiment the linker is a protease-cleavable linker. In one embodiment the peptide linker comprises at least one protease recognition site. In one embodiment the protease is selected from the group consisting of metalloproteinase, e.g., matrix metalloproteinase (MMP) 1-28 and A Disintegrin And Metalloproteinase (ADAM) 2, 7-12, 15, 17-23, 28-30 and 33, serine protease, e.g., urokinase-type plasminogen activator and Matriptase, cysteine protease, aspartic protease, and cathepsin protease. In one specific embodiment the protease is MMP9 or MMP2. In a further specific embodiment, the protease is Matriptase. In one embodiment the idiotypic-specific polypeptide is covalently attached to the molecule through more than one linker. In one embodiment the idiotypic-specific polypeptide is covalently attached to the molecule through two linkers.

In one embodiment the molecule which comprises the anti-CD3 antigen binding site is a T-cell activating bispecific molecule. In one particular embodiment the idiotype-specific polypeptide comprises a heavy chain variable region comprising at least one of:

- (a) a heavy chain complementarity determining region (CDR H) 1 amino acid sequence of DYSIH (SEQ ID NO:20);

In one particular embodiment the idiotype-specific polypeptide comprises a heavy chain variable region comprising:

- (a) a heavy chain complementarity determining region (CDR H) 1 amino acid sequence of SYGVS (SEQ ID NO:26);
- 5 (b) a CDR H2 amino acid sequence of IIWGDGSTNYHSALIS (SEQ ID NO:27);
- (c) a CDR H3 amino acid sequence of GITTVDYYAMDY (SEQ ID NO:28); and a light chain variable region comprising:
- (d) a light chain (CDR L)1 amino acid sequence of RASENIDSYLA (SEQ ID NO:29);
- 10 (e) a CDR L2 amino acid sequence of AATFLAD (SEQ ID NO:30); and
- (f) a CDR L3 amino acid sequence of QHYYSTPYT (SEQ ID NO:31).

According to the disclosure, an isolated polynucleotide encoding a protease-activatable T cell activating bispecific molecule of the disclosure or a fragment thereof is provided. In another aspect the disclosure relates to an isolated polynucleotide encoding the protease-activatable T cell activating bispecific antigen binding molecule of the disclosure or the idiotype-specific polypeptide of the disclosure. The disclosure also encompasses polypeptides encoded by the polynucleotides of the disclosure. In another aspect the disclosure further provides a vector, particularly an expression vector, comprising the isolated polynucleotide of the disclosure. In another aspect the disclosure provides a host cell comprising the polynucleotide or the expression of the disclosure. In some embodiments the host cell is a eukaryotic cell, particularly a mammalian cell.

In another aspect is provided a method of producing the protease-activated T cell molecule of the disclosure, comprising the steps of a) culturing the host cell of the disclosure under conditions suitable for the expression of the protease-activated T cell molecule and b) recovering the protease-activated T cell molecule. The disclosure also encompasses a protease-activated T cell molecule produced by the method of the disclosure.

In another aspect is provided a method of producing the idiotype-specific polypeptide of the disclosure, comprising the steps of a) culturing the host cell of the disclosure under conditions suitable for the expression of the protease-activated T cell molecule and b) recovering the idiotype-specific polypeptide. The disclosure also encompasses a idiotype-specific polypeptide produced by the method of the disclosure.

protease-activated T cell molecule or a pharmaceutical composition of the disclosure for use as a medicament. In one embodiment is provided a protease-activated T cell molecule or a pharmaceutical composition according to the disclosure for use in the treatment of a disease in an individual in need thereof. In a specific embodiment the disease is cancer.

- 5 Also provided is the use of a protease-activated T cell molecule of the disclosure for the manufacture of a medicament for the treatment of a disease in an individual in need thereof; as well as a method of treating a disease in an individual, comprising administering to said individual a therapeutically effective amount of a composition comprising the protease-activated T cell molecule according to the disclosure in a pharmaceutically acceptable form. In another
10 aspect, the disclosure provides use of the protease-activatable T cell activating bispecific molecule of the disclosure in the manufacture of a medicament for the treatment of or delaying the progression of cancer. In a specific embodiment the disease is cancer. In any of the above embodiments the individual preferably is a mammal, particularly a human.

- The disclosure also provides a method for inducing lysis of a target cell, particularly a tumor
15 cell, comprising contacting a target cell with a protease-activated T cell molecule of the disclosure in the presence of a T cell, particularly a cytotoxic T cell.

In another embodiment the disclosure also provides a composition comprising a protease-activatable T cell activating bispecific molecule described herein and a pharmaceutically acceptable carrier.

