METHODS OF TREATING CANCER USING ANTI-CD96/ANTI-TIGIT ANTIBODIES AND COMBINATION THERAPIES

RELATED APPLICATIONS

[0001] This application claims benefit to U.S. Provisional Application No. 63/561,131, filed on March 4, 2024, the entirety of which is herein incorporated by reference.

SEQUENCE LISTING

[0002] The content of the electronically submitted Sequence Listing XML (Name:216601_SL.xml; Size: 786,201 bytes; Created on March 3, 2025) is incorporated by reference herein in its entirety.

FIELD

[0003] The present disclosure relates to methods of treating cancer using multispecific molecules (*e.g.*, antibodies) that specifically bind to human CD96 and human TIGIT, and combination therapies with PD-1 inhibitors.

BACKGROUND

[0004] CD96 (Cluster of Differentiation 96), also known as TACTILE (T cell-activation, increased late expression), is a type I transmembrane protein in the immunoglobulin (Ig) superfamily. It has a single Ig domain, a type I transmembrane domain, a single intracellular immunoreceptor tyrosine-based inhibitory motif (ITIM), and a single YXXM phosphorylation motif, and is expressed on the surface of T cells and natural killer (NK) cells. CD96 is believed to play a role in the regulation of immune cells (*e.g.*, NK cells and T cells) and tumor metastasis. In particular, it has been shown that blockade of CD96 function suppressed primary tumor growth in several mouse tumor models in a CD8+ T cell-dependent manner.

[0005] The protein T-cell immunoreceptor with Ig and ITIM domains (TIGIT), also known as VSIG9 or VSTM3, is a type I transmembrane protein in the immunoglobulin (Ig) superfamily. It has a single Ig domain, a type I transmembrane domain, a single intracellular immunoreceptor tyrosine-based inhibitory motif (ITIM) and a single immunoglobulin tail tyrosine (ITT)-like phosphorylation motif and is expressed on activated CD4-positive/CD25-positive regulatory T cells (Tregs), memory CD45RO-positive T cells, and natural killer (NK) cells, but not naïve T cells.

[0006] CD155 (also known as poliovirus receptor (PVR)) is highly expressed on monocytes and dendritic cells, and is capable of activating effector T cells and NK cells, as

well as attenuating the activity of Tregs, through binding to its two receptors CD226 and CD96. TIGIT binds to CD155 and has been shown to antagonize the interaction of CD155 with CD226 and CD96, thereby suppressing T cell- and NK cell-mediated immune activity.

[0007] PD-1 is an inhibitory receptor that is expressed on activated B cells, T cells, and myeloid cells (Agata et al. (1996) Int Immunol 8:765-72; Okazaki et al. (2002) Curr. Opin. Immunol. 14: 391779-82; Bennett et al. (2003) J Immunol 170:711-8). Two ligands for PD-1 have been identified, PD-L1 and PD-L2, that have been shown to downregulate T cell activation upon binding to PD-1 (Freeman et al. (2000) J Exp Med 192:1027-34; Latchman et al. (2001) Nat Immunol 2:261-8; Carter et al. (2002) Eur J Immunol 32:634-43). The interaction between PD-1 and PD-L1 results in a decrease in tumor infiltrating lymphocytes, a decrease in T cell receptor mediated proliferation, and immune evasion by the cancerous cells (Dong et al. (2003) J. Mol. Med. 81:281-7; Blank et al. (2005) Cancer Immunol. Immunother. 54:307-314; Konishi et al. (2004) Clin. Cancer Res. 10:5094-100). This immune suppression can be reversed by inhibiting the local interaction of PD-1 with PD-L1 (Iwai et al. (2002) Proc. Nat'l. Acad. Sci. USA 99:12293-7; Brown et al. (2003) J. Immunol. 170:1257-66).

[0008] Given the important role of CD96, TIGIT, and PD-1 in modulating immune responses, therapies designed to antagonize signaling from these receptors hold great promise for enhancing anticancer immunity and effectively treating cancer.

SUMMARY

[0009] The present disclosure provides methods for treatment of cancer using multispecific molecules (*e.g.*, antibodies) that specifically bind to human CD96 and human TIGIT, and combinations of these antibodies with human PD-1 inhibitors. These methods are particularly advantageous in that the combination of anti-human CD96/TIGIT multispecific molecules and PD-1 inhibitors described herein potently increase immune cell responsiveness and activation, effectively enhancing anticancer immunity and overcoming tumor immunosuppression to treat cancer.

[0010] Accordingly, in one aspect, the present disclosure provides a method of treating cancer in a subject in need thereof, the method comprising administering to the subject: (a) a multispecific molecule at a dose of about 2 mg to about 2000 mg, wherein the multispecific molecule comprises: (i) a first antigen-binding region that specifically binds to human CD96, the first antigen-binding region comprising a first heavy chain variable region (VH) comprising CDRs CDRH1, CDRH2, and CDRH3, and a first light chain variable region (VL) comprising CDRs CDRL1, CDRL2, and CDRL3; wherein the first VH comprises the CDRH1, CDRH2,

and CDRH3 amino acid sequences of the VH amino acid sequence of SEQ ID NO: 7; and the first VL comprises the CDRL1, CDRL2, and CDRL3 amino acid sequences of the VL amino acid sequence of SEQ ID NO: 8; and (ii) a second antigen-binding region that specifically binds to human TIGIT, the second antigen-binding region comprising a second VH comprising CDRs CDRH1, CDRH2, and CDRH3, and a second VL comprising CDRs CDRL1, CDRL2, and CDRL3; wherein the second VH comprises the CDRH1, CDRH2, and CDRH3 amino acid sequences of the VH amino acid sequence of SEQ ID NO: 17; and the second VL comprises the CDRL1, CDRL2, and CDRL3 amino acid sequences of the VL amino acid sequence of SEQ ID NO: 18; and (b) a human PD-1 inhibitor.

[0011] In certain embodiments, the multispecific molecule is administered at a dose of about 20 mg to about 1200 mg. In certain embodiments, the multispecific molecule is administered at a dose of about 20 mg, about 60 mg, about 200 mg, about 600 mg, or about 1200 mg.

[0012] In certain embodiments, the multispecific molecule is administered intravenously. In certain embodiments, the multispecific molecule is administered by intravenous infusion over about 30 minutes. In certain embodiments, the multispecific molecule is administered by intravenous infusion over about 60 minutes. In certain embodiments, the multispecific molecule is administered by intravenous infusion over about 90 minutes.

[0013] In certain embodiments, the multispecific molecule is administered once weekly. In certain embodiments, the multispecific molecule is administered once every 2 weeks. In certain embodiments, the multispecific molecule is administered once every 3 weeks. In certain embodiments, the multispecific molecule is administered once every 4 weeks. In certain embodiments, the multispecific molecule is administered once every 5 weeks. In certain embodiments, the multispecific molecule is administered once every 6 weeks.

[0014] In certain embodiments, the multispecific molecule is administered intravenously at a dose of 20 mg once every 3 weeks. In certain embodiments, the multispecific molecule is administered intravenously at a dose of 60 mg once every 3 weeks. In certain embodiments, the multispecific molecule is administered intravenously at a dose of 200 mg once every 3 weeks. In certain embodiments, the multispecific molecule is administered intravenously at a dose of 600 mg once every 3 weeks. In certain embodiments, the multispecific molecule is administered intravenously at a dose of 1200 mg once every 3 weeks.

[0015] In certain embodiments, the cancer is a solid tumor. In certain embodiments, the solid tumor is metastatic or locally advanced. In certain embodiments, the cancer is selected from the group consisting of non-small cell lung cancer (NSCLC), gastric cancer,

gastroesophageal junction (GEJ) cancer, and squamous cell carcinoma of head and neck (SCCHN).

[0016] In certain embodiments, before administration of the multispecific molecule: (a) the subject is at least 18 years of age; (b) the subject has a histologically or cytologically confirmed diagnosis of metastatic or locally advanced solid tumor for which no acceptable standard therapy is available or progressed on or after standard therapies; (c) the subject has measurable disease as assessed on baseline imaging according to Response Evaluation Criteria in Solid Tumors Version 1.1 (RECIST 1.1) (d) the subject has a predicted life expectancy of at least 3 months and an Eastern Cooperative Oncology Group performance status of 0 or 1; and/or (e) the subject is not pregnant.

[0017] In certain embodiments, the first antigen-binding region comprises the CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3 amino acid sequences set forth in SEQ ID NOs: 1, 2, 3, 4, 5, and 6, respectively, and the second antigen-binding region comprises the CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3 amino acid sequences set forth in SEQ ID NOs: 11, 12, 13, 14, 15, and 16, respectively. In certain embodiments, the first antigen-binding region comprises the VH amino acid sequence of SEQ ID NO: 7 and the VL amino acid sequence of SEQ ID NO: 8, and the second antigen-binding region comprises the VH amino acid sequence of SEQ ID NO: 17 and the VL amino acid sequence of SEQ ID NO: 18. In certain embodiments, the first antigen-binding region and/or the second antigen-binding region comprises a heavy chain constant region selected from the group consisting of human IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, and IgA_2. In certain embodiments, (a) the first antigen-binding region comprises a first human IgG heavy chain constant region comprising aspartate, serine, alanine, and valine at amino acid positions 239, 366, 368, and 407, respectively, and the second antigen-binding region comprises a second human IgG heavy chain constant region comprising aspartate, leucine, glutamate, and tryptophan at amino acid positions 239, 330, 332, and 366, respectively; or (b) the first antigen-binding region comprises a first human IgG heavy chain constant region comprising aspartate, leucine, glutamate, and tryptophan at amino acid positions 239, 330, 332, and 366, respectively, and the second antigen-binding region comprises a second human IgG heavy chain constant region comprising aspartate, serine, alanine, and valine at amino acid positions 239, 366, 368, and 407, respectively, wherein the amino acid positions are numbered according to the EU numbering system. In certain embodiments, (a) the first antigen-binding region comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 9, and/or a light chain comprising the amino acid sequence of SEQ ID NO: 10; and/or (b) the second antigen-binding region comprises a heavy

chain comprising the amino acid sequence of SEQ ID NO: 19, and/or a light chain comprising the amino acid sequence of SEQ ID NO: 20. In certain embodiments, the first antigen-binding region comprises a heavy chain and a light chain comprising the amino acid sequences of SEQ ID NOs: 9 and 10, respectively, and the second antigen-binding region comprises a heavy chain and a light chain comprising the amino acid sequences of SEQ ID NOs: 19 and 20, respectively.

[0018] In certain embodiments, the human PD-1 inhibitor is a second antibody that specifically binds to human PD-1 or human PD-L1. In certain embodiments, the second antibody is selected from the group consisting of nivolumab, balstilimab, adebrelimab, atezolizumab, avelumab, camrelizumab, cemiplimab, cosibelimab, dostarlimab, durvalumab, enlonstobart, envafolimab, pembrolizumab, penpulimab, pidilizumab, prolgolimab, pucotenlimab, retifanlimab, serplulimab, sintilimab, socazolimab, sugemalimab, tagitanlimab, tislelizumab, toripalimab, and zimberelimab. In certain embodiments, the antibody is nivolumab or atezolizumab.

[0019] In certain embodiments, the method further comprises administering to the subject a chemotherapeutic agent. In certain embodiments, the chemotherapeutic agent is selected from the group consisting of docetaxel, pemetrexed, carboplatin, paclitaxel, and combinations thereof.

[0020] In another aspect, the present disclosure provides a multispecific molecule that specifically binds to human CD96 and human TIGIT for use in the treatment of cancer, wherein the treatment is performed according to a method described herein.

[0021] In another aspect, the present disclosure provides a multispecific molecule that specifically binds to human CD96 and human TIGIT for use in the manufacture of a medicament for the treatment of cancer, wherein the treatment is performed according to a method described herein.

[0022] In another aspect, the present disclosure provides use of a multispecific molecule that specifically binds to human CD96 and human TIGIT for the treatment of cancer, wherein the treatment is performed according to a method described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0023] **FIG. 1A** is a schematic of the superantigen T cell: APC priming assay described in Example 1. **FIG. 1B** is a graph showing the IL-2 cytokine level (pg/mL) produced from stimulation of primary healthy donor human PBMCs from 2 individual donors treated with SEA superantigen together with anti-CD96/anti-TIGIT bispecific antibody AGEN1777, anti-PD-1 antagonist balstilimab, a combination, or isotype control. **FIG. 1C-FIG. 1D** are graphs

showing IL-2 cytokine level (pg/mL) produced from stimulation of primary healthy donor human PBMCs from 2 individual donors treated with SEA superantigen together with AGEN1777, anti-TIGIT antibody tiragolumab, either alone or in combination with anti-PD-1 antibody nivolumab or anti-PD-L1 antibody atezolumab, or relevant isotype control antibody.

