METHODS OF CANCER TREATMENT USING ANTI-TIGIT ANTIBODIES

FIELD

[0001] The present application relates to antibodies that specifically bind to TIGIT (T cell immunoreceptor with Ig and ITIM domains) in combination with anti-PD1 antibodies for the treatment of cancer.

BACKGROUND

[0002] TIGIT (T cell immunoglobulin and ITIM domain) is a type I transmembrane protein, a member of the CD28 family of proteins that plays an important role in inhibiting T- and NK cell-mediated functional activities in anti-tumor immunity (Boles KS, et al., 2009 Eur. J Immunol, 39:695-703; Stanietsky N, et al., 2009 PNAS 106:17858-63; Yu X, et al. 2009 Nat. Immunol, 10:48-57).

[0003] The genes and cDNAs coding for TIGIT were cloned and characterized in mouse and human. Full length human TIGIT has a sequence of 244 amino acids (SEQ ID NO: 26) in length, in which the first 21 amino acids consist of a signal peptide. The amino acid sequence of the mature human TIGIT contains 223 amino acid (aa) residues (NCBI accession number: NM_173799). The extracellular domain (ECD) of mature human TIGIT consists of 120 amino acid residues, corresponding to amino acids 22-141 of SEQ ID NO: 26) with a V-type Ig-like domain (corresponding to amino acids 39-127 of SEQ ID NO: 26), followed by a 21 aa transmembrane sequence, and an 82 aa cytoplasmic domain with an immunoreceptor tyrosine-based inhibitory motif (ITIM) (Yu X, et al. 2009 Nat. Immunol, 10:48-57; Stengel KF, et al. 2012 PNAS 109:5399-04). Within the ECD, human TIGIT shares only 59% and 87% aa sequence identity with mouse and cynomolgus monkey, respectively.

[0004] TIGIT is expressed on T cells (including activated T cells, memory T cells, regulatory T (Treg) cells, and follicular T helper (Tfh) cells), and NK cells (Boles KS, et al., 2009 Eur J Immunol, 39:695-703; Joller N, et al., 2014 Immunity 40:569-81; Levin SD, et al., 2011 Eur J Immunol, 41:902-15; Stanietsky N, et al., 2009 PNAS 106:17858-63; Yu X, et al. 2009 Nat. Immunol, 10:48-57).

[0005] So far, two TIGIT ligands, CD155 (also known as poliovirus receptor or PVR) and CD112 (also known as poliovirus receptor-related 2, PVRL2, nectin-2), have been identified. These ligands

are primarily expressed on APCs (such as dendritic cells and macrophages) and tumor cells (Casado JG, et al., 2009 Cancer Immunol Immunother 58:1517-26; Levin SD, et al., 2011 Eur. J Immunol, 41:902-15; Mendelsohn CL et al., 1989 56:855-65; Stanietsky N, et al., 2009 PNAS 106:17858-63; Yu X, et al. 2009 Nat. Immunol, 10:48-57). As an immune "checkpoint" molecule, TIGIT initiates inhibitory signaling in immune cells when engaged by its ligands, CD155 and CD112. The binding affinity of TIGIT to CD155 (Kd: ~1 nM) is much higher than to CD112 and whether the TIGIT: CD112 interaction is functionally relevant in mediating inhibitory signals yet remain to be determined. A co-stimulatory receptor, CD226 (DNAM-1), binds to the same ligands with lower affinity (Kd:~100 nM), but delivers a positive signal (Bottino C, et al., 2003 J Exp Med 198:557-67). In addition, CD96 (TACTILE), a "TIGIT-like" receptor, also plays a similarly inhibitory role in the same pathway (Chan CJ, et al., 2014 Nat. Immunol 15:431-8).

[0006] Up-regulation of TIGIT expression in tumor-infiltrating lymphocytes (TILs) and peripheral blood mononuclear cells (PBMCs) has been reported in many types of cancers such as lung (Tassi, et al., Cancer Res. 2017 77: 851-861), esophageal (Xie J, et al., Oncotarget 2016 7:63669-63678), breast (Gil Del Alcazar CR, et al. 2017 Cancer Discov.), acute myeloid leukemia (AML) (Kong Y et al., Clin Cancer Res. 2016 22:3057-66) and melanoma (Chauvin JM, et al., J Clin Invest. 2015 125:2046-2058). The increased expression of TIGIT in AML is associated with poor prognosis of patient survival outcome (Kong Y et al., Clin Cancer Res. 2016 22:3057-66). Not only does up-regulation of TIGIT signaling play important roles in immune tolerance to cancer, but also to chronic viral infection. During HIV infection, expression of TIGIT on T cells was significantly higher and positively correlated with viral loads and disease progression (Chew GM, et al., 2016 PLoS Pathog. 12:e1005349). In addition, blockade of TIGIT receptor alone or in combination with other blockade could rescue functionally "exhausted" T cells both in vitro and in vivo (Chauvin JM, et al., J Clin Invest. 2015 125:2046-2058; Chew GM, et al., 2016 PLoS Pathog. 12:e1005349; Johnston RJ, et al. Cancer Cell 2014 26:923-937). In the cases of cancer and viral infections, activation of TIGIT signaling promotes immune cell dysfunction, leading to the cancer outgrowth or extended viral infection. Inhibition of TIGIT-mediated inhibitory signaling by therapeutic agents may restore the functional activities of immune cells including T cells, NK cells and dendritic cells (DCs), therefore enhancing immunity against cancer or chronic viral infection.

[0007] Therefore, anti-TIGIT antibodies with enhanced effector function can induce efficient

immune responses in the treatment of cancer or chronic viral infections.

SUMMARY

[0008] The present disclosure is directed methods of cancer treatment, administering anti-TIGIT antibodies.

[0009] A method of cancer treatment, the method comprising administering to a subject an effective amount of anti-TIGIT antibody or antigen-binding fragment thereof that is pH dependent.

[0010] The method, wherein the anti-TIGIT antibody binds to the TIGIT protein at amino acid histidine 76.

[0011] The method, wherein the anti-TIGIT antibody binds to the TIGIT protein at histidine 76 and leucine 73.

[0012] The method, wherein the method comprises administering to a subject an effective amount of a pH dependent antibody or antigen-binding fragment thereof, which specifically binds to human TIGIT and comprises: a heavy chain variable region that comprises a HCDR (Heavy Chain Complementarity Determining Region) 1 of SEQ ID NO:1, a HCDR2 of SEQ ID NO:2, and a HCDR3 of SEQ ID NO:3; and a light chain variable region that comprises a LCDR (Light Chain Complementarity Determining Region) 1 of SEQ ID NO:4, a LCDR2 of SEQ ID NO:5, and a LCDR3 of SEQ ID NO:6.

[0013] The method, wherein the anti-TIGIT antibody or antigen-binding fragment thereof comprises: a heavy chain variable region (VH) that comprises SEQ ID NO:7, and a light chain variable region (VL) that comprises SEQ ID NO: 8.

[0014] The method, wherein the anti-TIGIT antibody has enhanced antibody dependent cell mediated cytotoxicity (ADCC) activity.

[0015] The method, wherein the anti-TIGIT antibody has reduced fucosylation.

[0016] The method, wherein the anti-TIGIT antibody has Fc amino acid changes at S239D, and I332E (EU numbering).

[0017] The method, wherein the anti-TIGIT antibody has Fc amino acid changes at S239D, I332E and A330L (EU numbering).

[0018] The method of any one of the above, wherein the anti-TIGIT antibody has increased binding affinity to FcγRIIIA-V158 and FcγRIIIA-F158.

[0019] The method of any one of the above, wherein the anti-TIGIT antibody has enhanced ADCC in T-regulatory (Treg) cells.

[0020] The method of any one of the above, wherein the anti-TIGIT antibody activates Natural Killer (NK) cells.

[0021] The method of any one of the above, wherein the anti-TIGIT antibody has increased trogocytosis.

[0022] The method of the above, wherein the method further comprises administering an anti-PD1 antibody which specifically binds human PD1 and comprises: a heavy chain variable region that comprises a HCDR1 of SEQ ID NO:15, HCDR2 of SEQ ID NO:16, and HCDR3 of SEQ ID NO:17; and a light chain variable region that comprises LCDR1 of SEQ ID NO:18, LCDR2 of SEQ ID NO:19, and LCDR3 of SEQ ID NO:20.

[0023] The method of the above, wherein the anti-PD1 antibody or antigen binding fragment thereof which specifically binds human PD1 and comprises a heavy chain variable region (VH) comprising an amino acid sequence of SEQ ID NO:21 and a light chain variable region (VL) comprising an amino acid sequence of SEQ ID NO:22.

[0024] The method wherein the anti-PD1 antibody comprises an IgG4 constant domain comprising SEQ ID NO: 23.

[0025] The method, wherein the cancer is selected from the group consisting of breast cancer, colon cancer, pancreatic cancer, head and neck cancer, gastric cancer, kidney cancer, liver cancer, small cell lung cancer, non-small cell lung cancer, esophageal cancer, ovarian cancer, uterine cancer, cervical cancer, skin cancer, mesothelioma, lymphoma, leukemia, myeloma or sarcoma.

[0026] The method, wherein the cancer is non-small cell lung cancer.

[0027] The method, wherein the head and neck cancer is nasopharyngeal cancer.

[0028] The method, wherein the esophageal cancer is esophageal squamous cell carcinoma (ESCC).

[0029] The method, wherein the cancer is uterine cancer.

[0030] The method, wherein the gastric cancer is gastric or gastroesophageal junction cancer.

[0031] The method, wherein the cervical cancer is recurring or metastatic cervical cancer.

[0032] The method, wherein the cancer is kidney cancer.

[0033] The method, further comprising the administration of chemotherapy.

[0034] The method, wherein the chemotherapy is chemoradiotherapy.

[0035] The method, wherein the anti-PD1 antibody is dosed at 200mg every three weeks.

BRIEF DESCRIPTION OF THE DRAWINGS

[0036] **Figure 1A-D** shows the crystal structure of A1217 (Ociperlimab) Fab bound to human TIGIT. **Figure 1A** is the crystal structure of A1217 Fab bound to human TIGIT, with A1217 heavy chain (HC) and light chain (LC) regions colored in black and gray while TIGIT is represented by the white surface. **Figure 1B** shows the crystal structure of A1217 Fab bound to human TIGIT, superimposing with the known PVR/TIGIT complex structure (PDB 3UDW), showing that A1217 and PVR in gray ribbon have steric clash and competitive binding for TIGIT. **Figure 1C** is the atomic interactions on the binding surface of A1217/TIGIT complex, identifying certain key residues of A1217 (paratope residues shown in line) and TIGIT (epitope residues shown in stick with underlined text). For clarity, non-CDR regions of A1217 are removed with TIGIT represented in white transparent surface. The boxed residues were identified as functionally important epitopic residues by alanine scanning mutagenesis. **Figure 1D** shows the surrounding residues of $HIS76_{TIGIT}$ within A1217 to emphasize the interaction between $HIS76_{TIGIT}$ and $ASP103_{HCDR3}$ which is critical for pH-dependent binding for TIGIT. $HIS76_{TIGIT}$ and $ASP103_{HCDR3}$ are highlighted with underlined text.

[0037] **Figure 2A-C** shows the blockade comparison between A1217 (Ociperlimab) Fab and Tiragolumab Fab. Figure 2A shows superposition of Ociperlimab Fab/TIGIT with the Tiragolumab Fab/TIGIT complex. Ociperlimab Fab and Tiragolumab Fab are colored black and white, respectively. TIGIT is shown in white surface representation. Figure 2B shows the epitope by Ociperlimab Fab or Tiragolumab Fab. The overlapped epitope between these two antibodies is labeled with underlined black text. The unique epitope of Ociperlimab Fab is colored black with white text, whereas the unique epitope of Tiragolumab Fab is colored gray with black text. Figure 2C is Figure 2B viewed from the side (rotated 90°).

[0038] **Figure 3A-B** is a depiction of the atomic interaction on the binding surface of the Ociperlimab Fab/TIGIT and Tiragolumab Fab/TIGIT complex. Figure 3A depicts the binding interface between Ociperlimab Fab and TIGIT. HCDR1, HCDR2 and HCDR3 of the heavy chain are labelled with bold italics black text, black text and underlined black text, respectively. LCDR1, LCDR2 and LCDR3 of the light chain are labelled with bold italics gray text, gray text and underlined gray text, respectively. Figure 3B depicts the binding interface between Tiragolumab_Fab

and TIGIT. HCDR2 and HCDR3 of the heavy chain are labelled with black text and underlined black text, respectively. LCDR1 and LCDR3 of the light chain are labelled with gray text and underlined gray text, respectively. TIGIT is labelled with bold white text in both (a) and (b).

[0039] **Figure 4** is a table summarizing the production of afucosylated A1217 antibody.

[0040] **Figure 5A-B.** Figure 5A shows the binding of A1217AF antibodies to FcγRIIIA-F158, while Figure 5B depicts the binding of A1217AF antibodies to FcγRIIIA-V158.

[0041] **Figure 6A-D.** Figures 6A-B is a comparison of A1217WT with A1217AF and mutated effector variant antibodies (including an Fc silent antibody) and how they bind to FcγRIIIA-V158 and FcγRIIIA-F158. Figures 6C-D depicts the binding of A1217 antibodies to FcγRIIIA-V158 and FcγRIIIA-F158 expressed on HEK293 cells.