- 20 In another embodiment the disclosure also provides a composition comprising an idiotype-specific polypeptide as described herein and a pharmaceutically acceptable carrier.

- The disclosure also provides a protease-activatable T cell activating bispecific molecule or an idiotype-specific polypeptide as described herein, or the pharmaceutical composition described herein, for use as a medicament. In another aspect the disclosure also provides the protease-
25 activatable T cell activating bispecific molecule as described herein, or the pharmaceutical composition as described herein for use as a medicament. In one embodiment the medicament is for treating or delaying progression of cancer, treating or delaying progression of an immune related disease, and/or enhancing or stimulating an immune response or function in an individual.

Claims

1. A protease-activatable T cell activating bispecific molecule comprising
 - (a) a first antigen binding moiety capable of specific binding to CD3, wherein the first antigen binding moiety is an antibody or fragment thereof;
 - 5 (b) a second antigen binding moiety capable of specific binding to a target cell antigen wherein the second antigen binding moiety is an antibody or fragment thereof; and
 - (c) a masking moiety covalently attached to the protease-activatable T cell activating bispecific molecule through a protease-cleavable linker, wherein the masking moiety is capable of specific binding to the idiotype of the first or the second antigen binding moiety thereby reversibly concealing the first or the second antigen binding moiety,
10 wherein the masking moiety is an anti-idiotypic scFv.
2. The protease-activatable T cell activating bispecific molecule of claim 1, wherein the masking moiety is covalently attached to the first antigen binding moiety and reversibly conceals the first antigen binding moiety.
- 15 3. The protease-activatable T cell activating bispecific molecule of claim 1 or 2, wherein the masking moiety is covalently attached to the heavy chain variable region of the first antigen binding moiety.
4. The protease-activatable T cell activating bispecific molecule of any one of claims 1 to 3, wherein the second antigen binding moiety is capable of specific binding to a target cell
20 antigen selected from the group consisting of FolR1, HER1, HER2 and Mesothelin.
5. The protease-activatable T cell activating bispecific molecule of any one of claims 1 to 4, wherein the first and the second antigen binding moiety are fused to each other, optionally via a peptide linker.
6. The protease-activatable T cell activating bispecific molecule of any one of claims 1 to 5,
25 additionally comprising an Fc domain composed of a first and a second subunit capable of stable association.
7. The protease-activatable T cell activating bispecific molecule of claim 6, wherein the Fc domain is an IgG, specifically an IgG₁ or IgG₄, Fc domain.
8. The protease-activatable T cell activating bispecific molecule of any one of claims 6 or 7,
30 wherein the Fc domain exhibits reduced binding affinity to an Fc receptor and/or reduced effector function, as compared to a native IgG₁ Fc domain.
9. The protease-activatable T cell activating bispecific molecule of any one of claims 1 to 8, wherein the masking moiety comprises a heavy chain variable region comprising:

- (a) a heavy chain complementarity determining region (CDR H) 1 amino acid sequence of SYGVS (SEQ ID NO:26);
- (b) a CDR H2 amino acid sequence of IIWGDGSTNYHSALIS (SEQ ID NO:27);
- (c) a CDR H3 amino acid sequence of GITTVVDDYYAMDY (SEQ ID NO:28); and
- 5 a light chain variable region comprising:
- (d) a light chain complementary determining region (CDR L)1 amino acid sequence of RASENIDSYLA (SEQ ID NO:29);
- (e) a CDR L2 amino acid sequence of AATFLAD (SEQ ID NO:30); and
- (f) a CDR L3 amino acid sequence of QHYYSTPYT (SEQ ID NO:31).

10 10. The protease-activatable T cell activating bispecific molecule of any one of claims 1 to 9, wherein the protease cleavable linker comprises at least one protease recognition sequence.

11. The protease-activatable T cell activating bispecific molecule of claim 10, wherein the protease cleavable linker comprises the protease recognition sequence RQARVVNG
15 (SEQ ID NO:36).

12. The protease-activatable T cell activating bispecific molecule of any one of claims 1 to 11, wherein the first antigen binding moiety is capable of specific binding to CD3 and comprises a heavy chain variable region comprising:

- 20 a) a heavy chain complementarity determining region (CDR H) 1 amino acid sequence of TYAMN (SEQ ID NO:44);
- b) a CDR H2 amino acid sequence of RIRSKYNNYATYYADSVKG (SEQ ID NO:45); and
- c) a CDR H3 amino acid sequence of HGNFGNSYVSWFAY (SEQ ID NO:46); and a light chain variable region comprising:
- 25 d) a light chain complementary determining region (CDR L)1 amino acid sequence of GSSTGAVTTSNYAN (SEQ ID NO:17);
- e) a CDR L2 amino acid sequence of GTNKRAP (SEQ ID NO:18); and
- f) a CDR L3 amino acid sequence of ALWYSNLWV (SEQ ID NO:19).

