

2012;12:317–318). Localized or targeted use of anti-CD28 mAb can be used with less risk for promotion of antitumor immunity. Jung *et al.*, Int J Cancer. 2001 Jan 15;91(2):225-30.

[0006] Different families of growth factors and growth factor receptors have been shown to be involved in the autonomous growth of cancer cells. Among these, the epidermal growth factor receptor (EGFR) and the EGF-family of peptide growth factor have a central role in the pathogenesis and progression of different carcinoma types. EGFR belongs to a family of receptors that encompasses three additional proteins, ErbB-2, ErbB-3 and ErbB-4. These proteins and the growth factors of the EGF family form an integrated system in which a signal that hits an individual receptor type is often transmitted to other receptors of the same family.

[0007] Monoclonal antibodies (mAbs) aimed at enhancing T-cell activation are under clinical development as anti-tumor therapeutics. The majority of current treatments, however, have a difficult time overcoming the inhibitory nature of the tumor microenvironment, thus failing to generate efficient tumor-specific T-cell activation and subsequent tumor cell killing. Several blocking mAbs directed against checkpoint inhibitors such as CTLA-4 (cytotoxic T lymphocyte-associated protein) and programmed cell death 1(PD-1)/programmed cell death ligand 1 (PD-L1) have been clinically approved for melanoma, renal cell carcinoma, non-small lung cancer and advanced metastatic cutaneous squamous cell carcinoma. Blocking PD-1 releases the break on T-cell activation, but its efficacy as a single agent often it is not sufficient to get tumor clearance and durable anti-tumor responses.

SUMMARY

[0008] The present disclosure provides an immunotherapeutic modality using bi-specific antibodies targeted against Cluster of Differentiation 28 (CD28) and Epidermal Growth Factor Receptor (EGFRxCD28 or CD28xEGFR) that, when combined with a PD-1 blocking antibody, induce long lived anti-tumor immunity and promote robust intra-tumoral T-cell activation with no signs of systemic cytokine secretion, in both syngeneic and human tumor xenograft models. Toxicology studies in genetically-humanized immunocompetent mice and in cynomolgus monkeys demonstrate that these bi-specifics exhibited some single agent activity and no toxicity on their own or in combination with anti-PD-1 antibody. Collectively, these data suggest that combining this class of CD28-based bi-specifics with PD-1 inhibition may provide safe, biologics solutions with strikingly enhanced, specific and synergistic anti-tumor activity.

[0008a] In one aspect, the present disclosure refers to a bispecific antigen-binding molecule comprising a first antigen-binding domain that specifically binds human CD28, and a second antigen-binding domain that specifically binds human EGFR, [wherein the first antigen-binding domain that specifically binds human CD28 comprises three heavy chain complementarity determining regions \(HCDR1, HCDR2 and HCDR3\) and three light chain complementarity](#)

determining regions (LCDR1, LCDR2 and LCDR3), and wherein the second antigen-binding domain that specifically binds human EGFR comprises three heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3) and three light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3); wherein the first antigen-binding domain comprises a HCDR1 comprising an amino acid sequence of SEQ ID NO: 12; wherein HCDR2 comprises an amino acid sequence of SEQ ID NO: 6; wherein HCDR3 comprises an amino acid sequence of SEQ ID NO: 14, wherein LCDR1 comprises an amino acid sequence SEQ ID NO: 18, wherein LCDR2 comprises an amino acid sequence SEQ ID NO: 20, and wherein LCDR3 comprises an amino acid sequence SEQ ID NO: 22; and wherein the second antigen-binding domain comprises a HCDR1 comprising the amino acid sequence of SEQ ID NO: 4, wherein HCDR2 comprises the amino acid sequence of SEQ ID NO: 6, wherein HCDR3 comprises the amino acid sequence of SEQ ID NO: 8, wherein LCDR1 comprises an amino acid sequence of SEQ ID NOs: 18, wherein LCDR2 comprises an amino acid sequence of SEQ ID NO: 20, and wherein LCDR3 comprises an amino acid sequence of SEQ ID NO: 22.

[0008b] In another aspect, the present disclosure refers to a bispecific antigen-binding molecule comprising a first antigen-binding domain that specifically binds human CD28, and a

second antigen-binding domain that specifically binds human EGFR, wherein the first antigen-binding domain comprises a heavy chain variable region (HCVR) comprising SEQ ID NO: 10, and a light chain variable region (LCVR) comprising SEQ ID NO: 16; and wherein the second antigen-binding domain comprises a HCVR comprising SEQ ID NO: 2, and a LCVR comprising SEQ ID NO: 16.