[0024] **FIG. 2A** is a graph showing tumor volume over time in mice injected with CT26 colorectal carcinoma cells and treated intraperitoneally with AGEN1777 mouse surrogate antibody (AGEN1777^{ms}) alone or in combination with anti-PD-1 antibody (RMP1-14), anti-TIGIT mAb (Clone 10A7 mouse IgG2a), anti-CD96 mAb (Clone 6A6 mouse IgG2a), or isotype control bispecific antibody twice a week for two weeks post-tumor implantation. n=8 mice/group. **FIG. 2B** is a series of graphs showing individual tumor volume across the treatment groups in FIG. 2A. Mean tumor size at the start of treatment was approximately 45 mm^3. "CR x/y" indicates complete rejection, meaning the tumor volume reversed to zero mm^3 and were no longer palpable and did not reoccur, while "x" indicates number of CRs, and "y" is number of total mice used per treatment group. A 2-way ANOVA with repeated measures and adjusting p-values for multiple comparison was used to assess differences in tumor growth between treatment groups.

[0025] **FIG. 3A** is a graph showing tumor volume over time in mice injected with CT26 colorectal carcinoma cells and treated intraperitoneally with AGEN1777 mouse surrogate antibody (AGEN1777^{ms}) alone or in combination with anti-PD-1 antibody (RMP1-14), or isotype control bispecific antibody, twice a week for two weeks post-tumor implantation. **FIG. 3B** is a series of graphs showing individual tumor volume across the treatment groups in FIG. 3A. Mean tumor size at the start of treatment was approximately 100 mm^3. "CR x/y" indicates complete rejection, meaning the tumor volume reversed to zero mm^3 and were no longer palpable and did not reoccur, while "x" indicates number of CRs, and "y" is number of total mice used per treatment group. A 2-way ANOVA with repeated measures and adjusting p-values for multiple comparison was used to assess differences in tumor growth between treatment groups.

DETAILED DESCRIPTION

[0026] The present disclosure provides methods for treatment of cancer using multispecific molecules (*e.g.*, antibodies) that specifically bind to human CD96 and human TIGIT, and combinations of these molecules with human PD-1 inhibitors. These methods are particularly advantageous in that the combination of anti-human CD96/TIGIT multispecific molecules and PD-1 inhibitors described herein potently increase immune cell responsiveness and activation,

effectively enhancing anticancer immunity and overcoming tumor immunosuppression to treat cancer.

[0027] As used herein, the terms "about" and "approximately," when used to modify a numeric value or numeric range, indicate that deviations of 5% to 10% above and 5% to 10% below the value or range remain within the intended meaning of the recited value or range.

[0028] As used herein, the term "CD96" refers to Cluster of Differentiation 96, also known as TACTILE (T cell-activation, increased late expression), that in humans is encoded by the CD96 gene. As used herein, the term "human CD96" refers to a CD96 protein encoded by a wild-type human CD96 gene (*e.g.*, GenBank™ accession number NM_005816.5), a fragment, or a variant thereof. Exemplary amino acid sequences of immature human CD96 protein are provided as in RefSeq accession numbers NP_937839.1 (isoform 1) and NP_005807.1 (isoform 2).

[0029] As used herein, the term "TIGIT" refers to T-cell immunoreceptor with Ig and ITIM domains (also known as VSIG9 or VSTM3) that in humans is encoded by the TIGIT gene. As used herein, the term "human TIGIT" refers to a TIGIT protein encoded by a wild-type human TIGIT gene (*e.g.*, GenBank™ accession number NM_173799.4) or an extracellular domain of such a protein, a fragment, or a variant thereof. An exemplary amino acid sequence of immature human TIGIT protein is provided as in RefSeq accession number NP_776160.2.

[0030] As used herein, the term "PD-1" refers to programmed cell death protein 1. As used herein, the term "human PD-1" refers to a human PD-1 protein encoded by a wild-type human PD-1 gene, *e.g.*, RefSeq accession number NM_005018.3. An exemplary immature amino acid sequence of human PD-1 is provided as in RefSeq accession number NP_005009.2.

[0031] As used herein, the term "PD-L1" refers to the programmed cell death ligand 1. As used herein, the term "human PD-L1" refers to a human PD-L1 protein encoded by a wild-type human PD-L1 gene, e.g., RefSeq accession number NM_014143.4. An exemplary immature amino acid sequence of human PD-L1 is provided as in RefSeq accession number NP_054862.1.

[0032] As used herein, the terms "antibody" and "antibodies" include full-length antibodies, antigen-binding fragments of full-length antibodies, and molecules comprising antibody CDRs, VH regions, and/or VL regions. Examples of antibodies include, without limitation, monoclonal antibodies, recombinantly produced antibodies, monospecific antibodies, multispecific antibodies (including bispecific antibodies), human antibodies, humanized antibodies, chimeric antibodies, immunoglobulins, synthetic antibodies, tetrameric antibodies comprising two heavy chain and two light chain molecules, an antibody light chain

monomer, an antibody heavy chain monomer, an antibody light chain dimer, an antibody heavy chain dimer, an antibody light chain-antibody heavy chain pair, intrabodies, heteroconjugate antibodies, antibody-drug conjugates, single domain antibodies, monovalent antibodies, single-chain antibodies or single-chain Fvs (scFv), camelized antibodies, affibodies, Fab fragments, $F(ab')_2$ fragments, disulfide-linked Fvs (sdFv), anti-idiotypic (anti-Id) antibodies (including, *e.g.*, anti-anti-Id antibodies), and antigen-binding fragments of any of the above. In certain embodiments, antibodies described herein refer to polyclonal antibody populations. Antibodies can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA, or IgY), any class (*e.g.*, IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, or IgA_2), or any subclass (*e.g.*, IgG_2a or IgG_2b) of immunoglobulin molecule. In certain embodiments, antibodies described herein are IgG antibodies, or a class (*e.g.*, human IgG_1 or IgG_4) or subclass thereof. In a specific embodiment, the antibody is a humanized antibody. In another specific embodiment, the antibody is a human antibody.

[0033] As used herein, "multispecific molecules" are molecules that comprise two or more antigen-binding regions that specifically bind to different antigens. Multispecific molecules can include, for example, multispecific antibodies.

[0034] As used herein, "antigen-binding region" refers to the portion of a multispecific molecule or an antibody which comprises the amino acid residues that confer on the multispecific molecule or antibody its specificity for an antigen. Examples of antigen-binding regions include antibody complementarity determining regions (CDR), heavy chain variable regions, light chain variable regions, heavy chains, light chains, and any fragment thereof. The antigen-binding region can be derived from any animal species, such as rodents (*e.g.*, mouse, rat, or hamster) and humans.

[0035] As used herein, the term "CDR" or "complementarity determining region" means the noncontiguous antigen combining sites found within the variable regions of heavy and light chain polypeptides. In certain embodiments, CDRs of an antibody disclosed herein are determined according to Kabat et al., J. Biol. Chem. 252, 6609-6616 (1977) and Kabat et al, Sequences of protein of immunological interest (1991), each of which is herein incorporated by reference in its entirety. In certain embodiments, CDRs of an antibody disclosed herein are determined according to the Chothia numbering scheme (see, e.g., Chothia C & Lesk AM, (1987), J Mol Biol 196: 901-917; Al-Lazikani B et al., (1997) J Mol Biol 273: 927-948; Chothia C et al., (1992) J Mol Biol 227: 799-817; Tramontano A et al., (1990) J Mol Biol 215(1): 175-82; and U.S. Patent No. 7,709,226, all of which are herein incorporated by reference in their entireties). In certain embodiments, CDRs of an antibody disclosed herein are determined according to MacCallum RM et al., (1996) J Mol Biol 262: 732-745, herein incorporated by

reference in its entirety. See also, e.g., Martin A. "Protein Sequence and Structure Analysis of Antibody Variable Domains," in Antibody Engineering, Kontermann and Dribel, eds., Chapter 31, pp. 422-439, Springer-Verlag, Berlin (2001), herein incorporated by reference in its entirety. In certain embodiments, CDRs of an antibody disclosed herein are determined according to the IMGT numbering system as described in: Lefranc M-P, (1999) The Immunologist 7: 132-136; Lefranc M-P et al, (1999) Nucleic Acids Res 27: 209- 212, each of which is herein incorporated by reference in its entirety; and Lefranc M-P et al, (2009) Nucleic Acids Res 37: D1006-D1012. In certain embodiments, CDRs of an antibody disclosed herein are determined according to the AbM numbering scheme, which refers to AbM hypervariable regions, which represent a compromise between the Kabat CDRs and Chothia structural loops and are used by Oxford Molecular's AbM antibody modeling software (Oxford Molecular Group, Inc.), herein incorporated by reference in its entirety. In certain embodiments, CDRs of an antibody disclosed herein are determined according to the AHo numbering system, as described in Honegger and Pluckthun, J. Mol. Biol. 309:657-670 (2001), herein incorporated by reference in its entirety. In certain embodiments, CDRs of an antibody disclosed herein are each independently determined according to one of the Kabat, Chothia, MacCallum, IMGT, AHo, or AbM numbering schemes, or by structural analysis of the multispecific molecule, wherein the structural analysis identifies residues in the variable region(s) predicted to make contact with an epitope. CDRH1, CDRH2, and CDRH3 denote the heavy chain CDRs, and CDRL1, CDRL2, and CDRL3 denote the light chain CDRs.

[0036] As used herein, the terms "VH" and "VL" refer to antibody heavy and light chain variable regions, respectively, as described in Kabat *et al.*, (1991) Sequences of Proteins of Immunological Interest (NIH Publication No. 91-3242, Bethesda), which is herein incorporated by reference in its entirety.

[0037] As used herein, the term "constant region" is common in the art. The constant region is an antibody portion, *e.g.*, a carboxyl terminal portion of a light and/or heavy chain, which is not directly involved in binding of an antibody to antigen but which can exhibit various effector functions, such as interaction with an Fc receptor (*e.g.*, Fc gamma receptor).

[0038] As used herein, the term "heavy chain" when used in reference to an antibody can refer to any distinct type, *e.g.*, alpha (α), delta (δ), epsilon (ε), gamma (γ), and mu (μ), based on the amino acid sequence of the constant region, which give rise to IgA, IgD, IgE, IgG, and IgM classes of antibodies, respectively, including subclasses of IgG, *e.g.*, IgG_1, IgG_2, IgG_3, and IgG_4.

[0039] As used herein, the term "light chain" when used in reference to an antibody can

refer to any distinct type, *e.g.*, kappa (κ) or lambda (λ), based on the amino acid sequence of the constant region. Light chain amino acid sequences are well known in the art. In specific embodiments, the light chain is a human light chain.

[0040] As used herein, the term "EU numbering system" refers to the EU numbering convention for the constant regions of an antibody, as described in Edelman, G.M. et al., Proc. Natl. Acad. USA, 63, 78-85 (1969) and Kabat et al, Sequences of Proteins of Immunological Interest, U.S. Dept. Health and Human Services, 5th edition, 1991.

[0041] As used herein, the term "specifically binds" refers to the specificity of an antibody for an antigen, as is understood by one skilled in the art. Binding molecules that specifically bind to an antigen typically bind to the antigen with an equilibrium dissociation constant (KD) of less than 1×10^{-6} M, as measured by, *e.g.*, ELISA assay, surface plasmon resonance, or other suitable assays known in the art. The skilled worker will appreciate that, in certain embodiments, a binding molecule can specifically bind to different antigens, *e.g.*, different antigens that share a common epitope that is recognized by the binding molecule.

[0042] As used herein, the term "PD-1 inhibitor" refers to a molecule that can inhibit the binding of PD-1 to its ligand, Programmed death-ligand 1 (PD-L1).

