

[0009] CD137 (4-1BB or TNFRSF9) is a member of the TNF Receptor Superfamily. CD137 expresses on both innate and adaptive immune cells. It plays a multifaceted role in the tumor microenvironment (TME). It is prevalently upregulated on T cells in TME and provides co-stimulation to CD8 and CD4 T cell activation, proliferation, and survival. It also modulates other cell functions in TME, such as promoting NK cells to interact with CD8 T cells, enhancing tumor antigen presentation on DC cells. A CD137 agonist can enhance the anti-tumor immune response and may work synergistically with other anti-tumor agents, including PD1/PD-L1 blocking antibodies.

[0010] Secretion of TGF β is another leading contributor to immune evasion and tumor progression. TGF β promotes tumor progression and immune-suppressing by preventing T cell proliferation and decrease the effector function of both T and NK cells. It also enhances the function of T regulatory cells and induces epithelial-to-mesenchymal transition (EMT).

[0011] Since PD1, CD137, and TGF β regulate independent immune suppression or immune stimulative pathway, it is possible that targeting PD1 and CD137, PD1 and TGF β , or targeting PD1, CD137 and TGF β will increase the anti-tumor efficacy, especially for immune-excluded and immune-desert tumors.

SUMMARY

[0011a] In one aspect, the present disclosure relates to an anti-CD137 antibody or antigen binding fragment thereof comprising: (i) a heavy chain variable region comprising CDR1: SEQ ID NO: 5, CDR2: SEQ ID NO: 22, and CDR3: SEQ ID NO: 23; and a light chain variable region comprising CDR1: SEQ ID NO: 24, CDR2: SEQ ID NO: 25, and CDR3: SEQ ID NO: 26; (ii) a heavy chain variable region comprising CDR1: SEQ ID NO: 27, CDR2: SEQ ID NO: 28, and CDR3: SEQ ID NO: 29; and a light chain variable region comprising CDR1: SEQ ID NO: 30, CDR2: SEQ ID NO: 9, and CDR3: SEQ ID NO: 31; or (iii) a heavy chain variable region comprising CDR1: SEQ ID NO: 32, CDR2: SEQ ID NO: 33, and CDR3: SEQ ID NO: 34; and a light chain variable region comprising CDR1: SEQ ID NO: 35, CDR2: SEQ ID NO: 36, and CDR3: SEQ ID NO: 37.

[0011b] In another aspect, the present disclosure relates to an anti-PD-L1 antibody or antigen binding fragment thereof comprising: (i) a heavy chain variable region comprising CDR1: SEQ ID NO: 5, CDR2: SEQ ID NO: 6, and CDR3: SEQ ID NO: 7; and a light chain variable region comprising CDR1: SEQ ID NO: 8, CDR2: SEQ ID NO: 9, and CDR3: SEQ ID NO: 10; or (ii) a heavy chain variable region comprising CDR1: SEQ ID NO: 11, CDR2: SEQ ID NO: 12, and CDR3: SEQ ID NO: 13; and a light chain variable region comprising CDR1: SEQ ID NO: 14, CDR2: SEQ ID NO: 9, and CDR3: SEQ ID NO: 15.

[0011c] In another aspect, the present disclosure relates to a nucleic acid composition comprising one or more nucleic acids encoding the antibody or antigen binding fragment thereof as disclosed herein.

[0011d] In another aspect, the present disclosure relates to an expression vector composition comprising one or more expression vectors comprising the nucleic acid composition as disclosed herein.

[0011e] In another aspect, the present disclosure relates to a host cell comprising the nucleic acid composition as disclosed herein or the expression vector composition as disclosed herein.

[0011f] In another aspect, the present disclosure relates to a method for the production of the anti-CD137 antibody or antigen binding fragment thereof as disclosed herein, the method comprising culturing the host cell as disclosed herein.

[0011g] In another aspect, the present disclosure relates to a pharmaceutical composition comprising the anti-CD137 antibody or antigen binding fragment thereof as disclosed herein and a pharmaceutically acceptable carrier.

[0011h] In another aspect, the present disclosure relates to use of the antibody or antigen binding fragment thereof as disclosed herein or the pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating cancer in a subject.

[0012] The present disclosure addresses the above need by providing multispecific binding proteins including, for example, binding proteins that bind PD-L1 and CD137 (PD-L1/CD137 bispecific), binding proteins that bind PD-L1 and TGF β (PD-L1/TGF β bispecific), and binding proteins that bind PD-L1, TGF β , and CD137 (PD-L1/TGF β /CD137 trispecific). In an example, a PD-L1/CD137 bispecific is a binding protein that binds PD-L1 and CD137 and comprises: (a) an antibody scaffold module in an IgG format (e.g., a Y-shaped symmetrical or asymmetrical antibody comprising two heavy chains and two light chains) comprising a first antigen-binding site that binds PD-L1 and a second antigen-binding site that binds PD-L1; (b) at least one first binding module comprising a third antigen-binding site that binds CD137. In an example, a PD-L1/TGF β bispecific is a binding protein that binds PD-L1 and TGF β and comprises: (a) an antibody scaffold module in an IgG format comprising a first antigen-binding site that binds PD-L1 and a second antigen-binding site that binds PD-L1; (b) at least one

first binding module comprising a third antigen-binding site that binds TGF β . In an example, a PD-L1/TGF β /CD137 trispecific is a binding protein that comprises (a) an antibody scaffold module in an IgG format comprising a first antigen-binding site that binds PD-L1 and a second antigen-binding site that binds PD-L1; (b) at least one first binding module comprising a third antigen-binding site that binds TGF β ; and (c) at least one second binding module comprising a fourth antigen-binding site that binds CD137.

[0013] The present disclosure also relates to binding proteins which bind human CD137, including binding proteins that bind CD137, TGF β , and PD-L1 (CD137/TGF β /PD-L1 trispecific). More specifically, the disclosure relates to binding proteins that bind CD137 and to their use to stimulate the CD137 and to promote durable anti-tumor immune responses. In an example, a binding protein may bind CD137 and comprises an antibody scaffold module in an IgG format comprising a first antigen-binding site that binds CD137 and a second antigen-binding site that binds CD137. In an example, a CD137/TGF β /PD-L1 trispecific is a binding protein that comprises (a) an antibody scaffold module in an IgG format comprising a first antigen-binding site that binds CD137 and a second antigen-binding site that binds CD137; (b) at least one first binding module comprising a third antigen-binding site that binds TGF β ; and (c) at least one second binding module comprising a fourth antigen-binding site that binds PD-L1.

[0014] Despite the advances in immunotherapy attributed to the use of PD-1/PD-L1 blockers there is a need for binding proteins that can bind both PD-L1 and to a co-stimulatory target on T cells and/or reverse immunosuppression in the tumor microenvironment by neutralizing TGF β . The three TGF β isoforms, TGF β 1, TGF β 2 and TGF β 3 are highly expressed in many tumors, which promotes cancer progression primarily by suppressing both the innate and adaptive immune systems. And their serum concentrations correlate with poor prognosis. In the tumor microenvironment, TGF β promotes tumor progression by stromal modification, angiogenesis, and induction of epithelial-mesenchymal transition (EMT). TGF β signaling induces the differentiation of Treg cells and drives metastasis through myeloid cells. Moreover, TGF β 1 can directly inhibit T cell and NK cell function.

[0015] A binding protein can simultaneously bind two or three epitopes of a single antigen or to more than one target antigen, allowing for multiple mechanistic functions and potential synergistic effects that cannot be achieved by a monospecific therapeutic antibody or fusion protein.

Advantageously the use of a binding protein that binds two or three antigens may have a lower risk of toxicity than the risk associated with the use of multiple therapeutic agents.

[0016] Broadly speaking, the disclosed bispecifics and trispecifics disclosed herein are based on natural IgG scaffolds with or without modifications to promote heavy chain heterodimerization and are characterized by two or three binding specificities (e.g., PD-L1, CD137 and/or TGF β) contributed by antigen binding sites derived from antibody Fabs or scFv fragments and/or a fusion protein prepared from the extracellular domain (ECD) of TGF β RII receptor appended to the N-terminus or C-terminus of an IgG heavy or light chain in the IgG scaffold.

[0017] In some examples, the PD-L1/CD137 bispecifics, PD-L1/TGF β bispecifics or PD-L1/TGF β /CD137 trispecifics exhibit one or more of the following structural characteristics, alone or in combination:

- (a) have anti-CD137 in an scFv format,
- (b) have anti-PD-L1 in an scFv format,
- (c) have an scFv fused at the N-terminus of an antibody light chain in the IgG scaffold,
- (d) have an scFv fused at the N-terminus of an antibody heavy chain in the IgG scaffold,
- (e) have an scFv fused at the C-terminus of an antibody light chain in the IgG scaffold,
- (f) have an scFv fused at the C-terminus of an antibody heavy chain in the IgG scaffold,
- (g) have an scFv of CD137 existing in a monovalent or a divalent format,
- (h) contain human TGF β RII fused at the C-terminus of the light chain in the IgG scaffold,
or
- (i) contain human TGF β RII fused at the C-terminus of the heavy chain in the IgG scaffold.

[0018] In some examples, the PD-L1/CD137 bispecifics, PD-L1/TGF β bispecifics, and PD-L1/TGF β /CD137 trispecifics provide an immunotherapy that targets exhausted PD-1⁺ CD8 T cells and reinvigorates dysfunctional tumor infiltrating lymphocytes (TILs). Such binding proteins that bind PD-L1 may be particularly beneficial as therapeutics in solid tumors characterized by microenvironments enriched in exhausted TCD8⁺ cells and/or regulatory T cells that contribute to PD-1/PD-L1 resistance. In practice, blocking the PD-1/PD-L1 signaling axis reduces

immunosuppressive signals present in the TME and enhances anti-tumor immunity, which in turn produces durable clinical responses that prolong patient survival.

[0019] In some examples, the disclosed binding proteins that bind PD-L1 are bispecific, tetravalent molecules specific for PD-L1 and either CD137 or human TGF β .

[0020] In other examples, the disclosed binding proteins that bind PD-L1 are molecules specific for PD-L1 and both CD137 and human TGF β .

[0021] In some examples, the disclosed CD137/TGF β /PD-L1 trispecifics are designed to provide an immunotherapy that targets CD137 expressing immune cells in the tumor microenvironment. The binding proteins that bind CD137 may be particularly beneficial as therapeutics in solid tumors. In practice, activating the CD137 signaling axis will enhance the T effector function present in the TME and enhance anti-tumor immunity, which could in turn produce durable clinical responses that prolong patient survival.

[0022] In other examples, the disclosed binding proteins bind CD137 and both PD-L1 and human TGF β .

[0023] According to some examples, the disclosed binding proteins that bind PD-L1 comprise a set of six complementarity determining region (CDR) sequences selected from the group consisting of three CDRs of an anti-PD-L1 antibody heavy chain (HC) variable region sequence selected from SEQ ID NOS: 1 and 3 and three CDRs of a light chain variable region sequence selected from SEQ ID NOS: 2 and 4.

[0024] In some examples, the binding proteins that bind PD-L1 comprise a heavy chain variable region sequence comprising CDR1: SEQ ID NO: 5, CDR2: SEQ ID NO: 6, and CDR3: SEQ ID NO: 7; and/or a light chain variable region sequence comprising CDR1: SEQ ID NO: 8, CDR2: SEQ ID NO: 9, and CDR3: SEQ ID NO: 10.

[0025] In some examples, the binding proteins that bind PD-L1 comprise a heavy chain variable region sequence comprising CDR1: SEQ ID NO: 11, CDR2: SEQ ID NO: 12, and CDR3: SEQ ID NO: 13; and/or a light chain variable region sequence comprising CDR1: SEQ ID NO: 14, CDR2: SEQ ID NO: 9, and CDR3: SEQ ID NO: 15.

[0026] In some examples, the binding proteins that bind PD-L1 comprise a heavy chain variable region sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 3, or an analogue or derivative thereof having at least 90% sequence identity to SEQ ID NO: 1 or SEQ ID NO: 3.

[0027] In other examples, the binding proteins that bind PD-L1 comprise a light chain variable region sequence set forth in SEQ ID NOs: 2 or SEQ ID NO: 4 or an analogue or derivative thereof having at least 90% sequence identity to SEQ ID NO: 2 or SEQ ID NO: 4.

[0028] In other examples, the binding proteins that bind PD-L1 comprise a heavy chain variable region sequence set forth in SEQ ID NOs: 1 or SEQ ID NO: 3 and a light chain variable region sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4.

[0029] In some examples, the binding proteins that bind PD-L1 comprise a heavy chain variable region sequence and a light chain variable region sequence, selected from the following combinations:

(a) a heavy chain variable region sequence comprising SEQ ID NO: 1 and a light chain variable region sequence comprising SEQ ID NO: 2; or

(b) a heavy chain variable region sequence comprising SEQ ID NO: 3 and a light chain variable region sequence comprising SEQ ID NO: 4.

