SUMMARY

[0004] The present invention is based on the discovery that a bifunctional protein containing at least portion of TGFβ Receptor II (TGFβRII) that is capable of binding TGFβ and antibody or antigen-binding fragment that binds to an immune checkpoint protein such as human protein Programmed Death Ligand 1 (PD-L1) can be an effective anti-tumor and anti-cancer therapeutic. The protein can exhibit a synergistic effect in cancer treatment, as compared to the effect of administering the two agents separately.

[0004a] In one aspect, the present invention refers to a protein comprising: a) human TGFβRII, or a fragment thereof capable of binding TGFβ; and b) an antibody, or an antigen-binding fragment thereof, that binds human protein Programmed Death Ligand 1 (PD-L1).

[0004b] In another aspect, the present invention refers to a protein comprising a first polypeptide comprising: a) at least a variable domain of a heavy chain of an antibody that binds human protein Programmed Death Ligand 1 (PD-L1); and b) human TGFβRII, or a fragment thereof capable of binding TGFβ; and a second polypeptide comprising at least a variable region of a light chain of an antibody that binds PD-L1.

[0004c] In yet another aspect, the present invention refers to a nucleic acid comprising a first nucleotide sequence encoding the first polypeptide and a second nucleotide sequence encoding the second polypeptide according to the present invention.

[0004d] In yet another aspect, the present invention refers to a cell comprising the nucleic acid of the present invention.

[0004e] In yet another aspect, the present invention refers to a method of producing a protein comprising a) TGFβRII, or a fragment thereof capable of binding TGFβ, and b) an antibody, or an antigen-binding fragment thereof, that binds human protein Programmed Death Ligand 1 (PD-L1), the method comprising maintaining a cell according to the present invention under conditions permitting expression of the protein, and optionally further comprising harvesting the protein.

[0004f] In yet another aspect, the present invention refers to a protein according to the present invention for use in therapy.

[0004g] In yet another aspect, the present invention refers to the use of a protein according to the present invention in the manufacture of a medicament for inhibiting tumor growth or treating cancer in a patient.

[0005] Accordingly, in a first example, the present disclosure features a protein including (a) human TGFβRII, or a fragment thereof capable of binding TGFβ (*e.g.*, a soluble fragment); and (b) an antibody, or an antigen-binding fragment thereof, that binds PD-L1 (*e.g.*, any of the antibodies or antibody fragments described herein).

[0006] In a related example, the disclosure features a polypeptide including (a) at least a variable domain of a heavy chain of an antibody that binds PD-L1 (*e.g.*, amino acids 1-120 of SEQ ID NO: 2); and (b) human TGFβRII, or a soluble fragment thereof capable of binding TGFβ (*e.g.*, a human TGFβRII extra-cellular domain (ECD), amino acids 24-159 of SEQ ID NO: 9, or any of those described herein). The polypeptide may further include an amino acid linker connecting the C-terminus of the variable domain to the N-terminus of the human TGFβRII or soluble fragment thereof capable of binding TGFβ. The polypeptide may include the amino acid sequence of SEQ ID NO: 3 or an amino acid sequence substantially identical to SEQ ID NO: 3. The antibody fragment may be an scFv, Fab, $F(ab')_2$, or Fv fragment.

[0007] In certain examples, the protein or polypeptide includes an antibody or antigen-binding fragment thereof that includes SEQ ID NO: 2 and human TGFβRII. The antibody may optionally include a modified constant region (e.g., any described herein, including a C-terminal Lys→Ala substitution, a mutation of the Leu-Ser-Leu-Ser (SEQ ID NO: 19) sequence to Ala-Thr-Ala-Thr (SEQ ID NO: 20), or a hybrid constant region including an IgG1 hinge region and an IgG2 CH2 domain).

[0008] In certain examples, the protein or polypeptide includes an antibody or antigen-binding fragment thereof that includes SEQ ID NO: 2 and a fragment of human TGFβRII capable of binding TGFβ (e.g., a soluble fragment). The antibody may optionally include a modified constant region (e.g., any described herein, including a C-terminal Lys→Ala substitution, a mutation of the Leu-Ser-Leu-Ser (SEQ ID NO: 19) sequence to Ala-Thr-Ala-Thr

(SEQ ID NO: 20), or a hybrid constant region including an IgG1 hinge region and an IgG2 CH2 domain).

