

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.

[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.

[0008f] 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.

[0008g] 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.

[0010] In another example, the isolated bispecific antigen binding molecule demonstrates a costimulatory effect when used in conjunction with an anti-Mucine 16 (MUC16) X CD3 bispecific antibody and tested on target cells expressing EGFR. In one embodiment, the costimulatory effect is shown by one or more of the following: (a) the ability to activate and direct human T cells to kill a target cell expressing EGFR; (b) the ability to upregulate PD-1 on T cells; (c) the ability to increase the release of the cytokines IFN gamma and TNF from PBMC; (d) the ability to deplete tumor cells; or (f) the ability to enhance tumor clearance. In another example, the costimulatory effect is further shown by one or more of the following: (g) activation of NFκB activity in a T cell/APC luciferase-based reporter assay; or (h) measurement of IL-2 cytokine production using a primary CD4⁺T cell/APC functional assay.

[0011] The present disclosure provides a multispecific (*e.g.*, bi-specific) antigen-binding protein (*e.g.*, antibody or antigen-binding fragment thereof) that binds EGFR and CD28 comprising: (1) an EGFR binding arm comprising: (a) an immunoglobulin chain comprising the HCDRs of a heavy chain variable region that comprises an amino acid sequence set forth in a member selected from the group consisting of SEQ ID NOs: 2, 30, 40 and 50, or a variant thereof; and/or (b) an immunoglobulin chain comprising the LCDRs of a light chain variable region that comprises an amino acid sequence set forth in SEQ ID NO: 16, or a variant thereof; and a CD28 binding arm; or (2) a CD28 binding arm comprising: (c) an immunoglobulin chain comprising the HCDRs of a heavy chain variable region that comprises an amino acid sequence set forth in a member selected from the group consisting of SEQ ID NOs: 10, 59 and 63, or a variant thereof; and/or (d) an immunoglobulin chain comprising the LCDRs of a light chain variable region that comprises an amino acid sequence set forth in a member selected from the group consisting of SEQ ID NOs: 16 and 67, or a variant thereof; and an EGFR binding arm.

[0012] For example, in an example of the disclosure, the multispecific (*e.g.*, bi-specific) antigen-binding protein (*e.g.*, antibody or antigen-binding fragment thereof) that binds EGFR and CD28 includes: (1) an EGFR binding arm comprising: (a) a heavy chain immunoglobulin or variable region thereof comprising an amino acid sequence having at least 90% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NOs: 2, 24, 30, 40 and 50, or a variant thereof; and/or (b) a light chain immunoglobulin or variable region thereof comprising an amino acid sequence having at least 90% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NOs: 16 and 28, or a variant thereof; and a CD28 binding arm; or (2) a CD28 binding arm comprising: (c) a heavy chain immunoglobulin or variable region thereof comprising an amino acid sequence having at least 90% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NOs: 10, 26, 59 and 63, or a variant thereof; and/or (d) a light chain immunoglobulin or variable region thereof comprising an amino acid sequence having at least 90% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NOs: 16, 28, and 67, or a variant thereof; and an EGFR binding arm.

[0013] In an example of the disclosure, the multispecific antigen-binding protein (*e.g.*, antibody or antigen-binding fragment thereof) that binds EGFR and CD28 includes: (1) an EGFR binding arm comprising: (a) a heavy chain immunoglobulin or variable region thereof comprising the HCDR1, HCDR2 and HCDR3 of a heavy chain variable region comprising an amino acid sequence set forth in SEQ ID NO: 2, 30, 40 or 50, and at least 90% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO: 2, 30, 40 or 50, respectively; and/or a light chain immunoglobulin or variable region thereof comprising the LCDR1, LCDR2 and LCDR3 of a light chain variable region comprising an amino acid sequence set forth in SEQ ID NO: 16, and at least 90% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO: 16, respectively; and a CD28 binding arm; or (2) a CD28 binding arm comprising: (c) a heavy chain immunoglobulin or variable region thereof comprising the HCDR1, HCDR2 and HCDR3 of a heavy chain variable region comprising an amino acid sequence set forth in SEQ ID NO: 10, 59 or 63, and at least 90% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO: 10, 59 or 63, respectively; and/or (d) a light chain immunoglobulin or variable region thereof comprising the LCDR1, LCDR2 and LCDR3 of a light chain variable region comprising an amino acid sequence set forth in SEQ ID NO: 16, or 67, and at least 90% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO: 16, or 67, respectively; and an EGFR binding arm.

