

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.

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

- 20 (a) a first antigen binding moiety capable of specific binding to CD3;
- (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
25 concealing the first or second antigen binding moiety.

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
30 variable region of the first antigen binding moiety. In one embodiment the masking moiety is an anti-idiotype 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.

In one aspect, the disclosure relates to an idiotype-specific polypeptide for reversibly concealing an anti-CD3 antigen binding site of a molecule. In one embodiment the idiotype-specific polypeptide is an anti-idiotype scFv. In one embodiment the idiotype-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 idiotype-specific polypeptide is covalently attached to the molecule through more than one linker. In one embodiment the idiotype-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 GITTVDYDYAMDY (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 another embodiment of the disclosure, an isolated polynucleotide encoding a protease-activatable T cell activating bispecific molecule of the disclosure or a fragment thereof
- 15 is provided. The disclosure also encompasses polypeptides encoded by the polynucleotides of the disclosure. The disclosure further provides an expression vector comprising the isolated polynucleotide of the disclosure, and a host cell comprising the isolated polynucleotide or the expression vector of the disclosure. In some embodiments the host cell is a eukaryotic cell, particularly a mammalian cell.
- 20 In another embodiment 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.
- 25 In another embodiment 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.

In another aspect, the disclosure further provides a pharmaceutical composition comprising the protease-activatable T cell activating bispecific molecule of the disclosure or the idiotype-specific polypeptide of the disclosure and a pharmaceutically acceptable carrier.

Also encompassed by the disclosure are methods of using the protease-activated T cell molecule
5 and pharmaceutical composition of the disclosure. In one embodiment the disclosure provides a

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, or the idiotype-specific polypeptide of the disclosure in the manufacture of a medicament for the treatment of or delaying the progression of cancer, for the treatment of or delaying the progression of an immune related disease or in the enhancement or stimulation of an immune response of function. In a specific embodiment the disease is cancer. In any of the above
15 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 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-
20 activatable T cell activating bispecific molecule described herein and a pharmaceutically acceptable carrier.

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

- In another aspect the disclosure also provides a protease-activatable T cell activating bispecific
25 molecule or an idiotype-specific polypeptide as described herein, or the pharmaceutical composition 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.

In another embodiment the disclosure also provides a protease-activatable T cell activating bispecific molecule or idiotype-specific polypeptide as described herein for use in the treatment of a disease in an individual in need thereof. In one embodiment, the disease is a proliferative disorder, particularly cancer.

- 5 In another embodiment the disclosure also provides a method of treating a disease in an individual, comprising administering to said individual a therapeutically effective amount of a composition comprising the protease-activatable T cell activating bispecific molecule or composition as described herein.

In another embodiment the disclosure also provides a method for inducing lysis of a target cell,
10 comprising contacting a target cell with the protease-activatable T cell activating bispecific

molecule or composition as described herein in the presence of a T cell. In one embodiment the method for inducing lysis of a target cell is an in vitro method. In one embodiment the target cell is a cancer cell. In one embodiment the target cell expresses a protease capable of activating the protease-activatable T cell activating bispecific molecule.

- 5 In another embodiment the disclosure also provides an anti-idiotypic CD3 antibody or antigen-binding fragment thereof specific for an idiotype of an anti-CD3 antigen-binding molecule, wherein the anti-idiotypic CD3 antibody or fragment thereof when bound to the anti-CD3 antigen-binding molecule specifically blocks binding of the anti-CD3 antigen-binding molecule to CD3.
- 10 In one embodiment, the anti-idiotypic CD3 antibody or antigen-binding fragment thereof is reversibly associated with the anti-CD3 antigen-binding molecule through a peptide linker comprising a protease recognition site. In one embodiment, the CD3 is a mouse, monkey or human CD3.

In another embodiment the disclosure provides a method of reducing in vivo toxicity of a T cell activating bispecific molecule comprising attaching an idiotype-specific polypeptide as described hererin to the T cell activating bispecific molecule with a protease-cleavable linker to form a protease-activatable T cell activating bispecific molecule, wherein the protease-activatable T cell activating bispecific molecule has reduced in vivo toxicity compared to the T cell activating bispecific molecule.

20 SHORT DESCRIPTION OF THE FIGURES

Figures 1A-E depict schematics of different CD3 binders with masking moieties. FIG. 1A: 7859 anti-ID CH2527 scFv 4.15.64 MK062 Matriptase site CD3 Fc. FIG. 1B: 7860 anti-ID CH2527 scFv 4.32.63 MK062 Matriptase site CD3 Fc. FIG. 1C: 7857 anti-ID CH2527 scFv 4.15.64 non-cleavable linker CD3 Fc. FIG. 1D: ID 7858 anti-ID CH2527 scFv 4.32.63 non-cleavable linker CD3 Fc. FIG. 1E: 7861 monovalent CD3 Fc.

Figure 2 shows a table summarizing the affinities of the anti-idiotypic masking moieties to the CD3 binder (CH2527).

Figure 3A-D shows Capillary Electrophoresis-SDS analysis of the molecules depicted in Figures 1A and B. FIGs. 3A-B Capillary Electrophoresis-SDS analysis of the molecule depicted in FIG. 1A under non reducing (FIG. 3A) and reducing conditions (FIG. 3B). Comparison of the untreated (I) and treated molecule (III) shows complete cleavage of the anti-ID scFv after rhMatriptase/ST14 treatment for 48 h at 37°C. One sample (II) was untreated but incubated at 37°C for 48 h. FIGs. 3C-D Capillary Electrophoresis-SDS analysis of the molecule depicted in

- (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
a light chain variable region comprising:
- (d) a light chain (CDR L)1 amino acid sequence of RASENIDSYLA (SEQ ID
5 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).

- 33. A pharmaceutical composition comprising the protease-activatable T cell activating
10 bispecific molecule of any one of claims 1 to 23 or the idiotype-specific polypeptide of
any one of claims 24 to 32 and a pharmaceutically acceptable carrier.
- 34. A protease-activatable T cell activating bispecific molecule of any one of claims 1 to 23,
the idiotype-specific polypeptide of any one of claims 24 to 32 or the pharmaceutical
composition of claim 33 for use as a medicament.
- 35. The protease-activatable T cell activating bispecific molecule for use according to claim
15 34, wherein the medicament is for treating or delaying progression of cancer, treating or
delaying progression of an immune related disease, or enhancing or stimulating an
immune response or function in an individual.
- 36. Use of the protease-activatable T cell activating bispecific molecule of any one of claims
20 1 to 23 or the idiotype-specific polypeptide of any one of claims 24 to 32 in the
manufacture of a medicament for the treatment of or delaying the progression of cancer,
for the treatment of or delaying the progression of an immune related disease or in the
enhancement or stimulation of an immune response of function.