[0009] In certain examples, the protein or polypeptide includes an antibody or antigen-binding fragment thereof that includes SEQ ID NO: 2 and a human TGFβRII ECD. The antibody may include a modified constant region (e.g., any described herein, including a C-terminal Lys→Ala substitution, a mutation of the Leu-Ser-Leu-Ser (SEQ ID NO: 19) sequence to Ala-Thr-Ala-Thr (SEQ ID NO: 20), or a hybrid constant region including an IgG1 hinge region and an IgG2 CH2 domain).

[0010] In certain examples, the protein or polypeptide includes an antibody or antigen-binding fragment thereof that includes amino acids 1-120 of SEQ ID NO: 2 and human TGFβRII. The antibody may include a modified constant region (e.g., any described herein, including a C-terminal Lys→Ala substitution, a mutation of the Leu-Ser-Leu-Ser (SEQ ID NO: 19) sequence to Ala-Thr-Ala-Thr (SEQ ID NO: 20), or a hybrid constant region including an IgG1 hinge region and an IgG2 CH2 domain).

[0011] In certain examples, the protein or polypeptide includes an antibody or antigen-binding fragment thereof that includes amino acids 1-120 of SEQ ID NO: 2 and a fragment of human TGFβRII capable of binding TGFβ (e.g., a soluble fragment). The antibody may include a modified constant region (e.g., any described herein, including a C-terminal Lys→Ala substitution, a mutation of the Leu-Ser-Leu-Ser (SEQ ID NO: 19) sequence to Ala-Thr-Ala-Thr (SEQ ID NO: 20), or a hybrid constant region including an IgG1 hinge region and an IgG2 CH2 domain).

[0012] In certain examples, the protein or polypeptide includes an antibody or antigen-binding fragment thereof that includes amino acids 1-120 of SEQ ID NO: 2 and a human TGFβRII ECD. The antibody may include a modified constant region (e.g., any described herein, including a C-terminal Lys→Ala substitution, a mutation of the Leu-Ser-Leu-Ser (SEQ ID NO: 19) sequence to Ala-Thr-Ala-Thr (SEQ ID NO: 20), or a hybrid constant region including an IgG1 hinge region and an IgG2 CH2 domain).

[0013] In certain examples, the protein or polypeptide includes an antibody or antigen-binding fragment thereof that includes the hypervariable regions present in SEQ ID NO: 2 and human TGFβRII. The antibody may include a modified constant region (e.g., any described herein, including a C-terminal Lys→Ala substitution, a mutation of the Leu-Ser-Leu-Ser (SEQ

ID NO: 19) sequence to Ala-Thr-Ala-Thr (SEQ ID NO: 20), or a hybrid constant region including an IgG1 hinge region and an IgG2 CH2 domain).

[0014] In certain examples, the protein or polypeptide includes an antibody or antigen-binding fragment thereof that includes the hypervariable regions present in SEQ ID NO: 2 and a fragment of human TGFβRII capable of binding TGFβ (e.g., a soluble fragment). The antibody may include a modified constant region (e.g., any described herein, including a C-terminal Lys→Ala substitution, a mutation of the Leu-Ser-Leu-Ser (SEQ ID NO: 19) sequence to Ala-Thr-Ala-Thr (SEQ ID NO: 20), or a hybrid constant region including an IgG1 hinge region and an IgG2 CH2 domain).

[0015] In certain examples, the protein or polypeptide includes an antibody or antigen-binding fragment thereof that includes the hypervariable regions present in SEQ ID NO: 2 and a human TGFβRII ECD. The antibody may include a modified constant region (e.g., any described herein, including a C-terminal Lys→Ala substitution, a mutation of the Leu-Ser-Leu-Ser (SEQ ID NO: 19) sequence to Ala-Thr-Ala-Thr (SEQ ID NO: 20), or a hybrid constant region including an IgG1 hinge region and an IgG2 CH2 domain).