[0030] In some examples, a binding protein that binds PD-L1 comprises:

(a) a heavy chain variable region sequence comprising CDR1: SEQ ID NO: 5, CDR2: SEQ ID NO: 6, and CDR3: SEQ ID NO: 7; and/or a light chain variable region sequence comprising CDR1: SEQ ID NO: 8, CDR2: SEQ ID NO: 9, and CDR3: SEQ ID NO: 10; or

(b) a heavy chain variable region sequence comprising CDR1: SEQ ID NO: 11, CDR2: SEQ ID NO: 12, and CDR3: SEQ ID NO: 13; and/or a light chain variable region sequence comprising CDR1: SEQ ID NO: 14, CDR2: SEQ ID NO: 9, and CDR3: SEQ ID NO: 15.

[0031] In some examples, a binding protein that binds PD-L1 is provided wherein the first and second antigen-binding sites bind PD-L1, and wherein the antibody scaffold module comprises:

(i) a heavy chain variable region sequence as set forth in SEQ ID NO: 1, a heavy chain constant region sequence as set forth in SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62,

SEQ ID NO: 63, or SEQ ID NO: 64, a light chain variable region sequence as set forth in SEQ ID NO: 2, and a light chain constant region sequence as set forth in SEQ ID NO: 65 or SEQ ID NO: 66; or

(ii) a heavy chain variable region sequence as set forth in SEQ ID NO: 3, a heavy chain constant region sequence as set forth in SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, or SEQ ID NO: 64, a light chain variable region sequence as set forth in SEQ ID NO: 4, and a light chain constant region sequence as set forth in SEQ ID NO: 65 or SEQ ID NO: 66.

[0032] In some examples, a binding protein that binds PD-L1 is provided wherein the first and second antigen-binding sites bind PD-L1, and wherein the antibody scaffold module comprises:

a heavy chain sequence as set forth in SEQ ID NO: 45 and a light chain sequence as set forth in SEQ ID NO: 40.

[0033] In some examples, the binding proteins that bind PD-L1 comprise one or more heavy chain variable region CDRs disclosed in Table 1 and/or one or more light chain variable region CDRs disclosed in Table 2.

[0034] In some examples, the binding proteins that bind PD-L1 exhibit one or more of the following structural and functional characteristics, alone or in combination:

- (a) is specific for human PD-L1,
- (b) cross-reacts with cynomolgus PD-L1,
- (c) disrupts the interaction of PD-1 and PD-L1, or
- (d) dis-inhibits PD-1/PD-L1 checkpoint-mediated inhibition of T cells.

[0035] In some examples, the binding proteins that bind PD-L1 specifically bind to human cells expressing endogenous levels of PD-L1 and to host cells engineered to overexpress human PD-L1. The binding proteins that bind PD-L1 may also bind to cells overexpressing human or cyno PD-L1 with subnanomolar EC₅₀ values.

[0036] In some examples, the binding proteins that bind PD-L1 cross-react with cynomolgus monkey PD-L1 (cynoPD-L1) and do not demonstrate cross-reactive binding to mouse PD-L1 (mu-PD-L1).

[0037] In some examples, the binding proteins that bind PD-L1 disrupt the human PD-1/PD-L1 binding interaction.

[0038] In some examples, the binding proteins that bind PD-L1 dis-inhibits PD-1/PD-L1 checkpoint-mediated inhibition of T cells.

[0039] In some examples, the binding proteins that bind PD-L1 further comprise an Fc region that is engineered to abolish/minimize cross-linking activity with Fc γ Rs, which silence or eliminate Fc-mediated effector functions of T cells.

[0040] The present disclosure also provides isolated polynucleotide sequences encoding at least one of the above binding proteins that bind PD-L1.

[0041] The present disclosure also provides vectors comprising at least one of the above polynucleotide sequences.

[0042] The present disclosure also provides cells comprising one of the above polynucleotide sequences, or one of the above vectors.

[0043] The present disclosure also provides pharmaceutical compositions comprising or consisting of at least one of the binding proteins that bind PD-L1, and optionally a pharmaceutically acceptable diluent, carrier, vehicle and/or excipient. Such a pharmaceutical composition may be used for the treatment of cancer.

[0044] The present disclosure also relates to methods for treatment of cancer in a patient comprising administering to the patient a therapeutically effective amount of at least one of the disclosed binding proteins that bind PD-L1, alone or in combination with another therapeutic agent.

[0045] According to some examples, the binding proteins that bind CD137 comprise a set of six complementarity determining region (CDR) sequences selected from the group consisting of three CDRs of an anti-CD137 antibody heavy chain (HC) variable region sequence selected from SEQ ID NOS: 16, 18, and 20 and three CDRs of a light chain variable region sequence selected from SEQ ID NOS: 17, 19, and 21.

[0046] In some examples, the binding proteins that bind CD137 comprise a heavy chain variable region sequence comprising CDR1: SEQ ID NO: 5, CDR2: SEQ ID NO: 22, and CDR3:

SEQ ID NO: 23; and/or a light chain variable region sequence comprising CDR1: SEQ ID NO: 24, CDR2: SEQ ID NO: 25, and CDR3: SEQ ID NO: 26.

[0047] In some examples, the binding proteins that bind CD137 comprise a heavy chain variable region sequence comprising CDR1: SEQ ID NO: 27, CDR2: SEQ ID NO: 28, and CDR3: SEQ ID NO: 29; and/or a light chain variable region sequence comprising CDR1: SEQ ID NO: 30, CDR2: SEQ ID NO: 9, and CDR3: SEQ ID NO: 31.

[0048] In some examples, the binding proteins that bind CD137 comprise a heavy chain variable region sequence comprising CDR1: SEQ ID NO: 32, CDR2: SEQ ID NO: 33, and CDR3: SEQ ID NO: 34; and/or a light chain variable region sequence comprising CDR1: SEQ ID NO: 35, CDR2: SEQ ID NO: 36, and CDR3: SEQ ID NO: 37.

[0049] In some examples, the binding proteins that bind CD137 comprise a heavy chain variable region sequence as set forth in SEQ ID NOs: 16, 18, or 20, or an analogue or derivative thereof having at least 90% sequence identity to SEQ ID NOs: 16, 18, or 20.

[0050] In other examples, the binding proteins that bind CD137 comprise a light chain variable region sequence as set forth in SEQ ID NOs: 17, 19, or 21, or an analogue or derivative thereof having at least 90% sequence identity to SEQ ID NOs: 17, 19, or 21.

[0051] In other examples, the binding proteins that bind CD137 comprise a heavy chain variable region sequence as set forth in SEQ ID NOs: 16, 18 or 20 and a light chain variable region sequence as set forth in SEQ ID NOs: 17, 19 or 21.

[0052] In some examples, the binding proteins that bind comprise a heavy chain variable region sequence and a light chain variable region sequence, selected from the following combinations:

- (a) a heavy chain variable region sequence comprising SEQ ID NO: 16 and a light chain variable region sequence comprising SEQ ID NO: 17;
- (b) a heavy chain variable region sequence comprising SEQ ID NO: 18 and a light chain variable region sequence comprising SEQ ID NO: 19; and
- (c) a heavy chain variable region sequence comprising SEQ ID NO: 20 and a light chain variable region sequence comprising SEQ ID NO: 21.

[0053] In some examples, a binding protein that binds CD137 is provided, comprising:

(a) a heavy chain variable region sequence comprising CDR1: SEQ ID NO: 5, CDR2: SEQ ID NO: 22, and CDR3: SEQ ID NO: 23; and/or a light chain variable region sequence comprising CDR1: SEQ ID NO: 24, CDR2: SEQ ID NO: 25, and CDR3: SEQ ID NO: 26;

(b) a heavy chain variable region sequence comprising CDR1: SEQ ID NO: 27, CDR2: SEQ ID NO: 28, and CDR3: SEQ ID NO: 29; and/or a light chain variable region sequence comprising CDR1: SEQ ID NO: 30, CDR2: SEQ ID NO: 9, and CDR3: SEQ ID NO: 31;
or

(c) a heavy chain variable region sequence comprising CDR1: SEQ ID NO: 32, CDR2: SEQ ID NO: 33, and CDR3: SEQ ID NO: 34; and/or a light chain variable region sequence comprising CDR1: SEQ ID NO: 35, CDR2: SEQ ID NO: 36, and CDR3: SEQ ID NO: 37.

[0054] In some examples, a binding protein that binds CD137 is provided wherein the first and second antigen-binding sites bind CD137, and wherein the antibody scaffold module comprises:

(i) a heavy chain variable region sequence as set forth in SEQ ID NO: 16, a heavy chain constant region sequence as set forth in SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, or SEQ ID NO: 64, a light chain variable region sequence as set forth in SEQ ID NO: 17, and a light chain constant region sequence as set forth in SEQ ID NO: 65 or SEQ ID NO: 66;

(ii) a heavy chain variable region sequence as set forth in SEQ ID NO: 18, a heavy chain constant region sequence as set forth in SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, or SEQ ID NO: 64, a light chain variable region sequence as set forth in SEQ ID NO: 19, and a light chain constant region sequence as set forth in SEQ ID NO: 65 or SEQ ID NO: 66; or

(iii) a heavy chain variable region sequence as set forth in SEQ ID NO: 20, a heavy chain constant region sequence as set forth in SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, or SEQ ID NO: 64, a light chain variable region sequence as set forth in

SEQ ID NO: 21, and a light chain constant region sequence as set forth in SEQ ID NO: 65 or SEQ ID NO: 66.

[0055] In some examples, a binding protein that binds CD137 is provided wherein the first and second antigen-binding sites bind CD137, and wherein the antibody scaffold module comprises a heavy chain sequence as set forth in SEQ ID NO: 75 and a light chain sequence as set forth in SEQ ID NO: 76.

[0056] In some examples, the anti-CD137 antibodies comprise one or more heavy chain variable region CDRs disclosed in Table 3 and/or one or more light chain variable region CDRs disclosed in Table 4.

[0057] In some examples, the binding proteins that bind CD137 exhibit one or more of the following structural and functional characteristics, alone or in combination:

- (a) is specific for human CD137,
- (b) cross-reacts with cynomolgus CD137,
- (c) disrupts (e.g., reduces or prevents) human CD137L binding to CD137,
- (d) exhibits fast on and fast off properties to CD137;
- (e) possess crosslinking dependent agonistic activity for CD137 signaling, or
- (f) activates T cells in crosslinking dependent manner.

[0058] In some examples, the binding proteins that bind CD137 specifically bind to human cells expressing endogenous levels of CD137 and to host cells engineered to overexpress human CD137. In some examples, the binding proteins that bind CD137 bind to cells overexpressing human or cyno CD137 with EC₅₀ values ranging from 0.2 to 1.1 nM (e.g., 0.2 nM, 0.3 nM, 0.4 nM, 0.5 nM, 0.6 nM, 0.7 nM, 0.8 nM, 0.9 nM, 1.0 nM or 1.1 nM).

[0059] In some examples, the binding proteins that bind to CD137 have a fast on and fast off kinetic property.

[0060] In some examples, the binding proteins that bind CD137 cross-react with cynomolgus monkey CD137 (cynoCD137) and do not demonstrate cross-reactive binding to mouse CD137(mu-CD137).

[0061] In some examples, the binding proteins that bind CD137 disrupt the CD137 ligand/CD137 binding interaction.

[0062] In some examples, the binding proteins that bind CD137 possess crosslinking dependent agonistic activity for CD137 signaling.

[0063] In some examples, the binding proteins that bind CD137 activate T cells in crosslinking dependent manner.

[0064] In some examples, the binding proteins that bind CD137 further comprise an Fc region that is engineered to abolish/minimize cross-linking activity with Fc γ Rs, which silence or eliminate Fc-mediated effector functions of T cells.

[0065] The present disclosure also provides isolated polynucleotide sequences encoding at least one of the above binding proteins that bind CD137.

[0066] The present disclosure also provides vectors comprising at least one of the above polynucleotide sequences.

[0067] The present disclosure also provides cells comprising one of the above polynucleotide sequences, or one of the above vectors.

[0068] The present disclosure also provides pharmaceutical compositions comprising or consisting of at least one of the binding proteins that bind CD137, and optionally a pharmaceutically acceptable diluent, carrier, vehicle and/or excipient. Such a pharmaceutical composition may be used for the treatment of cancer.

[0069] The present disclosure also relates to methods for treatment of cancer in a patient comprising administering to the patient a therapeutically effective amount of at least one of the disclosed binding proteins that bind CD137, alone or in combination with another therapeutic agent.

[0070] In an example, a PD-L1/CD137 bispecific is a binding protein that binds PD-L1 and CD137 and comprises: (a) an antibody scaffold module in an IgG format comprising a first antigen-binding site that binds PD-L1 and a second antigen-binding site that binds PD-L1; (b) at least one first binding module comprising a third antigen-binding site that binds CD137.

