

Although the therapeutic efficacy of trastuzumab in breast carcinoma is well demonstrated, there are many patients who do not benefit from trastuzumab because of resistance. Given the lack of an effective anti-Her2 therapy in specific cancers expressing low levels of Her2, the resistance to the current therapies, and the prevalence of Her2-expressing cancers, new therapies are required to treat such cancers.

It has been found that a better T cell activation is achieved when limiting amounts of anti-CD3 bispecific antibodies, i.e. T cell bispecific antibodies (TCBs) such as a Her2-targeted CD3 antibody, are combined with agonistic anti-CD28 molecules. Given, that CD28 is expressed at baseline on T cells in various tumor indications (Lavin et al., 2017; Tirosh et al., 2016, Zheng et al., 2017) and activation of CD28 signaling enhances T cell receptor signals, the combination of a TCB molecule with a Her2-targeted CD28 antigen binding molecule is expected to act synergistically to induce strong and long-lasting anti-tumor responses. Thus, we herein describe novel Her2-targeted agonistic CD28 molecules which display synergy with TCBs and require CD28 binding monovalency for strict tumor target dependence in the presence of TCB signals.

SUMMARY

The present ~~invention~~disclosure describes Her2-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 ~~invention~~disclosure are characterized by monovalent binding to CD28 and in that they comprise a second antigen binding domain capable of specific binding to human epidermal growth factor receptor-2 (Her2). 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-linking through binding of the second antigen binding domain capable of specific binding to Her2.

Thus, the ~~invention~~disclosure provides a bispecific agonistic CD28 antigen binding molecule characterized by monovalent binding to CD28, comprising

- (a) a first antigen binding domain capable of specific binding to CD28,
- (b) a second antigen binding domain capable of specific binding to an antigen binding domain capable of specific binding to human epidermal growth factor receptor-2 (Her2), and
- (c) a 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.

In ~~particular~~one aspect, the ~~invention~~disclosure provides a bispecific agonistic CD28 antigen binding molecule characterized by monovalent binding to CD28, comprising

- (a) a first antigen binding domain capable of specific binding to CD28,
- (b) a second antigen binding domain capable of specific binding to an antigen binding domain
- 5 (c) a 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,

wherein said second antigen binding domain capable of specific binding to Her2 comprises

- 10 (i) a heavy chain variable region (V_HHer2) comprising a heavy chain complementary determining region CDR-H1 of SEQ ID NO: 2, a CDR-H2 of SEQ ID NO: 3, and a CDR-H3 of SEQ ID NO: 4, and a light chain variable region (V_LHer2) comprising a light chain complementary determining region CDR-L1 of SEQ ID NO: 5, a CDR-L2 of SEQ ID NO: 6 and a CDR-L3 of SEQ ID NO: 7; or
- 15 (ii) a heavy chain variable region (V_HHer2) comprising a heavy chain complementary determining region CDR-H1 of SEQ ID NO: 10, a CDR-H2 of SEQ ID NO: 11, and a CDR-H3 of SEQ ID NO: 12, and a light chain variable region (V_LHer2) comprising a light chain complementary determining region CDR-L1 of SEQ ID NO: 13, a CDR-L2 of SEQ ID NO: 14 and a CDR-L3 of SEQ ID NO: 15; or
- 20 (iii) a heavy chain variable region (V_HHer2) comprising a heavy chain complementary determining region CDR-H1 of SEQ ID NO: 132, a CDR-H2 of SEQ ID NO: 133, and a CDR-H3 of SEQ ID NO: 134, and a light chain variable region (V_LHer2) comprising a light chain complementary determining region CDR-L1 of SEQ ID NO: 135, a CDR-L2 of SEQ ID NO: 136 and a CDR-L3 of SEQ ID NO: 137, or
- 25 (iv) a heavy chain variable region (V_HHer2) comprising a heavy chain complementary determining region CDR-H1 of SEQ ID NO: 140, a CDR-H2 of SEQ ID NO: 141, and a CDR-H3 of SEQ ID NO: 142, and a light chain variable region (V_LHer2) comprising a light chain complementary determining region CDR-L1 of SEQ ID NO: 143, a CDR-L2 of SEQ ID NO: 144 and a CDR-L3 of SEQ ID NO: 145.

30 In one ~~aspect~~example, the ~~invention~~disclosure provides a bispecific agonistic CD28 antigen binding molecule characterized by monovalent binding to CD28, comprising

- (a) a first antigen binding domain capable of specific binding to CD28,
- (b) a second antigen binding domain capable of specific binding to an antigen binding domain
- 35 (c) a 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,

wherein said second antigen binding domain capable of specific binding to Her2 comprises

(i) a heavy chain variable region (V_HHer2) comprising a heavy chain complementary determining region CDR-H1 of SEQ ID NO: 2, a CDR-H2 of SEQ ID NO: 3, and a CDR-H3 of SEQ ID NO: 4, and a light chain variable region (V_LHer2) comprising a light chain

5 complementary determining region CDR-L1 of SEQ ID NO: 5, a CDR-L2 of SEQ ID NO: 6 and a CDR-L3 of SEQ ID NO: 7; or

(ii) a heavy chain variable region (V_HHer2) comprising a heavy chain complementary determining region CDR-H1 of SEQ ID NO: 10, a CDR-H2 of SEQ ID NO: 11, and a CDR-H3 of SEQ ID NO: 12, and a light chain variable region (V_LHer2) comprising a light chain

10 complementary determining region CDR-L1 of SEQ ID NO: 13, a CDR-L2 of SEQ ID NO: 14 and a CDR-L3 of SEQ ID NO: 15.

In another aspectexample, the inventiondisclosure provides a bispecific agonistic CD28 antigen binding molecule characterized by monovalent binding to CD28, comprising

(a) a first antigen binding domain capable of specific binding to CD28,

15 (b) a second antigen binding domain capable of specific binding to an antigen binding domain capable of specific binding to human epidermal growth factor receptor-2 (Her2), and

(c) a 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,

20 wherein said second antigen binding domain capable of specific binding to Her2 comprises

(iii) a heavy chain variable region (V_HHer2) comprising a heavy chain complementary determining region CDR-H1 of SEQ ID NO: 132, a CDR-H2 of SEQ ID NO: 133, and a CDR-H3 of SEQ ID NO: 134, and a light chain variable region (V_LHer2) comprising a light chain complementary determining region CDR-L1 of SEQ ID NO: 135, a CDR-L2 of SEQ ID NO:

25 136 and a CDR-L3 of SEQ ID NO: 137, or

(iv) a heavy chain variable region (V_HHer2) comprising a heavy chain complementary determining region CDR-H1 of SEQ ID NO: 140, a CDR-H2 of SEQ ID NO: 141, and a CDR-H3 of SEQ ID NO: 142, and a light chain variable region (V_LHer2) comprising a light chain complementary determining region CDR-L1 of SEQ ID NO: 143, a CDR-L2 of SEQ ID NO:

30 144 and a CDR-L3 of SEQ ID NO: 145.