[0016] In certain examples, the protein or polypeptide includes an antibody or antigen-binding fragment thereof that includes SEQ ID NO: 12 and human TGFβRII. The antibody may include a modified constant region (e.g., any described herein, including a C-terminal Lys→Ala substitution, a mutation of the Leu-Ser-Leu-Ser (SEQ ID NO: 19) sequence to Ala-Thr-Ala-Thr (SEQ ID NO: 20), or a hybrid constant region including an IgG1 hinge region and an IgG2 CH2 domain).

[0017] In certain examples, the protein or polypeptide includes an antibody or antigen-binding fragment thereof that includes SEQ ID NO: 12 and a fragment of human TGFβRII capable of binding TGFβ (e.g., a soluble fragment). The antibody may include a modified constant region (e.g., any described herein, including a C-terminal Lys→Ala substitution, a mutation of the Leu-Ser-Leu-Ser (SEQ ID NO: 19) sequence to Ala-Thr-Ala-Thr (SEQ ID NO: 20), or a hybrid constant region including an IgG1 hinge region and an IgG2 CH2 domain).

[0018] In certain examples, the protein or polypeptide includes an antibody or antigen-binding fragment thereof that includes SEQ ID NO: 12 and a human TGFβRII ECD. The antibody may include a modified constant region (e.g., any described herein, including a C-terminal Lys→Ala substitution, a mutation of the Leu-Ser-Leu-Ser (SEQ ID NO: 19) sequence

to Ala-Thr-Ala-Thr (SEQ ID NO: 20), or a hybrid constant region including an IgG1 hinge region and an IgG2 CH2 domain).

[0019] In certain examples, the protein or polypeptide includes an antibody or antigen-binding fragment thereof that includes the hypervariable regions present in SEQ ID NO: 12 and human TGFβRII. The antibody may include a modified constant region (e.g., any described herein, including a C-terminal Lys→Ala substitution, a mutation of the Leu-Ser-Leu-Ser (SEQ ID NO: 19) sequence to Ala-Thr-Ala-Thr (SEQ ID NO: 20), or a hybrid constant region including an IgG1 hinge region and an IgG2 CH2 domain).

[0020] In certain examples, the protein or polypeptide includes an antibody or antigen-binding fragment thereof that includes the hypervariable regions present in SEQ ID NO: 12 and a fragment of human TGFβRII capable of binding TGFβ (e.g., a soluble fragment). The antibody may include a modified constant region (e.g., any described herein, including a C-terminal Lys→Ala substitution, a mutation of the Leu-Ser-Leu-Ser (SEQ ID NO: 19) sequence to Ala-Thr-Ala-Thr (SEQ ID NO: 20), or a hybrid constant region including an IgG1 hinge region and an IgG2 CH2 domain).

[0021] In certain examples, the protein or polypeptide includes an antibody or antigen-binding fragment thereof that includes the hypervariable regions present in SEQ ID NO: 12 and a human TGFβRII ECD. The antibody may include a modified constant region (e.g., any described herein, including a C-terminal Lys→Ala substitution, a mutation of the Leu-Ser-Leu-Ser (SEQ ID NO: 19) sequence to Ala-Thr-Ala-Thr (SEQ ID NO: 20), or a hybrid constant region including an IgG1 hinge region and an IgG2 CH2 domain).

[0022] In certain examples, the protein or polypeptide includes an antibody or antigen-binding fragment thereof that includes SEQ ID NO: 14 and human TGFβRII. The antibody may include a modified constant region (e.g., any described herein, including a C-terminal Lys→Ala substitution, a mutation of the Leu-Ser-Leu-Ser (SEQ ID NO: 19) sequence to Ala-Thr-Ala-Thr (SEQ ID NO: 20), or a hybrid constant region including an IgG1 hinge region and an IgG2 CH2 domain).

[0023] In certain examples, the protein or polypeptide includes an antibody or antigen-binding fragment thereof that includes SEQ ID NO: 14 and a fragment of human TGFβRII capable of binding TGFβ (e.g., a soluble fragment). The antibody may include a modified constant region (e.g., any described herein, including a C-terminal Lys→Ala substitution, a

mutation of the Leu-Ser-Leu-Ser (SEQ ID NO: 19) sequence to Ala-Thr-Ala-Thr (SEQ ID NO: 20), or a hybrid constant region including an IgG1 hinge region and an IgG2 CH2 domain).

