

ANTI-TIGIT X PVRIG ANTIBODIES AND USES THEREOF

CROSS REFERENCE

This application claims the benefit of International patent application PCT/CN2023/098602, filed on June 6, 2023, which is incorporated by reference in its entirety.

5

SEQUENCE LISTING

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

10

FIELD

This application generally relates to antibodies. More specifically, the application relates to antibodies and polypeptide complexes that target TIGIT and PVRIG, a method of preparing the same, and the use of the antibodies.

15

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.

20

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) [1-4]. 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 [3, 5-8]. 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 [9-11]. TIGIT is upregulated and identified as an exhaustion marker in cancer and inflammatory diseases [12-14] as other exhaustion markers like PD-1, LAG3 and TIM3 [15, 16]. 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 [17, 18]. TIGIT could be a promising therapeutic target for tumor immunotherapy as a single agent or in combination with other immune modulators.

25

30

35

On the other hand, poliovirus receptor-related Ig domain containing (PVRIG, a.k.a CD112R) is one of the co-inhibitory immune-checkpoint proteins. It plays an important role in reversion of

T cell exhaustion and increasing NK cell activation [19-22]. PVRIG belongs to the nectin and nectin-like family, among which the family members also includes TIGIT, DNAM-1 (CD226) and CD96. PVRIG is expressed on NK and T cells and is further up-regulated in T cells upon activation [19, 20]. The interaction of PVRIG and its ligand PVRL2 (CD112) expressed on APC and multiple tumor cells results in suppression of T cell and NK cell activation [21, 22]. PVRL2 is also a ligand for CD226, which upon ligand interaction activates human T and NK cells [23-25]. Though PVRIG and TIGIT have many similarities, scientists have reported distinct differences that indicate the pathways are non-redundant [20, 22]. Targeting these pathways may synergistically enhance antitumor response.

Immune checkpoint blockade (anti-CTLA-4, PD-1, and PD-L1 antibodies) might offer the potential for durable remissions for patients across a broad range of cancers, such as non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC), triple negative breast cancer (TNBC) etc. Yet despite this broad applicability, the majority (well over 80%) of cancer patients will be eventually refractory or resistant to the CTLA-4, PD-1/PDL-1 immune checkpoint therapy.

Therefore, there is a need for novel antibodies that could effectively target immune checkpoint molecules and restore T cell functions for cancer therapies.

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.

In some aspects, the present disclosure provides a polypeptide complex, such as a bispecific polypeptide complex, which can bind to TIGIT and PVRIG proteins or antigens. The TIGIT and PVRIG proteins may be of human, cynomolgus monkey or mouse origin, among others. In some embodiments, the polypeptide complex has cross-species reactivity, e.g., can bind human TIGIT, cyno TIGIT and mouse TIGIT. In some embodiments, the polypeptide complex can bind to human PVRIG and cyno PVRIG.

In some embodiments, the polypeptide complex comprises a TIGIT-binding moiety and a PVRIG-binding moiety, wherein:

the TIGIT-binding moiety comprises a heavy chain CDR (HCDR) 1, HCDR2, HCDR3 and a light chain CDR (LCDR) 1, LCDR2 and LCDR3, wherein the HCDR1, HCDR2 and HCDR3 comprise, consists essentially of, or consists of the amino acid sequence of SEQ ID NOs: 1, 2 and 3, respectively; and the LCDR1, LCDR2 and LCDR3 comprise, consists essentially of, or consists of the amino acid sequence of SEQ ID NOs: 4, 5 and 6, respectively; and

the PVRIG-binding moiety comprises a HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, wherein the HCDR1, HCDR2 and HCDR3 comprise, consists essentially of, or

consists of the amino acid sequence of SEQ ID NOs: 7, 8 and 9, respectively; and the LCDR1, LCDR2 and LCDR3 comprise, consists essentially of, or consists of the amino acid sequence of SEQ ID NOs: 10, 11 and 12, respectively.

5 In some embodiments, the polypeptide complex comprises a TIGIT-binding moiety and a PVRIG-binding moiety, wherein:

the TIGIT-binding moiety 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 SEQ ID NO: 5; and a LCDR3 comprising the amino acid sequence of SEQ ID NO: 6; and

the PVRIG-binding moiety comprises:

a HCDR1 comprising the amino acid sequence of SEQ ID NO: 7; a HCDR2 comprising the amino acid sequence of SEQ ID NO: 8; a HCDR3 comprising the amino acid sequence of SEQ ID NO: 9; a LCDR1 comprising the amino acid sequence of SEQ ID NO: 10; a LCDR2 comprising the amino acid sequence of SEQ ID NO: 11; and a LCDR3 comprising the amino acid sequence of SEQ ID NO: 12.

In some embodiments, the TIGIT-binding moiety comprises:

(A) a heavy chain variable region (VH) comprising:

20 (i) the amino acid sequence as set forth in SEQ ID NO: 13;
(ii) an amino acid sequence at least 85%, 90%, or 95% identical to SEQ ID NO: 13; 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 or 1) amino acids compared with SEQ ID NO: 13; and

(B) a light chain variable region (VL) comprising:

25 (i) the amino acid sequence as set forth in SEQ ID NO: 14; or
(ii) an amino acid sequence at least 85%, 90%, or 95% identical to SEQ ID NO: 14; 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 or 1) amino acids compared with SEQ ID NO: 14.

In some embodiments, the PVRIG-binding moiety comprises:

30 (A) a heavy chain variable region (VH) comprising:

(i) the amino acid sequence as set forth in SEQ ID NO: 15;
(ii) an amino acid sequence at least 85%, 90%, or 95% identical to SEQ ID NO: 15; 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 or 1) amino acids compared with SEQ ID NO: 15; and

35 (B) a light chain variable region (VL) comprising:

(i) the amino acid sequence as set forth in SEQ ID NO: 16; or
(ii) an amino acid sequence at least 85%, 90%, or 95% identical to SEQ ID NO: 16; 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 or 1) amino acids compared with SEQ ID NO: 16.

In some embodiments, the TIGIT-binding moiety comprises the HCDR1, HCDR2 and HCDR3 of the VH region as set forth in SEQ ID NO: 13, and the LCDR1, LCDR2 and LCDR3 of the VL region as set forth in SEQ ID NO: 14. In some embodiments, the PVRIG-binding moiety comprises the HCDR1, HCDR2 and HCDR3 of the VH region as set forth in SEQ ID NO: 15, and the LCDR1, LCDR2 and LCDR3 of the VL region as set forth in SEQ ID NO: 16.

In some embodiments, the TIGIT-binding moiety comprises a VH region comprising the amino acid sequence of SEQ ID NO: 13 and a VL region comprising the amino acid sequence of SEQ ID NO: 14. In some embodiments, the PVRIG-binding moiety comprises a VH region comprising the amino acid sequence of SEQ ID NO: 15 and a VL region comprising the amino acid sequence of SEQ ID NO: 16.

In some embodiments, the TIGIT-binding moiety is in Fab or scFv format. In some embodiments, the PVRIG-binding moiety is in Fab or scFv format. In some embodiments, the TIGIT-binding moiety is in Fab format and the PVRIG-binding moiety is in scFv format. In some other embodiments, the TIGIT-binding moiety is in scFv format and the PVRIG-binding moiety is in Fab format.

In some embodiments, the polypeptide complex further comprises a human IgG constant region, such as human IgG1, IgG4, IgG2 or IgG3 constant region. The human IgG constant region may be a native IgG Fc region or a variant thereof. In some embodiments, the polypeptide complex comprises a native human IgG1 Fc region.

In some embodiments, the variant of the Fc region comprises one or more substitutions to modulate receptor binding or effector function, to promote dimerization, to prevent glycosylation, and/or to extend antibody half-life.

In some embodiments, the polypeptide complex comprises one, two or more TIGIT-binding moieties, as well as one, two or more PVRIG-binding moieties. The TIGIT-binding moieties may be identical or different, and/or the PVRIG-binding moieties are identical or different. The polypeptide complex may be a homodimer or heterodimer.

In some embodiments, the polypeptide complex comprises two heavy chains and two light chains, wherein the TIGIT-binding moiety is in Fab format and the PVRIG-binding moiety is in scFv format, and wherein from N-terminus to C-terminus:

the first and second heavy chains each comprises domains operably linked as in VH1-CH1-Fc-scFv or scFv-VH1-CH1-Fc,

the first and second light chains each comprises domains operably linked as in VL1-CL, wherein the VH1-CH1 and VL1-CL are from the TIGIT-binding moiety and the scFv is from the PVRIG-binding moiety. VH1 and VL1 refer to the first VH and first VL in the polypeptide complex, which constitute the TIGIT binding site. VH2 and VL2 refer to the second VH and second VL, which constitute the PVRIG binding site.

In some embodiments, the polypeptide complex comprises two heavy chains and two light chains, wherein the TIGIT-binding moiety is in scFv format and the PVRIG-binding moiety is in Fab format, and wherein from N-terminus to C-terminus:

the first and second heavy chains each comprises domains operably linked as in scFv-
5 VH2-CH1-Fc,
the first and second light chains each comprises domains operably linked as in VL2-CL,
wherein the scFv is from the TIGIT-binding moiety and the VH2-CH1 and VL2-CL are from the PVRIG-binding moiety.

In some embodiments, the polypeptide complex comprises two heavy chains and four light
10 chains, wherein the TIGIT-binding moiety and the PVRIG-binding moiety are in Fab format, and wherein from N-terminus to C-terminus:

the first and second heavy chains each comprises domains operably linked as in VH1-
CH1-Fc-VH2-CH1, VH2-CH1-Fc-VH1-CH1, VH1-CH1-VH2-CH1-Fc or VH2-CH1-VH1-
CH1-Fc,
15 the first and second light chains each comprises domains operably linked as in VL1-CL,
the third and fourth light chains each comprises domains operably linked as in VL2-CL,
wherein the VH1-CH1 and VL1-CL are from the TIGIT-binding moiety and the VH2-CH1
and VL2-CL are from the PVRIG-binding moiety.

In some embodiments, the polypeptide complex comprises two heavy chains and four light
20 chains, wherein the TIGIT-binding moiety and the PVRIG-binding moiety are in Fab format and the TIGIT-binding moiety is a chimeric Fab comprising VH1 operably linked to a first T cell receptor (TCR) constant region (C1), and VL1 operably linked to a second TCR constant region (C2), and wherein from N-terminus to C-terminus:

the first and second heavy chains each comprises domains operably linked as in VH1-C1-
25 Fc-VH2-CH1, VH2-CH1-Fc-VH1-C1, VH1-C1-VH2-CH1-Fc or VH2-CH1-VH1-C1-Fc,
the first and second light chains each comprises domains operably linked as in VL1-C2,
the third and fourth light chains each comprises domains operably linked as in VL2-CL,
wherein the VH1-C1 and VL1-C2 are from the TIGIT-binding moiety and the VH2-CH1
and VL2-CL are from the PVRIG-binding moiety. C1 and C2 refer to a pair of TCR constant
30 regions which can associate together to form a dimer or their engineered variants comprising one or more non-native disulfide bonds.