In one aspectexample, a bispecific agonistic CD28 antigen binding molecule as defined herein is provided, wherein the Fc domain is an IgG, particularly an IgG1 Fc domain or an IgG4 Fc domain. In one particular aspectexample, the Fc domain composed of a first and a second subunit capable of stable association is an IgG1 Fc domain. In one aspectexample, the Fc domain

35 comprises the amino acid substitutions L234A and L235A (numbering according to Kabat EU index). In one aspectexample, 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 [aspectexample](#), provided is a bispecific agonistic CD28 antigen binding molecule as defined herein before, wherein the first antigen binding domain capable of specific binding to CD28 comprises

(i) a heavy chain variable region (V_H CD28) comprising a heavy chain complementary determining region CDR-H1 of SEQ ID NO: 26, a CDR-H2 of SEQ ID NO: 27, and a CDR-H3 of SEQ ID NO: 28, and a light chain variable region (V_L CD28) comprising a light chain complementary determining region CDR-L1 of SEQ ID NO: 29, a CDR-L2 of SEQ ID NO: 30 and a CDR-L3 of SEQ ID NO: 31; or

(ii) a heavy chain variable region (V_H CD28) comprising a CDR-H1 of SEQ ID NO: 18, a CDR-H2 of SEQ ID NO: 19, and a CDR-H3 of SEQ ID NO: 20, and a light chain variable region (V_L CD28) comprising a CDR-L1 of SEQ ID NO: 21, a CDR-L2 of SEQ ID NO: 22 and a CDR-L3 of SEQ ID NO: 23.

In one [aspectexample](#), the antigen binding domain capable of specific binding to CD28 of the bispecific agonistic CD28 antigen binding molecule comprises a heavy chain variable region (V_H CD28) comprising a CDR-H1 of SEQ ID NO: 26, a CDR-H2 of SEQ ID NO: 27, and a CDR-H3 of SEQ ID NO: 28, and a light chain variable region (V_L CD28) comprising a CDR-L1 of SEQ ID NO: 29, a CDR-L2 of SEQ ID NO: 30 and a CDR-L3 of SEQ ID NO: 31.

In another [aspectexample](#), the antigen binding domains capable of specific binding to CD28 of the bispecific agonistic CD28 antigen binding molecule comprises a heavy chain variable region (V_H CD28) comprising a CDR-H1 of SEQ ID NO: 18, a CDR-H2 of SEQ ID NO: 19, and a CDR-H3 of SEQ ID NO: 20, and a light chain variable region (V_L CD28) comprising a CDR-L1 of SEQ ID NO: 21, a CDR-L2 of SEQ ID NO: 22 and a CDR-L3 of SEQ ID NO: 23.

In a further [aspectexample](#), provided is a bispecific agonistic CD28 antigen binding molecule as defined herein before, wherein the first antigen binding domain capable of specific binding to CD28 comprises

(i) a heavy chain variable region (V_H CD28) comprising a heavy chain complementary determining region CDR-H1 of SEQ ID NO: 52, a CDR-H2 of SEQ ID NO: 53, and a CDR-H3 of SEQ ID NO: 54, and a light chain variable region (V_L CD28) comprising a light chain complementary determining region CDR-L1 of SEQ ID NO: 55, a CDR-L2 of SEQ ID NO: 56 and a CDR-L3 of SEQ ID NO: 57; or

(ii) a heavy chain variable region (V_H CD28) comprising a CDR-H1 of SEQ ID NO: 58, a CDR-H2 of SEQ ID NO: 59, and a CDR-H3 of SEQ ID NO: 60, and a light chain variable region (V_L CD28) comprising a CDR-L1 of SEQ ID NO: 61, a CDR-L2 of SEQ ID NO: 62 and a CDR-L3 of SEQ ID NO: 63; or

(ii) a heavy chain variable region (V_H CD28) comprising a CDR-H1 of SEQ ID NO: 64, a CDR-H2 of SEQ ID NO: 65, and a CDR-H3 of SEQ ID NO: 66, and a light chain variable region

(V_LCD28) comprising a CDR-L1 of SEQ ID NO: 67, a CDR-L2 of SEQ ID NO: 68 and a CDR-L3 of SEQ ID NO: 69.

In one [aspectexample](#), the antigen binding domain capable of specific binding to CD28 of the bispecific agonistic CD28 antigen binding molecule comprises a heavy chain variable region (V_HCD28) comprising a heavy chain complementary determining region CDR-H1 of SEQ ID NO: 52, a CDR-H2 of SEQ ID NO: 53, and a CDR-H3 of SEQ ID NO: 54, and a light chain variable region (V_LCD28) comprising a light chain complementary determining region CDR-L1 of SEQ ID NO: 55, a CDR-L2 of SEQ ID NO: 56 and a CDR-L3 of SEQ ID NO: 57.

In another [aspectexample](#), the antigen binding domains capable of specific binding to CD28 of the bispecific agonistic CD28 antigen binding molecule comprises a heavy chain variable region (V_HCD28) comprising a CDR-H1 of SEQ ID NO: 58, a CDR-H2 of SEQ ID NO: 59, and a CDR-H3 of SEQ ID NO: 60, and a light chain variable region (V_LCD28) comprising a CDR-L1 of SEQ ID NO: 61, a CDR-L2 of SEQ ID NO: 62 and a CDR-L3 of SEQ ID NO: 63.

In yet another [aspectexample](#), the antigen binding domains capable of specific binding to CD28 of the bispecific agonistic CD28 antigen binding molecule comprises a heavy chain variable region (V_HCD28) comprising a CDR-H1 of SEQ ID NO: 64, a CDR-H2 of SEQ ID NO: 65, and a CDR-H3 of SEQ ID NO: 66, and a light chain variable region (V_LCD28) comprising a CDR-L1 of SEQ ID NO: 67, a CDR-L2 of SEQ ID NO: 68 and a CDR-L3 of SEQ ID NO: 69.

Furthermore, provided is a bispecific agonistic CD28 antigen binding molecule as defined herein before, wherein the first antigen binding domain capable of specific binding to CD28 comprises a heavy chain variable region (V_HCD28) comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:24, and a light chain variable region (V_LCD28) comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:25.

In a further [aspectexample](#), a bispecific agonistic CD28 antigen binding molecule is provided, wherein the first antigen binding domain capable of specific binding to CD28 comprises a heavy chain variable region (V_HCD28) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40 and SEQ ID NO:41, and a light chain variable region (V_LCD28) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:25, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50 and SEQ ID NO:51.