[0071] In an example, the first antigen-binding site and the second antigen-binding site of the PD-L1/CD137 bispecific comprise a heavy chain variable region sequence comprising CDR1: SEQ ID NO: 5, CDR2: SEQ ID NO: 6, and CDR3: SEQ ID NO: 7; and a light chain variable region sequence comprising CDR1: SEQ ID NO: 8, CDR2: SEQ ID NO: 9, and CDR3: SEQ ID NO: 10. In an example, the first antigen-binding site and the second antigen-binding site of the PD-L1/CD137 bispecific comprise a heavy chain variable region sequence comprising CDR1: SEQ ID NO: 11, CDR2: SEQ ID NO: 12, and CDR3: SEQ ID NO: 13; and a light chain variable region sequence comprising CDR1: SEQ ID NO: 14, CDR2: SEQ ID NO: 9, and CDR3: SEQ ID NO: 15.

[0072] In an example, the antibody scaffold module of the PD-L1/CD137 bispecific comprises a heavy chain variable region sequence as set forth in SEQ ID NO: 1 or SEQ ID NO: 3; and a light chain variable region sequence as set forth in SEQ ID NO: 2 or SEQ ID NO: 4.

[0073] In an example, the antibody scaffold module of the PD-L1/CD137 bispecific comprises, a heavy chain variable region sequence as set forth in SEQ ID NO: 1 and a light chain variable region sequence as set forth in SEQ ID NO: 2. In an example, the antibody scaffold module of the PD-L1/CD137 bispecific comprises, a heavy chain variable region sequence as set forth in SEQ ID NO: 3 and a light chain variable region sequence as set forth in SEQ ID NO: 4.

[0074] In an example, the antibody scaffold module of the PD-L1/CD137 bispecific comprises, a heavy chain variable region sequence as set forth in SEQ ID NO: 1, a heavy chain constant region sequence as set forth in SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, or SEQ ID NO: 64, a light chain variable region sequence as set forth in SEQ ID NO: 2, and a light chain constant region sequence as set forth in SEQ ID NO: 65 or SEQ ID NO: 66. In an example, the antibody scaffold module of the PD-L1/CD137 bispecific comprises a heavy chain variable region sequence as set forth in SEQ ID NO: 3, a heavy chain constant region sequence as set forth in SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, or SEQ ID NO: 64, a light chain variable region sequence as set forth in SEQ ID NO: 4, and a light chain constant region sequence as set forth in SEQ ID NO: 65 or SEQ ID NO: 66.

[0075] In an example, the antibody scaffold module of the PD-L1/CD137 bispecific comprises a heavy chain sequence as set forth in SEQ ID NO: 42 and a light chain sequence as set

forth in SEQ ID NO: 40. In an example, the antibody scaffold module of the PD-L1/CD137 bispecific comprises a heavy chain sequence as set forth in SEQ ID NO: 45 and a light chain sequence as set forth in SEQ ID NO: 40.

[0076] In an example, the PD-L1/CD137 bispecific has one first binding module. In an example, the PD-L1/CD137 bispecific has two first binding modules. In an example, the PD-L1/CD137 bispecific has an antibody scaffold module which comprises a heavy chain sequence which comprises a C-terminus and a N-terminus, and wherein this antibody scaffold module comprises a light chain sequence which comprises a C-terminus and an N-terminus, and the first binding module is covalently attached to the C-terminus of the antibody scaffold module heavy chain sequence, the C-terminus of the antibody scaffold module light chain sequence, the N-terminus of the antibody scaffold module heavy chain sequence, the N-terminus of the antibody scaffold module light chain sequence, or combinations thereof, optionally wherein the first binding module and the antibody scaffold module are covalently attached to each other directly or through an interlinker. In an example, the first binding module and the antibody scaffold module of the PD-L1/CD137 bispecific are covalently attached to each other through an interlinker, and the interlinker is the sequence, from N- to C-terminus, as set forth in SEQ ID NO: 58. In an example, the first binding module and the antibody scaffold module of the PD-L1/CD137 bispecific are covalently attached to each other through an interlinker, and the interlinker is the sequence, from N- to C-terminus, as set forth in SEQ ID NO: 59. In an example, the first binding module of the PD-L1/CD137 bispecific is covalently attached to the C-terminus of the antibody scaffold module heavy chain sequence. In an example, the first binding module of the PD-L1/CD137 bispecific is covalently attached to the C-terminus of the antibody scaffold module light chain sequence. In an example, when there is more than one first binding module in the PD-L1/CD137 bispecific, each is covalently attached to a different antibody scaffold module sequence or to a different end of the antibody scaffold module.

[0077] In an example, the first binding module in the PD-L1/CD137 bispecific is a scFv, which comprises a heavy chain variable region sequence and a light chain variable sequence, wherein the sequences are covalently attached to each other directly or through a scFv fusion linker. In an example, the scFv fusion linker comprises glycine and serine. In an example, the scFv fusion linker comprises the sequence Gly-Gly-Gly-Ser. In an

example, the scFv fusion linker comprises the sequence set forth in SEQ ID NO: 58. In an example, the scFv fusion linker is the sequence set forth in SEQ ID NO: 58. In an example, the scFv fusion linker comprises the sequence set forth in SEQ ID NO: 59. In an example, the scFv fusion linker is the sequence set forth in SEQ ID NO: 59. In an example, the first binding module in the PD-L1/CD137 bispecific comprises a sequence as set forth in SEQ ID NO: 53. In an example, the first binding module in the PD-L1/CD137 bispecific comprises a sequence, as set forth in SEQ ID NO: 54. In an example, the first binding module in the PD-L1/CD137 bispecific comprises a sequence as set forth in SEQ ID NO: 55. In an example, the first binding module in the PD-L1/CD137 bispecific comprises a sequence as set forth in SEQ ID NO: 56.

[0078] In an example, the first binding module in the PD-L1/CD137 bispecific comprises, from N- to C-terminus: a heavy chain variable region sequence comprising CDR1: SEQ ID NO: 5, CDR2: SEQ ID NO: 22, and CDR3: SEQ ID NO: 23; and a light chain variable region sequence comprising CDR1: SEQ ID NO: 24, CDR2: SEQ ID NO: 25, and CDR3: SEQ ID NO: 26. In an example, the first binding module in the PD-L1/CD137 bispecific comprises, from N- to C-terminus: a heavy chain variable region sequence comprising CDR1: SEQ ID NO: 27, CDR2: SEQ ID NO: 28, and CDR3: SEQ ID NO: 29; and a light chain variable region sequence comprising CDR1: SEQ ID NO: 30, CDR2: SEQ ID NO: 9, and CDR3: SEQ ID NO: 31. In an example, the first binding module in the PD-L1/CD137 bispecific comprises, from N- to C-terminus: a heavy chain variable region sequence comprising CDR1: SEQ ID NO: 32, CDR2: SEQ ID NO: 33, and CDR3: SEQ ID NO: 34; and a light chain variable region sequence comprising CDR1: SEQ ID NO: 35, CDR2: SEQ ID NO: 36, and CDR3: SEQ ID NO: 37.

[0079] In an example, the first binding module in the PD-L1/CD137 bispecific comprises, from N- to C-terminus: a heavy chain variable region sequence as set forth in SEQ ID NO: 16 and a light chain variable region sequence as set forth in SEQ ID NO: 17. In an example, the first binding module in the PD-L1/CD137 bispecific comprises, from N- to C-terminus: a heavy chain variable region sequence as set forth in SEQ ID NO: 18 and a light chain variable region sequence as set forth in SEQ ID NO: 19. In an example, the first binding module in the PD-L1/CD137 bispecific comprises, from N- to C-terminus: a heavy chain

variable region sequence as set forth in SEQ ID NO: 20 and a light chain variable region sequence as set forth in SEQ ID NO: 21.

[0080] In an example, the PD-L1/CD137 bispecific comprises, from N- to C-terminus: the heavy chain sequence of the antibody scaffold module and the first binding module as set forth in SEQ ID NO: 38; and the light chain sequence of the antibody scaffold module as set forth in SEQ ID NO: 40. In an example, the PD-L1/CD137 bispecific comprises, from N- to C-terminus: the heavy chain sequence of the antibody scaffold module and the first binding module as set forth in SEQ ID NO: 44; and the light chain sequence of the antibody scaffold module as set forth in SEQ ID NO: 40. In an example, the PD-L1/CD137 bispecific comprises, from N- to C-terminus: the heavy chain sequence of the antibody scaffold module and the first binding module as set forth in SEQ ID NO: 45; and the light chain sequence of the antibody scaffold module and the first binding module as set forth in SEQ ID NO: 46. In an example, the PD-L1/CD137 bispecific comprises, from N- to C-terminus: the heavy chain sequence of the antibody scaffold module and the first binding module as set forth in SEQ ID NO: 47; and the light chain sequence of the antibody scaffold module as set forth in SEQ ID NO: 40. In an example, the PD-L1/CD137 bispecific comprises, from N- to C-terminus: the heavy chain sequence of the antibody scaffold module and the first binding module as set forth in SEQ ID NO: 50; and the light chain sequence of the antibody scaffold module as set forth in SEQ ID NO: 40.

[0081] In an example, the antibody scaffold module of the PD-L1/CD137 bispecific further comprises a constant region. In an example, the constant region of the antibody scaffold module of the PD-L1/CD137 bispecific comprises at least one Fc silencing mutation. In an example, the Fc silencing mutation in the constant region of the antibody scaffold module of the PD-L1/CD137 bispecific is L234A/L235A or N297A. In an example, the constant region of the antibody scaffold module of the PD-L1/CD137 bispecific comprises knobs-in-holes (KiH) mutations.

[0082] According to some examples, the PD-L1/CD137 bispecifics (e.g., 1923Ab8, 1923Ab11, 1923Ab12, 1923Ab13, and 1923Ab18) are capable of effectively blocking the interactions between PD-L1 and its receptor PD-1 and between CD137 and its ligand. The disclosed PD-L1/CD137 bispecifics comprise amino acid sequences derived from one of the binding proteins that bind PD-L1 disclosed herein (e.g., 1923Ab2 or 1923Ab3) as a PD-L1

antibody scaffold module and amino acid sequences derived from one of the binding proteins that bind CD137 (e.g., 1923Ab4, 1923Ab5 or 1923Ab6) disclosed herein as a CD137 first binding module.

[0083] In some examples, the PD-L1/CD137 bispecific comprises a binding protein that binds PD-L1 and that disrupts the PD-1/PD-L1 binding interaction and dis-inhibits PD-1/PD-L1 checkpoint-mediated inhibition of T cells. As exemplified herein, in non-limiting examples, the antibody scaffold module that binds PD-L1 may comprise a single-chain variable fragment (e.g., a fusion protein of the variable regions of the heavy (VH) and light (VL) chains of one of the disclosed binding proteins that bind PD-L1, connected with a linker peptide) (scFv).

[0084] In some examples, the PD-L1/CD137 bispecific comprises a CD137-binding module comprised of a fragment derived from one of the disclosed binding proteins that bind CD137. In one example, the CD137 binding module may be in the form of a binding fragment derived from one of the binding proteins that bind CD137 disclosed herein. As exemplified herein, in non-limiting examples, the CD137 binding module may comprise a single-chain variable fragment (e.g., a fusion protein of the variable regions of the heavy (VH) and light (VL) chains of one of the disclosed binding proteins that bind CD137, connected with a linker peptide). Alternatively, the CD137 binding module may be in the form of an IgG molecule.

[0085] In some examples, the PD-L1/CD137 bispecific 1923Ab8 comprises an antibody scaffold module having two Fabs from 1923Ab3 that bind PD-L1, a human IgG1 Fc comprising two Fc constant chains with L234A L235A mutations (SEQ ID NO: 61), and an scFv fragment (VH precedes VL) (SEQ ID NO: 53) derived from 1923Ab4 attached to the C-terminus of each of the two Fc constant chains.

[0086] In some examples, the PD-L1/CD137 bispecific 1923Ab11 comprises an antibody scaffold module having two Fabs from 1923Ab3 that bind PD-L1, a human IgG1 Fc comprising two Fc constant chains with L234A L235A mutations (SEQ ID NO: 61), and scFv fragments (VL precedes VH) (SEQ ID NO: 54), derived from 1923Ab4 attached to the C-terminus of each of the two Fc constant chains.

[0087] In some examples, the PD-L1/CD137 bispecific 1923Ab12 comprises an antibody scaffold module having two Fabs from 1923Ab3 that bind PD-L1, a human IgG1 Fc comprising two constant chains with L234A L235A mutations (SEQ ID NO: 61), and disulfide bond-

stabilized scFv fragments (VH precedes VL) (SEQ ID NO: 56), derived from 1923Ab4 attached to the N-terminus of each of light chain in the Fabs.

