

TAAAs selected from BCMA, CD38 and GPRC5D and CD28 expressed on T-cells have the potency to treat multiple myeloma as single agent or in combination with other agents such as T cell bispecific antibodies (TCBs) targeting a human MM cell surface antigen.

SUMMARY

5 The present disclosure describes tumor-targeted bispecific agonistic CD28 antigen binding molecules which achieve a tumor-dependent T cell activation and tumor cell killing without the necessity to form multimers. The bispecific CD28 antigen binding molecules of the present disclosure are characterized by monovalent binding to CD28 and in that they comprise at least one antigen binding domain capable of specific binding to a tumor-associated antigen (such as
10 Fibroblast Activation Protein (FAP) or Carcinoembryonic Antigen (CEA), CD19 or GPRC5D). Furthermore, they possess an Fc domain composed of a first and a second subunit capable of stable association comprising one or more amino acid substitution that reduces the binding affinity of the antigen binding molecule to an Fc receptor and/or effector function. Fc receptor-mediated cross-linking is thereby abrogated and tumor-specific activation is achieved by cross-
15 linking through binding of the at least one antigen binding domain capable of specific binding to a tumor-associated antigen to its antigen.

 Thus, the disclosure provides a bispecific agonistic CD28 antigen binding molecule characterized by monovalent binding to CD28, comprising
(a) one antigen binding domain capable of specific binding to CD28,
20 (b) at least one antigen binding domain capable of specific binding to a tumor-associated antigen, and
(c) an Fc domain composed of a first and a second subunit capable of stable association comprising one or more amino acid substitution that reduces the binding affinity of the antigen binding molecule to an Fc receptor and/or effector function.

25 In one aspect, the present disclosure provides a bispecific agonistic CD28 antigen binding molecule characterized by monovalent binding to CD28, comprising
(a) one Fab fragment capable of specific binding to CD28 comprising
 (i) a heavy chain variable region (V_HCD28) comprising the amino acid sequence of SEQ ID NO:47 and a light chain variable region (V_LCD28) comprising the amino acid sequence
30 of SEQ ID NO:54, or

(ii) a heavy chain variable region (V_HCD28) comprising the amino acid sequence of SEQ ID NO:46 and a light chain variable region (V_LCD28) comprising the amino acid sequence of SEQ ID NO:53, or

(iii) a heavy chain variable region (V_HCD28) comprising the amino acid sequence of SEQ ID NO:42 and a light chain variable region (V_LCD28) comprising the amino acid sequence of SEQ ID NO:27,

(b) one crossFab fragment capable of specific binding to CD19 comprising

(i) a heavy chain variable region (V_HCD19) comprising the amino acid sequence of SEQ ID NO:412 and a light chain variable region (V_LCD19) comprising the amino acid sequence of SEQ ID NO:413, or

(ii) a heavy chain variable region (V_HCD19) comprising the amino acid sequence of SEQ ID NO:420 and a light chain variable region (V_LCD19) comprising the amino acid sequence of SEQ ID NO:421, and

(c) a Fc domain of human IgG1 subclass and comprises the amino acid mutations L234A,

L235A and P329G, numbered according to Kabat EU index.

In one example, a bispecific agonistic CD28 antigen binding molecule as defined below is provided, wherein the Fc domain is an IgG, particularly an IgG1 Fc domain or an IgG4 Fc domain. In one particular example, the Fc domain composed of a first and a second subunit capable of stable association is an IgG1 Fc domain. In one example, the Fc domain comprises the amino acid substitutions L234A and L235A (numbering according to Kabat EU index). In one example, the Fc domain is of human IgG1 subclass and comprises the amino acid mutations L234A, L235A and P329G (numbering according to Kabat EU index).

In one example, provided is a bispecific agonistic CD28 antigen binding molecule as defined herein before, wherein the antigen binding domain capable of specific binding to CD28 comprises

of producing a bispecific agonistic CD28 antigen binding molecule as described herein comprising culturing the host cell of the disclosure under conditions suitable for the expression of the bispecific agonistic CD28 antigen binding molecule. Optionally, the method also comprises recovering the bispecific agonistic CD28 antigen binding molecule. In another aspect,
5 provided is a method of producing a bispecific agonistic CD28 antigen binding molecule, comprising the steps of a) culturing the host cell of the disclosure under conditions suitable for the expression of the bispecific agonistic CD28 antigen binding molecule and b) optionally recovering the bispecific agonistic CD28 antigen binding molecule. The disclosure also encompasses a bispecific agonistic CD28 antigen binding molecule produced by the method of
10 the disclosure.

In another aspect, the disclosure further provides a pharmaceutical composition comprising a bispecific agonistic CD28 antigen binding molecule of the disclosure and at least one pharmaceutically

acceptable excipient. The pharmaceutical composition is for use in the treatment of cancer. In another aspect, the disclosure provides the pharmaceutical composition of the disclosure for use in the treatment of cancer.