[0042] **Figure 7** shows that A1217, A1217DE and A1217DEL have comparable binding to TIGIT over-expressing HEK293 cells.

[0043] **Figure 8** indicates that A1217AF and A1217 showed comparable binding to C1q.

[0044] **Figure 9A-B** demonstrate that A1217AF, A1217DEL and A1217DE have enhanced ADCC activity when compared with A1217 wild type.

[0045] **Figure 10A-C** shows that A1217AF has enhanced ADCC activity against Tregs.

[0046] **Figure 11A-B** indicates the activation of NK cells by A1217AF.

[0047] **Figure 12** demonstrates that TIGIT is down regulated by A1217AF.

[0048] **Figure 13** shows the trogocytosis properties of A1217.

[0049] **Figure 14** is a mouse model of A1217 in combination with an anti-PD1 antibody.

[0050] **Figure 15A-C** is a mouse model of A1217 in combination with an anti-PD1 antibody and Treg reduction.

DETAILED DESCRIPTION

Definitions

[0051] Conservative amino acid substitutions of amino acids are commonly known in the art. Generally, a conservative amino acid substitution means that an amino acid residue is replaced by another amino acid residue having a similar side chain.

[0052] Unless specifically defined elsewhere in this document, all other technical and scientific terms used herein have the meaning commonly understood by one of ordinary skill in the art to which this invention belongs.

[0053] As used herein, including the appended claims, the singular forms of words such as "a," "an," and "the" include their corresponding plural references unless the context clearly dictates otherwise.

[0054] The term "or" is used to mean, and is used interchangeably with, the term "and/or" unless the context clearly dictates otherwise.

[0055] Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise," and variations such as "comprises" and "comprising," will be understood to imply the inclusion of a stated amino acid sequence, DNA sequence, step or group thereof, but not the exclusion of any other amino acid sequence, DNA sequence, step. When used herein the term "comprising" can be substituted with the term "containing," "including" or sometimes "having."

[0056] The term "TIGIT" includes various mammalian isoforms, e.g., human TIGIT, orthologs of human TIGIT, and analogs comprising at least one epitope within TIGIT. The amino acid sequence of TIGIT, e.g., human TIGIT, and the nucleotide sequence encoding the same, is known in the art (see Genbank AAI01289). Human TIGIT sequence (SEQ ID NO:26)

MRWCLLLIWAQGLRQAPLASGMMTGTIETTGNISAEKGGSIILQCHLSSTTAQVTQVNWE
QQDQLLAICNADLGWHISPSFKDRVAPGPGLGLTLQSLTVNDTGEYFCIYHTYPDGTYTG
RIFLEVLESSVAEHGARFQIPLLGAMAATLVVICTAVIVVVALTRKKKALRIHSVEGDLR
RKSAGQEEWSPSAPSPPGSCVQAEAAPAGLCGEQRGEDCAELHDYFNVLSYRSLGNCSFF
TETG

[0057] The terms "administration," "administering," "treating" and "treatment" as used herein, when applied to an animal, human, experimental subject, cell, tissue, organ, or biological fluid, mean contact of an exogenous pharmaceutical, therapeutic, diagnostic agent, or composition to the animal, human, subject, cell, tissue, organ, or biological fluid. Treatment of a cell encompasses contact of a reagent to the cell, as well as contact of a reagent to a fluid, where the fluid is in contact with the cell. The term "administration" or "treatment" also includes *in vitro* and *ex vivo* treatments, e.g., of a cell, by a reagent, diagnostic, binding compound, or by another cell. The term "subject" herein refers to any organism, preferably an animal, more preferably a mammal (e.g., rat, mouse, dog, cat, rabbit) and most preferably a human.

[0058] The term "antibody" herein is used in the broadest sense and specifically covers antibodies (including full length monoclonal antibodies) and antibody fragments so long as they recognize

antigen, e.g., TIGIT. An antibody is usually monospecific, but may also be described as idiospecific, heterospecific, or polyspecific. Antibody molecules bind by means of specific binding sites to specific antigenic determinants or epitopes on antigens.

[0059] The term "monoclonal antibody" or "mAb" or "Mab" herein means a population of substantially homogeneous antibodies, i.e., the antibody molecules comprised in the population are identical in amino acid sequence except for possible naturally occurring mutations that may be present in minor amounts. In contrast, conventional (polyclonal) antibody preparations typically include a multitude of different antibodies having different amino acid sequences in their variable domains, particularly their complementarity determining regions (CDRs), which are often specific for different epitopes. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies and is not to be construed as requiring production of the antibody by any particular method. Monoclonal antibodies (mAbs) may be obtained by methods known to those skilled in the art. See, for example Kohler G et al., Nature 1975 256:495-497; U.S. Pat. No. 4,376,110; Ausubel FM et al., CURRENT PROTOCOLS IN MOLECULAR BIOLOGY 1992; Harlow E et al., ANTIBODIES: A LABORATORY MANUAL, Cold spring Harbor Laboratory 1988; and Colligan JE et al., CURRENT PROTOCOLS IN IMMUNOLOGY 1993. The mAbs disclosed herein may be of any immunoglobulin class including IgG, IgM, IgD, IgE, IgA, and any subclass thereof. A hybridoma producing a mAb may be cultivated *in vitro* or *in vivo*. High titers of mAbs can be obtained by *in vivo* production where cells from the individual hybridomas are injected intraperitoneally into mice, such as pristine-primed Balb/c mice to produce ascites fluid containing high concentrations of the desired mAbs. MAbs of isotype IgM or IgG may be purified from such ascites fluids, or from culture supernatants, using column chromatography methods well known to those of skill in the art.

[0060] In general, the basic antibody structural unit comprises a tetramer. Each tetramer includes two identical pairs of polypeptide chains, each pair having one "light chain" (about 25 kDa) and one "heavy chain" (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of the heavy chain may define a constant region primarily responsible for effector function. Typically, human light chains are classified as kappa and lambda light chains. Furthermore, human heavy chains are typically classified as α, δ, ε, γ, or μ, and define the antibody's

isotypes as IgA, IgD, IgE, IgG, and IgM, respectively. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 10 more amino acids.

[0061] The variable regions of each light/heavy chain (VL/VH) pair form the antibody binding site. Thus, in general, an intact antibody has two binding sites. Except in bifunctional or bispecific antibodies, the two binding sites are, in general, the same.

[0062] Typically, the variable domains of both the heavy and light chains comprise three hypervariable regions, also called "complementarity determining regions (CDRs)," which are located between relatively conserved framework regions (FR). The CDRs are usually aligned by the framework regions, enabling binding to a specific epitope. In general, from N-terminal to C-terminal, both light and heavy chain variable domains sequentially comprise FR-1 (or FR1), CDR-1 (or CDR1), FR-2 (FR2), CDR-2 (CDR2), FR-3 (or FR3), CDR-3 (CDR3), and FR-4 (or FR4). The assignment of amino acids to each domain is, generally, in accordance with the definitions of Sequences of Proteins of Immunological Interest, Kabat, et al., National Institutes of Health, Bethesda, Md. ; 5th ed.; NIH Publ. No. 91-3242 (1991); Kabat (1978) Adv. Prot. Chem. 32: 1-75; Kabat, et al., (1977) J. Biol. Chem. 252:6609-6616; Chothia, et al, (1987) J Mol. Biol. 196:901-917 or Chothia, et al., (1989) Nature 342:878-883.

[0063] The term "hypervariable region" means the amino acid residues of an antibody that are responsible for antigen-binding. The hypervariable region comprises amino acid residues from a "CDR" (i.e., VL-CDR1, VL-CDR2 and VL-CDR3 in the light chain variable domain and VH-CDR1, VH-CDR2 and VH-CDR3 in the heavy chain variable domain). See, Kabat et al. (1991) Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (defining the CDR regions of an antibody by sequence); see also Chothia and Lesk (1987) J. Mol. Biol. 196: 901-917 (defining the CDR regions of an antibody by structure). The term "framework" or "FR" residues mean those variable domain residues other than the hypervariable region residues defined herein as CDR residues.

[0064] Unless otherwise indicated, "antibody fragment" or "antigen-binding fragment" means antigen binding fragments of antibodies, i.e., antibody fragments that retain the ability to bind specifically to the antigen bound by the full-length antibody, e.g., fragments that retain one or more CDR regions. Examples of antigen binding fragments include, but not limited to, Fab, Fab', F(ab')2,

and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules, e.g., single chain Fv (ScFv); nanobodies and multispecific antibodies formed from antibody fragments.

[0065] An antibody that binds to a specified target protein with specificity is also described as specifically binding to a specified target protein. This means the antibody exhibits preferential binding to that target as compared to other proteins, but this specificity does not require absolute binding specificity. An antibody is considered "specific" for its intended target if its binding is determinative of the presence of the target protein in a sample, e.g., without producing undesired results such as false positives. Antibodies or binding fragments thereof, useful in the present invention will bind to the target protein with an affinity that is at least two-fold greater, preferably at least 10-times greater, more preferably at least 20-times greater, and most preferably at least 100-times greater than the affinity with non-target proteins. An antibody herein is said to bind specifically to a polypeptide comprising a given amino acid sequence, e.g., the amino acid sequence of a mature human TIGIT molecule, if it binds to polypeptides comprising that sequence but does not bind to proteins lacking that sequence.

[0066] The expressions "pH-dependent binding," "pH-dependent target binding" and "pH-dependent antigen binding" are interchangeable in the present disclosure, indicating that the antibody of the present application binds to its target/antigen, namely human TIGIT, in a pH-dependent manner. Specifically, the antibody of the present application shows a higher binding affinity and/or binding signal to its antigen at a mild acidic pH, e.g., pH 6.0, which is usually found in tumor microenvironment, as compared to the binding affinity and/or binding signal at physiologic pH, e.g., pH 7.4. The methods for determining the binding affinity and/or the intensity of binding signal of the antibody of the present application are well known in the art and include but not limited to surface plasmon resonance (Biacore) or similar technology. More specifically, the antibody of the present application has a K_D ratio at pH 7.4/pH 6.0 of greater than 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, or more, as measured by surface plasmon resonance (Biacore) or similar technology. Alternatively, or additionally, the antibody of the present application has a Rmax (RU) value at pH 6.0 which is at least 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50- fold higher than the Rmax at pH 7.4 as measured by surface plasmon resonance (Biacore) or similar technology. The binding affinity of the antibody can be measured at 25℃ or 37℃. Tumor microenvironment has been found to show a relatively more acidic pH than

physiological condition or normal tissues (Zhang et al., Focus on molecular Imaging 2010; Tannock and Rotin et al. Cancer Res 1989). Therefore, the antibody of the present application having above-mentioned pH-dependent binding is advantageous as an anti-TIGIT therapeutic agent for targeting TIGIT-positive lymphocytes in the tumor microenvironment with selectivity and having lower toxicity associated with periphery activation of lymphocytes.

[0067] The term "human antibody" herein means an antibody that comprises human immunoglobulin protein sequences only. A human antibody may contain murine carbohydrate chains if produced in a mouse, in a mouse cell, or in a hybridoma derived from a mouse cell. Similarly, "mouse antibody" or "rat antibody" means an antibody that comprises only mouse or rat immunoglobulin protein sequences, respectively.

[0068] The term "humanized antibody" means forms of antibodies that contain sequences from non-human (e.g., murine) antibodies as well as human antibodies. Such antibodies contain minimal sequence derived from non-human immunoglobulin. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. The prefix "hum," "hu," "Hu" or "h" is added to antibody clone designations when necessary to distinguish humanized antibodies from parental rodent antibodies. The humanized forms of rodent antibodies will generally comprise the same CDR sequences of the parental rodent antibodies, although certain amino acid substitutions may be included to increase affinity, increase stability of the humanized antibody, or for other reasons.

[0069] The antibody of the present application has potential therapeutic uses in treating cancer. The term "cancer" or "tumor" herein means or describes the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include but are not limited to, lung cancer (including small-cell lung cancer, or non-small cell lung cancer), adrenal cancer, liver cancer, stomach cancer, cervical cancer, melanoma, renal cancer, breast cancer, colorectal cancer, leukemia, bladder cancer, bone cancer, brain cancer, an endometrial cancer, head and neck cancer, lymphoma, ovarian cancer, skin cancer, thyroid tumor, or metastatic lesion of the

cancer.

[0070] Further, the antibody of the present application has potential therapeutic uses in controlling viral infections and other human diseases that are mechanistically involved in immune tolerance or "exhaustion." In the context of the present application, the term "exhaustion" refers to a process which leads to a depleted ability of immune cells to respond during to a cancer or a chronic viral infection.

[0071] The term "therapeutically effective amount" as herein used, refers to the amount of an antibody that, when administered to a subject for treating a disease or a disorder, or at least one of the clinical symptoms of a disease or disorder, is sufficient to affect such treatment for the disease, disorder, or symptom. The "therapeutically effective amount" can vary with the antibody, the disease, disorder, and/or symptoms of the disease or disorder, severity of the disease, disorder, and/or symptoms of the disease or disorder, the age of the subject to be treated, and/or the weight of the subject to be treated. An appropriate amount in any given instance can be apparent to those skilled in the art or can be determined by routine experiments. In the case of combination therapy, the "therapeutically effective amount" refers to the total amount of the active agents comprised in the combination for the effective treatment of a disease, a disorder or a condition.

