some examples, the present disclosure provides IFN-γ-Inducible Regulatory T Cell Convertible Anti-Cancer (IRTCA) antibodies and/or antigen-binding fragments thereof, including: (a) a heavy chain CDR1 comprising a sequence of SEQ ID NO: 8 or 24, a heavy chain CDR2 comprising a sequence of SEQ ID NO: 9 or 25, and a heavy chain CDR3 comprising at least one sequence selected from SEQ ID NO: 10, 14, 15, 16, and 17, and (b) a light chain CDR1 comprising a sequence of SEQ ID NO: 11, a light chain CDR2 comprising a sequence of SEQ ID NO: 12, and a light chain CDR3 comprising a sequence of SEQ ID NO: 13 or 18, wherein the IRTCA antibody or antigen-binding fragment thereof does not comprise each of a heavy chain CDR1 comprising a sequence of SEQ ID NO: 8, a heavy chain CDR2 comprising a sequence of SEQ ID NO: 9, a heavy chain CDR3 comprising a sequence of SEQ ID NO: 10, a light chain CDR1 comprising a sequence of SEQ ID NO: 11, a light chain CDR2 comprising a sequence of SEQ ID NO: 12 and a light chain CDR3 comprising a sequence of SEQ ID NO: 13.

[0005] In some examples, the present disclosure further provides IRTCA antibodies or antigen-binding fragments wherein the antibody or antigen-binding fragment include any one of the following: (a) a heavy chain variable domain comprising a sequence at least 90% identical to a sequence selected from SEQ ID NOs: 3, 4, 5, 6, 20, and 21; (b) a light chain variable domain comprising a sequence at least 90% identical to a sequence selected from SEQ ID NOs: 7, 22, and 23; or (c) a heavy chain variable domain comprising a sequence at least 90% identical to a sequence selected from SEQ ID NOs: 3, 4, 5, 6, 20, and 21 and a light chain variable domain comprising a sequence at least 98% identical to a sequence selected from SEQ ID NOs: 7, 22, and 23.

[0006] In some examples, the present disclosure also provides IRTCA antibodies or antigen-binding fragments wherein the antibody or antigen-binding fragment comprises any one of the following: (a) a heavy chain variable domain comprising a sequence at least 98% identical to a sequence selected from SEQ ID NOs: 3, 4, 5, 6, 20, and 21; (b) a light chain variable domain comprising a sequence at least 98% identical to a sequence selected from SEQ ID NOs: 7, 22, and 23; or (c) a heavy chain variable domain comprising a sequence at least 98% identical to a sequence selected from SEQ ID NOs: 3, 4, 5, 6, 20, and 21 and a light chain variable domain comprising a sequence at least 98% identical to a sequence selected from SEQ ID NOs: 7, 22, and 23.

[0007] In some examples, a provided IRTCA antibody or antigen-binding fragment includes any one of the following: (a) a heavy chain variable domain comprising a sequence selected from SEQ ID NOs: 3, 4, 5, 6, 20, and 21; (b) a light chain variable domain comprising a sequence selected from SEQ ID NOs: 7, 22, and 23; or (c) a heavy chain variable domain comprising a sequence selected from SEQ ID NOs: 3, 4, 5, 6, 20, and 21 and a light chain variable domain comprising a sequence selected from SEQ ID NOs: 7, 22, and 23.

[0008] In some examples, an IRTCA antibody or antigen-binding fragment thereof does not include each of a heavy chain CDR1 comprising a sequence of SEQ ID NO: 8, a heavy chain CDR2 comprising a sequence of SEQ ID NO: 9, a heavy chain CDR3 comprising a sequence of SEQ ID NO: 10, a light chain CDR1 comprising a sequence of SEQ ID NO: 11, a light chain CDR2 comprising a sequence of SEQ ID NO: 12 and a light chain CDR3 comprising a sequence of SEQ ID NO: 13

[0009] In accordance with any of a variety of examples, provided IRTCA antibodies or antigen-binding fragments thereof, and/or provided compositions may exhibit a range of binding affinities. For instance, in some examples, a provided IRTCA antibody or antigen-binding fragment has a binding affinity (K_D) for a human Activation-Inducible Tumor Necrosis Factor Receptor (TNFR) Family Receptor (AITR) molecule of $1x10^{-7}$ to $1x10^{-12}$ M. In some examples, a provided IRTCA antibody or antigen-binding fragment binds to an epitope within the extracellular domain of human AITR polypeptide. In some examples, an epitope within the extracellular domain of human AITR polypeptide comprises SEQ ID NO: 19.