In another [aspectexample](#), provided is a bispecific agonistic CD28 antigen binding molecule, wherein the first antigen binding domain capable of specific binding to CD28 comprises

(a) a heavy chain variable region (V_HCD28) comprising the amino acid sequence of SEQ ID NO:37 and a light chain variable region (V_LCD28) comprising the amino acid sequence of

5 SEQ ID NO:44, or

(b) a heavy chain variable region (V_HCD28) comprising the amino acid sequence of SEQ ID NO:37 and a light chain variable region (V_LCD28) comprising the amino acid sequence of SEQ ID NO:25, or

(c) a heavy chain variable region (V_HCD28) comprising the amino acid sequence of SEQ ID NO:41 and a light chain variable region (V_LCD28) comprising the amino acid sequence of SEQ ID NO:51, or

(d) a heavy chain variable region (V_HCD28) comprising the amino acid sequence of SEQ ID NO:36 and a light chain variable region (V_LCD28) comprising the amino acid sequence of SEQ ID NO:43, or

15 (e) a heavy chain variable region (V_HCD28) comprising the amino acid sequence of SEQ ID NO:36 and a light chain variable region (V_LCD28) comprising the amino acid sequence of SEQ ID NO:44, or

(f) a heavy chain variable region (V_HCD28) comprising the amino acid sequence of SEQ ID NO:36 and a light chain variable region (V_LCD28) comprising the amino acid sequence of

20 SEQ ID NO:49, or

(g) a heavy chain variable region (V_HCD28) comprising the amino acid sequence of SEQ ID NO:36 and a light chain variable region (V_LCD28) comprising the amino acid sequence of SEQ ID NO:25, or

(h) a heavy chain variable region (V_HCD28) comprising the amino acid sequence of SEQ ID NO:33 and a light chain variable region (V_LCD28) comprising the amino acid sequence of SEQ ID NO:25, or

25 (i) a heavy chain variable region (V_HCD28) comprising the amino acid sequence of SEQ ID NO:32 and a light chain variable region (V_LCD28) comprising the amino acid sequence of SEQ ID NO:43, or

30 (j) a heavy chain variable region (V_HCD28) comprising the amino acid sequence of SEQ ID NO:32 and a light chain variable region (V_LCD28) comprising the amino acid sequence of SEQ ID NO:49, or

(k) a heavy chain variable region (V_HCD28) comprising the amino acid sequence of SEQ ID NO:32 and a light chain variable region (V_LCD28) comprising the amino acid sequence of SEQ ID NO:25.

In one particular [aspectexample](#), a bispecific agonistic CD28 antigen binding molecule is provided, wherein the first antigen binding domain capable of specific binding to CD28 comprises

(i) a heavy chain variable region (V_HCD28) comprising a heavy chain complementary determining region CDR-H1 of SEQ ID NO: 52, a CDR-H2 of SEQ ID NO: 53, and a CDR-H3 of SEQ ID NO: 54, and a light chain variable region (V_LCD28) comprising a light chain complementary determining region CDR-L1 of SEQ ID NO: 55, a CDR-L2 of SEQ ID NO: 56 and a CDR-L3 of SEQ ID NO: 57. In one [aspectexample](#), the first antigen binding domain capable of specific binding to CD28 comprises the CDRs of the heavy chain variable region (V_HCD28) comprising the amino acid sequence of SEQ ID NO:37 and the CDRs of the light chain variable region (V_LCD28) comprising the amino acid sequence of SEQ ID NO:44.

In another particular [aspectexample](#), a bispecific agonistic CD28 antigen binding molecule is provided, wherein the antigen binding domain capable of specific binding to CD28 comprises a heavy chain variable region (V_HCD28) comprising the amino acid sequence of SEQ ID NO:36 and a light chain variable region (V_LCD28) comprising the amino acid sequence of SEQ ID NO:43. In a further particular [aspectexample](#), a bispecific agonistic CD28 antigen binding molecule is provided, wherein the antigen binding domain capable of specific binding to CD28 comprises a heavy chain variable region (V_HCD28) comprising the amino acid sequence of SEQ ID NO:32 and a light chain variable region (V_LCD28) comprising the amino acid sequence of SEQ ID NO:25.

In one [aspectexample](#), a bispecific agonistic CD28 antigen binding molecule is provided, wherein the antigen binding domain capable of specific binding to Her2 comprises a heavy chain variable region (V_HHer2) comprising a heavy chain complementary determining region CDR-H1 of SEQ ID NO: 2, a CDR-H2 of SEQ ID NO: 3, and a CDR-H3 of SEQ ID NO: 4, and a light chain variable region (V_LHer2) comprising a light chain complementary determining region CDR-L1 of SEQ ID NO: 5, a CDR-L2 of SEQ ID NO: 6 and a CDR-L3 of SEQ ID NO: 7. In one [aspectexample](#), the antigen binding domain capable of specific binding to Her2 comprises the CDRs of the heavy chain variable region (V_HHer2) comprising the amino acid sequence of SEQ ID NO:8 and the CDRs of the light chain variable region (V_LHer2) comprising the amino acid sequence of SEQ ID NO:9. In one particular [aspectexample](#), the the antigen binding domain capable of specific binding to Her2 comprises a heavy chain variable region (V_HHer2) comprising the amino acid sequence of SEQ ID NO:8, and a light chain variable region (V_LHer2) comprising the amino acid sequence of SEQ ID NO:9.

In another [aspectexample](#), provided is a bispecific agonistic CD28 antigen binding molecule as described herein, wherein the antigen binding domain capable of specific binding to Her2 comprises a heavy chain variable region (V_HHer2) comprising a heavy chain complementary determining region CDR-H1 of SEQ ID NO: 10, a CDR-H2 of SEQ ID NO: 11, and a CDR-H3 of SEQ ID NO: 12, and a light chain variable region (V_LHer2) comprising a light chain complementary determining region CDR-L1 of SEQ ID NO: 13, a CDR-L2 of SEQ ID NO:

and a CDR-L3 of SEQ ID NO: 15. In one [aspectexample](#), the antigen binding domain capable of specific binding to Her2 comprises the CDRs of the heavy chain variable region (V_HHer2) comprising the amino acid sequence of SEQ ID NO:16 and the CDRs of the light chain variable region (V_LHer2) comprising the amino acid sequence of SEQ ID NO:17. In one particular

5 [aspectexample](#), the antigen binding domain capable of specific binding to Her2 comprises a heavy chain variable region (V_HHer2) comprising the amino acid sequence of SEQ ID NO:16, and a light chain variable region (V_LHer2) comprising the amino acid sequence of SEQ ID NO:17.

In yet another [aspectexample](#), provided is a bispecific agonistic CD28 antigen binding molecule as described herein, wherein the antigen binding domain capable of specific binding to Her2 comprises a heavy chain variable region (V_HHer2) comprising a heavy chain complementary determining region CDR-H1 of SEQ ID NO: 132, a CDR-H2 of SEQ ID NO: 133, and a CDR-H3 of SEQ ID NO: 134, and a light chain variable region (V_LHer2) comprising a light chain complementary determining region CDR-L1 of SEQ ID NO: 135, a CDR-L2 of

10 SEQ ID NO: 136 and a CDR-L3 of SEQ ID NO: 137. In one [aspectexample](#), the antigen binding domain capable of specific binding to Her2 comprises the CDRs of the heavy chain variable region (V_HHer2) comprising the amino acid sequence of SEQ ID NO:138 and the CDRs of the light chain variable region (V_LHer2) comprising the amino acid sequence of SEQ ID NO:139. In one particular [aspectexample](#), the antigen binding domain capable of specific binding to Her2

15 comprises a heavy chain variable region (V_HHer2) comprising the amino acid sequence of SEQ ID NO:138, and a light chain variable region (V_LHer2) comprising the amino acid sequence of SEQ ID NO:139.