Also encompassed by the disclosure are methods of using the bispecific agonistic CD28 antigen binding molecule and the pharmaceutical composition of the disclosure. In one aspect the disclosure provides a bispecific agonistic CD28 antigen binding molecule or a pharmaceutical composition according to the disclosure for use as a medicament. Provided is a bispecific agonistic CD28 antigen binding molecule as described herein for use in enhancing (a) cell activation or (b) T cell effector functions. Provided is a bispecific agonistic CD28 antigen binding molecule or a pharmaceutical composition according to the disclosure for use in the treatment of a disease. In a specific example, the disease is cancer. In another aspect, provided is the bispecific agonistic CD28 antigen binding molecule of the disclosure for use in the treatment of cancer. In another aspect, provided is the bispecific agonistic CD28 antigen binding molecule of the disclosure for use in treating a B-cell proliferative disorder selected from the group consisting of Non-Hodgkin lymphoma (NHL), acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL), diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), mantle-cell lymphoma (MCL), marginal zone lymphoma (MZL), Multiple myeloma (MM) and Hodgkin lymphoma (HL). Provided is a bispecific agonistic CD28 antigen binding molecule or pharmaceutical composition according to the disclosure is for use in the treatment of cancer, wherein the bispecific agonistic CD28 antigen binding molecule is administered in combination with a chemotherapeutic agent, radiation therapy and/ or other agents for use in cancer immunotherapy. In another aspect, provided is the bispecific agonistic CD28 antigen binding molecule of the disclosure for use in the treatment of cancer, wherein the agonistic CD28 antigen binding molecule is to be administered in combination with a chemotherapeutic agent, radiation therapy and/ or other agents for use in cancer immunotherapy. Provided is a bispecific agonistic CD28 antigen binding molecule or a pharmaceutical composition for use in the treatment of cancer, wherein the bispecific agonistic CD28 antigen binding molecule is administered in combination with a T-cell activating anti-CD3 bispecific antibody. In another aspect, provided is the bispecific agonistic CD28 antigen binding molecule of the disclosure for use in the treatment of cancer, wherein the agonistic CD28 antigen binding molecule is to be administered in combination with a T-cell activating anti-CD3 bispecific antibody. In another aspect, provided is the bispecific agonistic CD28 antigen binding molecule of the disclosure for use in the treatment of cancer, wherein the agonistic CD28 antigen

binding molecule is to be administered in combination with an anti-CD20/anti-CD3 bispecific antibody. In another aspect, provided is the bispecific agonistic CD28 antigen binding molecule of the disclosure for use in the treatment of cancer, wherein the agonistic CD28 antigen binding molecule is to be administered in combination with an anti-CD20/anti-CD3 bispecific antibody comprising a polypeptide sequence of SEQ ID NO: 455, a polypeptide sequence of SEQ ID NO: 456, a polypeptide sequence of SEQ ID NO: 457 and a polypeptide sequence of SEQ ID NO: 458. Provided is a bispecific agonistic CD28 antigen binding molecule or a pharmaceutical composition for use in the treatment of cancer, wherein the bispecific agonistic CD28 antigen binding molecule is administered in combination with an anti-PD-L1 antibody or an anti-PD-1 antibody. In another aspect, provided is the bispecific agonistic CD28 antigen binding molecule of the disclosure for use in the treatment of cancer, wherein the agonistic CD28 antigen binding molecule is to be administered in combination with an anti-PD-L1 antibody or an anti-PD-1 antibody.

Also provided is the use of a bispecific agonistic CD28 antigen binding molecule according to the disclosure in the manufacture of a medicament for the treatment of a disease; as well as a method of treating a disease in an individual, comprising administering to said individual a therapeutically effective amount of a bispecific agonistic CD28 antigen binding molecule according to the disclosure or a composition comprising the bispecific agonistic CD28 antigen binding molecule according to the disclosure in a pharmaceutically acceptable form. In a specific example, the disease is cancer. In another aspect, provided is the use of the bispecific agonistic CD28 antigen binding molecule of the disclosure, or the pharmaceutical composition of the disclosure, in the manufacture of a medicament for the treatment of cancer. In another aspect, provided is use of of the bispecific agonistic CD28 antigen binding molecule of the disclosure in the manufacture of a medicament for treating a B-cell proliferative disorder selected from the group consisting of Non-Hodgkin lymphoma (NHL), acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL), diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), mantle-cell lymphoma (MCL), marginal zone lymphoma (MZL), Multiple myeloma (MM) and Hodgkin lymphoma (HL). In another aspect, provided is the use of the bispecific agonistic CD28 antigen binding molecule of the disclosure in the manufacture of a medicament for the treatment of cancer, wherein the agonistic CD28 antigen binding molecule is to be administered in combination with a chemotherapeutic agent, radiation therapy and/ or other agents for use in cancer immunotherapy. In another aspect, provided is the use of the bispecific agonistic CD28 antigen binding molecule of the disclosure in the manufacture of a medicament for the treatment of cancer, wherein the agonistic CD28 antigen binding molecule is to be