[0072] The "subject" as used herein is a mammal, e.g., a rodent or a primate, preferably a higher primate, e.g., a human (e.g., a patient having, or at risk of having, a disorder described herein).

Anti-TIGIT antibodies

[0073] The present disclosure provides for antibodies and antigen-binding fragments thereof, that specifically bind human TIGIT. Furthermore, the present disclosure provides antibodies that have desirable pharmacokinetic characteristics and other desirable attributes, and thus can be used for reducing the likelihood of or treating cancer. The present disclosure further provides pharmaceutical compositions comprising the antibodies and methods of making and using such pharmaceutical compositions for the prevention and treatment of cancer and associated disorders.

[0074] The present disclosure provides for antibodies or antigen-binding fragments thereof that specifically bind to TIGIT. Antibodies or antigen-binding fragments of the present disclosure include, but are not limited to, the antibodies or antigen-binding fragments thereof, generated as described in Table 1 below.

Table 1. A1217 (Ociperlimab) sequence

SEQ ID NO:	Domain	Sequence
SEQ ID NO:1	HCDR1	DYYMY
SEQ ID NO:2	HCDR2	YITKGGGSTYYPDSVKG
SEQ ID NO:3	HCDR3	QTNYDFTMDY
SEQ ID NO:4	LCDR1	KASQDVGTSVA
SEQ ID NO:5	LCDR2	WASARHT
SEQ ID NO:6	LCDR3	QQYSSYPLT
SEQ ID NO:7	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYYMYWVRQAPG KGLEWVAYITKGGGSTYYPDSVKGRFTISRDNAKNTLYLQMN SLRAEDTAVYYCARQTNYDFTMDYWGQGTLVTVSS
SEQ ID NO:8	VL	EIVMTQSPATLSVSPGERATLSCKASQDVGTSVAWYQQKPGQA PRLLIYWASARHTGIPARFSGSGSGTEFTLTISSLQSEDFAVYYC QQYSSYPLTFGGGTKVEIK
SEQ ID NO:9	Heavy chain	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYYMYWVRQAPG KGLEWVAYITKGGGSTYYPDSVKGRFTISRDNAKNTLYLQMN SLRAEDTAVYYCARQTNYDFTMDYWGQGTLVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV DKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPGK
SEQ ID NO:10	light chain	EIVMTQSPATLSVSPGERATLSCKASQDVGTSVAWYQQKPGQA PRLLIYWASARHTGIPARFSGSGSGTEFTLTISSLQSEDFAVYYC QQYSSYPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO:11	IgG1 WT	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNH KPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:12	IgG1 MF	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNH KPSNTKVDKKVEPKSCDKTHTCPPCPAPPAAGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALAAPIE KTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK
SEQ ID NO:13	IgG1 DE	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNH KPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPDVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA PEEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK
SEQ ID NO:14	IgG1 DEL	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNH KPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPDVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPL PEEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[0075] The present disclosure provides antibodies or antigen-binding fragments that specifically bind to TIGIT, wherein said antibodies or antibody fragments (e.g., antigen-binding fragments) comprise a VH domain having an amino acid sequence of SEQ ID NO:7. The present disclosure also provides antibodies or antigen-binding fragments that specifically bind TIGIT, wherein said antibodies or antigen-binding fragments comprise a VH CDR having an amino acid sequence of any one of the VH CDRs provided herein. In one aspect, the present disclosure provides antibodies or antigen-binding fragments that specifically bind to TIGIT, wherein said antibodies comprise (or alternatively, consist of) one, two, three, or more VH CDRs having an amino acid sequence of any of the VH CDRs provided in the present disclosure.

[0076] The present disclosure provides for antibodies or antigen-binding fragments that specifically bind to TIGIT, wherein said antibodies or antigen-binding fragments comprise a VL domain having an amino acid sequence of SEQ ID NO:8. The present disclosure also provides antibodies or antigen-

binding fragments that specifically bind to TIGIT, wherein said antibodies or antigen-binding fragments comprise a VL CDR having an amino acid sequence of any one of the VL CDRs listed herein. In particular, the disclosure provides for antibodies or antigen-binding fragments that specifically bind to TIGIT, said antibodies or antigen-binding fragments comprise (or alternatively, consist of) one, two, three or more VL CDRs having an amino acid sequence of any of the VL CDRs of the current disclosure.

[0077] Other antibodies or antigen-binding fragments thereof of the present disclosure include amino acids that have been mutated, yet have at least 60%, 70%, 80%, 90%, 95% or 99% percent identity in the CDR regions with the CDR regions depicted in the sequences described herein. In some aspects, it includes mutant amino acid sequences wherein no more than 1, 2, 3, 4 or 5 amino acids have been mutated in the CDR regions when compared with the CDR regions disclosed in the sequences provided.

[0078] Other antibodies of the present disclosure include those where the amino acids or nucleic acids encoding the amino acids have been mutated; yet have at least 60%, 70%, 80%, 90%, 95% or 99% percent identity to the sequences described in Table 1. In some aspects, it includes mutant amino acid sequences wherein no more than 1, 2, 3, 4 or 5 amino acids have been mutated in the variable regions when compared with the variable regions depicted in the sequence described herein, while retaining substantially the same therapeutic activity.

Further Alteration of the Framework of Fc Region

[0079] In yet other aspects, the Fc region is altered by replacing at least one amino acid residue with a different amino acid residue to alter the effector functions of the antibody. For example, one or more amino acids can be replaced with a different amino acid residue such that the antibody has an altered affinity for an effector ligand but retains the antigen-binding ability of the parent antibody. The effector ligand to which affinity is altered can be, for example, an Fc receptor or the C1 component of complement. This approach is described in, e.g., U.S. Pat. Nos. 5,624,821 and 5,648,260, both by Winter et al.

[0080] In another aspect, one or more amino acid residues can be replaced with one or more different amino acid residues such that the antibody has altered C1q binding and/or reduced or abolished complement dependent cytotoxicity (CDC). This approach is described in, e.g., U.S. Pat. No. 6,194,551 by Idusogie et al.

[0081] In yet another aspect, one or more amino acid residues are altered to thereby alter the ability of the antibody to fix complement. This approach is described in, e.g., the PCT Publication WO 94/29351 by Bodmer et al. In a specific aspect, one or more amino acids of an antibody or antigen-binding fragment thereof of the present disclosure are replaced by one or more allotypic amino acid residues, for the IgG1 subclass and the kappa isotype. Allotypic amino acid residues also include, but are not limited to, the constant region of the heavy chain of the IgG1, IgG2, and IgG3 subclasses as well as the constant region of the light chain of the kappa isotype as described by Jefferis et al., MAbs. 1:332-338 (2009).

[0082] In another aspect, the Fc region is modified to increase the ability of the antibody to mediate antibody dependent cellular cytotoxicity (ADCC) and/or to increase the affinity of the antibody for an Fcγ receptor by modifying one or more amino acids. This approach is described in, e.g., the PCT publication WO00/42072 by Presta. Moreover, the binding sites on human IgG1 for FcγRI, FcγRII, FcγRIII and FcRn have been mapped and variants with improved binding have been described (see Shields et al., J. Biol. Chem. 276:6591-6604, 2001).

[0083] In still another aspect, the glycosylation of an antibody is modified. For example, an aglycosylated antibody can be made (i.e., the antibody lacks or has reduced glycosylation). Glycosylation can be altered to, for example, increase the affinity of the antibody for "antigen." Such carbohydrate modifications can be accomplished by, for example, altering one or more sites of glycosylation within the antibody sequence. For example, one or more amino acid substitutions can be made that result in elimination of one or more variable region framework glycosylation sites to thereby eliminate glycosylation at that site. Such aglycosylation can increase the affinity of the antibody for antigen. Such an approach is described in, e.g., U.S. Pat. Nos. 5,714,350 and 6,350,861 by Co et al.

[0084] Additionally, or alternatively, an antibody can be made that has an altered type of glycosylation, such as a hypofucosylated antibody having reduced amounts of fucosyl residues or an antibody having increased bisecting GlcNac structures. Such altered glycosylation patterns have been demonstrated to increase the ADCC ability of antibodies. Such carbohydrate modifications can be accomplished by, for example, expressing the antibody in a host cell with altered glycosylation machinery. Cells with altered glycosylation machinery have been described in the art and can be used as host cells in which to express recombinant antibodies to thereby produce an antibody with altered

glycosylation. For example, EP 1,176,195 by Hang et al., describes a cell line with a functionally disrupted FUT8 gene, which encodes a fucosyl transferase, such that antibodies expressed in such a cell line exhibit hypofucosylation. PCT Publication WO 03/035835 by Presta describes a variant CHO cell line, Lecl3 cells, with reduced ability to attach fucose to Asn (297)-linked carbohydrates, also resulting in hypofucosylation of antibodies expressed in that host cell (see also Shields et al., (2002) J. Biol. Chem. 277:26733-26740). PCT publication WO 99/54342 by Umana et al., describes cell lines engineered to express glycoprotein-modifying glycosyl transferases (e.g., beta(1,4)-N acetylglucosaminyltransferase III (GnTIII)) such that antibodies expressed in the engineered cell lines exhibit increased bisecting GlcNac structures which results in increased ADCC activity of the antibodies (see also Umana et al., Nat. Biotech. 17:176-180, 1999).

[0085] In another aspect, if a reduction of ADCC is desired, human antibody subclass IgG4 was shown in many previous reports to have only modest ADCC and almost no CDC effector function (Moore G L, et al. 2010 MAbs, 2:181-189). On the other hand, natural IgG4 was found less stable in stress conditions such as in acidic buffer or under increasing temperature (Angal 1993 Mol Immunol, 30:105-108; Dall'Acqua, et al., 1998 Biochemistry, 37:9266-9273; Aalberse et al., 2002 Immunol, 105:9-19). Reduced ADCC can be achieved by operably linking the antibody to IgG4 engineered with combinations of alterations to have reduced or null FcγR binding or C1q binding activities, thereby reducing or eliminating ADCC and CDC effector functions. Considering physicochemical properties of antibody as a biological drug, one of the less desirable, intrinsic properties of IgG4 is dynamic separation of its two heavy chains in solution to form half antibody, which lead to bi-specific antibodies generated *in vivo* via a process called "Fab arm exchange" (Van der Neut Kolfschoten et al., 2007 Science, 317:1554-157). The mutation of serine to proline at position 228 (EU numbering system) appeared inhibitory to the IgG4 heavy chain separation (Angal 1993 Mol Immunol, 30:105-108; Aalberse et al., 2002 Immunol, 105:9-19). Some of the amino acid residues in the hinge and γFc region were reported to have impact on antibody interaction with Fcγ receptors (Chappel et al., 1991 Proc. Natl. Acad. Sci. USA, 88:9036-9040; Mukherjee et al., 1995 FASEB J, 9:115-119; Armour et al., 1999 Eur J Immunol, 29:2613-2624; Clynes et al., 2000 Nature Medicine, 6:443-446; Arnold 2007 Annu Rev immunol, 25:21-50). Furthermore, some rarely occurring IgG4 isoforms in human population can also elicit different physicochemical properties (Brusco et al., 1998 Eur J Immunogenet, 25:349-55; Aalberse et al., 2002 Immunol, 105:9-19). To generate TIGIT

antibodies with low ADCC, CDC and instability, it is possible to modify the hinge and Fc region of human IgG4 and introduce a number of alterations. These modified IgG4 Fc molecules can be found disclosed in SEQ ID NOs: 83-88, U.S. Patent No. 8,735,553.

Antibody Production

[0086] Anti-TIGIT antibodies and antigen-binding fragments thereof can be produced by any means known in the art, including but not limited to, recombinant expression, chemical synthesis, and enzymatic digestion of antibody tetramers, whereas full-length monoclonal antibodies can be obtained by, e.g., hybridoma or recombinant production. Recombinant expression can be from any appropriate host cells known in the art, for example, mammalian host cells, bacterial host cells, yeast host cells, insect host cells, etc.

[0087] The disclosure further provides polynucleotides encoding the antibodies described herein, e.g., polynucleotides encoding heavy or light chain variable regions or segments comprising the complementarity determining regions as described herein. In some aspects, the polynucleotide encoding the heavy chain variable regions has at least 85%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% nucleic acid sequence identity with a polynucleotide that encodes for the polypeptides of SEQ ID NO: 7. In some aspects, the polynucleotide encoding the light chain variable regions has at least 85%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% nucleic acid sequence identity with a polynucleotide that encodes for the polypeptides of SEQ ID NO:8.

[0088] The polynucleotides of the present disclosure can encode the variable region sequence of an anti-TIGIT antibody. They can also encode both a variable region and a constant region of the antibody. Some of the polynucleotide sequences encode a polypeptide that comprises variable regions of both the heavy chain and the light chain of one of the exemplified anti-TIGIT antibodies. Some other polynucleotides encode two polypeptide segments that respectively are substantially identical to the variable regions of the heavy chain and the light chain of one of the murine antibodies.