[0014] In an example of the disclosure, the multispecific (*e.g.*, bi-specific) antigen-binding protein (*e.g.*, antibody or antigen-binding fragment thereof) that binds EGFR and CD28 includes: (1) an EGFR binding arm that comprises: a CDR-H1 that comprises the amino acid sequence: GDSIITFY (SEQ ID NO: 4; or a variant thereof); a CDR-H2 that comprises the amino acid sequence: IYYSGIT (SEQ ID NO: 6; or a variant thereof); and a CDR-H3 that comprises the amino acid sequence: ARVSEDSYFHYGMDV (SEQ ID NO: 8; or a variant thereof); and a CDR-L1 that comprises the amino acid sequence: QSVSSSY (SEQ ID NO: 18; or a variant thereof); a CDR-L2 that comprises the amino acid sequence: GAS (SEQ ID NO: 20; or a variant thereof); and a CDR-L3 that comprises the amino acid sequence: QQYGSSPWT (SEQ ID NO: 22; or a variant thereof); and a CD28 binding arm that comprises: a CDR-H1 that comprises the amino acid sequence: GGSISSYY (SEQ ID NO: 12; or a variant thereof); a CDR-H2 that comprises the amino acid sequence: IYYSGIT (SEQ ID NO: 6; or a variant thereof); and a CDR-H3 that comprises the amino acid sequence: ARWGVRRDYYYYGMDV (SEQ ID NO: 14; or a variant thereof); and a CDR-L1 that comprises the amino acid sequence: QSVSSSY (SEQ ID NO: 18; or a variant thereof); a CDR-L2 that comprises the amino acid sequence: GAS (SEQ ID NO: 20; or a variant thereof); a CDR-L3 that comprises the amino acid sequence: QQYGSSPWT (SEQ ID NO: 22; or a variant thereof);

(2) an EGFR binding arm that comprises: a CDR-H1 that comprises the amino acid sequence: GFTFSTFI (SEQ ID NO: 32; or a variant thereof); a CDR-H2 that comprises the amino acid sequence: ISSNGGTI (SEQ ID NO: 34; or a variant thereof); and a CDR-H3 that comprises the amino acid sequence: TRGGDFWSGYYPFDY (SEQ ID NO: 36; or a variant thereof); a CDR-L1 that comprises the amino acid sequence: QSVSSSY (SEQ ID NO: 18; or a variant thereof); a CDR-L2 that comprises the amino acid sequence: GAS (SEQ ID NO: 20; or a variant thereof); and a CDR-L3 that comprises the amino acid sequence: QQYGSSPWT (SEQ ID NO: 22; or a variant thereof); and

a CD28 binding arm that comprises: a CDR-H1 that comprises the amino acid sequence: GGSISSYY (SEQ ID NO: 12; or a variant thereof); a CDR-H2 that comprises the amino acid sequence: IYYSGIT (SEQ ID NO: 6; or a variant thereof); a CDR-H3 that comprises the amino acid sequence: ARWGVRRDYYYYGMDV (SEQ ID NO: 14; or a variant thereof); and a CDR-L1 that comprises the amino acid sequence: QSVSSSY (SEQ ID NO: 18; or a variant thereof); a CDR-L2 that comprises the amino acid sequence: GAS (SEQ ID NO: 20; or a variant thereof); and a CDR-L3 that comprises the amino acid sequence: QQYGSSPWT (SEQ ID NO: 22; or a variant thereof);

(3) an EGFR binding arm that comprises: a CDR-H1 that comprises the amino acid sequence: GFSFRDAW (SEQ ID NO: 42; or a variant thereof); a CDR-H2 that comprises the amino acid sequence: IRNKIDGGTT (SEQ ID NO: 44; or a variant thereof); and a CDR-H3 that comprises the amino acid sequence: TTDIWNVYVLFYYYGLDV (SEQ ID NO: 46; or a variant thereof); and a CDR-L1 that comprises the amino acid sequence: QSVSSSY (SEQ ID NO: 18; or a variant thereof); a CDR-L2 that comprises the amino acid sequence: GAS (SEQ ID NO: 20; or a variant thereof); and a CDR-L3 that comprises the amino acid sequence: QQYGSSPWT (SEQ ID NO: 22; or a variant thereof) and

a CD28 binding arm that comprises: a CDR-H1 that comprises the amino acid sequence: GGSISSYY (SEQ ID NO: 12; or a variant thereof); a CDR-H2 that comprises the amino acid sequence: IYYSGIT (SEQ ID NO: 6; or a variant thereof); and a CDR-H3 that comprises the amino acid sequence: ARWGVRRDYYYYGMDV (SEQ ID NO: 14; or a variant thereof); and a CDR-L1 that comprises the amino acid sequence: QSVSSSY (SEQ ID NO: 18; or a variant thereof); a CDR-L2 that comprises the amino acid sequence: GAS (SEQ ID NO: 20; or a variant thereof); and a CDR-L3 that comprises the amino acid sequence: QQYGSSPWT (SEQ ID NO: 22; or a variant thereof);

