

ANTI-TIGIT ANTIBODIES AND USES THEREOF

CROSS REFERENCE

This application claims the benefit of International application PCT/CN2023/098574, filed on June 6, 2023, which is incorporated herein by reference in its entirety.

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SEQUENCE LISTING

The instant application contains a sequence listing which is hereby incorporated by reference in its entirety.

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FIELD

This application generally relates to antibodies. More specifically, the application relates to monoclonal antibodies against TIGIT, a method of preparing the same, and the use of the antibodies.

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BACKGROUND

In the tumor microenvironment, persisting antigenic stimulation can result in T cell exhaustion, a state of T cell dysfunction, and high expression of co-inhibitory receptors including PD-1, LAG-3, TIM3, and TIGIT. Currently, multiple strategies are being explored to reinvigorate exhausted T cells by either small molecule or therapeutic antibody approaches alone or in combination.

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TIGIT (T cell immune receptor with Ig and ITIM domains), also known as Vstm3 and WUCAM, is a co-inhibitory receptor that is expressed on NK and CD8+ T cells, as well as a subset CD4+ T cells including immunosuppressive regulatory T cells (Tregs). There are four known ligands of TIGIT, poliovirus receptor (PVR), PVRL2, PVRL3 and PVRL4, all of which are over-expressed by tumors and antigen-presenting cells, resulting in immune suppression. These ligands also bind the co-stimulatory molecule CD226 and the co-inhibitory molecule PVRIG and CD96 (the latter of which is sometimes considered co-stimulator). Antagonistic antibodies of TIGIT disrupt binding of TIGIT to its ligands and block its inhibitory signals, shifting the balance in favor of CD226-mediated activating signals, which induces a strong anti-tumor immune response.

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TIGIT is upregulated and identified as an exhaustion marker in cancer and inflammatory diseases as other exhaustion markers like PD-1, LAG3 and TIM3. Moreover, TIGIT is identified as a key inhibitory receptor of a new population of T cells, stem-like memory T cells, that may be the preferred targets for anti-PD-(L)1 efficacy.

TIGIT could be a promising therapeutic target for tumor immunotherapy as single agent or in combination with other immune modulators.

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SUMMARY

These and other objectives are provided for by the present disclosure which, in a broad sense, is directed to compounds, methods, compositions and articles of manufacture that provide antibodies with improved efficacy. The benefits provided by the present disclosure are broadly applicable in the field of antibody therapeutics and diagnostics and may be used in conjunction with other antibodies that react with a variety of targets.

The present disclosure provides chimeric and humanized monoclonal antibodies against TIGIT. Further provided are methods for validating the function of antibodies in vitro and in vivo, and methods of treating a subject having cancer or immune disorder by administering the anti-TIGIT antibodies as disclosed herein, alone or combined with a PD-1/PD-L1 antagonist.

In some aspects, the present disclosure provides an isolated antibody or an antigen-binding portion thereof against TIGIT. In some embodiments, the isolated antibody or the antigen-binding portion thereof comprises:

- a heavy chain CDR (HCDR) 1 comprising the amino acid sequence of SEQ ID NO: 1;
- a HCDR2 comprising the amino acid sequence of SEQ ID NO: 2;
- a HCDR3 comprising the amino acid sequence of SEQ ID NO: 3;
- a light chain CDR (LCDR) 1 comprising the amino acid sequence of SEQ ID NO: 4;
- a LCDR2 comprising the amino acid sequence of any of SEQ ID NOs: 7, 5, 8 and 9; and
- a LCDR3 comprising the amino acid sequence of SEQ ID NO: 6.

In some embodiments, the isolated antibody or the antigen-binding portion thereof comprises a heavy chain variable region (VH) and a light chain variable region (VL), wherein the VH comprises or consists of:

- (i) the amino acid sequence as set forth in any of SEQ ID NOs: 10-11;
- (ii) an amino acid sequence at least 85%, 90%, or 95% identical to any of SEQ ID NOs: 10-11; or

(iii) an amino acid sequence with addition, deletion and/or substitution of one or more (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10) amino acids compared with any of SEQ ID NOs: 10-11; and/or the VL comprises or consists of:

- (i) the amino acid sequence as set forth in any of SEQ ID NOs: 12-18;
- (ii) an amino acid sequence at least 85%, at least 90%, or at least 95% identical to any of SEQ ID NOs: 12-18; or

(iii) an amino acid sequence with addition, deletion and/or substitution of one or more (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10) amino acids compared with any of SEQ ID NOs: 12-18.

In some embodiments, the isolated antibody or the antigen-binding portion thereof comprises the HCDR1, HCDR2 and HCDR3 of the VH region as set forth in any of SEQ ID NOs: 10-11, and the LCDR1, LCDR2 and LCDR3 of the VL region as set forth in any of SEQ ID NOs: 12-18.

In some embodiments, the isolated antibody or the antigen-binding portion thereof comprises: a HCDR1 as set forth in SEQ ID NO: 1; a HCDR2 as set forth in SEQ ID NO: 2; a

HCDR3 as set forth in SEQ ID NO: 3; a LCDR1 as set forth in SEQ ID NO: 4; a LCDR2 as set forth in SEQ ID NO: 7; and a LCDR3 as set forth in SEQ ID NO: 6.

In some embodiments, the isolated antibody or the antigen-binding portion thereof comprises a VH region comprising the amino acid sequence of SEQ ID NO: 11 and a VL region
5 comprising the amino acid sequence of SEQ ID NO: 15.

In some embodiments, the isolated antibody or the antigen-binding portion thereof further comprises a human IgG constant region, such as a human IgG1, IgG4, IgG2 or IgG3 constant region, which may be native or a variant thereof. Specifically, the antibody may comprise a human IgG1 Fc region or a human IgG4 Fc region with a S228P substitution.

10 In some embodiments, the anti-TIGIT antibody as disclosed herein is a murine antibody, a chimeric antibody or a humanized antibody. In some embodiments, the antibodies herein are anti-TIGIT antagonist antibodies.

In some aspects, the present disclosure provides an isolated nucleic acid molecule, comprising a nucleic acid sequence encoding the heavy chain variable region and/or the light chain
15 variable region of the isolated antibody or the antigen-binding portion thereof as disclosed herein. In some embodiments, the nucleic acid molecule comprises the nucleic acid sequence as set forth in SEQ ID NO: 21, and/or the nucleic acid sequence as set forth in SEQ ID NO: 22.

In some aspects, the present disclosure provides an expression vector(s) comprising the nucleic acid molecule(s) as disclosed herein.

20 In some aspects, the present disclosure provides a host cell comprising the expression vector as disclosed herein.

In some aspects, the present disclosure provides a pharmaceutical composition comprising the antibody or antigen-binding portion thereof as disclosed herein and a pharmaceutically acceptable carrier.

25 In some aspects, the present disclosure provides a method for preparing the antibody or antigen-binding portion thereof which comprises expressing the antibody or antigen-binding portion thereof in a host cell and isolating the antibody or antigen-binding portion thereof from the host cell. In some embodiments, the host cell has been transfected or transformed with an expression vector(s) encoding the heavy chain and light chain of the antibody disclosed herein.
30 The heavy chain encoding nucleic acid sequence and the light chain encoding nucleic acid sequence may be in the same vector or in separate vectors.

In some aspects, the present disclosure provides a method of modulating a TIGIT related immune response in a subject, comprising administering the antibody or antigen-binding portion thereof as disclosed herein to the subject.

35 In some aspects, the present disclosure provides a method for inhibiting growth of tumor cells in a subject, comprising administering an effective amount of the antibody or antigen-binding portion thereof or the pharmaceutical composition as disclosed herein, alone or combined with another anti-cancer agent such as an anti-PD-1 antibody, to the subject.

In some aspects, the present disclosure provides a method for treating or preventing a cancer or an immune related disorder in a subject, comprising administering an effective amount of the antibody or antigen-binding portion thereof as disclosed herein, alone or combined with another anti-cancer agent, to the subject.

5 The anti-cancer agent may be a chemotherapeutic agent(s), a monoclonal antibody, an antibody-drug conjugate etc. In some embodiments, the anti-cancer agent is an anti-PD-1 antibody, anti-PD-L1 antibody or an anti-CTLA-4 antibody.

10 Said cancer can be selected from colon cancer, lung cancer (such as NSCLC), breast cancer, ovarian cancer, melanoma, bladder cancer, renal cell carcinoma, liver cancer, prostate cancer, stomach cancer, pancreatic cancer, lymphoma, leukemia, uterine cancer, cervical cancer, testicular cancer, esophageal cancer, gastrointestinal cancer, gastric cancer, colorectal cancer, kidney cancer, clear cell renal carcinoma, head and neck cancer, germ cell cancer, bone cancer, thyroid cancer, skin cancer, neoplasm of the central nervous system, mesothelioma, chronic lymphocytic leukemia, diffuse large B cell lymphoma, follicular lymphoma, Hodgkin lymphoma, myeloma, soft-tissue
15 cancer and sarcoma. The immune related disorder may be a T cell dysfunctional disorder, an infection, or an inflammatory disease.

In some embodiments, the antibody or antigen-binding portion thereof as disclosed herein is administered in combination with an anti-PD-1 antibody.

20 In some aspects, the present disclosure provides a combination of the isolated antibody or the antigen-binding portion thereof as disclosed herein with an anti-PD-1 antibody.

In some aspects, the present disclosure provides use of the antibody or antigen-binding portion thereof as disclosed herein, alone or combined with another anti-cancer agent, in the manufacture of a medicament for treating or preventing diseases such as cancers and immune disorders. In some embodiments, the anti-cancer agent is an anti-PD-1 antibody, anti-PD-L1
25 antibody or an anti-CTLA-4 antibody.

In some aspects, the present disclosure provides the use of the antibody or antigen-binding portion thereof as disclosed herein in the manufacture of a diagnostic agent for diagnosing diseases related to TIGIT overexpression.

30 In some aspects, the present disclosure provides the antibody or antigen-binding portion thereof as disclosed herein for use in treating or preventing cancers and immune disorders. In some embodiments, the antibody as disclosed herein is used in combination with an PD-1/PD-L1 antagonist, such as an PD-1 antibody.

In some aspects, the present disclosure provides a method for detecting the presence of TIGIT antigen in a sample or measuring the amount of TIGIT antigen, comprising contacting the
35 sample with the anti-TIGIT antibody or an antigen binding portion thereof as disclosed herein.

In some aspects, the present disclosure provides kits or devices that comprise the antibody or antigen-binding portion thereof as disclosed herein in one or more containers.

The foregoing is a summary and thus contains, by necessity, simplifications, generalizations, and omissions of detail; consequently, those skilled in the art will appreciate that the summary is illustrative only and is not intended to be in any way limiting. This summary is not intended to identify key features or essential features of the claimed subject matter, nor is it intended to be used as an aid in determining the scope of the claimed subject matter.

BRIEF DESCRIPTION OF THE FIGURES

Figures 1-3 show the binding of antibodies to human TIGIT (Figure 1), cynomolgus monkey TIGIT (Figure 2) and mouse TIGIT (Figure 3), as determined by FACS.

Figures 4 shows the binding of antibodies to TIGIT paralogous proteins, as determined by ELISA.

Figure 5 shows the result of antibodies blocking CD155 binding to TIGIT, as determined by FACS.

Figure 6 shows the result of antibodies blocking CD112 binding to TIGIT, as determined by FACS.

Figure 7 shows the result of antibodies blocking CD113 binding to TIGIT, as determined by FACS.

Figure 8 shows the result of antibodies in NFAT reporter gene assay.

Figure 9 shows the effect of antibodies in stimulation of IL-2 release by Jurkat cells.

Figures 10 and 11 show the effect of antibodies in NK cell activation assay and ADCC assay, respectively.

Figure 12 shows the stability of the antibodies in human serum.

Figure 13 shows the result of tumor volume changes after treatment with antibodies in MC38 xenograft study.

Figure 14 shows the result of body weight changes after treatment with antibodies in the MC38 xenograft study.

DETAILED DESCRIPTION

While the present invention may be embodied in many different forms, disclosed herein are specific illustrative embodiments thereof that exemplify the principles of the invention. It should be emphasized that the present invention is not limited to the specific embodiments illustrated. Moreover, any section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

Unless otherwise defined herein, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. More specifically, as used in this specification and the appended claims, the singular forms “a”, “an” and “the” include plural referents unless the context

clearly dictates otherwise. Thus, for example, reference to “a protein” includes a plurality of proteins; reference to “a cell” includes mixtures of cells, and the like. In this application, the use of “or” means “and/or” unless stated otherwise. Furthermore, the use of the term “comprising,” as well as other forms, such as “comprises” and “comprised,” is not limiting. In addition, ranges
5 provided in the specification and appended claims include both end points and all points between the end points.

Generally, nomenclature used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well-known and commonly used in the art. The
10 methods and techniques of the present disclosure are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. See, e.g., Abbas *et al.*, *Cellular and Molecular Immunology*, 6th ed., W.B. Saunders Company (2010); Sambrook J. & Russell D. *Molecular Cloning: A Laboratory Manual*, 3rd ed.,
15 Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2000); Ausubel *et al.*, *Short Protocols in Molecular Biology: A Compendium of Methods from Current Protocols in Molecular Biology*, Wiley, John & Sons, Inc. (2002); Harlow and Lane *Using Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1998); and Coligan *et al.*, *Short Protocols in Protein Science*, Wiley, John & Sons, Inc. (2003). The nomenclature used
20 in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well-known and commonly used in the art.

Definitions

25 In order to better understand the disclosure, the definitions and explanations of the relevant terms are provided as follows.

The term “antibody” or “Ab”, as used herein, generally refers to a Y-shaped tetrameric protein comprising two heavy (H) and two light (L) polypeptide chains held together by covalent disulfide bonds and non-covalent interactions. Light chains of an antibody may be classified
30 into κ and λ light chain. Heavy chains may be classified into μ , δ , γ , α and ϵ , which define isotypes of an antibody as IgM, IgD, IgG, IgA and IgE, respectively. In a light chain and a heavy chain, a variable region is linked to a constant region via a “J” region of about 12 or more amino acids, and a heavy chain further comprises a “D” region of about 3 or more amino acids. Each heavy chain consists of a heavy chain variable region (V_H) and a heavy
35 chain constant region (C_H). A heavy chain constant region consists of 3 domains (C_{H1} , C_{H2} and C_{H3}). Each light chain consists of a light chain variable region (V_L) and a light chain constant region (C_L). V_H and V_L regions can further be divided into hypervariable regions (called complementary determining regions (CDR)), which are interspaced by relatively

conservative regions (called framework region (FR)). Each V_H and V_L consists of 3 CDRs and 4 FRs in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4 from N-terminal to C-terminal. The variable region (V_H and V_L) of each heavy/light chain pair forms antigen binding sites, respectively. Antibodies may be of different antibody isotypes, for example, IgG (e.g., IgG1, IgG2, IgG3 or IgG4 subtype), IgA1, IgA2, IgD, IgE or IgM antibody.

The term “antigen-binding portion” or “antigen-binding fragment” of an antibody, which can be interchangeably used in the context of the application, refers to polypeptides comprising fragments of a full-length antibody, which retain the ability of specifically binding to an antigen that the full-length antibody specifically binds to, and/or compete with the full-length antibody for binding to the same antigen. Generally, see Fundamental Immunology, Ch. 7 (Paul, W., ed., the second edition, Raven Press, N.Y. (1989), which is incorporated herein by reference for all purposes. Antigen binding fragments of an antibody may be produced by recombinant DNA techniques or by enzymatic or chemical cleavage of an intact antibody. Under some conditions, antigen binding fragments include Fab, Fab', $F(ab')_2$, Fd, Fv, dAb and complementary determining region (CDR) fragments, single chain antibody (e.g. scFv), chimeric antibody, diabody and such polypeptides that comprise at least part of antibody sufficient to confer the specific antigen binding ability on the polypeptides. Antigen binding fragments of an antibody may be obtained from a given antibody (e.g., the monoclonal anti-human TIGIT antibody provided herein) by conventional techniques known by a person skilled in the art (e.g., recombinant DNA technique or enzymatic or chemical cleavage methods), and may be screened for specificity in the same manner by which intact antibodies are screened.

The term “monoclonal antibody” or “mAb”, as used herein, refer to a preparation of antibody molecules of single molecular composition. A monoclonal antibody displays a single binding specificity and affinity for a particular epitope.

The term “chimeric antibody”, as used herein, refers to an antibody in which the variable region sequences are derived from one species and the constant region sequences are derived from another species, such as an antibody in which the variable region sequences are derived from a murine antibody and the constant region sequences are derived from a human antibody. An exemplary chimeric antibody as disclosed herein is W3642-1.433.11-xIgG4.SP, which comprises a heavy chain with rat V_H fused to human IgG4 constant region and a light chain with rat V_L fused to human Ig lambda.

The term “humanized antibody”, as used herein, refers to antibodies in which CDR sequences derived from the germline of another mammalian species, such as a rat or mouse, have been grafted onto human framework sequences. Additional framework region modifications may be made within the human framework sequences. The humanized antibody optionally will also comprise at least a portion of an immunoglobulin constant region (e.g. Fc), typically that of a

human immunoglobulin. Exemplary humanized antibodies as disclosed herein are W3642-1.433.11-z10-p1-IgG4.SP and W3642-1.433.11-z11-p1-IgG4.SP, which comprise a heavy chain with human germlined VH fused to human IgG4 constant region and a light chain with human germlined VL fused to human Ig lambda.

5 The term “PTM” or “post-translational modification”, as used herein, refers to a process that occurs to one or more amino acids on a protein (e.g., antibody) after the protein has been translated. Proteins are usually produced by ribosomes that translate mRNA into polypeptide chains and then form mature protein products via PTM. PTM process includes phosphorylation, glycosylation, ubiquitination, S-nitrosylation, methylation, N-acetylation and lipidation. Preferably, potential
10 PTM sites are removed during antibody optimization to avoid structural and functional heterogeneity brought by PTM process.