[0024] In certain examples, the protein or polypeptide includes an antibody or antigen-binding fragment thereof that includes SEQ ID NO: 14 and a human TGFβRII ECD. The antibody may include a modified constant region (e.g., any described herein, including a C-terminal Lys→Ala substitution, a mutation of the Leu-Ser-Leu-Ser (SEQ ID NO: 19) sequence to Ala-Thr-Ala-Thr (SEQ ID NO: 20), or a hybrid constant region including an IgG1 hinge region and an IgG2 CH2 domain).

[0025] The disclosure also features a nucleic acid that includes a nucleotide sequence that encodes a polypeptide described above. In certain examples, the nucleic acid further includes a second nucleotide sequence encoding at least a variable domain of a light chain of an antibody which, when combined with the polypeptide, forms an antigen-binding site that binds PD-L1 (*e.g.*, including amino acids 1-110 of SEQ ID NO: 1). The second nucleotide sequence may encode the amino acid sequence of SEQ ID NO: 1 (secreted anti-PD-L1 lambda light chain) or an amino acid sequence substantially identical to SEQ ID NO: 1. The disclosure also features a cell including any of the nucleic acids described above.

[0026] The disclosure also features a method of producing a protein including (a) the extracellular domain of the human TGFβRII, or a fragment thereof capable of binding TGFβ (*e.g.*, a soluble fragment), and (b) an antibody, or an antigen-binding fragment thereof, that binds human PD-L1. The method includes maintaining a cell described under conditions that permit expression of the protein. The method may further include harvesting the protein.

[0027] The disclosure also features a protein including the polypeptide described above and at least a variable domain of a light chain of an antibody which, when combined with the polypeptide, forms an antigen-binding site that binds PD-L1. The protein may include (a) two polypeptides, each having an amino acid sequence consisting of the amino acid sequence of SEQ ID NO: 3, and (b) two additional polypeptides each having an amino acid sequence consisting of the amino acid sequence of SEQ ID NO: 1.

[0028] The disclosure also features a protein described above for use in therapy. The therapy may include administration of radiation or administration of a chemotherapeutic, a biologic, or a vaccine.

[0029] The disclosure also features a protein described above for use in promoting local depletion of TGFβ at a tumor.

[0030] The disclosure also features a protein described above for use in inhibiting SMAD3 phosphorylation in a cell (*e.g.*, a tumor cell or an immune cell).

[0031] The disclosure also features a protein described above for use in treating cancer or for use in inhibiting tumor growth. The cancer or tumor may be selected from the group consisting of colorectal, breast, ovarian, pancreatic, gastric, prostate, renal, cervical, myeloma, lymphoma, leukemia, thyroid, endometrial, uterine, bladder, neuroendocrine, head and neck, liver, nasopharyngeal, testicular, small cell lung cancer, non-small cell lung cancer, melanoma, basal cell skin cancer, squamous cell skin cancer, dermatofibrosarcoma protuberans, Merkel cell carcinoma, glioblastoma, glioma, sarcoma, mesothelioma, and myelodisplastic syndromes. The use may further include administration of radiation or administration of a chemotherapeutic, a biologic, or a vaccine.

[0032] The disclosure also features a method of promoting local depletion of TGFβ. The method includes administering a protein described above, where the protein binds TGFβ in solution, binds PD-L1 on a cell surface, and carries the bound TGFβ into the cell (*e.g.*, a cancer cell).

[0033] The disclosure also features a method of inhibiting SMAD3 phosphorylation in a cell (*e.g.*, a cancer cell or an immune cell), the method including exposing the cell in the tumor microenvironment to a protein described above.

[0034] The disclosure also features a method of inhibiting tumor growth or treating cancer. The method includes exposing the tumor to a protein described above. The method may further include exposing the tumor to radiation or to a chemotherapeutic, a biologic, or a vaccine. In certain examples, the tumor or cancer is selected from the group consisting of colorectal, breast, ovarian, pancreatic, gastric, prostate, renal, cervical, myeloma, lymphoma, leukemia, thyroid, endometrial, uterine, bladder, neuroendocrine, head and neck, liver, nasopharyngeal, testicular, small cell lung cancer, non-small cell lung cancer, melanoma, basal cell skin cancer, squamous cell skin cancer, dermatofibrosarcoma protuberans, Merkel cell carcinoma, glioblastoma, glioma, sarcoma, mesothelioma, and myelodisplastic syndromes.