[0043] As used herein, an "epitope" is a term in the art and refers to a localized region of an antigen to which an antibody can specifically bind. An epitope can be, for example, contiguous amino acids of a polypeptide (linear or contiguous epitope) or an epitope can, for example, come together from two or more non-contiguous regions of a polypeptide or polypeptides (conformational, non-linear, discontinuous, or non-contiguous epitope). In certain embodiments, the epitope to which an antibody binds can be determined by, *e.g.*, NMR spectroscopy, X-ray diffraction crystallography studies, ELISA assays, hydrogen/deuterium exchange coupled with mass spectrometry (*e.g.*, liquid chromatography electrospray mass spectrometry), array-based oligo-peptide scanning assays, and/or mutagenesis mapping (*e.g.*, site-directed mutagenesis mapping). For X-ray crystallography, crystallization may be accomplished using any of the known methods in the art (*e.g.*, Giegé R *et al.*, (1994) Acta Crystallogr D Biol Crystallogr 50(Pt 4): 339-350; McPherson A (1990) Eur J Biochem 189: 1-23; Chayen NE (1997) Structure 5: 1269-1274; McPherson A (1976) J Biol Chem 251: 6300-6303). Antibody:antigen crystals may be studied using well known X-ray diffraction techniques and may be refined using computer software such as X-PLOR (Yale University, 1992, distributed by Molecular Simulations, Inc.; see *e.g.* Meth Enzymol (1985) volumes 114 & 115, eds Wyckoff HW *et al.*; U.S. 2004/0014194), and BUSTER (Bricogne G (1993) Acta Crystallogr D Biol Crystallogr 49(Pt 1): 37-60; Bricogne G (1997) Meth Enzymol 276A: 361-

423, ed Carter CW; Roversi P *et al.*, (2000) Acta Crystallogr D Biol Crystallogr 56(Pt 10): 1316-1323). Mutagenesis mapping studies may be accomplished using any method known to one of skill in the art. *See*, *e.g.*, Champe M *et al.*, (1995) J Biol Chem 270: 1388-1394 and Cunningham BC & Wells JA (1989) Science 244: 1081-1085 for a description of mutagenesis techniques, including alanine scanning mutagenesis techniques. In a specific embodiment, the epitope of an antibody is determined using alanine scanning mutagenesis studies.

[0044] As used herein, the term "treat," "treating," and "treatment" refer to therapeutic or preventative measures described herein. The methods of "treatment" employ administration of an antibody to a subject having a disease or disorder, or predisposed to having such a disease or disorder, in order to prevent, cure, delay, reduce the severity of, or ameliorate one or more symptoms of the disease or disorder or recurring disease or disorder, or in order to prolong the survival of a subject beyond that expected in the absence of such treatment.

[0045] As used herein, the term "therapeutic combination" refers to the combination of a first therapy and the second therapy administered to a subject. The first therapy and the second therapy can be administered simultaneously (in the same pharmaceutical composition or in separate pharmaceutical compositions) or sequentially in any order.

[0046] As used herein, the term "effective amount" in the context of the administration of a therapy to a subject refers to the amount of a therapy that achieves a desired prophylactic or therapeutic effect. The effective amount of a therapeutic combination of a first therapy and a second therapy includes a first amount of the first therapy and a second amount of the second therapy, wherein the administration of the therapeutic combination achieves a desired prophylactic or therapeutic effect.

[0047] As used herein, the term "subject" includes any human or non-human animal. In certain embodiments, the subject is a human or non-human mammal. In certain embodiments, the subject is a human.

[0048] The determination of "percent identity" between two sequences (*e.g.*, amino acid sequences or nucleic acid sequences) can be accomplished using a mathematical algorithm. A specific, non-limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin S & Altschul SF (1990) PNAS 87: 2264-2268, modified as in Karlin S & Altschul SF (1993) PNAS 90: 5873-5877, each of which is herein incorporated by reference in its entirety. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul SF *et al.*, (1990) J Mol Biol 215: 403, which is herein incorporated by reference in its entirety. BLAST nucleotide searches can be performed with the NBLAST nucleotide program parameters set, *e.g.*, for score=100, wordlength=12 to obtain nucleotide

sequences homologous to a nucleic acid molecule described herein. BLAST protein searches can be performed with the XBLAST program parameters set, *e.g.*, to score 50, wordlength=3 to obtain amino acid sequences homologous to a protein molecule described herein. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul SF *et al.*, (1997) Nuc Acids Res 25: 3389-3402, which is herein incorporated by reference in its entirety. Alternatively, PSI BLAST can be used to perform an iterated search which detects distant relationships between molecules (*Id.*). When utilizing BLAST, Gapped BLAST, and PSI Blast programs, the default parameters of the respective programs (*e.g.*, of XBLAST and NBLAST) can be used (*see, e.g.*, National Center for Biotechnology Information (NCBI) on the worldwide web, ncbi.nlm.nih.gov). Another specific, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, 1988, CABIOS 4:11-17, which is herein incorporated by reference in its entirety. Such an algorithm is incorporated in the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used. The percent identity between two sequences can be determined using techniques similar to those described above, with or without allowing gaps. In calculating percent identity, typically only exact matches are counted.

[0049] As used herein, the term "Eastern Cooperative Oncology Group performance status" refers to the grade on the Eastern Cooperative Oncology Group Performance Status Scale determined for a subject prior to treatment. The Eastern Cooperative Oncology Group Performance Status Scale is well known in the art and describes a patient's level of function in terms of ability to care for themself, daily activity, and physical ability (walking, working, etc.).

[0050] As used herein, the term "Cockcroft-Gault method" refers to a method of calculating creatinine clearance (CrCl) from serum creatinine, age, and weight of a subject. The method is well known in the art and described in Cockcroft DW, Gault MH. Nephron. 1976;16(1):31-41), which is herein incorporated by reference in its entirety.

[0051] As used herein, the terms "Response Evaluation Criteria in Solid Tumors Version 1.1" and "RECIST 1.1" refer to a standardized method of radiological imaging to measure the response of a tumor to treatment. RECIST 1.1 is well known in the art and described in, *e.g.*, Therasse P, et al. JNCI. 2000;92(3):205-216.

Multispecific Molecules That Bind to CD96 and TIGIT

[0052] Multispecific molecules (*e.g.*, antibodies) that are useful in the methods and uses described herein include, but are not limited to, those described below.

[0053] In one aspect, the instant disclosure provides multispecific molecules (*e.g.*, antibodies) that specifically bind to CD96 (*e.g.*, human CD96) and TIGIT (*e.g.*, human TIGIT). For example, a multispecific molecule (*e.g.*, antibody) provided herein can comprise a first antigen-binding region that binds to CD96 and a second antigen-binding region that binds to TIGIT. The amino acid sequences of exemplary anti-CD96 antigen-binding regions and anti-TIGIT antigen-binding regions are set forth in Table 1 and Table 2, respectively.

Table 1. Amino acid sequences of exemplary anti-CD96 antigen-binding regions.

Description	SEQ ID NO:	Amino acid sequence
CDRH1	1	TYALH
CDRH2	2	WINTGSGDTKYSQKFQG
CDRH3	3	SLGVYYGMDV
CDRL1	4	RASQSISSYLN
CDRL2	5	AASSLQS
CDRL3	6	QQSYSTPKIT
VH	7	QVQLVQSGAEVKKPGASVKVSCKASGYTFTTYALHWV RQAPGQRLEWMGWINTGSGDTKYSQKFQGRVTITRDT SASTAYMELSSLRSEDTAVYYCARSLGVYYGMDVWGQ GTLVTVSS
VL	8	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQK PGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQ PEDFATYYCQQSYSTPKITFGQGTKLEIK
Heavy chain	9	QVQLVQSGAEVKKPGASVKVSCKASGYTFTTYALHWV RQAPGQRLEWMGWINTGSGDTKYSQKFQGRVTITRDT SASTAYMELSSLRSEDTAVYYCARSLGVYYGMDVWGQ GTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKD YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT VPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHT CPPCPAPELLGGPDVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPSREEMTKNQVSLSCAVKGFYPSDIA VEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG
Light chain	10	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQK PGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQ PEDFATYYCQQSYSTPKITFGQGTKLEIKRTVAAPSVFIF

		PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQ SGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA CEVTHQGLSSPVTKSFNRGEC

Table 2. Amino acid sequences of exemplary anti-TIGIT antigen-binding regions.

Description	SEQ ID NO:	Amino acid sequence
CDRH1	11	SYGIS
CDRH2	12	GITPFFNRVDVAEKFQG
CDRH3	13	DLRRGGVGDAFDI
CDRL1	14	TGTSPDVGSHAYRS
CDRL2	15	EVSYRPS
CDRL3	16	SSYPPSSATV
VH	17	EVQLVQSGAEVKKPGSSVKVSCKASGYTFASYGISWVR QAPGQGLEWMGGITPFFNRVDVAEKFQGRVTITADKST STAYIELSSLRSEDTAVYYCARDLRRGGVGDAFDIWGR GTLVTVSS
VL	18	QSALTQPRSVSGSPGQSVTISCTGTSPDVGSHAYRSWYQ QHPGKAPKLMIYEVSYRPSGVSNRFSGSKSGNTASLTIS GLQAEDEADYYCSSYPPSSATVFGAGTKLTVL
Heavy chain	19	EVQLVQSGAEVKKPGSSVKVSCKASGYTFASYGISWVR QAPGQGLEWMGGITPFFNRVDVAEKFQGRVTITADKST STAYIELSSLRSEDTAVYYCARDLRRGGVGDAFDIWGR GTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKD YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT VPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHT CPPCPAPELLGGPDVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPLPEEKTISKA KGQPREPQVYTLPPSREEMTKNQVSLWCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
Light chain	20	QSALTQPRSVSGSPGQSVTISCTGTSPDVGSHAYRSWYQ QHPGKAPKLMIYEVSYRPSGVSNRFSGSKSGNTASLTIS GLQAEDEADYYCSSYPPSSATVFGAGTKLTVLGQPKAA PSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKAD SSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHR SYSCQVTHEGSTVEKTVAPTEC

[0054] In certain embodiments, the multispecific molecule (*e.g.*, antibody) comprises a first antigen-binding region that specifically binds to human CD96, the first antigen-binding region comprising a first heavy chain variable region (VH) comprising CDRs CDRH1,

CDRH2, and CDRH3, and a first light chain variable region (VL) comprising CDRs CDRL1, CDRL2, and CDRL3; wherein the first VH comprises the CDRH1, CDRH2, and CDRH3 amino acid sequences of the VH amino acid sequence of SEQ ID NO: 7; and the first VL comprises the CDRL1, CDRL2, and CDRL3 amino acid sequences of the VL amino acid sequence of SEQ ID NO: 8. In certain embodiments, the multispecific molecule (*e.g.*, antibody) comprises a second antigen-binding region that specifically binds to human TIGIT, the second antigen-binding region comprising a second VH comprising CDRs CDRH1, CDRH2, and CDRH3, and a second VL comprising CDRs CDRL1, CDRL2, and CDRL3; wherein the second VH comprises the CDRH1, CDRH2, and CDRH3 amino acid sequences of the VH amino acid sequence of SEQ ID NO: 17; and the second VL comprises the CDRL1, CDRL2, and CDRL3 amino acid sequences of the VL amino acid sequence of SEQ ID NO: 18. In certain embodiments, the multispecific molecule (*e.g.*, antibody) comprises a first antigen-binding region that specifically binds to human CD96, the first antigen-binding region comprising a first VH comprising CDRs CDRH1, CDRH2, and CDRH3, and a first VL comprising CDRs CDRL1, CDRL2, and CDRL3; wherein the first VH comprises the CDRH1, CDRH2, and CDRH3 amino acid sequences of the VH amino acid sequence of SEQ ID NO: 7; and the first VL comprises the CDRL1, CDRL2, and CDRL3 amino acid sequences of the VL amino acid sequence of SEQ ID NO: 8; and a second antigen-binding region that specifically binds to human TIGIT, the second antigen-binding region comprising a second VH comprising CDRs CDRH1, CDRH2, and CDRH3, and a second VL comprising CDRs CDRL1, CDRL2, and CDRL3; wherein the second VH comprises the CDRH1, CDRH2, and CDRH3 amino acid sequences of the VH amino acid sequence of SEQ ID NO: 17; and the second VL comprises the CDRL1, CDRL2, and CDRL3 amino acid sequences of the VL amino acid sequence of SEQ ID NO: 18.