The term “TIGIT” or “T-cell immunoreceptor with Ig and ITIM domains” as used herein encompasses any native TIGIT from any vertebrate source, including mammals such as primates (e.g., humans) and rodents (e.g., mice and rats), unless otherwise indicated. TIGIT is also known
15 in the art as DKFZp667A205, FLJ39873, V-set and immunoglobulin domain-containing protein 9, V-set and transmembrane domain-containing protein 3, VSIG9, VSTM3, and WUCAM. The term encompasses full-length unprocessed TIGIT, extracellular domain of TIGIT as well as any form of TIGIT that results from processing in the cell. The term also encompasses naturally occurring variants of TIGIT, e.g., splice variants or allelic variants. An exemplary amino acid
20 sequence of full length human TIGIT is shown in SEQ ID NO: 23 (MRWCLLLIWAQGLRQAPLASGMMTGTIETGNI SA EKGGSIILQCHLSSTTAQVTQVN WEQQDQLLAICNADLGWHISPSFKDRVAPGPGLGLTLQSLTVNDTGEYFCIYHTYPDGT YTGRIFLEVLESSVAEHGARFQIPLLGAMAATLVVICTAVIVVVALTRKKKALRIHSVEG DLRRKSAGQEEWSPSAPSPPGSCVQAEAAPAGLCGEQRGEDCAELHDYFNVLSYRSLG
25 NCSFFTETG).

The term “PD-1/PD-L1 antagonist”, as used herein, includes PD-L1 antagonists (such as anti-PD-L1 antibodies) that decrease, block, inhibit, abrogate, or interfere with signal transduction resulting from the interaction of PD-L1 with either one or more of its binding partners, such as PD-1 or B7-1, as well as PD-1 antagonists (such as anti-PD-1 antibodies) that decrease, block,
30 inhibit, abrogate, or interfere with signal transduction resulting from the interaction of PD-1 with one or more of its binding partners, such as PD-L1, PD-L2. In some embodiments, the PD-1 antagonist is an anti-PD-1 antagonist antibody selected from, but not limited to, nivolumab (MDX-1106) or pembrolizumab (formerly lambrolizumab (MK-3475), MED1-0680, PDR001 (spartalizumab), REGN2810 (cemiplimab), BGB-108, prolgolimab, camrelizumab, sintilimab, tislelizumab, toripalimab, dostarlimab, retifanlimab, spartalizumab, sasanlimab, penpulimab,
35 CS1003, HLX10, SCT-110A, SHR-1316, CS1001, envafolimab, TQB2450, ZKAB001, LP- 002, zimberelimab, balstilimab, genolimzumab, BI 754091, cetrelimab, YBL-006, BAT1306, HX008, CX- 072, IMC-001, KL-A167, budigalimab, AMG 404, CX-188, JTX-4014, 609A, Sym021,

LZM009, F520, SG001, APL-502, cosibelimab, lodapolimab, GS-4224, INCB086550, FAZ053, TG-1501, BGB-A333, BCD-135, AK-106, LDP, GR1405, HLX20, MSB2311, MAX-10181, RC98, BION-004, AM0001, CB201, ENUM 244C8, ENUM 388D4, AUNP-012, STI-1110, ADG104, AK-103, LBL-006, hAb21, AVA-004, PDL- GEX, INCB090244, KD036, KY1003, LYN192, MT-6035, VXM10, YBL-007, ABSK041, GB7003, JS-003, and HS-636. In some embodiments, the PD-L1 antagonist is an anti-PD-L1 antagonist antibody selected from, but not limited to, MPDL3280A (atezolizumab), MDX-1105, MEDI4736 (durvalumab), or MSB0010718C (avelumab). PD-1/PD-L1 antagonists include known antibodies and in house developed antibodies.

10 The term “binding affinity” is herein used as a measure of the strength of a non-covalent interaction between two molecules, e.g., an antibody or antigen-portion thereof, and an antigen. Binding affinity between two molecules may be quantified by determination of the equilibrium dissociation constant (KD). In turn, KD can be determined by measurement of the kinetics of complex formation and dissociation using, as a nonlimiting example, the surface plasmon resonance (SPR) method (Biacore™). The rate constants corresponding to the association and the dissociation of a monovalent complex are referred to as the association rate constants k_a (or k_{on}) and dissociation rate constant k_d (or k_{off}), respectively. The term k_a (or k_{on}) refers to the association rate of a particular antibody-antigen interaction, whereas the term k_d (or k_{off}) refers to the dissociation rate of a particular antibody-antigen interaction. KD is related to k_a and k_d through the equation $KD = k_d/k_a$ or k_{off}/k_{on} . The binding kinetics and binding affinity of the antibody can be assessed by standard assays known in the art or as described in the Example section below.

25 The term “high affinity”, as used herein, refers to an antibody having a K_D of 1×10^{-9} M or less, more preferably 5×10^{-10} M or less, even more preferably 1×10^{-10} M or less, even more preferably 5×10^{-11} M or less for a target antigen.

The term “ EC_{50} ,” as used herein, which is also termed as “half maximal effective concentration” refers to the concentration of a drug, antibody or toxicant which induces a response halfway between the baseline and maximum after a specified exposure time. In the context of the application, EC_{50} is expressed in the unit of “nM” or “M”.

30 The term “isolated,” as used herein, refers to a state obtained from natural state by artificial means. If a certain “isolated” substance or component is present in nature, it is possible because its natural environment changes, or the substance is isolated from natural environment, or both. For example, a certain un-isolated polynucleotide or polypeptide naturally exists in a certain living animal body, and the same polynucleotide or polypeptide with a high purity isolated from such a natural state is called isolated polynucleotide or polypeptide. The term “isolated” excludes neither the mixed artificial or synthesized substance nor other impure substances that do not affect the activity of the isolated substance.

The term “isolated antibody,” as used herein, is intended to refer to an antibody that is substantially free of other antibodies having different antigenic specificities (e.g., an isolated antibody that specifically binds a TIGIT protein is substantially free of antibodies that specifically bind antigens other than TIGIT proteins). An isolated antibody that specifically binds a human
5 TIGIT protein may, however, have cross- reactivity to other antigens, such as TIGIT proteins from other species. Moreover, an isolated antibody can be substantially free of other cellular material and/or chemicals.

The term “vector,” as used herein, refers to a nucleic acid vehicle which can have a polynucleotide inserted therein. When the vector allows for the expression of the protein encoded
10 by the polynucleotide inserted therein, the vector is called an expression vector. The vector can have the carried genetic material elements expressed in a host cell by transformation, transduction, or transfection into the host cell. Vectors are well known by a person skilled in the art, including, but not limited to plasmids, phages, cosmids, artificial chromosome such as yeast artificial chromosome (YAC), bacterial artificial chromosome (BAC) or P1-derived artificial chromosome
15 (PAC); phage such as λ phage or M13 phage and animal virus. The animal viruses that can be used as vectors, include, but are not limited to, retrovirus (including lentivirus), adenovirus, adeno-associated virus, herpes virus (such as herpes simplex virus), pox virus, baculovirus, papillomavirus, papova virus (such as SV40). A vector may comprise multiple elements for controlling expression, including, but not limited to, a promoter sequence, a transcription initiation
20 sequence, an enhancer sequence, a selection element and a reporter gene. In addition, a vector may comprise origin of replication.

The term “host cell,” as used herein, refers to a cellular system which can be engineered to generate proteins, protein fragments, or peptides of interest. Host cells include, without limitation, cultured cells, e.g., mammalian cultured cells derived from rodents (rats, mice, guinea pigs, or
25 hamsters) such as CHO, BHK, NSO, SP2/0, YB2/0; or human tissues or hybridoma cells, yeast cells, and insect cells, and cells comprised within a transgenic animal or cultured tissue. The term encompasses not only the particular subject cell but also the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not be identical to the parent cell, but are still included within the
30 scope of the term “host cell.”

The term “identity,” as used herein, refers to a relationship between the sequences of two or more polypeptide molecules or two or more nucleic acid molecules, as determined by aligning and comparing the sequences. “Percent identity” means the percent of identical residues between the amino acids or nucleotides in the compared molecules and is calculated based on the size of the
35 smallest of the molecules being compared. For these calculations, gaps in alignments (if any) are preferably addressed by a particular mathematical model or computer program (i.e., an “algorithm”). Methods that can be used to calculate the identity of the aligned nucleic acids or polypeptides include those described in Computational Molecular Biology, (Lesk, A. M., ed.),

1988, New York: Oxford University Press; Biocomputing Informatics and Genome Projects, (Smith, D. W., ed.), 1993, New York: Academic Press; Computer Analysis of Sequence Data, Part I, (Griffin, A. M., and Griffin, H. G., eds.), 1994, New Jersey: Humana Press; von Heinje, G., 1987, Sequence Analysis in Molecular Biology, New York: Academic Press; Sequence Analysis Primer,
5 (Gribskov, M. and Devereux, J., eds.), 1991, New York: M. Stockton Press; and Carillo et al, 1988, SIAMJ. Applied Math. 48:1073.

The term “immunogenicity,” as used herein, refers to ability of stimulating the formation of specific antibodies or sensitized lymphocytes in organisms. It not only refers to the property of an antigen to stimulate a specific immunocyte to activate, proliferate and
10 differentiate so as to finally generate immunologic effector substance such as antibody and sensitized lymphocyte, but also refers to the specific immune response that antibody or sensitized T lymphocyte can be formed in immune system of an organism after stimulating the organism with an antigen. Immunogenicity is the most important property of an antigen. Whether an antigen can successfully induce the generation of an immune response in a host
15 depends on three factors, properties of an antigen, reactivity of a host, and immunization means.

The term “transfection,” as used herein, refers to the process by which nucleic acids are introduced into eukaryotic cells, particularly mammalian cells. Protocols and techniques for transfection include but not limited to lipid transfection and chemical and physical
20 methods such as electroporation. A number of transfection techniques are well known in the art and are disclosed herein. See, e.g., Graham et al., 1973, Virology 52:456; Sambrook et al., 2001, Molecular Cloning: A Laboratory Manual, supra; Davis et al., 1986, Basic Methods in Molecular Biology, Elsevier; Chu et al, 1981, Gene 13:197. In a specific embodiment of the invention, human TIGIT gene was transfected into 293F cells.

25 The term “SPR” or “surface plasmon resonance,” as used herein, refers to and includes an optical phenomenon that allows for the analysis of real-time biospecific interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIAcore system (Pharmacia Biosensor AB, Uppsala, Sweden and Piscataway, N.J.). For further descriptions, see Example 5 and Jönsson, U., et al. (1993) *Ann. Biol. Clin.* 51:19-26; Jönsson, U.,
30 et al. (1991) *Biotechniques* 11:620-627; Johnsson, B., et al. (1995) *J. Mol. Recognit.* 8:125-131; and Johnson, B., et al. (1991) *Anal. Biochem.* 198:268-277.

The term “fluorescence-activated cell sorting” or “FACS,” as used herein, refers to a specialized type of flow cytometry. It provides a method for sorting a heterogeneous mixture of biological cells into two or more containers, one cell at a time, based upon the specific light
35 scattering and fluorescent characteristics of each cell (FlowMetric. “Sorting Out Fluorescence Activated Cell Sorting”. Retrieved 2017-11-09.). Instruments for carrying out FACS are known to those of skill in the art and are commercially available to the public. Examples of such instruments include FACS Star Plus, FACScan and FACSort instruments from Becton Dickinson (Foster City,

Calif.) Epics C from Coulter Epics Division (Hialeah, Fla.) and MoFlo from Cytomation (Colorado Springs, Colo.).

The term “antibody-dependent cell-mediated cytotoxicity” or “ADCC,” as used herein, refers to a form of cytotoxicity in which secreted Ig bound onto Fc receptors (FcRs) present on
5 certain cytotoxic cells (e.g. Natural Killer (NK) cells, neutrophils, and macrophages) enable these cytotoxic effector cells to bind specifically to an antigen-bearing target cell and subsequently kill the target cell with cytotoxins. The antibodies “arm” the cytotoxic cells and are absolutely required for such killing. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is
10 summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol* 9:457-92 (1991). To assess ADCC activity of a molecule of interest, an in vitro ADCC assay, such as that described in US Patent No. 5,500,362 or 5,821,337 may be performed. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed in vivo, e.g., in an
15 animal model such as that disclosed in Clynes et al. *PNAS (USA)* 95:652-656 (1998).

The terms “subject” and “patient” are used interchangeably and include mammals such as humans and non-human primates, as well as rabbits, rats, mice, goats, pigs, and other mammalian species. The term does not necessarily indicate that the subject has been diagnosed with a particular disease, but typically refers to an individual under medical supervision.

20 The term “prevent,” “prevention” or “preventing,” as used herein, with reference to a certain disease condition in a mammal, refers to preventing or delaying the onset of the disease, or preventing the manifestation of clinical or subclinical symptoms thereof.

The term “treatment,” “treating” or “treated,” as used herein in the context of treating a condition, pertains generally to treatment and therapy, whether of a human or an animal, in which
25 some desired therapeutic effect is achieved, for example, the inhibition of the progress of the condition, and includes a reduction in the rate of progress, a halt in the rate of progress, regression of the condition, amelioration of the condition, and cure of the condition. For cancer, “treating” may refer to dampen or slow the tumor or malignant cell growth, proliferation, or metastasis, or some combination thereof.

30 The term “an effective amount,” as used herein, pertains to that amount of an active compound, or a material, composition or dosage form comprising an active compound, which is effective for producing some desired therapeutic effect, commensurate with a reasonable benefit/risk ratio, when administered in accordance with a desired treatment regimen. For instance, the “an effective amount,” when used in connection with treatment of a disease or condition, refers
35 to an antibody or antigen-binding portion thereof in an amount or concentration effective to treat the said disease or condition.

The term “pharmaceutically acceptable,” as used herein, means that the vehicle, diluent, excipient and/or salts thereof, are chemically and/or physically is compatible with other ingredients in the formulation, and the physiologically compatible with the recipient.

As used herein, the term “a pharmaceutically acceptable carrier and/or excipient” refers to a carrier and/or excipient pharmacologically and/or physiologically compatible with a subject and an active agent, which is well known in the art (see, e.g., Remington's Pharmaceutical Sciences. Edited by Gennaro AR, 19th ed. Pennsylvania: Mack Publishing Company, 1995), and includes, but is not limited to pH adjuster, surfactant, adjuvant and ionic strength enhancer. For example, the pH adjuster includes, but is not limited to, phosphate buffer; the surfactant includes, but is not limited to, cationic, anionic, or non-ionic surfactant, e.g., Tween-80; the ionic strength enhancer includes, but is not limited to, sodium chloride.

As used herein, the term “adjuvant” refers to a non-specific immunopotentiator, which can enhance immune response to an antigen or change the type of immune response in an organism when it is delivered together with the antigen to the organism or is delivered to the organism in advance. There are a variety of adjuvants, including, but not limited to, aluminium adjuvants (for example, aluminum hydroxide), Freund's adjuvants (for example, Freund's complete adjuvant and Freund's incomplete adjuvant), coryne bacterium parvum, lipopolysaccharide, cytokines, and the like. Freund's adjuvant is the most commonly used adjuvant in animal experiments now. Aluminum hydroxide adjuvant is more commonly used in clinical trials.

20

Anti-TIGIT Antibodies

In some aspects, the disclosure provides an antibody or antigen-binding portion or variant thereof capable of binding TIGIT (such as human, mouse or cyno TIGIT) with sufficient affinity such that it substantially or completely inhibits the biological activity of TIGIT.

In some embodiments, the anti-TIGIT antibody as disclosed herein are antibodies produced in rat immunized with a TIGIT antigen protein. In some embodiments, the anti-TIGIT antibody as disclosed herein are chimeric antibodies. In some embodiments, the anti-TIGIT antibody as disclosed herein are humanized antibodies. The antigen-binding portion of the antibody may be a Fab, a Fab', a F(ab')₂, a single chain variable fragment (scFv), or a diabody.

In some embodiments, provided herein is a humanized anti-TIGIT antibody in which HCDR and LCDR sequences of a human immunoglobulin are replaced by HCDR and LCDR sequences obtained from a non-human species (such as a rat) having the desired specificity, affinity, and/or capacity. In some further embodiments, certain framework (“FR”) residues of the human immunoglobulin are back mutated to corresponding non-human residues. In some other embodiments, humanized antibodies may comprise residues that are not found in the parental rat antibodies or human immunoglobulins. Further modifications can be made to CDR residues and framework residues to remove potential post translational modifications, to refine antibody performance, such as binding affinity.

Various methods for humanizing non-human antibodies are known in the art. For example, a humanized antibody can have one or more amino acid residues introduced into it from a source that is non-human. These non-human amino acid residues are often referred to as “import” residues, which are typically taken from an “import” variable domain. Humanized antibodies that bind
5 TIGIT may be produced using techniques known to those skilled in the art (e.g., Zhang et al., Molecular Immunology, 42(12): 1445-1451, 2005; Hwang et al., Methods, 36(1): 35-42, 2005; Dall’Acqua et al., Methods, 36(1): 43-60, 2005; Clark, Immunology Today, 21(8): 397-402, 2000, and U.S. Patent Nos. 6,180,370; 6,054,927; 5,869,619; 5,861,155; 5,712,120; and 4,816,567).

The antibodies as disclosed herein can bind to at least one of human, mouse and cynomolgus
10 monkey TIGIT with high affinity. The binding of an antibody of the disclosure to TIGIT can be assessed using one or more techniques well established in the art, for instance, ELISA. The binding specificity of an antibody of the disclosure can also be determined by monitoring binding of the antibody to cells expressing an TIGIT protein, e.g., flow cytometry. In some embodiments, the antibody is tested by a flow cytometry assay in which the antibody is reacted with a cell line that
15 expresses human TIGIT, such as HEK293 cells that have been transfected to express TIGIT on their cell surface. Additionally or alternatively, the binding of the antibody, including the binding kinetics (e.g., K_D value) can be tested in BIAcore binding assays.

In some embodiments, the antibody or antigen-binding portion thereof of the disclosure binds to human TIGIT with a K_D of 1×10^{-9} M or less, 5×10^{-10} M or less, 1×10^{-10} M or less, $5 \times$
20 10^{-11} M or less, 4×10^{-11} M or less, 3×10^{-11} M or less, 2.5×10^{-11} M or less, or 2×10^{-11} M or less, as measured by SPR. In some embodiments, the antibody or antigen-binding portion thereof is capable of specifically binding to human TIGIT as well as cynomolgus TIGIT and mouse TIGIT. For instance, the antibody or antigen-binding portion thereof can bind to human TIGIT expressing cells with an EC_{50} of no more than 0.5 nM, no more than 0.4 nM, no more than 0.3 nM, such as
25 no more than 0.23 nM; bind to cyno TIGIT expressing cells with an EC_{50} of no more than 0.5 nM, no more than 0.4 nM, no more than 0.3 nM, no more than 0.2 nM, such as no more than 0.18 nM; bind to mouse TIGIT expressing cells with an EC_{50} of no more than 0.5 nM, no more than 0.4 nM, no more than 0.3 nM, such as no more than 0.33 nM; as measured by FACS.