In some embodiments, the polypeptide complex comprises two heavy chains and four light
chains, wherein the TIGIT-binding moiety and the PVRIG-binding moiety are in Fab format and the PVRIG-binding moiety is a chimeric Fab comprising VH2 operably linked to C1, and VL2
35 operably linked to C2, and wherein from N-terminus to C-terminus:

the first and second heavy chains each comprises domains operably linked as in VH1-
CH1-Fc-VH2-C1, VH2-C1-Fc-VH1-CH1, VH1-CH1-VH2-C1-Fc or VH2-C1-VH1-CH1-Fc,
the first and second light chains each comprises domains operably linked as in VL1-CL,

the third and fourth light chains each comprises domains operably linked as in VL2-C2, wherein the VH1-CH1 and VL1-CL are from the TIGIT-binding moiety and the VH2-C1 and VL2-C2 are from the PVRIG-binding moiety.

In some embodiments, the scFv comprises a VH region operably linked to a VL region
5 directly or via a linker, and the VH region is at the N terminal of the VL region or the VL region is at the N terminal of the VH region.

In some embodiments, the moiety or moieties are operably linked to the Fc region directly or via a linker. The linker may be a peptide linker such as an immunoglobulin whole or partial hinge region (indicated as “hinge” herein), or a conventionally used artificial linker, such as a GS
10 linker. The GS linker as used herein may be (GS)_n, (GGS)_n, (GGGS)_n, (GGGGS)_n, (GGSG)_n, or (GGGSS)_n, wherein n is an integer of 1-9. Preferably, the CH1 or C1 region as disclosed above is operably linked to the Fc region via a hinge region.

In some embodiments, the polypeptide complex comprises:

(i) a first and a second heavy chain that comprise SEQ ID NO: 17, and a first and a second
15 light chain that comprise SEQ ID NO: 18; or

(ii) a first and a second heavy chain that comprise SEQ ID NO: 19, and a first and a second light chain that comprise SEQ ID NO: 20.

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
20 chain variable region of the polypeptide complex as disclosed herein.

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

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

25 In some aspects, the present disclosure provides a pharmaceutical composition comprising the polypeptide complex as disclosed herein and a pharmaceutically acceptable carrier.

In some aspects, the present disclosure provides a method for preparing the polypeptide complex which comprises expressing the polypeptide complex in a host cell comprising an expression vector(s) encoding the polypeptide complex and isolating the polypeptide complex
30 from the culture supernatant.

In some aspects, the present disclosure provides a method of modulating a TIGIT/PVRIG related immune response in a subject, comprising administering the polypeptide complex as disclosed herein to the subject.

In some aspects, the present disclosure provides a method for inhibiting growth of tumor
35 cells in a subject, comprising administering an effective amount of the polypeptide complex or the pharmaceutical composition as disclosed herein, alone or combined with another anti-cancer agent such as an anti-PD-L1 antibody, to the subject.

In some aspects, the present disclosure provides a method for treating or preventing a cancer or an immune disorder in a subject, comprising administering an effective amount of the polypeptide complex 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 or anti-PD-L1 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, 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, leukemia such as chronic lymphocytic leukemia, lymphoma such as diffuse large B cell lymphoma, follicular lymphoma, 15 Hodgkin lymphoma, myeloma, soft-tissue cancer and sarcoma. The immune disorder may be a T cell dysfunctional disorder or an infection.

In some embodiments, the polypeptide complex as disclosed herein is administered in combination with an anti-PD-L1 antibody.

20 In some aspects, the present disclosure provides a combination of the polypeptide complex as disclosed herein with an anti-PD-L1 antibody such as Atezolizumab.

In some aspects, the present disclosure provides use of the polypeptide complex 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 or anti-PD-L1 antibody.

25 In some aspects, the present disclosure provides the use of the polypeptide complex as disclosed herein in the manufacture of a diagnostic agent for diagnosing diseases related to TIGIT/PVRIG overexpression.

30 In some aspects, the present disclosure provides the polypeptide complex 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-L1 antibody.

In some aspects, the present disclosure provides kits or devices that comprise the polypeptide complex as disclosed herein in one or more containers.

35 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

Figure 1 shows a schematic graph of the structure of W3XX104-T4U1.G15-2.uIgG1 and W3XX104-T4U1.G17-2.uIgG1.

5 Figures 2-7 show the binding of antibodies to human TIGIT (Figure 2) and human PVRIG (Figure 3), cynomolgus monkey TIGIT (Figure 4), cynomolgus monkey PVRIG (Figure 5), mouse TIGIT (Figure 6) and mouse PVRIG (Figure 7), as determined by FACS.

Figures 8 shows SPR sensorgrams of antibodies binding to human TIGIT.

Figures 9 shows SPR sensorgrams of antibodies binding to human PVRIG.

10 Figure 10 shows the binding of antibodies to TIGIT and PVRIG paralog proteins, as determined by ELISA.

Figure 11 shows dual binding result of antibodies to human TIGIT and PVRIG.

Figure 12 shows the result of antibodies blocking PVR binding to TIGIT, as determined by FACS.

15 Figure 13 shows the result of antibodies blocking PVRL2 binding to PVRIG, as determined by ELISA.

Figure 14 shows the result of antibodies in Jurkat TIGIT/PVRIG/NFAT-luciferase reporter gene assay.

20 Figures 15-16 show the effect of antibodies in NK cell killing assay (Fig. 15) and T cell activation assay (Fig. 16).

Figures 17A-17B show the melt curve of W3XX104-T4U1.G17-2.uIgG1 (Fig. 17A) and W3XX104-T4U1.G15-2.uIgG1 (Fig. 17B).

Figures 18A-18B show the plotting of diffusion coefficient against concentration of W3XX104-T4U1.G17-2.uIgG1 (Fig. 18A) and W3XX104-T4U1.G15-2.uIgG1 (Fig. 18B).

25 Figures 19A-19B show the retention of W3XX104-T4U1.G17-2.uIgG1 (Fig. 19A) and W3XX104-T4U1.G15-2.uIgG1 (Fig. 19B) in HIC-HPLC.

Figure 20 shows the body weight changes of monkeys in pharmacokinetics study.

Figures 21-24 show the PK profiles of W3XX104-T4U1.G17-2.uIgG1 (Fig. 21 and Fig. 22) and W3XX104-T4U1.G15-2.uIgG1 (Fig. 23 and Fig. 24) in pharmacokinetics study in monkeys.

30 Figure 25 shows the average change in tumor volume in CT26 mouse model.

Figure 26 shows the individual tumor volume changes upon treatment in different groups.

Figure 27 shows tumor weight of different groups at study endpoint (D17) in CT26 mouse model.

35 Figure 28 shows average body weight change of different groups after administration in CT26 mouse model.

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 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 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., 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 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

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, is used in the broadest sense, and encompasses any form of antibody that exhibits the desired biological or binding activity. It

covers, but is not limited to, humanized antibodies, fully human antibodies, chimeric antibodies and single-domain antibodies. The bispecific polypeptide complexes as disclosed herein also belong to antibodies. A common antibody generally comprises heavy chain(s) and light chain(s). Heavy chains may be classified into μ , δ , γ , α and ϵ , which define isotypes of an antibody as IgM, 5 IgD, IgG, IgA and IgE, respectively. Each heavy chain consists of a heavy chain variable region (VH) and a heavy chain constant region (CH). A heavy chain constant region consists of 3 domains (CH1, CH2 and CH3). Each light chain consists of a light chain variable region (VL) and a light chain constant region (CL). As demonstrated herein, various modifications may be made to the constant regions or they may be replaced with 10 other immunoglobulin-derived constant regions. VH and VL region can further be divided into hypervariable regions (called complementary determining regions (CDR)), which are interspaced by relatively conservative regions (called framework region (FR)). Each VH and VL consists of 3 CDRs and 4 FRs in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4 from the N-terminus to the C-terminus. The variable region (VH and VL) 15 of each heavy/light chain pair forms antigen binding sites, respectively. 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 EU definition, and/or the Contact definition, all of which are well known in the art. See, *e.g.*, Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of 20 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.* 1969 May, 63(1):78-85; and Almagro, *J. Mol. Recognit.* 17:132-143 (2004). See also hgmp.mrc.ac.uk and bioinf.org.uk/abs. Correspondence or alignments between numberings according to different definitions can for 25 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 cell receptor variable domains and Ig superfamily V-like domains. *Dev Comp Immunol.* (2003) 27:55–77). Antibodies may be of different antibody isotypes, for example, IgG (*e.g.*, IgG1, IgG2, IgG3 or IgG4 subtype), IgA1, IgA2, 30 IgD, IgE or IgM antibody.

The term “antigen-binding moiety” as used herein refers to an antibody fragment formed from a portion of an antibody comprising one or more CDRs, or any other antibody fragment that binds to an antigen but does not comprise an intact native antibody structure. Examples of antigen-binding moiety include, without limitation, a variable domain, a variable region, a 35 diabody, a Fab, a Fab', a F(ab')₂, an Fv fragment, a single chain Fv fragment (scFv), a disulfide

stabilized Fv fragment (dsFv), a (dsFv)₂, a bispecific dsFv (dsFv-dsFv'), a disulfide stabilized diabody (ds diabody), a multispecific antibody, a camelized single domain antibody, a single variable domain (i.e. VHH), a nanobody, a domain antibody, and a bivalent domain antibody. An antigen-binding moiety is capable of binding to the same antigen to which the parent antibody binds. In certain embodiments, an antigen-binding moiety may comprise one or more CDRs from a particular antibody grafted to a framework region(s) from different antibodies. More detailed formats of antigen-binding moiety are described in Spiess et al, (2015) Molecular Immunology 67: 95-106, and Brinkman et al., mAbs, 9(2), pp.182-212 (2017), which are incorporated herein by their entirety.

“Fab” with regard to an antibody refers to that portion of the antibody consisting of a single light chain (both variable and constant regions) associating to the variable region and first constant region of a single heavy chain by a disulfide bond. In certain embodiments, the constant regions of both the light chain and heavy chain of the Fab may be replaced with TCR constant regions C α and C β or engineered variants thereof.