In another [aspectexample](#), provided is a bispecific agonistic CD28 antigen binding molecule as described herein, wherein the antigen binding domain capable of specific binding to

25 Her2 comprises a heavy chain variable region (V_HHer2) comprising a heavy chain complementary determining region CDR-H1 of SEQ ID NO: 140, a CDR-H2 of SEQ ID NO: 141, and a CDR-H3 of SEQ ID NO: 142, and a light chain variable region (V_LHer2) comprising a light chain complementary determining region CDR-L1 of SEQ ID NO: 143, a CDR-L2 of SEQ ID NO: 144 and a CDR-L3 of SEQ ID NO: 145. In one [aspectexample](#), the antigen binding domain capable of specific binding to Her2 comprises the CDRs of the heavy chain variable region (V_HHer2) comprising the amino acid sequence of SEQ ID NO:146 and the CDRs of the light chain variable region (V_LHer2) comprising the amino acid sequence of SEQ ID NO:147. In one particular [aspectexample](#), the antigen binding domain capable of specific binding to Her2

30 comprises a heavy chain variable region (V_HHer2) comprising the amino acid sequence of SEQ ID NO:146, and a light chain variable region (V_LHer2) comprising the amino acid sequence of SEQ ID NO:147.

In a further [aspectexample](#), provided is a bispecific agonistic CD28 antigen binding molecule as defined herein before, wherein the first antigen binding domain capable of specific binding to CD28 and/or the second antigen binding domain capable of specific binding to Her2

35 is a Fab

40

fragment or a crossFab fragment. In one [aspectexample](#), provided is a bispecific agonistic CD28 antigen binding molecule as described herein, comprising (a) a Fab fragment capable of specific binding to CD28, (b) a crossFab fragment capable of specific binding to Her2, and (c) a 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. In another [aspectexample](#), provided is a bispecific agonistic CD28 antigen binding molecule as described herein, comprising (a) a crossFab fragment capable of specific binding to CD28, (b) a Fab fragment capable of specific binding to Her2, and (c) a 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.

In one [aspectexample](#), the first antigen binding domain capable of specific binding to CD28 is a Fab fragment wherein the variable domains VL and VH or the constant domains CL and CH1, particularly the variable domains VL and VH, of the Fab light chain and the Fab heavy chain are replaced by each other. In one [aspectexample](#), the second antigen binding domain capable of specific binding to Her2 is a conventional Fab fragment. In one [aspectexample](#), the second antigen binding domain capable of specific binding to Her2 is a Fab molecule wherein in the constant domain CL the amino acid at position 123 (numbering according to Kabat EU index) is substituted by an amino acid selected from lysine (K), arginine (R) or histidine (H) and the amino acid at position 124 (numbering according to Kabat EU index) is substituted independently by lysine (K), arginine (R) or histidine (H), and wherein in the constant domain CH1 the amino acid at position 147 (numbering according to Kabat EU index) is substituted independently by glutamic acid (E) or aspartic acid (D) and the amino acid at position 213 (numbering according to Kabat EU index) is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

In one particular [aspectexample](#), provided is a bispecific agonistic CD28 antigen binding molecule comprising

- (i) a first light chain comprising the amino acid sequence of SEQ ID NO:92, a first heavy chain comprising the amino acid sequence of SEQ ID NO:91, a second heavy chain comprising the amino acid sequence of SEQ ID NO:96 and a second light chain comprising the amino acid sequence of SEQ ID NO:97, or
- (ii) a first light chain comprising the amino acid sequence of SEQ ID NO:92, a first heavy chain comprising the amino acid sequence of SEQ ID NO:91, a second heavy chain comprising the amino acid sequence of SEQ ID NO:98 and a second light chain comprising the amino acid sequence of SEQ ID NO:97, or
- (iii) a first light chain comprising the amino acid sequence of SEQ ID NO:92, a first heavy chain comprising the amino acid sequence of SEQ ID NO:91, a second heavy chain comprising the

amino acid sequence of SEQ ID NO:101 and a second light chain comprising the amino acid sequence of SEQ ID NO:97.

In another particular [aspectexample](#), provided is a bispecific agonistic CD28 antigen binding molecule comprising

- 5 (i) a first light chain comprising the amino acid sequence of SEQ ID NO:179, a first heavy chain comprising the amino acid sequence of SEQ ID NO:178, a second heavy chain comprising the amino acid sequence of SEQ ID NO:180 and a second light chain comprising the amino acid sequence of SEQ ID NO:181, or
- 10 (ii) a first light chain comprising the amino acid sequence of SEQ ID NO:179, a first heavy chain comprising the amino acid sequence of SEQ ID NO:178, a second heavy chain comprising the amino acid sequence of SEQ ID NO:182 and a second light chain comprising the amino acid sequence of SEQ ID NO:183.

In a further [aspectexample](#), provided is a bispecific agonistic CD28 antigen binding molecule as described herein, wherein the second antigen binding domain capable of specific
15 binding to Her2 is a Fab molecule wherein the variable domains VL and VH or the constant domains CL and CH1, particularly the variable domains VL and VH, of the Fab light chain and the Fab heavy chain are replaced by each other. In one [aspectexample](#), the first antigen binding domain capable of specific binding to CD28 is a conventional Fab molecule. In one
20 [aspectexample](#), the first antigen binding domain capable of specific binding to CD28 is a Fab molecule wherein in the constant domain CL the amino acid at position 123 (numbering according to Kabat EU index) is substituted by an amino acid selected from lysine (K), arginine (R) or histidine (H) and the amino acid at position 124 (numbering according to Kabat EU index) is substituted independently by lysine (K), arginine (R) or histidine (H), and wherein in the constant domain CH1 the amino acid at position 147 (numbering according to Kabat EU index)
25 is substituted independently by glutamic acid (E) or aspartic acid (D) and the amino acid at position 213 (numbering according to Kabat EU index) is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

In one particular [aspectexample](#), provided is a bispecific agonistic CD28 antigen binding molecule comprising

- 30 (i) a first light chain comprising the amino acid sequence of SEQ ID NO:83, a first heavy chain comprising the amino acid sequence of SEQ ID NO:74, a second heavy chain comprising the amino acid sequence of SEQ ID NO:99 and a second light chain comprising the amino acid sequence of SEQ ID NO:100, or
- 35 (ii) a first light chain comprising the amino acid sequence of SEQ ID NO:83, a first heavy chain comprising the amino acid sequence of SEQ ID NO:74, a second heavy chain comprising the

amino acid sequence of SEQ ID NO:102 and a second light chain comprising the amino acid sequence of SEQ ID NO:100.

In another aspectexample, a bispecific agonistic CD28 antigen binding molecule as disclosed herein is provided, wherein the first and the second antigen binding domain are each a Fab molecule and the Fc domain is composed of a first and a second subunit capable of stable association; and wherein (i) the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain and the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the second subunit of the Fc domain, or (ii) the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain and the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the second subunit of the Fc domain. In one aspectexample, the Fc domain comprises a modification promoting the association of the first and the second subunit of the Fc domain. In one aspectexample, the first subunit of the Fc domain comprises the amino acid substitutions S354C and T366W (EU numbering) and the second subunit of the Fc domain comprises the amino acid substitutions Y349C, T366S and Y407V (numbering according to Kabat EU index).