[0055] In certain embodiments, the multispecific molecule (*e.g.*, antibody) comprises a first antigen-binding region that specifically binds to human CD96, the first antigen-binding region comprising the CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3 amino acid sequences set forth in SEQ ID NOs: 1, 2, 3, 4, 5, and 6, respectively. In certain embodiments, the multispecific molecule (*e.g.*, antibody) comprises a second antigen-binding region that specifically binds to human TIGIT, the second antigen-binding region comprising the CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3 amino acid sequences set forth in SEQ ID NOs: 11, 12, 13, 14, 15, and 16, respectively. In certain embodiments, the multispecific molecule (*e.g.*, antibody) comprises a first antigen-binding region that specifically binds to human CD96, the first antigen-binding region comprising the CDRH1, CDRH2, CDRH3,

CDRL1, CDRL2, and CDRL3 amino acid sequences set forth in SEQ ID NOs: 1, 2, 3, 4, 5, and 6, respectively, and a second antigen-binding region that specifically binds to human TIGIT, the second antigen-binding region comprising the CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3 amino acid sequences set forth in SEQ ID NOs: 11, 12, 13, 14, 15, and 16, respectively.

[0056] In certain embodiments, the first antigen-binding region that specifically binds to human CD96 comprises a VH comprising an amino acid sequence which is at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence set forth in SEQ ID NO: 7. In certain embodiments, the first antigen-binding region comprises a VH comprising the amino acid sequence set forth in SEQ ID NO: 7.

[0057] In certain embodiments, the first antigen-binding region that specifically binds to human CD96 comprises a VL comprising an amino acid sequence which is at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence set forth in SEQ ID NO: 8. In certain embodiments, the first antigen-binding region comprises a VL comprising the amino acid sequence set forth in SEQ ID NO: 8.

[0058] In certain embodiments, the second antigen-binding region that specifically binds to human TIGIT comprises a VH comprising an amino acid sequence which is at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence set forth in SEQ ID NO: 17. In certain embodiments, the second antigen-binding region comprises a VH comprising the amino acid sequence set forth in SEQ ID NO: 17.

[0059] In certain embodiments, the second antigen-binding region that specifically binds to human TIGIT comprises a VL comprising an amino acid sequence which is at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence set forth in SEQ ID NO: 18. In certain embodiments, the second antigen-binding region comprises a VL comprising the amino acid sequence set forth in SEQ ID NO: 18.

[0060] In certain embodiments, the multispecific molecule (*e.g.*, antibody) comprises: a first antigen-binding region that specifically binds to human CD96, the first antigen-binding region comprising a VH comprising an amino acid sequence which is at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence set forth in SEQ ID NO: 7, and a VL comprising an amino acid sequence which is at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence set forth in SEQ ID NO: 8; and a second antigen-binding region that specifically binds to human TIGIT, the second antigen-binding region comprising a VH comprising an amino acid sequence which is at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence set forth in SEQ ID NO: 17, and a

VL comprising an amino acid sequence which is at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence set forth in SEQ ID NO: 18. In certain embodiments, the multispecific molecule (*e.g.*, antibody) comprises: a first antigen-binding region that specifically binds to human CD96, the first antigen-binding region comprising a VH comprising the amino acid sequence set forth in SEQ ID NO: 7 and a VL comprising the amino acid sequence set forth in SEQ ID NO: 8; and a second antigen-binding region that specifically binds to human TIGIT, the second antigen-binding region comprising a VH comprising the amino acid sequence set forth in SEQ ID NO: 17 and a VL comprising the amino acid sequence set forth in SEQ ID NO: 18.

[0061] In certain embodiments, the multispecific molecule (*e.g.*, antibody) comprises a heavy chain constant region selected from the group consisting of human IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, and IgA_2. In certain embodiments, the first antigen-binding region that specifically binds to human CD96 comprises a heavy chain constant region selected from the group consisting of human IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, and IgA_2. In certain embodiments, the second antigen-binding region that specifically binds to human TIGIT comprises a heavy chain constant region selected from the group consisting of human IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, and IgA_2. In certain embodiments, the first antigen-binding region comprises a heavy chain constant region selected from the group consisting of human IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, and IgA_2, and the second antigen-binding region comprises a heavy chain constant region selected from the group consisting of human IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, and IgA_2.

[0062] In certain embodiments, the multispecific molecule (*e.g.*, antibody) comprises a light chain constant region selected from the group consisting of a human kappa light chain constant region and a human lambda light chain constant region. In certain embodiments, the first antigen-binding region that specifically binds to human CD96 and/or the second antigen-binding region that specifically binds to human TIGIT comprises a light chain constant region selected from the group consisting of a human kappa light chain constant region and a human lambda light chain constant region.

[0063] In certain embodiments, the multispecific molecule (*e.g.*, antibody) comprises a first antigen-binding region that specifically binds to human CD96 comprising a first human IgG heavy chain constant region comprising aspartate, serine, alanine, and valine at amino acid positions 239, 366, 368, and 407, respectively, and a second antigen-binding region that specifically binds to human TIGIT comprising a second human IgG heavy chain constant region comprising aspartate, leucine, glutamate, and tryptophan at amino acid positions 239, 330, 332, and 366, respectively, wherein the amino acid positions are numbered according to

the EU numbering system. In certain embodiments, the multispecific molecule (*e.g.*, antibody) comprises a first antigen-binding region that specifically binds to human CD96 comprising a first human IgG heavy chain constant region comprising aspartate, leucine, glutamate, and tryptophan at amino acid positions 239, 330, 332, and 366, respectively, and a second antigen-binding region that specifically binds to human TIGIT comprising a second antigen-binding region comprising a second human IgG heavy chain constant region comprising aspartate, serine, alanine, and valine at amino acid positions 239, 366, 368, and 407, respectively, wherein the amino acid positions are numbered according to the EU numbering system.

[0064] In certain embodiments, the multispecific molecule (*e.g.*, antibody) comprises a first antigen-binding region that specifically binds to human CD96 comprising a heavy chain comprising an amino acid sequence which is at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence set forth in SEQ ID NO: 9. In certain embodiments, the first antigen-binding region comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 9.

[0065] In certain embodiments, the multispecific molecule (*e.g.*, antibody) comprises a first antigen-binding region that specifically binds to human CD96 comprising a light chain comprising an amino acid sequence which is at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence set forth in SEQ ID NO: 10. In certain embodiments, the first antigen-binding region comprises a light chain comprising the amino acid sequence set forth in SEQ ID NO: 10.

[0066] In certain embodiments, the multispecific molecule (*e.g.*, antibody) comprises a second antigen-binding region that specifically binds to human TIGIT comprising a heavy chain comprising an amino acid sequence which is at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence set forth in SEQ ID NO: 19. In certain embodiments, the second antigen-binding region comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 19.

[0067] In certain embodiments, the multispecific molecule (*e.g.*, antibody) comprises a second antigen-binding region that specifically binds to human TIGIT comprising a light chain comprising an amino acid sequence which is at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence set forth in SEQ ID NO: 20. In certain embodiments, the second antigen-binding region comprises a light chain comprising the amino acid sequence set forth in SEQ ID NO: 20.

[0068] In certain embodiments, the multispecific molecule (*e.g.*, antibody) comprises a first antigen-binding region that specifically binds to human CD96 comprising a heavy chain

comprising an amino acid sequence which is at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence set forth in SEQ ID NO: 9, and a light chain comprising an amino acid sequence which is at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence set forth in SEQ ID NO: 10. In certain embodiments, the first antigen-binding region comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 9 and a light chain comprising the amino acid sequence set forth in SEQ ID NO: 10. In certain embodiments, the first antigen-binding region comprises a heavy chain consisting of the amino acid sequence set forth in SEQ ID NO: 9 and a light chain consisting of the amino acid sequence set forth in SEQ ID NO: 10.

[0069] In certain embodiments, the multispecific molecule (*e.g.*, antibody) comprises a second antigen-binding region that specifically binds to human TIGIT comprising a heavy chain comprising an amino acid sequence which is at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence set forth in SEQ ID NO: 19, and a light chain comprising an amino acid sequence which is at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence set forth in SEQ ID NO: 20. In certain embodiments, the second antigen-binding region comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 19 and a light chain comprising the amino acid sequence set forth in SEQ ID NO: 20. In certain embodiments, the second antigen-binding region comprises a heavy chain consisting of the amino acid sequence set forth in SEQ ID NO: 19 and a light chain consisting of the amino acid sequence set forth in SEQ ID NO: 20.

[0070] In certain embodiments, the multispecific molecule (*e.g.*, antibody) comprises a first antigen-binding region that specifically binds to human CD96 comprising a heavy chain and a light chain comprising the amino acid sequences of SEQ ID NOs: 9 and 10, respectively, and a second antigen-binding region that specifically binds to human TIGIT comprising a heavy chain and a light chain comprising the amino acid sequences of SEQ ID NOs: 19 and 20, respectively. In certain embodiments, the first antigen-binding region comprises a heavy chain and a light chain consisting of the amino acid sequences of SEQ ID NOs: 9 and 10, respectively, and the second antigen-binding region comprises a heavy chain and a light chain consisting of the amino acid sequences of SEQ ID NOs: 19 and 20, respectively.

[0071] In certain embodiments, multispecific molecule is AGEN1777.

PD-1 Inhibitors

[0072] Human PD-1 inhibitors that are useful in the methods and uses described herein include but are not limited to those described below.

[0073] In certain embodiments, the human PD-1 inhibitor is an antibody that specifically binds to human PD-1 or human PD-L1.

[0074] In certain embodiments, the antibody that specifically binds to human PD-1 or human PD-L1 is nivolumab, balstilimab, adebrelimab, atezolizumab, avelumab, camrelizumab, cemiplimab, cosibelimab, dostarlimab, durvalumab, enlonstobart, envafolimab, pembrolizumab, penpulimab, pidilizumab, prolgolimab, pucotenlimab, retifanlimab, serplulimab, sintilimab, socazolimab, sugemalimab, tagitanlimab, tislelizumab, toripalimab, and zimberelimab.

[0075] In certain embodiments, the antibody that specifically binds to human PD-1 is nivolumab. Nivolumab (also known as "OPDIVO®"; formerly designated 5C4, BMS-936558, MDX-1106, or ONO-4538) is a fully human IgG4 (S228P) PD-1 immune checkpoint inhibitor antibody that selectively prevents interaction with PD-1 ligands (PD-L1 and PD-L2), thereby blocking the down-regulation of antitumor T-cell functions (U.S. Patent No. 8,008,449; Wang et al., 2014 Cancer Immunol Res. 2(9): 846-56). In another embodiment, the anti-PD-1 antibody or fragment thereof cross-competes with nivolumab. In other embodiments, the anti-PD-1 antibody or fragment thereof binds to the same epitope as nivolumab. In certain embodiments, the anti-PD-1 antibody has the same CDRs as nivolumab.

[0076] In certain embodiments, the antibody that specifically binds to human PD-1 is atezolizumab. In another embodiment, the anti-PD-1 antibody or fragment thereof cross-competes with atezolizumab. In other embodiments, the anti-PD-1 antibody or fragment thereof binds to the same epitope as atezolizumab. In certain embodiments, the anti-PD-1 antibody has the same CDRs as atezolizumab.

[0077] Further non-limiting examples of anti-PD-1 antibodies that may be used in treatment methods described herein are disclosed in the following patents and patent applications, which are incorporated herein by reference in their entireties for all purposes: U.S. Patent No. 6,808,710; U.S. Patent No. 7,332,582; U.S. Patent No. 7,488,802; U.S. Patent No. 8,008,449; U.S. Patent No. 8,114,845; U.S. Patent No. 8,168,757; U.S. Patent No. 8,354,509; U.S. Patent No. 8,686,119; U.S. Patent No. 8,735,553; U.S. Patent No. 8,747,847; U.S. Patent No. 8,779,105; U.S. Patent No. 8,927,697; U.S. Patent No. 8,993,731; U.S. Patent No. 9,102,727; U.S. Patent No. 9,205,148; U.S. Publication No. US 2013/0202623 A1; U.S. Publication No. US 2013/0291136 A1; U.S. Publication No. US 2014/0044738 A1; U.S. Publication No. US 2014/0356363 A1; U.S. Publication No. US 2016/0075783 A1; and PCT Publication No. WO 2013/033091 A1; PCT Publication No. WO 2015/036394 A1; PCT Publication No. WO 2014/179664 A2; PCT Publication No. WO 2014/209804 A1; PCT

Publication No. WO 2014/206107 A1; PCT Publication No. WO 2015/058573 A1; PCT Publication No. WO 2015/085847 A1; PCT Publication No. WO 2015/200119 A1; PCT Publication No. WO 2016/015685 A1; and PCT Publication No. WO 2016/020856 A1.