The anti-TIGIT antibody as disclosed herein can inhibit interaction between TIGIT and
30 one or more of its ligands PVR (CD155), PVRL2 (CD112) and PVRL3 (CD113). For example, the anti-TIGIT antibodies can block signaling through PVR, PVRL2, and/or PVRL3 so as to restore a functional response by T-cells (e.g., proliferation, cytokine production, target cell killing) from a dysfunctional state to antigen stimulation.

In some embodiments, the anti-TIGIT antibodies provided herein inhibit interaction
35 between TIGIT and the ligand CD155. In some embodiments, the anti-TIGIT antibodies provided herein inhibit interaction between TIGIT and the ligand CD112. In some embodiments, the anti-TIGIT antibodies provided herein inhibit interaction between TIGIT and the ligand CD113. In

some embodiments, the anti-TIGIT antibodies provided herein inhibit interaction between TIGIT and one or more of the ligands CD155, CD112 and CD113.

In some embodiments, the ability of an anti-TIGIT antibody to inhibit interactions between TIGIT and CD155, CD112 or CD113 is evaluated by measuring whether physical interactions
5 between TIGIT and CD155, CD112 or CD113 decrease in a binding assay. In some embodiments, the binding assay is a competitive binding assay. The assay may be performed in various formats, such as but not limited to an ELISA assay, flow cytometry, a surface plasmon resonance (SPR) assay (e.g., Biacore™), or BioLayer interferometry (e.g., ForteBio Octet™).

10 ***Anti-TIGIT antibodies comprising CDRs***

In some embodiments, the present disclosure provides an isolated antibody or the antigen-binding portion thereof comprising:

A) one or more heavy chain CDRs (HCDRs) selected from the group consisting of:

a HCDR1 comprising SEQ ID NO: 1 or an amino acid sequence that differs from SEQ ID NO:
15 1 by an amino acid addition, deletion or substitution of not more than 2 amino acids; a HCDR2 comprising SEQ ID NO: 2 or an amino acid sequence that differs from SEQ ID NO: 2 by an amino acid addition, deletion or substitution of not more than 2 amino acids; and a HCDR3 comprising SEQ ID NO: 3 or an amino acid sequence that differs from SEQ ID NO: 3 by an amino acid addition, deletion or substitution of not more than 2 amino acids;

20 B) one or more light chain CDRs (LCDRs) selected from the group consisting of:

a LCDR1 comprising SEQ ID NO: 4 or an amino acid sequence that differs from SEQ ID NO:
4 by an amino acid addition, deletion or substitution of not more than 2 amino acids; a LCDR2 comprising any of SEQ ID NOs: 5, 7, 8 and 9 or an amino acid sequence that differs from any of SEQ ID NOs: 5, 7, 8 and 9 by an amino acid addition, deletion or substitution of not more than 2
25 amino acids; and a LCDR3 comprising SEQ ID NO: 6 or an amino acid sequence that differs from SEQ ID NO: 6 by an amino acid addition, deletion or substitution of not more than 2 amino acids;
or

C) one or more HCDRs of A) and one or more LCDRs of B).

In some embodiments, the CDR identification is according to the Contact definition
30 introduced by Dr. Andrew C.R. Martin's group (<http://www.bioinf.org.uk/abs/>).

In some embodiments, the present disclosure provides an isolated antibody or antigen-binding portion thereof comprising: a HCDR1 as set forth in SEQ ID NO: 1, a HCDR2 as set forth in SEQ ID NO: 2, a HCDR3 as set forth in SEQ ID NO: 3, a LCDR1 as set forth in SEQ ID NO: 4, a LCDR2 as set forth in any of SEQ ID NOs: 5, 7, 8 and 9, and a LCDR3 as set forth in SEQ ID
35 NO: 6.

The extent of the framework region and CDRs can be precisely identified using methodology known in the art, for example, by the Kabat definition, the Chothia definition, the AbM definition, the contact definition, the IMGT definition (all of which are well known in the art) and any

combinations thereof. See, e.g., Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242, Chothia *et al.*, (1989) *Nature* 342:877; Chothia, C. *et al.* (1987) *J. Mol. Biol.* 196:901-917, Al-lazikani *et al.* (1997) *J. Molec. Biol.* 273:927-948; Edelman *et al.*, *Proc Natl Acad Sci U S A.* 5 1969 May, 63(1):78-85; and Martin and Allen, in “*Handbook of Therapeutic Antibodies*”, chapter 5, 2007. See also hgmp.mrc.ac.uk and bioinf.org.uk/abs. Correspondence or alignments between numberings according to different definitions can for example be found at www.imgt.org/ (see also Giudicelli V *et al.* IMGT, the international ImMunoGeneTics database. *Nucleic Acids Res.* (1997) 25:206–11; and Lefranc MP *et al.*, IMGT unique numbering for immunoglobulin and T 10 cell receptor variable domains and Ig superfamily V-like domains. *Dev Comp Immunol.* (2003) 27:55–77).

As will be appreciated by those in the art, the exact numbering and placement of the CDRs can be different among different numbering systems. However, it should be understood that the disclosure of a variable heavy sequence and/or a variable light sequence includes the disclosure of 15 the associated (inherent) CDRs, regardless of which numbering approach is adopted. Accordingly, the disclosure of each variable region is a disclosure of the CDRs (e.g., HCDR1, HCDR2 and HCDR3). Two antibodies having the same VH and VL means that their CDRs are identical when determined by the same approach (e.g., the Kabat, AbM, Chothia, Contact, and IMGT numbering approaches as known in the art). The same antibody as disclosed herein may have a different set 20 of CDRs when determined by a different numbering approach.

Variable regions and CDRs in an antibody sequence can be identified according to general rules that have been developed in the art (for example, the Kabat, AbM, Chothia, Contact, and IMGT numbering system) or by aligning the sequences against a database of known variable regions. Methods for identifying these regions are described in Kontermann and Dubel, eds., 25 *Antibody Engineering*, Springer, New York, NY, 2001 and Dinarello *et al.*, *Current Protocols in Immunology*, John Wiley and Sons Inc., Hoboken, NJ, 2000. Exemplary databases of antibody sequences are described in, and can be accessed through, the “Abysis” website at www.bioinf.org.uk/abs (maintained by A.C. Martin in the Department of Biochemistry & Molecular Biology University College London, London, England) and the VBASE2 website at 30 www.vbase2.org, as described in Retter *et al.*, *Nucl. Acids Res.*, 33 (Database issue): D671 -D674 (2005). Sequences may be analyzed using the Abysis database, which integrates sequence data from Kabat, IMGT and the Protein Data Bank (PDB) with structural data from the PDB. See Dr. Andrew C. R. Martin's book chapter *Protein Sequence and Structure Analysis of Antibody Variable Domains*. In: *Antibody Engineering Lab Manual* (Ed.: Duebel, S. and Kontermann, R., 35 Springer-Verlag, Heidelberg, ISBN-13: 978-3540413547, also available on the website bioinforg.uk/abs). The Abysis database website further includes general rules that have been developed for identifying CDRs which can be used in accordance with the teachings herein.

In some embodiments, the anti-TIGIT antibodies as disclosed herein comprise a VH region and a VL region, wherein the VH region comprises FRW1-HCDR1-FRW2-HCDR2-FRW3-HCDR3-FRW4, and wherein HCDR1 has an amino acid sequence as set forth in SEQ ID NO: 1, HCDR2 has an amino acid sequence as set forth in SEQ ID NO: 2, and HCDR3 has an amino acid sequence as set forth in SEQ ID NO: 3, and/or wherein the VL region comprises FRW1-LCDR1-FRW2-LCDR2-FRW3-LCDR3-FRW4, and wherein LCDR1 has an amino acid sequence as set forth in SEQ ID NO: 4, LCDR2 has an amino acid sequence as set forth in SEQ ID NO: 5, 7, 8 or 9, and LCDR3 has an amino acid sequence as set forth in SEQ ID NO: 6.

In some embodiments, the framework regions are derived from human germline (i.e. human germlined), e.g. a human immunoglobulin. In some embodiments, certain residues in the human germlined framework regions are back mutated to corresponding residues in the parental non-human antibody. In some embodiments, the FR regions may include one or more individual FR residue substitutions that improve antibody performance, such as binding affinity, isomerization, immunogenicity, etc. In some specific embodiments, FRW3 in the human germlined VH region comprises Val and Trp at positions 78 and 94 (according to Kabat numbering), respectively. In some specific embodiments, FRW1 in the human germlined VL region comprise one or more of Gln at position 1, Ala at position 2 and Val at position 3 (according to Kabat numbering). The Kabat numbering system is generally used when referring to a residue in the variable region (approximately residues 1-107 of the light chain and residues 1-113 of the heavy chain) (e.g., Kabat et al., Sequences of Immunological Interest. 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)). In some specific embodiments, the FRW1 and FRW4 at the N and C terminal of the VH and/or VL region may be truncated such that it comprises only a partial FRW1 and/or FRW4. In some embodiments, the CDRs and FR regions have undergone a PTM-removal optimization.

In some embodiments, provided herein is an anti-TIGIT antibody comprising one, two, or all three HCDRs of the amino acid sequence as set forth in SEQ ID NO: 10, and one, two, or all three LCDRs of the amino acid sequence as set forth in SEQ ID NO: 12. In some embodiments, provided herein is an anti-TIGIT antibody comprising one, two, or all three HCDRs of the amino acid sequence as set forth in SEQ ID NO: 11, and one, two, or all three LCDRs of the amino acid sequence as set forth in SEQ ID NO: 13 or 16. In some embodiments, provided herein is an anti-TIGIT antibody comprising one, two, or all three HCDRs of the amino acid sequence as set forth in SEQ ID NO: 10, and one, two, or all three LCDRs of the amino acid sequence as set forth in SEQ ID NO: 14, 17 or 18. In some embodiments, provided herein is an anti-TIGIT antibody comprising one, two, or all three HCDRs of the amino acid sequence as set forth in SEQ ID NO: 11, and one, two, or all three LCDRs of the amino acid sequence as set forth in SEQ ID NO: 15.

In some embodiments, provided herein is an anti-TIGIT antibody comprising at least one of the FRW1, FRW2, FRW3 and FRW4 of the amino acid sequence as set forth in SEQ ID NO: 10, and at least one of the FRW1, FRW2, FRW3 and FRW4 of the amino acid sequence as set forth

in SEQ ID NO: 12. In some embodiments, provided herein is an anti-TIGIT antibody comprising at least one of the FRW1, FRW2, FRW3 and FRW4 of the amino acid sequence as set forth in SEQ ID NO: 11, and at least one of the FRW1, FRW2, FRW3 and FRW4 of the amino acid sequence as set forth in SEQ ID NO: 13. In some embodiments, provided herein is an anti-TIGIT antibody comprising at least one of the FRW1, FRW2, FRW3 and FRW4 of the amino acid sequence as set forth in SEQ ID NO: 10, and at least one of the FRW1, FRW2, FRW3 and FRW4 of the amino acid sequence as set forth in SEQ ID NO: 14. In some embodiments, provided herein is an anti-TIGIT antibody comprising at least one of the FRW1, FRW2, FRW3 and FRW4 of the amino acid sequence as set forth in SEQ ID NO: 11, and at least one of the FRW1, FRW2, FRW3 and FRW4 of the amino acid sequence as set forth in SEQ ID NO: 15. In some embodiments, provided herein is an anti-TIGIT antibody comprising at least one of the FRW1, FRW2, FRW3 and FRW4 of the amino acid sequence as set forth in SEQ ID NO: 11, and at least one of the FRW1, FRW2, FRW3 and FRW4 of the amino acid sequence as set forth in SEQ ID NO: 16. In some embodiments, provided herein is an anti-TIGIT antibody comprising at least one of the FRW1, FRW2, FRW3 and FRW4 of the amino acid sequence as set forth in SEQ ID NO: 10, and at least one of the FRW1, FRW2, FRW3 and FRW4 of the amino acid sequence as set forth in SEQ ID NO: 17. In some embodiments, provided herein is an anti-TIGIT antibody comprising at least one of the FRW1, FRW2, FRW3 and FRW4 of the amino acid sequence as set forth in SEQ ID NO: 10, and at least one of the FRW1, FRW2, FRW3 and FRW4 of the amino acid sequence as set forth in SEQ ID NO: 18.

In some specific embodiments, the VH region comprises amino acid Val (V) at position 78 and Trp (W) at position 94. Additionally or alternatively, the VL region comprises amino acids "QAV" (Gln-Ala-Val) at positions 1-3.

Anti-TIGIT antibodies comprising a heavy chain variable region and a light chain variable region

In some embodiments, the isolated antibody or the antigen-binding portion thereof comprises a heavy chain variable region (VH) and a light chain variable region (VL), wherein: the VH comprises:

- (i) the amino acid sequence of one of SEQ ID NOs: 10-11;
- (ii) an amino acid sequence having the same set of CDRs as one of SEQ ID NOs: 10-11 and with at least 85%, 90%, or 95% identity in the framework regions; or
- (iii) an amino acid sequence with addition, deletion and/or substitution of one or more (e.g. 10, 9, 8, 7, 6, 5, 4, 3, 2) amino acids in the framework regions compared with the amino acid sequence of one of SEQ ID NOs: 10-11; and/or

the VL comprises:

- (i) the amino acid sequence of one of SEQ ID NOs: 12-18;

(ii) an amino acid sequence having the same set of CDRs as one of SEQ ID NOs: 12-18 and with at least 85%, 90%, or 95% identity in the framework regions; or

(iii) an amino acid sequence with addition, deletion and/or substitution of one or more (e.g. 10, 9, 8, 7, 6, 5, 4, 3, 2) amino acids in the framework regions compared with the amino acid sequence of one of SEQ ID NOs: 12-18.

The percent identity between two amino acid sequences can be determined using the algorithm of E. Meyers and W. Miller (Comput. Appl. Biosci., 4:11-17 (1988)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. In addition, the percentage of identity between two amino acid sequences can be determined by the algorithm of Needleman and Wunsch (J. Mol. Biol. 48:444-453 (1970)) which has been incorporated into the GAP program in the GCG software package (available at <http://www.gcg.com>), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6.

Additionally or alternatively, the protein sequences of the present disclosure can further be used as a "query sequence" to perform a search against public databases to, for example, identify related sequences. Such searches can be performed using the XBLAST program (version 2.0) of Altschul, et al. (1990) J. Mol. Biol. 215:403-10. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to the antibody molecules of the disclosure. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al, (1997) Nucleic Acids Res. 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. See www.ncbi.nlm.nih.gov.

In some further embodiments, the isolated antibody or the antigen-binding portion thereof may contain conservative substitution or modification of amino acids in the variable regions of the heavy chain and/or light chain. It is understood in the art that certain conservative sequence modification can be made which do not remove antigen binding. See, e.g., Brummell et al. (1993) Biochem 32:1180-8; de Wildt et al. (1997) Prot. Eng. 10:835-41; Komissarov et al. (1997) J. Biol. Chem. 272:26864- 26870; Hall et al. (1992) J. Immunol. 149:1605-12; Kelley and O'Connell (1993) Biochem. 32:6862-35; Adib-Conquy et al. (1998) Int. Immunol. 10:341-6 and Beers et al. (2000) Clin. Can. Res. 6:2835-43.

The term "conservative substitution," as used herein, refers to amino acid substitutions which would not disadvantageously affect or change the essential properties of a protein/polypeptide comprising the amino acid sequence. For example, a conservative substitution may be introduced by standard techniques known in the art such as site-directed mutagenesis and PCR-mediated mutagenesis. Conservative amino acid substitutions include substitutions wherein an amino acid residue is substituted with another amino acid residue having a similar side chain, for example, a residue physically or functionally similar (such as, having similar size, shape, charge, chemical property including the capability of forming

covalent bond or hydrogen bond, etc.) to the corresponding amino acid residue. The families of amino acid residues having similar side chains have been defined in the art. These families include amino acids having alkaline side chains (for example, lysine, arginine and histidine), amino acids having acidic side chains (for example, aspartic acid and glutamic acid), amino acids having uncharged polar side chains (for example, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), amino acids having nonpolar side chains (for example, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), amino acids having β -branched side chains (such as threonine, valine, isoleucine) and amino acids having aromatic side chains (for example, tyrosine, phenylalanine, tryptophan, histidine). Therefore, a corresponding amino acid residue is preferably substituted with another amino acid residue from the same side-chain family. Methods for identifying amino acid conservative substitutions are well known in the art (see, for example, Brummell et al., Biochem. 32: 1180-1187 (1993); Kobayashi et al., Protein Eng. 12(10): 879-884 (1999); and Burks et al., Proc. Natl. Acad. Sci. USA 94: 412-417 (1997), which are incorporated herein by reference).

In a specific embodiment, the isolated antibody or the antigen-binding portion thereof comprises: a heavy chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 11 and a light chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 15.

In other embodiments, the amino acid sequences of the heavy chain variable region and/or the light chain variable region can be at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the respective sequences set forth above.

Anti-TIGIT antibodies with certain properties

The antibodies of the present disclosure are characterized by particular functional features or properties of the antibodies. Based on the action mechanism against the target, the *in vitro* functional properties and pharmacological activity of the antibody were fully evaluated at both the molecular and cellular levels. In some embodiments, the isolated antibody or the antigen-binding portion thereof has one or more of the following properties:

- (a) specifically binding to at least one of human TIGIT protein, cyno TIGIT protein and mouse TIGIT protein, e.g. could bind to human TIGIT with a K_D lower than 1 nM, as determined by SPR;
- (b) having no cross binding to TIGIT homologous proteins;
- (c) block binding between TIGIT and its ligands, CD155, CD112 and CD113;
- (d) activation of immune cells such as T cells and NK cells;
- (e) induce ADCC effect on TIGIT-expressing CHOK1 cells; and
- (f) significantly better efficacy in treating cancer than benchmark antibodies, when combined with an anti-PD-1 agent, as demonstrated in in vivo mouse model.

The dual-blockade of TIGIT and PD-1 could reverse immune suppression. As shown herein, the anti-TIGIT antibody of the present disclosure exhibits synergistic effect with an anti-PD-1 agent (e.g., an anti-PD-1 antibody) or an anti-PD-L1 agent (e.g., an anti-PD-L1 antibody).