According to another aspect of the inventiondisclosure, there is provided one or more isolated polynucleotide(s) encoding the bispecific agonistic CD28 antigen binding molecule of the inventiondisclosure. The inventiondisclosure further provides one or more vector(s), particularly expression vector(s), comprising the isolated polynucleotide(s) of the inventiondisclosure, and a host cell comprising the isolated polynucleotide(s) or the expression vector(s) of the inventiondisclosure. In another aspect, the present disclosure provides one or more vector, particularly expression vector, comprising the polynucleotide(s) of the disclosure. In another aspect, the present disclosure provides a host cell comprising the polynucleotide(s) of the disclosure or the vector(s) of the disclosure. In some aspectexamples, the host cell is a eukaryotic cell, particularly a mammalian cell. In some aspectexamples, the host cell is a prokaryotic cell. In another aspect, provided is The present disclosure also provides a method of producing a bispecific agonistic CD28 antigen binding molecule as described herein comprising culturing the host cell of the inventiondisclosure 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, the present disclosure provides 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 invention~~In another aspect, the present disclosure also encompasses a bispecific agonistic CD28 antigen binding molecule produced by the method of the ~~invention~~disclosure.

5 ~~The invention~~In another aspect, the present disclosure further provides a pharmaceutical composition comprising ~~a~~the bispecific agonistic CD28 antigen binding molecule of the ~~invention~~disclosure and at least one pharmaceutically acceptable excipient. In ~~one aspect~~the present disclosure, the pharmaceutical composition is for use in the treatment of a disease, particularly cancer. In one ~~aspect~~example, the pharmaceutical composition is for use in the treatment of Her2-positive cancer.

10 Also encompassed by the ~~invention~~disclosure are methods of using the bispecific agonistic CD28 antigen binding molecule or the pharmaceutical composition of the ~~invention~~disclosure. In ~~one~~another aspect, the

~~invention~~disclosure provides ~~a~~the bispecific agonistic CD28 antigen binding molecule according to the disclosure, or ~~a~~the pharmaceutical composition according to the ~~invention~~disclosure, for use as a medicament. ~~In one aspect, provided is~~The present disclosure provides a bispecific agonistic CD28 antigen binding molecule as described herein for use in (a) enhancing cell activation or (b) enhancing T cell effector functions. In another aspect, the present disclosure provides the bispecific agonistic CD28 antigen binding molecule of the disclosure, or the pharmaceutical composition of the disclosure, for use in enhancing (a) T cell activation or (b) T cell effector functions. ~~In one aspect, provided is~~The present disclosure provides a bispecific agonistic CD28 antigen binding molecule or a pharmaceutical composition according to the ~~invention~~disclosure for use in the treatment of a disease. In a specific ~~aspect~~example, the disease is cancer, in particular Her2-positive cancer. In another aspect, the present disclosure provides the bispecific agonistic CD28 antigen binding molecule of the disclosure, or the pharmaceutical composition of the disclosure, for use in the treatment of cancer. ~~In another aspect is provided~~The present disclosure provides a bispecific agonistic CD28 antigen binding molecule or pharmaceutical composition according to the ~~invention~~disclosure ~~is~~ for use in the treatment of cancer, wherein the bispecific agonistic CD28 antigen binding molecule is for administration in combination with a chemotherapeutic agent, radiation therapy and/ or other agents for use in cancer immunotherapy. In another aspect, the present disclosure provides the bispecific agonistic CD28 antigen binding molecule of the disclosure, or the pharmaceutical composition of the disclosure, for use in the treatment of cancer, wherein the use is for administration in combination with a chemotherapeutic agent, radiation therapy and/ or other agents for use in cancer immunotherapy. ~~In a further aspect, provided is~~The present disclosure provides 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 for administration in combination with a T-cell activating anti-CD3 bispecific antibody. In another aspect, the present disclosure provides the bispecific agonistic CD28 antigen binding molecule of the disclosure, or the pharmaceutical composition of the disclosure, for use in the treatment of cancer, wherein the use is for administration in combination with a T-cell activating anti-CD3 bispecific antibody. In one ~~aspect~~example, the T-cell activating anti-CD3 bispecific antibody is an anti-Her2/anti-CD3 antibody. ~~In yet another aspect, provided is~~The present disclosure provides 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 for administration in

combination with an anti-PD-L1 antibody or an anti-PD-1 antibody. In another aspect, the present disclosure provides the bispecific agonistic CD28 antigen binding molecule of the disclosure, or the pharmaceutical composition of the disclosure, for use in the treatment of cancer, wherein the use is for administration 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 or the pharmaceutical composition according to the ~~invention~~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 ~~invention~~disclosure or a composition comprising the bispecific agonistic CD28 antigen binding molecule according to the ~~invention~~disclosure in a pharmaceutically acceptable form. In a specific ~~aspect~~example, the disease is cancer. In another aspect, the present disclosure provides 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 a disease, particularly for the treatment of cancer. ~~In one aspect, provided is~~The present disclosure provides a method (a) enhancing T cell activation or (b) enhancing T cell effector functions in an individual, comprising administering a bispecific agonistic CD28 antigen binding molecule according to the ~~invention~~disclosure or a composition comprising the bispecific agonistic CD28 antigen binding molecule according to the ~~invention~~disclosure in a pharmaceutically acceptable form to said individual. In another ~~aspect~~example, provided is the use of a bispecific agonistic CD28 antigen binding molecule according to the ~~invention~~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. In a further ~~aspect~~example, provided is 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 ~~invention~~disclosure or a composition comprising the bispecific agonistic CD28

antigen binding molecule according to the ~~invention~~disclosure in a pharmaceutically acceptable form, wherein the method comprises co-administration with a chemotherapeutic agent, radiation therapy and/ or other agents for use in cancer immunotherapy. In a further ~~aspect~~example, provided is 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 ~~invention~~disclosure or a composition comprising the bispecific agonistic CD28 antigen binding molecule according to the ~~invention~~disclosure in a pharmaceutically acceptable form, wherein the method comprises co-administration of a T-cell activating anti-CD3 bispecific antibody. In one ~~aspect~~example, the T-cell activating anti-CD3 bispecific antibody is an anti-Her2/anti-CD3 antibody. In another ~~aspect~~example, provided is 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 ~~invention~~disclosure or a composition comprising the bispecific agonistic CD28 antigen binding molecule according to the ~~invention~~disclosure in a pharmaceutically acceptable form, wherein the method comprises co-administration of an anti-PD-L1 antibody or an anti-PD-1 antibody. Also provided is a method of inhibiting the growth of tumor cells in an individual comprising administering to the individual an effective amount of the bispecific agonistic CD28 antigen binding molecule according to the ~~invention~~disclosure, or or a composition comprising the bispecific agonistic CD28 antigen binding molecule according to the ~~invention~~disclosure in a pharmaceutically acceptable form, to inhibit the growth of the tumor cells. ~~In any of the above aspects~~Where mentioned above, the individual preferably is a mammal, particularly a human.