~~[0008c]—In another aspect, the present disclosure refers to a bispecific antigen-binding molecule comprising a first antigen-binding domain that specifically binds human CD28, and a second antigen-binding domain that specifically binds human EGFR, wherein the first antigen-binding domain comprises a heavy chain variable region (HCVR) comprising SEQ ID NO: 10, and a light chain variable region (LCVR) comprising SEQ ID NO: 16; and wherein the second antigen-binding domain comprises a HCVR comprising SEQ ID NO: 30, and a LCVR comprising SEQ ID NO: 16.~~

~~[0008d]—In another aspect, the present disclosure refers to a bispecific antigen-binding molecule comprising a first antigen-binding domain that specifically binds human CD28, and a second antigen-binding domain that specifically binds human EGFR, wherein the first antigen-binding domain comprises a heavy chain variable region (HCVR) comprising SEQ ID NO: 10, and a light chain variable region (LCVR) comprising SEQ ID NO: 16; and wherein the second antigen-binding domain comprises a HCVR comprising SEQ ID NO: 40, and a LCVR comprising SEQ ID NO: 16.~~

~~[0008e]—In another aspect, the present disclosure refers to a bispecific antigen-binding molecule comprising a first antigen-binding domain that specifically binds human CD28, and a second antigen-binding domain that specifically binds human EGFR, wherein the first antigen-binding domain comprises a heavy chain variable region (HCVR) comprising SEQ ID NO: 10, and a light chain variable region (LCVR) comprising SEQ ID NO: 16; and wherein the second antigen-binding domain comprises a HCVR comprising SEQ ID NO: 50, and a LCVR comprising SEQ ID NO: 16.~~

[0008f0008c] In another aspect, the present disclosure refers to a pharmaceutical composition comprising the bispecific antigen-binding molecule as disclosed herein and a pharmaceutically acceptable carrier or diluent.

[0008g0008d] In another aspect, the present disclosure refers to the use of the bispecific antigen-binding molecule as disclosed herein in the manufacture of a medicament for treating a cancer in a subject.

[0009] In one example, the present disclosure provides an isolated bispecific antigen binding molecule comprising a first antigen-binding domain that binds human CD28 with a K_D of less than about $10^{-6}M$ as measured by surface plasmon resonance at 25° C; and a second antigen-binding domain that specifically binds a human epidermal growth factor receptor (EGFR) on a target tumor cell, with a K_D of less than about $10^{-9}M$ as measured by surface plasmon resonance at 25° C.

We Claim:

1. A bispecific antigen-binding molecule comprising a first antigen-binding domain that specifically binds human CD28, and a second antigen-binding domain that specifically binds human EGFR,

wherein the first antigen-binding domain that specifically binds human CD28 comprises three heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3) and three light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3), and wherein the second antigen-binding domain that specifically binds human EGFR comprises three heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3) and three light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3);

wherein the first antigen-binding domain comprises a HCDR1 comprising an amino acid sequence of SEQ ID NO: 12; wherein HCDR2 comprises an amino acid sequence of SEQ ID NO: 6; wherein HCDR3 comprises an amino acid sequence of SEQ ID NO: 14, wherein LCDR1 comprises an amino acid sequence SEQ ID NO: 18, wherein LCDR2 comprises an amino acid sequence SEQ ID NO: 20, and wherein LCDR3 comprises an amino acid sequence SEQ ID NO: 22; and

wherein the second antigen-binding domain comprises a HCDR1 comprising the amino acid sequence of SEQ ID NO: 4, wherein HCDR2 comprises the amino acid sequence of SEQ ID NO: 6, wherein HCDR3 comprises the amino acid sequence of SEQ ID NO: 8, wherein LCDR1 comprises an amino acid sequence of SEQ ID NOs: 18, wherein LCDR2 comprises an amino acid sequence of SEQ ID NO: 20, and wherein LCDR3 comprises an amino acid sequence of SEQ ID NO: 22.

2. The bispecific antigen-binding molecule of claim 1, wherein the antigen-binding molecule binds to CD28-expressing human T-cells with an EC50 value of between 1×10^{-12} M and 1×10^{-5} M.

3. The bispecific antigen-binding molecule of claim 2, wherein the antigen-binding molecule binds to CD28-expressing human T-cells with an EC50 value of between 1×10^{-9} M to 1×10^{-5} M.