“Fc” (short for fragment, crystallizable) with regard to an antibody refers to that portion of the antibody comprising the second (CH₂) and third (CH₃) constant regions of a first heavy chain bound to the second and third constant regions of a second heavy chain via disulfide bonding. Fc is a specific dimerization domain. The Fc region as used herein may also comprise part or whole of the hinge region. The Fc region of the antibody is responsible for various effector functions such as ADCC and CDC, but generally does not function in antigen binding. The capacity of antibodies to initiate and regulate effector functions through their Fc domain is a key component of their in vivo protective activity.

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 “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.

The term “human antibody” or “fully human antibody,” as used herein, is intended to include antibodies having variable regions in which both the framework and CDR regions are derived from human germline immunoglobulin sequences. Furthermore, if the antibody contains a constant region, the constant region also is derived from human germline immunoglobulin sequences.

The term “TIGIT” or “T-cell immunoreceptor with Ig and ITIM domains” as used herein refers to 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 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.

The term “PVRIG” or “Poliovirus Receptor Related Immunoglobulin Domain Containing Protein” includes known or wild type PVRIG, or variants, conjugates or fragments (in particular the extracellular domain fragment) thereof. PVRIG is a transmembrane domain protein with a signal peptide, an extracellular domain, a transmembrane domain and a cytoplasmic domain. PVRIG is expressed on the cell surface of NK and T-cells and shares several similarities to other known immune checkpoints. The identification and methods used to show that PVRIG is a checkpoint receptor can be found in WO2016/134333, incorporated herein by reference.

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, 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, CS1003, HLX10, SCT-I10A, 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.

The terms “operably link” and “operably linked” refer to a juxtaposition, with or without a spacer or linker, of two or more biological sequences of interest in such a way that they are in a relationship permitting them to function in an intended manner. When used with respect to polypeptides, it is intended to mean that the polypeptide sequences are linked in such a way that permits the linked product to have the intended biological function. For example, an antibody variable region may be operably linked to a constant region so as to provide for a stable product with antigen-binding activity. An antigen-binding moiety comprising two amino acid chains (such as antibody Fab) may be said to be “operably linked” to a Fc region when one chain of the moiety is linked to one chain of the Fc region in such a way that they can both perform the respective function. The term may also be used with respect to polynucleotides. For one instance, when a polynucleotide encoding a polypeptide is operably linked to a regulatory sequence (e.g., promoter, enhancer, silencer sequence, etc.), it is intended to mean that the polynucleotide sequences are linked in such a way that permits regulated expression of the polypeptide from the polynucleotide. The term operably linked, when used herein to describe domains linked to form a polypeptide, can be represented by a “-”, and can refer to a direct linkage between domains or linkage via a linker comprising 1-30 amino acids in length, such as a single amino acid or a series of (G4S)_n linker, with n=1-5 (1, 2, 3, 4, or 5).

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.

The term “EC₅₀”, 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. The term “IC₅₀”, as used herein, which is also termed as “half maximal inhibitory concentration” refers to the half maximal inhibitory concentration of a drug, antibody or other substances. It is a measure of the effectiveness of the drug, antibody or other substances in inhibiting biological or

biochemical function. In the context of the application, EC₅₀ and IC₅₀ are expressed in the unit of “nM” or “M”.

The ability to “inhibit binding” or “block binding”, as used herein, refers to the ability of an antibody to inhibit the binding interaction between two molecules (e.g., human TIGIT and PVR, human PVRL2) to any detectable degree. In some embodiments, the bispecific polypeptide complexes as disclosed herein block binding between human TIGIT and PVR with an IC₅₀ of no more than 1 nM, no more than 0.8 nM, no more than 0.6 nM, no more than 0.4 nM, or no more than 0.3 nM. In some embodiments, the bispecific polypeptide complexes as disclosed herein block binding between human PVRL2 and PVRL2 with an IC₅₀ of no more than 1 nM, no more than 0.9 nM, or no more than 0.8 nM.

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 “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 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 (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 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 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 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 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, (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 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 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 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.

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., et al. (1991) *Biotechniques* 11:620-627; Johnsson, B., et al. (1995) *J. Mol. Recognit.* 8:125-131; and Johnsson, B., et al. (1991) *Anal. Biochem.* 198:268-277.

5 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 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
10 to those of skill in the art and are commercially available to the public. Examples of such instruments include FACS Star Plus, FACScan and FACSsort instruments from Becton Dickinson (Foster City, Calif.) Epics C from Coulter Epics Division (Hialeah, Fla.) and MoFlo from Cytomation (Colorado Springs, Colo.).

 The terms “subject” and “patient” are used interchangeably and include mammals such as
15 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.

 The term “prevent”, “preventing”, or “prevention”, as used herein in the context of preventing a condition, refers generally to preventing or delaying the onset of the disease, or
20 preventing the manifestation of clinical or subclinical symptoms thereof in a subject (whether a human or animal), for example, preventing the disease from occurring in a subject predisposed to the condition or disease but has not yet been diagnosed as having it.

 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
25 which 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
35 condition, refers 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
5 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,
10 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
15 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

Polypeptide Complexes targeting TIGIT and PVRIG

The polypeptide complexes provided herein include bispecific antibodies and antigen-binding portions thereof. As used herein, the term “polypeptide complex” can be used interchangeably with “antibody”. In some embodiments, the bispecific antibodies and antigen-
25 binding portions thereof have a first specificity for TIGIT (e.g., human, cynomolgus monkey and mouse TIGIT), and a second specificity for PVRIG (e.g., human and cynomolgus monkey PVRIG). Such antibodies may be referred to herein as, e.g., “anti-TIGIT/anti-PVRIG,” or “anti-PVRIG/TIGIT,” or “anti-PVRIGxTIGIT” or “PVRIGxTIGIT” bispecific antibodies, or other similar terminology.

30 In some embodiments, the bispecific antibodies herein comprise a first antigen-binding moiety that specifically binds to PVRIG (PVRIG-binding moiety) and a second antigen-binding moiety that specifically binds to TIGIT (TIGIT-binding moiety). For constructing a bispecific antibody, the first and second antigen-binding moiety may be in Fab, scFv, or VHH format etc., considering the stability, expression level, binding capacity and other functions of the assembled
35 antibody. For example, the TIGIT-binding moiety may be in Fab format, and the PVRIG-binding moiety may be in scFv format; alternatively, the PVRIG-binding moiety is in Fab format, and the TIGIT-binding moiety is in scFv format. In some embodiments, both the TIGIT-binding moiety

and the PVRIG-binding moiety are in Fab format.

In some embodiments, the bispecific antibodies as disclosed herein comprise more than one antigen-binding moieties that specifically bind to PVRIG and/or more than one antigen-binding moieties that specifically binds to TIGIT. Usually, for a bispecific antibody, said more than one
5 antigen-binding moieties have the same variable regions (thus targeting the same antigen/epitope), or are completely the same in the variable regions and constant regions (if present). For example, the antibodies may comprise two same PVRIG-binding moieties and one TIGIT-binding moiety, or one PVRIG-binding moiety and two same TIGIT-binding moieties, or two same PVRIG-binding moieties and two same TIGIT-binding moieties. In addition, where
10 two PVRIG-binding moieties or two TIGIT-binding moieties are present, these moieties may adopt the following formats: (i) the TIGIT-binding moieties are Fabs while the PVRIG-binding moieties are scFvs, (ii) the TIGIT-binding moieties are scFvs while the PVRIG-binding moieties are Fabs, or (iii) the TIGIT-binding moieties and PVRIG-binding moieties are all Fabs.

In some specific embodiments, the TIGIT-binding moiety is in Fab format comprising a
15 first heavy chain variable domain (VH1) operably linked to an antibody heavy chain CH1 domain (VH1-CH1), and a first light chain variable domain (VL1) operably linked to an antibody light chain constant (CL) domain (VL1-CL), and the PVRIG-binding moiety is in scFv format comprising a second heavy chain variable domain (VH2) operably linked to a second light chain variable domain (VL2) (VH2-VL2). The PVRIG-binding scFv may be located at the
20 N-terminal or C-terminal of the heavy chain or light chain of the bispecific polypeptide complex, and within the scFv, VH2 may be at the N-terminal of VL2 or vice versa.

In some specific embodiments, the TIGIT-binding moiety is in scFv format comprising a first VH operably linked to a first VL (VH1-VL1), and the PVRIG-binding moiety is in Fab format comprising a second VH operably linked to an antibody heavy chain CH1 domain (VH2-
25 CH1), and a second VL operably linked to an antibody light chain constant (CL) domain (VL2-CL). The TIGIT-binding scFv may be located at the N-terminal or C-terminal of the heavy chain or light chain of the bispecific polypeptide complex, and within the scFv, VH1 may be at the N-terminal of VL1 or vice versa.

As one example, the bispecific polypeptide complex as disclosed herein may comprise two
30 heavy chains and two light chains, wherein the TIGIT-binding moiety is in Fab format and the PVRIG-binding moiety is in scFv format, and wherein from N-terminus to C-terminus:

- (a) the first and second heavy chains each comprises domains operably linked as in VH1-CH1-hinge-Fc-scFv, the first and second light chains each comprises domains operably linked as in VL1-CL; or
35 (b) the first and second heavy chains each comprises domains operably linked as in scFv-

VH1-CH1-hinge-Fc, the first and second light chains each comprises domains operably linked as in VL1-CL;

wherein the VH1-CH1 and VL1-CL are from the TIGIT-binding moiety and the scFv (VH2-VL2 or VL2-VH2 from N to C terminus) is from the PVRIG-binding moiety.

5 As another example, the bispecific polypeptide complex as disclosed herein may comprise two heavy chains and two light chains, wherein the TIGIT-binding moiety is in scFv format and the PVRIG-binding moiety is in Fab format, and wherein from N-terminus to C-terminus:

(a) the first and second heavy chains each comprises domains operably linked as in VH2-CH1-hinge-Fc-scFv, the first and second light chains each comprises domains operably linked as
10 in VL2-CL; or

(b) the first and second heavy chains each comprises domains operably linked as in scFv-VH2-CH1-hinge-Fc, the first and second light chains each comprises domains operably linked as in VL2-CL;

wherein the scFv (VH1-VL1 or VL1-VH1 from N to C terminus) is from the TIGIT-binding moiety and the VH2-CH1 and VL2-CL are from the PVRIG-binding moiety.
15

In some specific embodiments, the TIGIT-binding moiety is in Fab format comprising a first VH operably linked to an antibody heavy chain CH1 domain (VH1-CH1), and a first VL operably linked to an antibody light chain constant (CL) domain (VL1-CL), and the PVRIG-binding moiety is also in Fab format comprising a second heavy chain variable domain operably
20 linked to an antibody heavy chain CH1 domain (VH2-CH1), and a second VL operably linked to an antibody light chain constant (CL) domain (VL2-CL). The TIGIT-binding moieties may be located on the N-terminal and the PVRIG-binding moieties on the C-terminal of the Fc region, or the TIGIT-binding moieties may be located on the C-terminal and the PVRIG-binding moieties on the N-terminal of the Fc region, i.e. the Fc region is located between the TIGIT-binding
25 moieties and the PVRIG-binding moieties. In some other embodiments, the TIGIT-binding moieties and the PVRIG-binding moieties are all located at the N terminal of the Fc region. For stability of dimerization, the CH1 and CL of the TIGIT-binding moieties or the PVRIG-binding moieties may be replaced by a pair of TCR constant regions, to distinguish between the TIGIT-binding Fabs and PVRIG-binding Fabs. Thus, in some embodiments, the CH1 domain is
30 replaced by a first TCR constant region (C1, i.e. wild-type or engineered TCR beta chain constant region) and the CL domain is replaced by a second TCR constant region (C2, i.e. wild-type or engineered TCR alpha chain constant region). Alternatively, they may be exchanged, with TCR beta chain constant region in the light chain and TCR alpha chain constant region in the heavy chain.