[0010] In some examples, a provided antibody or antigen-binding fragment is or comprises a humanized antibody. In some examples, a provided IRTCA antibody or antigen-binding fragment thereof is or comprises a monoclonal antibody. In some examples, a provided IRTCA antibody or antigen-binding fragment thereof includes an immunoglobulin constant domain, wherein the constant domain is selected from an IgG1 or a variant thereof, an IgG2 or a variant thereof, an IgG4 or a variant thereof, an IgA or a variant thereof, an IgE or a variant thereof, an IgM or a variant thereof, and an IgD or a variant thereof. In some examples, a provided IRTCA antibody or antigen-binding fragment thereof is or comprises a human IgG1. In some examples, the IgG1 is or comprises a sequence that is at least 95% identical to SEQ ID NO: 26. In some examples, a provided IRTCA antibody or antigen-

binding fragment thereof is or comprises a Fab fragment, a Fab' fragment, a F(ab')2 fragment, a Fv fragment, a disulfide-bonded Fv fragment, a scFv fragment, a single domain antibody, humabody, nanobody, or a diabody.

[0011] In accordance with various examples, the present disclosure provides a variety of molecules and compositions for, *inter alia*, facilitating delivery and/or expression of a provided composition that comprises an amount of a provided IRTCA antibody or antigen-binding fragment thereof. For instance, in some examples, the present disclosure provides nucleic acid molecules encoding an IRTCA antibody or antigen-binding fragment thereof. In some examples, the present disclosure also provides recombinant vectors including such nucleic acid molecules. In some examples, the present disclosure also provides host cells including a provided recombinant vector and/or a provided nucleic acid molecule. In some examples, a host cell may be selected from a bacterial, yeast, insect or mammalian cell. In some examples a host cell is selected from the group consisting of *E.coli*, *P.pastoris*, Sf9, COS, HEK293, Expi293, CHO-S, CHO-DG44, CHO-K1, and a mammalian lymphocyte.

[0012] In some examples, the present disclosure provides pharmaceutical compositions including: (a) one or more provided IRTCA antibodies or antigen-binding fragments thereof, one or more provided nucleic acid molecules, one or more provided recombinant vectors, and/or one or more provided host cells, and (b) a pharmaceutically acceptable carrier. Any of a variety of pharmaceutically acceptable carriers may be used in accordance with various examples.

[0013] In addition to the variety of powerful new antibodies, antigen-binding fragments thereof, and compositions provided herein, the present disclosure also provides, in various examples, a variety of new therapeutic methods as well. For example, and in accordance with various examples, the present disclosure provides methods of treating a subject in need thereof, the method including the step of: administering to the subject a composition that comprises or delivers a provided IRTCA antibody or antigen-binding fragment thereof, a provided nucleic acid molecule, and/or a provided recombinant vector.

[0013a] In one aspect, the present disclosure refers to use of an IFN-γ-Inducible Regulatory T Cell Convertible Anti-Cancer (IRTCA) antibody or antigen-binding fragment thereof in the manufacture of a medicament for treating cancer, wherein the IRTCA antibody comprises:

> (a) a heavy chain CDR1 comprising a sequence of SEQ ID NO: 8 or 24, a heavy chain CDR2 comprising a sequence of SEQ ID NO: 9 or 25, and a heavy chain CDR3 comprising at least one sequence selected from SEQ ID NO: 14, 15, 16, and 17; and
> (b) a light chain CDR1 comprising a sequence of SEQ ID NO: 11, a light chain CDR2 comprising a sequence of SEQ ID NO: 12 and a light chain CDR3 comprising a sequence of SEQ ID NO: 13 or 18,
> wherein the IRTCA antibody or antigen-binding fragment thereof does not comprise each of a heavy chain CDR1 comprising a sequence of SEQ ID NO: 8, a heavy chain CDR2 comprising a sequence of SEQ ID NO: 9, a heavy chain CDR3 comprising a sequence of SEQ ID NO: 10, a light chain CDR1 comprising a sequence of SEQ ID NO: 11, a light chain CDR2 comprising a sequence of SEQ ID NO: 12 and a light chain CDR3 comprising a sequence of SEQ ID NO: 13.

[0014] By way of additional example, the present disclosure also provides, methods of inducing an immune response in a subject in need thereof, the method

including the step of: administering to the subject a composition that comprises or delivers a provided IRTCA antibody or antigen-binding fragment thereof, a provided nucleic acid molecule, and/or a provided recombinant vector.