(4) an EGFR binding arm that comprises: a CDR-H1 that comprises the amino acid sequence: DDSIISYY (SEQ ID NO: 52; or a variant thereof); a CDR-H2 that comprises the amino acid sequence: IYYSGRT (SEQ ID NO: 54; or a variant thereof); and a CDR-H3 that comprises the amino acid sequence: ARVSEDSYHYHGMVDV (SEQ ID NO: 56; or a variant thereof); and a CDR-L1 that comprises the amino acid sequence: QSVSSSY (SEQ ID NO: 18; or a variant thereof); a CDR-L2 that comprises the amino acid sequence: GAS (SEQ ID NO: 20; or a variant thereof);

thereof); and a CDR-L3 that comprises the amino acid sequence: QQYGSSPWT (SEQ ID NO: 22; or a variant thereof); and
a CD28 binding arm that comprises: a CDR-H1 that comprises the amino acid sequence: GGSISSYY (SEQ ID NO: 12; or a variant thereof); a CDR-H2 that comprises the amino acid sequence: IYYSGIT (SEQ ID NO: 6; or a variant thereof); and a CDR-H3 that comprises the amino acid sequence: ARWGVRRDYYYYGMDV (SEQ ID NO: 14; or a variant thereof); and a CDR-L1 that comprises the amino acid sequence: QSVSSSY (SEQ ID NO: 18; or a variant thereof); a CDR-L2 that comprises the amino acid sequence: GAS (SEQ ID NO: 20; or a variant thereof); and a CDR-L3 that comprises the amino acid sequence: QQYGSSPWT (SEQ ID NO: 22; or a variant thereof).

[0015] In an example of the disclosure, the multispecific (*e.g.*, bi-specific) antigen-binding protein (*e.g.*, antibody or antigen-binding fragment thereof) that binds EGFR and CD28 includes: (1) an EGFR binding arm that comprises: a heavy chain variable region that comprises the amino acid sequence set forth in SEQ ID NO: 2 (or a variant thereof); and a light chain variable region that comprises the amino acid sequence set forth in SEQ ID NO: 16 (or a variant thereof); and a CD28 binding arm that comprises: a heavy chain variable region that comprises the amino acid sequence set forth in SEQ ID NO: 10 (or a variant thereof); and a light chain variable region that comprises the amino acid sequence set forth in SEQ ID NO: 16 (or a variant thereof); (2) an EGFR binding arm that comprises: a heavy chain variable region that comprises the amino acid sequence set forth in SEQ ID NO: 30 (or a variant thereof); and a light chain variable region that comprises the amino acid sequence set forth in SEQ ID NO: 16 (or a variant thereof); and a CD28 binding arm that comprises: a heavy chain variable region that comprises the amino acid sequence set forth in SEQ ID NO: 10 (or a variant thereof); and a light chain variable region that comprises the amino acid sequence set forth in SEQ ID NO: 16 (or a variant thereof); (3) an EGFR binding arm that comprises: a heavy chain variable region that comprises the amino acid sequence set forth in SEQ ID NO: 40 (or a variant thereof); and a light chain variable region that comprises the amino acid sequence set forth in SEQ ID NO: 16 (or a variant thereof); and a CD28 binding arm that comprises: a heavy chain variable region that comprises the amino acid sequence set forth in SEQ ID NO: 10 (or a variant thereof); and a light chain variable region that comprises the amino acid sequence set forth in SEQ ID NO: 16 (or a variant thereof); (4) an EGFR binding arm that comprises: a heavy chain variable region that comprises the amino acid sequence set forth in SEQ ID NO: 50 (or a variant thereof); and a light chain variable region that comprises the amino acid sequence set forth in SEQ ID NO: 16 (or a variant thereof); and a CD28 binding arm that comprises: a heavy chain variable region that comprises the amino acid sequence set forth in SEQ ID NO: 10 (or a variant thereof); and a light chain variable region that comprises the amino acid sequence set forth in SEQ ID NO: 16 (or a variant thereof).