35 The TCR (T cell receptor) is a heterodimeric T cell surface protein that belongs to the

immunoglobulin superfamily and is similar to a half antibody with a single heavy chain and a single light chain. A native TCR has an extracellular portion, a transmembrane portion, and an intracellular portion. The extracellular domain of a TCR has a membrane-proximal constant region and a membrane-distal variable region. The introduction of TCR constant regions to replace the commonly used CH1 and CL domains have been shown to increase the stability and expression level of the generated antibody format. A detailed description of the utility of TCR constant regions in assembling two parental antibodies into a bispecific molecule with desired valency and functionality have been disclosed in WO2019/057122, the full content of which is herein incorporated by reference. The replacement by TCR constant regions results a chimeric Fab that possesses a unique light-heavy chain interface orthogonal to that of a regular antibody Fab. The assembly of the chimeric and regular Fabs in different formats can create various bispecific molecules with different structures and valences.

The pair of TCR constant regions in the chimeric Fab includes TCR alpha and beta constant regions (wild-type or preferably engineered) in the light chain and heavy chain respectively. In some embodiments, the chimeric Fab comprises a first TCR constant region and a second TCR constant region that are associated via a non-native interchain disulfide bond. The TCR constant regions may be engineered to introduce a non-native disulfide bonds between the light-heavy chain interface. In some other embodiments, the chimeric Fab comprises a pair of wild type TCR constant regions, such as wild type human TCR beta and alpha constant regions.

The sequences of constant regions of wild type human TCR beta and alpha chain can be found in NCBI accession number A0A5B9 (www.uniprot.org/uniprot/A0A5B9) and NCBI accession number P01848 (www.uniprot.org/uniprot/P01848). The pair of TCR constant regions for constructing the bispecific antibodies herein are derived from the wild type TCR constant regions, with one or more substitutions, additions or deletions of one or more amino acids. The bispecific antibody may comprise an engineered TCR beta chain constant region and an engineered TCR alpha chain constant region (referred to as C1 and C2 herein). As illustrated in the present application, the bispecific antibody may comprise an engineered TCR beta chain constant region (C1) with the sequence as shown in SEQ ID NO: 21 (LEDLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFYDPDHVELSWWNGKEVHSGVC TDPQPLKEQPALQDSRYALSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAK PVTQIVSAEAWGR) and an engineered TCR alpha chain constant region (C2) with the sequence as shown in SEQ ID NO: 22 (PDIQNPDCAVYQLRDSKSSDKSVCLFTDFDSQTQVSQSKSDVYITDKCVLDMRSMDF KSNSAWAWSQKSDFACANAFQNSIIPECTFFPS). Multiple TCR constant region variants for constructing bispecific antibody formats have been disclosed in PCT/CN2021/072601, the full

content of which is incorporated herein by reference.

As one example, the bispecific polypeptide complex as disclosed herein comprise two heavy chains and four light chains, wherein the TIGIT-binding moiety is in Fab format and the PVRIG-binding moiety is in Fab format, and wherein from N-terminus to C-terminus:

5 the first and second heavy chains each comprises domains operably linked as in VH1-C1-VH2-CH1-hinge-Fc or VH2-CH1-VH1-C1-hinge-Fc (VH1-C1 is from the TIGIT-binding moiety, VH2-CH1 is from the PVRIG-binding moiety); two light chains comprising domains operably linked as in VL1-C2 (VL1-C2 is from the TIGIT-binding moiety); and the other two light chains comprising domains operably linked as in VL2-CL (VL2-CL is from the PVRIG-binding moiety).

As another example, the bispecific polypeptide complex as disclosed herein comprise two heavy chains and four light chains, wherein the TIGIT-binding moiety is in Fab format and the PVRIG-binding moiety is in Fab format, and wherein from N-terminus to C-terminus:

15 the first and second heavy chains each comprises domains operably linked as in VH1-C1-hinge-Fc-VH2-CH1 or VH2-CH1-hinge-Fc-VH1-C1 (VH1-C1 is from the TIGIT-binding moiety, VH2-CH1 is from the PVRIG-binding moiety); two light chains comprising domains operably linked as in VL1-C2 (VL1-C2 is from the TIGIT-binding moiety); and the other two light chains comprising domains operably linked as in VL2-CL (VL2-CL is from the PVRIG-binding moiety).

20 As a further example, the bispecific polypeptide complex as disclosed herein comprise two heavy chains and four light chains, wherein the TIGIT-binding moiety is in Fab format and the PVRIG-binding moiety is in Fab format, and wherein from N-terminus to C-terminus:

25 the first and second heavy chains each comprises domains operably linked as in VH1-CH1-VH2-C1-hinge-Fc or VH2-C1-VH1-CH1-hinge-Fc (VH1-CH1 is from the TIGIT-binding moiety, VH2-C1 is from the PVRIG-binding moiety); two light chains comprising domains operably linked as in VL1-CL (VL1-CL is from the TIGIT-binding moiety); and the other two light chains comprising domains operably linked as in VL2-C2 (VL2-C2 is from the PVRIG-binding moiety).

30 Still as a further example, the bispecific polypeptide complex as disclosed herein comprise two heavy chains and four light chains, wherein the TIGIT-binding moiety is in Fab format and the PVRIG-binding moiety is in Fab format, and wherein from N-terminus to C-terminus:

35 the first and second heavy chains each comprises domains operably linked as in VH1-CH1-hinge-Fc-VH2-C1 or VH2-C1-hinge-Fc-VH1-CH1 (VH1-CH1 is from the TIGIT-binding moiety, VH2-C1 is from the PVRIG-binding moiety); two light chains comprising domains operably linked as in VL1-CL (VL1-CL is from the TIGIT-binding moiety); and the other two

light chains comprising domains operably linked as in VL2-C2 (VL2-C2 is from the PVRIG-binding moiety).

In some embodiments, the bispecific antibodies as disclosed herein comprises one TIGIT-binding moiety and two PVRIG-binding moieties per antibody, or two TIGIT-binding moieties
5 and one PVRIG-binding moiety per antibody. These moieties may be in Fab or scFv format.

As one example, the bispecific polypeptide complex herein comprises two heavy chains and two light chains, wherein the TIGIT-binding moiety is in Fab format and the PVRIG-binding moiety is in scFv format and wherein from N-terminus to C-terminus:

the first heavy chain comprises domains operably linked as in VH1-CH1-hinge-Fc-scFv or
10 scFv-VH1-CH1-hinge-Fc, the second heavy chain comprises domains operably linked as in VH1-CH1-hinge-Fc; the two light chain comprises domains operably linked as in VL1-CL,

wherein the VH1-CH1 and VL1-CL are from the TIGIT-binding moiety and the scFv (VH2-VL2 or VL2-VH2 from N to C terminus) is from the PVRIG-binding moiety.

As another example, the bispecific polypeptide complex herein comprises two heavy chains
15 and two light chains, wherein the TIGIT-binding moiety is in scFv format and the PVRIG-binding moiety is in Fab format and wherein from N-terminus to C-terminus:

the first heavy chain comprises domains operably linked as in VH2-CH1-hinge-Fc-scFv or scFv-VH2-CH1-hinge-Fc, the second heavy chain comprises domains operably linked as in VH2-CH1-hinge-Fc; the two light chain comprises domains operably linked as in VL2-CL,

20 wherein the VH2-CH1 and VL2-CL are from the PVRIG-binding moiety and the scFv (VH1-VL1 or VL1-VH1 from N to C terminus) is from the TIGIT-binding moiety.

Depending on the bispecific format and/or numbering convenience, the numbering sequences as disclosed herein, such as first or second, could be different. For example, the first VH may be numbered as the second VH, and the first VL may be numbered as the second VL.

25 As another example, the second antigen-binding moiety may be numbered as the first antigen-binding moiety, and the first antigen-binding moiety may be numbered as the second antigen-binding moiety, depending on preference and/or convenience.

CDRs and variable regions of the bispecific polypeptide complex

30 In some embodiments, the bispecific polypeptide complex or antigen-binding portion thereof comprises a first antigen-binding moiety that specifically binds to TIGIT (preferably human TIGIT) and a second antigen-binding moiety that specifically binds to PVRIG (preferably human PVRIG), wherein the first and second antigen-binding moieties are derived from an anti-TIGIT antibody and anti-PVRIG antibody, respectively. The parental antibodies may be already
35 developed and known to the public, or developed de novo. By “derived from”, it is generally

meant herein that the antigen-binding moiety comprises the CDR sequences or highly homologous CDR sequences of the parent antibody, and preferably, comprises the variable regions of the parent antibody. The antigen-binding moiety may also comprise the variants of the CDR sequences of the parent antibody which retain the antigen-binding specificity. For example,
5 compared to the original CDR sequences of parent antibody, one or two amino acids in one or more CDR regions may be modified to reduce glycosylation and deamidation risk.