In some preferable embodiments, the antibodies of the present disclosure may be combined with an additional therapeutic agent, such as an anti-cancer agent, including an anti-cancer antibody and a chemotherapeutic agent. The additional therapeutic agent may also be an antagonist or an inhibitor of a T cell coinhibitor, an agonist of a T cell coactivator or an immune stimulatory cytokine.

In some embodiments, the additional therapeutic agent is an antibody that binds a protein selected from CD25, PD-1, PD-L1, Tim3, Lag3, CTLA4, 41BB, OX40, CD3, CD40, CD47M, GM-CSF, CSF1R, TLR, STING, RIGI, TAM receptor kinase, NKG2A, NKG2D, GD2, TIGIT, EGFR, PDGFR α , SLAMF7, VEGF, CTLA-4, CD20, cCLB8, KIR, and CD52. In some embodiments, the additional therapeutic agent is selected from an anti-CD25 antibody, anti-PD-1 antibody, anti-PD-L1 antibody, anti-Tim3 antibody, anti-Lag3 antibody, anti-CTLA4 antibody, anti-4-1BB antibody, anti-OX40 antibody, anti-CD3 antibody, anti-CD40 antibody, anti-CD47M antibody, anti-CSF1R antibody, anti-TLR antibody, anti-STING antibody, anti-RIGI antibody, anti-TAM receptor kinase antibody, anti-NKG2A antibody, an anti-NKG2D antibody, an anti-GD2 antibody, an anti-EGFR antibody, an anti-PDGFR- α antibody, an anti-SLAMF7 antibody, an anti-VEGF antibody, an anti-CTLA-4 antibody, an anti-CD20 antibody, an anti-cCLB8 antibody, an anti-KIR antibody, and an anti-CD52 antibody. In some embodiments, the additional therapeutic agent is selected from SEA-CD40, avelumab, durvalumab, nivolumab, pembrolizumab, pidilizumab, atezolizumab, Hul4.18K322A, Hu3F8, dinituximab, trastuzumab, cetuximab, olaratumab, necitumumab, elotuzumab, ramucirumab, pertuzumab, ipilimumab, bevacizumab, rituximab, obinutuzumab, siltuximab, ofatumumab, lirilumab, and alemtuzumab.

Fc region

Anti-TIGIT antibodies and antigen-binding portions provided herein further comprise an immunoglobulin constant region comprising a Fc region, such as a human IgG1, IgG2, IgG3 or IgG4 Fc region (native or variant thereof), and optionally a hinge region. In some embodiments, the Fc region is a human IgG1 Fc region, such as a wild-type Fc region or an Fc variant. An Fc variant can possess at least about 80% homology with a native sequence Fc region, or at least about 90% homology therewith, for example, at least about 95% homology therewith. In some embodiments, the Fc region is a human IgG4 Fc region, such as a wild-type Fc region or a Fc variant comprising a S228P substitution. In certain embodiments, the anti-TIGIT antibodies disclosed herein comprise wild-type human IgG1 Fc region. The variant Fc region may comprise one or more amino acid modifications (e.g. Leu234Ala/Leu235Ala or LALA) that alters the antibody-dependent cellular cytotoxicity (ADCC) or other effector functions. In some embodiments, the Fc region may comprise one or more amino acid changes (e.g., insertions,

deletions or substitutions) that results in a modified Fc region having a modified binding interaction between Fc and FcRn or FcγR.

In certain embodiments, the Fc region is a IgG4 Fc region comprising a S228P mutation (according to EU numbering as in Kabat et al.) that prevents Fab arm exchange and stabilizes IgG4 molecule. In certain embodiments, the Fc region is a IgG1 Fc region and comprises a LALA mutation, i.e. mutations of L234A and L235A. LALA mutation is perhaps the most commonly used mutation for disrupting antibody effector function, e.g. eliminate Fc binding to specific FcγRs, reduce ADCC activity mediated by PBMCs and monocytes. The “EU numbering system” or “EU index” is generally used when referring to a residue in an immunoglobulin heavy chain constant region (e.g., the EU index reported in Kabat et al., supra). The “EU numbering as in Kabat” or “EU index as in Kabat” refers to the residue numbering of the human IgG1 EU antibody. Unless stated otherwise herein, references to residue numbers in the constant domain of antibodies means residue numbering by the EU numbering system.

Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including hybridoma techniques, recombinant techniques, phage display technologies, transgenic animals (e.g., a XenoMouse[®]) or some combination thereof. For example, monoclonal antibodies can be produced using hybridoma and art-recognized biochemical and genetic engineering techniques such as described in more detail in An, Zhigiang (ed.) *Therapeutic Monoclonal Antibodies: From Bench to Clinic*, John Wiley and Sons, 1st ed. 2009; Shire et. al. (eds.) *Current Trends in Monoclonal Antibody Development and Manufacturing*, Springer Science + Business Media LLC, 1st ed. 2010; Harlow et al., *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, 2nd ed. 1988; Hammerling, et al., in: *Monoclonal Antibodies and T-Cell Hybridomas* 563-681 (Elsevier, N.Y., 1981) each of which is incorporated herein in its entirety by reference. It should be understood that a selected binding sequence can be further altered, for example, to improve affinity for the target, to humanize the target binding sequence, to improve its production in cell culture, to reduce its immunogenicity in vivo, to create a multispecific antibody, etc., and that an antibody comprising the altered target binding sequence is also an antibody of this invention. In some embodiments, the anti-human TIGIT monoclonal antibody is prepared by using hybridoma techniques. Generation of hybridomas is well-known in the art. See, e.g., Harlow and Lane (1988) *Antibodies, A Laboratory Manual*, Cold Spring Harbor Publications, New York.

Nucleic Acid Molecules Encoding Antibodies of the Disclosure

In some aspects, the disclosure is directed to an isolated nucleic acid molecule, comprising a nucleic acid sequence encoding the heavy chain variable region and/or the light chain variable region of the isolated antibody as disclosed herein.

Nucleic acids of the disclosure can be obtained using standard molecular biology techniques. For antibodies expressed by hybridomas (e.g., hybridomas prepared from transgenic mice carrying

human immunoglobulin genes), cDNAs encoding the light and heavy chains of the antibody made by the hybridoma can be obtained by standard PCR amplification or cDNA cloning techniques. For antibodies obtained from an immunoglobulin gene library (e.g., using phage display techniques), a nucleic acid encoding such antibodies can be recovered from the gene library.

5 The isolated nucleic acid encoding the VH region can be converted to a full-length heavy chain gene by operatively linking the VH-encoding nucleic acid to another DNA molecule encoding heavy chain constant regions (CH1, CH2 and CH3). The sequences of human heavy chain constant region genes are known in the art (see e.g., Kabat et al. (1991), supra) and DNA fragments encompassing these regions can be obtained by standard PCR amplification. The heavy
10 chain constant region can be an IgG1, IgG2, IgG3, IgG4, IgA, IgE, IgM or IgD constant region, but more preferably is an IgG1 or IgG4 constant region.

 The isolated nucleic acid encoding the VL region can be converted to a full-length light chain gene (as well as a Fab light chain gene) by operatively linking the VL-encoding DNA to another DNA molecule encoding the light chain constant region, CL. The sequences of human
15 light chain constant region genes are known in the art (see e.g., Kabat et al., supra) and DNA fragments encompassing these regions can be obtained by standard PCR amplification. In preferred embodiments, the light chain constant region can be a kappa or lambda constant region.

 Once DNA fragments encoding VH and VL segments are obtained, these DNA fragments can be further manipulated by standard recombinant DNA techniques, for example to convert the variable region genes to full-length antibody chain genes, to Fab fragment genes or to a scFv gene.
20 In these manipulations, a VL- or VH-encoding DNA fragment is operatively linked to another DNA fragment encoding another protein, such as an antibody constant region or a flexible linker. The term “operatively linked”, as used in this context, is intended to mean that the two DNA fragments are joined such that the amino acid sequences encoded by the two DNA fragments
25 remain in-frame.

 In some embodiments, the disclosure is directed to an isolated nucleic acid molecule, comprising a nucleic acid sequence encoding the heavy chain variable region of the isolated antibody as disclosed herein.

 In some specific embodiments, the isolated nucleic acid molecule encodes the heavy chain variable region of the isolated antibody and comprises a nucleic acid sequence selected from the group consisting of:

 (A) a nucleic acid sequence that encodes a heavy chain variable region as set forth in any one of SEQ ID NOs: 10-11;

 (B) a nucleic acid sequence as set forth in SEQ ID NO: 21; or

35 (C) a nucleic acid sequence that hybridized under high stringency conditions to the complementary strand of the nucleic acid sequence of (A) or (B).

In some embodiments, the disclosure is directed to an isolated nucleic acid molecule, comprising a nucleic acid sequence encoding the light chain variable region of the isolated antibody as disclosed herein.

In some specific embodiments, the isolated nucleic acid molecule encodes the light chain variable region of the isolated antibody comprises a nucleic acid sequence selected from the group consisting of:

(A) a nucleic acid sequence that encodes a light chain variable region as set forth in any one of SEQ ID NO: 12-18;

(B) a nucleic acid sequence as set forth in SEQ ID NO: 22; or

(C) a nucleic acid sequence that hybridized under high stringency conditions to the complementary strand of the nucleic acid sequence of (A) or (B).

For example, the nucleic acid molecule comprises SEQ ID NO: 21 and 22. In some other embodiments, the nucleic acid molecule shares at least 80% (e.g. at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 21 or 22. In some specific embodiments, the percentage of identity is derived from the degeneracy of the genetic code, and the encoded protein sequences remain unchanged.

Exemplary high stringency conditions include hybridization at 45°C in 5X SSPE and 45% formamide, and a final wash at 65°C in 0.1 X SSC. It is understood in the art that conditions of equivalent stringency can be achieved through variation of temperature and buffer, or salt concentration as described Ausubel, et al. (Eds.), *Protocols in Molecular Biology*, John Wiley & Sons (1994), pp. 6.0.3 to 6.4.10. Modifications in hybridization conditions can be empirically determined or precisely calculated based on the length and the percentage of guanosine/cytosine (GC) base pairing of the probe. The hybridization conditions can be calculated as described in Sambrook, et al. (Eds.), *Molecular Cloning: A laboratory Manual*. Cold Spring Harbor Laboratory Press: Cold Spring Harbor, New York (1989), pp. 9.47 to 9.51.

Host Cells

Host cells as disclosed in the present disclosure may be any cell which is suitable for expressing the antibodies of the present disclosure, for example, yeast, bacterial, fungal, plant and animal cells, preferably mammalian cells. Mammalian host cells for expressing the antibodies of the present disclosure include Chinese Hamster Ovary (CHO cells) (including dhfr CHO cells, described in Urlaub and Chasin, (1980) *Proc. Natl. Acad. Sci. USA* 77:4216-4220, used with a DHFR selectable marker, e.g., as described in R. J. Kaufman and P. A. Sharp (1982) *J. Mol. Biol.* 159:601-621), 293F cells, NSO myeloma cells, COS cells and SP2 cells. In particular, for use with NSO myeloma cells, another expression system is the GS gene expression system disclosed in WO 87/04462, WO 89/01036 and EP 338,841. Also included are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham et al., *J. Gen. Virol.* 36:59 (1977)); baby hamster kidney

cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO, Urlaub et al., 1980, Proc. Natl. Acad. Sci. USA 77:4216); mouse sertoli cells (TM4, Mather, 1980, Biol. Reprod. 23:243-251); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather et al., 1982, Annals N.Y. Acad. Sci. 383:44-68); MRC 5 cells; FS4 cells; mouse myeloma cells, such as NSO (e.g. RCB0213, 1992, Bio/Technology 10:169) and SP2/0 cells (e.g. SP2/0-Ag14 cells, ATCC CRL 1581); rat myeloma cells, such as YB2/0 cells (e.g. YB2/3HL.P2.G11.16Ag.20 cells, ATCC CRL 1662); PER.C6 cells; and a human hepatoma line (Hep G2). CHO cells are one of the cell lines that can be used herein, with CHO-K1, DUK-B11, CHO-DP12, CHO-DG44 (Somatic Cell and Molecular Genetics 12:555 (1986)), and Lec13 being exemplary host cell lines. In the case of CHO-K1, DUK-B11, DG44 or CHO-DP12 host cells, these may be altered such that they are deficient in their ability to fucosylate proteins expressed therein. In some embodiments, the host cells herein are selected from CHO, CHO-S, HEK, HEK293, HEK-293F, Expi293F, PER.C6, NSO cells and lymphocytic cells.

Suitable prokaryotes for this purpose include eubacteria, such as Gram-negative or Gram-positive organisms, for example, Enterobacteriaceae such as Escherichia, e.g., E. coli, Enterobacter, Erwinia, Klebsiella, Proteus, Salmonella, e.g., Salmonella typhimurium, Serratia, e.g., Serratia marcescans, and Shigella, as well as Bacilli such as B. subtilis and B. licheniformis, Pseudomonas such as P. aeruginosa, and Streptomyces.

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are also suitable cloning or expression hosts for antibody-encoding vectors. Saccharomyces cerevisiae, or common baker's yeast, is the most commonly used among lower eukaryotic host microorganisms. However, a number of other genera, species, and strains are commonly available and useful herein, such as Schizosaccharomyces pombe; Kluyveromyces hosts such as, e.g., K. lactis, K. fragilis (ATCC 12,424), K. bulgaricus (ATCC 16,045), K. wickerhamii (ATCC 24,178), K. waltii (ATCC 56,500), K. drosophilae (ATCC 36,906), K. thermotolerans, and K. marxianus; Yarrowia (EP 402,226); Pichia pastoris (EP 183,070); Candida; Trichoderma reesei (EP 244,234); Neurospora crassa; Schwanniomyces such as Schwanniomyces occidentalis; and filamentous fungi such as, e.g., Neurospora, Penicillium, Tolypocladium, and Aspergillus hosts such as A. nidulans and A. niger.

When recombinant expression vectors encoding an antibody are introduced into mammalian host cells, the antibody is produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or, secretion of the antibody into the culture medium in which the host cells are grown. Antibodies can be recovered from the culture medium using standard protein purification methods.

Pharmaceutical Compositions

In some aspects, the disclosure is directed to a pharmaceutical composition comprising at least one antibody or antigen-binding portion thereof as disclosed herein and a pharmaceutically acceptable carrier. In some aspects, the present disclosure provides a pharmaceutical composition comprising a nucleic acid encoding the antibody or antigen-binding portion thereof as disclosed herein and a pharmaceutically acceptable carrier. In some aspects, the present disclosure provides a pharmaceutical composition comprising a cell expressing the antibody or antigen-binding portion thereof as disclosed herein and a pharmaceutically acceptable carrier.

Components of the compositions

The pharmaceutical composition may optionally contain one or more additional pharmaceutically active ingredients, such as another antibody or a drug. The pharmaceutical compositions of the disclosure also can be administered in a combination therapy with, for example, another immune-stimulatory agent, anti-cancer agent, an antiviral agent, or a vaccine. A pharmaceutically acceptable carrier can include, for example, a pharmaceutically acceptable liquid, gel or solid carriers, an aqueous medium, a non-aqueous medium, an anti-microbial agent, isotonic agents, buffers, antioxidants, anesthetics, suspending/dispersing agent, a chelating agent, a diluent, adjuvant, excipient or a nontoxic auxiliary substance, other components known in the art, various combinations thereof etc.

Suitable components may include, for example, antioxidants, fillers, binders, disintegrating agents, buffers, preservatives, lubricants, flavorings, thickening agents, coloring agents, emulsifiers or stabilizers such as sugars and cyclodextrin. Suitable anti-oxidants may include, for example, methionine, ascorbic acid, EDTA, sodium thiosulfate, platinum, catalase, citric acid, cysteine, mercapto glycerol, thioglycolic acid, Mercapto sorbitol, butyl methyl anisole, butylated hydroxy toluene and/or propyl gallate. For example, a composition containing an antibody or an antigen-binding fragment of the present disclosure may include one or more anti-oxidants such as methionine, reducing antibody or antigen binding fragment thereof may be oxidized. The oxidation reduction may prevent or reduce a decrease in binding affinity, thereby enhancing antibody stability and extended shelf life. Thus, in some embodiments, the present disclosure provides a composition comprising one or more antibodies or antigen binding fragment thereof and one or more anti-oxidants such as methionine. The present disclosure further provides a variety of methods, wherein an antibody or antigen binding fragment thereof is mixed with one or more anti-oxidants, such as methionine, so that the antibody or antigen binding fragment thereof can be prevented from oxidation, to extend their shelf life and/or increase activity.

To further illustrate, pharmaceutical acceptable carriers may include, for example, aqueous vehicles such as sodium chloride injection, Ringer's injection, isotonic dextrose injection, sterile water injection, or dextrose and lactated Ringer's injection, nonaqueous vehicles such as fixed oils of vegetable origin, cottonseed oil, corn oil, sesame oil, or peanut oil, antimicrobial agents at bacteriostatic or fungistatic concentrations, isotonic agents such as sodium chloride or dextrose,

5 buffers such as phosphate or citrate buffers, antioxidants such as sodium bisulfate, local anesthetics such as procaine hydrochloride, suspending and dispersing agents such as sodium carboxymethylcellulose, hydroxypropyl methylcellulose, or polyvinylpyrrolidone, emulsifying agents such as Polysorbate 80 (TWEEN-80), sequestering or chelating agents such as EDTA (ethylenediaminetetraacetic acid) or EGTA (ethylene glycol tetraacetic acid), ethyl alcohol, polyethylene glycol, propylene glycol, sodium hydroxide, hydrochloric acid, citric acid, or lactic acid. Antimicrobial agents utilized as carriers may be added to pharmaceutical compositions in multiple-dose containers that include phenols or cresols, mercurials, benzyl alcohol, chlorobutanol, methyl and propyl p-hydroxybenzoic acid esters, thimerosal, benzalkonium chloride and benzethonium chloride. Suitable excipients may include, for example, water, saline, dextrose, glycerol, or ethanol. Suitable non-toxic auxiliary substances may include, for example, wetting or emulsifying agents, pH buffering agents, stabilizers, solubility enhancers, or agents such as sodium acetate, sorbitan monolaurate, triethanolamine oleate, or cyclodextrin.