[0089] Also provided in the present disclosure are expression vectors and host cells for producing the anti-TIGIT antibodies. The choice of expression vector depends on the intended host cells in which the vector is to be expressed. Typically, the expression vectors contain a promoter and other regulatory sequences (e.g., enhancers) that are operably linked to the polynucleotides encoding an

anti-TIGIT antibody chain or antigen binding fragment thereof. In some aspects, an inducible promoter is employed to prevent expression of inserted sequences except under the control of inducing conditions. Inducible promoters include, e.g., arabinose, lacZ, metallothionein promoter or a heat shock promoter. Cultures of transformed organisms can be expanded under non-inducing conditions without biasing the population for coding sequences whose expression products are better tolerated by the host cells. In addition to promoters, other regulatory elements can also be required or desired for efficient expression of an anti-TIGIT antibody or antigen binding fragment thereof. These elements typically include an ATG initiation codon and adjacent ribosome binding site or other sequences. In addition, the efficiency of expression can be enhanced by the inclusion of enhancers appropriate to the cell system in use (see, e.g., Scharf et al., Results Probl. Cell Differ. 20:125, 1994; and Bittner et al., Meth. Enzymol., 153:516, 1987). For example, the SV40 enhancer or CMV enhancer can be used to increase expression in mammalian host cells.

[0090] The host cells for harboring and expressing the anti-TIGIT antibody chains can be either prokaryotic or eukaryotic. *E. coli* is one prokaryotic host useful for cloning and expressing the polynucleotides of the present disclosure. Other microbial hosts suitable for use include bacilli, such as Bacillus subtilis, and other enterobacteriaceae, such as Salmonella, Serratia, and various Pseudomonas species. In these prokaryotic hosts, one can also make expression vectors, which typically contain expression control sequences compatible with the host cell (e.g., an origin of replication). In addition, any number of a variety of well-known promoters will be present, such as the lactose promoter system, a tryptophan (trp) promoter system, a beta-lactamase promoter system, or a promoter system from phage lambda. The promoters typically control expression, optionally with an operator sequence, and have ribosome binding site sequences and the like, for initiating and completing transcription and translation. Other microbes, such as yeast, can also be employed to express anti-TIGIT polypeptides. Insect cells in combination with baculovirus vectors can also be used.

[0091] In other aspects, mammalian host cells are used to express and produce the anti-TIGIT antibodies of the present disclosure. For example, they can be either a hybridoma cell line expressing endogenous immunoglobulin genes or a mammalian cell line harboring an exogenous expression vector. These include any normal mortal or normal or abnormal immortal animal or human cell. For example, a number of suitable host cell lines capable of secreting intact immunoglobulins have been

developed, including the CHO cell lines, various COS cell lines, HEK 293 cells, myeloma cell lines, transformed B-cells and hybridomas. The use of mammalian tissue cell culture to express polypeptides is discussed generally in, e.g., Winnacker, From Genes to Clones, VCH Publishers, NY, N.Y., 1987. Expression vectors for mammalian host cells can include expression control sequences, such as an origin of replication, a promoter, and an enhancer (see, e.g., Queen et al., Immunol. Rev. 89:49-68, 1986), and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences. These expression vectors usually contain promoters derived from mammalian genes or from mammalian viruses. Suitable promoters can be constitutive, cell type-specific, stage-specific, and/or modulatable or regulatable. Useful promoters include, but are not limited to, the metallothionein promoter, the constitutive adenovirus major late promoter, the dexamethasone-inducible MMTV promoter, the SV40 promoter, the MRP polIII promoter, the constitutive MPSV promoter, the tetracycline-inducible CMV promoter (such as the human immediate-early CMV promoter), the constitutive CMV promoter, and promoter-enhancer combinations known in the art.

Methods of Detection and Diagnosis

[0092] The antibodies or antigen-binding fragments of the present disclosure are useful in a variety of applications including, but not limited to, methods for the detection of TIGIT. In one aspect, the antibodies or antigen-binding fragments are useful for detecting the presence of TIGIT in a biological sample. The term "detecting" as used herein includes quantitative or qualitative detection. In certain aspects, a biological sample comprises a cell or tissue. In other aspects, such tissues include normal and/or cancerous tissues that express TIGIT at higher levels relative to other tissues.

[0093] In one aspect, the present disclosure provides a method of detecting the presence of TIGIT in a biological sample. In certain aspects, the method comprises contacting the biological sample with an anti-TIGIT antibody under conditions permissive for binding of the antibody to the antigen and detecting whether a complex is formed between the antibody and the antigen. The biological sample can include, without limitation, urine or blood samples.

[0094] Also included is a method of diagnosing a disorder associated with expression of TIGIT. In certain aspects, the method comprises contacting a test cell with an anti-TIGIT antibody; determining the level of expression (either quantitatively or qualitatively) of TIGIT in the test cell by detecting binding of the anti-TIGIT antibody to the TIGIT polypeptide; and comparing the level of expression

in the test cell with the level of TIGIT expression in a control cell (e.g., a normal cell of the same tissue origin as the test cell or a non-TIGIT expressing cell), wherein a higher level of TIGIT expression in the test cell as compared to the control cell indicates the presence of a disorder associated with expression of TIGIT.

Methods of Treatment

[0095] The antibodies or antigen-binding fragments of the present disclosure are useful in a variety of applications including, but not limited to, methods for the treatment of an TIGIT-associated disorder or disease. In one aspect, the TIGIT-associated disorder or disease is a cancer.

[0096] In one aspect, the present disclosure provides a method of treating cancer. In certain aspects, the method comprises administering to a patient in need an effective amount of an anti-TIGIT antibody or antigen-binding fragment. The cancer can include, without limitation, breast cancer, head and neck cancer, gastric cancer, kidney cancer, liver cancer, small cell lung cancer, non-small cell lung cancer, ovarian cancer, skin cancer, mesothelioma, lymphoma, leukemia, myeloma and sarcoma.

[0097] An antibody or antigen-binding fragment of the invention can be administered by any suitable means, including parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. Dosing can be by any suitable route, e.g., by injections, such as intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic. Various dosing schedules including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.

[0098] Antibodies or antigen-binding fragments of the invention would be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The antibody need not be but is optionally formulated with one or more agents currently used to prevent or treat the disorder in question. The effective amount of such other agents depends on the amount of antibody present in the formulation, the type of disorder or treatment, and

other factors discussed above. These are generally used in the same dosages and with administration routes as described herein, or about from 1 to 99% of the dosages described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate.

[0099] For the prevention or treatment of disease, the appropriate dosage of an antibody or antigen-binding fragment of the invention will depend on the type of disease to be treated, the type of antibody, the severity and course of the disease, whether the antibody is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody, and the discretion of the attending physician. The antibody is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 1 μg/kg to 100 mg/kg of antibody can be an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. One typical daily dosage might range from about 1 μg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment would generally be sustained until a desired suppression of disease symptoms occurs. Such doses can be administered intermittently, e.g., every week or every three weeks (e.g., such that the patient receives from about two to about twenty, or e.g., about six doses of the antibody). An initial higher loading dose, followed by one or more lower doses can be administered. However, other dosage regimens can be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

Combination Therapy

[00100] In one aspect, TIGIT antibodies of the present disclosure can be used in combination with other therapeutic agents, for example anti-PD1 antibodies. Other therapeutic agents that can be used with the TIGIT antibodies of the present disclosure include: but are not limited to, a chemotherapeutic agent (e.g., paclitaxel or a paclitaxel agent; (e.g. Abraxane®), docetaxel; carboplatin; topotecan; cisplatin; irinotecan, doxorubicin, lenalidomide, 5-azacytidine, ifosfamide, oxaliplatin, pemetrexed disodium, cyclophosphamide, etoposide, decitabine, fludarabine, vincristine, bendamustine, chlorambucil, busulfan, gemcitabine, melphalan, pentostatin, mitoxantrone, pemetrexed disodium), tyrosine kinase inhibitor (e.g., EGFR inhibitor (e.g., erlotinib), multikinase inhibitor (e.g., MGCD265, RGB-286638), CD-20 targeting agent (e.g., rituximab, ofatumumab, RO5072759, LFB-R603), CD52 targeting agent (e.g., alemtuzumab), prednisolone, darbepoetin alfa,

lenalidomide, Bcl-2 inhibitor (e.g., oblimersen sodium), aurora kinase inhibitor (e.g., MLN8237, TAK-901), proteasome inhibitor (e.g., bortezomib), CD-19 targeting agent (e.g., MEDI-551, MOR208), MEK inhibitor (e.g., ABT-348), JAK-2 inhibitor (e.g., INCB018424), mTOR inhibitor (e.g., temsirolimus, everolimus), BCR/ABL inhibitor (e.g., imatinib), ET-A receptor antagonist (e.g., ZD4054), TRAIL receptor 2 (TR-2) agonist (e.g., CS-1008), HGF/SF inhibitor (e.g., AMG 102), EGEN-001, Polo-like kinase 1 inhibitor (e.g., BI 672).

[00101] TIGIT antibodies of the present disclosure can be used in combination with other therapeutics, for example, anti-PD1 antibodies. Anti-PD1 antibodies can include, without limitation, Tislelizumab, Pembrolizumab or Nivolumab. Tislelizumab is disclosed in US 8,735,553, and in Table 2 below.

Table 2. – Tislelizumab sequences

BGB-A317 (Tislelizumab)		
Domain	**SEQ ID NO:**	**Amino Acid Sequence**
HCDR1	SEQ ID NO:15	GFSLTSYGVH
HCDR1	SEQ ID NO:16	VIYADGSTNYNPSLKS
HCDR3	SEQ ID NO:17	ARAYGNYWYIDV
LCDR1	SEQ ID NO:18	KSSESVSNDVA
LCDR2	SEQ ID NO:19	YAFHRFT
LCDR3	SEQ ID NO:20	HQAYSSPYT
VH	SEQ ID NO:21	QVQLQESGPGLVKPSETLSLTCTVSGFSLTSYGVHWIRQPP GKGLEWIGVIYADGSTNYNPSLKSRVTISKDTSKNQVSLKL SSVTAADTAVYYCARAYGNYWYIDVWGQGTTVTVSS
VL	SEQ ID NO:22	DIVMTQSPDSLAVSLGERATINCKSSESVSNDVAWYQQKP GQPPKLLINYAFHRFTGVPDRFSGSGYGTDFTLTISSLQAE DVAVYYCHQAYSSPYTFGQGTKLEIK
IgG4 constant domain	SEQ ID NO:23	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYT CNVDHKPSNTKVDKRVESKYGPPCPPCPAPPVAGGPSVFL FPPKPKDTLMISRTPEVTCVVVAVSQEDPEVQFNWYVDGV EVHNAKTKPREEQFNSTYRVVSVLTVVHQDWLNGKEYKC KVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFLYSKLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLS LSLGK
Heavy Chain	SEQ ID NO:24	QVQLQESGPGLVKPSETLSLTCTVSGFSLTSYGVHWIRQPP GKGLEWIGVIYADGSTNYNPSLKSRVTISKDTSKNQVSLKL SSVTAADTAVYYCARAYGNYWYIDVWGQGTTVTVSSAST KGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSG

		ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNV DHKPSNTKVDKRVESKYGPPCPPCPAPPVAGGPSVFLFPPK PKDTLMISRTPEVTCVVVAVSQEDPEVQFNWYVDGVEVH NAKTKPREEQFNSTYRVVSVLTVVHQDWLNGKEYKCKVS NKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL YSKLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSL GK
Light chain	SEQ ID NO:25	DIVMTQSPDSLAVSLGERATINCKSSESVSNDVAWYQQKP GQPPKLLINYAFHRFTGVPDRFSGSGYGTDFTLTISSLQAE DVAVYYCHQAYSSPYTFGQGTKLEIKRTVAAPSVFIFPPSD EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQE SVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGL SSPVTKSFNRGEC

[00102] Pembrolizumab (formerly MK-3475), as disclosed by Merck, in US 8,354,509 and US 8,900,587 is a humanized lgG4-K immunoglobulin which targets the PD1 receptor and inhibits binding of the PD1 receptor ligands PD-L1 and PD-L2. Pembrolizumab has been approved for the indications of metastatic melanoma and metastatic non-small cell lung cancer (NSCLC) and is under clinical investigation for the treatment of head and neck squamous cell carcinoma (HNSCC), and refractory Hodgkin's lymphoma (cHL). Nivolumab (as disclosed by Bristol-Meyers Squibb) is a fully human lgG4-K monoclonal antibody. Nivolumab (clone 5C4) is disclosed in US Patent No. US 8,008,449 and WO 2006/121168. Nivolumab is approved for the treatment of melanoma, lung cancer, kidney cancer, and Hodgkin's lymphoma.

Pharmaceutical compositions and formulations

[00103] Also provided are compositions, including pharmaceutical formulations, comprising an anti-TIGIT antibody or antigen-binding fragment, or polynucleotides comprising sequences encoding an anti-TIGIT antibody or antigen-binding fragment. In certain embodiments, compositions comprise one or more antibodies or antigen-binding fragments that bind to TIGIT, or one or more polynucleotides comprising sequences encoding one or more antibodies or antigen-binding fragments that bind to TIGIT. These compositions can further comprise suitable carriers, such as pharmaceutically acceptable excipients including buffers, which are well known in the art.