4. The bispecific antigen-binding molecule of claim 1, wherein the antigen-binding molecule binds human cells expressing human CD28 and cynomolgus monkey cells expressing cynomolgus CD28.
5. The bispecific antigen-binding molecule of claim 1, wherein the antigen-binding molecule induces cytokine release and CD25 up-regulation in human whole blood.
6. The bispecific antigen-binding molecule of claim 1, wherein the antigen-binding molecule induces T-cell mediated cytotoxicity of human cancer cells.
7. The bispecific antigen-binding molecule of claim 6, wherein the cancer cells express EGFR.
8. The bispecific antigen-binding molecule of claim 6, wherein the cancer cells are selected from the group consisting of esophageal carcinoma, lung squamous cell carcinoma, lung adenocarcinoma, cervical squamous cell carcinoma, endometrial adenocarcinoma, bladder urothelial carcinoma, lung cancer (e.g., non-small cell lung cancer), colorectal cancer, rectal cancer, endometrial cancer, skin cancer (e.g., head & neck squamous cell carcinoma), brain cancer (e.g., glioblastoma multiforme), breast cancer, gastroesophageal cancer, (e.g., gastroesophageal adenocarcinoma), prostate cancer and ovarian cancer cells.
- ~~9. The bispecific antigen-binding molecule of claim 1, wherein the first antigen-binding domain that specifically binds human CD28 comprises the heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3) from a heavy chain variable region (HCVR) comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 59, and 63, and the light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3) from a light chain variable region (LCVR) comprising an amino acid sequence SEQ ID NO: 16.~~
- ~~10. The bispecific antigen-binding molecule of claim 1, wherein the second antigen-binding domain that specifically binds human EGFR comprises the heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3) from a heavy chain variable region (HCVR) comprising SEQ ID NOs: 2, 30, 40, and 50, and the light chain~~

~~complementarity determining regions (LCDR1, LCDR2 and LCDR3) from a light chain variable region (LCVR) comprising an amino acid sequence SEQ ID NO: 16.~~

~~11. —The bispecific antigen-binding molecule of claim 1, wherein the first antigen-binding domain that specifically binds human CD28 comprises three heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3) and three light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3), wherein HCDR1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 12, 60, and 64; wherein HCDR2 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 6, 61, and 65; wherein HCDR3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 14, 62, and 66, wherein LCDR1 comprises an amino acid sequence SEQ ID No: 18, wherein LCDR2 comprises an amino acid sequence SEQ ID NO: 20, wherein LCDR3 comprises an amino acid sequence SEQ ID No: 22.~~

~~12. —The bispecific antigen-binding molecule of claim 1, wherein the second antigen-binding domain that specifically binds human EGFR comprises three heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3) and three light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3), wherein HCDR1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 32, 42, and 52; wherein HCDR2 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 6, 34, 44, and 54; wherein HCDR3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 36, 46, and 56, wherein LCDR1 comprises an amino acid sequence SEQ ID No: 18, wherein LCDR2 comprises an amino acid sequence SEQ ID NO: 20, and wherein LCDR3 comprises an amino acid sequence SEQ ID NO: 22.~~

~~13. —The bispecific antigen-binding molecule of claim 1, wherein the first antigen-binding domain that specifically binds human CD28 comprises three heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3) and three light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3), and wherein the second antigen-binding domain that specifically binds human EGFR comprises three heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3) and three light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3);~~

~~wherein the first antigen-binding domain comprises a HCDR1 comprising an amino acid sequence of SEQ ID NO: 12; wherein HCDR2 comprises an amino acid sequence of SEQ ID NO: 6; wherein HCDR3 comprises an amino acid sequence of SEQ ID NO: 14, wherein LCDR1 comprises an amino acid sequence SEQ ID NO: 18, wherein LCDR2 comprises an amino acid sequence SEQ ID NO: 20, and wherein LCDR3 comprises an amino acid sequence SEQ ID NO: 22; and~~

~~wherein the second antigen-binding domain comprises a HCDR1 comprising the amino acid sequence of SEQ ID NO: 4, wherein HCDR2 comprises the amino acid sequence of SEQ ID NO: 6, wherein HCDR3 comprises the amino acid sequence of SEQ ID NO: 8, wherein LCDR1 comprises an amino acid sequence of SEQ ID NOs: 18, wherein LCDR2 comprises an amino acid sequence of SEQ ID NO: 20, and wherein LCDR3 comprises an amino acid sequence of SEQ ID NO: 22.~~