15 ***Administration, Formulation and Dosage***

The pharmaceutical composition of the disclosure may be administered *in vivo*, to a subject in need thereof, by various routes, including, but not limited to, oral, intravenous, intra-arterial, subcutaneous, parenteral, intranasal, intramuscular, intracranial, intracardiac, intraventricular, intratracheal, buccal, rectal, intraperitoneal, intradermal, topical, transdermal, and intrathecal, or otherwise by implantation or inhalation. The subject compositions may be formulated into preparations in solid, semi-solid, liquid, or gaseous forms; including, but not limited to, tablets, capsules, powders, granules, ointments, solutions, suppositories, enemas, injections, inhalants, and aerosols. The appropriate formulation and route of administration may be selected according to the intended application and therapeutic regimen.

25 Suitable formulations for enteral administration include hard or soft gelatin capsules, pills, tablets, including coated tablets, elixirs, suspensions, syrups or inhalations and controlled release forms thereof.

Formulations suitable for parenteral administration (e.g., by injection), include aqueous or non-aqueous, isotonic, pyrogen-free, sterile liquids (e.g., solutions, suspensions), in which the active ingredient is dissolved, suspended, or otherwise provided (e.g., in a liposome or other microparticulate). Such liquids may additionally contain other pharmaceutically acceptable ingredients, such as anti-oxidants, buffers, preservatives, stabilizers, bacteriostats, suspending agents, thickening agents, and solutes which render the formulation isotonic with the blood (or other relevant bodily fluid) of the intended recipient. Examples of excipients include, for example, water, alcohols, polyols, glycerol, vegetable oils, and the like. Examples of suitable isotonic carriers for use in such formulations include Sodium Chloride Injection, Ringer's Solution, or Lactated Ringer's Injection. Similarly, the particular dosage regimen, including dose, timing and

repetition, will depend on the particular individual and that individual's medical history, as well as empirical considerations such as pharmacokinetics (e.g., half-life, clearance rate, etc.).

Frequency of administration may be determined and adjusted over the course of therapy, and is based on reducing the number of proliferative or tumorigenic cells, maintaining the reduction of such neoplastic cells, reducing the proliferation of neoplastic cells, or delaying the development of metastasis. In some embodiments, the dosage administered may be adjusted or attenuated to manage potential side effects and/or toxicity. Alternatively, sustained continuous release formulations of a subject therapeutic composition may be appropriate.

It will be appreciated by one of skill in the art that appropriate dosages can vary from patient to patient. Determining the optimal dosage will generally involve the balancing of the level of therapeutic benefit against any risk or deleterious side effects. The selected dosage level will depend on a variety of factors including, but not limited to, the activity of the particular compound, the route of administration, the time of administration, the rate of excretion of the compound, the duration of the treatment, other drugs, compounds, and/or materials used in combination, the severity of the condition, and the species, sex, age, weight, condition, general health, and prior medical history of the patient. The amount of compound and route of administration will ultimately be at the discretion of the physician, veterinarian, or clinician, although generally the dosage will be selected to achieve local concentrations at the site of action that achieve the desired effect without causing substantial harmful or deleterious side-effects.

In general, the antibody or the antigen binding portion thereof of the disclosure may be administered in various ranges. These include about 5 µg/kg body weight to about 40 mg/kg body weight per dose; about 50 µg/kg body weight to about 5 mg/kg body weight per dose; about 100 µg/kg body weight to about 10 mg/kg body weight per dose. Other ranges include about 100 µg/kg body weight to about 20 mg/kg body weight per dose and about 0.5 mg/kg body weight to about 20 mg/kg body weight per dose. In certain embodiments, the dosage is at least about 100 µg/kg body weight, at least about 250 µg/kg body weight, at least about 750 µg/kg body weight, at least about 3 mg/kg body weight, at least about 5 mg/kg body weight, at least about 10 mg/kg body weight.

In any event, the antibody or the antigen binding portion thereof of the disclosure is preferably administered as needed to subjects in need thereof. Determination of the frequency of administration may be made by persons skilled in the art, such as an attending physician based on considerations of the condition being treated, age of the subject being treated, severity of the condition being treated, general state of health of the subject being treated and the like.

In certain preferred embodiments, the course of treatment involving the antibody or the antigen-binding portion thereof of the present disclosure will comprise multiple doses of the selected drug product over a period of weeks or months. More specifically, the antibody or the antigen-binding portion thereof of the present disclosure may be administered once every day, every two days, every four days, every week, every ten days, every two weeks, every three weeks,

every month, every six weeks, every two months, every ten weeks or every three months. In this regard, it will be appreciated that the dosages may be altered or the interval may be adjusted based on patient response and clinical practices.

Dosages and regimens may also be determined empirically for the disclosed therapeutic compositions in individuals who have been given one or more administration(s). For example, individuals may be given incremental dosages of a therapeutic composition produced as described herein. In selected embodiments, the dosage may be gradually increased or reduced or attenuated based respectively on empirically determined or observed side effects or toxicity. To assess efficacy of the selected composition, a marker of the specific disease, disorder or condition can be followed as described previously. For cancer, these include direct measurements of tumor size via palpation or visual observation, indirect measurement of tumor size by x-ray or other imaging techniques; an improvement as assessed by direct tumor biopsy and microscopic examination of the tumor sample; the measurement of an indirect tumor marker (e.g., PSA for prostate cancer) or a tumorigenic antigen identified according to the methods described herein, a decrease in pain or paralysis; improved speech, vision, breathing or other disability associated with the tumor; increased appetite; or an increase in quality of life as measured by accepted tests or prolongation of survival.

Compatible formulations for parenteral administration (e.g., intravenous injection) may comprise the antibody or antigen-binding portion thereof as disclosed herein in concentrations of from about 10 µg/ml to about 100 mg/ml. It will be apparent to one of skill in the art that the dosage of the antibody or antigen-binding portion thereof as disclosed herein may vary depending on the individual, the type of neoplastic condition, the stage of neoplastic condition, whether the neoplastic condition has begun to metastasize to other location in the individual, the past and concurrent treatments being used, and the dosage of therapeutic agents used in combination with the antibody as disclosed herein.

Applications of the Disclosure

The antibodies, antibody compositions and methods of the present disclosure have numerous in vitro and in vivo utilities involving, for example, detection of TIGIT or enhancement of immune response. For example, these molecules can be administered to cells in culture, in vitro or ex vivo, or to human subjects, e.g., in vivo, to enhance immunity in a variety of situations. The immune response can be modulated, for instance, augmented, stimulated or up-regulated.

For instance, the subjects include human patients in need of enhancement of an immune response. The methods are particularly suitable for treating human patients having a disorder that can be treated by augmenting an immune response (e.g., the T-cell mediated immune response). In a particular embodiment, the methods are particularly suitable for treatment of cancer in vivo. When the anti-TIGIT antibodies are administered together with another agent such as an anti-PD-1 agent, the two can be administered in either order or simultaneously.

The present disclosure further provides methods for detecting the presence of TIGIT antigen in a sample, or measuring the amount of TIGIT antigen, comprising contacting the sample, and a control sample, with the anti-TIGIT antibody or an antigen binding portion thereof, under conditions that allow for formation of a complex between the antibody or portion thereof and TIGIT. The formation of a complex is then detected, wherein a difference in complex formation between the sample compared to the control sample is indicative of the presence of TIGIT antigen in the sample. Moreover, the anti-TIGIT antibodies of the disclosure can be used to purify TIGIT via immunoaffinity purification.

10 ***Treatment of disorders including cancers***

In some aspects, the present disclosure provides a method of treating a disorder or a disease in a mammal, which comprises administering to the subject (for example, a human) in need of treatment a therapeutically effective amount of the anti-TIGIT antibody or antigen-binding portion thereof as disclosed herein, preferably with an PD-1/PD-L1 antagonist. The disorder or disease comprises but not limited to, proliferative disorders (such as cancers), immune disorders, inflammatory disease or infectious diseases. For example, the disorder may be a cancer.

In some embodiments, the cancer is a cancer that is enriched for expression of CD112, CD113 or CD155. In some embodiments, the cancer is a cancer that is enriched for T cells or natural killer (NK) cells that express TIGIT.

20 Examples of cancer include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include, but are not limited to, lung cancer, such as non-small cell lung cancer (NSCLC), which includes squamous NSCLC or non-squamous NSCLC, including locally advanced unresectable NSCLC (e.g., Stage IIIB NSCLC), or recurrent or metastatic NSCLC (e.g., Stage IV NSCLC),
25 adenocarcinoma of the lung, or squamous cell cancer (e.g., epithelial squamous cell cancer); esophageal cancer; cancer of the peritoneum; hepatocellular cancer; gastric or stomach cancer, including gastrointestinal cancer and gastrointestinal stromal cancer; pancreatic cancer; glioblastoma; cervical cancer; ovarian cancer; liver cancer; bladder cancer (e.g., urothelial bladder cancer (UBC), muscle invasive bladder cancer (MIBC), and BCG-refractory non-muscle invasive
30 bladder cancer (NMIBC)); cancer of the urinary tract; hepatoma; breast cancer (e.g., TIGIT+ breast cancer and triple-negative breast cancer (TNBC), which are estrogen receptors (ER-), progesterone receptors (PR-), and TIGIT (TIGIT-) negative); colon cancer; rectal cancer; colorectal cancer; endometrial or uterine carcinoma; salivary gland carcinoma; kidney or renal cancer (e.g., renal cell carcinoma (RCC)); prostate cancer; vulval cancer; thyroid cancer; hepatic carcinoma; anal
35 carcinoma; penile carcinoma; melanoma, including superficial spreading melanoma, lentigo maligna melanoma, acral lentiginous melanomas, and nodular melanomas; multiple myeloma and B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL)); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high

grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non- cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenstrom's Macroglobulinemia); chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); acute myelogenous leukemia (AML); hairy cell leukemia; chronic myeloblastic leukemia (CML);
5 post-transplant lymphoproliferative disorder (PTLD); and myelodysplastic syndromes (MDS), as well as abnormal vascular proliferation associated with phakomatoses, edema (such as that associated with brain tumors), Meigs' syndrome, brain cancer, head and neck cancer, and associated metastases.

As a co-inhibitory receptor on a wide variety of immune cells, TIGIT is implicated in a
10 variety of cancers, whether malignant or benign and whether primary or secondary, which may be treated or prevented with a method provided by the disclosure. Preferably, the anti-TIGIT antibody as disclosed herein is administered in combination with another anti-cancer agent, preferably an PD-1/PD-L1 antagonist, such as an anti-PD-1 antibody. The cancers may be solid cancers or hematologic malignancies. Examples of such cancers include lung cancers such as bronchogenic
15 carcinoma (e.g., non-small cell lung cancer, squamous cell carcinoma, small cell carcinoma, large cell carcinoma, and adenocarcinoma), alveolar cell carcinoma, bronchial adenoma, chondromatous hamartoma (noncancerous), and sarcoma (cancerous); heart cancer such as myxoma, fibromas, and rhabdomyomas; bone cancers such as osteochondromas, condromas, chondroblastomas, chondromyxoid fibromas, osteoid osteomas, giant cell tumors,
20 chondrosarcoma, multiple myeloma, osteosarcoma, fibrosarcomas, malignant fibrous histiocyctomas, Ewing's tumor (Ewing's sarcoma), and reticulum cell sarcoma; brain cancer such as gliomas (e.g., glioblastoma multiforme), anaplastic astrocytomas, astrocytomas, oligodendrogliomas, medulloblastomas, Schwannomas, ependymomas, meningiomas, pituitary adenoma, pinealoma, osteomas, hemangioblastomas, craniopharyngiomas, chordomas,
25 germinomas, teratomas, dermoid cysts, and angiomas; cancers in digestive system such as colon cancer, leiomyoma, epidermoid carcinoma, adenocarcinoma, leiomyosarcoma, stomach adenocarcinomas, intestinal lipomas, intestinal neurofibromas, intestinal fibromas, polyps in large intestine, and colorectal cancers; liver cancers such as hepatocellular adenomas, hemangioma, hepatocellular carcinoma, fibrolamellar carcinoma, cholangiocarcinoma, hepatoblastoma, and
30 angiosarcoma; kidney cancers such as kidney adenocarcinoma, renal cell carcinoma, hypernephroma, and transitional cell carcinoma of the renal pelvis; bladder cancers; skin cancers such as basal cell carcinoma, squamous cell carcinoma, melanoma, Kaposi's sarcoma, and Paget's disease; head and neck cancers; eye-related cancers such as retinoblastoma and intraocular melanocarcinoma; male reproductive system cancers such as benign prostatic hyperplasia, prostate
35 cancer, and testicular cancers (e.g., seminoma, teratoma, embryonal carcinoma, and choriocarcinoma); breast cancer; female reproductive system cancers such as uterine cancer (endometrial carcinoma), cervical cancer (cervical carcinoma), cancer of the ovaries (ovarian carcinoma), vulvar carcinoma, vaginal carcinoma, fallopian tube cancer, and hydatidiform mole;

thyroid cancer (including papillary, follicular, anaplastic, or medullary cancer); pheochromocytomas (adrenal gland); noncancerous growths of the parathyroid glands; pancreatic cancers. In a specific embodiment, the cancer is colon cancer.

5 In some other embodiments, the disorder or a disease to be treated or prevented is an immune related disease. The immune related disease may be associated with a T cell dysfunctional disorder. In some embodiments, the T cell dysfunctional disorder is characterized by decreased responsiveness to antigenic stimulation. In some embodiments, the T cell dysfunctional disorder is characterized by T cell anergy, or decreased ability to secrete cytokines, proliferate or execute cytolytic activity. In some embodiments, the T cell dysfunctional disorder is characterized by T
10 cell exhaustion. In some embodiments, the T cells are CD4⁺ and CD8⁺ T cells. In some embodiments, the immune related disease is selected from the group consisting of unresolved acute infection, chronic infection and reduced tumor immunity.

Stimulation of an immune response

15 In some aspects, the disclosure also provides a method of enhancing (for example, stimulating) an immune response in a subject comprising administering an antibody or an antigen binding portion thereof of the disclosure to the subject such that an immune response in the subject is enhanced. For example, the subject is a mammal. In a specific embodiment, the subject is a human.

20 The term “enhancing an immune response” or its grammatical variations, means stimulating, evoking, increasing, improving, or augmenting any response of a mammal’s immune system. The immune response may be a cellular response (i.e. cell-mediated, such as cytotoxic T lymphocyte mediated) or a humoral response (i.e. antibody mediated response), and may be a primary or secondary immune response. Examples of enhancement of immune response include increased
25 CD4⁺ helper T cell activity and generation of cytolytic T cells. The enhancement of immune response can be assessed using a number of in vitro or in vivo measurements known to those skilled in the art, including, but not limited to, cytotoxic T lymphocyte assays, release of cytokines (for example IL-2 production or IFN- γ production), regression of tumors, survival of tumor bearing animals, antibody production, immune cell proliferation, expression of cell surface
30 markers, and cytotoxicity. Typically, methods of the disclosure enhance the immune response by a mammal when compared to the immune response by an untreated mammal or a mammal not treated using the methods as disclosed herein. In one embodiment, the antibody or an antigen binding portion thereof is used to enhance the immune response of a human to a microbial pathogen (such as a virus). In another embodiment, the antibody or an antigen binding portion
35 thereof is used to enhance the immune response of a human to a vaccine. In one embodiment, the method enhances a cellular immune response, particularly a cytotoxic T cell response. In another embodiment, the cellular immune response is a T helper cell response. In still another embodiment, the immune response is a cytokine production, particularly IFN- γ production or IL-2 production.

The antibody or an antigen binding portion thereof may be used to enhance the immune response of a human to a microbial pathogen (such as a virus) or to a vaccine.

The antibody or the antigen-binding portion thereof may be used alone as a monotherapy, or may be used in combination with chemical therapies, radiotherapies, targeting therapy or cell immunotherapy etc.

Combined use with chemotherapies

The antibody or the antigen-binding portion thereof may be used in combination with an anti-cancer agent, a cytotoxic agent or chemotherapeutic agent.

10 The term “anti-cancer agent” or “anti-proliferative agent” means any agent that can be used to treat a cell proliferative disorder such as cancer, and includes, but is not limited to, cytotoxic agents, cytostatic agents, anti-angiogenic agents, debulking agents, chemotherapeutic agents, radiotherapy and radiotherapeutic agents, targeted anti-cancer agents, BRMs, therapeutic antibodies, cancer vaccines, cytokines, hormone therapies, radiation therapy and anti-metastatic agents and immunotherapeutic agents. It will be appreciated that, such anti-cancer agents may comprise conjugates and may be associated with the disclosed antibodies prior to administration. More specifically, in certain embodiments selected anti-cancer agents will be linked to the unpaired cysteines of the engineered antibodies to provide engineered conjugates. Accordingly, such engineered conjugates are expressly contemplated as being within the scope of the present disclosure. In some other embodiments, the anti-cancer agents will be given in combination with antibody-drug conjugates comprising a different therapeutic agent.

As used herein the term “cytotoxic agent” means a substance that is toxic to the cells and decreases or inhibits the function of cells and/or causes destruction of cells. In certain embodiments, the substance is a naturally occurring molecule derived from a living organism. Examples of cytotoxic agents include, but are not limited to, small molecule toxins or enzymatically active toxins of bacteria (e.g., Diphtheria toxin, Pseudomonas endotoxin and exotoxin, Staphylococcal enterotoxin A), fungal (e.g., α -sarcin, restrictocin), plants (e.g., abrin, ricin, modeccin, viscumin, pokeweed anti-viral protein, saporin, gelonin, momoridin, trichosanthin, barley toxin, Aleurites fordii proteins, dianthin proteins, Phytolacca mericana proteins (PAPI, PAPII, and PAP-S), Momordica charantia inhibitor, curcin, croton, saponaria officinalis inhibitor, gelonin, mitegellin, restrictocin, phenomycin, neomycin, and the tricothecenes) or animals, (e.g., cytotoxic RNases, such as extracellular pancreatic RNases; DNase I, including fragments and/or variants thereof).

For the purposes of the present disclosure a “chemotherapeutic agent” comprises a chemical compound that non-specifically decreases or inhibits the growth, proliferation, and/or survival of cancer cells (e.g., cytotoxic or cytostatic agents). Such chemical agents are often directed to intracellular processes necessary for cell growth or division, and are thus particularly effective against cancerous cells, which generally grow and divide rapidly. For example,

vincristine depolymerizes microtubules, and thus inhibits cells from entering mitosis. In general, chemotherapeutic agents can include any chemical agent that inhibits, or is designed to inhibit, a cancerous cell or a cell likely to become cancerous or generate tumorigenic progeny (e.g., TIC). Such agents are often administered, and are often most effective, in combination, e.g., in regimens such as CHOP or FOLFIRI.