[0016] In an example of the disclosure, the multispecific (*e.g.*, bi-specific) antigen-binding protein (*e.g.*, antibody or antigen-binding fragment thereof) that binds EGFR and CD28 includes:

(1) an EGFR binding arm that comprises: a heavy chain that comprises the amino acid sequence set forth in SEQ ID NO: 24 (or a variant thereof); and a light chain that comprises the amino acid sequence set forth in SEQ ID NO: 28 (or a variant thereof); and a CD28 binding arm that comprises: a heavy chain that comprises the amino acid sequence set forth in SEQ ID NO: 26 (or a variant thereof); and a light chain that comprises the amino acid sequence set forth in SEQ ID NO: 28 (or a variant thereof);

(2) an EGFR binding arm that comprises: a heavy chain that comprises the amino acid sequence set forth in SEQ ID NO: 38 (or a variant thereof); and a light chain that comprises the amino acid sequence set forth in SEQ ID NO: 28 (or a variant thereof); and a CD28 binding arm that comprises: a heavy chain that comprises the amino acid sequence set forth in SEQ ID NO: 26 (or a variant thereof); and a light chain that comprises the amino acid sequence set forth in SEQ ID NO: 28 (or a variant thereof);

(3) an EGFR binding arm that comprises: a heavy chain that comprises the amino acid sequence set forth in SEQ ID NO: 48 (or a variant thereof); and a light chain that comprises the amino acid sequence set forth in SEQ ID NO: 28 (or a variant thereof); and a CD28 binding arm that comprises: a heavy chain that comprises the amino acid sequence set forth in SEQ ID NO: 26 (or a variant thereof); and a light chain that comprises the amino acid sequence set forth in SEQ ID NO: 28 (or a variant thereof);

(4) an EGFR binding arm that comprises: a heavy chain that comprises the amino acid sequence set forth in SEQ ID NO: 58 (or a variant thereof); and a light chain that comprises the amino acid sequence set forth in SEQ ID NO: 28 (or a variant thereof); and a CD28 binding arm that comprises: a heavy chain that comprises the amino acid sequence set forth in SEQ ID NO: 26 (or a variant thereof); and a light chain that comprises the amino acid sequence set forth in SEQ ID NO: 28 (or a variant thereof).

[0017] In an example of the disclosure, the multispecific (e.g., bispecific) antigen-binding protein (e.g., an antibody or antigen-binding fragment thereof) is REGN7075 (also referred to herein as bsAb7075); REGN6321 (also referred to herein as bsAb6321); REGN6322 (also referred to herein as bsAb6322); REGN6323 (also referred to herein as bsAb6323). Other bispecific antibodies may be generated using the parental EGFR antibodies described herein in **Tables 9A, 9B and 9C**, whereby the sequences of the HCVR arms of the EGFR parental antibodies may be found in WO2014/004427 and the HCVR amino acid sequences of the parental CD28 antibodies may be found in **Table 3** herein. Any EGFR HCVR arm may be paired with any of the CD28 HCVR arms described herein.

[0018] The present disclosure also provides an antigen-binding protein (e.g., antibody or antigen-binding fragment thereof) that specifically binds to epidermal growth factor receptor comprising: (i) a heavy chain immunoglobulin or variable region thereof that comprises HCDR1, HCDR2 and HCDR3 of a heavy chain immunoglobulin or variable region thereof that comprises the amino acid sequence set forth in SEQ ID NO: 2, 24, 30, 38, 40, 48, 50 or 58; or a variant

thereof; and/or (ii) a light chain immunoglobulin or variable region thereof that comprises LCDR1, LCDR2 and LCDR3 of a light chain immunoglobulin or variable region thereof that comprises the amino acid sequence set forth in SEQ ID NO: 16 or 28; or a variant thereof. In an example of the disclosure, the antigen-binding protein is multispecific (e.g., bispecific).

[0019] The present disclosure also provides a multispecific antigen-binding protein set forth herein bound to an EGFR polypeptide or an antigenic fragment thereof (e.g., on the surface of a tumor cell) and a CD28 polypeptide or an antigenic fragment thereof (e.g., on the surface of an immune cells such as a T-cell).

[0020] The present disclosure also provides a method for making a multispecific (e.g., bi-specific) antigen-binding protein set forth herein comprising: (a) introducing one or more polynucleotides encoding the immunoglobulin chains of said antigen-binding protein into a host cell (e.g., a CHO cell); (b) culturing the host cell under conditions favorable to expression of the polynucleotide; and (c) optionally, isolating the antigen-binding protein or immunoglobulin chain from the host cell and/or medium in which the host cell is grown. Any antigen-binding protein or immunoglobulin chain which is a product of such a method is part of the present disclosure.