[0015] By way of further example, the present disclosure also provides methods of enhancing an immune response or increasing the activity of an immune cell in a subject in need thereof, the method including the step of: administering to the subject a composition that comprises or delivers a provided IRTCA antibody or antigen-binding fragment thereof, a provided nucleic acid molecule, and/or a provided recombinant vector.

[0016] In some examples, the present disclosure also provides methods for increasing secretion of IFN-γ by a T cell *in vivo* or *in vitro*, the method comprising: contacting the cell with a composition that comprises or delivers an amount of a provided IRTCA antibody or antigen-binding fragment thereof. In some examples, the present disclosure also provides methods for decreasing secretion of TGF-β by a T cell *in vivo* or *in vitro*, the method comprising: contacting the cell with a composition that comprises or delivers an amount of a provided IRTCA antibody or antigen-binding fragment thereof. In some examples, the present disclosure also provides methods for converting a T cell into a Type 1 helper T (T_H1) cell, the method comprising: contacting the cell with a composition that comprises or delivers an amount of a provided IRTCA antibody or antigen-binding fragment thereof.

[0017] In some examples, the subject has, or is at risk for developing, cancer. In some examples, the cancer is selected from a bladder cancer, breast cancer, cervical cancer, colon cancer, endometrial cancer, esophageal cancer, fallopian tube cancer, gall bladder cancer, gastrointestinal cancer, head and neck cancer, hematological cancer, laryngeal cancer, liver cancer, lung cancer, lymphoma, melanoma, mesothelioma, ovarian cancer, primary peritoneal cancer, salivary gland cancer, sarcoma, stomach cancer, thyroid cancer, pancreatic cancer, renal cell carcinoma, glioblastoma, and prostate cancer.

[0018] It is specifically contemplated that various examples are suitable for us in one or more combination therapy regimen. For instance, in some examples, the subject has been administered or will be administered one or more additional anticancer therapies. In some examples, the one or more additional cancer therapies is selected from ionizing radiation, a

chemotherapeutic agent, an antibody agent, and a cell-based therapy, such that the subject receives treatment with both. In some examples, the one or more additional anticancer therapies comprise an immune checkpoint inhibitor, IL-12, GM-CSF, an anti-CD4 agent, cisplatin, fluorouracil, doxorubicin, irinotecan, paclitaxel, indoleamine 2,3-dioxygenase-1 (IDO1) inhibitor, or cyclophosphamide.

[0019] Various examples of the present disclosure may also be useful for determining an advantageous dosing regimen for one or more provided IRTCA antibodies and/or antigen binding fragments thereof, nucleic acid molecules, recombinant vectors, and/or host cells. For instance, in some examples, the present disclosure provides methods of determining a dose of an IRTCA antibody or antigen binding fragment thereof for therapeutic treatment of a subject in need thereof, the method including the steps of: (a) providing or obtaining a measurement of secreted IFN-γ in a biological sample from the subject, wherein the subject has been administered a composition that comprises or delivers an amount of a provided IRTCA antibody or antigen-binding fragment thereof, and (b) comparing the measurement of secreted IFN-γ to a reference value, wherein if the measurement of secreted IFN-γ is higher or lower than the reference value, adjusting the amount of the IRTCA antibody or antigen binding fragment thereof to be administered, thereby determining a dose for therapeutic treatment of a subject.

[0020] In some examples, a reference value may be a level of IFN-γ in a biological sample from the subject prior to administration of the provided IRTCA antibody or antigen-binding fragment thereof. In some examples, if a measured amount of secreted IFN-γ in a biological sample from the subject is higher than a reference value, then treatment is either maintained at the same level given previously, or treatment is ceased for a period of time.

[0021] By way of additional example, in some examples, the present disclosure provides methods of determining a dose of an IRTCA antibody or antigen binding fragment thereof for therapeutic treatment of a subject in need thereof, including the steps of: (a) providing or obtaining a measurement of T_{reg} cell population in a biological sample from the subject, wherein the subject has been administered a composition that comprises or delivers an amount of a provided IRTCA antibody or antigen-binding fragment thereof; and (b) comparing the measurement of T_{reg} cell population to a reference value, wherein if the measurement of T_{reg} cell population is higher or lower than the reference value, adjusting the amount of the IRTCA

antibody or antigen binding fragment thereof to be administered, thereby determining a dose for therapeutic treatment of a subject. In some examples, a reference value may be a level of T_{reg} cell population in a biological sample from the subject prior to administration of the provided IRTCA antibody or antigen-binding fragment thereof. In some examples, if a measured amount of T_{reg} cell population in a biological sample from the subject is lower than a reference value, then treatment is either maintained at the same level given previously, or treatment is ceased for a period of time.