administered in combination with a T-cell activating anti-CD3 bispecific antibody. In another aspect, provided is use of the bispecific agonistic CD28 antigen binding molecule of the disclosure in the manufacture of a medicament for the treatment of cancer, wherein the agonistic CD28 antigen binding molecule is to be administered in combination with an anti-CD20/anti-CD3 bispecific antibody. In another aspect, provided is use of the bispecific agonistic CD28 antigen binding molecule of the disclosure in the manufacture of a medicament for the treatment of cancer, wherein the agonistic CD28 antigen binding molecule is to be administered in combination with an anti-CD20/anti-CD3 bispecific antibody comprising a polypeptide sequence of SEQ ID NO: 455, a polypeptide sequence of SEQ ID NO: 456, a polypeptide sequence of SEQ ID NO: 457 and a polypeptide sequence of SEQ ID NO: 458. In another aspect, provided is the use of the bispecific agonistic CD28 antigen binding molecule of the disclosure in the manufacture of a medicament for the treatment of cancer, wherein the agonistic CD28 antigen binding molecule is to be administered in combination with an anti-PD-L1 antibody or an anti-PD-1 antibody. Provided is a method (a) enhancing cell activation or (b) enhancing T cell effector functions in an individual, comprising administering a bispecific agonistic CD28 antigen binding molecule according to the disclosure or a composition comprising the bispecific agonistic CD28 antigen binding molecule according to the disclosure in a pharmaceutically acceptable form to said individual. Provided is the use of a bispecific agonistic CD28 antigen binding molecule according to the disclosure in the manufacture of a medicament for the treatment of a disease, wherein the treatment comprises co-administration with a chemotherapeutic agent, radiation therapy and/ or other agents for use in cancer immunotherapy. Provided is a method of treating a disease in an individual, comprising administering to said individual a therapeutically effective amount of a

Claims

1. A bispecific agonistic CD28 antigen binding molecule characterized by monovalent binding to CD28, comprising

(a) one Fab fragment capable of specific binding to CD28 comprising

5 (i) a heavy chain variable region (V_H CD28) comprising the amino acid sequence of SEQ ID NO:47 and a light chain variable region (V_L CD28) comprising the amino acid sequence of SEQ ID NO:54, or

(ii) a heavy chain variable region (V_H CD28) comprising the amino acid sequence of SEQ ID NO:46 and a light chain variable region (V_L CD28) comprising the amino acid sequence
10 of SEQ ID NO:53, or

(iii) a heavy chain variable region (V_H CD28) comprising the amino acid sequence of SEQ ID NO:42 and a light chain variable region (V_L CD28) comprising the amino acid sequence of SEQ ID NO:27,

(b) one crossFab fragment capable of specific binding to CD19 comprising

15 (i) a heavy chain variable region (V_H CD19) comprising the amino acid sequence of SEQ ID NO:412 and a light chain variable region (V_L CD19) comprising the amino acid sequence of SEQ ID NO:413, or

(ii) a heavy chain variable region (V_H CD19) comprising the amino acid sequence of SEQ ID NO:420 and a light chain variable region (V_L CD19) comprising the amino acid
20 sequence of SEQ ID NO:421, and

(c) a Fc domain of human IgG1 subclass and comprises the amino acid mutations L234A, L235A and P329G, numbered according to Kabat EU index.

2. The bispecific agonistic CD28 antigen binding molecule of claim 1, wherein the Fab fragment capable of specific binding to CD28 comprises a heavy chain variable region (V_H CD28)
25 comprising the amino acid sequence of SEQ ID NO:47 and a light chain variable region (V_L CD28) comprising the amino acid sequence of SEQ ID NO:54.

3. The bispecific agonistic CD28 antigen binding molecule of claim 1, wherein the Fab fragment capable of specific binding to CD28 comprises a heavy chain variable region (V_H CD28) comprising the amino acid sequence of SEQ ID NO:46 and a light chain variable region
30 (V_L CD28) comprising the amino acid sequence of SEQ ID NO:53.

4. The bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 3, wherein the antigen binding domain capable of specific binding to CD19 comprises a heavy chain variable region (V_H CD19) comprising an amino acid sequence of SEQ ID NO:412 and a light chain variable region (V_L CD19) comprising an amino acid sequence of SEQ ID NO:413.