~~14. — The bispecific antigen-binding molecule of claim 1, wherein the first antigen-binding domain competes for binding to human CD28 with a reference antigen-binding protein comprising three heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3) and three light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3), wherein HCDR1 comprises an amino acid sequence SEQ ID NO: 12; wherein HCDR2 comprises an amino acid sequence SEQ ID NO: 6; wherein HCDR3 comprises an amino acid sequence SEQ ID NO: 14, wherein LCDR1 comprises an amino acid sequence SEQ ID NO: 18, wherein LCDR2 comprises an amino acid sequence SEQ ID NO: 20 and wherein LCDR3 comprises an amino acid sequence SEQ ID NO: 22.~~

~~15. — The bispecific antigen-binding molecule of claim 1, wherein the first antigen-binding domain competes for binding to human CD28 with a reference antigen-binding protein comprising a heavy chain variable region (HCVR) comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 59 and 63, and a light chain variable region (LCVR) comprising an amino acid sequence of SEQ ID NOs: 16.~~

~~16. — The bispecific antigen-binding molecule of claim 1, wherein the second antigen-binding domain competes for binding to human EGFR with a reference antigen-binding protein comprising three heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3) and three light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3), wherein HCDR1 comprises the amino acid sequence selected from~~

~~the group consisting of SEQ ID NOs: 4, 32, 42, and 52, wherein HCDR2 comprises the amino acid sequence selected from the group consisting of SEQ ID NOs: 6, 34, 44, and 54, wherein HCDR3 comprises the amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 36, 46, and 56, wherein LCDR1 comprises an amino acid sequence SEQ ID NO: 18, wherein LCDR2 comprises an amino acid sequence SEQ ID NO: 20 and wherein LCDR3 comprises an amino acid sequence SEQ ID NO: 22.~~

~~17. —The bispecific antigen-binding molecule of claim 1, wherein the second antigen-binding domain competes for binding to human EGFR with a reference antigen binding protein comprising a heavy chain variable region (HCVR) comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 30, 40, and 50, and a light chain variable region (LCVR) comprising an amino acid sequence SEQ ID NO: 16.~~

~~18. —The bispecific antigen-binding molecule of claim 1, wherein the first antigen-binding domain competes for binding to human CD28 with a reference antigen binding protein comprising a heavy chain variable region (HCVR) comprising an amino acid sequence SEQ ID NO: 10, and a light chain variable region (LCVR) comprising an amino acid sequence SEQ ID NO: 16, and wherein the second antigen-binding domain competes for binding to human EGFR with a reference antigen binding protein comprising a heavy chain variable region (HCVR) comprising the amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 30, 40, and 50, and a light chain variable region (LCVR) comprising an amino acid sequence SEQ ID NO: 16.~~

19. A bispecific antigen-binding molecule comprising a first antigen-binding domain that specifically binds human CD28, and a second antigen-binding domain that specifically binds human EGFR,
wherein the first antigen-binding domain comprises a heavy chain variable region (HCVR) comprising SEQ ID NO: 10, and a light chain variable region (LCVR) comprising SEQ ID NO: 16; and
wherein the second antigen-binding domain comprises a HCVR comprising SEQ ID NO: 2, and a LCVR comprising SEQ ID NO: 16.

~~20. — A bispecific antigen-binding molecule comprising a first antigen-binding domain that specifically binds human CD28, and a second antigen-binding domain that specifically binds human EGFR,~~

~~wherein the first antigen-binding domain comprises a heavy chain variable region (HCVR) comprising SEQ ID NO: 10, and a light chain variable region (LCVR) comprising SEQ ID NO: 16; and~~

~~wherein the second antigen-binding domain comprises a HCVR comprising SEQ ID NO: 30, and a LCVR comprising SEQ ID NO: 16.~~

~~21. — A bispecific antigen-binding molecule comprising a first antigen-binding domain that specifically binds human CD28, and a second antigen-binding domain that specifically binds human EGFR,~~

~~wherein the first antigen-binding domain comprises a heavy chain variable region (HCVR) comprising SEQ ID NO: 10, and a light chain variable region (LCVR) comprising SEQ ID NO: 16; and~~

~~wherein the second antigen-binding domain comprises a HCVR comprising SEQ ID NO: 40, and a LCVR comprising SEQ ID NO: 16.~~

~~22. — A bispecific antigen-binding molecule comprising a first antigen-binding domain that specifically binds human CD28, and a second antigen-binding domain that specifically binds human EGFR,~~

~~wherein the first antigen-binding domain comprises a heavy chain variable region (HCVR) comprising SEQ ID NO: 10, and a light chain variable region (LCVR) comprising SEQ ID NO: 16; and~~

~~wherein the second antigen-binding domain comprises a HCVR comprising SEQ ID NO: 50, and a LCVR comprising SEQ ID NO: 16.~~

10. The bispecific antigen-binding molecule of any one of claims 1-9 that is a bispecific antibody comprising a heavy chain of IgG1 isotype.