Examples of anti-cancer agents that may be used in combination with the antibody of the present disclosure (either as a component of a site specific conjugate or in an unconjugated state) include, but are not limited to, alkylating agents, alkyl sulfonates, aziridines, ethylenimines and methylamelamines, acetogenins, a camptothecin, bryostatin, calystatin, CC-1065, cryptophycins, dolastatin, duocarmycin, eleutherobin, pancratistatin, a sarcodictyin, spongistatin, nitrogen mustards, antibiotics, enediyne antibiotics, dynemicin, bisphosphonates, esperamicin, chromoprotein enediyne antibiotic chromophores, aclacinomysins, actinomycin, anthramycin, azaserine, bleomycins, cactinomycin, carabycin, carminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, ADRIAMYCIN[®] doxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites, erlotinib, vemurafenib, crizotinib, sorafenib, ibrutinib, enzalutamide, folic acid analogues, purine analogs, androgens, anti-adrenals, folic acid replenisher such as frolinic acid, aceglatone, aldophosphamide glycoside, aminolevulinic acid, eniluracil, amsacrine, bestabucil, bisantrene, edatraxate, defofamine, demecolcine, diaziquone, elfornithine, elliptinium acetate, an epothilone, etoglucid, gallium nitrate, hydroxyurea, lentinan, lonidainine, maytansinoids, mitoguazone, mitoxantrone, mopidanmol, nitraerine, pentostatin, phenamet, pirarubicin, losoxantrone, podophyllinic acid, 2-ethylhydrazide, procarbazine, PSK[®] polysaccharide complex (JHS Natural Products, Eugene, OR), razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, chloranbucil; GEMZAR[®] gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs, vinblastine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; NAVELBINE[®] vinorelbine; novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; irinotecan (Camptosar, CPT-11), topoisomerase inhibitor RFS 2000; difluoromethylornithine; retinoids; capecitabine; combretastatin; leucovorin; oxaliplatin; inhibitors of PKC-alpha, Raf, H-Ras, EGFR and VEGF-A that reduce cell proliferation; and pharmaceutically acceptable salts, acids or derivatives of any of the above. Also included in this definition are anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens and selective estrogen receptor modulators, aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the adrenal glands, and anti-androgens; as well as troxacitabine

(a 1,3- dioxolane nucleoside cytosine analog); antisense oligonucleotides, ribozymes such as a VEGF expression inhibitor and a TIGIT expression inhibitor; vaccines, PROLEUKIN[®] rIL-2; LURTOTECAN[®] topoisomerase 1 inhibitor; ABARELIX[®] rmRH; Vinorelbine and Esperamicins; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

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Combined use with radiotherapies

The present disclosure also provides for the combination of the antibody or the antigen-binding portion thereof with radiotherapy (i.e., any mechanism for inducing DNA damage locally within tumor cells such as gamma-irradiation, X-rays, UV-irradiation, microwaves, electronic
10 emissions and the like). Combination therapy using the directed delivery of radioisotopes to tumor cells is also contemplated, and the disclosed antibodies may be used in connection with a targeted anti-cancer agent or other targeting means. Typically, radiation therapy is administered in pulses over a period of time from about 1 to about 2 weeks. The radiation therapy may be administered to subjects having head and neck cancer for about 6 to 7 weeks. Optionally, the radiation therapy
15 may be administered as a single dose or as multiple, sequential doses.

Pharmaceutical packs and kits

Pharmaceutical packs and kits comprising one or more containers, comprising one or more doses of the antibody or the antigen-binding portion thereof are also provided. In certain
20 embodiments, a unit dosage is provided wherein the unit dosage contains a predetermined amount of a composition comprising, for example, the antibody or the antigen-binding portion thereof, with or without one or more additional agents. For other embodiments, such a unit dosage is supplied in single-use prefilled syringe for injection. In still other embodiments, the composition contained in the unit dosage may comprise saline, sucrose, or the like; a buffer, such as phosphate,
25 or the like; and/or be formulated within a stable and effective pH range. Alternatively, in certain embodiments, the composition may be provided as a lyophilized powder that may be reconstituted upon addition of an appropriate liquid, for example, sterile water or saline solution. In certain preferred embodiments, the composition comprises one or more substances that inhibit protein aggregation, including, but not limited to, sucrose and arginine. Any label on, or associated with,
30 the container(s) indicates that the enclosed antibody is used for treating the neoplastic disease condition of choice.

The present disclosure also provides kits comprising single-dose or multi-dose administration units of antibodies and, optionally, one or more anti-cancer agents. The kit comprises a container and a label or package insert on or associated with the container. Suitable
35 containers include, for example, bottles, vials, syringes, etc. The containers may be formed from a variety of materials such as glass or plastic and contain a pharmaceutically effective amount of the disclosed antibodies. In some embodiments, the container(s) comprise a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable

by a hypodermic injection needle). Such kits will generally contain in a suitable container a pharmaceutically acceptable formulation of the antibodies and, optionally, one or more anti-cancer agents in the same or different containers. The kits may also contain other pharmaceutically acceptable formulations, either for diagnosis or combined therapy. For example, in addition to the antibody or the antigen-binding portion thereof of the disclosure such kits may contain any one or more of a range of anti-cancer agents such as chemotherapeutic or radiotherapeutic drugs; anti-angiogenic agents; anti-metastatic agents; targeted anti-cancer agents; cytotoxic agents; and/or other anti-cancer agents. In some embodiments, the kit may contain an anti-PD-1 antibody.

More specifically the kits may have a single container that contains the antibody or the antigen-binding portion thereof, with or without additional components, or they may have distinct containers for each desired agent. Where combined therapeutics are provided for conjugation, a single solution may be pre-mixed, either in a molar equivalent combination, or with one component in excess of the other. Alternatively, the antibody and any optional anti-cancer agent of the kit may be maintained separately within distinct containers prior to administration to a patient. The kits may also comprise a second/third container means for containing a sterile, pharmaceutically acceptable buffer or other diluents such as bacteriostatic water for injection (BWFI), phosphate-buffered saline (PBS), Ringer's solution and dextrose solution.

When the components of the kit are provided in one or more liquid solutions, the liquid solution is preferably an aqueous solution, with a sterile aqueous or saline solution being particularly preferred. However, the components of the kit may be provided as dried powder(s). When reagents or components are provided as a dry powder, the powder can be reconstituted by the addition of a suitable solvent.

As indicated briefly above the kits may also contain a means by which to administer the antibody or the antigen-binding portion thereof and any optional components to a patient, e.g., one or more needles, I.V. bags or syringes, or even an eye dropper, pipette, or other such like apparatus, from which the formulation may be injected or introduced into the animal or applied to a diseased area of the body. The kits of the present disclosure will also typically include a means for containing the vials, or such like, and other component in close confinement for commercial sale, such as, e.g., injection or blow-molded plastic containers into which the desired vials and other apparatus are placed and retained.

Sequence Listing Summary

Appended to the instant application is a sequence listing comprising a number of nucleic acid and amino acid sequences. The following Table A, B and C provide a summary of the included sequences.

W3642-1.433.11 is the parental hybridoma clone which is humanized to obtain the W3642-1.433.11-z11 clone and PTM removed to obtain the W3642-1.433.11-p1 clone. W3642-1.433.11-xIgG4.SP is a chimeric antibody; W3642-1.433.11-z10-p1-IgG4.SP and W3642-1.433.11-z11-p1-

IgG4.SP are humanized antibodies comprising a same set of CDRs; W3642-1.433.11-z11-p1-IgG4.SP and W3642-1.433.11-z11-p1-uIgG1L are only different in the constant regions; W3642-1.433.11-p1-xIgG4.SP, W3642-1.433.11-p2-xIgG4.SP and W3642-1.433.11-p3-xIgG4.SP are antibodies comprising different substitutions in the LCDR2 for PTM removal.

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Table A: CDR sequences of antibodies/clones

	HCDR1	HCDR2	HCDR3	LCDR1	LCDR2	LCDR3
W3642-1.433.11-xIgG4.SP; W3642-1.433.11; W3642-1.433.11-z11	GFTFSVA WIH (SEQ ID NO: 1)	LIKAGSNNYA TDYAESVKG (SEQ ID NO: 2)	TLSHGSLN WFAS (SEQ ID NO: 3)	TLSSGNIENKY VH (SEQ ID NO: 4)	NDDKRPD (SEQ ID NO: 5)	HSYVSSIN V (SEQ ID NO: 6)
W3642-1.433.11-p1-xIgG4.SP; W3642-1.433.11-z10-p1-IgG4.SP; W3642-1.433.11-z11-p1-IgG4.SP; W3642-1.433.11-z11-p1-uIgG1L	SEQ ID NO: 1	SEQ ID NO: 2	SEQ ID NO: 3	SEQ ID NO: 4	NDDKRPE (SEQ ID NO: 7, with D56E)	SEQ ID NO: 6
W3642-1.433.11-p2-xIgG4.SP	SEQ ID NO: 1	SEQ ID NO: 2	SEQ ID NO: 3	SEQ ID NO: 4	NDDKRPO (SEQ ID NO: 8, with D56Q)	SEQ ID NO: 6
W3642-1.433.11-p3-xIgG4.SP	SEQ ID NO: 1	SEQ ID NO: 2	SEQ ID NO: 3	SEQ ID NO: 4	NDDKRPS (SEQ ID NO: 9, with D56S)	SEQ ID NO: 6

Table B: Amino acid sequences of the variable regions

Clone/Antibody		Amino acid sequence
W3642-1.433.11-xIgG4.SP	VH	EVQLVETGGSLVQPGKSLKLTVCVSSGFTFSVAWIHWVRQSPDKQLEWIGLIKAG SNNYATDYAESVKGRFTMSRDDSKSSVYLMNSLREEDTAIYYCAWTLSHGSL NWFASWGRGTLVTVSS (SEQ ID NO: 10)
	VL	QAVLTQPDVSTSLGGTVRLSCTLSSGNIENKYVHWYQQHEGRSPTTMIYNDDK RPGVPRDFSGSIDSSNSAFLTITNVEIEDEAIYFCHSYVSSINVFGGGKLTVL (SEQ ID NO: 12)
W3642-1.433.11-z11	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSVAWIHWVRQAPGKGLEWVGLIKA GSNNYATDYAESVKGRFTISRDDSKNSVYLMNSLKTEDTAVYYCAWTLSHGS LWFWASWGRGTLVTVSS (SEQ ID NO: 11)
	VL	QAVLTQPHSVSESPGKTVTISC TLSSGNIENKYVHWYQRPQSSPTTVIYNDDKR PDGVPDRFSGSIDSSNSASLTISGLKTEDEADYYCHSYVSSINVFGGGKLTVL (SEQ ID NO: 13)
W3642-1.433.11-p1-xIgG4.SP	VH	EVQLVETGGSLVQPGKSLKLTVCVSSGFTFSVAWIHWVRQSPDKQLEWIGLIKAG SNNYATDYAESVKGRFTMSRDDSKSSVYLMNSLREEDTAIYYCAWTLSHGSL NWFASWGRGTLVTVSS (SEQ ID NO: 10)

	VL	QAVLTQPD SV STSLGGTVRLSCTLSSGNIENKYVHWYQQHEGRSPTTMIYNDDK RPEGV P DRFSGSIDSSSNSAFLTITNVEIEDEAIYFCHSYVSSINVF GG GKLT VL (SEQ ID NO: 14)
W3642- 1.433.11- z11-p1- uIgG1L; W3642- 1.433.11- z11-p1- IgG4.SP	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSVAWIHWVRQAPGKGLEWVGLIKA GSNNYATDYAESVKGRFTISRDDSKNSVYLQMNSLKTEDTAVYYCAWTL SHGS LNWFASWGRGTLVT VSS (SEQ ID NO: 11) GAGGTGCAGCTGGTGGAGAGCGGAGGAGGACTGGTGCAGCCCGGTGGATCT TTAAGACTGAGCTGCGCCGCTAGCGGCTTCACCTTCAGCGTGGCTTGGATCC ACTGGGTGAGGCAAGCTCCCGGTAAGGGTTTAGAGTGGGTGGGTTTAATCA AGGCCGGCAGCAACAACACGCCACAGACTACGCCGAGAGCGTGAAGGGTC GTTTCACCATCTCTCGTGACGACAGCAAGAACTCTGTGTATTTACAGATGAA CTCTTTAAAGACCGAGGACACCGCCGTGTACTACTGCGCTTGGACTTTAAGC CATGGCTCTTTAACTGGTTCGCCAGCTGGGGCAGAGGCACTTTAGTGACCG TGAGCAGC (SEQ ID NO: 21, encoding nucleic acid sequence)
	VL	QAVLTQPHSVSESPGKTVTISCTLSSGNIENKYVHWYQRP GSS PTTVIYNDDKR PEGV P DRFSGSIDSSSNSASLTISGLKTEDEADYYCHSYVSSINVF GG GKLT VL (SEQ ID NO: 15) CAAGCTGTGCTGACCCAGCCTCACAGCGTGAGCGAGAGCCCCGCAAGACC GTGACCATCAGCTGCACTTTAAGCAGCGGCAACATCGAGAACAAGTACGTG CACTGGTACCAGCAGAGGCCCGGTAGCAGCCCCACCACAGTGATCTACAAC GACGACAAGAGGCCCGAGGGCGTGCCCGATCGTTTCAGCGGCAGCATCGAC AGCAGCAGCAACAGCGCCTCTTTAACCATCAGCGGTTTAAAGACCGAGGAC GAGGCCGACTACTACTGCCACAGCTACGTGAGCAGCATCAACGTGTTCTGGCG GCGGCACCAAGCTGACCGTGCTG (SEQ ID NO: 22, encoding nucleic acid sequence)
W3642- 1.433.11- z10-p1- IgG4.SP	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSVAWIHWVRQAPGKGLEWVGLIKA GSNNYATDYAESVKGRFTISRDDSKNSVYLQMNSLKTEDTAVYYCAWTL SHGS LNWFASWGRGTLVT VSS (SEQ ID NO: 11)
	VL	NFMLTQPHSVSESPGKTVTISCTLSSGNIENKYVHWYQRP GSS PTTVIYNDDKR PEGV P DRFSGSIDSSSNSASLTISGLKTEDEADYYCHSYVSSINVF GG GKLT VL (SEQ ID NO: 16)
W3642- 1.433.11-p2- xIgG4.SP	VH	EVQLVETGGSLVQPGKSLKLT CVSS GFTFSVAWIHWVRQSPDKQLEWIGLIKAG SNNYATDYAESVKGRFTMSRDDSKSSVYLQMNSLREEDTAIYYCAWTL SHGSL NWFASWGRGTLVT VSS (SEQ ID NO: 10)
	VL	QAVLTQPD SV STSLGGTVRLSCTLSSGNIENKYVHWYQQHEGRSPTTMIYNDDK RPQGV P DRFSGSIDSSSNSAFLTITNVEIEDEAIYFCHSYVSSINVF GG GKLT VL (SEQ ID NO: 17)
W3642- 1.433.11-p3- xIgG4.SP	VH	EVQLVETGGSLVQPGKSLKLT CVSS GFTFSVAWIHWVRQSPDKQLEWIGLIKAG SNNYATDYAESVKGRFTMSRDDSKSSVYLQMNSLREEDTAIYYCAWTL SHGSL NWFASWGRGTLVT VSS (SEQ ID NO: 10)
	VL	QAVLTQPD SV STSLGGTVRLSCTLSSGNIENKYVHWYQQHEGRSPTTMIYNDDK RPSGV P DRFSGSIDSSSNSAFLTITNVEIEDEAIYFCHSYVSSINVF GG GKLT VL (SEQ ID NO: 18)

Table C: Sequences of the heavy and light chains

Antibody		Amino acid sequence
W3642-1.433.11-z11-p1-uIgG1L	Heavy chain Amino acid sequence	EVQLVESGGGLVQPGGSLRLSCAASGFTFSVAWIHWVRQAPGKGLEWVGLIKA GSNNYATDYAESVKGRFTISRDDSKNSVYLQMNSLKTEDTAVYYCAWTLSHGS LNWFASWGRGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT KVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPETCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 19)
	Light chain Amino acid sequence	QAVLTQPHSVSESPGKTVTISCTLSSGNIENKYVHWYQRPGSSPTTVIYNDDKR PEGVPDRFSGSIDSSSNSASLTISGLKTEDEADYYCHSYVSSINVFGGGTKLTVLG QPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVET TTPSKQSNKYAASSYLSLTPEQWKSHRYSQCQVTHEGSTVEKTVAPTECS (SEQ ID NO: 20)

EXAMPLES

The present disclosure, thus generally described, will be understood more readily by reference to the following Examples, which are provided by way of illustration and are not intended to be limiting of the present disclosure. The Examples are not intended to represent that the experiments below are all or the only experiments performed.

EXAMPLE 1

Preparation of Antigens, Benchmark Antibodies and Cell Lines

1.1 Generation of antigens

W364-hPro1.ECD.His is the extracellular domain of human TIGIT (NP_776160.2) with a C-terminal polyhistidine tag; W364-hPro1.ECD.hFc is the extracellular domain of human TIGIT (NP_776160.2) with the Fc region of human IgG1 at the C-terminus; W364-mPro1.ECD.His is the extracellular domain of mouse TIGIT (NP_001139797.1) with a C-terminal polyhistidine tag; W364-mPro1.ECD.hFc is the extracellular domain of mouse TIGIT (NP_001139797.1) with the Fc region of human IgG1 at the C-terminus; W364-hPro1L1.ECD.hFc is the extracellular domain of human CD155 (NP_006496.3) with the Fc region of human IgG1 at the C-terminus. W364-hPro1L1.ECD.mFc is the extracellular domain of human CD155 (NP_006496.3) with the Fc region of mouse IgG1 at the C-terminus. These antigens were purchased from vendors or prepared in house.

1.2 Preparation of benchmark antibodies (BMKs)

The amino acid sequences encoding the variable domain of the anti-TIGIT reference antibodies WBP364-BMK1, WBP364-BMK4 and WBP364-BMK6 were synthesized according

to the disclosed sequences in respective patent, the information of which is summarized in Table 1.