[0021] The present disclosure also provides a polynucleotide encoding one or more of the immunoglobulin chains of a multispecific (e.g., bi-specific) antigen-binding protein of the present disclosure. Vectors comprising the polynucleotides of the present disclosure are also part of the present disclosure as well as host cells (e.g., CHO) comprising a polynucleotide, vector or antigen-binding protein of the present disclosure.

[0022] The present disclosure also provides a composition or kit comprising one or more of the multispecific antigen-binding proteins of the present disclosure in association with one or more other substances or items, optionally, in association with a further therapeutic agent (e.g., PD-1 inhibitor, an anti-PD-1 antibody or antigen-binding fragment thereof, a PD-L1 inhibitor, an anti-PD-L1 antibody or antigen-binding fragment thereof, a platinum anti-cancer chemotherapeutic agent, paclitaxel, docetaxel, vincristine, cisplatin, carboplatin, oxaliplatin, an anti-cancer antibody or antigen-binding fragment thereof, pembrolizumab, nivolumab, trastuzumab, cetuximab, bevacizumab and/or cemiplimab). Pharmaceutical formulations comprising the multispecific antigen-binding protein of the present disclosure and a pharmaceutically acceptable carrier or excipient and, optionally, a further therapeutic agent are also part of the present disclosure.

[0023] The present disclosure also provides vessels and injection devices (e.g., syringe, pre-filled syringe or autoinjector) including the multispecific antigen-binding proteins, compositions and/or formulations of the present disclosure.

[0024] The present disclosure also provides a method for administering a multispecific antigen-binding protein of the present disclosure, composition or formulation thereof to a subject (e.g. a human) comprising injecting said antigen-binding protein, composition or formulation into the body of the subject, e.g., with a syringe, pre-filled syringe or autoinjector. Optionally a further

therapeutic agent (*e.g.*, cemiplimab, pembrolizumab or nivolumab) is also administered to the subject.

[0025] The present disclosure also provides a method for treating or preventing a hyperproliferative disease (*e.g.*, EGFR-expressing cancer), in a subject (*e.g.*, a human) in need thereof, comprising administering (*e.g.*, subcutaneously, intravenously or intramuscularly) an effective amount of multispecific antigen-binding protein or composition or formulation, optionally in association with a further therapeutic agent (*e.g.*, cemiplimab, pembrolizumab or nivolumab). In an example of the disclosure, the EGFR-expressing cancer is 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 or ovarian cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

[0026] **Figures 1A – 1E** show that, in cancer cell lines with endogenous TSA, EGFRxCD28 bispecific antibody (REGN7075) potentiates T-cell activation in the presence of TCR stimulation by TSAxCD3 (MUC16xCD3). In Figures 1A and 1B, flow cytometry analysis shows that EGFRxCD28 bispecific antibody binds to CD28⁺ and EGFR⁺ cells. (**Figure 1A**) Jurkat cells (CD28⁺ cells). (**Figure 1B**) PEO1 cells (EGFR⁺ cells). In **Figures 1C – 1E**, human T-cells were cultured with cancer target cells expressing endogenous MUC16 and EGFR (Ovarian cancer cell line PEO1) and the indicated bispecifics for 96 hours. (**Figure 1C**) Tumor cell kill, % viable cells. (**Figure 1D**) frequency of CD25⁺ T cells (% of CD2). (**Figure 1E**) Supernatants from cytotoxicity assay were analyzed using a Cytometric Bead Array (CBA) kit. IFN γ released is plotted as pg/ml

[0027] **Figures 2A to 2D** show that expression of a CD28 ligand (CD86) on tumor cells synergizes with anti-PD1 treatment to induce CD8 dependent anti-tumor immunity. MC38 tumor cells were transduced with the ligand for CD28, CD86 (MC38/CD86), or empty vector control (MC38/EV). WT C57BL6 mice were initially implanted with 1x10⁶ tumor cells per

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.
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 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 (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.

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.

23. A pharmaceutical composition comprising the bispecific antigen-binding molecule of claim 1 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. Use of the bispecific antigen-binding molecule of any one of claims 1-22 in the manufacture of a medicament for treating a cancer in a subject.

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

32. The use of claim 31, 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. The use of claim 30, wherein the medicament further comprises a second therapeutic agent.

34. The use of claim 33, 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.

35. The use of claim 34, wherein the checkpoint inhibitor is cemiplimab.

36. The use of claim 34, 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.