[0022] By way of additional example, in some examples, the present disclosure provides methods of determining a dose of an IRTCA antibody or antigen binding fragment thereof for therapeutic treatment of a subject in need thereof, including the steps of: (a) providing or obtaining a measurement of IFN-γ-secreting T cell population in a biological sample from the subject, wherein the subject has been administered a composition that comprises or delivers an amount of a provided IRTCA antibody or antigen-binding fragment thereof; (b) providing or obtaining a measurement of T_{reg} cell population in a biological sample from the subject, wherein the subject has been administered a composition that comprises or delivers an amount of a provided IRTCA antibody or antigen-binding fragment thereof; (c) calculating the ratio of the measurement of IFN-γ-secreting T cell population to the measurement of T_{reg} cell population; and (d) comparing the ratio to a reference value, wherein if ratio is higher or lower than the reference value, adjusting the amount of the IRTCA antibody or antigen binding fragment thereof to be administered, thereby determining a dose for therapeutic treatment of a subject. In some examples, a reference value may be a ratio of a level of IFN-γ-secreting T cell population in a biological sample from a subject to a level of T_{reg} cell population in the same biological sample from the subject prior to administration of the provided IRTCA antibody or antigen-binding fragment thereof. In some examples, if a calculated ratio in a biological sample from the subject is higher than a reference value, then treatment is either maintained at the same level given previously, or treatment is ceased for a period of time.

[0023] In some examples, a reference value comprises an index value which includes a value derived from one or more healthy subjects or a value derived from one or more cancer-diagnosed subjects. In some examples, a biological sample is a sample of whole blood, plasma, tumor tissue, or serum.

[0024] In some examples, the present disclosure also provides methods of validating affinity of an antibody or antigen-binding fragment thereof for AITR, the method including the steps of: contacting a T cell with a composition that comprises or delivers an amount of a provided IRTCA antibody or antigen-binding fragment thereof, and measuring cytokine secretion from the T cell, wherein cytokine secretion correlates with immune response enhancement, anti-cancer effects and/or anti-tumor effects of the IRTCA antibody. In some examples, the T cell expresses AITR protein. In some examples, the T cell is a regulatory T cell (T_{reg} cell). In some examples, the T cell is an effector T cell (T_{eff} cell).

[0025] As used in this application, the terms "about" and "approximately" are used as equivalents. Any citations to publications, patents, or patent applications herein are incorporated by reference in their entirety. Any numerals used in this application with or without about/approximately are meant to cover any normal fluctuations appreciated by one of ordinary skill in the relevant art.

[0026] Other features, objects, and advantages of the present disclosure are apparent in the detailed description that follows. It should be understood, however, that the detailed description, while indicating examples of the present disclosure, is given by way of illustration only, not limitation. Various changes and modifications within the scope of the disclosure will become apparent to those skilled in the art from the detailed description.

BRIEF DESCRIPTION OF THE DRAWING

[0027] The Drawing included herein, which is comprised of the following Figures, is for illustration purposes only and not for limitation. The foregoing and other objects, aspects, features, and advantages of the present disclosure will become more apparent and better understood by referring to the following description taken in conjunction with the accompanying figures in which:

[0028] **FIG. 1** shows Fab type amino acid sequences of IRTCA-A, wherein a and b respectively represent the amino acid sequence of the IRTCA-A Fab type and the IRTCA-A amino acid sequence into which a stop codon is inserted (asterisk indicates stop codon).

[0029] **FIG. 2** shows the amino acid regions of IRTCA-A in which randomized PCR configuration and mutation were induced. Panel a shows the IRTCA-A light chain, and panel b

CLAIMS

What is claimed is:

1. Use of an IFN-γ-Inducible Regulatory T Cell Convertible Anti-Cancer (IRTCA) antibody or antigen-binding fragment thereof in the manufacture of a medicament for treating cancer, wherein the IRTCA antibody comprises:

(a) a heavy chain CDR1 comprising a sequence of SEQ ID NO: 8 or 24, a heavy chain CDR2 comprising a sequence of SEQ ID NO: 9 or 25, and a heavy chain CDR3 comprising at least one sequence selected from SEQ ID NO: 14, 15, 16, and 17; and

(b) a light chain CDR1 comprising a sequence of SEQ ID NO: 11, a light chain CDR2 comprising a sequence of SEQ ID NO: 12 and a light chain CDR3 comprising a sequence of SEQ ID NO: 13 or 18,

wherein the IRTCA antibody or antigen-binding fragment thereof does not comprise each of a heavy chain CDR1 comprising a sequence of SEQ ID NO: 8, a heavy chain CDR2 comprising a sequence of SEQ ID NO: 9, a heavy chain CDR3 comprising a sequence of SEQ ID NO: 10, a light chain CDR1 comprising a sequence of SEQ ID NO: 11, a light chain CDR2 comprising a sequence of SEQ ID NO: 12 and a light chain CDR3 comprising a sequence of SEQ ID NO: 13.