Table 1 Reference antibody information

Antibody Code	Company	Patent NO.	Sequence ID
WBP364-BMK1	Roche (Genentech)	US20170088613	4.1D3
WBP364-BMK4	BMS (Ono)	US20160176963	22G2
WBP364-BMK6	Astellas (Potenza)	WO2017059095	MAB10

5 1.3 Cell Pool/Line Generation

Human TIGIT-expressing cell pool W364-293F.hPro1.pool was generated using 293F cells transfected with full-length human TIGIT (NP_776160.2). Human TIGIT-expressing cell line W364-CHOK1.hPro1.2A11 was generated using CHOK1 cells transfected with full-length human TIGIT (NP_776160.2). Cynomolgus monkey TIGIT-expressing cell pool W364-FlpinCHO.cynoPro1.pool was generated using FlpinCHO cells transfected with full-length cynomolgus monkey TIGIT (XP_015300911.1). Mouse TIGIT-expressing cell pool W364-FlpinCHO.mPro1.pool was generated using FlpinCHO cells transfected with full-length mouse TIGIT (NP_001139797.1).

EXAMPLE 2

Generation of Chimeric and Humanized Antibodies

2.1 Immunization

Two SD rats, 6~8 weeks of age, both female, were purchased from Shanghai SLAC Experiment Animal Co., Ltd. and housed in an IACUC approved animal facility. The two animals were immunized with W364-hPro1.ECD.His and W364-mPro1.ECD.His alternately.

2.2 Serum titer detection

Anti-human/mouse TIGIT antibody titers in serum samples were determined by ELISA. Microplates were coated with W364-hPro1.ECD.hFc or W364-mPro1.ECD.hFc at 1 μ g/mL in 100 μ L of coating buffer ($\text{Na}_2\text{CO}_3/\text{NaHCO}_3$, pH9.2) per well and incubated at 4 $^\circ\text{C}$ overnight. On the day of assay, diluted rat serum samples (first 1:100, then 3-fold dilution in 1 $\times$ PBS/2%BSA) and negative control were added into the plates post 1-hour block with 1 $\times$ PBS/2%BSA, and then the plates were incubated at ambient temperature for 1 hour. After washing with 1 $\times$ PBST (PBS containing 0.05% Tween-20) for 3 times, HRP-labeled goat anti-rat IgG Fc (Bethyl, cat#A110-236P) was added and incubated at ambient temperature for 1 hour. After removing of the unbound substance, TMB (3,3',5,5'-Tetramethylbenzidine) substrate was added and the reaction was stopped by 2M HCl. Absorbance at 450nm was detected by a microplate spectrophotometer.

The serum titers of the immunized SD rats are shown in Table 2. After the last boost with W364-hPro1.ECD.His and W364-mPro1.ECD.His, the two animals were euthanized and the lymph nodes were collected for fusion.

Table 2 Serum titer of anti-TIGIT antibodies

Animal ID #	Antigen	Titer information		
		Pre-bleed	1 st bleed	2 nd bleed
1	W364-	100	1968300	656100
2	hPro1.ECD.hFc	100	1968300	656100
1	W364-	/	/	1968300
2	mPro1.ECD.hFc	/	/	1968300

5 **2.3 Hybridoma generation**

Lymph nodes were collected from immunized rats under sterile condition and dissociated into single cell suspension. B cells were isolated from lymph nodes, and then mixed with myeloma cell SP2/0 at a ratio of 1:1.2. Electro cell fusion was performed using BTX 2001 Electro cell manipulator according to an optimized electro-fusion procedure. After fusion, the cells were transferred into 96-well plates (1.2×10⁴ cells/well) with DMEM medium supplemented with 20% FBS and 1% HAT selective reagents. The plates were cultured at 37 °C, 5% CO₂, and were monitored periodically. When the clones reached about 80% confluence in a well, 100 μL of supernatant were transferred from the tissue culture plates to 96-well assay plates for antibody screening.

15 **2.4 Antibody screening and subcloning**

The process of high-throughput screening included primary screening for cynomolgus TIGIT binder by cell-based ELISA, secondary screening for human/cynomolgus/mouse binder by cell-based FACS, and functional screening for TIGIT/PVR blocker by cell-based FACS.

The positive lines in logarithmic growth was diluted to 200~300 cells per 1.5 mL semi-solid HAT media. The cell suspensions were mixed gently on vortex oscillator for 5 to 10 seconds and then seeded in 6-well plates. The plates were kept at 37 °C, 5% CO₂ for 7-8 days. When the cell clusters grew up, each visible single colony was picked and seeded into 96-well plates with DMEM medium supplemented with 10% fetal bovine serum. After 2-3 days, the supernatant of each clone was collected and screened again to obtain positive hybridoma single clones.

25 **2.5 Hybridoma sequencing**

RNAs were isolated from monoclonal hybridoma cells using TaKaRa MiniBEST Universal RNA Extraction Kit (TaKaRa Bio Inc.) according to the manufacturer's instructions. The cDNAs were amplified using SMART RACE cDNA Amplification Kit (Clontech Laboratories, Inc.) according to the manufacturer's instructions. The resulting cDNA was used as templates for subsequent PCR amplification using primers specific for the interested genes. PCR product was inserted into the pMD18-T vectors, and the ligation products were sent to GENEWIZ for sequencing.

Through primary and secondary binding screening, as well as TIGIT/PVR blockade, 50 positive cell lines were selected for subcloning. After confirmation of the monoclonal antibodies, 39 positive hits were selected for sequencing and 3 of them were followed by human IgG conversion.

5 2.6 Sequence optimization

2.6.1 Chimeric antibody production

The amino acid sequences of the VH and VL domains were codon optimized for mammalian expression. The codon optimized DNA sequences were synthesized by GENEWIZ and then subcloned into pcDNA expression vectors with constant region of human IgG1 or IgG4. The plasmids containing VH and VL genes were co-transfected into Expi293 cells, and the cells were cultured for ~5 days until the supernatants were harvested. The antibodies were purified with Protein A column from the supernatants.

W3642-1.433.11 clone was identified and its variable domain sequences are shown in Table B. The chimeric antibody of W3642-1.433.11 comprising a heavy chain with rat VH fused to human IgG4 constant region and a light chain with rat VL fused to human Ig lambda was named as W3642-1.433.11-xIgG4.SP.

2.6.2 PTM removal

For the parental antibody W3642-1.433.11, amino acid residues “DG” at the border of VLCDR2 was identified as potentially unstable residues with isomerization risk. Mutations were made to remove the risk of post-translational modification (PTM), and SPR analysis was used to measure the binding kinetics of antibodies binding to human TIGIT. Mutations of residues “DG” to either EG, QG or SG did not significantly alter binding affinity. The results are shown in Table 3.

Table 3. Koff rates for PTM removed variants binding to human TIGIT

Antibody	VH domain	VL domain	k_{off} (1/s)
W3642-1.433.11-xIgG4.SP	rVH	rVL	5.95E-05
W3642-1.433.11-p1-xIgG4.SP	rVH	rVL+D056E	5.27E-05
W3642-1.433.11-p2-xIgG4.SP	rVH	rVL+D056Q	5.30E-05
W3642-1.433.11-p3-xIgG4.SP	rVH	rVL+D056S	5.31E-05

2.6.3 Humanization of the rat antibody

Humanization of W3642-1.433.11 was carried out in principle by CDR grafting. The CDRs are identified according to the contact definition introduced by Dr. Andrew C.R. Martin's group

(<http://www.bioinf.org.uk/abs/>). The VH and VL sequences of W3642-1.433.11 were blasted against human germline V-gene database, and the human IGVH and IGVL with the highest homology to W3642-1.433.11 were selected as the template for humanization. CDRs of W3642-1.433.11 VH and VL domains were grafted into the frameworks of the humanization template, to constitute the germlined VH and VL domain sequences.

Several “back mutation” positions in the frameworks were performed to convert the amino acids in the germlined sequence to their counterpart amino acids in the original rat sequence. We also generated a hybrid chimeric antibody consisting of rat VH fused to human IgG4 constant region and germlined VL fused to human Ig lambda (W3642-1.433.11-hyAbL).

To determine whether the humanized variant retained antigen binding activity, surface plasmon resonance (SPR) was performed using a Biacore 8K instrument (Cytiva). Briefly, goat anti-human IgG Fc antibody (JacksonImmunoResearch, cat#109-005-098) was immobilized on CM5 biosensor chips (GE, cat#29-1496-03). Antibodies in the supernatants of the pilot scale transfection were captured by goat anti-human IgG Fc antibody. The antigen W364-hPro1.ECD.His was injected in running buffer (0.01 M HEPES, 0.15 M NaCl, 3 mM EDTA, 0.05% surfactant P20, pH 7.4) with a flow rate of 100 uL/min for an association phase of 120s, followed by a dissociation phase of 900s. The dissociation rate (*k_{off}*) was calculated using a simple one-to-one Langmuir binding model. The results are shown in Table 4.

Table 4. Koff rates for antibody variants binding to human TIGIT

Antibody	VH domain	VL domain	<i>k_{off}</i> (1/s)
W3642-1.433.11-xIgG4.SP	rVH	rVL	5.95E-05
W3642-1.433.11-hyAbL	rVH	GL	9.83E-05

Note: rVH=rat hybridoma sequence of variable heavy chain, rVL=rat hybridoma sequence of variable light chain, GH=human germlined sequence of variable heavy chain, GL=human germlined sequence of variable light chain.

Two variants W3642-1.433.11-z10-p1-IgG4.SP and W3642-1.433.11-z11-p1-IgG4.SP, that combined humanization and PTM removal, showed high affinity binding to human TIGIT as measured by Biacore. The affinity of W3642-1.433.11-z11-p1-IgG4.SP is about 2-fold higher than that of W3642-1.433.11-z10-p1-IgG4.SP. The results are shown in Table 5.

Table 5 Affinity of humanized and PTM removed variants binding to human TIGIT

Antibody	VH domain	VL domain	<i>k_{on}</i> (1/Ms)	<i>k_{off}</i> (1/s)	KD (M)
W3642-1.433.11-xIgG4.SP	rVH	rVL	1.68E+06	2.58E-05	1.54E-11

W3642-1.433.11-z10-p1-IgG4.SP	GH+L078 V+R094W	GL+D056E	1.55E+06	6.15E-05	3.96E-11
W3642-1.433.11-z11-p1-IgG4.SP	GH+L078 V+R094W	GL+N001Q+F002 A+M003V+D056E	1.72E+06	4.11E-05	2.39E-11

The corresponding IgG1 formatted antibody of W3642-1.433.11-z11-p1-IgG4.SP was generated and named as W3642-1.433.11-z11-p1-uIgG1L, or W3642 for short.

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EXAMPLE 3

In vitro Characterization

3.1 Human TIGIT binding assay

W364-293F.hPro1.pool (1×10^5 cells/well) cells were incubated with various concentrations of anti-TIGIT antibodies at 4 °C for 1 hour. After washing with 1×PBS/1%BSA, the secondary antibody, AlexaFluor647-labeled goat anti-human IgG (JacksonImmunoResearch cat#109-605-098) was added and incubated with cells at 4 °C in dark for 1 hour. Anti-human TIGIT antibodies WBP364-BMK1 and WBP364-BMK4 were used as positive controls. Human IgG1 isotype antibody was used as isotype control. The cells were then washed and re-suspended in 1×PBS/1%BSA. MFI of the cells was measured by a flow cytometer (BD) and analyzed by FlowJo.

The binding results of W3642-1.433.11-z11-p1-uIgG1L on W364-293F.hPro1.pool cells are shown in Figure 1, which demonstrates that W3642-1.433.11-z11-p1-uIgG1L can strongly bind to human TIGIT-expressing cells, and the binding potency is equivalent to the reference antibody. A summary of antibody binding is shown in Table 7.

3.2 Cynomolgus monkey TIGIT binding assay

W364-FlpinCHO.cynoPro1.pool (1×10^5 cells/well) cells were incubated with various concentrations of anti-TIGIT antibodies at 4 °C for 1 hour. After washing with 1×PBS/1%BSA, the secondary antibody, AlexaFluor647-labeled goat anti-human IgG (JacksonImmunoResearch cat#109-605-098) was added and incubated with cells at 4 °C in dark for 1 hour. Anti-human TIGIT antibodies WBP364-BMK1 and WBP364-BMK4 were used as positive controls. Human IgG1 isotype antibody was used as isotype control. The cells were then washed and re-suspended in 1×PBS/1%BSA. MFI of the cells was measured by a flow cytometer (BD) and analyzed by FlowJo.

The binding result of W3642-1.433.11-z11-p1-uIgG1L on W364-FlpinCHO.cynoPro1.pool cells is shown in Figure 2, which demonstrates that W3642-1.433.11-z11-p1-uIgG1L can strongly bind to cynomolgus monkey TIGIT-expressing cells, and the binding potency is equivalent to the reference antibody. A summary of antibody binding is shown in Table 7.

3.3 Mouse TIGIT binding assay

W364-FlpinCHO.mPro1.pool (1×10^5 cells/well) cells were incubated with various concentrations of anti-TIGIT antibodies at 4 °C for 1 hour. After washing with 1×PBS/1%BSA, the secondary antibody, AlexaFluor647-labeled goat anti-human IgG (JacksonImmunoResearch cat#109-605-098) was added and incubated with cells at 4 °C in dark for 1 hour. Anti-human TIGIT antibody WBP364-BMK6, which was known to have cross-reactivity to mouse TIGIT, was used as positive control. Human IgG1 isotype antibody was used as isotype control. The cells were then washed and re-suspended in 1×PBS/1%BSA. MFI of the cells was measured by a flow cytometer (BD) and analyzed by FlowJo.

The binding result of W3642-1.433.11-z11-p1-uIgG1L to mouse TIGIT extracellular domain is shown in Figure 3, which demonstrates that W3642-1.433.11-z11-p1-uIgG1L can strongly bind to mouse TIGIT-expressing cells, and the binding potency is higher than that of the reference antibody WBP364-BMK6. WBP364-BMK1 and WBP364-BMK4 show no binding to mouse TIGIT-expressing cells. A summary of antibody binding is shown in Table 7.

3.4 Human TIGIT affinity assay

Affinity determination of anti-TIGIT antibodies against recombinant human TIGIT was performed by surface plasmon resonance (SPR) using a Biacore 8K instrument (Cytiva). Goat anti-human IgG Fc antibody (JacksonImmunoResearch cat#109-005-098) was immobilized on CM5 biosensor chips (GE cat#29-1496-03), and anti-TIGIT antibody was captured by goat anti-human IgG Fc antibody. For kinetics measurements, a series concentration of W364-hPro1.ECD.His was injected in running buffer (0.01 M HEPES, 0.15 M NaCl, 3 mM EDTA, 0.05% surfactant P20, pH 7.4) at 25 °C with a flow rate of 30 uL/min for an association phase, followed by a period of dissociation phase. Association rates (*kon*) and dissociation rate (*koff*) were calculated using a simple one-to-one Langmuir binding model. The equilibrium dissociation constant (kD) was calculated as the ratio *koff/kon*.

The binding affinity result of W3642-1.433.11-z11-p1-uIgG1L to human TIGIT extracellular domain is shown in Table 6. W3642-1.433.11-z11-p1-uIgG1L binds to human TIGIT with an affinity at a KD of 2.39E-11 M.

Table 6 Affinity constant of anti-TIGIT antibodies to human TIGIT

Antibody	ka (1/Ms)	kd (1/s)	KD (M)
WBP364-BMK1	5.95E+05	2.14E-05	3.59E-11
WBP364-BMK4	1.46E+06	7.19E-05	4.92E-11
W3642-1.433.11-z11-p1-uIgG1L	1.72E+06	4.11E-05	2.39E-11

3.5 TIGIT paralogous proteins binding assay

Plate was pre-coated with 1 µg/mL of W364-hPro1.ECD.His, recombinant human CD28, CTLA-4, PD-1, ICOS or PVRIG extracellular domain in 50 µL coating buffer per well at 4 °C overnight. After blocking with 200 µL of 1×PBS/2%BSA, 50 µL of testing antibodies were added to the plate at the concentration of 1 nM, 0.1 nM or 0.01 nM, and the plate was incubated at ambient temperature for 1 hour. After incubation, the plate was washed using 1×PBST for 3 times. HRP-labeled goat anti-human IgG antibody (Bethyl cat#A80-304P) diluted in 1×PBS/2%BSA was added and incubated for 1 hour at ambient temperature. Anti-CD28 antibody (US7585960, TGN1412), anti-CTLA-4 antibody (US8784815, 10D1), anti-PD-1 antibody (US9084776, 5C4), anti-ICOS antibody (US10023635, 37A10) and anti-PVRIG antibody (US20180244774, CHA.7.518.1) were used as positive controls to CD28, CTLA-4, PD-1, ICOS and PVRIG, respectively. Human IgG1 isotype antibody was used as isotype control. After washing with 1×PBST for 6 times, the color was developed by dispensing 100 µL of TMB substrate, and then reaction was stopped by adding 100 µL of 2M HCl. Absorbance was read at 450nm using M5e microplate reader (Molecule Devices).

The binding results of W3642-1.433.11-z11-p1-uIgG1L to TIGIT paralogous proteins are shown in Figure 4, which demonstrate that W3642-1.433.11-z11-p1-uIgG1L specifically binds to TIGIT without cross-reactivity to human CD28, ICOS, PVRIG, PD-1 or CTLA-4.

3.6 Human TIGIT/ligands blocking assay

The ability of anti-TIGIT antibodies to block human TIGIT/PVR interaction was tested by FACS. W364-CHOK1.hPro1.2A11 cells were washed with 1×PBS/1%BSA and seeded at 1×10^5 cells per well in a 96-well round bottom plate. Excess buffer in the wells was removed by centrifugation. The serially diluted anti-TIGIT antibodies (2× concentration) were pre-mixed with 4 µg/mL (2× concentration) of W364-hPro1L1.ECD.mFc at a volume ratio of 1:1, and added 100 µL of antibody/ligand mixture to each well. The plate was incubated at 4 °C for 1 hour. After washing with 1×PBS/1%BSA, the secondary antibody, PE-labeled goat anti-mouse IgG (Bethyl cat#A90-239PE) was added and incubated with cells at 4 °C in dark for 1 hour. Anti-human TIGIT antibodies WBP364-BMK1 and WBP364-BMK4 were used as positive controls. Human IgG1 isotype antibody was used as isotype control. The cells were then washed and re-suspended in 1×PBS/1%BSA. MFI of the cells was measured by a flow cytometer (BD) and analyzed by FlowJo.