BRIEF DESCRIPTION OF THE DRAWINGS

In **Fig. 1A to 1D** schematic illustrations of exemplary molecules as described herein are shown. **Fig. 1A** shows a schematic illustration of the CD28 agonistic antibody variants as monovalent hu IgG1 PGLALA isotype (“Fc silent”). **Fig. 1B** shows a bispecific Her2-CD28 antigen binding molecule in 1+1 format, wherein in the Fab molecule comprising the CD28 antigen binding domain the VH and VL domains are exchanged with each other (VH/VL crossfab) and wherein in the Fab molecule comprising the Her2 antigen binding domain certain amino acids in the CH1 and CL domain are exchanged (Charged variants) to allow better pairing with the light chain. The Fc domain is a hu IgG1 PGLALA version. **Fig. 1C** shows a bispecific Her2-CD28 antigen binding molecule in 1+1 format with both heavy chains comprising the N297G mutations, wherein both Fab molecules are expressed in different cell lines, purified and assembled into the intact bispecific. **Fig. 1D** shows a bispecific Her2-CD28 antigen binding molecule in 1+1 format, wherein in the Fab molecule comprising the Her2 antigen binding domain the VH and VL domains are exchanged with each other (VH/VL crossfab) and wherein in the Fab molecule comprising the CD28 antigen binding domain certain amino acids in the CH1 and CL domain are exchanged (Charged variants) to allow better pairing with the light chain.

Claims

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

(a) a first antigen binding domain capable of specific binding to CD28,

5 (b) a second antigen binding domain capable of specific binding to an antigen binding domain capable of specific binding to human epidermal growth factor receptor-2 (Her2), and

(c) a 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,

10 wherein said second antigen binding domain capable of specific binding to Her2 comprises

(i) a heavy chain variable region ($V_{H\text{Her2}}$) comprising a heavy chain complementary determining region CDR-H1 of SEQ ID NO: 2, a CDR-H2 of SEQ ID NO: 3, and a CDR-H3 of SEQ ID NO: 4, and a light chain variable region ($V_{L\text{Her2}}$) comprising a light chain complementary determining region CDR-L1 of SEQ ID NO: 5, a CDR-L2 of SEQ ID NO: 6 and

15 a CDR-L3 of SEQ ID NO: 7; or

(ii) a heavy chain variable region ($V_{H\text{Her2}}$) comprising a heavy chain complementary determining region CDR-H1 of SEQ ID NO: 10, a CDR-H2 of SEQ ID NO: 11, and a CDR-H3 of SEQ ID NO: 12, and a light chain variable region ($V_{L\text{Her2}}$) comprising a light chain complementary determining region CDR-L1 of SEQ ID NO: 13, a CDR-L2 of SEQ ID NO: 14

20 and a CDR-L3 of SEQ ID NO: 15; or

(iii) a heavy chain variable region ($V_{H\text{Her2}}$) comprising a heavy chain complementary determining region CDR-H1 of SEQ ID NO: 132, a CDR-H2 of SEQ ID NO: 133, and a CDR-H3 of SEQ ID NO: 134, and a light chain variable region ($V_{L\text{Her2}}$) comprising a light chain complementary determining region CDR-L1 of SEQ ID NO: 135, a CDR-L2 of SEQ ID NO:

25 136 and a CDR-L3 of SEQ ID NO: 137, or

(iv) a heavy chain variable region ($V_{H\text{Her2}}$) comprising a heavy chain complementary determining region CDR-H1 of SEQ ID NO: 140, a CDR-H2 of SEQ ID NO: 141, and a CDR-H3 of SEQ ID NO: 142, and a light chain variable region ($V_{L\text{Her2}}$) comprising a light chain complementary determining region CDR-L1 of SEQ ID NO: 143, a CDR-L2 of SEQ ID NO:

30 144 and a CDR-L3 of SEQ ID NO: 145.

2. The bispecific agonistic CD28 antigen binding molecule of claim 1, wherein the Fc domain is of human IgG1 subclass and comprises the amino acid mutations L234A, L235A and P329G (numbering according to Kabat EU index).

3. The bispecific agonistic CD28 antigen binding molecule of claims 1 or 2, wherein the first antigen binding domain capable of specific binding to CD28 comprises

35 (i) a heavy chain variable region ($V_{H\text{CD28}}$) comprising a heavy chain complementary

determining region CDR-H1 of SEQ ID NO: 26, a CDR-H2 of SEQ ID NO: 27, and a CDR-H3 of SEQ ID NO: 28, and a light chain variable region (V_LCD28) comprising a light chain complementary determining region CDR-L1 of SEQ ID NO: 29, a CDR-L2 of SEQ ID NO: 30 and a CDR-L3 of SEQ ID NO: 31; or

- 5 (ii) a heavy chain variable region (V_HCD28) comprising a CDR-H1 of SEQ ID NO: 18, a CDR-H2 of SEQ ID NO: 19, and a CDR-H3 of SEQ ID NO: 20, and a light chain variable region (V_LCD28) comprising a CDR-L1 of SEQ ID NO: 21, a CDR-L2 of SEQ ID NO: 22 and a CDR-L3 of SEQ ID NO: 23.

- 10 4. The bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 3, wherein the first antigen binding domain capable of specific binding to CD28 comprises
- (i) a heavy chain variable region (V_HCD28) comprising a heavy chain complementary determining region CDR-H1 of SEQ ID NO: 52, a CDR-H2 of SEQ ID NO: 53, and a CDR-H3 of SEQ ID NO: 54, and a light chain variable region (V_LCD28) comprising a light chain complementary determining region CDR-L1 of SEQ ID NO: 55, a CDR-L2 of SEQ ID NO: 56
- 15 and a CDR-L3 of SEQ ID NO: 57; or
- (ii) a heavy chain variable region (V_HCD28) comprising a CDR-H1 of SEQ ID NO: 58, a CDR-H2 of SEQ ID NO: 59, and a CDR-H3 of SEQ ID NO: 60, and a light chain variable region (V_LCD28) comprising a CDR-L1 of SEQ ID NO: 61, a CDR-L2 of SEQ ID NO: 62 and a CDR-L3 of SEQ ID NO: 63; or
- 20 (ii) a heavy chain variable region (V_HCD28) comprising a CDR-H1 of SEQ ID NO: 64, a CDR-H2 of SEQ ID NO: 65, and a CDR-H3 of SEQ ID NO: 66, and a light chain variable region (V_LCD28) comprising a CDR-L1 of SEQ ID NO: 67, a CDR-L2 of SEQ ID NO: 68 and a CDR-L3 of SEQ ID NO: 69.

- 25 5. The bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 4, wherein the first antigen binding domain capable of specific binding to CD28 comprises a heavy chain variable region (V_HCD28) comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:24, and a light chain variable region (V_LCD28) comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:25.