[0088] In some examples, the PD-L1/CD137 bispecific 1923Ab13 comprises an antibody scaffold module having two Fabs from 1923Ab3 that bind PD-L1, a human IgG1 Fc comprising two constant chains with L234A L235A mutations (SEQ ID NO: 61), and disulfide bond-stabilized scFv fragments (VH precedes VL) (SEQ ID NO: 56), derived from 1923Ab4 attached to the N-terminus of each of the heavy chains in the Fabs.

[0089] In some examples, the PD-L1/CD137 bispecific 1923Ab18 comprises an antibody scaffold module having two Fabs from 1923Ab3 that bind PD-L1, a human IgG1 Fc comprising two Fc constant chains with L234A L235A mutations (SEQ ID NO: 61), and disulfide bond-stabilized scFv fragments (VH precedes VL) (SEQ ID NO: 55) derived from 1923Ab4 attached to the C-terminus of each of the Fc constant chains.

[0090] In some examples, the PD-L1/CD137 bispecific comprise a variable heavy chain sequence and a variable light chain sequence, selected from the following combinations:

- (a) a heavy chain sequence comprising SEQ ID NO: 38 and a light chain sequence comprising SEQ ID NO: 40;
- (b) a heavy chain sequence comprising SEQ ID NO: 44 and a light chain sequence comprising SEQ ID NO: 40;
- (c) a heavy chain sequence comprising SEQ ID NO: 45 and a light chain sequence comprising SEQ ID NO: 46;
- (d) a heavy chain sequence comprising SEQ ID NO: 47 and a light chain sequence comprising SEQ ID NO: 40; and
- (e) a heavy chain sequence comprising SEQ ID NO: 50 and a light chain sequence comprising SEQ ID NO: 40.

[0091] In some examples, the PD-L1/CD137 bispecific comprises a heavy chain sequence selected from the group consisting of SEQ ID NOs: 38, 42, 44, 45, 47, and 50 or a fragment thereof having at least 90% sequence identity with SEQ ID NOs: 38, 42, 44, 45, 47, or 50.

[0092] In other examples, the PD-L1/CD137 bispecific comprises a light chain sequence selected from the group consisting of SEQ ID NOs: 40 and 46 or a fragment derivative thereof having at least 90% sequence identity SEQ ID NOs: 40 or 46.

[0093] In some examples, the CD137 binding module is a scFv subunit that blocks the CD137/CD137 ligand interaction and possesses crosslinking dependent agonistic activity for CD137 signaling and T cell activation. In some examples, the CD137 binding module is a scFv subunit stabilized with a disulfide bond.

[0094] In some examples, the PD-L1/CD137 bispecific comprises an antibody scaffold module having an IgG format in which a CD137 scFv binding module is fused to the C-terminus of the heavy chain or the light chain of the antibody scaffold module to create a PD-L1/CD137 bispecific.

[0095] In some examples, the disclosed PD-L1/CD137 bispecific further comprises a Fc region that is engineered to abolish/minimize cross-linking activity with FcγRs, which silence or eliminate Fc-mediated effector functions of T cells.

[0096] The present disclosure also provides pharmaceutical compositions comprising or consisting of at least one of the PD-L1/CD137 bispecifics disclosed herein, and optionally a pharmaceutically acceptable diluent, carrier, vehicle and/or excipient. Such a pharmaceutical composition may be used for the treatment of cancer.

[0097] The present disclosure also relates to methods for treatment of cancer in a patient comprising administering to the patient a therapeutically effective amount of at least one of the disclosed PD-L1/CD137 bispecifics, alone or in combination with another therapeutic agent.

[0098] The present disclosure also provides isolated polynucleotide sequences encoding at least one of the PD-L1/CD137 bispecifics described herein. The present disclosure also provides isolated polynucleotide sequences encoding at least one of the PD-L1/CD137 bispecific sequences described herein.

[0099] The present disclosure also provides vectors comprising a polynucleotide of a PD-L1/CD137 bispecific described herein.

[0100] The present disclosure also provides vectors comprising at least one of the PD-L1/CD137 bispecific polynucleotide sequences described herein.

[0101] The present disclosure also provides cells comprising one of the PD-L1/CD137 bispecific polynucleotide sequences described herein, or one of the above vectors.

[0102] In an example, a PD-L1/TGF β bispecific is a binding protein that binds PD-L1 and TGF β and comprises: (a) an antibody scaffold module in an IgG format comprising a first antigen-binding site that binds PD-L1 and a second antigen-binding site that binds PD-L1; (b) at least one first binding module comprising a third antigen-binding site that binds TGF β .

[0103] In an example, the first antigen-binding site and the second antigen-binding site of the PD-L1/TGF β bispecific comprise a heavy chain variable region sequence comprising CDR1: SEQ ID NO: 5, CDR2: SEQ ID NO: 6, and CDR3: SEQ ID NO: 7; and a light chain variable region sequence comprising CDR1: SEQ ID NO: 8, CDR2: SEQ ID NO: 9, and CDR3: SEQ ID NO: 10. In an example, the first antigen-binding site and the second antigen-binding site of the PD-L1/TGF β bispecific comprise a heavy chain variable region sequence comprising CDR1: SEQ ID NO: 11, CDR2: SEQ ID NO: 12, and CDR3: SEQ ID NO: 13; and a light chain variable region sequence comprising CDR1: SEQ ID NO: 14, CDR2: SEQ ID NO: 9, and CDR3: SEQ ID NO: 15.

[0104] In an example, the antibody scaffold module of the PD-L1/TGF β bispecific comprises a heavy chain variable region sequence as set forth in SEQ ID NO: 1 or SEQ ID NO: 3; and a light chain variable region sequence as set forth in SEQ ID NO: 2 or SEQ ID NO: 4.

[0105] In an example, the antibody scaffold module of the PD-L1/TGF β bispecific comprises a heavy chain variable region sequence as set forth in SEQ ID NO: 1 and a light chain variable region sequence as set forth in SEQ ID NO: 2. In an example, the antibody scaffold module of the PD-L1/TGF β bispecific comprises a heavy chain variable region sequence as set forth in SEQ ID NO: 3 and a light chain variable region sequence as set forth in SEQ ID NO: 4.

[0106] In an example, the antibody scaffold module of the PD-L1/TGF β bispecific comprises a heavy chain variable region sequence as set forth in SEQ ID NO: 1, a heavy chain constant region sequence as set forth in SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, or SEQ ID NO: 64, a light chain variable region sequence as set forth in SEQ ID NO: 2, and a light chain constant region sequence as set forth in SEQ ID NO: 65 or SEQ ID NO: 66. In an example, the antibody scaffold module of the PD-L1/TGF β bispecific comprises

a heavy chain variable region sequence as set forth in SEQ ID NO: 3, a heavy chain constant region sequence as set forth in SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, or SEQ ID NO: 64, a light chain variable region sequence as set forth in SEQ ID NO: 4, and a light chain constant region sequence as set forth in SEQ ID NO: 65 or SEQ ID NO: 66.

[0107] In an example, the antibody scaffold module of the PD-L1/TGF β bispecific comprises a heavy chain sequence as set forth in SEQ ID NO: 42 and a light chain sequence as set forth in SEQ ID NO: 40. In an example, the antibody scaffold module of the PD-L1/TGF β bispecific comprises a heavy chain sequence as set forth in SEQ ID NO: 45 and a light chain sequence as set forth in SEQ ID NO: 40.

[0108] In an example, the PD-L1/TGF β bispecific has one first binding module. In an example, the PD-L1/TGF β bispecific has two first binding modules. In an example, the PD-L1/TGF β bispecific has an antibody scaffold module which comprises a heavy chain sequence which comprises a C-terminus and a N-terminus, and wherein this antibody scaffold module comprises a light chain sequence which comprises a C-terminus and a N-terminus, and the first binding module is covalently attached to the C-terminus of the antibody scaffold module heavy chain sequence, the C-terminus of the antibody scaffold module light chain sequence, the N-terminus of the antibody scaffold module heavy chain sequence, the N-terminus of the antibody scaffold module light chain sequence, or combinations thereof, optionally wherein the first binding module and the antibody scaffold module are covalently attached to each other directly or through an interlinker. In an example, the first binding module and the antibody scaffold module of the PD-L1/TGF β bispecific are covalently attached to each other through an interlinker, and the interlinker is the sequence, from N- to C-terminus, as set forth in SEQ ID NO: 58. In an example, the first binding module and the antibody scaffold module of the PD-L1/TGF β bispecific are covalently attached to each other through an interlinker, and the interlinker is the sequence, from N- to C-terminus, as set forth in SEQ ID NO: 59. In an example, the first binding module of the PD-L1/TGF β bispecific is covalently attached to the C-terminus of the antibody scaffold module heavy chain sequence. In an example, the first binding module of the PD-L1/TGF β bispecific is covalently attached to the C-terminus of the antibody scaffold module light chain sequence. In an example, when there is more than one first binding module in the PD-L1/TGF β bispecific, each is covalently

attached to a different antibody scaffold module sequence or to a different end of the antibody scaffold module.

[0109] In an example, the first binding module in the PD-L1/TGF β bispecific comprises the extracellular domain of TGF β RII. In an example, the extracellular domain of TGF β RII sequence is set forth in SEQ ID NO: 67.

[0110] In an example, the PD-L1/TGF β bispecific has two first binding modules. In an example, the heavy chain sequence of the antibody scaffold module and the first binding module of the PD-L1/TGF β bispecific comprise a sequence set forth in SEQ ID NO: 51; and wherein the light chain sequence of the antibody scaffold module of the PD-L1/TGF β bispecific comprises a sequence, from N- to C-terminus, as set forth in SEQ ID NO: 40.

[0111] In an example, the antibody scaffold module of the PD-L1/TGF β bispecific further comprises a constant region. In an example, the constant region of the antibody scaffold module of the PD-L1/TGF β bispecific comprises at least one Fc silencing mutation. In an example, the Fc silencing mutation in the constant region of the antibody scaffold module of the PD-L1/TGF β bispecific is L234A L235A or N297A. In an example, the constant region of the antibody scaffold module of the PD-L1/TGF β bispecific comprises knobs-in-holes (KiH) mutations.

[0112] According to some examples, the present disclosure provides PD-L1/TGF β bispecifics (e.g., 1923Ab20) capable of effectively blocking the interactions between PD-L1 and its receptor PD-1 and sequesters TGF β . The disclosed PD-L1/TGF β bispecifics comprise all or a part of the amino acid sequences derived from one of the binding proteins that bind PD-L1 disclosed herein (e.g., 1923Ab2 or 1923Ab3) as a PD-L1 antibody scaffold module and an amino acid sequence comprising the full length extracellular domain of TGF β RII or truncations of TGF β RII (e.g., N-terminal or C-terminal truncations) provided that the sequence can bind and neutralize the biological activities of TGF β . In some examples the first binding module appended to the binding protein that binds PD-L1 is a recombinant TGF β binding protein derived from the ECD of the human TNF β RII receptor.

[0113] In some examples, the PD-L1/TGF β bispecific 1923Ab20 comprises an antibody scaffold module having two Fabs from 1923Ab3 that bind PD-L1, a human IgG1 Fc having two Fc constant chains with L234A L235A mutations (SEQ ID NO: 61), and two polypeptides

encoding the extracellular domain of TGF β RII (SEQ ID NO: 67) each attached to the C-terminus of each of the Fc constant chains.

[0114] In some examples, the PD-L1/TGF β bispecific comprises a heavy and/or light chain sequence disclosed in Table 5. In other examples, the PD-L1/TGF β bispecific comprises a heavy and/or light chain sequence disclosed in Table 6.

[0115] In some examples, the PD-L1/TGF β bispecific comprises heavy chain sequence comprising SEQ ID NO: 51 and a light chain sequence comprising SEQ ID NO: 40.

[0116] In some examples, the PD-L1/TGF β bispecific comprises a heavy chain sequence selected from the group consisting of SEQ ID NOs: 42, 45, and 51 or a fragment thereof having at least 90% sequence identity to SEQ ID NOs: 42, 45, or 51.

[0117] In other examples, the PD-L1/TGF β bispecific comprises a light chain sequence selected from the group consisting of SEQ ID NOs: 39 and 40 or a fragment derivative thereof having at least 90% sequence identity to SEQ ID NOs: 39 and 40.

[0118] In some examples, the PD-L1/TGF β bispecifics exhibit one or more of the following characteristics, alone or in combination:

- (a) is specific for human PD-L1 and binds human TGF β ;
- (b) cross-reacts with cynomolgus PD-L1;
- (c) disrupts interaction of PD-1 and PD-L1;
- (d) dis-inhibits T cell PD-L1 mediated check-point inhibitory signal; or
- (e) sequesters human TGF β ;

[0119] The present disclosure also provides pharmaceutical compositions comprising or consisting of at least one of the PD-L1/TGF β bispecifics disclosed herein, and optionally a pharmaceutically acceptable diluent, carrier, vehicle and/or excipient. Such a pharmaceutical composition may be used for the antibody-based immunotherapy of cancer.