The ability of anti-TIGIT antibodies to block human TIGIT/CD112 and TIGIT/CD113 interactions were also tested by FACS.

The human TIGIT and its ligands (CD155, CD112 and CD113) binding blockade results are shown in Figures 5-7. The results demonstrate that W3642-1.433.11-z11-p1-uIgG1L can effectively block human CD155, CD112 and CD113 binding to TIGIT. A summary of antibody blocking activity is shown in Table 7. The ligand binding rate was calculated as $\text{binding\%} = \text{MFI}_{\text{sample}} / \text{MFI}_{\text{max}} \times 100\%$, where MFI_{max} was defined as MFI in the absence of antibody.

3.7 Jurkat TIGIT/NFAT-luciferase reporter gene assay (RGA)

Jurkat cells co-expressing human TIGIT and NFAT-luciferase reporter was stimulated by engagement of T cell receptor by co-culturing with CHOK1 cells co-expressing human PVR and TCR activator. CHOK1/PVR/TCR-activator cells were seeded at a density of 2×10^4 cells/well in a 96-well plate overnight at 37 °C, 5% CO₂. The second day, following removal of the supernatants and non-adherent cells, serially diluted anti-TIGIT antibodies and Jurkat/TIGIT/NFAT-luciferase cells (2×10^4 cells/well) were added to the plate, and were co-cultured at 37 °C, 5% CO₂ for 5-6 hours. After incubation, reconstituted luciferase substrate (Promega cat#E6130) was added to each well and mixed well. The luciferase intensity was read using EnVision microplate reader (PerkinElmer).

The result of W3642-1.433.11-z11-p1-uIgG1L reversing suppression of NFAT signaling induced by TIGIT/PVR interaction in RGA is shown in Figure 8, which demonstrates that W3642-1.433.11-z11-p1-uIgG1L can enhance TCR/NFAT activation by suppressing TIGIT/PVR pathway. A summary of antibody RGA activity is shown in Table 7.

3.8 Jurkat functional assay

HT1080 is a human fibrosarcoma cell line, which expresses human PVR, PVRL2 and PVRL3. Jurkat cells over-expressing human TIGIT was stimulated by engagement of T cell receptor by co-culturing with HT1080 cells over-expressing human TCR activator. 1×10^4 Jurkat/TIGIT cells were co-cultured with 5×10^3 HT1080/TCR-activator cells in the presence of serially diluted anti-TIGIT antibodies at 37 °C, 5% CO₂ for 2 days. After the incubation, the supernatants were collected for IL-2 measurement by ELISA (capture antibody R&D cat#MAB602, detection antibody R&D cat#BAF202). The absorbance was detected using M5e microplate reader (Molecule Devices).

TIGIT-expressing Jurkat cells were co-cultured with HT1080/TCR-activator cells in the presence of antibodies, and IL-2 in the supernatant was quantitated by ELISA. The result demonstrates that W3642-1.433.11-z11-p1-uIgG1L can dose-dependently enhance Jurkat cell activation. The data is shown in Figure 9. A summary of antibody promoting IL-2 secretion is shown in Table 7.

Table 7 Summary of antibody characterizations

Assay	W3642-1.433.11-z11-p1-uIgG1L	WBP364-BMK1	WBP364-BMK4
Binding to human TIGIT engineered cells, EC50 (nM)	0.23	0.50	0.32
Binding to cynomolgus TIGIT engineered cells, EC50 (nM)	0.18	0.47	0.20
Binding to mouse TIGIT engineered cells, EC50 (nM)	0.33	No binding	No binding
Blocking CD155 binding to TIGIT, IC50 (nM)	0.13	0.28	0.21

Blocking CD112 binding to TIGIT, IC50 (nM)	0.52	0.31	0.34
Blocking CD113 binding to TIGIT, IC50 (nM)	0.42	0.28	0.28
NFAT reporter gene assay, EC50 (nM)	2.5	3.0	2.1
IL-2 secretion by Jurkat cells, EC50 (nM)	0.50	~2.58	~0.77

3.9 Human primary NK cell activation assay

HT1080 expressing human PVR, PVRL2 and PVRL3 was used as target cell, while human primary NK cell isolated from human PBMC was used as effector cell. 1×10^4 HT1080 cells pre-loaded with EuTDA (PerkinElmer cat#AD0116) were co-cultured with 1×10^4 human primary NK cells in the presence of serially diluted anti-TIGIT antibodies at 37 °C, 5% CO₂ for 2 hours. Culture supernatants were then tested for target cell lysis by time-resolved fluorescence according to the manufacturer's instructions (PerkinElmer cat#AD0116). Fluorescence was detected using EnVision microplate reader (PerkinElmer).

Human NK cells were co-cultured with HT1080 cells in the presence of antibodies. The result demonstrates that W3642-1.433.11-z11-p1-uIgG1L can dose-dependently enhance NK cell killing activity. The data is shown in Figure 10.

3.10 Antibody-dependent cellular cytotoxicity assay

To evaluate the ADCC effect of anti-TIGIT antibodies on TIGIT-expressing cells, human TIGIT engineered W364-CHOK1.hPro1.2A11 cell was used as target cell, and human primary NK cell isolated from human PBMC was used as effector cell. 1×10^4 target cells and various concentrations of anti-TIGIT antibodies were pre-incubated in a 96-well plate at 37 °C, 5% CO₂ for half an hour, and then 5×10^4 freshly isolated human NK cells were added to each well. The plate was incubated at 37 °C, 5% CO₂ for 6 hours. Target cell lysis was determined by LDH-based cytotoxicity detection kit (Roche cat#04744926001) according to the manufacturer's instruction. The absorbance was detected using M5e microplate reader (Molecule Devices).

The result of ADCC assay is shown in Figure 11. The result displays that W3642-1.433.11-z11-p1-uIgG1L can induce ADCC effect on TIGIT-expressing CHOK1 cells in a dose-dependent manner, the potency of which is similar to that of the reference antibodies.

3.11 Antibody serum stability assay

Human serum was freshly isolated from healthy donor. Anti-TIGIT antibody was diluted in the serum. The samples were aliquoted to 5 tubes and incubated at 37 °C. Samples were then collected on day 0, day 1, day 4, day 7 and day 14, respectively, quick-frozen and stored at a

freezer set to -70 °C until analysis. The binding activity of the samples was evaluated by FACS according to the method described in section 3.1.

The binding of serum incubated W3642-1.433.11-z11-p1-uIgG1L to W364-293F.hPro1.pool cells is shown in Figure 12. The antibody incubated with serum up to two weeks has maintained the similar binding activity and very similar EC50 as the fresh antibody. The result demonstrates that W3642-1.433.11-z11-p1-uIgG1L is stable in human serum at 37 °C for at least two weeks.

3.12 Antibody thermal stability assay

The conformational stability is a very important property for a successful antibody. Conformational stability can be assessed by measuring thermal stability using differential scanning fluorimetry (DSF), which is sensitive to changes in protein folding. DSF measures the temperature of the protein unfolding transition (Tm) based on the change in fluorescence intensity of the environmentally sensitive dye SYPRO Orange.

DSF was carried out in a Quant Studio 7 Flex Real-Time PCR instrument (Applied Biosystems) in the respective formulation buffer. The SYPRO orange dye (Invitrogen cat#S6651) was added to the antibody and transfer the mixture to a 96-well plate. Put the plate in a Quant Studio® 7 Flex Real-Time PCR system, and set up the temperature range from 26 °C to 95 °C with a heating rate of 0.9 °C/min. The first two temperatures of protein unfolding transitions were recorded as Tm1 and Tm2. The two values were calculated according to the melt curve using QuantStudio® Real Time PCR software (v1.3).

DSF thermogram for the W3642-1.433.11-z11-p1-uIgG1L antibody displays 2 transitions: first with a lower (Tm1) and second with a higher (Tm2) melting temperature, 69.0 °C and 79.0 °C, respectively. The result is shown in Table 8.

Table 8 Tm values of antibody

Antibody	Tm1 (°C)	Tm2 (°C)
W3642-1.433.11-z11-p1-uIgG1L	69.0	79.0

EXAMPLE 4

In vivo Characterization

4.1 In-vivo Efficacy of Combo-Therapy of Anti-TIGIT and Anti-PD-1 Antibody

In order to investigate the anti-tumor activity of anti-TIGIT antibodies in MC38 syngeneic mouse model, human TIGIT transgenic h-TIGIT C57BL/6 mice (Jiangsu GemPharmatech Co., Ltd) were used for tumor cell inoculation. h-TIGIT C57BL/6 mice were subcutaneously injected with wild type MC38 tumor cells (1×10⁶) suspended in 0.1 mL DPBS at the right forearm armpit for tumor development. Tumor-bearing animals were randomly enrolled into six study groups when the mean tumor size reached about 73 mm³. Each group consisted of 8 mice. The study

design is shown in Table 9. A monoclonal anti-PD-1 antibody (“anti-mPD-1”, as disclosed in WO2018053709 with clone ID 2E5) was used in combination with the anti-TIGIT antibodies in several groups.

5 All antibodies were intraperitoneally administrated to tumor-bearing mice at a frequency of twice per week. Body weight and tumor volume were measured twice per week. All the procedures related to animal handling, care and the treatment in the study were performed according to the guidelines approved by the Institutional Animal Care and Use Committee (IACUC) of Shanghai Bio-model following the guidance of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). Mice were euthanized according to pre-defined health
10 criteria and the study was terminated twenty-four days post the first dosing.

Table 9 Study design of MC38 model

Groups	Treatment	N/group	Dosage (mg/kg)	Dosing Route	Dosing Frequency	Dosing times
G1	PBS	8	/	IP	BIW	6 times
G2	Anti-mPD-1	8	0.3	IP	BIW	6 times
G3	WBP364-BMK1	8	10	IP	BIW	6 times
G4	W3642-1.433.11-z11-p1-uIgG1L	8	10	IP	BIW	6 times
G5	Anti-mPD-1 + WBP364-BMK1	8	0.3 + 10	IP	BIW	6 times
G6	Anti-mPD-1 + W3642-1.433.11-z11-p1-uIgG1L	8	0.3 + 10	IP	BIW	6 times

This study was to determine the anti-tumor activity of W3642-1.433.11-z11-p1-uIgG1L in h-TIGIT mice inoculated with MC38 tumor cells. All mice were closely monitored for tumor growth and body weight during the entire experiment, with tumor size measured and recorded twice a
15 week. The tumor growth inhibition (TGI) was calculated and analyzed at the best therapeutic time-point (24 days post grouping, the first dosing was performed on the same day as grouping). The results of tumor volume are shown in Figure 13 and summarized in Table 10 and Table 11. The results of body weight change are shown in Figure 14 and indicate that there was no abnormal body weight change in different groups.

20 According to the results, W3642-1.433.11-z11-p1-uIgG1L alone showed weak tumor inhibition effect, but it enhanced the efficacy of anti-mPD-1 antibody in combination. Compared to the combination of WBP364-BMK1 and anti-mPD-1 antibody, the combination of W3642 and the anti-mPD-1 antibody showed an obviously better tumor inhibition result from the beginning of the treatment to the end.

25 Table 10 Summary of tumor volume

Days post grouping	Tumor volume (mm ³) ^a					
	G1	G2	G3	G4	G5	G6
0	73.08±6.68	72.65±6.22	73.98±6.09	73.09±4.64	73.80±5.39	73.51±5.98
3	138.65±21.51	128.98±8.86	137.49±14.18	125.63±12.12	132.34±19.30	112.16±9.37
7	228.22±46.78	165.75±18.54	221.80±39.92	227.21±29.54	141.45±44.44	94.99±19.15
10	394.86±106.40	194.46±26.00	372.44±75.42	330.66±48.02	216.14±81.90	100.59±31.80
14	645.15±160.86	308.91±49.83	611.54±110.48	533.45±80.47	288.89±119.78	121.24±44.96
17	1069.40±253.4 8	435.83±74.73	959.99±213.11	825.23±147.30	450.50±182.32	207.56±84.20
21	1814.22±403.0 6	655.85±146.33	1527.09±310.1 0	1293.32±229.9 6	383.82±122.20	286.65±114.30
24	2176.32±532.1 3	907.09±215.22	2404.98±530.7 3	1834.10±326.1 0	723.05±251.46	432.77±181.64

Note: a, Mean ± SEM.

Table 11 Summary of tumor growth inhibition

Group	Test articles	Dosages (mg/kg)	Tumor volume (mm ³) ^a		TGI (%)	<i>P</i> ^b
			Before treatment	Day 24 post treatment		
G1	PBS	/	73.08±6.68	2176.32±532.13	/	/
G2	Anti-mPD-1	0.3	72.65±6.22	907.09±215.22	60.33	0.037
G3	WBP364-BMK1	10	73.98±6.09	2404.98±530.73	-10.83	0.768
G4	W3642-1.433.11-z11-p1-uIgG1L	10	73.09±4.64	1834.10±326.10	16.27	0.582
G5	Anti-mPD-1 + WBP364-BMK1	0.3 + 10	73.80±5.39	723.05±251.46	69.13	0.030
G6	Anti-mPD-1 + W3642-1.433.11-z11-p1-uIgG1L	0.3 + 10	73.51±5.98	432.77±181.64	82.92	0.006

Note:

5

a, Mean ± SEM.

b, Statistical analysis via independent sample t-test on mean tumor volume of the treatment group versus PBS group on day 24 post grouping.

Those skilled in the art will further appreciate that the present disclosure may be embodied in other specific forms without departing from the spirit or central attributes thereof. In that the foregoing description of the present disclosure discloses only exemplary embodiments thereof, it is to be understood that other variations are contemplated as being within the scope of the present disclosure. Accordingly, the present invention is not limited to the particular embodiments that

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have been described in detail herein. Rather, reference should be made to the appended claims as indicative of the scope and content of the invention.

CLAIMS

1. An isolated antibody or an antigen-binding portion thereof, comprising:
 - a heavy chain CDR (HCDR) 1 comprising the amino acid sequence of SEQ ID NO: 1;
 - a HCDR2 comprising the amino acid sequence of SEQ ID NO: 2;
 - a HCDR3 comprising the amino acid sequence of SEQ ID NO: 3;
 - a light chain CDR (LCDR) 1 comprising the amino acid sequence of SEQ ID NO: 4;
 - a LCDR2 comprising the amino acid sequence of any of SEQ ID NOs: 7, 5, 8 and 9; and
 - a LCDR3 comprising the amino acid sequence of SEQ ID NO: 6.
2. The isolated antibody or the antigen-binding portion thereof of claim 1, comprising a heavy chain variable region (VH) and a light chain variable region (VL), wherein:
 - the VH comprises:
 - (i) the amino acid sequence as set forth in any of SEQ ID NOs: 10-11; or
 - (ii) an amino acid sequence at least 85%, 90%, or 95% identical to any of SEQ ID NOs: 10-11; and
 - the VL comprises:
 - (i) the amino acid sequence as set forth in any of SEQ ID NOs: 12-18; or
 - (ii) an amino acid sequence at least 85%, at least 90%, or at least 95% identical to any of SEQ ID NOs: 12-18.
3. The isolated antibody or the antigen-binding portion thereof of claim 1 or 2, comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 11 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 15.
4. The isolated antibody or the antigen-binding portion thereof of any of the preceding claims, wherein the isolated antibody further comprises a human IgG constant region.
5. The isolated antibody or the antigen-binding portion thereof of claim 4, wherein the human IgG constant region is a human IgG1, IgG4, IgG2 or IgG3 constant region or a variant thereof.
6. The isolated antibody or the antigen-binding portion thereof of any of the preceding claims, wherein the antibody comprises a human IgG1 Fc region or a human IgG4 Fc region with a S228P substitution.

7. The isolated antibody or the antigen-binding portion thereof of any of the preceding claims, wherein the antibody is a chimeric antibody or a humanized antibody.
8. An isolated nucleic acid molecule, comprising a nucleic acid sequence encoding the heavy chain variable region and/or the light chain variable region of the isolated antibody or the antigen-binding portion thereof of any of claims 1-7.
9. A vector comprising the isolated nucleic acid molecule of claim 8.
10. A host cell comprising the vector of claim 9.
11. A pharmaceutical composition comprising the antibody or the antigen-binding portion thereof as defined in any of claims 1-7 and a pharmaceutically acceptable carrier.
12. A method for producing the antibody or the antigen-binding portion thereof as defined in any of claims 1-7 comprising the steps of:
- culturing a host cell comprising an expression vector(s) encoding the antibody or the antigen-binding portion thereof under suitable conditions; and
 - harvesting the antibody or antigen-binding portion thereof from the cell culture.
13. A method for inhibiting growth of tumor cells in a subject, comprising administering an effective amount of the antibody or the antigen-binding portion thereof as defined in any of claims 1-7 or the pharmaceutical composition of claim 11 to the subject.
14. A method for treating or preventing a cancer or an immune related disorder in a subject, comprising administering an effective amount of the antibody or the antigen-binding portion thereof as defined in any of claims 1-7 or the pharmaceutical composition of claim 11 to the subject.
15. The method of claim 14, wherein the cancer is selected from colon cancer, lung cancer, breast cancer, ovarian cancer, melanoma, bladder cancer, renal cell carcinoma, liver cancer, prostate cancer, stomach cancer, pancreatic cancer, lymphoma, leukemia, uterine cancer, cervical cancer, testicular cancer, esophageal cancer, gastrointestinal cancer, gastric cancer, colorectal cancer, kidney cancer, clear cell renal carcinoma, head and neck cancer, germ cell cancer, bone cancer, thyroid cancer, skin cancer, neoplasm of the central nervous system, mesothelioma, myeloma, and sarcoma.

16. The method of claim 14, wherein the immune related disorder is a T cell dysfunctional disorder or an infection.
17. The method of any of claims 14-16, wherein the method further comprises administering an additional therapeutic agent, such as an anti-PD-1 antibody.
18. A combination of the isolated antibody or the antigen-binding portion thereof of any of claims 1-7 with an anti-PD-1 antibody.
19. Use of the antibody or the antigen-binding portion thereof as defined in any of claims 1-7, alone or combined with an anti-PD-1 antibody, in the manufacture of a medicament for treating or preventing cancer or an immune related disorder.
20. The use of claim 19, wherein the cancer is colon cancer or lung cancer.
21. The antibody or the antigen-binding portion thereof as defined in any of claims 1-7 for use in treating or preventing cancer or an immune related disorder.
22. A kit comprising a container comprising the antibody or the antigen-binding portion thereof as defined in any of claims 1-7.