Specifically, in the bispecific antibodies as exemplified herein, the TIGIT-binding moiety comprises:

- A) one or more heavy chain CDRs (HCDRs) selected from the group consisting of:
- 10 (i) a HCDR1 comprising the amino acid sequence of SEQ ID NO: 1 or an amino acid sequence with addition, deletion and/or substitution of no more than 1, 2 or 3 amino acids compared with SEQ ID NO: 1;
- (ii) a HCDR2 comprising the amino acid sequence of SEQ ID NO: 2 or an amino acid sequence with addition, deletion and/or substitution of no more than 1, 2 or 3 amino acids
15 compared with SEQ ID NO: 2; and
- (iii) a HCDR3 comprising the amino acid sequence of SEQ ID NO: 3 or an amino acid sequence with addition, deletion and/or substitution of no more than 1, 2 or 3 amino acids compared with SEQ ID NO: 3;
- B) one or more light chain CDRs (LCDRs) selected from the group consisting of:
- 20 (i) a LCDR1 comprising the amino acid sequence of SEQ ID NO: 4 or an amino acid sequence with addition, deletion and/or substitution of no more than 1, 2 or 3 amino acids compared with SEQ ID NO: 4;
- (ii) a LCDR2 comprising the amino acid sequence of SEQ ID NO: 5 or an amino acid sequence with addition, deletion and/or substitution of no more than 1, 2 or 3 amino acids
25 compared with SEQ ID NO: 5; and
- (iii) a LCDR3 comprising the amino acid sequence of SEQ ID NO: 6 or an amino acid sequence with addition, deletion and/or substitution of no more than 1, 2 or 3 amino acids compared with SEQ ID NO: 6; or
- C) one or more HCDRs of A) and one or more LCDRs of B); and
- 30 the PVRIG-binding moiety comprises:
- A') one or more HCDRs selected from the group consisting of:
- (i) a HCDR1 comprising the amino acid sequence of SEQ ID NO: 7 or an amino acid sequence with addition, deletion and/or substitution of no more than 1, 2 or 3 amino acids compared with SEQ ID NO: 7;

- (ii) a HCDR2 comprising the amino acid sequence of SEQ ID NO: 8 or an amino acid sequence with addition, deletion and/or substitution of no more than 1, 2 or 3 amino acids compared with SEQ ID NO: 8; and
- (iii) a HCDR3 comprising the amino acid sequence of SEQ ID NO: 9 or an amino acid sequence with addition, deletion and/or substitution of no more than 1, 2 or 3 amino acids compared with SEQ ID NO: 9;
- B') one or more LCDRs selected from the group consisting of:
- (i) a LCDR1 comprising the amino acid sequence of SEQ ID NO: 10 or an amino acid sequence with addition, deletion and/or substitution of no more than 1, 2 or 3 amino acids compared with SEQ ID NO: 10;
- (ii) a LCDR2 comprising the amino acid sequence of SEQ ID NO: 11 or an amino acid sequence with addition, deletion and/or substitution of no more than 1, 2 or 3 amino acids compared with SEQ ID NO: 11; and
- (iii) a LCDR3 comprising the amino acid sequence of SEQ ID NO: 12 or an amino acid sequence with addition, deletion and/or substitution of no more than 1, 2 or 3 amino acids compared with SEQ ID NO: 12; or
- C') one or more HCDRs of A') and one or more LCDRs of B').

In some embodiments, the above described CDRs of the anti-TIGIT moiety are identified according to Contact definition, while the CDRs of the anti-PVRIG moiety are identified according to IMGT/Kabat definition. Variable regions and CDRs in an antibody sequence can be identified according to general rules that have been developed in the art or by aligning the sequences against a database of known variable regions. CDRs have been described by Kabat et al., J. Biol. Chem. 252:6609-6616 (1977); Kabat et al., U.S. Dept. of Health and Human Services, "Sequences of proteins of immunological interest" (1991); by Chothia et al., J. Mol. Biol. 196:901-917 (1987); and MacCallum et al., J. Mol. Biol. 262:732-745 (1996), where the definitions include overlapping or subsets of amino acid residues when compared against each other. Nevertheless, application of any definition to refer to a CDR of the antibody as disclosed herein is intended to be within the scope of present application. Although CDRs indicated in Table A below are defined by the Contact, or Kabat and IMGT numbering system, the Chothia, MacCallum, and other methods known in the art could also be used to define the CDRs. Methods for identifying these regions are described in e.g., Kontermann and Dubel, eds., 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 www.vbase2.org, as described in Retter *et al.*, Nucl. Acids Res., 33 (Database issue): D671 - D674 (2005). Preferably sequences are 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., 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.

10 In some specific embodiments, the TIGIT-binding moiety comprises a heavy chain variable region (VH) and a light chain variable region (VL), wherein

(a) the VH comprises: a HCDR1 as set forth in SEQ ID NO: 1; a HCDR2 as set forth in SEQ ID NO: 2; and a HCDR3 as set forth in SEQ ID NO: 3; and

(b) the VL comprises: a LCDR1 as set forth in SEQ ID NO: 4; a LCDR2 as set forth in SEQ ID NO: 5; and a LCDR3 as set forth in SEQ ID NO: 6.

In some specific embodiments, the PVRIG-binding moiety comprises a heavy chain variable region (VH) and a light chain variable region (VL), wherein

(a) the VH comprises: a HCDR1 as set forth in SEQ ID NO: 7; a HCDR2 as set forth in SEQ ID NO: 8; and a HCDR3 as set forth in SEQ ID NO: 9; and

20 (b) the VL comprises: a LCDR1 as set forth in SEQ ID NO: 10; a LCDR2 as set forth in SEQ ID NO: 11; and a LCDR3 as set forth in SEQ ID NO: 12.

In some embodiments, the bispecific antibody or antigen-binding portion thereof comprises a first antigen-binding moiety that specifically binds to TIGIT, wherein the first antigen-binding moiety comprises:

25 (A) a heavy chain variable region:

(i) comprising the amino acid sequence of SEQ ID NO: 13;

(ii) comprising an amino acid sequence at least 85%, 90%, or 95% identical (preferably, at least 90%, more preferably, at least 95% (e.g., 95%, 96%, 97%, 98%, or 99%)) to SEQ ID NO: 13; or

30 (iii) comprising an amino acid sequence with addition, deletion and/or substitution of one or more (e.g., one, two, three, or more, preferably one, two, or three, more preferably, one or two) amino acids compared with SEQ ID NO: 13; and/or

(B) a light chain variable region:

(i) comprising the amino acid sequence of SEQ ID NO: 14;

(ii) comprising an amino acid sequence at least 85%, at least 90%, or at least 95% identical (preferably, at least 90%, more preferably, at least 95% (e.g., 95%, 96%, 97%, 98%, or 99%)) to SEQ ID NO: 14; or

5 (iii) comprising an amino acid sequence with addition, deletion and/or substitution of one or more (e.g., one, two, three, or more, preferably one, two, or three, more preferably, one or two) amino acids compared with SEQ ID NO: 14.

In some further embodiments, the bispecific antibody or antigen-binding portion thereof comprises a second antigen-binding moiety that specifically binds to PVRIG, wherein the second antigen-binding moiety comprises:

10 (A) a heavy chain variable region:

(i) comprising the amino acid sequence of SEQ ID NO: 15;

(ii) comprising an amino acid sequence at least 85%, 90%, or 95% identical (preferably, at least 90%, more preferably, at least 95% (e.g., 95%, 96%, 97%, 98%, or 99%)) to SEQ ID NO: 15; or

15 (iii) comprising an amino acid sequence with addition, deletion and/or substitution of one or more (e.g., one, two, three, or more, preferably one, two, or three, more preferably, one or two) amino acids compared with SEQ ID NO: 15; and/or

(B) a light chain variable region:

(i) comprising the amino acid sequence of SEQ ID NO: 16;

20 (ii) comprising an amino acid sequence at least 85%, at least 90%, or at least 95% identical (preferably, at least 90%, more preferably, at least 95% (e.g., 95%, 96%, 97%, 98%, or 99%)) to SEQ ID NO: 16; or

(iii) comprising an amino acid sequence with addition, deletion and/or substitution of one or more (e.g., one, two, three, or more, preferably one, two, or three, more preferably, one or two) amino acids compared with SEQ ID NO: 16.

25 Preferably, an amino acid sequence at least 85%, at least 90%, or at least 95% identical to SEQ ID NO: 13, 14, 15 or 16 has the same CDR sequences as SEQ ID NO: 13, 14, 15 or 16, and the amino acid changes only take place in the framework regions. 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
30 <http://www.gcg.com>), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight
35

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 present 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 specific embodiments, the heavy chain variable region of the TIGIT-binding moiety is consisted of the amino acid sequence of SEQ ID NO: 13, and the light chain variable region of the TIGIT-binding moiety is consisted of the amino acid sequence of SEQ ID NO: 14, and/or the heavy chain variable region of the PVRIG-binding moiety is consisted of the amino acid sequence of SEQ ID NO: 15, and the light chain variable region of the PVRIG-binding moiety is consisted of the amino acid sequence of SEQ ID NO: 16.

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

In some further embodiments, the bispecific 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.

As described above, the term “conservative substitution” 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).

20

Antigen binding of the bispecific polypeptide complex

Preferably, the bispecific polypeptide complex of the disclosure is capable of binding to human TIGIT and PVRIG. The binding of an antibody to TIGIT and PVRIG can be assessed using one or more techniques well established in the art, for instance, ELISA or FACS, which measures binding of the antibody to soluble TIGIT/PVRIG protein or TIGIT/PVRIG protein expressed on cell surfaces, respectively. For example, an antibody can be tested by a flow cytometry assay in which the antibody is reacted with a cell line that expresses human TIGIT or human PVRIG, such as CHO cells that have been transfected to express TIGIT or PVRIG on their cell surface, or a TIGIT or PVRIG positive cell line, or a TIGIT and PVRIG double positive cell line.

30

Additionally or alternatively, the binding of the antibody, including the binding kinetics (e.g., K_D value) can be tested in BIAcore binding assays. For instance, the bispecific antibodies of the disclosure could bind to a human TIGIT protein with a K_D of 1×10^{-10} M or less, a K_D of 8×10^{-11} M or less, a K_D of 6×10^{-11} M or less, a K_D of 4×10^{-11} M or less, or a K_D of 2×10^{-11} M or less, and could bind to a human PVRIG protein with a K_D of 5×10^{-9} M or less, a K_D of 4×10^{-9} M

35

or less, or a K_D of 3×10^{-9} M or less, as measured by Surface Plasmon Resonance.

Functionality of the BsAbs

The bispecific antibodies as disclosed herein are characterized by particular functional
5 features or properties. In some embodiments, the antibodies have one or more of the following properties:

- (a) specifically bind to human, cyno and mouse TIGIT;
- (b) specifically bind to human and cyno PVRIG;
- (c) could simultaneously bind to both TIGIT and PVRIG;
- 10 (d) efficiently block the binding of PVRIG to PVRL2 and TIGIT to PVR;
- (e) have no cross-activity to paralog proteins of TIGIT and PVRIG;
- (f) show better effect in Reporter Gene assay, NK cell killing and T cell activation assays than control antibodies (WBP364-BMK1 and WBPT117-BMK1) and even the combination of the control antibodies;
- 15 (g) have good antibody developability, including thermal stability, solubility, hydrophobicity and stress stability;
- (h) have acceptable pharmacokinetic profiles in monkeys; and
- (i) show significant anti-tumor efficacy in CT26 mouse model.