5. The bispecific agonistic CD28 antigen binding molecule of claim 1 comprising a first light chain comprising the amino acid sequence of SEQ ID NO:122, a first heavy chain comprising the amino acid sequence of SEQ ID NO:114, a second heavy chain comprising the amino acid sequence of SEQ ID NO:430 and a second light chain comprising the amino acid sequence of SEQ ID NO:431.
6. The bispecific agonistic CD28 antigen binding molecule of claim 1 comprising the amino acid sequence of SEQ ID NO:121, a first heavy chain comprising the amino acid sequence of SEQ ID NO:116, a second heavy chain comprising the amino acid sequence of SEQ ID NO:430 and a second light chain comprising the amino acid sequence of SEQ ID NO:431.
7. One or more isolated polynucleotide encoding the bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 6.
8. One or more vector, particularly expression vector, comprising the polynucleotide(s) of claim 7.
9. A host cell comprising the polynucleotide(s) of claim 7 or the vector(s) of claim 8.
10. A method of producing a bispecific agonistic CD28 antigen binding molecule, comprising the steps of a) culturing the host cell of claim 9 under conditions suitable for the expression of the bispecific agonistic CD28 antigen binding molecule and b) optionally recovering the bispecific agonistic CD28 antigen binding molecule.
11. A pharmaceutical composition comprising the bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 6 and at least one pharmaceutically acceptable excipient.
12. The pharmaceutical composition of claim 11 for use in the treatment of cancer.
13. The bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 6, or the pharmaceutical composition of claim 11, for use as a medicament.
14. The bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 6 for use in the treatment of cancer.
15. The bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 6 for use in treating a B-cell proliferative disorder selected from the group consisting of Non-Hodgkin lymphoma (NHL), acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL), diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), mantle-cell lymphoma

(MCL), marginal zone lymphoma (MZL), Multiple myeloma (MM) and Hodgkin lymphoma (HL).

16. The bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 6 for use in the treatment of cancer, wherein the agonistic CD28 antigen binding molecule is to be administered in combination with a chemotherapeutic agent, radiation therapy and/ or other agents for use in cancer immunotherapy.

17. The bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 6 for use in the treatment of cancer, wherein the agonistic CD28 antigen binding molecule is to be administered in combination with a T-cell activating anti-CD3 bispecific antibody.

18. The bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 6 for use in the treatment of cancer, wherein the agonistic CD28 antigen binding molecule is to be administered in combination with an anti-CD20/anti-CD3 bispecific antibody.

19. The bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 6 for use in the treatment of cancer, wherein the agonistic CD28 antigen binding molecule is to be administered in combination with an anti-CD20/anti-CD3 bispecific antibody comprising a polypeptide sequence of SEQ ID NO: 455, a polypeptide sequence of SEQ ID NO: 456, a polypeptide sequence of SEQ ID NO: 457 and a polypeptide sequence of SEQ ID NO: 458.

20. The bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 6 for use in the treatment of cancer, wherein the agonistic CD28 antigen binding molecule is to be administered in combination with an anti-PD-L1 antibody or an anti-PD-1 antibody.

21. Use of the bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 6, or the pharmaceutical composition of claim 11, in the manufacture of a medicament for the treatment of cancer.

22. Use of the bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 6 in the manufacture of a medicament for treating a B-cell proliferative disorder selected from the group consisting of Non-Hodgkin lymphoma (NHL), acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL), diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), mantle-cell lymphoma (MCL), marginal zone lymphoma (MZL), Multiple myeloma (MM) and Hodgkin lymphoma (HL).

23. Use of the bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 6 in the manufacture of a medicament for the treatment of cancer, wherein the agonistic CD28 antigen binding molecule is to be administered in combination with a chemotherapeutic agent, radiation therapy and/ or other agents for use in cancer immunotherapy.

24. Use of the bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 6 in the manufacture of a medicament for the treatment of cancer, wherein the agonistic CD28 antigen binding molecule is to be administered in combination with a T-cell activating anti-CD3 bispecific antibody.

5 25. Use of the bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 6 in the manufacture of a medicament for the treatment of cancer, wherein the agonistic CD28 antigen binding molecule is to be administered in combination with an anti-CD20/anti-CD3 bispecific antibody.

10 26. Use of the bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 6 in the manufacture of a medicament for the treatment of cancer, wherein the agonistic CD28 antigen binding molecule is to be administered in combination with an anti-CD20/anti-CD3 bispecific antibody comprising a polypeptide sequence of SEQ ID NO: 455, a polypeptide sequence of SEQ ID NO: 456, a polypeptide sequence of SEQ ID NO: 457 and a polypeptide sequence of SEQ ID NO: 458.

15 27. Use of the bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 6 in the manufacture of a medicament for the treatment of cancer, wherein the agonistic CD28 antigen binding molecule is to be administered in combination with an anti-PD-L1 antibody or an anti-PD-1 antibody.