[00104] Pharmaceutical formulations of an TIGIT antibody or antigen-binding fragment as described herein are prepared by mixing such antibody or antigen-binding fragment having the desired degree

of purity with one or more optional pharmaceutically acceptable carriers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3- pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG).

[00105] Exemplary lyophilized antibody formulations are described in US Patent No. 6,267,958. Aqueous antibody formulations include those described in US Patent No. 6,171,586 and WO2006/044908, the latter formulations including a histidine-acetate buffer.

[00106] Sustained-release preparations can be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g., films, or microcapsules.

[00107] The formulations to be used for in vivo administration are generally sterile. Sterility can be readily accomplished, e.g., by filtration through sterile filtration membranes.

Pharmaceutical Compositions and Kits

[00108] In some aspects, this disclosure provides compositions, e.g., pharmaceutically acceptable compositions, which include an anti-TIGIT antibody described herein, formulated together with at least one pharmaceutically acceptable excipient. As used herein, the term "pharmaceutically acceptable excipient" includes any and all solvents, dispersion media, isotonic and absorption delaying agents, and the like that are physiologically compatible. The excipient can be suitable for intravenous, intramuscular, subcutaneous, parenteral, rectal, spinal or epidermal administration (e.g.,

by injection or infusion).

[00109] The compositions herein may be in a variety of forms. These include, for example, liquid, semi-solid and solid dosage forms, such as liquid solutions (e.g., injectable and infusion solutions), dispersions or suspensions, liposomes, and suppositories. A suitable form depends on the intended mode of administration and therapeutic application. Typical suitable compositions are in the form of injectable or infusion solutions. One suitable mode of administration is parenteral (e.g., intravenous, subcutaneous, intraperitoneal, intramuscular). In some embodiments, the antibody is administered by intravenous infusion or injection. In certain embodiments, the antibody is administered by intramuscular or subcutaneous injection.

EXAMPLES

Example 1: Generation of anti-TIGIT monoclonal antibody

[00110] Anti-TIGIT monoclonal antibodies (mAbs) were generated based on conventional hybridoma fusion technology (de St Groth and Sheidegger, 1980 J Immunol Methods 35:1; Mechetner, 2007 Methods Mol Biol 378:1) with minor modifications. The mAbs with high binding activity in enzyme-linked immunosorbent assay (ELISA) and fluorescence-activated cell sorting (FACS) assay were selected for further characterization.

Cloning and sequence analysis of TIGIT Antibodies

[00111] Murine hybridoma clones were harvested to prepare total cellular RNAs using Ultrapure RNA kit (Cat.: 74104, QIAGEN, Germany) based on the manufacturer's protocol. The 1st strand cDNAs were synthesized using a cDNA synthesis kit from Invitrogen (Cat.: 18080-051) and PCR amplification of the nucleotide sequences coding for heavy chain variable region (*Vh)* and kappa chain variable region (*Vk)* of murine mAbs was performed using a PCR kit (Cat.: CW0686, CWBio, Beijing, China). The oligo primers used for antibody cDNAs cloning of *Vh* and *Vk* were synthesized by Invitrogen (Beijing, China) based on the sequences reported previously (Brocks et al., 2001 Mol Med 7:461). PCR products were then subcloned into the pEASY-Blunt cloning vector (Cat.:C B101-02, TransGen, China) and sequenced by Genewiz (Beijing, China). The amino acid sequences of *Vh* and *Vk* regions were deduced from the DNA sequencing results. Mu1217 was identified as a specific clone of interest.

Humanization of the murine anti-human TIGIT mAb mu1217

[0082] For humanization of the mu1217, human germline IgG genes were searched for sequences

that share high degrees of homology to the cDNA sequences of mu1217 variable regions by running a comparison with the human immunoglobulin gene database in IMGT. The human IGVH and IGVK genes that are present in human antibody repertoires with high frequencies (Glanville et al., PNAS 106:20216-20221 2009) and are highly homologous to mu1217 were selected as the templates for humanization.

[0083] Humanization was carried out by CDR-grafting (Methods in Molecular Biology, Vol 248: Antibody Engineering, Methods and Protocols, Humana Press) and the humanized antibody (A1217) was engineered as the human IgG1MF format using an in-house developed expression vector and the sequences of this antibody are shown in Table 1.

Example 2: Binding activities of A1217 to TIGIT, structure and function

[0084] To better understand how the A1217 antibody is capable of high affinity for TIGIT, robust blocking of TIGIT-PVR interaction, and in particular, pH-dependent binding on TIGIT while exhibiting pharmacokinetic and molecular assessment properties, the crystal structure of A1217 in complex with TIGIT was determined as described in detail below. Mutagenesis experiments of the TIGIT interface were also performed to identify functional epitopic residues, especially HIS76 of TIGIT for pH-dependent binding.

TIGIT and Fab expression, purification, and crystallization

[0085] Human TIGIT residues 23-128 with C-terminal HIS tag was expressed as inclusion bodies in E. coli BL21(DE3) pLysS using pET21a vector (Novagen). Site-directed mutations of TIGIT were introduced by using QuickChange-based procedures (Xia et al., Nucleic Acids Res, 2015. 43(2): p. e12) employing Q5 DNA polymerase (New England Biolabs). Protein expression in BL21(DE3) pLysS host strain was induced at OD600 of 0.6-1.0 with 1mM IPTG for 4h at 37℃. The cells were harvested by centrifugation, re-suspended in lysis buffer (50 mM sodium phosphate pH 7.0, 300mM sodium chloride). The cells were lysed under sonication on ice. The inclusion bodies were recovered by centrifugation (20,000rpm for 30min at 4℃) and solubilized in 8M urea, 20mM Tris pH 8.0, 200mM NaCl, 1mM DTT followed by stirring overnight. After removing the undissolved pellet by centrifugation (20,000rpm for 30min at 4℃), the solubilized fraction was applied to a Ni-Penta™ affinity column (Marvelgent Biosciences Inc.) and washed with 10 column volumes of wash buffer (8M urea, 20mM Tris pH 8.0, 200mM NaCl, 5mM imidazole). The protein was then eluted with elution buffer (8M urea, 20mM Tris pH 8.0, 200mM NaCl, 200mM imidazole). The eluted protein

was refolded by dialyzing against buffer containing 20mM Tris pH 8.0, 200mM NaCl, 0.4M L-Arginine, 1mM oxidized glutathione, 5mM reduced glutathione and further purified by gel filtration in buffer (20 mM Tris pH 8.0, 100 mM NaCl) using a HiLoad 16/600 Superdex™ 75pg column (GE Healthcare Life Sciences). The TIGIT mutants were purified similarly with the wild-type protein.

[0086] DNA sequences for the Fab fragments of A1217 were synthesized with codon-optimization in mammalian cell. The sequences of heavy and light chain of Fabs were cloned into pMAX vector, respectively, with a C-terminal 6xHIS tag of heavy chain. The plasmids harboring heavy chain and light chain of Fabs were transiently co-transfected into HEK293G cells for protein expression. The supernatant containing secreted Fabs was purified by TALON affinity resin (Clontech Laboratories), followed by further purification using a HiLoad 16/600 Superdex™ 75pg column (GE Healthcare Life Sciences). Similarly, the plasmids of full-length heavy chain and light chain of A1217 were transiently co-transfected into HEK293G cells for protein expression. The full-length antibodies were purified by Mab Select SuRe™ affinity resin (GE Healthcare), followed by further polished with a HiLoad 16/600 Superdex™ 200pg column (GE Healthcare Life Sciences).

[0087] The Fabs of A1217 were concentrated to around 10 mg/ml in 20 mM Tris pH 8.0, 100 mM NaCl for initial crystallization screening. A1217 Fab was incubated with 1.5 molar excess of TIGIT on ice for 30 minutes and purified by Superdex™ 75 Increase 10/300 GL column (GE Healthcare) in 20mM Tris pH 8.0, 100 mM NaCl. Pooled fractions were concentrated to approximately 10 mg/ml and used for initial crystal screening. Crystals of A1217 Fab/TIGIT was grown in 0.1M Citric acid pH4.6, 1M Lithium chloride, 7% PEG6000. Crystals cryoprotected with stepwise 5% glycerol to a final 20% concentration were flash frozen in liquid nitrogen. The X-ray diffraction data was collected at beamline BL45XU at Spring-8 (Japan Synchrotron Radiation Research Institute). In order to compare, Tiragolumab Fabs were generated by a similar method.

Data collection and structure solution

[0088] The diffraction data of A1217 Fab/TIGIT and Tiragolumab Fab/TIGIT complexes were collected in BL45XU with automatic data collection system ZOO (Hirata et al., Acta Crystallogr D Struct Biol, 2019. 75(Pt 2): p. 138-150) in Spring-8 (Japan) and processed by KAMO (Yamashita et al., Acta Crystallographica Section D, Structural biology, 2018. 74(Pt 5): p. 441-449). Molecular replacement using PHASER (McCoy et al., J Appl Crystallogr, 2007. 40(Pt 4): p. 658-674) employed rigid body search models from Fabs solved in-house and TIGIT (PDB: 3UCR). Structure refinement

was performed with the programs REFMAC (Murshudov et al., Acta Crystallogr D Biol Crystallogr, 1997. 53(Pt 3): p. 240-55) and PHENIX (Adams et al., Acta Crystallogr D Biol Crystallogr, 2010. 66(Pt 2): p. 213-21) together with manual model building in COOT (Emsley et al., Acta Crystallogr D Biol Crystallogr, 2004. 60(Pt 12 Pt 1): p. 2126-32). Crystallographic data statistics are summarized in Table 3. All molecular graphics were prepared with PyMOL (Schrodinger, LLC, The PyMOL Molecular Graphics System, Version 1.8. 2015).

Table 3. Data collection and refinement statistics

Crystals	A1217_Fab/TIGIT (soak0257 ccc_refine_8.pdb)	Tiragolumab_Fab/TIGIT (soak0182 XDS, soak0182_2_refine_7.pdb)
Data collection		
X-ray source	BL45XU	BL45XU
Wavelength (Å)	1.0	1.0
Space group	P2 2_1 2_1	I4
Cell Dimensions		
a (Å)	135.53	214.16
b (Å)	138.31	214.16
c (Å)	196.80	66.89
α (°)	90	90
ß(°)	90	90
ϒ (°)	90	90
Resolution (Å)	2.45 (2.60-2.45)	2.06 (2.18-2.06)
Rsym (%)	20.8 (282.4)	21.9 (163.3)
<I/σ>	5.23 (0.43)	6.20 (0.94)
$CC_{1/2}$	99.4 (56.1)	99.1 (41.6)
Redundancy	10.1 (10.2)	6.2 (5.9)

Unique reflection	136106 (21993)	183793 (29063)
Completeness (%)	100 (100)	99.5 (97.2)
Refinement		
Resolution (Å)	2.45	2.06
No. reflections	135976	94078
Rwork/Rfree [b](%)	19.17/24.92	17.22/20.55
r.m.s.d. [c]bonds/angles	0.008/1.068	0.007/0.912
Number of atoms		
Protein	16276	8400
Water	818	682
B factors		
Protein	79.87	50.96
Water	73.91	53.03
Ramachandran		
Favored (%)	93.16	96.67
Allowed (%)	5.80	3.24
Outlier (%)	1.05	0.09

[a] Values in parentheses are those of the highest resolution shell.
[b] Calculated from about 5% of the reflection set aside during refinement
[c] r.m.s.d., root mean square deviation

Example 3: The structure of A1217 bound to human TIGIT

[0089] The A1217 Fab in complex with TIGIT crystallized in the P 2 21 21 space group, with four complexes in the asymmetric unit, and diffracted to 2.45Å. Overlay of the four individual complexes

in the asymmetric unit shows only minor changes in main chain positioning between the individual copies. The structure of A1217 bound to human TIGIT (Figure 1A) shows that A1217 sterically interfaces with PVR binding (Figure 1B). The buried surface area between A1217 and TIGIT is approximately 1556 $Å^2$. The epitope of TIGIT by A1217 consists of numerous discontinuous regions. Eighteen residues of A1217 Fab (paratope) and fifteen residues of TIGIT (epitope) are involved in the paratope-epitope formation (Figure 1C). The interactions at the A1217/TIGIT interface are primarily non-polar in nature, with a total of eleven hydrogen bond and three salt bridges. The paratope of A1217 consists of TYR33 of HCDR1, THR52, LYS53, GLY54, GLY56, SER57, TYR59 of HCDR2, ASN101, TYR102, ASP103, PHE104 of HCDR3, THR31, SER32 of LCDR1, TYR49, TRP50 of LCDR2, TYR91, SER92, TYR94 of LCDR3 (Figure 1C). The A1217 epitope of TIGIT contains GLN56, GLU60, ASP63, GLN64, LEU65, ILE68, ASN70, LEU73, GLY74, TRP75, HIS76, SER78, PRO79, SER80, LYS82 (Figure 1C). The hydrogen bonds between TIGIT and A1217 involves side chain atoms of $GLN56_{TIGIT}$, $ASP63_{TIGIT}$, $ASN70_{TIGIT}$, $HIS76_{TIGIT}$, $SER80_{TIGIT}$, $LYS82_{TIGIT}$, $TYR33_{HCDR1}$, $THR52_{HCDR2}$, $ASP103_{HCR3}$, $THR31_{LCDR1}$, $TRP50_{LCDR2}$, $SER92_{LCDR3}$, $TYR94_{LCDR3}$ and main chain atoms of $LEU65_{TIGIT}$, $LEU73_{TIGIT}$, $LYS53_{HCDR2}$, $GLY54_{HCDR2}$, $GLY56_{HCDR2}$, $ASN101_{HCDR3}$, $TYR102_{HCDR3}$, whereas two salt bridges formed between $HIS76_{TIGIT}$ and $ASP103_{HCDR3}$ and one salt bridge found between $GLU60_{TIGIT}$ and $LYS53_{HCDR2}$. In addition, the residues of TIGIT only participated in van der Waals interactions are GLN64, ILE68, GLY74, TRP75, SER78, PRO79 while the residues of A1217 involved in these interactions are SER57, TYR59, PHE104 of heavy chain, SER32, TYR49, TYR91 of light chain.