In some embodiments, the control antibody is a monoclonal antibody, such as a monoclonal
20 anti-TIGIT antibody. In some embodiments, the control antibody is WBP364-BMK1 as shown in Table 1. In some embodiments, the control antibody is a monoclonal anti-PVRIG antibody. In some embodiments, the control antibody is WBPT117-BMK1 as shown in Table 1. In some embodiments, the control antibody is the parental antibody from which the bispecific antibodies are derived and constructed. In some embodiments, the control antibody is W3642 and/or
25 WT1175.

In some embodiments, the bispecific polypeptide complexes as disclosed herein have a higher binding affinity to TIGIT as compared to monospecific anti-TIGIT antibodies or other anti-TIGIT/PVRIG bispecific antibodies. In some embodiments, the bispecific polypeptide complexes as disclosed herein have binding affinity to TIGIT that is at least 10%, 20%, 30%,
30 40%, 50%, 60%, 70%, 80%, 90%, or 100% higher than monospecific anti-TIGIT antibodies, as measured by SPR or FACS.

In some embodiments, the bispecific polypeptide complexes as disclosed herein have a higher or comparable binding affinity to PVRIG as compared to monospecific anti-PVRIG antibodies or other anti-TIGIT/PVRIG bispecific antibodies. In some embodiments, the
35 bispecific polypeptide complexes as disclosed herein have binding affinity to PVRIG that is at

least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% higher than monospecific anti-PVRIG antibodies or other anti-TIGIT/PVRIG bispecific antibodies, as measured by FACS.

The anti-TIGIT/PVRIG antibodies as disclosed herein can inhibit interaction between TIGIT and its ligand PVR (CD155) and between PVRIG and PVRL2 (CD112). The blockade of these signaling pathways can e.g., restore a functional response by T-cells (e.g., proliferation, cytokine production, target cell killing) from a dysfunctional state to antigen stimulation.

The ability of an antibody to inhibit interactions between antigens and their ligands may be evaluated by measuring whether physical interactions between e.g. TIGIT and PVR 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™).

The multiple blockade of TIGIT, PVRIG and PD-1/PD-L1 could reverse immune suppression. The bispecific antibodies of the present disclosure have the potential to exhibit 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 PD-1/PD-L1, CD47M, GM-CSF, CSF1R, TLR, RIGI, TAM receptor kinase, NKG2A, NKG2D, GD2, EGFR, PDGFR α , SLAMF7, VEGF, CTLA-4, CD20, cCLB8, KIR, and CD52. In some embodiments, the additional therapeutic agent is selected from an anti-PD-1 antibody, anti-PD-L1 antibody, anti-CD47M antibody, anti-CSF1R antibody, anti-TLR antibody, anti-RIGI antibody, anti-TAM receptor kinase antibody, anti-NKG2A antibody, anti-CD25 antibody, an anti-NKG2D antibody, an anti-GD2 antibody, an anti-PDGFR- α antibody, an anti-SLAMF7 antibody, an anti-VEGF 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 Hul4.18K322A, Hu3F8, dinituximab, olaratumab, elotuzumab, ramucirumab, bevacizumab, rituximab, siltuximab, lirilumab, and alemtuzumab.

Fc region

The present disclosure provides polypeptide complexes comprising a TIGIT-binding moiety, a PVRIG-binding moiety, and a dimerization pair comprising two dimerization domains that

promote assembly of at least two polypeptide chains. The dimerization pair may comprise, for example and without limitation, constant regions of an immunoglobulin, including for example a Fc, CH2 and/or CH3 domain of a heavy chain immunoglobulin.

In certain embodiments, the bispecific antibodies and antigen-binding portions thereof provided herein may 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 IgG1 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 an Fc variant. In certain embodiments, the bispecific polypeptide complexes disclosed herein comprise a variant Fc region possessing one or more amino acid modifications (e.g. Leu234Ala/Leu235Ala or LALA, according to EU numbering as in Kabat et al.) 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 some embodiments, the Fc region may comprise one or more amino acid changes (e.g., insertions, deletions or substitutions) to obtain the desired functionality, e.g., a “knob into hole” structure to promote heterodimerization (if necessary).

The term “knob into hole”, as used herein, refers to engineering the CH3 domain of antibody Fc region to create either a “knob” or a “hole” in each heavy chain to promote heterodimerization. A knob can be obtained by replacement of a small amino acid residue with a larger one in the first CH2/CH3 polypeptide, and a hole can be obtained by replacement of a large residue with a smaller one. For details of the mutation sites for knobs into holes please see Spiess et al., 2015, supra and Brinkmann et al., 2017, supra. Generally, a “knob” is created by replacing T366 with a bulky residue W on one heavy chain, and the corresponding “hole” is made by triple mutations of T366S, L368A and Y407V on the other heavy chain, according to EU numbering as in Kabat et al. In some embodiments, the heavy chain of the bispecific antibody comprising C1 has the “hole” structure, while the heavy chain comprising C2 has the “knob” structure. Alternatively, the heavy chain of the bispecific antibody comprising C1 has the “knob” structure, while the heavy chain comprising C2 has the “hole” structure.

In certain embodiments, the Fc region of the bispecific antibodies herein is an 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 (according to EU numbering as in Kabat et al). 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.

In some embodiments, the Fc region may be modified so as to prevent glycosylation, to extend its half-life, to modulate receptor binding or effector function. Exemplary mutations are discussed in Saunders K.O. (Front. Immunol. 10:1296, 2019 the entire content of which is incorporated herein by reference) and include for example mutation of asparagine 297 (e.g., N297, according to EU numbering as in Kabat et al).

In exemplary embodiments, the polypeptide complexes as disclosed herein may have a mutated constant region or Fc region having a sequence which is from 80% to 99% identical with that of a natural IgG1, IgG2, IgG3 or IgG4 constant region or Fc region. For example, a mutated Fc region that is from 85% to 99% identical, from 90% to 99% identical, from 95% to 99% identical with that of a natural IgG1, IgG2, IgG3 or IgG4 Fc region is encompassed herein.

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.

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 bispecific antibodies as disclosed herein. In some embodiments, the nucleic acid molecule comprises a nucleic acid sequence encoding the heavy chain of the bispecific antibodies as disclosed herein. In some embodiments, the nucleic acid molecule comprises a nucleic acid sequence encoding the light chain of the bispecific antibodies 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.

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 chain constant region can be an IgG1, IgG2, IgG3, IgG4, IgA, IgE, IgM or IgD constant region, but 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 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. 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 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 TIGIT-binding moiety or the PVRIG-binding moiety) or heavy chain of the bispecific antibodies as disclosed herein.

In some specific embodiments, the isolated nucleic acid molecule comprises:

(A) a nucleic acid sequence that encodes an amino acid sequence as set forth in SEQ ID NO: 13, 15, 17 or 19;

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

(C) a nucleic acid sequence with 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 the nucleic acid sequence of (A).

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 TIGIT-binding moiety or the PVRIG-binding moiety) or light chain of the bispecific antibodies as disclosed herein.

In some specific embodiments, the isolated nucleic acid molecule comprises:

(A) a nucleic acid sequence that encodes a light chain variable region as set forth in SEQ ID NO: 14, 16, 18 or 20;

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

(C) a nucleic acid sequence with 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 the nucleic acid sequence of (A).

In some 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.

15

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, such as 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

35

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
5 embodiments, the host cells herein are selected from CHO, CHO-S, HEK, HEK293, HEK-293F, Expi293F, PER.C6 or NSO cells or 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,
10 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
15 microorganisms. However, a number of other genera, species, and strains are commonly available and useful herein, such as Schizo saccharomyces 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. drosophilarum (ATCC 36,906), K. thermotolerans, and K. marxianus; yarrowia (EP 402,226); Pichia pastoris (EP 183,070); Candida; Trichoderma reesia
20 (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
25 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 (DNA or RNA) encoding the antibody as disclosed herein and a pharmaceutically acceptable carrier. In some aspects, the present disclosure provides a
35 pharmaceutical composition comprising a cell expressing the antibody 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.
- 5 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 known in the art various combinations of components or more.
- 10 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
- 15 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
- 20 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 increased activity.
- 25 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,
- 30 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,
- 35 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.

5

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, 10 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 15 according to the intended application and therapeutic regimen.

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 20 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 25 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 30 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 35 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
5 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,
10 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;
15 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
20 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
25 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,
30 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
35 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.

In some embodiments, compatible formulations for parenteral administration (e.g., intravenous injection) 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/PVRIG 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 bispecific antibodies are administered together with another agent such as an anti-PD-L1 agent, the two can be administered in either order or simultaneously.

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 bispecific antibodies or antigen-binding portion thereof as disclosed herein, e.g. in combination 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
 5 natural killer (NK) cells that express TIGIT. In some embodiments, the cancer is a cancer that is enriched for expression of PRVL2. In some embodiments, the cancer is a cancer that is enriched for T cells or natural killer (NK) cells that express PVRIG.

Examples of cancer include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies. More particular examples of such cancers
 10 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), 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,
 15 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 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
 20 (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 carcinoma; penile carcinoma; melanoma, including superficial spreading melanoma, lentigo maligna melanoma, acral lentiginous melanomas, and nodular melanomas;
 25 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
 30 leukemia (CLL); acute lymphoblastic leukemia (ALL); acute myelogenous leukemia (AML); hairy cell leukemia; chronic myeloblastic leukemia (CML); 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.

35 As a co-inhibitory receptor on a wide variety of immune cells, TIGIT or PVRIG is implicated in a 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. The cancers may be solid cancers or hematologic malignancies. Examples of such cancers include

lung cancers such as bronchogenic 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

5 osteochondromas, condromas, chondroblastomas, chondromyxoid fibromas, osteoid osteomas, giant cell tumors, 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, chordoma, Schwannomas, ependymomas, meningiomas,

10 pituitary adenoma, pinealoma, osteomas, hemangioblastomas, craniopharyngiomas, chordomas, 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,

15 hemangioma, hepatocellular carcinoma, fibrolamellar carcinoma, cholangiocarcinoma, hepatoblastoma, and 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

20 and intraocular melanocarcinoma; male reproductive system cancers such as benign prostatic hyperplasia, prostate 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

25 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.

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

30 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 cell exhaustion. In some embodiments, the T cells are CD4⁺ and CD8⁺ T

35 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

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.

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 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 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 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.

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
5 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
10 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,
15 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;
20 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
25 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
30 regimens such as CHOP or FOLFIRI.