[0078] Further examples of anti-PD-L1 antibodies that may be used in treatment methods described herein are disclosed in the following patents and patent applications, which are incorporated herein by reference in their entireties for all purposes: U.S. Patent No. 7,943,743; U.S. Patent No. 8,168,179; U.S. Patent No. 8,217,149; U.S. Patent No. 8,552,154; U.S. Patent No. 8,779,108; U.S. Patent No. 8,981,063; U.S. Patent No. 9,175,082; U.S. Publication No. US 2010/0203056 A1; U.S. Publication No. US 2003/0232323 A1; U.S. Publication No. US 2013/0323249 A1; U.S. Publication No. US 2014/0341917 A1; U.S. Publication No. US 2014/0044738 A1; U.S. Publication No. US 2015/0203580 A1; U.S. Publication No. US 2015/0225483 A1; U.S. Publication No. US 2015/0346208 A1; U.S. Publication No. US 2015/0355184 A1; PCT Publication No. WO 2014/100079 A1; PCT Publication No. WO 2014/022758 A1; PCT Publication No. WO 2014/055897 A2; PCT Publication No. WO 2015/061668 A1; PCT Publication No. WO 2015/109124 A1; PCT Publication No. WO 2015/195163 A1; PCT Publication No. WO 2016/000619 A1; and PCT Publication No. WO 2016/030350 A1.

Methods of Treating Cancer

[0079] The present disclosure provides methods for the treatment of cancer using a multispecific molecule (*e.g.*, antibody) that specifically binds to human CD96 and human TIGIT, and combinations of the multispecific molecule with a human PD-1 inhibitor (*e.g.*, an antibody that specifically binds to human PD-1).

[0080] In one aspect, the present disclosure provides a method of treating cancer in a subject in need thereof, the method comprising administering to the subject: (a) a multispecific molecule at a dose of about 2 mg to about 2000 mg, wherein the multispecific molecule comprises: a first antigen-binding region that specifically binds to human CD96, the first antigen-binding region comprising a first heavy chain variable region (VH) comprising CDRs CDRH1, CDRH2, and CDRH3, and a first light chain variable region (VL) comprising CDRs CDRL1, CDRL2, and CDRL3; wherein the first VH comprises the CDRH1, CDRH2, and CDRH3 amino acid sequences of the VH amino acid sequence of SEQ ID NO: 7; and the first VL comprises the CDRL1, CDRL2, and CDRL3 amino acid sequences of the VL amino acid sequence of SEQ ID NO: 8; and a second antigen-binding region that specifically binds to human TIGIT, the second antigen-binding region comprising a second VH comprising CDRs

CDRH1, CDRH2, and CDRH3, and a second VL comprising CDRs CDRL1, CDRL2, and CDRL3; wherein the second VH comprises the CDRH1, CDRH2, and CDRH3 amino acid sequences of the VH amino acid sequence of SEQ ID NO: 17; and the second VL comprises the CDRL1, CDRL2, and CDRL3 amino acid sequences of the VL amino acid sequence of SEQ ID NO: 18; and (b) a human PD-1 inhibitor.

[0081] In certain embodiments, the methods described herein comprise any combination of a multispecific molecule (*e.g.*, antibody) that specifically binds to human CD96 and human TIGIT described herein, and a human PD-1 inhibitor described herein.

[0082] In certain embodiments, the method comprises administration of a multispecific molecule (*e.g.*, antibody) that specifically binds to human CD96 and human TIGIT, and a human PD-1 inhibitor that is an antibody that specifically binds to human PD-1 or human PD-L1. In certain embodiments, the antibody specifically binds to human PD-1. In certain embodiments, the antibody specifically binds to human PD-L1. In certain embodiments, the antibody is selected from the group consisting of nivolumab, balstilimab, adebrelimab, atezolizumab, avelumab, camrelizumab, cemiplimab, cosibelimab, dostarlimab, durvalumab, enlonstobart, envafolimab, pembrolizumab, penpulimab, pidilizumab, prolgolimab, pucotenlimab, retifanlimab, serplulimab, sintilimab, socazolimab, sugemalimab, tagitanlimab, tislelizumab, toripalimab, and zimberelimab. In certain embodiments, the second antibody is nivolumab or atezolizumab.

[0083] In certain embodiments, the multispecific molecule (*e.g.*, antibody) is administered at a dose of about 2 mg to about 2000 mg, about 2 mg to about 1200 mg, about 2 mg to about 800 mg, about 2 mg to about 600 mg, about 2 mg to about 400 mg, about 2 mg to about 200 mg, about 2 mg to about 60 mg, about 2 mg to about 20 mg, about 2 mg to about 6 mg, 6 mg to about 2000 mg, about 6 mg to about 1200 mg, about 6 mg to about 800 mg, about 6 mg to about 600 mg, about 6 mg to about 400 mg, about 6 mg to about 200 mg, about 6 mg to about 60 mg, about 6 mg to about 20 mg, about 20 mg to about 2000 mg, about 20 mg to about 1200 mg, about 20 mg to about 800 mg, about 20 mg to about 600 mg, about 20 mg to about 400 mg, about 20 mg to about 200 mg, about 20 mg to about 60 mg, about 60 mg to about 2000 mg, about 60 mg to about 1200 mg, about 60 mg to about 800 mg, about 60 mg to about 600 mg, about 60 mg to about 400 mg, about 60 mg to about 200 mg, about 200 mg to about 2000 mg, about 200 mg to about 1200 mg, about 200 mg to about 800 mg, about 200 mg to about 600 mg, about 200 mg to about 400 mg, about 400 mg to about 2000 mg, about 400 mg to about 1200 mg, about 400 mg to about 800 mg, about 400 mg to about 600 mg, about 600 mg to about 2000 mg, about 600 mg to about 1200 mg, about 600 mg to about 800 mg, about 800

to about 2000 mg, or about 800 to about 1200 mg.

[0084] In certain embodiments, the multispecific molecule (*e.g.*, antibody) is administered at a dose of about 2 mg, about 6 mg, about 20 mg, about 60 mg, about 200 mg, about 400 mg, about 600 mg, about 800 mg, about 1200 mg, or about 2000.

[0085] In certain embodiments, the multispecific molecule (*e.g.*, antibody) is administered once every week. In certain embodiments, the multispecific molecule (*e.g.*, antibody) is administered once every 2 weeks. In certain embodiments, the multispecific molecule (*e.g.*, antibody) is administered once every 3 weeks. In certain embodiments, the multispecific molecule (*e.g.*, antibody) is administered once every 4 weeks. In certain embodiments, the multispecific molecule (*e.g.*, antibody) is administered once every 5 weeks. In certain embodiments, the multispecific molecule (*e.g.*, antibody) is administered once every 6 weeks.

[0086] In certain embodiments, the multispecific molecule (*e.g.*, antibody) is administered at a dose of about 2 mg once every 3 weeks. In certain embodiments, the multispecific molecule (*e.g.*, antibody) is administered at a dose of about 6 mg once every 3 weeks. In certain embodiments, the multispecific molecule (*e.g.*, antibody) is administered at a dose of about 20 mg once every 3 weeks. In certain embodiments, the multispecific molecule (*e.g.*, antibody) is administered at a dose of about 60 mg once every 3 weeks. In certain embodiments, the multispecific molecule (*e.g.*, antibody) is administered at a dose of about 200 mg once every 3 weeks. In certain embodiments, the multispecific molecule (*e.g.*, antibody) is administered at a dose of about 600 mg once every 3 weeks. In certain embodiments, the multispecific molecule (*e.g.*, antibody) is administered at a dose of about 1200 mg once every 3 weeks. In certain embodiments, the multispecific molecule (*e.g.*, antibody) is administered at a dose of about 2000 mg once every 3 weeks. In certain embodiments, the multispecific molecule (*e.g.*, antibody) is administered at a dose of about 400 mg once every 2 weeks. In certain embodiments, the multispecific molecule (*e.g.*, antibody) is administered at a dose of about 800 mg once every 2 weeks.

[0087] In certain embodiments, the multispecific molecule (*e.g.*, antibody) is administered intravenously. In certain embodiments, the multispecific molecule (*e.g.*, antibody) is administered intratumorally. In certain embodiments, the multispecific molecule (*e.g.*, antibody) is administered peritumorally. In certain embodiments, the human PD-1 inhibitor is administered intravenously. In certain embodiments, the human PD-1 inhibitor is administered intratumorally. In certain embodiments, the human PD-1 inhibitor is administered peritumorally.

[0088] In certain embodiments, the multispecific molecule (*e.g.*, antibody) and the human

PD-1 inhibitor are administered intravenously. In certain embodiments, the multispecific molecule (*e.g.*, antibody) and the human PD-1 inhibitor are administered intratumorally. In certain embodiments, the multispecific molecule (*e.g.*, antibody) and the human PD-1 inhibitor are administered peritumorally.

[0089] In certain embodiments, the multispecific molecule (*e.g.*, antibody) or the human PD-1 inhibitor are administered by intravenous infusion over about 30 minutes. In certain embodiments, multispecific molecule (*e.g.*, antibody) or the human PD-1 inhibitor are administered by intravenous infusion over about 45 minutes. In certain embodiments, multispecific molecule (*e.g.*, antibody) or the human PD-1 inhibitor are administered by intravenous infusion over about 60 minutes. In certain embodiments, multispecific molecule (*e.g.*, antibody) or the human PD-1 inhibitor are administered by intravenous infusion over about 90 minutes.

[0090] In certain embodiments, the multispecific molecule (*e.g.*, antibody) and the human PD-1 inhibitor are each administered by intravenous infusion over about 30 minutes. In certain embodiments, multispecific molecule (*e.g.*, antibody) and the human PD-1 inhibitor are each administered by intravenous infusion over about 45 minutes. In certain embodiments, the multispecific molecule (*e.g.*, antibody) and the human PD-1 inhibitor are each administered by intravenous infusion over about 60 minutes. In certain embodiments, the multispecific molecule (*e.g.*, antibody) and the human PD-1 inhibitor are each administered by intravenous infusion over about 90 minutes.

[0091] In certain embodiments, the human PD-1 inhibitor is administered to the subject prior to administration of the multispecific molecule (*e.g.*, antibody). In certain embodiments, the multispecific molecule (*e.g.*, antibody) is administered to the subject prior to administration of the human PD-1 inhibitor.

[0092] In certain embodiments, the dose of the multispecific molecule (*e.g.*, antibody) and the dose of the human PD-1 inhibitor are administered on the same day. In certain embodiments, the dose of the multispecific molecule (*e.g.*, antibody) and the dose of the human PD-1 inhibitor are administered simultaneously. In certain embodiments, the dose of the multispecific molecule (*e.g.*, antibody) is administered prior to (e.g., 30 minutes before, 1 hour before, 2 hours before, 4 hours before, 8 hours before, 12 hours before, 16 hours before, 20 hours before, 1 day before, 1.5 days before, 2 days before, 3 days before, etc.) the dose of the human PD-1 inhibitor. In certain embodiments, the dose of the multispecific molecule (*e.g.*, antibody) is administered after (e.g., 30 minutes after, 1 hour after, 2 hours after, 4 hours after, 8 hours after, 12 hours after, 16 hours after, 20 hours after, 1 day after, 1.5 days after, 2 days

after, 3 days after, etc.) the dose of the human PD-1 inhibitor. In certain embodiments, each administration of the multispecific molecule (*e.g.*, antibody) is on the same day as the human PD-1 inhibitor.

[0093] The multispecific molecule (*e.g.*, antibody) and human PD-1 inhibitor can be administered simultaneously or sequentially by the same or different routes of administration (*e.g.*, as described herein). In certain embodiments, the multispecific molecule (*e.g.*, antibody) and human PD-1 inhibitor are administered simultaneously via intravenous injection (*e.g.*, intravenous infusion). In certain embodiments, the multispecific molecule (*e.g.*, antibody) and human PD-1 inhibitor are administered sequentially via intravenous injection (*e.g.*, intravenous infusion).