11. The bispecific antigen-binding molecule of any one of claims 1-9 that is a bispecific antibody comprising a heavy chain of IgG4 isotype.

12. The bispecific antigen-binding molecule of any one of claims 1-9 that is a bispecific antibody, wherein (a) the first antigen-binding domain that specifically binds human CD28

comprises a heavy chain comprising the sequence of SEQ ID NO: 26, and a light chain comprising the sequence of SEQ ID NO: 28; and

(b) the second antigen-binding domain that specifically binds human EGFR comprises a heavy chain comprising the sequence of SEQ ID NO: 24, and a light chain comprising the sequence of SEQ ID NO: 28.

13. The bispecific antigen-binding molecule of any one of claims 1-12, wherein the bispecific antigen-binding molecule interacts with one or more of amino acid residues 345-368 of EGFR as set forth in SEQ ID NO:70, or amino acid residues 399-416 of EGFR as set forth in SEQ ID NO: 71.

2314. A pharmaceutical composition comprising the bispecific antigen-binding molecule of any one of claims 1-13 and a pharmaceutically acceptable carrier or diluent.

~~24. The pharmaceutical composition of claim 23, further comprising a checkpoint inhibitor.~~

~~25. The pharmaceutical composition of claim 24, wherein the checkpoint inhibitor is selected from the group consisting of pembrolizumab, nivolumab and cemiplimab.~~

~~26. The pharmaceutical composition of claim 25, wherein the checkpoint inhibitor is cemiplimab.~~

~~27. The pharmaceutical composition of claim 23, further comprising another different bispecific antigen-binding molecule comprising a first antigen-binding domain that binds to the same tumor target antigen and a second antigen-binding domain that binds to CD3 on T cells.~~

~~28. The pharmaceutical composition of claim 23, further comprising another different bispecific antigen-binding molecule comprising a first antigen-binding domain that binds to a different tumor target antigen and a second antigen-binding domain that binds to CD3 on T cells.~~

~~29. The pharmaceutical composition of claim 28, wherein the different tumor target antigen is selected from the group consisting of PSMA, MUC16, and STEAP2.~~

~~30~~15. Use of the bispecific antigen-binding molecule of any one of claims 1-~~22~~13 in the manufacture of a medicament for treating a cancer in a subject.

~~31~~16. The use of claim ~~30~~15, wherein the cancer is an EGFR expressing cancer.

~~32~~17. The use of claim ~~31~~16, wherein the EGFR expressing cancer is selected from the group consisting of esophageal carcinoma, lung squamous cell carcinoma, lung adenocarcinoma, cervical squamous cell carcinoma, endometrial adenocarcinoma, bladder urothelial carcinoma, lung cancer, non-small cell lung cancer, colorectal cancer, rectal cancer, endometrial cancer, skin cancer, head & neck squamous cell carcinoma, brain cancer, glioblastoma multiforme, breast cancer, gastroesophageal cancer, gastroesophageal adenocarcinoma, prostate cancer, and ovarian cancer.

~~33~~18. The use of claim ~~30~~15, wherein the medicament further comprises a second therapeutic agent.

~~34~~19. The use of claim ~~33~~18, wherein the second therapeutic agent is an anti-tumor agent, radiotherapy, an antibody drug conjugate, a checkpoint inhibitor, another different bispecific antibody comprising a first antigen-binding domain that binds to the same tumor target antigen and a second antigen-binding domain that binds to CD3 on T cells, another different bispecific antibody comprising a first antigen-binding domain that binds to a different tumor target antigen and a second antigen-binding domain that binds to CD3 on T cells, or combinations thereof.

~~2035~~. The use of claim ~~34~~19, wherein the checkpoint inhibitor is cemiplimab.

~~3621~~. The use of claim ~~34~~19, wherein the different bispecific antibody comprises a first antigen-binding domain that binds to the different tumor antigen selected from the group consisting of PSMA, MUC16, and STEAP2 and a second antigen-binding domain that binds to CD3 on T cells.

37. ~~The bispecific antigen-binding molecule of claim 1, wherein the bispecific antigen-binding molecule interacts with one or more of amino acid residues 133-154 of EGFR as set forth in SEQ ID NO: 72, or amino acid residues 345-368 of EGFR as set forth in SEQ ID NO:70, or amino acid residues 399-416 of EGFR as set forth in SEQ ID NO: 71.~~