Examples of anti-cancer agents that may be used in combination with the bispecific antibodies 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-
35 1065, cryptophycins, dolastatin, duocarmycin, eleutherobin, pancratistatin, a sarcodictyin, spongistatin, nitrogen mustards, antibiotics, enediyne antibiotics, dynemicin, bisphosphonates, esperamicin, chromoprotein enediyne antibiotic chromophores, aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabacin, 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;

5 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, bestrabucil, bisantrene, edatraxate, defofamine, demecolcine, diaziquone, elfornithine, elliptinium acetate, an

10 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;

15 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

20 inhibitor RFS 2000; difluoromethylornithine; retinoids; capecitabine; combretastatin; leucovorin; oxaliplatin; inhibitors of PKC-alpha, Raf, and VEGF-A that reduce cell proliferation and pharmaceutically acceptable salts, acids or derivatives of any of the above. Also included in this

25 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; 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.

30

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,

35 electronic 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 may be administered as a single dose or as multiple, sequential doses.

5 **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 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
10 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, 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
15 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, the container(s) indicates that the enclosed antibody is used for treating the neoplastic disease condition of choice.

20 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 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
25 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
30 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
35 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
 5 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
 10 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. It is envisioned that the solvent may also be provided in another container.

As indicated briefly above the kits may also contain a means by which to administer the
 15 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
 20 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 amino acid
 25 sequences. The following Table A, B, C and D provide a summary of the included sequences. The CDRs of the anti-TIGIT moiety or anti-TIGIT antibody are identified according to Contact definition scheme, while the CDRs of the anti-PVRIG moiety or anti-PVRIG antibody are identified according to IMGT/Kabat definition scheme (HCDR1 defined by a combination of IMGT and Kabat schemes; other CDRs defined by Kabat scheme). W3XX104-T4U1.G15-
 30 2.uIgG1 and W3XX104-T4U1.G17-2.uIgG1 are bispecific antibodies constructed from parental antibodies W3642 (anti-TIGIT) and WT1175 (anti-PVRIG).

Table A: CDR sequences of bispecific antibodies W3XX104-T4U1.G15-2.uIgG1 and W3XX104-T4U1.G17-2.uIgG1

	HCDR1	HCDR2	HCDR3	LCDR1	LCDR2	LCDR3
anti-TIGIT moiety (derived from W3642 parental antibody)	SEQ ID NO: 1	SEQ ID NO: 2	SEQ ID NO: 3	SEQ ID NO: 4	SEQ ID NO: 5	SEQ ID NO: 6
	GFTFSVA WIH	LIKAGSNNY ATDYAESVK G	TLSHGSLN WFAS	TLSSGNIENKY VH	NDDKRPE	HSYVSSI NV

anti-PVRIG moiety (derived from WT1175 parental antibody)	SEQ ID NO: 7	SEQ ID NO: 8	SEQ ID NO: 9	SEQ ID NO: 10	SEQ ID NO: 11	SEQ ID NO: 12
	GGTFSSY TIS	GIIPIFGSADY AQKFQG	EGLTGYSS FDY	TGTSSDIGSYK FVS	EGSKRPS	SSYLGSG TVV

Table B: Amino acid sequences of the variable regions of BsAbs (CDR underlined)

		Amino acid sequence
anti-TIGIT moiety (derived from W3642 parental antibody)	VH	EVQLVESGGGLVQPGGSLRLSCAASGTFESVAWIHWVRQAPGKGLEWVGLIKA GSNNYATDYAESVKGRFTISRDDSKNSVYLQMNSLKTEDTAVYYCAWTLSHGS LNWFASWGRGTLTVTVSS (SEQ ID NO: 13)
	VL	QAVLTQPHSVSESPGKTVTISCTLSSGNIENKYVHWYQRPQSSPTTVIYNDDKR PEGVPDRFSGSIDSSSNSASLTISGLKTEDEADYYCHSYVSSINVFGGGKLTVL (SEQ ID NO: 14)
anti-PVRIG moiety (derived from WT1175 parental antibody)	VH	QVQLVQSGAEVKKPGSSSLKVSCKASGGTFSSYTISWVRQAPGQGLEWMGGIPI FGSADY AQKFQGRVTITADESTAYMELSSLRSED TAVYYCAREGLTGYSSFD YWGQGT LTVTVSS (SEQ ID NO: 15)
	VL	QSALTQPASVSGSPGQSITISCTGTSSDIGSYKFVSWYQHPGKAPKLMIEGSK RPSGVSNRFGSGKSGNTASLTISGLQTEDEADYYCSSYLGSGTVVFGGGTKLTVL (SEQ ID NO: 16)

Table C: Sequences of the heavy and light chains of BsAbs (CDR underlined)

Antibody	Amino acid sequence	
W3XX104-T4U1.G15-2. uIgG1	Heavy chain	SEQ ID NO: 17 EVQLVESGGGLVQPGGSLRLSCAASGTFESVAWIHWVRQAPGKGLEWVGLIKA GSNNYATDYAESVKGRFTISRDDSKNSVYLQMNSLKTEDTAVYYCAWTLSHGS LNWFASWGRGTLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNT KVDKRVPEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVTV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTSKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGGGGGSGGGGSGGGGSGGGGSGVQLVQSGA EVKKPGSSSLKVSCKASGGTFSSYTISWVRQAPGQGLEWMGGIPIFGSADY AOKF QGRVTITADESTAYMELSSLRSED TAVYYCAREGLTGYSSFDYWGQGT LTV SSGGGGSGGGGSGGGGSGGGGSGSALTQPASVSGSPGQSITISCTGTSSDIGSY KFVSWYQHPGKAPKLMIEGSKRPSGVSNRFGSGKSGNTASLTISGLQTEDEA DYYCSSYLGSGTVVFGGGTKLTVL
	Light chain	SEQ ID NO: 18 QAVLTQPHSVSESPGKTVTISCTLSSGNIENKYVHWYQRPQSSPTTVIYNDDKR PEGVPDRFSGSIDSSSNSASLTISGLKTEDEADYYCHSYVSSINVFGGGKLTVLG QPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVET TTPSKQSNNKYAASSYLSLTPEQWWSHKSYSCQVTHEGSTVEKTVAPTECS