[0120] The present disclosure also relates to methods for treatment of cancer in a patient comprising administering to the patient a therapeutically effective amount of at least one of the disclosed PD-L1/TGF β bispecifics, alone or in combination with another therapeutic agent.

[0121] The present disclosure also provides isolated polynucleotide sequences encoding at least one of the PD-L1/TGF β bispecifics described herein. The present disclosure also provides isolated polynucleotide sequences encoding at least one of the PD-L1/TGF β bispecific sequences described herein.

[0122] The present disclosure also provides vectors comprising a polynucleotide of a PD-L1/TGF β bispecific described herein.

[0123] The present disclosure also provides vectors comprising at least one of the PD-L1/TGF β bispecific polynucleotide sequences described herein.

[0124] The present disclosure also provides cells comprising one of the PD-L1/TGF β bispecific polynucleotide sequences described herein, or one of the above vectors.

[0125] The present disclosure also provides a binding protein that binds PD-L1, TGF β , and CD137, comprising: (a) an antibody scaffold module in an IgG format comprising a first antigen-binding site that binds PD-L1 and a second antigen-binding site that binds PD-L1; (b) at least one first binding module comprising a third antigen-binding site that binds TGF β ; and (c) at least one second binding module comprising a fourth antigen-binding site that binds CD137. In an example, the PD-L1/TGF β /CD137 trispecific is constructed in the form of a recombinant protein comprising an antibody scaffold module that binds PD-L1, a first binding module that comprises an amino acid sequence derived from the TGF β receptor II binding protein that is capable of binding to human TGF β and neutralizing its activity, and a second binding module that binds CD137.

[0126] In an example, the first antigen-binding site and the second antigen-binding site of the PD-L1/TGF β /CD137 trispecific comprise (i) a heavy chain variable region sequence comprising CDR1: SEQ ID NO: 5, CDR2: SEQ ID NO: 6, and CDR3: SEQ ID NO: 7; and a light chain variable region sequence comprising CDR1: SEQ ID NO: 8, CDR2: SEQ ID NO: 9, and CDR3: SEQ ID NO: 10. In an example, the first antigen-binding site and the second antigen-binding site of the PD-L1/TGF β /CD137 trispecific comprise a heavy chain variable region sequence comprising CDR1: SEQ ID NO: 11, CDR2: SEQ ID NO: 12, and CDR3: SEQ ID NO: 13; and a light chain variable region sequence comprising CDR1: SEQ ID NO: 14, CDR2: SEQ ID NO: 9, and CDR3: SEQ ID NO: 15.

[0127] In an example, the antibody scaffold module of the PD-L1/TGFβ/CD137 trispecific comprises a heavy chain variable region sequence as set forth in SEQ ID NO: 1 or SEQ ID NO: 3; and a light chain variable region sequence as set forth in SEQ ID NO: 2 or SEQ ID NO: 4.

[0128] In an example, the antibody scaffold module of the PD-L1/TGFβ/CD137 trispecific comprises a heavy chain variable region sequence as set forth in SEQ ID NO: 1 and a light chain variable region sequence as set forth in SEQ ID NO: 2. In an example, the antibody scaffold module of the PD-L1/TGFβ/CD137 trispecific comprises a heavy chain variable region sequence as set forth in SEQ ID NO: 3 and a light chain variable region sequence as set forth in SEQ ID NO: 4.

[0129] In an example, the antibody scaffold module of the PD-L1/TGFβ/CD137 trispecific comprises a heavy chain variable region sequence as set forth in SEQ ID NO: 1, a heavy chain constant region sequence as set forth in SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, or SEQ ID NO: 64, a light chain variable region sequence as set forth in SEQ ID NO: 2, and a light chain constant region sequence as set forth in SEQ ID NO: 65 or SEQ ID NO: 66. In an example, the antibody scaffold module of the PD-L1/TGFβ/CD137 trispecific comprises a heavy chain variable region sequence as set forth in SEQ ID NO: 3, a heavy chain constant region sequence as set forth in SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, or SEQ ID NO: 64, a light chain variable region sequence as set forth in SEQ ID NO: 4, and a light chain constant region sequence as set forth in SEQ ID NO: 65 or SEQ ID NO: 66.

[0130] In an example, the antibody scaffold module of the PD-L1/TGFβ/CD137 trispecific comprises a heavy chain sequence as set forth in SEQ ID NO: 42 and a light chain sequence as set forth in SEQ ID NO: 40. In an example, the antibody scaffold module of the PD-L1/TGFβ/CD137 trispecific comprises a heavy chain sequence as set forth in SEQ ID NO: 45 and a light chain sequence as set forth in SEQ ID NO: 40.

[0131] In an example, the PD-L1/TGFβ/CD137 trispecific has one first binding module. In an example, the PD-L1/TGFβ/CD137 trispecific has two first binding modules. In an example, the PD-L1/TGFβ/CD137 trispecific has an antibody scaffold module which comprises a heavy chain sequence which comprises a C-terminus and an

N-terminus, and wherein this antibody scaffold module comprises a light chain sequence which comprises a C-terminus and an N-terminus, and the first binding module is covalently attached to the C-terminus of the antibody scaffold module heavy chain sequence, the C-terminus of the antibody scaffold module light chain sequence, the N-terminus of the antibody scaffold module heavy chain sequence, the N-terminus of the antibody scaffold module light chain sequence, or combinations thereof, optionally wherein the first binding module and the antibody scaffold module are covalently attached to each other directly or through a first binding module interlinker. In an example, the first binding module and the antibody scaffold module of the PD-L1/TGF β /CD137 trispecific are covalently attached to each other through a first binding module interlinker, and the first binding module interlinker is the sequence, from N- to C-terminus, as set forth in SEQ ID NO: 58. In an example, the first binding module and the antibody scaffold module of the PD-L1/TGF β /CD137 trispecific are covalently attached to each other through a first binding module interlinker, and the first binding module interlinker is the sequence, from N- to C-terminus, as set forth in SEQ ID NO: 59. In an example, the first binding module of the PD-L1/TGF β /CD137 trispecific is covalently attached to the C-terminus of the antibody scaffold module heavy chain sequence. In an example, the first binding module of the PD-L1/TGF β /CD137 trispecific is covalently attached to the C-terminus of the antibody scaffold module light chain sequence. In an example, when there is more than one first binding module in the PD-L1/TGF β /CD137 trispecific, each is covalently attached to a different antibody scaffold module sequence or to a different end of the antibody scaffold module.

[0132] In an example, the first binding module in the PD-L1/TGF β /CD137 trispecific comprises the extracellular domain of TGF β RII. In an example, the extracellular domain of TGF β RII in the PD-L1/TGF β /CD137 trispecific comprises the sequence set forth in SEQ ID NO: 67.

[0133] In an example, the second binding module in the PD-L1/TGF β /CD137 trispecific is a scFv, which comprises a heavy chain variable region sequence and a light chain variable sequence, wherein the sequences are covalently attached to each other directly or through a scFv fusion linker. In an example, the scFv fusion linker comprises glycine and serine. In an example, the scFv fusion linker comprises the sequence Gly-Gly-Gly-Ser. In an example, the scFv fusion linker comprises the sequence set forth in SEQ

ID NO: 58. In an example, the scFv fusion linker is the sequence set forth in SEQ ID NO: 58. In an example, the scFv fusion linker comprises the sequence set forth in SEQ ID NO: 59. In an example, the scFv fusion linker is the sequence set forth in SEQ ID NO: 59. In an example, the second binding module in the PD-L1/TGF β /CD137 trispecific comprises a sequence as set forth in SEQ ID NO: 53. In an example, the second binding module in the PD-L1/TGF β /CD137 trispecific comprises a sequence as set forth in SEQ ID NO: 54. In an example, the second binding module in the PD-L1/TGF β /CD137 trispecific comprises a sequence as set forth in SEQ ID NO: 55. In an example, the second binding module in the PD-L1/TGF β /CD137 trispecific comprises a sequence as set forth in SEQ ID NO: 56.

[0134] In an example, the PD-L1/TGF β /CD137 trispecific has one second binding module. In an example, the PD-L1/TGF β /CD137 trispecific has two second binding modules. In an example, the PD-L1/TGF β /CD137 trispecific has an antibody scaffold module which comprises a heavy chain sequence which comprises a C-terminus and a N-terminus, and wherein the antibody scaffold module comprises a light chain sequence which comprises a C-terminus and a N-terminus, and the second binding module is covalently attached to the C-terminus of the antibody scaffold module heavy chain sequence, the C-terminus of the antibody scaffold module light chain sequence, the N-terminus of the antibody scaffold module heavy chain sequence, the N-terminus of the antibody scaffold module light chain sequence, or combinations thereof, optionally wherein the second binding module and the antibody scaffold module are covalently attached to each other directly or through a second binding module interlinker.

[0135] In an example, the second binding module and the antibody scaffold module of the PD-L1/TGF β /CD137 trispecific are covalently attached to each other through a second binding module interlinker, and the second binding module interlinker is set forth in SEQ ID NO: 58. In an example, the second binding module and the antibody scaffold module of the PD-L1/TGF β /CD137 trispecific are covalently attached to each other through a

second binding module interlinker, and the second binding module interlinker is set forth in SEQ ID NO: 59.

[0136] In an example, the second binding module of the PD-L1/TGF β /CD137 trispecific is covalently attached to the C-terminus of the antibody scaffold module heavy chain sequence. In an example, the first binding module of the PD-L1/TGF β /CD137 trispecific is covalently attached to the C-terminus of the antibody scaffold module light chain sequence. In an example, the second binding module of the PD-L1/TGF β /CD137 trispecific is covalently attached to the C-terminus of the antibody scaffold module heavy chain sequence, and the first binding module of the PD-L1/TGF β /CD137 trispecific is covalently attached to the C-terminus of the antibody scaffold module light chain sequence.

[0137] In an example, the second binding module of the PD-L1/TGF β /CD137 trispecific is covalently attached to the C-terminus of the antibody scaffold module light chain sequence. In an example, the first binding module of the PD-L1/TGF β /CD137 trispecific is covalently attached to the C-terminus of the antibody scaffold module heavy chain sequence. In an example, the second binding module of the PD-L1/TGF β /CD137 trispecific is covalently attached to the C-terminus of the antibody scaffold module light chain sequence, and the first binding module of the PD-L1/TGF β /CD137 trispecific is covalently attached to the C-terminus of the antibody scaffold module heavy chain sequence.

[0138] In an example, when there is more than one second binding module in the PD-L1/TGF β /CD137 trispecific, each is covalently attached to a different antibody scaffold module sequence or to a different end of the antibody scaffold module (e.g., one binding module may be attached to the C-terminus of the heavy chain in the antibody scaffold module and the other binding module may be attached to the N-terminus of the same heavy chain). In an example, the PD-L1/TGF β /CD137 trispecific has one second binding module, and wherein the one second binding module is covalently attached to the C-terminus of the antibody scaffold module heavy chain sequence. In an example, the PD-L1/TGF β /CD137 trispecific has one second binding module, and wherein the one second binding module is covalently attached to the C-terminus of the antibody scaffold module light chain sequence. In an example, the PD-L1/TGF β /CD137 trispecific has two second binding modules, and wherein one second binding module is covalently attached to the C-terminus of the antibody scaffold

module heavy chain sequence, and the other second binding module is covalently attached to the C-terminus of the antibody scaffold module light chain sequence. In an example, the second binding module of the PD-L1/TGFβ/CD137 trispecific is a scFv.

[0139] In an example, the second binding module of the PD-L1/TGFβ/CD137 trispecific comprises a heavy chain variable region sequence comprising CDR1: SEQ ID NO: 5, CDR2: SEQ ID NO: 22, and CDR3: SEQ ID NO: 23; and a light chain variable region sequence comprising CDR1: SEQ ID NO: 24, CDR2: SEQ ID NO: 25, and CDR3: SEQ ID NO: 26. In an example, the second binding module of the PD-L1/TGFβ/CD137 trispecific comprises, from a heavy chain variable region sequence comprising CDR1: SEQ ID NO: 27, CDR2: SEQ ID NO: 28, and CDR3: SEQ ID NO: 29; and a light chain variable region sequence comprising CDR1: SEQ ID NO: 30, CDR2: SEQ ID NO: 9, and CDR3: SEQ ID NO: 31. In an example, the second binding module of the PD-L1/TGFβ/CD137 trispecific comprises a heavy chain variable region sequence comprising CDR1: SEQ ID NO: 32, CDR2: SEQ ID NO: 33, and CDR3: SEQ ID NO: 34; and a light chain variable region sequence comprising CDR1: SEQ ID NO: 35, CDR2: SEQ ID NO: 36, and CDR3: SEQ ID NO: 37. In an example, the second binding module of the PD-L1/TGFβ/CD137 trispecific comprises a heavy chain variable region sequence as set forth in SEQ ID NO: 16 and a light chain variable region sequence as set forth in SEQ ID NO: 17. In an example, the second binding module of the PD-L1/TGFβ/CD137 trispecific comprises a heavy chain variable region sequence as set forth in SEQ ID NO: 18 and a light chain variable region sequence as set forth in SEQ ID NO: 19. In an example, the second binding module of the PD-L1/TGFβ/CD137 trispecific comprises a heavy chain variable region sequence as set forth in SEQ ID NO: 20 and a light chain variable region sequence as set forth in SEQ ID NO: 21.