[0035] By "TGFβRII" or "TGFβ Receptor II" is meant a polypeptide having the wild-type human TGFβ Receptor Type 2 Isoform A sequence (*e.g.*, the amino acid sequence of NCBI

WHAT IS CLAIMED IS:

1. A protein comprising:

a) human TGFβRII, or a fragment thereof capable of binding TGFβ; and

b) an antibody, or an antigen-binding fragment thereof, that binds human protein Programmed Death Ligand 1 (PD-L1).

2. A protein comprising a first polypeptide comprising:

a) at least a variable domain of a heavy chain of an antibody that binds human protein Programmed Death Ligand 1 (PD-L1); and

b) human TGFβRII, or a fragment thereof capable of binding TGFβ; and a second polypeptide comprising at least a variable region of a light chain of an antibody that binds PD-L1.

3. The protein of claim 2, wherein the first polypeptide further comprises an amino acid linker connecting the C-terminus of the heavy chain to the N-terminus of the human TGFβRII or fragment thereof.

4. The protein of claim 2 or claim 3, wherein the first polypeptide comprises an amino acid sequence of SEQ ID NO: 10.

5. The protein of any one of claims 2-4, wherein the first polypeptide comprises an HVR-H1 sequence, an HVR-H2 sequence, and an HVR-H3 sequence of SYIMM, SIYPSGGITFYADTVKG, and IKLGTVTTVDY, respectively, and wherein the second polypeptide comprises an HVR-L1 sequence, an HVR-L2 sequence, and an HVR-L3 sequence of TGTSSDVGGYNYVS, DVSNRPS, and SSYTSSSTRV, respectively.

6. The protein of any one of claims 2-5, wherein the first polypeptide comprises the amino acid sequence of SEQ ID NO: 2.

7. The protein of any one of claims 2-5, wherein the first polypeptide comprises the amino acid sequence of SEQ ID NO: 3.

8. The protein of any one of claims 2-7, wherein the second polypeptide comprises the amino acid sequence of SEQ ID NO: 1.

9. The protein of any one of claims 2-5 and 7 wherein the first polypeptide comprises the amino acid sequence of SEQ ID NO: 3 and the second polypeptide comprises the amino acid sequence of SEQ ID NO: 1.

10. A nucleic acid comprising a first nucleotide sequence encoding the first polypeptide and a second nucleotide sequence encoding the second polypeptide according to any one of claims 2-9.

11. A cell comprising the nucleic acid of claim 10.

12. A method of producing a protein comprising a) TGFβRII, or a fragment thereof capable of binding TGFβ, and b) an antibody, or an antigen-binding fragment thereof, that binds human protein Programmed Death Ligand 1 (PD-L1), the method comprising maintaining a cell according to claim 11 under conditions permitting expression of the protein, and optionally further comprising harvesting the protein.

13. The protein of claim 9, comprising two polypeptides each having an amino acid sequence consisting of the amino acid sequence of SEQ ID NO: 3, and two additional polypeptides each having an amino acid sequence consisting of the amino acid sequence of SEQ ID NO: 1.

14. A protein according to claim 1, 2 to 9, or 13, for use in therapy.

15. Use of a protein according to claim 1, 2 to 9 or 13 in the manufacture of a medicament for inhibiting tumor growth or treating cancer in a patient

16. The use according to claim 15, wherein the use further comprises that the patient is to be exposed to radiation and/or is to be administered a chemotherapeutic.

17. The use according to claim 15 or 16, wherein the cancer or tumor is selected from the group consisting of colorectal, breast, ovarian, pancreatic, gastric, prostate, renal, cervical, myeloma, lymphoma, leukemia, thyroid, endometrial, uterine, bladder, neuroendocrine, head and neck, liver, nasopharyngeal, testicular, small cell lung cancer, non-small cell lung cancer, melanoma, basal cell skin cancer, squamous cell skin cancer, dermatofibrosarcoma protuberans, Merkel cell carcinoma, glioblastoma, glioma, sarcoma, mesothelioma, and myelodisplastic syndromes.