13. The protease-activatable T cell activating bispecific molecule of any one of claims 1 to
30 12, wherein the first antigen binding moiety is capable of specific binding to CD3 and comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 43 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 55.

14. The protease-activatable T cell activating bispecific molecule of any one of claims 1 to 13, wherein the second antigen binding moiety is capable of specific binding to FolR1 and comprises a heavy chain variable region comprising:

- a) a heavy chain complementarity determining region (CDR H) 1 amino acid sequence of NAWMS (SEQ ID NO:14);
- b) a CDR H2 amino acid sequence of RIKSKTDGGTTDYAAPVKG (SEQ ID NO:15); and
- c) a CDR H3 amino acid sequence of PWEWSWYDY (SEQ ID NO:16); and a light chain variable region comprising:
- d) a light chain complementary determining region (CDR L)1 amino acid sequence of GSSTGAVTTSNYAN (SEQ ID NO:17);
- e) a CDR L2 amino acid sequence of GTNKRAP (SEQ ID NO:18); and
- f) a CDR L3 amino acid sequence of ALWYSNLWV (SEQ ID NO:19).

15. An idiotype-specific polypeptide for reversibly concealing an anti-CD3 antigen binding site of a molecule, wherein the idiotype-specific polypeptide is an anti-idiotypic scFv wherein the idiotype-specific polypeptide is covalently attached to the molecule through a linker.

16. The idiotype-specific polypeptide of claim 15, wherein the linker is a peptide linker.

17. The idiotype-specific polypeptide of claim 15 or 16, wherein the linker is a protease-cleavable linker.

18. The idiotype-specific polypeptide of any one of claims 15 to 17, wherein the peptide linker comprises at least one protease recognition site.

19. The idiotype-specific polypeptide of claim 18, wherein the protease cleavable linker comprises the protease recognition sequence RQARVVNG (SEQ ID NO:36).

20. The idiotype-specific polypeptide of any one of claims 18 or 19, wherein the molecule is a T-cell activating bispecific molecule.

21. The idiotype-specific polypeptide of any one of claims 15 to 20, comprising a heavy chain variable region comprising:

- (a) a heavy chain complementarity determining region (CDR H) 1 amino acid sequence of SYGVS (SEQ ID NO:26);
- (b) a CDR H2 amino acid sequence of IIWGDGSTNYHSALIS (SEQ ID NO:27);
- (c) a CDR H3 amino acid sequence of GITTVDYDYAMDY (SEQ ID NO:28); and
- a light chain variable region comprising:

(d) a light chain complementary determining region (CDR L)1 amino acid sequence of RASENIDSYLA (SEQ ID NO:29);

(e) a CDR L2 amino acid sequence of AATFLAD (SEQ ID NO:30); and

(f) a CDR L3 amino acid sequence of QHYYSTPYT (SEQ ID NO:31).

- 5 22. A pharmaceutical composition comprising the protease-activatable T cell activating bispecific molecule of any one of claims 1 to 14 or the idiotype-specific polypeptide of any one of claims 15 to 21 and a pharmaceutically acceptable carrier.
23. The protease-activatable T cell activating bispecific molecule of any one of claims 1 to 14, or the pharmaceutical composition of claim 22 for use as a medicament.
- 10 24. The protease-activatable T cell activating bispecific molecule for use according to claim 23, wherein the medicament is for treating or delaying progression of cancer.
25. An isolated polynucleotide encoding the protease-activatable T cell activating bispecific antigen binding molecule of any one of claims 1 to 14 or the idiotype-specific polypeptide of any one of claims 15 to 21.
- 15 26. A vector, particularly an expression vector, comprising the polynucleotide of claim 25.
27. A host cell comprising the polynucleotide of claim 25 or the vector of claim 26.
28. A method of producing a protease-activatable T cell activating bispecific molecule or an idiotype-specific polypeptide, comprising the steps of a) culturing the host cell of claim 27 under conditions suitable for the expression of the protease-activatable T cell activating bispecific molecule or the idiotype-specific polypeptide and b) recovering the
- 20 protease-activatable T cell activating bispecific molecule or the idiotype-specific polypeptide.
29. Use of the protease-activatable T cell activating bispecific molecule of any one of claims 1 to 14 in the manufacture of a medicament for the treatment of or delaying the
- 25 progression of cancer.