[0090] A crystal structure of Tiragolumab was also generated by a similar method as described above. The epitope mapping of TIGIT by Tiragolumab Fab shows many disconnected areas. Thirteen residues of Tiragolumab Fab (paratope) and ten residues of TIGIT (epitope) are involved in paratope-epitope formation calculated with a 3.7 Å cutoff distance. The paratope of Tiragolumab Fab consists of ARG56, PHE57, LYS58, and TYR60 of HCDR2; TYR106, ASP107, LEU108, and LEU109 of HCDR3; TYR31 and TYR38 of LCDR1; and TYR98, SER99, and THR100 of LCDR3. Therefore, the HCDR1 and LCDR2 of Tiragolumab Fab do not directly participate in the epitope-paratope interaction. The Tiragolumab epitope consists of GLN56, ASN58, GLU60, HIS76, ILE77, SER78, PRO79, SER80, LYS82, and HIS111. Eight hydrogen bonds, four salt bridges and van der Waals

forces contribute to the binding interface formation. The hydrogen bonds between TIGIT and Tiragolumab Fab involve side chain atoms of ASN58$_{TIGIT}$, GLU60$_{TIGIT}$, HIS76$_{TIGIT}$, SER80$_{TIGIT}$, LYS82$_{TIGIT}$, LYS58$_{HCDR2}$, TYR60$_{HCDR2}$, ASP107$_{HCDR3}$, and THR100$_{LCDR3}$ and main chain atoms of PRO79$_{TIGIT}$, ARG56$_{HCDR2}$, and THR100$_{LCDR3}$, whereas two salt bridges formed between GLU60$_{TIGIT}$ and LYS58$_{HCDR2}$, two salt bridges formed between LYS82$_{TIGIT}$ and ASP107$_{HCDR3}$, and one salt bridge formed between HIS76$_{TIGIT}$ and ASP107$_{HCDR3}$. In addition, the residues of TIGIT that participated only in van der Waals interactions were GLN56, ILE77, SER78, and HIS111, while the residues of Tiragolumab Fab involved in this interaction were PHE57, TYR106, LEU108, and LEU109 of the heavy chain and TYR31, TYR38, TYR98, and SER99 of the light chain. The amino acids ARG56, PHE57, LYS58 and TYR60 of HCDR2 in the Tiragolumab Fab covered the exposed hydrophobic surface formed by THR55, GLN56, ASN58, GLU60, ASP63, GLN64, LEU65, ALA67, ILE68, ILE109 and HIS111 of TIGIT. LEU109 of HCDR3 in Tiragolumab Fab inserts into the hydrophobic pocket formed by ALA67, ILE68, HIS76, ILE77 and SER78 of TIGIT. The comparison data between the A1217 (Ociperlimab) and Tiragolumab interaction with TIGIT is shown in Figures 2A-C and Figure 3A-B.

[0091] Based on the crystal structure of the A1217/TIGIT complex, the residues of TIGIT that are contacted by A1217 (i.e., the epitopic residues of TIGIT bound by A1217) and the residues of A1217 that are contacted by TIGIT (i.e., the paratopic residues of A1217 contacted by TIGIT) were determined. Tables 4 and 5, below, show the residues of TIGIT and the light or heavy chain residues of A1217 to which they contact, as assessed using a contact distance stringency of 3.7 Å, a point at which van der Walls (non-polar) interaction forces are highest.

Table 4. Epitopic residues of TIGIT and their corresponding paratopic residues on the light chain of A1217

TIGIT	residue number	A1217 Light Chain	residue number
ILE	68	TRP	50
ASN	70	TRP	50
LEU	73	TYR	49
		TRP	50
GLY	74	TRP	50
TRP	75	TRP	50
HIS	76	THR	31

		SER	32
		TRP	50
PRO	79	TYR	91
		SER	92
SER	80	TYR	94
LYS	82	SER	92

Table 5. Epitopic residues of TIGIT and their corresponding paratopic residues on the heavy chain of A1217

TIGIT	residue number	A1217 Heavy chain	residue number
GLN	56	ASN	101
		TYR	102
GLU	60	LYS	53
ASP	63	TYR	33
		THR	52
		GLY	54
		GLY	56
GLN	64	TYR	33
		SER	57
LEU	65	TYR	33
ILE	68	ASP	103
ASN	70	TYR	102
HIS	76	ASP	103
		PHE	104
SER	78	PHE	104
SER	80	TYR	59

[0092] Alanine scanning of the human TIGIT interface was also performed, with alanine mutations of TIGIT residues made for LEU73/HIS76, HIS76, ILE68, ASP63, LEU73, PRO79, LEU65, GLN56, ASN70, GLU60, LYS82, SER80, GLN64 (Table 6). The capture immobilization strategy in SPR was employed to test the binding kinetics of A1217 towards TIGIT variants. In this experiment, mutation of TIGIT residues LEU73/HIS76, HIS76, ILE68 reduced A1217 binding greater than 10-fold (Table 6), with the HIS76 mutation nearly abolishing A1217 binding, suggesting that this residue is critical for the interaction. The LEU73A/HIS76A double mutant completely abolished A1217 binding, consistent with the structural observation that LEU73 and HIS76 of TIGIT sandwiches the TRP50 of LCDR2 with strong hydrophobic interactions supplemented with several hydrogen bonds ($H76_{TIGIT}$-$D103_{HCDR3}$, $H76_{TIGIT}$-$T31_{LCDR1}$, $L73_{TIGIT}$-$W50_{LCDR2}$) and salt bridges

(H76$_{TIGIT}$-D103$_{HCDR3}$).The LEU73A mutation in TIGIT had a similar association rate but a slightly faster disassociation rate to WT, resulting in an approximately 7-fold reduction in binding affinity to A1217. Furthermore, the ILE68A mutation in TIGIT had a similar association rate but a much faster disassociation rate than WT, resulting in an approximately 30-fold decrease in binding affinity with A1217. The complex structure revealed that ILE68 of TIGIT lies in the hydrophobic core of the IgV domain and interacts extensively with HCDR3 of A1217. Moreover, the ASP63A mutation showed an approximately 7-fold decrease in binding affinity with A1217, as ASP63 of TIGIT formed three hydrogen bonds with THR52, GLY54 and GLY56 of HCDR2 in A1217. In addition, the PRO79A mutation in TIGIT had a slower association rate and faster disassociation rate than WT, resulting in an approximately 3-fold lower binding affinity with A1217. The complex structure indicates that PRO79 of TIGIT formed a strong hydrophobic interaction with PHE104 of HCDR3 and TYR91, SER92 and TYR94 of LCDR3 in A1217. Mutation of TIGIT at SER80, GLN64 did not affect A1217 binding (Table 6). This analysis agreed with the crystal structure analysis, with the TIGIT residues that most affected A1217 binding found to interact with A1217 in the structure.

[0093] For Tiragolumab interactions, the LYS82A mutant, which had extensive hydrophobic interactions with TYR31/TYR 38/TYR 98 of the light chain and formed a strong hydrogen bond/salt bridge with ASP107 of the heavy chain, completely abolished Tiragolumab binding. The ILE68A mutant formed a strong hydrophobic interaction with LEU108 and LEU109 of HCDR3 in Tiragolumab, resulting in an approximately 40-fold lower binding affinity with Tiragolumab (Table 6), a site which also influences A1217 binding. The LEU65A mutation in TIGIT resulted in about 25-fold lower binding affinity with Tiragolumab, leading to a slower association rate and a faster disassociation rate when compared with WT TIGIT. The HIS76A mutant had a mild effect on Tiragolumab binding, whereas this mutation nearly abolished A1217 binding. Similarly, the P79A/S80A single mutant, which formed hydrogen bonds with THR100, had only a mild effect on Tiragolumab binding. The mutagenesis experiments show that A1217 and Tiragolumab have distinct epitopes when binding to TIGIT, implying that the antagonistic mechanism of the two antibodies can be different.

Table 6. TIGIT binding kinetics with A1217 and Tiragolumab

Analyte	kon ($M^{-1}s^{-1}$)	koff (s^{-1})	A1217 KD(M)	A1217 KDmut /KDwt	HB/ SB[a]	kon ($M^{-1}s^{-1}$)	koff (s^{-1})	Tiragolumab KD(M)	Tiragolumab KDmut/ KDwt	HB/SB
TIGIT-WT	2.14E+6	4.49E-4	2.10E-10	N.A.		1.79E+6	3.47E-4	1.93E-10	N.A.	
TIGIT-L73/H76A	N.A.	N.A.	No binding	No binding	HB/ SB	1.36E+6	4.04E-4	2.98E-10	1.5	
TIGIT-H76A	3.22E+6	6.63E-2	2.06E-08	98.1	HB/ SB	2.00E+6	4.19E-4	2.10E-10	1.1	HB/SB
TIGIT-I68A	3.63E+6	2.22E-2	6.12E-09	29.1		9.92E+5	7.55E-3	7.61E-09	39.4	
TIGIT-D63A	5.74E+5	8.24E-4	1.44-E9	6.9	HB	1.07E+6	3.67E-4	3.43E-10	1.8	
TIGIT-L73A	2.44E+6	3.46E-3	1.42E-09	6.8	HB	1.42E+6	3.80E-4	2.69E-10	1.4	
TIGIT-P79A	1.67E+6	1.09E-3	6.52E-10	3.1		1.71E+6	4.39E-4	2.56E-10	1.3	HB
TIGIT-L65A	8.09E+5	5.06E-4	6.25E-10	3	HB	6.22E+5	2.94E-3	4.72E-09	24.5	
TIGIT-Q56A	1.12E+6	5.59E-4	5.00E-10	2.4	HB	1.39E+6	6.42E-4	4.63E-10	2.4	
TIGIT-N70A	9.43E+5	4.52E-4	4.80E-10	2.3	HB	8.53E+5	4.00E-4	4.69E-10	2.4	
TIGIT-R84A	7.56E+6	3.30E-4	4.37E-10	2.08		2.03E+6	6.64E-4	3.26E-10	1.69	
TIGIT-N58A	2.21E+6	9.26E-4	4.19E-10	2		1.50E+6	1.22E-3	8.10E-10	4.2	HB
TIGIT-E60A	1.43E+6	5.34E-4	3.74E-10	1.8	SB	9.99E+5	2.37E-3	2.37E-09	12.3	HB/SB
TIGIT-D83A	1.29E+6	4.88E-4	3.78E-10	1.8		8.56E+5	3.91E-4	4.56E-10	2.4	
TIGIT-K82A	1.22E+6	4.00E-4	3.26E-10	1.6	HB	N.A.	N.A.	No binding	No binding	HB/SB
TIGIT-I109A	1.91E+6	4.63E-4	2.42E-10	1.2		1.70E+6	3.77E-4	2.22E-10	1.2	
TIGIT-P87A	1.89E+6	4.57E-4	2.42E-10	1.2		1.63E+6	3.89E-4	2.38E-10	1.2	
TIGIT-T55A	2.10E+6	4.66E-4	2.22E-10	1.1		1.50E+6	3.89E-4	2.59E-10	1.3	
TIGIT-Q61A	1.75E+6	3.91E-4	2.23E-10	1.06		2.29E+6	6.76E-4	2.96E-10	1.53	
TIGIT-	1.93E+6	4.23E-4	2.19E-10	1	HB	1.50+E6	4.04E-4	2.70E-10	1.4	HB

S80A										

[a] HB/SB denote hydrogen bond and salt bridge, respectively.

A1217 has the property of pH-dependent binding for TIGIT

[0094] Based on our SPR data, we observed that the binding affinity (KD) of A1217 towards TIGIT increases around 17-fold when pH decreases from 7.4 to 6.0 (Table 7). The amine coupling method was employed to measure the binding affinity between A1217 and TIGIT under different pHs. In our complex structure, ASP103$_{HCDR3}$ is well positioned towards HIS76$_{TIGIT}$ at 2.7 Å distance which forms strong electrostatic interaction (Figure 1D). In tumor microenvironment, the imidazole ring of HIS76$_{TIGIT}$ could be mostly protonated and carry positive charge under this acidic environment which strengthen the electrostatic interaction with ASP103$_{HCDR3}$. However, in physiological condition HIS76$_{TIGIT}$ would carry less positive charge and weaken this interaction. In the A1217/TIGIT complex structure, ASP103$_{HCDR3}$ is well positioned with respect to HIS76$_{TIGIT}$ at a 2.7 Å distance and forms a strong electrostatic interaction, whereas no such favorable architecture surrounding HIS76$_{TIGIT}$ in Tiragolumab /TIGIT was observed. We found that the binding affinity (KD) of A1217 toward TIGIT increased approximately 17-fold when the pH decreased from 7.4 to 6.0, whereas Tiragolumab did not show obvious pH-dependent binding to TIGIT (Table 7). In conclusion, pH-sensitive antibodies could be better at mitigating the balance between efficacy on tumor cells versus safety on normal cells than non-pH-sensitive antibodies.