[0094] In certain embodiments, the method comprises administering to the subject a multispecific molecule, *e.g.*, a bispecific antibody comprising a first antigen-binding region comprising a heavy chain and a light chain comprising the amino acid sequences of SEQ ID NOs: 9 and 10, respectively, and a second antigen-binding region comprising a heavy chain and a light chain comprising the amino acid sequences of SEQ ID NOs: 19 and 20, respectively; and a human PD-1 inhibitor, *e.g.*, nivolumab or atezolizumab.

[0095] In certain embodiments, the dose of the multispecific molecule (*e.g.*, antibody) is a therapeutically effective amount. In certain embodiments, the dose of the multispecific molecule (*e.g.*, antibody) and the dose of the human PD-1 inhibitor are a therapeutically effective amount.

[0096] Any disease or disorder in a subject that would benefit from inhibition of CD96, TIGIT, and/or PD-1 function can be treated using the methods, molecules, antibodies, or inhibitors described herein. The methods, molecules, antibodies, or inhibitors described herein are particularly useful for inhibiting immune system tolerance to tumors, and accordingly can be used as an immunotherapy for subjects with cancer. For example, in certain embodiments, the present disclosure provides a method of treating cancer in a subject, the method comprising administering to the subject an effective amount of a multispecific molecule and inhibitor as described herein.

[0097] In certain embodiments, the cancer treated in accordance with the methods described herein is a metastatic or locally advanced cancer (*e.g.*, solid tumor). In certain embodiments, the cancer is treated in accordance with a method described herein as a first cancer therapy after diagnosis of the metastatic or locally advanced tumor (*e.g.*, within 1, 2, 3, 4, 5, or 6 days; 1, 2, 3, 4, 6, 8, or 12 weeks; or, 1, 2, 3, 4, 6, 8, or 12 months after diagnosis). In certain embodiments, the cancer is treated in accordance with a method described herein as the

first cancer therapy after diagnosis of tumor progression (*e.g.*, within 1, 2, 3, 4, 5, or 6 days; 1, 2, 3, 4, 6, 8, or 12 weeks; or, 1, 2, 3, 4, 6, 8, or 12 months after diagnosis of tumor progression) that has occurred despite previous treatment of the tumor with a different cancer therapy, optionally wherein the method described herein is provided as the second cancer therapy administered. In certain embodiments, the cancer is treated in accordance with a method described herein as the first cancer therapy after diagnosis of toxicity of a different cancer therapy (*e.g.*, within 1, 2, 3, 4, 5, or 6 days; 1, 2, 3, 4, 6, 8, or 12 weeks; or, 1, 2, 3, 4, 6, 8, or 12 months after diagnosis of toxicity of the different cancer therapy), optionally wherein the method described herein is provided as the second cancer therapy administered. In certain embodiments, the cancer treated in accordance with the methods described herein is a metastatic or locally advanced cancer (*e.g.*, solid tumor) for which no standard therapy is available. In other embodiments, the cancer treated in accordance with the methods described herein is a metastatic or locally advanced cancer (*e.g.*, solid tumor) for which a standard therapy has failed (*i.e.*, the cancer has progressed after the standard therapy). In certain embodiments, a therapy fails if the cancer is refractory to the therapy. In certain embodiments, a therapy fails if the cancer relapses after responding, fully or partially, to the therapy. In certain embodiments, metastatic or locally advanced cancer (e.g., solid tumor) has been confirmed histologically or cytologically.

[0098] In certain embodiments, the cancer is a solid tumor. In certain embodiments, the cancer (*e.g.*, solid tumor) expresses CD96. In certain embodiments, the metastatic or locally advanced cancer (*e.g.*, solid tumor) expresses CD96. In certain embodiments, the cancer (*e.g.*, solid tumor) expresses TIGIT. In certain embodiments, the metastatic or locally advanced cancer (*e.g.*, solid tumor) expresses TIGIT. In certain embodiments, the cancer (*e.g.*, solid tumor) expresses PD-1. In certain embodiments, the metastatic or locally advanced cancer (*e.g.*, solid tumor) expresses PD-1. In certain embodiments, the cancer (*e.g.*, solid tumor) expresses PD-L1. In certain embodiments, the metastatic or locally advanced cancer (*e.g.*, solid tumor) expresses PD-L1.

[0099] Cancers that can be treated according to the methods described herein include, without limitation, solid cancer (*e.g.*, relapsed or refractory solid cancer, and advanced or metastatic solid cancer).

[00100] In certain embodiments, the subject has previously received an immunotherapy. In certain embodiments, the subject has not previously received any immunotherapy. In certain embodiments, the cancer is an advanced or metastatic cancer.

[00101] In certain embodiments, the subject is at least 18 years of age. In certain

embodiments, the subject has documented histologically or cytologically confirmed advanced unresectable or metastatic solid malignancy of any histology. In certain embodiments, no standard therapy is available or standard therapy has failed. In certain embodiments, before administration of the multispecific molecule (*e.g.*, antibody) and/or the human PD-1 inhibitor, the subject has measurable disease on baseline imaging as assessed according to Response Evaluation Criteria in Solid Tumors Version 1.1 (RECIST 1.1). In certain embodiments, the subject has a predicted life expectancy of at least 3 months. In certain embodiments, before administration of the multispecific molecule (*e.g.*, antibody) and/or the human PD-1 inhibitor, the subject has an Eastern Cooperative Oncology Group performance status of 0 or 1. In certain embodiments, the subject is not pregnant and/or is not breastfeeding.

[00102] In certain embodiments, the method comprises further administering to the subject a chemotherapeutic agent. In certain embodiments, the chemotherapeutic agent is selected from the group consisting of docetaxel, pemetrexed, carboplatin, paclitaxel, FOLFOX, CAPOX, and combinations thereof.

[00103] In certain embodiments, the dose of the multispecific molecule (*e.g.*, antibody), the dose of the human PD-1 inhibitor, and the dose of the chemotherapeutic agent are a therapeutically effective amount.

[00104] In an aspect, provided herein is a multispecific molecule (*e.g.*, antibody) that specifically binds to human CD96 and human TIGIT, and a human PD-1 inhibitor, for use in the treatment of cancer, wherein the treatment is performed according to a method described herein.

[00105] In an aspect, provided herein is a multispecific molecule (*e.g.*, antibody) that specifically binds to human CD96 and human TIGIT, and a human PD-1 inhibitor, for use in the manufacture of a medicament for the treatment of cancer, wherein the treatment is performed according to a method described herein.

[00106] In an aspect, provided herein is a multispecific molecule (*e.g.*, antibody) that specifically binds to human CD96 and human TIGIT, and a human PD-1 inhibitor, for the treatment of cancer, wherein the treatment is performed according to a method described herein.

EXAMPLES

[00107] The examples in this section are offered by way of illustration, and not by way of limitation.

Example 1: AGEN1777 combines with an anti-PD-1 antagonist to enhance T cell responsiveness

[00108] The ability of anti-TIGIT/anti-CD96 bispecific antibody AGEN1777, alone and combination with anti-PD-1 antibody balstilimab, to enhance T cell responsiveness was investigated using a superantigen T cell : antigen presenting cell (APC) priming assay.

[00109] Staphylococcal enterotoxin A (SEA) peptide simultaneously binds certain Vβ chains of the TCR on CD4+ or CD8+ T cells and the MHC class II complex on APCs (FIG. 1A). This interaction results in the stimulation of both APC and T cells leading to the production of cytokines into the cultured media. To facilitate the stimulation of T cells by APCs, human PBMCs from three healthy donors were cultured for four days with a pre-established sub-optimal concentration of SEA peptide (1 or 10 ng/mL) and a fixed dose (2.5 μg/mL) of AGEN1777, anti-TIGIT antibody tiragolumab, relevant isotype control, or bivalent isotype control, in the presence or absence of anti-PD-1 (balstilimab or nivolumab) or anti-PD-L1 (atezolizumab). Following the four-day incubation, cell-free supernatants from the assay system were assessed for IL-2 levels by AlphaLISA®.

[00110] In two independent experiments, SEA-stimulated human PBMC cultures treated with the combination of AGEN1777 and anti-PD-1, or AGEN1777 and anti-PD-L1, exhibited significantly increased IL-2 cytokine secretion compared to either therapy alone (FIGs. 1B-1D). Furthermore, AGEN1777 demonstrated superior IL-2 responses compared to tiragolumab or isotype control. Notably, the combination of AGEN1777 with anti-PD-1 or anti-PD-L1 resulted in superior IL-2 responses compared to combinations of tiragolumab with anti-PD-1 or anti-PD-L1.

Example 2: AGEN1777 mouse surrogate controls tumor growth alone and in combination with anti-PD-1 in an early intervention CT26 tumor-bearing mouse model

[00111] The ability of AGEN1777 mouse surrogate antibody (AGEN1777^{ms}), alone and combination with an anti-PD-1 antibody, to control tumor growth was investigated in an early intervention model.

[00112] Balb/c mice were injected with 10^5 CT26 colorectal carcinoma tumor cells (subcutaneous, n=8 mice/group) and treated intraperitoneally with 200 μg of TIGIT/CD96 bispecific antibody (AGEN1777^{ms}) alone or in combination with anti-PD-1 antibody (RMP1-14), anti-TIGIT mAb (Clone 10A7 mouse IgG2a), anti-CD96 mAb (Clone 6A6 mouse IgG2a), or isotype control bispecific antibody, twice a week for two weeks post-tumor implantation. Tumor growth was monitored bi-weekly using a digital caliper. Mean tumor size at the start of

treatment was approximately 45 mm^3. Historical data shown for the effect of anti-PD-1 vs. isotype control in CT26 tumor-bearing mice. A 2-way ANOVA with repeated measures and adjusting p-values for multiple comparison was used to assess differences in tumor growth between treatment groups.

[00113] Treatment with AGEN1777^{ms} resulted in superior tumor growth control compared to treatment with anti-TIGIT, anti-CD96, anti-PD-1, or isotype control antibodies (FIG. 2A). Complete responses were observed in 4 of 8 mice treated with AGEN1777^{ms}, while there was only 1 complete response out of the mice treated with anti-TIGIT and no complete responses in the anti-CD96, anti-PD-1 or isotype control groups (FIG. 2B). Notably, AGEN1777^{ms} in combination with anti-PD-1 demonstrated superior tumor growth control compared to either monotherapy, with complete responses observed in 5 of 8 treated mice.

Example 3: AGEN1777 mouse surrogate in combination with anti-PD-1 promotes robust tumor control in a late intervention CT26 tumor-bearing mouse model

[00114] The ability of AGEN1777^{ms}, either alone or combination with anti-PD-1 murine antibody RMP1-14, to control tumor growth was investigated in a late intervention model.

[00115] Balb/c mice were injected with 10^5 CT26 tumor cells (subcutaneous, n=8 mice/group) and treated intraperitoneally with 200 μg of TIGIT/CD96 bispecific antibody (AGEN1777^{ms}) alone or in combination with anti-PD-1 (RMP1-14), or isotype control bispecific antibody, twice a week for two weeks post-tumor implantation. Tumor growth was monitored bi-weekly using a digital caliper. Mean tumor size at the start of treatment was approximately 100 mm^3. A 2-way ANOVA with repeated measures and adjusting p-values for multiple comparison was used to assess differences in tumor growth between treatment groups.

[00116] Treatment with AGEN1777^{ms} resulted in superior tumor growth control compared to treatment with isotype control, with complete responses observed in 2 of 8 mice (FIGs. 3A-3B). Notably, AGEN1777^{ms} in combination with anti-PD-1 demonstrated superior tumor growth control compared to AGEN1777^{ms} alone, with complete responses observed in 5 of 8 treated mice.

Example 4: A Phase 1 Study of AGEN1777 as a Single-Agent and in Combination with PD-1 Inhibition in Patients with Advanced Solid Tumors

[00117] AGEN1777 is a fragment crystallizable-engineered bispecific human immunoglobulin gamma 1 antibody that selectively binds to T cell immunoreceptor with Ig and immunoreceptor tyrosine-based inhibitory motif domains (TIGIT) and cluster of

differentiation (CD)96 on certain populations of T cells and natural killer (NK) cells. As a dual antagonist, AGEN1777 is designed to block TIGIT and CD96 binding to their shared ligand (poliovirus receptor [PVR/CD155]) and facilitate T-cell and NK cell activation by relieving inhibitory signals and enabling CD155 to bind to the stimulatory receptor CD226.