- 30 6. The bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 5, wherein the first antigen binding domain capable of specific binding to CD28 comprises a heavy chain variable region (V_HCD28) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40 and SEQ ID NO:41, and a
- 35 light chain variable region (V_LCD28) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:25, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID

NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50 and SEQ ID NO:51.

7. The bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 4 or 6, wherein the first antigen binding domain capable of specific binding to CD28 comprises

5 (a) a heavy chain variable region (V_HCD28) comprising the amino acid sequence of SEQ ID NO:37 and a light chain variable region (V_LCD28) comprising the amino acid sequence of SEQ ID NO:44, or

(b) a heavy chain variable region (V_HCD28) comprising the amino acid sequence of SEQ ID NO:37 and a light chain variable region (V_LCD28) comprising the amino acid sequence of
10 SEQ ID NO:25, or

(c) a heavy chain variable region (V_HCD28) comprising the amino acid sequence of SEQ ID NO:41 and a light chain variable region (V_LCD28) comprising the amino acid sequence of SEQ ID NO:51, or

(d) a heavy chain variable region (V_HCD28) comprising the amino acid sequence of SEQ
15 ID NO:36 and a light chain variable region (V_LCD28) comprising the amino acid sequence of SEQ ID NO:43, or

(e) a heavy chain variable region (V_HCD28) comprising the amino acid sequence of SEQ ID NO:36 and a light chain variable region (V_LCD28) comprising the amino acid sequence of SEQ ID NO:44, or

20 (f) a heavy chain variable region (V_HCD28) comprising the amino acid sequence of SEQ ID NO:36 and a light chain variable region (V_LCD28) comprising the amino acid sequence of SEQ ID NO:49, or

(g) a heavy chain variable region (V_HCD28) comprising the amino acid sequence of SEQ ID NO:36 and a light chain variable region (V_LCD28) comprising the amino acid sequence of
25 SEQ ID NO:25, or

(h) a heavy chain variable region (V_HCD28) comprising the amino acid sequence of SEQ ID NO:33 and a light chain variable region (V_LCD28) comprising the amino acid sequence of SEQ ID NO:25, or

(i) a heavy chain variable region (V_HCD28) comprising the amino acid sequence of SEQ
30 ID NO:32 and a light chain variable region (V_LCD28) comprising the amino acid sequence of SEQ ID NO:43, or

(j) a heavy chain variable region (V_HCD28) comprising the amino acid sequence of SEQ ID NO:32 and a light chain variable region (V_LCD28) comprising the amino acid sequence of SEQ ID NO:49, or

35 (k) a heavy chain variable region (V_HCD28) comprising the amino acid sequence of SEQ ID NO:32 and a light chain variable region (V_LCD28) comprising the amino acid sequence of SEQ ID NO:25.

8. The bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 4, wherein the first antigen binding domain capable of specific binding to CD28 comprises (i) a heavy chain variable region (V_HCD28) comprising a heavy chain complementary determining region CDR-H1 of SEQ ID NO: 52, a CDR-H2 of SEQ ID NO: 53, and a CDR-H3 of SEQ ID NO: 54, and a light chain variable region (V_LCD28) comprising a light chain complementary determining region CDR-L1 of SEQ ID NO: 55, a CDR-L2 of SEQ ID NO: 56 and a CDR-L3 of SEQ ID NO: 57.

9. The bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 4 or 8, wherein the first antigen binding domain capable of specific binding to CD28 comprises the CDRs of the heavy chain variable region (V_HCD28) comprising the amino acid sequence of SEQ ID NO:37 and the CDRs of the light chain variable region (V_LCD28) comprising the amino acid sequence of SEQ ID NO:44.

10. The bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 9, wherein the antigen binding domain capable of specific binding to Her2 comprises the CDRs of the heavy chain variable region (V_HHer2) comprising the amino acid sequence of SEQ ID NO:8 and the CDRs of the light chain variable region (V_LHer2) comprising the amino acid sequence of SEQ ID NO:9.

11. The bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 10 wherein the antigen binding domain capable of specific binding to Her2 comprises a heavy chain variable region (V_HHer2) comprising the amino acid sequence of SEQ ID NO:8, and a light chain variable region (V_LHer2) comprising the amino acid sequence of SEQ ID NO:9.

12. The bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 9, wherein the antigen binding domain capable of specific binding to Her2 comprises the CDRs of the heavy chain variable region (V_HHer2) comprising the amino acid sequence of SEQ ID NO:16 and the CDRs of the light chain variable region (V_LHer2) comprising the amino acid sequence of SEQ ID NO:17.

13. The bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 9 or 12, wherein the antigen binding domain capable of specific binding to Her2 comprises a heavy chain variable region (V_HHer2) comprising the amino acid sequence of SEQ ID NO:16, and a light chain variable region (V_LHer2) comprising the amino acid sequence of SEQ ID NO:17.

14. The bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 9, wherein the antigen binding domain capable of specific binding to Her2 comprises the CDRs of the heavy chain variable region (V_HHer2) comprising the amino acid sequence of SEQ ID

NO:138 and the CDRs of the light chain variable region (V_LHer2) comprising the amino acid sequence of SEQ ID NO:139.

15 15. The bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 9 or 14, wherein the antigen binding domain capable of specific binding to Her2 comprises a heavy chain variable region (V_HHer2) comprising the amino acid sequence of SEQ ID NO:138, and a light chain variable region (V_LHer2) comprising the amino acid sequence of SEQ ID NO:139.

10 16. The bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 9, wherein the antigen binding domain capable of specific binding to Her2 comprises the CDRs of the heavy chain variable region (V_HHer2) comprising the amino acid sequence of SEQ ID NO:146 and the CDRs of the light chain variable region (V_LHer2) comprising the amino acid sequence of SEQ ID NO:147.

15 17. The bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 9 or 16, wherein the antigen binding domain capable of specific binding to Her2 comprises a heavy chain variable region (V_HHer2) comprising the amino acid sequence of SEQ ID NO:146, and a light chain variable region (V_LHer2) comprising the amino acid sequence of SEQ ID NO:147.

18. The bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 17, wherein the first antigen binding domain capable of specific binding to CD28 and/or the second antigen binding domain capable of specific binding to Her2 is a Fab molecule.

20 19. The bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 18, wherein the first antigen binding domain capable of specific binding to CD28 is a Fab molecule wherein the variable domains VL and VH or the constant domains CL and CH1, particularly the variable domains VL and VH, of the Fab light chain and the Fab heavy chain are replaced by each other.

25 20. The bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 19, wherein the second antigen binding domain capable of specific binding to Her2 is a conventional Fab molecule.