[0140] In an example, the second binding module of the PD-L1/TGFβ/CD137 trispecific comprises a sequence as set forth in SEQ ID NO: 53. In an example, the second binding module of the PD-L1/TGFβ/CD137 trispecific comprises a sequence as set forth in SEQ ID NO: 54. In an example, the second binding module of the PD-L1/TGFβ/CD137 trispecific comprises a sequence as set forth in SEQ ID NO: 55. In an

example, the second binding module of the PD-L1/TGF β /CD137 trispecific comprises a sequence as set forth in SEQ ID NO: 56.

[0141] In an example, the PD-L1/TGF β /CD137 trispecific has two first binding modules and two second binding modules. In an example, the PD-L1/TGF β /CD137 trispecific comprises the heavy chain sequence of the antibody scaffold module and the second binding module as set forth in SEQ ID NO: 38; and the light chain sequence of the antibody scaffold module and the first binding module as set forth in SEQ ID NO: 39. In an example, the PD-L1/TGF β /CD137 trispecific comprises the heavy chain sequence of the antibody scaffold module and the second binding module as set forth in SEQ ID NO: 50; and the light chain sequence of the antibody scaffold module and the first binding module as set forth in SEQ ID NO: 39. In an example, the PD-L1/TGF β /CD137 trispecific comprises the heavy chain sequence of the antibody scaffold module and the first binding module as set forth in SEQ ID NO: 51; and the light chain sequence of the antibody scaffold module and the second binding module as set forth in SEQ ID NO: 52.

[0142] In an example, the PD-L1/TGF β /CD137 trispecific has two first binding modules and one second binding module. In an example, the PD-L1/TGF β /CD137 trispecific comprises: the heavy chain sequence of the antibody scaffold module and the second binding module as set forth in SEQ ID NO: 41; the light chain sequence of the antibody scaffold module and the first binding module as set forth in SEQ ID NO: 39, the heavy chain sequence of the antibody scaffold module comprises a sequence set forth in SEQ ID NO: 42; the light chain sequence of the antibody scaffold module and the first binding module comprise a sequence set forth in SEQ ID NO: 39. In an example, the PD-L1/TGF β /CD137 trispecific has a structure which is, from N- to C-terminus: the heavy chain sequence of the antibody scaffold module and the second binding module comprise a sequence set forth in SEQ ID NO: 43; the light chain sequence of the antibody scaffold module and the first binding module comprise a sequence set forth in SEQ ID NO: 39, the heavy chain sequence of the antibody scaffold module comprise a sequence set forth in SEQ ID NO: 42; the light chain sequence of the antibody scaffold module and the first binding module comprise a sequence set forth in SEQ ID NO: 39.

[0143] In an example, the antibody scaffold module of the PD-L1/TGF β /CD137 trispecific further comprises a constant region. In an example, the constant region

of the antibody scaffold module of the PD-L1/TGF β /CD137 trispecific comprises at least one Fc silencing mutation. In an example, the Fc silencing mutation in the constant region of the antibody scaffold module of the PD-L1/TGF β /CD137 trispecific is L234A L235A or N297A. In an example, the constant region of the antibody scaffold module of the PD-L1/TGF β /CD137 trispecific comprises a knobs-in-holes (KiH) mutation.

[0144] According to some examples, the present disclosure provides PD-L1/TGF β /CD137 trispecifics (e.g., 1923Ab7, 1923Ab9, 1923Ab10, 1923Ab17 and 1923Ab19) that: 1) block the interaction between PD-L1 and its receptor PD-1; 2) possess crosslinking dependent agonistic activity for CD137 signaling and/or 3) neutralizes the immunosuppressive activities of TGF β .

[0145] In some examples, the disclosed PD-L1/TGF β /CD137 trispecifics comprise amino acid sequences derived from one of the binding protein that binds PD-L1 (e.g., 1923Ab2 or 1923Ab3) as an antibody scaffold module and TGF β binding amino acid sequence derived from the ECD of the human TGF β RII receptor disclosed herein as a first binding module and amino acid sequences derived from one of the binding protein that binds CD137 (e.g., 1923Ab4, 1923Ab5, or 1923Ab6) disclosed herein as a CD137 second binding module.

[0146] In some examples, the PD-L1/TGF β /CD137 trispecifics comprise an antibody scaffold module that disrupts the PD-1/PD-L1 binding interaction and dis-inhibits PD-1/PD-L1 checkpoint-mediated inhibition of T cells. The PD-L1 antibody scaffold module may be in the form of an IgG molecule (e.g., 1923Ab7, 1923Ab9, 1923Ab10, 1923Ab17, 1923Ab19).

[0147] In some examples, the disclosed PD-L1/TGF β /CD137 trispecifics further comprise a CD137 second binding module that blocks the CD137/CD137 ligand interaction and possesses agonistic activity for CD137 signaling. In alternative examples, the CD137 second binding module may comprise a scFv (e.g., 1923Ab7, 1923Ab9, 1923Ab10, 1923Ab17 and 1923Ab19). In alternative examples, the disclosed PD-L1/TGF β /CD137 trispecifics may comprise a second binding module (e.g., a CD137 scFv) fused to the C-terminus of the heavy chain or the light chain of a PD-L1 antibody scaffold module. In alternative examples, the disclosed PD-L1/TGF β /CD137 trispecifics may comprise a second binding module (e.g., a CD137 scFv) fused to the N-terminus of the heavy chain or the light chain of a PD-L1 antibody scaffold module.

[0148] In some examples, the second binding module that binds CD137 contributes monovalent binding to CD137. In alternative examples, the second binding module that binds CD137 contributes bivalent binding to CD137.

[0149] In some examples, the second binding module is a CD137 scFv and may be stabilized with a disulfide bond.

[0150] In some examples, the PD-L1/TGF β /CD137 trispecifics comprise a TGF β first binding module. In some examples the disclosed TGF β first binding module comprises the extracellular domain of the human TGF β RII receptor. The TGF β first binding module functions to neutralize the biological activities of TGF β present in the tumor microenvironment. In alternative examples, the TGF β first binding module comprises truncated versions (e.g., N-terminal or C-terminal truncations) of the human TNF β RII receptor that are capable of binding to human TGF β .

[0151] In alternative examples, the disclosed PD-L1/TGF β /CD137 trispecifics comprise a PD-L1 antibody scaffold module in which TGF β first binding module(s) are attached via a linker to the C-terminus or to the N-terminus of the heavy chains or the light chains of the PD-L1 antibody scaffold module.

[0152] In some examples, disclosed PD-L1/TGF β /CD137 trispecifics comprise an antibody scaffold module having a molecular design that is symmetrical (e.g., 1923Ab7, 1923Ab17 and 1923Ab19). In alternative examples, the disclosed PD-L1/TGF β /CD137 trispecifics are characterized by an asymmetrical design (e.g., 1923Ab9 and 1923Ab10). In order to direct the heterodimerization of the heavy chains of the asymmetrical disclosed PD-L1/TGF β /CD137 trispecifics, knobs-into-holes (KIHs) technology can be applied.

[0153] In some examples, the PD-L1/TGF β /CD137 trispecific 1923Ab7 comprises a PD-L1 antibody scaffold module having two Fabs from 1923Ab3, a human IgG1 Fc having two Fc constant chains with L234A L235A mutations (SEQ ID NO: 61), two second binding modules in the form of a CD137 scFv (VH precedes VL) (SEQ ID NO: 53) derived from 1923Ab4 each separately attached to the C-terminus of each of the two Fc constant chains, and two TGF β first binding modules as polypeptides encoding the extracellular domain of TGF β RII (SEQ ID NO: 67) each separately attached to the C-terminus of each light chain in the Fabs.

[0154] In some examples, the PD-L1/TGF β /CD137 trispecific 1923Ab9 comprises a PD-L1 antibody scaffold module having two Fabs from 1923Ab3, a heterodimeric human IgG1 Fc having two Fc constant chains with L234A L235A mutations and knobs-in-holes (KiH) mutations (e.g., the Fc constant chains are set forth in SEQ ID NOS: 62 and 63), one second binding module in the form of a CD137 scFv (VH precedes VL) (SEQ ID NO: 53) derived from 1923Ab4 attached to the C-terminus of the knob Fc constant chain, and two TGF β first binding modules as polypeptides encoding the extracellular domain of TGF β RII (SEQ ID NO: 67) each separately attached to the C-terminus of each light chain in the Fabs.

[0155] In some examples, the PD-L1/TGF β /CD137 trispecific 1923Ab10 comprises a PD-L1 antibody scaffold module having two Fabs from 1923Ab3, a heterodimeric human IgG1 Fc having two Fc chains with L234A L235A mutations and knobs-in-holes (KiH) mutations (e.g., the Fc constant chains are set forth in SEQ ID NOS: 62 and 63), one second binding module in the form of a CD137 scFv (VL precedes VH) (SEQ ID NO: 54) derived from 1923Ab4 attached to the C-terminus of the knob Fc constant chain, and two TGF β first binding modules as polypeptides encoding the extracellular domain of TGF β RII (SEQ ID NO: 67) each separately attached to the C-terminus of each light chain in the Fabs.

[0156] In some examples, the PD-L1/TGF β /CD137 trispecific 1923Ab17 comprises a PD-L1 antibody scaffold module having two Fabs from 1923Ab3, a human IgG1 Fc having two Fc chains with L234A L235A mutations (SEQ ID NO: 61), two second binding modules in the form of a disulfide bond-stabilized CD137 scFv (VH precedes VL) (SEQ ID NO: 55) derived from 1923Ab4 each separately attached to the C-terminus of each of the Fc chains, and two TGF β first binding modules as polypeptides encoding the extracellular domain of TGF β RII (SEQ ID NO: 67) each separately attached to the C-terminus of each light chain in the Fabs.

[0157] In some examples, the PD-L1/TGF β /CD137 trispecific 1923Ab19 comprises a PD-L1 antibody scaffold module having two Fabs from 1923Ab3, a human IgG1 Fc having two Fc chains with L234A L235A mutations (SEQ ID NO: 61), two TGF β first binding modules as polypeptides encoding the extracellular domain of TGF β RII (SEQ ID NO: 67) each separately attached to the C-terminus of each light chain in the Fabs, and two CD137 second binding module disulfide bond-stabilized scFvs (VH precedes VL) (SEQ ID NO: 56) derived from 1923Ab4 each separately attached to the C-terminus of each of the Fc chains.

[0158] In some examples, the PD-L1/TGFβ/CD137 trispecific comprises a PD-L1 antibody scaffold module derived from the heavy and/or light chain sequences disclosed in Table 7. In alternative examples the PD-L1/TGFβ/CD137 trispecific comprises a combination of two heavy chains and a light chain sequence paired according to Table 7.

[0159] In some examples, the PD-L1/TGFβ/CD137 trispecific comprises a heavy chain sequence and a light chain sequence, selected from the following combinations:

- (a) a heavy chain sequence comprising SEQ ID NO: 38 and a light chain sequence comprising SEQ ID NO: 39;
- (b) a heavy chain sequence comprising SEQ ID NO: 48 and a light chain sequence comprising SEQ ID NO: 49;
- (c) a heavy chain sequence comprising SEQ ID NO: 50 and a light chain sequence comprising SEQ ID NO: 39; and
- (d) a heavy chain sequence comprising SEQ ID NO: 51 and a light chain sequence comprising SEQ ID NO: 52.

[0160] In some examples, the PD-L1/TGFβ/CD137 trispecifics comprise two different variable heavy chain sequence (design to heterodimerize using a knob-into-hole format) and a variable light chain sequence, selected from the following combinations: (a) a first heavy chain sequence comprising SEQ ID NO: 41, a second heavy chain sequence comprising SEQ ID NO: 42 and a light chain sequence comprising SEQ ID NO: 39; and (b) a first heavy chain sequence comprising SEQ ID NO: 43, a second heavy chain sequence comprising SEQ ID NO: 42 and a light chain sequence comprising SEQ ID NO: 39.

[0161] In some examples, the PD-L1/TGFβ/CD137 trispecific comprises a heavy chain sequence selected from the group consisting of SEQ ID NOs: 38, 41, 42, 43, 48, 50 and 51 or an analogue or derivative thereof having at least 90% sequence identity to SEQ ID NOs: 38, 41, 42, 43, 48, 50 or 51.

[0162] In other examples, the PD-L1/TGFβ/CD137 trispecific comprises a light chain sequence selected from the group consisting of SEQ ID NOs: 39, 49 and 52 or an analogue or derivative thereof having at least 90% sequence identity to SEQ ID NOs: 39, 49 or 52.