Table 7. TIGIT binding kinetics with A1217 compared to Tiragolumab under different pHs

Analyte	**kon ($M^{-1}s^{-1}$)**	**koff (s^{-1})**	**A1217 KD(M)**
TIGIT-WT pH 7.4	2.40E+5	1.27E-4	5.32E-10
TIGIT-WT pH 6.0	2.94E+6	9.28E-5	3.16E-11
Analyte	**kon ($M^{-1}s^{-1}$)**	**koff (s^{-1})**	**Tiragolumab KD(M)**
TIGIT-WT pH 7.4	1.75E+5	1.03E-4	5.88E-10
TIGIT-WT pH 6.0	9.05E+5	4.60E-4	5.08E-10

Example 4: Generation of reduced fucosylated anti-TIGIT antibodies

[0095] Removal of core fucose from N-glycans attached to human IgG1 significantly enhances the antibody dependent cell cytotoxicity (ADCC) response (Shields, et al., (2002) J Biol Chem 277, 26733-26740); Shinkawa et al., (2003) J Biol Chem 278, 3466-3473). There are many approaches to reduce core fucosylation. The work presented here utilized the approach of a fucosyltransferase (FUT) inhibitor, 2F-peracetyl-fucose.

[0096] A1217 P5-2 3G11 is a stably transfected CHO-K1 cell line that expresses the A1217 antibody. A1217 P5-2 3G11 cells were cultured in shaker flasks and passaged every 3-4 days for maintenance and seeded at $5x10^5$cells/mL in Hyclone ActiPro™ medium with or without the addition of 1.25% Glycosylation Adjust (Gal +) (Sigma-Aldrich,14701C0). 2F-peracetyl-fucose concentrations of 0, 50, 100, 150, 200 μM were added to the culture. Cell supernatant was harvested on day 14 and filtered through an 0.2 μm filter for further analysis.

[0097] A1217 antibodies purified with a Protein A chromatography capture step under platform conditions and glycan profiles were analyzed by HILIC-UPLC oligo-glycans (Figure 4). Glycan analysis showed that the inhibitor reduced total fucoslyation to 11% at 50 μM 2F-peracetyl-fucose, and to less than 7% at 100, 150, or 200 μM 2F-peracetyl-fucose.

Example 5: Generation of anti-TIGIT effector variants

[0098] Fc mutation combinations of S239D, I332E or S239D, I332E, A330L (EU numbering) were generated on the plasmid containing the DNA fragment encoding the heavy chain of A1217 according to the methods provided by the Fast MultiSite Mutagenesis System™ (FM201-01, Transgenbiotech). Variant A1217DE contains the amino acid changes S239D, I332E (DE). Variant A1217DEL contains the amino acid changes S239D, I332E, A330L (DEL). Expi-CHO cells were transfected by the corresponding plasmids for A1217 effector variants (A1217DE or A1217DEL) and cultured for 10 days at 30℃ with 5% CO2. After that the supernatant was harvested, and the proteins were purified using MabSelect SuRe™ (17543802, Cytiva).

Example 6: Binding affinities of TIGIT antibodies to FcγRs

[0099] For determination of FcγRI binding, protein A was coupled to activated CM5 biosensor chips (Cat. No. BR100530, GE Life Sciences), 30 nM of A1217 or A1217 variants were flowed over the chip and captured by Protein A. A series of concentrations of FcγRI (from 0.0586 nM to 15 nM) were injected in the SPR running buffer (10 mM HEPES, 150 mM NaCl, 3 mM EDTA, 0.05%

Tween20, pH7.4) at 30μL/minute. Binding responses to FcγRI of A1217 or mutants was calculated by subtracting of RU from a reference flow-cell without injection of A1217 or mutants. For determination of FcγRI binding affinities, *kon* and *koff* were calculated using the one-to-one Langmuir binding model; KD was calculated as the ratio of *koff /kon*.

[00100] Anti-Human Kappa antibody was coupled to activated CM5 biosensor chips (Cat. No. BR100530, GE Life Sciences), A1217 or A1217 variants were flowed over the chip and captured by the anti-human Kappa surface. A series concentration of different FcγR(FcγRIIA, FcγRIIB , FcγRIIIA) were injected in the SPR running buffer (10 mM HEPES, 150 mM NaCl, 3 mM EDTA, 0.05% Tween20, pH7.4) at 30μL/minute. Binding responses to FcγR of A1217 or mutants was calculated by subtracting of RU from a reference flow-cell without injection of A1217 or mutants. For determination of FcγRIIA, FcγRIIB, and FcγRIIIA binding affinities, the data from all the concentrations were fitted using a steady-state affinity model.

[00101] As shown in Table 8, compared with A1217, A1217AF demonstrated around 10-fold increased binding affinity to FcγRIIIA-V158 and FcγRIIIA-F158. Compared with A1217, A1217DEL and A1217DE also increased the binding affinity to FcγRIIIA-V158 and FcγRIIIA-F158. In addition, A1217DEL and A1217DE increased the binding affinity to FcγRI and FcγRII.

Table 8. Binding affinities of TIGIT antibodies to FcγRs by SPR

Sample	FcγRI	FcγRIIA		FcγRIIB	FcγRIIIA	
		H131	R131		V158	F158
A1217	8.27E-11	9.59E-7	1.13E-6	4.43E-6	1.57E-7	8.71E-7
A1217AF	5.58E-11	1.10E-6	7.43E-7	2.94E-6	1.7E-8	6.15E-8
A1217 DEL	2.81E-12	9.37E-7	4.75E-7	8.00E-7	6.71E-9	4.42E-9
A1217 DE	4.90E-12	3.22E-7	1.57E-7	2.81E-7	7.97E-9	7.40E-9

Example 7: Binding of reduced fucosylated TIGIT antibodies to FcγRIII

[00102] FcγRIIIA-V158 or FcγRIIIA-F158 over-expressing HEK293 cells were first incubated with A1217 or A1217AF, followed by staining with secondary Ab, Alexa Fluor™ 647 anti-human IgG Fc, Biolegend, REF#409320). Cell samples were washed and fixed with 1% paraformaldehyde in DPBS. Immunofluorescence was detected using NovoCyte® Flow Cytometry (ACEA) and analyzed using

Guava Soft™ 3.1.1 software. The result of this analysis is shown in Figure 5A-B. A1217AF showed increased binding to FcγRIIIA-V158 or FcγRIIIA-F158 over-expressing HEK293 cells compared to A1217, indicating enhanced Fc-effector functions of A1217AF compared to A1217 through both variants of FcγRIIIA.

Example 8: Binding of anti-TIGIT effector variants to FcγRIII

[00103] FcγRIIIA-V158 or FcγRIIIA-F158 over-expressing HEK293 cells were first incubated with A1217, A1217DE or A1217DEL, followed by staining with secondary Ab, Alexa Fluor® 488 $F(ab')_2$ fragment goat anti-human IgG ($F(ab')_2$ fragment specific, Jackson ImmunoResearch, catalog no. 109-546-097). Cell samples were washed and fixed with 1% paraformaldehyde in DPBS. Immunofluorescence was detected using Guava easyCyte™ 6HT (Merck-Millipore, USA) and analyzed using Guava Soft™ 3.1.1 software. The results are shown in Figure 6A-D, that A1217DE and A1217DEL showed increased binding to FcγRIIIA-V158 or FcγRIIIA-F158 over-expressing HEK293 cells compared to wild type A1217, indicating enhanced Fc-effector functions of A1217DE and A1217DEL compared to A1217 through both variants of FcγRIIIA.

Example 9: Binding of anti-TIGIT effector variants to TIGIT

[00104] TIGIT over-expressing BW5147.3 cells were first incubated with A1217, A1217DE or A1217DEL, followed by staining with secondary antibody, Alexa Fluor® 488 $F(ab')_2$ fragment goat anti-human IgG [$F(ab')_2$ fragment specific, Jackson ImmunoResearch, catalog no. 109-546-097]. Cell samples were washed and fixed with 1% paraformaldehyde in DPBS. Immunofluorescence was detected using Guava easyCyte™ 6HT (Merck-Millipore, USA) and analyzed using Guava Soft 3.1.1 software. This data is shown in Figure 7. In this result, A1217, A1217DE and A1217DEL showed comparable binding to TIGIT over-expressing HEK293 cells, thus indicating that the deletions produced no change in TIGIT binding via FACS.

Example 10: Binding of reduced fucoslyation anti-TIGIT antibodies to complement C1q

[00105] The C1q binding activity of A1217 or A1217AF was determined by a sandwich-type ELISA. Briefly, serial dilutions of indicated A1217 or A1217AF variants were coated on MaxiSorp immuno plates. C1q binding was tested by incubating human C1q with the antibody coated wells. After washing, bound C1q was detected by an anti-C1q monoclonal antibody, followed by an HRP-conjugated secondary antibody. Binding signals were measured by absorbance at 450 nm using TMB (3,3′,5,5′-Tetramethylbenzidine) substrate. The results are shown in Figure 8, wherein both A1217AF

and A1217 showed comparable binding to C1q.

Example 11: Anti-TIGIT antibodies with reduced fucoslyation and effector variants have enhanced ADCC

[00106] Antibody-dependent cell-mediated cytotoxicity (ADCC) is a mechanism for killing target cells. The antibody binds to target antigens on the surface of target cells. When the Fc portion of target-bound antibodies also binds to FcγRIIIA receptors on the cell surface of effector cells, such as NK cells, crosslinking and activation of FcγRIIIA leads to ADCC Hogarth and Pietersz, (2012) Nat Rev Drug Discov 11, 311-331. The human FcγRIIIA gene displays a dimorphism in the position coding for amino acid residue 158. The FcγRIIIA variant with a valine at amino acid residue 158 (V158) has a high affinity for IgG1 Fc portion, and the FcγRIIIA variant with phenylalanine (F158) has a lower affinity for IgG1 Fc portion. Killing of target cells by NK cells is usually used as a readout for ADCC activity in classic ADCC assays. Due to the variability of different donors and the polymorphism of FcγRIIIA, these cells can be highly variable in response. To use the FcγRIIIA activation as a surrogate readout for ADCC, we monitor the FcγRIIIA activation in a more simplified and stable setting. Briefly, in the surrogate ADCC assay, Jurkat cells were engineered to stably express the FcγRIIIA-V158 or FcγRIIIA-F158 variant, and an NFAT response element driving expression of firefly luciferase (NFAT-reporter-Luciferase). The resulting Jurkat/NFAT-reporter-Luciferase/FcγRIIIA-V158 or Jurkat/NFAT-reporter-Luciferase/FcγRIIIA-F158 cells were used as effector cells. BW5147.3 cells engineered to stably express TIGIT (BW5147.3/TIGIT cells) were used as target cells. Antibody biological activity in the surrogate ADCC assay is quantified through the luciferase as a result of NFAT pathway activation. Luciferase activity in the effector cell is quantified with luminescence readout.

[00107] (A) Jurkat/NFAT-reporter-Luciferase/FcγRIIIA-V158 cells (1 x 10^4 cells/well) as effector cells were co-cultured with BW5147.3/TIGIT cells (1 x 10^4 cells/well) overnight. The NFAT reporter activity were detected using One-Step™ luciferase assay system, BPS Bioscience.

[00108] This experiment demonstrated that A1217AF, A1217DEL and A1217DE had enhanced ADCC activity when compared with A1217 wild type (Figure 9A).

[00109] (B) Jurkat/NFAT-reporter-Luciferase/FcγRIIIA-F158 cells (5 x 10^4 cells/well) were co-cultured with BW5147.3/TIGIT cells (1 x 10^4 cells/well) overnight. The NFAT reporter activity were detected using One-glo™ luciferase assay system (Promega). A1217AF, A1217DEL and A1217DE

had enhanced ADCC activity when compared to A1217 wild type (Figure 9B).

Example 12: Anti-TIGIT reduced fucoslyation and effector variants have enhanced ADCC in Tregs

[00110] $TIGIT^+$ Tregs represent a functionally distinct Treg subsets which are highly immunosuppressive (Joller et al., (2014) Immunity 40, 569-581)). Since TIGIT expression is higher on intra-tumoral Tregs than on effector T cells, and higher on PBMC derived Tregs in cancer patients than on PBMC derived Tregs in healthy donors (Preillon et al., (2021) Mol Cancer Ther 20, 121-131.), it is reasonable to reckon that Fc function-competent TIGIT antibodyA1217 can induce ADCC in $TIGIT^+$ Treg cells.

[00111] To measure the A1217 or A1217AF induced ADCC activity against T cells, particularly Tregs, PBMCs from lung cancer patients were used as target cells. NK cells isolated from PBMCs from healthy donors were used as effector cells. A1217MF contains a silent Fc, which reduces the amount of ADCC (SEQ ID NO:12) Anti-TIGIT antibodies A1217, A1217AF or A1217MF, were incubated with target cells ($5x10^4$ cells/well) and NK effector cells ($5x10^4$ cells/well, purified using NK Cell isolation kit, Miltenyi Biotec, catalog no. 130-092-657) in 96-well plates overnight. Cell samples were subjected to flow cytometry analysis.