30 21. The bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 20, wherein the second antigen binding domain capable of specific binding to Her2 is a Fab molecule wherein in the constant domain CL the amino acid at position 123 (numbering according to Kabat EU index) is substituted by an amino acid selected from lysine (K), arginine (R) or histidine (H) and the amino acid at position 124 (numbering according to Kabat EU index) is substituted independently by lysine (K), arginine (R) or histidine (H), and wherein in the constant domain CH1 the amino acid at position 147 (numbering according to Kabat EU index)

is substituted independently by glutamic acid (E) or aspartic acid (D) and the amino acid at position 213 (numbering according to Kabat EU index) is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

22. The bispecific agonistic CD28 antigen binding molecule of claim 1, comprising

- 5 (i) a first light chain comprising the amino acid sequence of SEQ ID NO:92, a first heavy chain comprising the amino acid sequence of SEQ ID NO:91, a second heavy chain comprising the amino acid sequence of SEQ ID NO:96 and a second light chain comprising the amino acid sequence of SEQ ID NO:97, or
- 10 (ii) a first light chain comprising the amino acid sequence of SEQ ID NO:92, a first heavy chain comprising the amino acid sequence of SEQ ID NO:91, a second heavy chain comprising the amino acid sequence of SEQ ID NO:98 and a second light chain comprising the amino acid sequence of SEQ ID NO:97, or
- 15 (iii) a first light chain comprising the amino acid sequence of SEQ ID NO:92, a first heavy chain comprising the amino acid sequence of SEQ ID NO:91, a second heavy chain comprising the amino acid sequence of SEQ ID NO:101 and a second light chain comprising the amino acid sequence of SEQ ID NO:97.

23. The bispecific agonistic CD28 antigen binding molecule of claim 1, comprising

- 20 (i) a first light chain comprising the amino acid sequence of SEQ ID NO:179, a first heavy chain comprising the amino acid sequence of SEQ ID NO:178, a second heavy chain comprising the amino acid sequence of SEQ ID NO:180 and a second light chain comprising the amino acid sequence of SEQ ID NO:181, or
- (ii) a first light chain comprising the amino acid sequence of SEQ ID NO:179, a first heavy chain comprising the amino acid sequence of SEQ ID NO:178, a second heavy chain comprising the amino acid sequence of SEQ ID NO:182 and a second light chain comprising the amino acid
- 25 sequence of SEQ ID NO:183.

24. The bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 18, wherein the second antigen binding domain capable of specific binding to Her2 is a Fab molecule wherein the variable domains VL and VH or the constant domains CL and CH1, particularly the variable domains VL and VH, of the Fab light chain and the Fab heavy chain are

30 replaced by each other.

25. The bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 18 or 24, wherein the first antigen binding domain capable of specific binding to CD28 is a conventional Fab molecule.

26. The bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 18

35 or 24 or 25, wherein the first antigen binding domain capable of specific binding to CD28 is a

Fab molecule wherein in the constant domain CL the amino acid at position 123 (numbering according to Kabat EU index) is substituted by an amino acid selected from lysine (K), arginine (R) or histidine (H) and the amino acid at position 124 (numbering according to Kabat EU index) is substituted independently by lysine (K), arginine (R) or histidine (H), and wherein in the
5 constant domain CH1 the amino acid at position 147 (numbering according to Kabat EU index) is substituted independently by glutamic acid (E) or aspartic acid (D) and the amino acid at position 213 (numbering according to Kabat EU index) is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

27. The bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 13
10 or 24 to 26, comprising

(i) a first light chain comprising the amino acid sequence of SEQ ID NO:83, a first heavy chain comprising the amino acid sequence of SEQ ID NO:74, a second heavy chain comprising the amino acid sequence of SEQ ID NO:99 and a second light chain comprising the amino acid sequence of SEQ ID NO:100, or

15 (ii) a first light chain comprising the amino acid sequence of SEQ ID NO:83, a first heavy chain comprising the amino acid sequence of SEQ ID NO:74, a second heavy chain comprising the amino acid sequence of SEQ ID NO:102 and a second light chain comprising the amino acid sequence of SEQ ID NO:100.

28. The bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 27,
20 wherein the first and the second antigen binding domain are each a Fab molecule and the Fc domain is composed of a first and a second subunit capable of stable association; and wherein (i) the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain and the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the second subunit of the Fc domain,
25 or (ii) the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain and the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the second subunit of the Fc domain.

29. The bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 22,
wherein the Fc domain comprises a modification promoting the association of the first and the
30 second subunit of the Fc domain.

30. The bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 23,
wherein the first subunit of the Fc domain comprises the amino acid substitutions S354C and/or T366W (EU numbering) and the second subunit of the Fc domain comprises the amino acid substitutions Y349C, T366S and Y407V (numbering according to Kabat EU index).

31. One or more isolated polynucleotide encoding the bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 30.

32. One or more vector, particularly expression vector, comprising the polynucleotide(s) of claim 31.

5 33. A host cell comprising the polynucleotide(s) of claim 31 or the vector(s) of claim 32.

34. A method of producing a bispecific agonistic CD28 antigen binding molecule, comprising the steps of a) culturing the host cell of claim 33 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.

10 35. A bispecific agonistic CD28 antigen binding molecule produced by the method of claim 34.

36. A pharmaceutical composition comprising the bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 30 and at least one pharmaceutically acceptable excipient.

15 37. The bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 30, or the pharmaceutical composition of claim 36, for use as a medicament.

38. The bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 30, or the pharmaceutical composition of claim 36, for use in enhancing (a) T cell activation or (b) T cell effector functions.

20 39. The bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 30, or the pharmaceutical composition of claim 36, for use in the treatment of cancer.

40. The bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 30, or the pharmaceutical composition of claim 36, for use in the treatment of cancer, wherein the use is for administration in combination with a chemotherapeutic agent, radiation therapy and/ or
25 other agents for use in cancer immunotherapy.

41. The bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 30, or the pharmaceutical composition of claim 36, for use in the treatment of cancer, wherein the use is for administration in combination with a T-cell activating anti-CD3 bispecific antibody.

42. The bispecific agonistic CD28 antigen binding molecule or the pharmaceutical
30 composition [for use](#) of claim 41, wherein the T-cell activating anti-CD3 bispecific antibody is an anti-Her2/anti-CD3 antibody.

43. The bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 30, or the pharmaceutical composition of claim 36, for use in the treatment of cancer, wherein the use is for administration in combination with an anti-PD-L1 antibody or an anti-PD-1 antibody.

44. Use of the bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 30, or the pharmaceutical composition of claim 36, in the manufacture of a medicament for the treatment of a disease, particularly for the treatment of cancer.

45. The use of claim 44, wherein the bispecific agonistic CD28 antigen binding molecule or the pharmaceutical composition is to be administered in combination with a chemotherapeutic agent, radiation therapy and/ or other agents for use in cancer immunotherapy, particularly in combination with a T-cell activating anti-CD3 bispecific antibody or an anti-PD-L1 antibody or an anti-PD-1 antibody.

~~45. A method of treating a disease, particularly cancer, in an individual, comprising administering to said individual an effective amount of the bispecific agonistic CD28 antigen binding molecule of any one of claims 1 to 30, or the pharmaceutical composition of claim 36.~~

~~46. The method of claim 45, further comprising administration in combination with a chemotherapeutic agent, radiation therapy and/ or other agents for use in cancer immunotherapy, particularly in combination with a T-cell activating anti-CD3 bispecific antibody or an anti-PD-L1 antibody or an anti-PD-1 antibody.~~