[0163] In some examples, the disclosed PD-L1/TGF β /CD137 trispecific further comprises a Fc region that is engineered to abolish/minimize cross-linking activity with Fc γ Rs, which silence or eliminate Fc-mediated effector functions of T cells.

[0164] In some examples, the PD-L1/TGF β /CD137 trispecific exhibits one or more of the following functional characteristics, alone or in combination:

- (a) capable of binding to human PD-L1, CD137 and TGF β ;
- (b) cross-reacts with cynomolgus PD-L1 and CD137;
- (c) disrupts (e.g., reduces or prevents) interaction of PD-1 and PD-L1;
- (d) disrupts (e.g., reduces or prevents) human CD137L binding to CD137;(e) exhibits fast on and fast off properties to CD137;
- (f) dis-inhibits T cell PD-L1 mediated check-point inhibitory signal;
- (g) inhibits TGF β signaling and neutralizes its biological activities;
- (h) possess PD-L1 dependent agonistic activity to CD137 signaling;
- (i) activates T cells in PD-L1 dependent manner; and
- (j) kills PD-L1 expressing tumor cells by activating CD8 T cells.

[0165] The present disclosure also provides pharmaceutical compositions comprising or consisting of at least one of the PD-L1/TGF β /CD137 trispecifics disclosed herein, and optionally a pharmaceutically acceptable diluent, carrier, vehicle and/or excipient. Such a pharmaceutical composition may be used for the treatment of cancer.

[0166] The present disclosure also relates to methods for treatment of cancer in a patient comprising administering to the patient a therapeutically effective amount of at least one of the PD-L1/TGF β /CD137 trispecifics disclosed herein, alone or in combination with another therapeutic agent.

[0167] The present disclosure also provides isolated polynucleotide sequences encoding at least one of the PD-L1/TGF β /CD137 trispecifics described herein. The present disclosure also provides isolated polynucleotide sequences encoding at least one of the PD-L1/TGF β /CD137 trispecific sequences described herein.

[0168] The present disclosure also provides vectors comprising a polynucleotide of a PD-L1/TGF β /CD137 trispecific described herein.

[0169] The present disclosure also provides vectors comprising at least one of the PD-L1/TGF β /CD137 trispecific polynucleotide sequences described herein.

[0170] The present disclosure also provides cells comprising one of the PD-L1/TGF β /CD137 trispecific polynucleotide sequences described herein, or one of the above vectors.

[0171] The present disclosure also provides a binding protein that binds CD137, TGF β , and PD-L1, comprising: (a) an antibody scaffold module in an IgG format comprising a first antigen-binding site that binds CD137 and a second antigen-binding site that binds CD137; (b) at least one first binding module comprising a third antigen-binding site that binds TGF β ; and (c) at least one second binding module comprising a fourth antigen-binding site that binds PD-L1. In some examples, the present disclosure also provides CD137/TGF β /PD-L1 trispecifics constructed in the form of a recombinant protein comprising an antibody scaffold module that binds CD137, a first binding module that comprises an amino acid sequence derived from the TGF β receptor II binding protein that is capable of binding to human TGF β and neutralizing its activity, and a second binding module that binds PD-L1.

[0172] In an example, the first antigen-binding site and the second antigen-binding site of the CD137/TGF β /PD-L1 trispecific comprises a heavy chain variable region sequence comprising CDR1: SEQ ID NO: 5, CDR2: SEQ ID NO: 22, and CDR3: SEQ ID NO: 23; and a light chain variable region sequence comprising CDR1: SEQ ID NO: 24, CDR2: SEQ ID NO: 25, and CDR3: SEQ ID NO: 26. In an example, the first antigen-binding site and the second antigen-binding site of the CD137/TGF β /PD-L1 trispecific comprises a heavy chain variable region sequence comprising CDR1: SEQ ID NO: 27, CDR2: SEQ ID NO: 28, and CDR3: SEQ ID NO: 29; and a light chain variable region sequence comprising CDR1: SEQ ID NO: 30, CDR2: SEQ ID NO: 9, and CDR3: SEQ ID NO: 31. In an example, the first antigen-binding site and the second antigen-binding site of the CD137/TGF β /PD-L1 trispecific comprises a heavy chain variable region sequence comprising CDR1: SEQ ID NO: 32, CDR2: SEQ ID NO: 33, and CDR3: SEQ ID NO: 34; and a light chain variable region sequence comprising CDR1: SEQ ID NO: 35, CDR2: SEQ ID NO: 36, and CDR3: SEQ ID NO: 37.

[0173] In an example, the antibody scaffold module of the CD137/TGF β /PD-L1 trispecific comprises a heavy chain variable region sequence as set forth in SEQ ID NO: 16, SEQ ID NO: 18, or SEQ ID NO: 20. In an example, the antibody scaffold module of the CD137/TGF β /PD-L1 trispecific comprises a light chain variable region sequence as set forth in SEQ ID NO: 17; SEQ ID NO: 19, or SEQ ID NO: 21.

[0174] In an example, the antibody scaffold module of the CD137/TGF β /PD-L1 trispecific comprises a heavy chain variable region sequence as set forth in SEQ ID NO: 16 and a light chain variable region sequence as set forth in SEQ ID NO: 17. In an example, the antibody scaffold module of the CD137/TGF β /PD-L1 trispecific comprises a heavy chain variable region sequence as set forth in SEQ ID NO: 18 and a light chain variable region sequence as set forth in SEQ ID NO: 19. In an example, the antibody scaffold module of the CD137/TGF β /PD-L1 trispecific comprises a heavy chain variable region sequence as set forth in SEQ ID NO: 20 and a light chain variable region sequence as set forth in SEQ ID NO: 21.

[0175] In an example, the antibody scaffold module of the CD137/TGF β /PD-L1 trispecific comprises a heavy chain variable region sequence as set forth in SEQ ID NO: 16, a heavy chain constant region sequence as set forth in SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, or SEQ ID NO: 64, a light chain variable region sequence as set forth in SEQ ID NO: 17, and a light chain constant region sequence as set forth in SEQ ID NO: 65 or SEQ ID NO: 66. In an example, the antibody scaffold module of the CD137/TGF β /PD-L1 trispecific comprises a heavy chain variable region sequence as set forth in SEQ ID NO: 18, a heavy chain constant region sequence as set forth in SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, or SEQ ID NO: 64, a light chain variable region sequence as set forth in SEQ ID NO: 19, and a light chain constant region sequence as set forth in SEQ ID NO: 65 or SEQ ID NO: 66. In an example, the antibody scaffold module of the CD137/TGF β /PD-L1 trispecific comprises a heavy chain variable region sequence as set forth in SEQ ID NO: 20, a heavy chain constant region sequence as set forth in SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, or SEQ ID NO: 64, a light chain variable region sequence as set forth in

SEQ ID NO: 21, and a light chain constant region sequence as set forth in SEQ ID NO: 65 or SEQ ID NO: 66.

[0176] In an example, the antibody scaffold moiety of the CD137/TGF β /PD-L1 trispecific comprises a heavy chain sequence as set forth in SEQ ID NO: 75 and a light chain sequence as set forth in SEQ ID NO: 76.

[0177] In an example, the CD137/TGF β /PD-L1 trispecific has one first binding module. In an example, the CD137/TGF β /PD-L1 trispecific has two first binding modules. In an example, the CD137/TGF β /PD-L1 trispecific has an antibody scaffold module which comprises a heavy chain sequence which comprises a C-terminus and a N-terminus, and wherein the antibody scaffold module comprises a light chain sequence which comprises a C-terminus and a N-terminus, and the first binding module is covalently attached to the C-terminus of the antibody scaffold module heavy chain sequence, the C-terminus of the antibody scaffold module light chain sequence, the N-terminus of the antibody scaffold module heavy chain sequence, the N-terminus of the antibody scaffold module light chain sequence, or combinations thereof, optionally wherein the first binding module and the antibody scaffold module are covalently attached to each other directly or through a first binding module interlinker. In an example, the first binding module and the antibody scaffold module of the CD137/TGF β /PD-L1 trispecific are covalently attached to each other through a first binding module interlinker, and the first binding module interlinker is set forth in SEQ ID NO: 58. In an example, the first binding module and the antibody scaffold module of the CD137/TGF β /PD-L1 trispecific are covalently attached to each other through a first binding module interlinker as set forth in SEQ ID NO: 59.

[0178] In an example, the first binding module of the CD137/TGF β /PD-L1 trispecific is covalently attached to the C-terminus of the antibody scaffold module heavy chain sequence. In an example, the first binding module of the CD137/TGF β /PD-L1 trispecific is covalently attached to the C-terminus of the antibody scaffold module light chain sequence. In an example, when there is more than one first binding module in the

CD137/TGFβ/PD-L1 trispecific, each is covalently attached to a different antibody scaffold module sequence or to a different end of the antibody scaffold module sequence.

[0179] In an example, the first binding module in the CD137/TGFβ/PD-L1 trispecific comprises the extracellular domain of TGFβRII. In an example, the extracellular domain of TGFβRII in the CD137/TGFβ/PD-L1 trispecific comprises the sequence set forth in SEQ ID NO: 67.

[0180] In an example, the second binding module in the CD137/TGFβ/PD-L1 trispecific is a scFv, which comprises a heavy chain variable region sequence and a light chain variable sequence, wherein the sequences are covalently attached to each other directly or through a scFv fusion linker. In an example, the scFv fusion linker comprises glycine and serine. In an example, the scFv fusion linker comprises the sequence Gly-Gly-Gly-Ser. In an example, the scFv fusion linker comprises the sequence set forth in SEQ ID NO: 58. In an example, the scFv fusion linker is the sequence set forth in SEQ ID NO: 58. In an example, the scFv fusion linker comprises the sequence set forth in SEQ ID NO: 59. In an exemplary CD137/TGFβ/PD-L1 trispecific example in which a CD137 antibody provides the scaffold moiety, the second binding module is an scFv that binds to PD-L1 comprising SEQ ID NO: 57.

[0181] In an example, the CD137/TGFβ/PD-L1 trispecific has one second binding module. In an example, the CD137/TGFβ/PD-L1 trispecific has two second binding modules. In an example, the antibody scaffold module of the CD137/TGFβ/PD-L1 trispecific comprises a heavy chain sequence which comprises a C-terminus and a N-terminus, and wherein the antibody scaffold module comprises a light chain sequence which comprises a C-terminus and a N-terminus, and the second binding module is covalently attached to the C-terminus of the antibody scaffold module heavy chain sequence, the C-terminus of the antibody scaffold module light chain sequence, the N-terminus of the antibody scaffold module heavy chain sequence, the N-terminus of the antibody scaffold module light chain sequence, or combinations thereof, optionally wherein the second binding module and the antibody

scaffold module are covalently attached to each other directly or through a second binding module interlinker.

[0182] In an example, the second binding module and the antibody scaffold module of the CD137/TGF β /PD-L1 trispecific are covalently attached to each other through a second binding module interlinker, and the second binding module interlinker as set forth in SEQ ID NO: 58. In an example, the second binding module and the antibody scaffold module of the CD137/TGF β /PD-L1 trispecific are covalently attached to each other through a second binding module interlinker as set forth in SEQ ID NO: 59.

[0183] In an example, the second binding module of the CD137/TGF β /PD-L1 trispecific is covalently attached to the C-terminus of the antibody scaffold module heavy chain sequence. In an example, the first binding module of the CD137/TGF β /PD-L1 trispecific is covalently attached to the C-terminus of the antibody scaffold module light chain sequence. In an example, the second binding module of the CD137/TGF β /PD-L1 trispecific is covalently attached to the C-terminus of the antibody scaffold module heavy chain sequence, and the first binding module of the CD137/TGF β /PD-L1 trispecific is covalently attached to the C-terminus of the antibody scaffold module light chain sequence.

[0184] In an example, the second binding module of the CD137/TGF β /PD-L1 trispecific is covalently attached to the C-terminus of the antibody scaffold module light chain sequence. In an example, the first binding module of the CD137/TGF β /PD-L1 trispecific is covalently attached to the C-terminus of the antibody scaffold module heavy chain sequence. In an example, the second binding module of the CD137/TGF β /PD-L1 trispecific is covalently attached to the C-terminus of the antibody scaffold module light chain sequence, and the first binding module of the CD137/TGF β /PD-L1 trispecific is covalently attached to the C-terminus of the antibody scaffold module heavy chain sequence.

[0185] In an example, when there is more than one second binding module in the CD137/TGF β /PD-L1 trispecific, each is covalently attached to a different antibody scaffold module sequence or to a different end of the antibody scaffold module. In an example, the CD137/TGF β /PD-L1 trispecific has one second binding module, and the one

second binding module is covalently attached to the C-terminus of the antibody scaffold module heavy chain sequence.