[00118] This Example describes a multicenter, Phase 1 study to evaluate the safety, tolerability, PK, and pharmacodynamics of AGEN1777 as a single agent and when used in combination with an anti-PD-1 antibody in patients with advanced, metastatic solid tumors to determine the recommended Phase 2 doses (RP2D).

Overall Study Design

[00119] A standard 3+3 dose-escalation design will be used to evaluate AGEN1777 in 2 arms, as a single-agent administered every 3 weeks (Q3W), and in combination with an anti-PD-1 antibody administered Q3W. Patients will be assigned to available treatment arms. Alternate dosing schedules of AGEN1777, such as every 4 weeks (Q4W), may also be explored depending on PK, pharmacodynamic, and safety results. The treatment will be in 21-day cycles for both arms. For AGEN1777, the maximum recommended starting dose (MRSD) of 2 mg flat dose (approximately 0.03 mg/kg in 70 kg patient) administered intravenously (IV) Q3W or Q4W was derived from a 20% to 80% receptor occupancy approach.

Single-Agent AGEN1777

[00120] AGEN1777 will be administered on Day 1 of each 3-week cycle. Patients will receive AGEN1777 for up to 2 years or until progressive disease (PD), unacceptable toxicity, or withdrawal of consent.

AGEN1777 Combination with Anti-PD-1 Antibody

[00121] The starting dose for AGEN1777 in combination with an anti-PD-1 antibody will be 1 to 2 dose levels below the expected active dose determined by the Safety Monitoring Committee (SMC) based on available safety, pharmacodynamic, and PK data from single-agent AGEN1777 administration. AGEN1777 in combination with anti-PD-1 antibody will be administered on Day 1 of each 3-week cycle followed by a fixed dose of 300 mg Q3W PD-1 inhibitor administered on Day 1 of each 3-week cycle for up to 2 years of treatment or until PD or unacceptable toxicity is reported.

[00122] The study will enroll up to approximately 75 evaluable patients with measurable advanced/metastatic solid tumor.

Objectives and Endpoints

Table 3. Objectives and Endpoints

Objectives	Endpoints
Primary	
• To assess the safety and tolerability of AGEN1777 as a single-agent and in combination with an anti-PD-1 antibody to determine the maximum tolerated dose (MTD), if feasible • To determine the RP2D of AGEN1777 as a single-agent and AGEN1777 in combination with an anti-PD-1 antibody	• Occurrence of dose–limiting toxicities (DLT) within 21 days after the first administration of AGEN1777 as a single-agent and in combination with an anti-PD-1 antibody • Frequency and severity of TEAEs, and laboratory abnormalities by dose level, according to CTCAE version 5.0
Secondary	
• To characterize the pharmacokinetics (PK) of AGEN1777 as a single-agent and in combination with an anti-PD-1 antibody • To evaluate immunogenicity of AGEN1777 as a single-agent and in combination with an anti-PD-1 antibody and assess potential impact on PK and biological activity • To assess complete response (CR), partial response (PR), duration of response (DOR), and stable disease (SD) per Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST 1.1)	• Serum AGEN1777 and anti-PD-1 antibody concentrations • Serum AGEN1777 and anti-PD-1 antibody anti-drug antibody (ADA) determinations • CR per RECIST 1.1 based on Investigator assessment • PR per RECIST 1.1 based on Investigator assessment • DOR per RECIST 1.1 based on Investigator assessment • SD per RECIST 1.1 based on Investigator assessment Version 1.1 (RECIST 1.1) by Investigator
Exploratory	
• To explore the association of systemic exposure of AGEN1777 and changes in select biomarkers of interest • To explore dose and exposure-related effects of AGEN1777 administered as a single-agent and in combination with an anti-PD-1 antibody on QT interval corrected using Fridericia's formula (QTcF) • To explore biomarkers that may predict pharmacologic activity or response to AGEN1777 as a single-agent and when combined with an anti-PD-1 antibody	• Associated measures between systemic AGEN1777 PK measures and select biomarkers of interest • Summary measures of changes in QTc from baseline when AGEN1777 is administered as a single-agent and in combination with an anti-PD-1 antibody • Molecular determinants of response to treatment including but not limited to assays on blood and tissue including immune cell activation markers, immune cell subsets, genomics, gene expression, circulating tumor DNA, and proteomics

Objectives	Endpoints
• To explore the effect of AGEN1777 as a single-agent and in combination with an anti-PD-1 antibody on immune biomarkers in peripheral blood and tumor tissue	• PFS median and/or rate as defined in the Statistical Analysis Plan (SAP) • OS median and/or rate as defined in the SAP

Investigational product, dosage, and mode of administration

[00123] AGEN1777 will be administered via IV infusion over 60 (± 5) minutes Q3W. The anti-PD-1 antibody will be administered via continuous IV infusion over 30 (± 15) minutes Q3W.

AGEN1777 as Single-Agent:

[00124] AGEN1777 will be evaluated at fixed doses of 2, 6, 20, 60, 200, 600, and 1200 mg as a single-agent, Q3W for up to 2 years or until PD or unacceptable toxicity is reported.

Cohort	Number of Patients	Escalation Type	Dose of AGEN1777 (mg) Q3W[1]
1 (Starting dose level)	3-6	3+3	2
2	3-6	3+3	6
3	3-6	3+3	20
4	3-6	3+3	60
5	3-6	3+3	200
6	3-6	3+3	600
7	3-6	3+3	1200

Abbreviations: DLT: dose-limiting toxicity; MTD: maximum tolerated dose; PK: pharmacokinetic; Q3W: every 3 weeks; RP2D: recommended Phase 2 dose; SMC, Safety Monitoring Committee

[1] Based on the totality of safety data, planned dose levels of single-agent AGEN1777 may be reduced, or intermediate dose levels may be added after discussion and agreement between the Sponsor and Investigators. If a patient experiences a DLT or 2 or more patients experience Grade ≥ 3 treatment related adverse events, dose escalation may proceed using approximately 2-fold increments (instead of approximately 3-fold increments) until the MTD is reached. The SMC may also suggest measures to manage emerging toxicities for future dosing cohorts and / or ongoing subjects.

Note: Alternate dosing schedules of AGEN1777, such as administered Q4W, may be explored depending on safety, preliminary efficacy, PK, and pharmacodynamic data.

Note: Following the identification of an RP2D, up to 15 additional patients may be treated at the RP2D for single-agent AGEN1777 and combination from a safety/PK/pharmacodynamic perspective.

AGEN1777 Combination with an Anti-PD-1 Antibody:

[00125] AGEN1777 in combination with an anti-PD-1 antibody will be administered on

Day 1 of each 3-week cycle, for up to 2 years, or until PD or unacceptable toxicity. The starting dose for AGEN1777 in combination with the anti-PD-1 antibody will be 1 to 2 dose levels below the expected active dose determined by the SMC based on available safety, pharmacodynamic, and PK data from single-agent AGEN1777.

[00126] Following AGEN1777 infusion, a fixed dose of 300 mg of the anti-PD-1 antibody will be administered on Day 1 of each 3 -week cycle via IV infusion over 30 (±15) minutes.

Cohort	Number of Patients	Escalation Type	Dose of AGEN1777 Q3W	Dose of anti-PD-1 antibody (mg) Q3W
1	3-6	3+3	2 dose levels below expected active dose	300
2	3-6	3+3	1 dose level below expected active dose	300
3	3-6	3+3	expected active dose	300

Abbreviations: PK: pharmacokinetic; Q3W: every 3 weeks; RP2D: recommended Phase 2 dose
Note: For the combination, AGEN1777 should be administered before the anti-PD-1 antibody with 30-minute interval between the infusion.
Note: Alternate dosing schedules of AGEN1777 and anti-PD-1 antibody, such as administered Q4W, may be explored depending on safety, preliminary efficacy, PK, and pharmacodynamic data.
Note: Following the identification of an RP2D, up to 15 additional patients may be treated at the RP2D for single-agent AGEN1777 and combination from a safety/PK/pharmacodynamic perspective.

Inclusion Criteria

[00127] In order to participate in the study, patient should meet the following inclusion criteria:

1. Voluntarily agree to participate by giving signed, dated, and written informed consent prior to any study specific procedures (participation in genetic testing is optional)
2. ≥ 18 years of age
3. Histologically or cytologically confirmed diagnosis of metastatic or locally advanced solid tumor for which no acceptable standard therapy available or progressed on or after standard therapies
4. Measurable disease on baseline imaging based on RECIST 1.1
5. Life expectancy of at least 3 months and an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1
6. Women of child-bearing potential (WOCBP) must have a negative urine or serum pregnancy test at screening (within 72 hours of first dose of study medication) with repeat urine or serum pregnancy test of Day 1 of each cycle and at the end of treatment visit. Non-childbearing potential is defined as:

- ≥ 45 years of age and has not had menses for greater than 1 year
- Amenorrheic for ≥ 2 years without a hysterectomy and oophorectomy and a follicle-stimulating hormone value in the postmenopausal range upon pre-study (screening) evaluation
- Status is post hysterectomy, oophorectomy, or tubal ligation

WOCBP must agree to use highly effective contraceptive measures starting with the screening visit through 90 days after the last dose of study treatment.

Note: Abstinence is acceptable if this is the established and preferred contraception for the patient.

WOCBP must agree not to donate eggs (ova, oocytes) during the treatment period and for at least 90 days after the last dose of study drug.

7. Patients must provide a sufficient and adequate formalin-fixed paraffin-embedded (FFPE) tumor tissue sample preferably from the most recent biopsy of a tumor lesion, collected either at the time of or after the diagnosis of advanced or metastatic disease has been made AND from a site not previously irradiated. If no suitable tumor tissue is available, a fresh biopsy will be required
8. Willing and able to comply with the requirements of the protocol

Exclusion Criteria

[00128] Patient will not be enrolled in the study, if the following criteria are met:

1. Active infection requiring treatment
2. Lack of recovery for patients who had major surgical procedure within 4 weeks prior to first dose of protocol therapy
3. Clinically significant (i.e., active) cardiovascular disease: cerebral vascular accident/stroke or myocardial infarction within 6 months of enrollment, unstable angina, congestive heart failure (New York Heart Association class ≥ II), or serious uncontrolled cardiac arrhythmia requiring medication.
4. QTc interval (corrected for heart rate using Fridericia's formula prolongation) >480 msec at screening except for right bundle branch block
5. Known central nervous system (CNS) involvement:
   - Untreated CNS metastases
   - Patients are eligible if CNS metastases have been treated and patients have neurologically returned to baseline (except for residual signs and symptoms related to the CNS treatment)

- In addition, patients must have been either off corticosteroids, or on a stable or decreasing dose of ≤ 10 mg daily prednisone or equivalent for at least 2 weeks prior to the first dose of study treatment

6. Patients with leptomeningeal metastases
7. Prior radiation therapy within 2 weeks prior to first treatment. Patients must have recovered (i.e., Grade ≤ 1 or at baseline) from radiation-related toxicities prior to first study treatment
8. Concurrent malignancy (present during screening) requiring treatment or history of prior malignancy active within 2 years prior to the first dose of study treatment (i.e., patients with a history of prior malignancy are eligible if treatment was completed at least 2 years before the first dose of study treatment and the patient has no evidence of disease). Patients with history of prior early-stage basal/squamous cell skin cancer or noninvasive or in situ cancers who have undergone definitive treatment at any time are also eligible

   The following washout windows are accepted:

   - Cytotoxic agent after 3 weeks
   - Monoclonal antibodies after 5 half-lives or 4 weeks, whichever is less
   - Investigational therapies after 14 days or following 5 circulating half-lives of investigational drug
   - Previous SARS-CoV-2 vaccine > 7 days before administration
9. Known allergy or hypersensitivity to any of the study drugs or any of the study drug excipients
10. Any evidence of current interstitial lung disease (ILD) or pneumonitis or a prior history of ILD or non-infectious pneumonitis requiring high-dose glucocorticoids
11. History of allogeneic organ transplant
12. Psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the study
13. Uncontrolled hypertension or controlled hypertension (< 140/90 mmHg) on more than 3 antihypertensive agents
14. Patients with a condition requiring systemic treatment with either corticosteroids (> 10 mg/day prednisone equivalent) within 14 days or another immunosuppressive medication within 30 days of the first dose of study treatment. Inhaled or topical steroids, and adrenal replacement steroid doses > 10 mg/day prednisone equivalent, are permitted in the absence of active autoimmune disease