The result was that A1217AF enhanced ADCC activity against Tregs when compared with A1217 wild type as shown in Figure 10A-C. As shown in Figure 10A, Treg frequencies in $CD3^+$ T cells were significantly reduced in a dose-dependent manner upon A1217 or A1217 AF treatment, whereas the percentage remained largely unchanged in the A1217MF-treated group. Compared to A1217 wild type, A1217 AF induced significantly higher ADCC against Tregs. On the other hand, neither A1217 wild type, A1217MF or A1217AF treatment altered the frequencies of effector CD4+ T cells or CD8+ T cells (Figure 10B and 10C). The results clearly indicate that the A1217AF is able to induce stronger ADCC against Treg cells in cancer patient derived PBMCs, compared to A1217 wild type. Enhanced Fc function may augment TIGIT antibody's activity in the anti-tumor immune response through Treg reduction.

Example 13: Anti-TIGIT antibodies with reduced fucoslyation activate NK cells

[00112] TIGIT is constitutively expressed on natural killer (NK) cells and the interaction between TIGIT and its ligands PVR and PVR-L2 inhibits NK cell-mediated cytotoxicity (Stanietsky et al., (2009) Proc Natl Acad Sci U S A 106, 17858-17863; Wang et al., (2015) Eur J Immunol 45, 2886-

2897). The dominant FcγR expressed on NK cells is FcγRIIIA (Bruhns, (2012) Blood 119, 5640-5649). Additive and/or synergistic effects upon co-engagement of TIGIT and FcγRIIIA on NK cells can converge downstream, leading to stronger signals. To confirm whether the capability of FcγR co-engagement is required for optimal NK activation by TIGIT antibodies, and whether an Fc enhanced A1217 could further promote NK cell activation, purified primary NK cells were co-cultured with a human breast cancer cell line SK-BR-3 (ATCC HTB-30) expressing high level of PVR in the presence of TIGIT antibodies with different Fc format. NK cell activation was determined by measuring the NK cell degranulation marker CD107a by flow cytometry. The result is that A1217AF had a greater activation of NK cells when compared to A1217 wild type or A1217MF (Figure 11A-B).

[00113] In the co-culture assay, anti-TIGIT antibodies A1217 wild type, A1217AF or A1217MF, were added to the co-culture of SK-BR-3 ($5x10^4$ cells/well) with primary NK cells isolated from healthy donor-derived PBMCs ($5x10^4$ cells/well) in 96-well plates overnight. NK cells were pre-stimulated with 25 U/mL recombinant human IL-2 (Novoprotein, China, catalog no. C013) overnight before the co-culture assay. CD107a expression on NK cells was determined by FACS. NK activation was significantly increased by A1217AF when compared to A1217 wild type, suggesting the enhanced FcγR co-engagement through afucosylation of A1217 induced optimal NK cell activation in the presence of tumor cells.

Example 14: Trogocytosis properties of anti-TIGIT antibodies with reduced fucoslyation

[00114] Trogocytosis is a process in which cell surface molecules are transferred from donor cells to acceptor cells (Beum et al., (2008) J Immunol 181, 8120-8132; Joly and Hudrisier, (2003) Immunity 40, 569-581; Machlenkin et al., (2008) Cancer Res 68, 2006-2013; Rossi et al., (2013) Blood 122, 3020-3029). Antibody mediated trogocytosis via Fcγ receptors (FcγRs) leads to down-regulation of receptors on the cell surface(Taylor and Lindorfer, (2015) Blood 125, 762-766). Down-regulation of target receptor by trogocytosis can cause dampened signaling. To investigate whether TIGIT antibodies with Fc function can induce FcγR-mediated trogocytosis to remove TIGIT from cell surface, and whether the enhanced Fc binding to FcγR through DE/DEL mutation or reduced fucosylation can further promote trogocytosis, a trogocytosis assay was performed.

[00115] Jurkat/TIGIT/DNAM-1 cells ($2x10^4$ cells/well) were used as donor cells, and CFSE (Invitrogen, catalog no. C34554) labeled HEK293 cells expressing different FcγRs ($4x10^4$ cells/well)

were used as acceptor cells. Donor cells were pre-incubated with 10 μg/mL CF633-labeled A1217 wild type, A1217AF or A1217MF for 30 minutes and washed. The donor cells were then incubated with acceptor cells in 96-well plates overnight. Changes of TIGIT (CF633) mean fluorescence intensity (MFI) on donor cells were measured by FACS.

[00116] As shown in Figure 12, when compared to A1217 wild type, A1217AF significantly induced TIGIT down-regulation on Jurkat/TIGIT/DNAM-1 cells when the acceptor cells express FcγRIIIA-F158 and FcγRIIIA-V158, indicating that the Fc-enhancement of A1217 through afucosylation can induce a maximal TIGIT downregulation through FcγRIIIA-binding-dependent trogocytosis. Interestingly, when acceptor cells express FcγRI, A1217AF induced comparable level of trogocytosis to A1217 wild type, which is consistent with the comparable binding affinity of A1217AF and A1217 to FcγRI.

Example 15: Trogocytosis properties of anti-TIGIT antibody mutants

[00117] Jurkat/TIGIT/DNAM-1 ($2x10^4$ cells/well) cells were used as donor cells, and CFSE (Invitrogen, catalog no. C34554) labeled HEK293 cells expressing different FcγRs ($4x10^4$ cells/well) were used as acceptor cells. Donor cells were pre-incubated with 10 μg/mL CF633-labeled A1217 wild type, A1217DE, A1217DEL or A1217MF for 30 minutes and washed. The donor cells were then incubated with acceptor cells in 96-well plates overnight. Changes of TIGIT (CF633) MFI on donor cells were measured by FACS.

[00118] The results of the A1217DE and DEL effector variants were similar to that of A1217AF with reduced fucoslyation. On cells expressing FcγRIIIA-F158 or FcγRIIIA-V158, trogocytosis was greater for A1217DE and A1217DEL when compared with A1217 wild type. The Fc silent A1217MF had little to no activity as expected and this data is shown graphically in Figure 13.

Example 16: A1217 antibodies with reduced fucoslyation in combination with anti-PD1 antibodies in a mouse tumor model

[00119] For this experiment, $2x10^5$ Renca cells were implanted subcutaneously in the right flank in humanized TIGIT mice on BABL/c background. Renca cells are of murine renal adenocarcinoma model. The mice were then administered PBS as a control, 3 mg/kg mPD-1 Ab, 10 mg/kg A1217 wild type or 10 mg/kg A1217AF as single agent therapy. For combination therapy the mice were administered 3 mg/kg mPD-1 antibody in combination with 10 mg/kg A1217 wild type, or 3 mg/kg mPD-1 Ab and 10 mg/kg A1217AF. This treatment was administered intraperitoneally at day 5

post tumor cell inoculation. The tumor volume was determined twice weekly using the formula: V = 0.5 (a x b^2) where a and b are the long and short diameter of the tumor, respectively. *p<0.05.

[00120] This data is shown graphically in Figure 14. In comparing A1217 wild type and A1217AF, there was little difference when administered as a single agent. However, the A1217AF antibody when administered in combination with an anti-PD1 antibody had improved tumor reduction compared to A1217 wild type administered in combination with an anti-PD1 antibody.

Example 17: A1217 antibodies with reduced fucoslyation in combination with anti-PD1 antibodies induces Treg reduction in a mouse tumor model

[00121] To investigate whether the A1217AF antibodies with reduced fucoslyation in combination with anti-PD-1 antibodies reduce Treg cells in the Renca model, female hTIGIT Balb/c mice (6-8 weeks old) were subcutaneously implanted with 0.3 million RENCA tumor cells in the right flank on day 0 and randomized into 8 groups according to body weight and tumor volume when mean tumor volume reached 100~200cm^3. The mice were treated with anti-TIGIT antibody A1217 with different Fc formats (10 mg/kg) as a single agent, murine PD-1 blockade antibody Ch15mt (3 mg/kg) as single agent, or the combination of the two antibodies at the dose indicated. Tumor samples were collected at 48 hours after dosing for tumor infiltrating lymphocytes (TILs) isolation and FACS analysis. Treg cells were gated with $CD45^+CD4^+FOXP3^+$. As shown in Figures 15A-C, when comparing A1217 wild type and A1217AF, there was not a significant difference when administered as a single agent. However, when administered in combination with an anti-PD1 antibody, the A1217AF antibody reduced intra-tumor Tregs significantly when compared to A1217 wild type administered in combination with an anti-PD1 antibody, indicating that afucosylation of A1217 can induce more significant anti-tumor effects in combination with PD-1 antibody by decreasing Treg cells in the tumor microenvironment.

CLAIMS

1. A method of cancer treatment, the method comprising administering to a subject an effective amount of anti-TIGIT antibody or antigen-binding fragment thereof that is pH dependent.

2. The method of claim 1, wherein the anti-TIGIT antibody binds to the TIGIT protein at amino acid histidine 76.

3. The method of claim 2, wherein the anti-TIGIT antibody binds to the TIGIT protein at histidine 76 and leucine 73.

4. The method of claim 3, wherein the method comprises administering to a subject an effective amount of a pH dependent antibody or antigen-binding fragment thereof, which specifically binds to human TIGIT and comprises: a heavy chain variable region that comprises a HCDR (Heavy Chain Complementarity Determining Region) 1 of SEQ ID NO: 1, a HCDR2 of SEQ ID NO: 2, and a HCDR3 of SEQ ID NO:3; and a light chain variable region that comprises a LCDR (Light Chain Complementarity Determining Region) 1 of SEQ ID NO:4, a LCDR2 of SEQ ID NO:5, and a LCDR3 of SEQ ID NO:6.

5. The method of claim 4, wherein the anti-TIGIT antibody or antigen-binding fragment thereof comprises: a heavy chain variable region (VH) that comprises SEQ ID NO:7, and a light chain variable region (VL) that comprises SEQ ID NO: 8.

6. The method of claim 1, wherein the anti-TIGIT antibody has enhanced antibody dependent cell mediated cytotoxicity (ADCC) activity.

7. The method of claim 6, wherein the anti-TIGIT antibody has reduced fucosylation.

8. The method of claim 6, wherein the anti-TIGIT antibody has Fc amino acid changes at S239D, and I332E (EU numbering).

9. The method of claim 6, wherein the anti-TIGIT antibody has Fc amino acid changes at S239D,

I332E and A330L (EU numbering).

10. The method of any one of claims 7-9, wherein the anti-TIGIT antibody has increased binding affinity to FcγRIIIA-V158 and FcγRIIIA-F158.

11. The method of any one of claims 7-9, wherein the anti-TIGIT antibody has enhanced ADCC in T-regulatory (Treg) cells.

12. The method of any one of claims 7-9, wherein the anti-TIGIT antibody activates Natural Killer (NK) cells.

13. The method of any one of claims 7-9, wherein the anti-TIGIT antibody has increased trogocytosis.

14. The method of claim 1, wherein the method further comprises administering an anti-PD1 antibody which specifically binds human PD1 and comprises: a heavy chain variable region that comprises a HCDR1 of SEQ ID NO: 15, HCDR2 of SEQ ID NO: 16, and HCDR3 of SEQ ID NO: 17; and a light chain variable region that comprises LCDR1 of SEQ ID NO: 18, LCDR2 of SEQ ID NO: 19, and LCDR3 of SEQ ID NO: 20.

15. The method of claim 14, wherein the anti-PD1 antibody or antigen binding fragment thereof which specifically binds human PD1 and comprises a heavy chain variable region (VH) comprising an amino acid sequence of SEQ ID NO:21 and a light chain variable region (VL) comprising an amino acid sequence of SEQ ID NO: 22.

16. The method of claim 14 or 15 wherein the anti-PD1 antibody comprises an IgG4 constant domain comprising SEQ ID NO: 23.

17. The method of claim 1, wherein the cancer is selected from the group consisting of breast cancer, colon cancer, pancreatic cancer, head and neck cancer, gastric cancer, kidney cancer, liver cancer,

small cell lung cancer, non-small cell lung cancer, esophageal cancer, ovarian cancer, uterine cancer, cervical cancer, skin cancer, mesothelioma, lymphoma, leukemia, myeloma or sarcoma.

18. The method of claim 17, wherein the cancer is non-small cell lung cancer.

19. The method of claim 17, wherein the head and neck cancer is nasopharyngeal cancer.

20. The method of claim 17, wherein the esophageal cancer is esophageal squamous cell carcinoma (ESCC).

21. The method of claim 17, wherein the cancer is uterine cancer.

22. The method of claim 17, wherein the gastric cancer is gastric or gastroesophageal junction cancer.

23. The method of claim 17, wherein the cervical cancer is recurring or metastatic cervical cancer.

24. The method of claim 17, wherein the cancer is kidney cancer.

25. The method of claim 14, further comprising the administration of chemotherapy.

26. The method of claim 20, wherein the chemotherapy is chemoradiotherapy.

27. The method of claim 1, wherein the anti-PD1 antibody is dosed at 200mg every three weeks.