2. The use of claim 1, wherein the IRTCA antibody comprises:

(a) a heavy chain variable domain comprising a sequence at least 90% identical to a sequence selected from SEQ ID NOs: 3, 4, 5, 6, 20, and 21;

(b) a light chain variable domain comprising a sequence at least 90% identical to a sequence selected from SEQ ID NOs: 7, 22, and 23; or

(c) a heavy chain variable domain comprising a sequence at least 90% identical to a sequence selected from SEQ ID NOs: 3, 4, 5, 6, 20, and 21 and a light chain variable domain comprising a sequence at least 90% identical to a sequence selected from SEQ ID NOs: 7, 22, and 23.

3. The use of any of claims 1-2, wherein the IRTCA antibody comprises:

(a) a heavy chain variable domain comprising a sequence at least 98% identical to a sequence selected from SEQ ID NOs: 3, 4, 5, 6, 20, and 21;
(b) a light chain variable domain comprising a sequence at least 98% identical to a sequence selected from SEQ ID NOs: 7, 22, and 23; or
(c) a heavy chain variable domain comprising a sequence at least 98% identical to a sequence selected from SEQ ID NOs: 3, 4, 5, 6, 20, and 21 and a light chain variable domain comprising a sequence at least 98% identical to a sequence selected from SEQ ID NOs: 7, 22, and 23.

4. The use of any of claims 1-3, wherein the IRTCA antibody comprises:
(a) a heavy chain variable domain comprising a sequence selected from SEQ ID NOs: 3, 4, 5, 6, 20, and 21;
(b) a light chain variable domain comprising a sequence selected from SEQ ID NOs: 7, 22, and 23; or
(c) a heavy chain variable domain comprising a sequence selected from SEQ ID NOs: 3, 4, 5, 6, 20, and 21 and a light chain variable domain comprising a sequence selected from SEQ ID NOs: 7, 22, and 23.

5. The use of any of claims 1-4, wherein the IRTCA antibody includes an immunoglobulin constant domain, wherein the constant domain is selected from an IgG1 or a variant thereof, an IgG2 or a variant thereof, an IgG4 or a variant thereof, an IgA or a variant thereof, an IgE or a variant thereof, an IgM or a variant thereof, and an IgD or a variant thereof.

6. The use of any of claims 1-5, wherein the IgG1 is or comprises a sequence that is at least 95% identical to SEQ ID NO: 26.

7. The use of any of claims 1-6, wherein the cancer is selected from a bladder cancer, breast cancer, cervical cancer, colon cancer, endometrial cancer, esophageal cancer, fallopian tube cancer, gall bladder cancer, gastrointestinal cancer, head and neck cancer, hematological cancer, laryngeal cancer, liver cancer, lung cancer, lymphoma, melanoma, mesothelioma,

ovarian cancer, primary peritoneal cancer, salivary gland cancer, sarcoma, stomach cancer, thyroid cancer, pancreatic cancer, renal cell carcinoma, glioblastoma, and prostate cancer.

8. The use of any of claims 1-7, wherein the medicament further comprises one or more additional anticancer therapies selected from ionizing radiation, a chemotherapeutic agent, an antibody agent, and a cell-based therapy.

9. The use of any of claims 1-8, wherein the one or more additional anticancer therapies comprise an immune checkpoint inhibitor, IL-12, GM-CSF, an anti-CD4 agent, cisplatin, fluorouracil, doxorubicin, irinotecan, paclitaxel, indoleamine 2,3-dioxygenase-1 (IDO1) inhibitor, or cyclophosphamide.

10. The use of any of claims 1-9, wherein treating the subject comprises inducing an immune response.

11. The use of any of claims 1-10, wherein treating the subject comprises enhancing an immune response.

12. The use of any of claims 1-11, wherein treating the subject comprises increasing secretion of IFN-γ by a T cell *in vivo* or *in vitro*.

13. The use of any of claims 1-12, wherein treating the subject comprises decreasing secretion of TGF-β by a T cell *in vivo* or *in vitro*.

14. The use of any of claims 1-13, wherein treating the subject comprises converting a T cell into a Type 1 helper T cell (T_H1).