[0186] In an example, the CD137/TGFβ/PD-L1 trispecific has one second binding module, and the one second binding module is covalently attached to the C-terminus of the antibody scaffold module light chain sequence. In an example, the CD137/TGFβ/PD-L1 trispecific has two second binding modules, and one second binding module is covalently attached to the C-terminus of the antibody scaffold module heavy chain sequence, and the other second binding module is covalently attached to the C-terminus of the antibody scaffold module light chain sequence. In an example, the second binding module of the CD137/TGFβ/PD-L1 trispecific is a scFv.

[0187] In an example, the second binding module of the CD137/TGFβ/PD-L1 trispecific comprises, from N- to C-terminus: a heavy chain variable region sequence comprising CDR1: SEQ ID NO: 5, CDR2: SEQ ID NO: 6, and CDR3: SEQ ID NO: 7; and a light chain variable region sequence comprising CDR1: SEQ ID NO: 8, CDR2: SEQ ID NO: 9, and CDR3: SEQ ID NO: 10. In an example, the second binding module of the CD137/TGFβ/PD-L1 trispecific comprises, from N- to C-terminus: a heavy chain variable region sequence comprising CDR1: SEQ ID NO: 11, CDR2: SEQ ID NO: 12, and CDR3: SEQ ID NO: 13; and a light chain variable region sequence comprising CDR1: SEQ ID NO: 14, CDR2: SEQ ID NO: 9, and CDR3: SEQ ID NO: 15.

[0188] In an example, the second binding module of the CD137/TGFβ/PD-L1 trispecific comprises, from N- to C-terminus: a heavy chain variable region sequence as set forth in SEQ ID NO: 1 and a light chain variable region sequence as set forth in SEQ ID NO: 2. In an example, the second binding module of the CD137/TGFβ/PD-L1 trispecific comprises, from N- to C-terminus: a heavy chain variable region sequence as set forth in SEQ ID NO: 3 and a light chain variable region sequence as set forth in SEQ ID NO: 4. In an example, the second binding module of the CD137/TGFβ/PD-L1 trispecific comprises a sequence as set forth in SEQ ID NO: 57.

[0189] In an example, the CD137/TGFβ/PD-L1 trispecific has two first binding modules and two second binding modules. In an example, the CD137/TGFβ/PD-L1 trispecific has a structure in which the heavy chain sequence of the antibody scaffold module

and the second binding module comprise SEQ ID NO: 48; and the light chain sequence of the antibody scaffold module and the first binding module comprise SEQ ID NO: 49.

[0190] In an example, the antibody scaffold module of the CD137/TGF β /PD-L1 trispecific further comprises a constant region. In an example, the constant region of the antibody scaffold module of the CD137/TGF β /PD-L1 trispecific comprises at least one Fc silencing mutation. In an example, the Fc silencing mutation in the constant region of the antibody scaffold module of the CD137/TGF β /PD-L1 trispecific is L234A L235A or N297A. In an example, the constant region of the antibody scaffold module of the CD137/TGF β /PD-L1 trispecific comprises a knobs-in-holes (KiH) mutation.

[0191] According to some examples, the present disclosure provides CD137/TGF β /PD-L1 trispecifics (1923Ab16) that: 1) block the interaction between CD137 and its ligand; 2) disrupts (e.g., reduces or prevents the interaction of PD-1 and PD-L1; and 3) neutralize the immunosuppressive activities of TGF β .

[0192] In some examples, the disclosed CD137/TGF β /PD-L1 trispecifics comprise amino acid sequences derived from one of the binding protein that binds CD137 (e.g., 1923Ab4, 1923Ab5 or 1923Ab6) as an antibody scaffold module and TGF β binding amino acid sequence derived from the ECD of the human TGF β RII receptor disclosed herein as a first binding module and amino acid sequences derived from one of the binding proteins that binds PD-L1 (e.g., 1923Ab2, or 1923Ab3) disclosed herein as a PD-L1 second binding module.

[0193] In some examples, the CD137/TGF β /PD-L1 trispecifics comprise an antibody scaffold module that blocks the CD137/CD137 ligand interaction and possess agonistic activity for CD137 signaling. The CD137 antibody scaffold module may be in the form of an IgG molecule (e.g., 1923Ab16) or a binding fragment thereof.

[0194] In some examples, the disclosed CD137/TGF β /PD-L1 trispecifics further comprise a PD-L1 second binding module that disrupts the PD-1/PD-L1 binding interaction and dis-inhibits PD-1/PD-L1 checkpoint-mediated inhibition of T cells. In alternative examples, the PD-L1 second binding module may comprise a scFv (e.g., 1923Ab16). In alternative examples, the disclosed CD137/TGF β /PD-L1 trispecifics may comprise a PD-L1 scFv second binding module fused to the C-terminus of the heavy chain or the light chain of a CD137 antibody scaffold module.

[0195] In some examples, the PD-L1 second binding module contributes monovalent binding to PD-L1. In alternative examples, the PD-L1 second binding module contributes bivalent binding to PD-L1.

[0196] In some examples, the PD-L1 scFv second binding module may be stabilized with a disulfide bond.

[0197] In some examples, the disclosed CD137/TGF β /PD-L1 trispecifics comprise a tumor microenvironment modulator, exemplified herein as a TGF β first binding module. In some examples the disclosed TGF β first binding module comprises the extracellular domain of the human TGF β RII receptor. The TGF β first binding module functions to neutralize the biological activities of TGF β present in the tumor microenvironment. In alternative examples, the TGF β first binding module may comprise truncated versions (e.g., N-terminal or C-terminal truncations) of the human TNF β RII receptor that are capable of binding to human TGF β .

[0198] In alternative examples, the disclosed CD137/TGF β /PD-L1 trispecifics comprise a CD137 antibody binding module in which TGF β first binding module(s) are attached via a linker to the C-terminus or to the N-terminus of the heavy chains or the light chains of the CD137 antibody binding module.

[0199] In some examples, CD137/TGF β /PD-L1 trispecifics have a molecular design that is symmetrical. In alternative examples the CD137/TGF β /PD-L1 trispecifics are characterized by an asymmetrical design. In order to direct the heterodimerization of the heavy chains of the asymmetrical disclosed CD137/TGF β /PD-L1 trispecifics, knobs-into-holes (KIHS) technology can be applied.

[0200] In some examples, the CD137/TGF β /PD-L1 trispecific 1923Ab16 comprises a CD137 antibody scaffold module having two Fabs from 1923Ab4, a human IgG1 Fc having two Fc chains with L234A L235A mutations (SEQ ID NO: 61), two PD-L1 second binding module scFvs (VH precedes VL) (SEQ ID NO: 57) derived from 1923Ab3 each separately attached to the C-terminus of each of the Fc chains, and two TGF β first binding modules as polypeptides encoding the extracellular domain of TGF β RII (SEQ ID NO: 67) each separately attached to the C-terminus of each light chain in the Fabs.

[0201] In some examples, the disclosed CD137/TGF β /PD-L1 trispecific further comprises a Fc region that is engineered to abolish/minimize cross-linking activity with Fc γ Rs, which silence or eliminate Fc-mediated effector functions of T cells.

[0202] In some examples, the CD137/TGF β /PD-L1 trispecific exhibits one or more of the following functional characteristics, alone or in combination:

- (a) capable of binding to human PD-L1, CD137 and TGF β ;
- (b) cross-reacts with cynomolgus PD-L1 and CD137;
- (c) disrupts (e.g., reduces or prevents) interaction of PD-1 and PD-L1;
- (d) disrupts (e.g., reduces or prevents) human CD137L binding to CD137;
- (e) exhibits fast on and fast off properties to CD137;
- (f) dis-inhibits T cell PD-L1 mediated check-point inhibitory signal;
- (g) inhibits TGF β signaling and neutralizes its biological activities;
- (h) possess PD-L1 dependent agonistic activity to CD137 signaling;
- (i) activates T cells in PD-L1 dependent manner; and
- (j) kills PD-L1 expressing tumor cells by activating CD8 T cells.

[0203] The present disclosure also provides pharmaceutical compositions comprising or consisting of at least one of the CD137/TGF β /PD-L1 trispecifics disclosed herein, and optionally a pharmaceutically acceptable diluent, carrier, vehicle and/or excipient. Such a pharmaceutical composition may be used for the antibody-based immunotherapy of cancer.

[0204] The present disclosure also relates to methods for treatment of cancer in a patient comprising administering to the patient a therapeutically effective amount of at least one of the CD137/TGF β /PD-L1 trispecifics disclosed herein, alone or in combination with another therapeutic agent.

[0205] The present disclosure also provides isolated polynucleotide sequences encoding at least one of the CD137/TGF β /PD-L1 trispecifics described herein. The present disclosure also provides isolated polynucleotide sequences encoding at least one of the CD137/TGF β /PD-L1 trispecific sequences described herein.

WHAT IS CLAIMED IS:

1. An anti-CD137 antibody or antigen binding fragment thereof comprising:
 - (i) a heavy chain variable region comprising CDR1: SEQ ID NO: 5, CDR2: SEQ ID NO: 22, and CDR3: SEQ ID NO: 23; and a light chain variable region comprising CDR1: SEQ ID NO: 24, CDR2: SEQ ID NO: 25, and CDR3: SEQ ID NO: 26;
 - (ii) a heavy chain variable region comprising CDR1: SEQ ID NO: 27, CDR2: SEQ ID NO: 28, and CDR3: SEQ ID NO: 29; and a light chain variable region comprising CDR1: SEQ ID NO: 30, CDR2: SEQ ID NO: 9, and CDR3: SEQ ID NO: 31; or
 - (iii) a heavy chain variable region comprising CDR1: SEQ ID NO: 32, CDR2: SEQ ID NO: 33, and CDR3: SEQ ID NO: 34; and a light chain variable region comprising CDR1: SEQ ID NO: 35, CDR2: SEQ ID NO: 36, and CDR3: SEQ ID NO: 37.
2. The anti-CD137 antibody or antigen binding fragment thereof of claim 1, wherein the anti-CD137 antibody or antigen binding fragment thereof comprises:
 - (i) a heavy chain variable region comprising SEQ ID NO: 16; and a light chain variable region comprising SEQ ID NO: 17;
 - (ii) a heavy chain variable region comprising SEQ ID NO: 18; and a light chain variable region comprising SEQ ID NO: 19; or
 - (iii) a heavy chain variable region comprising SEQ ID NO: 20; and a light chain variable region comprising SEQ ID NO: 21.
3. The anti-CD137 antibody or antigen binding fragment thereof of claim 1, wherein the binding protein or binding fragment thereof comprises a heavy chain constant region.
4. The anti-CD137 antibody or antigen binding fragment thereof of claim 3, wherein the constant region comprises SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, or SEQ ID NO: 64.
5. The anti-CD137 antibody of claim 1, wherein the anti-CD137 antibody comprises a heavy chain comprising SEQ ID NO: 75; and a light chain comprising SEQ ID NO: 76.

- 6.** The anti-CD137 antibody or antigen binding fragment thereof of claim 1 comprising a light chain constant region comprising SEQ ID NO: 65 or SEQ ID NO: 66.
- 7.** The anti-CD137 antibody of claim 1, wherein the anti-CD137 antibody is a multi-specific antibody, preferably a bispecific or tri-specific antibody.
- 8.** The antigen binding fragment thereof of claim 1, wherein the binding fragment thereof is a Fab, a Fab', a F(ab)₂, a F(ab')₂, a Fd, a Fv, a scFv, a single-chain antibody, a minibody, or a diabody.
- 9.** An anti-PD-L1 antibody or antigen binding fragment thereof comprising:
- (i) a heavy chain variable region comprising CDR1: SEQ ID NO: 5, CDR2: SEQ ID NO: 6, and CDR3: SEQ ID NO: 7; and a light chain variable region comprising CDR1: SEQ ID NO: 8, CDR2: SEQ ID NO: 9, and CDR3: SEQ ID NO: 10; or
 - (ii) a heavy chain variable region comprising CDR1: SEQ ID NO: 11, CDR2: SEQ ID NO: 12, and CDR3: SEQ ID NO: 13; and a light chain variable region comprising CDR1: SEQ ID NO: 14, CDR2: SEQ ID NO: 9, and CDR3: SEQ ID NO: 15.
- 10.** A nucleic acid composition comprising one or more nucleic acids encoding the antibody or antigen binding fragment thereof of claim 1 or claim 9.
- 11.** An expression vector composition comprising one or more expression vectors comprising the nucleic acid composition of claim **10**.
- 12.** A host cell comprising the nucleic acid composition of claim **10** or the expression vector composition of claim **11**.

13. A method for the production of the anti-CD137 antibody or antigen binding fragment thereof of claim **1**, the method comprising culturing the host cell of claim **12**.

14. A pharmaceutical composition comprising the anti-CD137 antibody or antigen binding fragment thereof of claim **1** and a pharmaceutically acceptable carrier.

15. Use of the antibody or antigen binding fragment thereof of any of claims **1-9** or the pharmaceutical composition of claim **14** in the manufacture of a medicament for treating cancer in a subject.