15. Active or history of autoimmune disease that requires systemic treatment within 2 years of the start of study treatment (i.e., with use of disease-modifying agents, systemic corticosteroids, or immunosuppressive drugs)
16. History or current evidence of any condition, therapy, any active infections, or laboratory abnormality that might confound the results of the study, interfere with the patient's participation for the full duration of the study, or is not in the best interest of the patient to participate, in the opinion of the treating Investigator
17. Legally incapacitated or has limited legal capacity except if patient's legal representative provides written signature for inform consent and study documents
18. Pregnant or breastfeeding or female patients of reproductive potential who are not willing to employ effective birth control from screening to 90 days after the last dose of AGEN1777 and/or anti-PD-1 antibody (whichever is later)
19. HIV positive except CD4 > 200 and HIV viral load undetectable
20. Previous SARS-CoV-2 infection within 10 days for mild or asymptomatic infections or 20 days for severe/critical illness prior to Cycle 1 Day 1
21. Laboratory test finding:
    - Cytopenia's due to bone marrow suppression with the following specific lower limits. (Individual cases of cytopenia's due to tumor bleeding may be discussed with the Medical Monitor). Regardless of origin of cytopenia patients must be clinically stable. Patients with the following will be excluded:
        - White blood cell count (WBC) < 2000/μL
        - Neutrophils < 1500/μL (stable off any growth factor within 4 weeks of first study treatment administration)
        - Platelets < 75 ×μL (transfusion to achieve this level is not permitted within 2 weeks of first study treatment administration)
        - Hemoglobin < 8.0 g/dL (transfusion to achieve this level is not permitted within 2 weeks of first study treatment administration
    - Serum creatinine > 1.5 × upper limit of normal (ULN), unless creatinine clearance ≥ 40 mL/min (measured or calculated using the Cockcroft-Gault formula)
    - Aspartate aminotransferase (AST) or alanine aminotransferase (ALT): > 3.0 × ULN
    - Total bilirubin > 1.5 × ULN (except patients with Gilbert syndrome who must have a total bilirubin level of < 3.0 × ULN)
    - Albumin < 3 g/dL
    - Any positive test result for hepatitis B virus (HBV) indicating presence of virus,

e.g., Hepatitis B surface antigen (HBsAg, Australia antigen) positive

- Any positive test result for hepatitis C virus (HCV) indicating presence of active viral replication (detectable HCV-RNA). Note: Patients with positive HCV antibody and an undetectable HCV RNA are eligible to enroll

Criteria for evaluation

[00129] **Safety:** Safety will be assessed by evaluating adverse events (AEs) graded according to CTCAE version 5.0, vital signs (temperature, pulse, respiratory rate, and blood pressure); physical examinations, 12-lead electrocardiogram (ECG), ECOG performance status, and clinical laboratory assessments. DLT will be assessed during the dose escalation phase. An SMC will be established to assess safety, decide on dose escalation, and define the RP2D.

[00130] **Pharmacokinetics:** Serum AGEN1777 and anti-PD-1 antibody PK parameters may include (but are not limited to) maximum observed drug concentration at steady state ($C_{max\text{-}ss}$), minimum observed drug concentration at steady state ($C_{min\text{-}ss}$), area under the drug concentration-time curve within time span t1 to t2 at steady state ($AUC_{(t1\text{-}t2)\text{-}ss}$), area under the drug concentration-time curve from time zero to time t ($AUC_{(0\text{-}t)}$), area under the drug concentration-time curve from time zero to infinity ($AUC_{(0\text{-}\infty)}$), time to maximum observed drug concentration (t_{max}), elimination rate constant (λz), terminal elimination half-life ($t_{1/2}$), systemic drug clearance (CL), and volume of distribution (Vd). Both noncompartmental analysis (NCA) and compartmental modeling (e.g., population PK) will be used to analyze PK.

[00131] **Immunogenicity:** Serum ADA and potential impact on AGEN1777 and anti-PD-1 antibody PK exposure metrics and biological activity will be assessed.

[00132] **Pharmacodynamics:** Blood samples and tumor biopsies will be collected for exploratory biomarker analysis.

[00133] **Efficacy:** Response assessments will be performed according to RECIST 1.1. On-study imaging will be performed every 9 weeks (±7 days) after initiation of treatment for 12 months, and every 12 weeks (±7 days) thereafter.

* * *

[00134] The invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

[00135] All references (*e.g.*, publications or patents or patent applications) cited herein are incorporated herein by reference in their entireties and for all purposes to the same extent as if each individual reference (*e.g.*, publication or patent or patent application) was specifically and individually indicated to be incorporated by reference in its entirety for all purposes.
Other embodiments are within the following claims.

WHAT IS CLAIMED:

1. A method of treating cancer in a subject in need thereof, the method comprising administering to the subject:

(a) a multispecific molecule at a dose of about 2 mg to about 2000 mg, wherein the multispecific molecule comprises:

(i) a first antigen-binding region that specifically binds to human CD96, the first antigen-binding region comprising a first heavy chain variable region (VH) comprising CDRs CDRH1, CDRH2, and CDRH3, and a first light chain variable region (VL) comprising CDRs CDRL1, CDRL2, and CDRL3; wherein the first VH comprises the CDRH1, CDRH2, and CDRH3 amino acid sequences of the VH amino acid sequence of SEQ ID NO: 7; and the first VL comprises the CDRL1, CDRL2, and CDRL3 amino acid sequences of the VL amino acid sequence of SEQ ID NO: 8; and

(ii) a second antigen-binding region that specifically binds to human TIGIT, the second antigen-binding region comprising a second VH comprising CDRs CDRH1, CDRH2, and CDRH3, and a second VL comprising CDRs CDRL1, CDRL2, and CDRL3; wherein the second VH comprises the CDRH1, CDRH2, and CDRH3 amino acid sequences of the VH amino acid sequence of SEQ ID NO: 9; and the second VL comprises the CDRL1, CDRL2, and CDRL3 amino acid sequences of the VL amino acid sequence of SEQ ID NO: 10; and

(b) a human PD-1 inhibitor.

2. The method of claim 1, wherein the multispecific molecule is administered at a dose of about 20 mg to about 1200 mg.

3. The method of claim 1 or 2, wherein the multispecific molecule is administered at a dose of about 20 mg, about 60 mg, about 200 mg, about 600 mg, or about 1200 mg.

4. The method of any one of the preceding claims, wherein the multispecific molecule is administered intravenously.

5. The method of any one of the preceding claims, wherein the multispecific molecule is administered by intravenous infusion over about 30 minutes.

6. The method of any one of the preceding claims, wherein the multispecific molecule is administered by intravenous infusion over about 60 minutes.

7. The method of any one of the preceding claims, wherein the multispecific molecule is administered by intravenous infusion over about 90 minutes.

8. The method of any one of the preceding claims, wherein the multispecific molecule is administered once weekly.

9. The method of any one of the preceding claims, wherein the multispecific molecule is administered once every 2 weeks.

10. The method of any one of the preceding claims, wherein the multispecific molecule is administered once every 3 weeks.

11. The method of any one of the preceding claims, wherein the multispecific molecule is administered once every 4 weeks.

12. The method of any one of the preceding claims, wherein the multispecific molecule is administered once every 5 weeks.

13. The method of any one of the preceding claims, wherein the multispecific molecule is administered once every 6 weeks.

14. The method of any one of the preceding claims, wherein the multispecific molecule is administered intravenously at a dose of 20 mg once every 3 weeks.

15. The method of any one of the preceding claims, wherein the multispecific molecule is administered intravenously at a dose of 60 mg once every 3 weeks.

16. The method of any one of the preceding claims, wherein the multispecific molecule is administered intravenously at a dose of 200 mg once every 3 weeks.

17. The method of any one of the preceding claims, wherein the multispecific molecule is administered intravenously at a dose of 600 mg once every 3 weeks.

18. The method of any one of the preceding claims, wherein the multispecific molecule is administered intravenously at a dose of 1200 mg once every 3 weeks.

19. The method of any one of the preceding claims, wherein the cancer is a solid tumor.

20. The method of claim 19, wherein the solid tumor is metastatic or locally advanced.

21. The method of any one of the preceding claims, wherein before administration of the multispecific molecule:

(a) the subject is at least 18 years of age;

(b) the subject has a histologically or cytologically confirmed diagnosis of metastatic or locally advanced solid tumor for which no acceptable standard therapy is available or progressed on or after standard therapies;

(c) the subject has measurable disease as assessed on baseline imaging according to Response Evaluation Criteria in Solid Tumors Version 1.1 (RECIST 1.1);

(d) the subject has a predicted life expectancy of at least 3 months and an Eastern Cooperative Oncology Group performance status of 0 or 1; and/or

(e) the subject is not pregnant.

22. The method of any one of the preceding claims, wherein the first antigen-binding region comprises the CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3 amino acid sequences set forth in SEQ ID NOs: 1, 2, 3, 4, 5, and 6, respectively, and the second antigen-binding region comprises the CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3 amino acid sequences set forth in SEQ ID NOs: 11, 12, 13, 14, 15, and 16, respectively.

23. The method of any one of the preceding claims, wherein the first antigen-binding region comprises the VH amino acid sequence of SEQ ID NO: 7 and the VL amino acid sequence of SEQ ID NO: 8, and the second antigen-binding region comprises the VH amino acid sequence of SEQ ID NO: 17 and the VL amino acid sequence of SEQ ID NO: 18.

24. The method of any one of the preceding claims, wherein the first antigen-binding region and/or the second antigen-binding region comprises a heavy chain constant region selected from the group consisting of human IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, and IgA_2.

25. The method of claim 24, wherein:

(a) the first antigen-binding region comprises a first human IgG heavy chain constant region comprising aspartate, serine, alanine, and valine at amino acid positions 239, 366, 368, and 407, respectively, and the second antigen-binding region comprises a second human IgG heavy chain constant region comprising aspartate, leucine, glutamate, and tryptophan at amino acid positions 239, 330, 332, and 366, respectively; or

(b) the first antigen-binding region comprises a first human IgG heavy chain constant region comprising aspartate, leucine, glutamate, and tryptophan at amino acid positions 239, 330, 332, and 366, respectively, and the second antigen-binding region comprises a second human IgG heavy chain constant region comprising aspartate, serine, alanine, and valine at amino acid positions 239, 366, 368, and 407, respectively,

wherein the amino acid positions are numbered according to the EU numbering system.

26. The method of any one of the preceding claims, wherein:

(a) the first antigen-binding region comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 9, and/or a light chain comprising the amino acid sequence of SEQ ID NO: 10; and/or

(b) the second antigen-binding region comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 19, and/or a light chain comprising the amino acid sequence of SEQ ID NO: 20.

27. The method of any one of the preceding claims, wherein the first antigen-binding region comprises a heavy chain and a light chain comprising the amino acid sequences of SEQ ID NOs: 9 and 10, respectively, and the second antigen-binding region comprises a heavy chain and a light chain comprising the amino acid sequences of SEQ ID NOs: 19 and 20, respectively.

28. The method of any one of the preceding claims, wherein the human PD-1 inhibitor is an antibody that specifically binds to human PD-1 or human PD-L1.

29. The method of claim 28, wherein the antibody is selected from the group consisting of nivolumab, adebrelimab, atezolizumab, avelumab, camrelizumab, cemiplimab, cosibelimab, dostarlimab, durvalumab, enlonstobart, envafolimab, pembrolizumab, penpulimab, pidilizumab, prolgolimab, pucotenlimab, retifanlimab, serplulimab, sintilimab, socazolimab, sugemalimab, tagitanlimab, tislelizumab, toripalimab, and zimberelimab.

30. The method of claim 28 or 29, wherein the antibody is nivolumab or atezolizumab.

31. The method of any one of the previous claims, further comprising administering to the subject a chemotherapeutic agent.

32. A multispecific molecule that specifically binds to human CD96 and human TIGIT for use in the treatment of cancer, wherein the treatment is performed according to the method of any one of the preceding claims.

33. A multispecific molecule that specifically binds to human CD96 and human TIGIT for use in the manufacture of a medicament for the treatment of cancer, wherein the treatment is performed according to the method of any one of the preceding claims.

34. Use of a multispecific molecule that specifically binds to human CD96 and human TIGIT for the treatment of cancer, wherein the treatment is performed according to the method of any one of the previous claims.