W3XX104-T4U1.G17-2.uIgG1	Heavy chain	SEQ ID NO: 19 EVQLVESGGGLVQPGGSLRLSCAAS<u>GF</u><u>TFSVA</u><u>W</u><u>I</u><u>H</u>WVRQAPGKGLEWV<u>G</u><u>L</u><u>I</u><u>K</u><u>A</u> <u>G</u><u>S</u><u>N</u><u>N</u><u>Y</u><u>A</u><u>T</u><u>D</u><u>Y</u><u>A</u><u>E</u><u>S</u><u>V</u><u>K</u><u>G</u><u>R</u><u>F</u><u>T</u><u>I</u><u>S</u><u>R</u><u>D</u><u>D</u><u>S</u><u>K</u><u>N</u><u>S</u><u>V</u><u>Y</u><u>L</u><u>Q</u><u>M</u><u>N</u><u>S</u><u>L</u><u>K</u><u>T</u><u>E</u><u>D</u><u>T</u><u>A</u><u>V</u><u>Y</u><u>Y</u><u>C</u><u>A</u><u>W</u><u>T</u><u>L</u><u>S</u><u>H</u><u>G</u><u>S</u> <u>L</u><u>N</u><u>W</u><u>F</u><u>A</u><u>S</u><u>W</u><u>G</u><u>R</u><u>G</u><u>T</u><u>L</u><u>V</u><u>T</u><u>V</u><u>S</u><u>S</u><u>G</u><u>G</u><u>G</u><u>G</u><u>S</u><u>G</u><u>G</u><u>G</u><u>S</u><u>G</u><u>G</u><u>G</u><u>S</u><u>G</u><u>G</u><u>G</u><u>S</u><u>Q</u><u>A</u><u>V</u><u>L</u><u>T</u><u>Q</u><u>P</u><u>H</u><u>S</u><u>V</u><u>S</u><u>E</u><u>S</u><u>P</u> <u>G</u><u>K</u><u>T</u><u>V</u><u>T</u><u>I</u><u>S</u><u>C</u><u>L</u><u>S</u><u>S</u><u>G</u><u>N</u><u>I</u><u>E</u><u>N</u><u>K</u><u>Y</u><u>V</u><u>H</u><u>W</u><u>Y</u><u>Q</u><u>Q</u><u>R</u><u>P</u><u>G</u><u>S</u><u>S</u><u>P</u><u>T</u><u>T</u><u>V</u><u>I</u><u>Y</u><u>N</u><u>D</u><u>D</u><u>K</u><u>R</u><u>P</u><u>E</u><u>G</u><u>V</u><u>P</u><u>D</u><u>R</u><u>F</u><u>S</u><u>G</u><u>I</u><u>D</u><u>S</u><u>S</u> <u>S</u><u>N</u><u>S</u><u>A</u><u>S</u><u>L</u><u>T</u><u>I</u><u>S</u><u>G</u><u>L</u><u>K</u><u>T</u><u>E</u><u>D</u><u>E</u><u>A</u><u>D</u><u>Y</u><u>Y</u><u>C</u><u>H</u><u>S</u><u>V</u><u>S</u><u>S</u><u>I</u><u>N</u><u>V</u><u>F</u><u>G</u><u>G</u><u>G</u><u>T</u><u>K</u><u>L</u><u>T</u><u>V</u><u>L</u><u>G</u><u>G</u><u>G</u><u>G</u><u>S</u><u>G</u><u>G</u><u>G</u><u>S</u><u>G</u><u>G</u> <u>G</u><u>S</u><u>G</u><u>G</u><u>G</u><u>G</u><u>S</u><u>Q</u><u>V</u><u>Q</u><u>L</u><u>V</u><u>Q</u><u>S</u><u>G</u><u>A</u><u>E</u><u>V</u><u>K</u><u>K</u><u>P</u><u>G</u><u>S</u><u>S</u><u>L</u><u>K</u><u>V</u><u>S</u><u>C</u><u>K</u><u>A</u><u>S</u><u>G</u><u>G</u><u>T</u><u>E</u><u>S</u><u>S</u><u>Y</u><u>T</u><u>I</u><u>S</u><u>W</u><u>V</u><u>R</u><u>Q</u><u>A</u><u>P</u><u>G</u><u>Q</u><u>G</u><u>L</u> <u>W</u><u>M</u><u>G</u><u>G</u><u>I</u><u>P</u><u>I</u><u>F</u><u>G</u><u>S</u><u>A</u><u>D</u><u>Y</u><u>A</u><u>Q</u><u>K</u><u>F</u><u>O</u><u>G</u><u>R</u><u>V</u><u>T</u><u>I</u><u>T</u><u>A</u><u>D</u><u>E</u><u>S</u><u>T</u><u>S</u><u>T</u><u>A</u><u>Y</u><u>M</u><u>E</u><u>L</u><u>S</u><u>S</u><u>L</u><u>R</u><u>S</u><u>E</u><u>D</u><u>T</u><u>A</u><u>V</u><u>Y</u><u>Y</u><u>C</u><u>A</u><u>R</u><u>E</u><u>G</u><u>L</u> <u>T</u><u>G</u><u>Y</u><u>S</u><u>S</u><u>F</u><u>D</u><u>Y</u><u>W</u><u>G</u><u>Q</u><u>G</u><u>T</u><u>L</u><u>V</u><u>T</u><u>V</u><u>S</u><u>S</u><u>A</u><u>S</u><u>T</u><u>K</u><u>G</u><u>P</u><u>S</u><u>V</u><u>F</u><u>L</u><u>A</u><u>P</u><u>S</u><u>S</u><u>K</u><u>S</u><u>T</u><u>S</u><u>G</u><u>G</u><u>T</u><u>A</u><u>A</u><u>L</u><u>G</u><u>C</u><u>L</u><u>V</u><u>K</u><u>D</u><u>Y</u><u>F</u><u>E</u><u>P</u> <u>V</u><u>T</u><u>V</u><u>S</u><u>W</u><u>N</u><u>S</u><u>G</u><u>A</u><u>L</u><u>T</u><u>S</u><u>G</u><u>V</u><u>H</u><u>T</u><u>F</u><u>A</u><u>V</u><u>L</u><u>Q</u><u>S</u><u>S</u><u>G</u><u>L</u><u>Y</u><u>S</u><u>L</u><u>S</u><u>S</u><u>V</u><u>T</u><u>V</u><u>P</u><u>S</u><u>S</u><u>L</u><u>G</u><u>T</u><u>Q</u><u>T</u><u>Y</u><u>I</u><u>C</u><u>N</u><u>V</u><u>N</u><u>H</u><u>K</u><u>P</u><u>S</u><u>N</u> <u>T</u><u>K</u><u>V</u><u>D</u><u>K</u><u>R</u><u>V</u><u>E</u><u>P</u><u>K</u><u>S</u><u>C</u><u>D</u><u>K</u><u>T</u><u>H</u><u>T</u><u>C</u><u>P</u><u>P</u><u>C</u><u>A</u><u>P</u><u>E</u><u>L</u><u>L</u><u>G</u><u>G</u><u>P</u><u>S</u><u>V</u><u>F</u><u>L</u><u>F</u><u>P</u><u>P</u><u>K</u><u>P</u><u>K</u><u>D</u><u>T</u><u>L</u><u>M</u><u>I</u><u>S</u><u>R</u><u>T</u><u>P</u><u>E</u><u>V</u><u>T</u><u>C</u><u>V</u><u>V</u> <u>V</u><u>D</u><u>V</u><u>S</u><u>H</u><u>E</u><u>D</u><u>P</u><u>E</u><u>V</u><u>K</u><u>F</u><u>N</u><u>W</u><u>Y</u><u>V</u><u>D</u><u>G</u><u>V</u><u>E</u><u>V</u><u>H</u><u>N</u><u>A</u><u>K</u><u>T</u><u>K</u><u>P</u><u>R</u><u>E</u><u>E</u><u>Q</u><u>Y</u><u>N</u><u>S</u><u>T</u><u>Y</u><u>R</u><u>V</u><u>S</u><u>V</u><u>L</u><u>T</u><u>V</u><u>L</u><u>H</u><u>Q</u><u>D</u><u>W</u><u>L</u><u>N</u> <u>G</u><u>K</u><u>E</u><u>Y</u><u>K</u><u>C</u><u>K</u><u>V</u><u>S</u><u>N</u><u>K</u><u>A</u><u>L</u><u>P</u><u>A</u><u>P</u><u>I</u><u>E</u><u>K</u><u>T</u><u>I</u><u>S</u><u>K</u><u>A</u><u>K</u><u>G</u><u>Q</u><u>P</u><u>R</u><u>E</u><u>P</u><u>Q</u><u>V</u><u>Y</u><u>T</u><u>L</u><u>P</u><u>P</u><u>S</u><u>R</u><u>E</u><u>E</u><u>M</u><u>T</u><u>K</u><u>N</u><u>Q</u><u>V</u><u>S</u><u>L</u><u>T</u><u>C</u><u>L</u><u>V</u> <u>K</u><u>G</u><u>F</u><u>Y</u><u>P</u><u>S</u><u>D</u><u>I</u><u>A</u><u>V</u><u>E</u><u>W</u><u>E</u><u>S</u><u>N</u><u>G</u><u>P</u><u>E</u><u>N</u><u>N</u><u>Y</u><u>K</u><u>T</u><u>T</u><u>P</u><u>P</u><u>V</u><u>L</u><u>D</u><u>S</u><u>D</u><u>G</u><u>S</u><u>F</u><u>F</u><u>L</u><u>Y</u><u>S</u><u>K</u><u>L</u><u>T</u><u>V</u><u>D</u><u>K</u><u>S</u><u>R</u><u>W</u><u>Q</u><u>Q</u><u>G</u><u>N</u><u>V</u> <u>S</u><u>C</u><u>S</u><u>V</u><u>M</u><u>H</u><u>E</u><u>A</u><u>L</u><u>H</u><u>N</u><u>H</u><u>Y</u><u>T</u><u>Q</u><u>K</u><u>S</u><u>L</u><u>S</u><u>L</u><u>P</u><u>G</u><u>K</u>
	Light chain	SEQ ID NO: 20 QSALTQPASVSGSPGQSITISC<u>T</u><u>G</u><u>T</u><u>S</u><u>S</u><u>D</u><u>I</u><u>G</u><u>S</u><u>Y</u><u>K</u><u>F</u><u>V</u><u>S</u><u>W</u><u>Y</u><u>Q</u><u>Q</u><u>H</u><u>P</u><u>G</u><u>K</u><u>A</u><u>P</u><u>K</u><u>L</u><u>M</u><u>I</u><u>Y</u><u>E</u><u>G</u><u>S</u><u>K</u> <u>R</u><u>P</u><u>S</u><u>G</u><u>V</u><u>S</u><u>N</u><u>R</u><u>F</u><u>S</u><u>G</u><u>S</u><u>K</u><u>S</u><u>G</u><u>N</u><u>T</u><u>A</u><u>S</u><u>L</u><u>T</u><u>I</u><u>S</u><u>G</u><u>L</u><u>Q</u><u>T</u><u>E</u><u>D</u><u>E</u><u>A</u><u>D</u><u>Y</u><u>Y</u><u>C</u><u>S</u><u>S</u><u>Y</u><u>L</u><u>G</u><u>S</u><u>G</u><u>T</u><u>V</u><u>V</u><u>F</u><u>G</u><u>G</u><u>G</u><u>T</u><u>K</u><u>L</u><u>T</u><u>V</u> <u>G</u><u>Q</u><u>P</u><u>K</u><u>A</u><u>A</u><u>P</u><u>S</u><u>V</u><u>T</u><u>L</u><u>F</</u>

Table D: Sequences of the antigens

Full length of human TIGIT	SEQ ID NO: 23 MRWCLLLIWAQGLRQAPLASGMMTGTIETTGNISAEKGGSIILQCHLSSTTAQVTQVNWEQQD QLLAICNADLGWHSFSFKDRVAPGPGGLGLTLQSLTVNDTGEYFCIYHTYPDGTYTGRIFLEVL ESSVAEHGARFQIPLLGAMAATLVVICTAVIVVVALTRKKKALRIHSVEGDLRRKSAGQEEWS PSAPSPPGSCVQAEAAPAGLCGEQRGEDCAELHDYFNVLSYRSLGNCSEFTTETG
Full length of cynomolgus TIGIT	SEQ ID NO: 24 MRWCLFLIWAQGLRQAPLASGMMTGTIETTGNISAKKGGSVILQCHLSSTMAQVTQVNWEQHH DHSLLAIRNAELGWHIYPAFKDRVAPGPGGLGLTLQSLTMNDTGEYFCTYHTYPDGTYRGRIFL EVLESSVAEHSARFQIPLLGAMAMMLVVICIAVIVVVVLARKKKSLRIHSVESGLQRKSTGQEE QIPSAAPSPPGSCVQAEAAPAGLCGEQGGDDCAELHDYFNVLSYRSLGSCSEFTTETG
Full length of mouse TIGIT	SEQ ID NO: 25 MHGWLLLVVWVQGLIQAFLATGATAGTIDTKRNISAEEGGSVILQCHFSSDTAEVTQVDWKQ QDQLLAITYVDLGWHVASVFSRDRVVPGPSLGLTFQSLTMNDTGEYFCTYHTYPGGIYKGRIFL KVQESSVAQFQTAPLGGTMAAVLGLICLMVTGTVLARKKSIRMHSIESGLGRTEAEPQEWNL RSLSSPGSPVQTQTAPAGPCGEQAEDDYADPQEYFNVLSYRSLESFIAVSKTG
Full length of human PVRIG	SEQ ID NO: 26 MRTEAQVPALQPPEPGLEGAMGHRTLVLPPWVLLTLCVTAGTPEVWVQVRMEATELSSFTIRC GFLGSGSISLVTVSWGPNAGAGGTTLAVLHPERGIRQWAPARQARWETQSSSISLILEGSGASSP CANTTFCKKFASFPEGSWEACGSLPPSSDPGLSAPPTAPILRADLAGILGVSGVLLFGCVYLLH

	LLRRHKHRPAPRLQPSRTSPQAPRARAWAPSQASQAALHVPYATINTSCRPATLDTAHPHGGP SWWASLPHTAAHRPQGPAAWASTPIPARGSFVSVENGLYAQAGERPPHTGPGLTLFPDPRGPR AMEGPLGVR
Full length of cynomolgus PVRIG	SEQ ID NO: 27 MRTEAQVLALQSPEPGLEGAMGRRTLALPWVLLTLCVTAGTPEVWVQVQMEATELSSFTVH CGFLGPGSISLVTVSWGPGDGAGGTKLAVLHPELGTRQWAPARQARWETQSSISLALED SGAS SPFANTTFCCKFASFPEGSWESCGSLPPSSDPGLSAPPTVPILRADLAGILGLSGVLLFGCGYLL HLLRRQKHRPTPRLQPSHTNSQALTAQAWAPSQASQAALHDPYATINTSFCPATLDTAHPNG WASLPHTAAHQPGPAASASTPILARGSFVSVENGLYTQAGERPPHTGPGLTLFPDCRGPRAV EGRFGVR
Full length of mouse PVRIG	SEQ ID NO: 28 MRTGNTQAAHATNMGMQMTLVLFSTLLTLCVSEASPEVWVQVQMEATNLSSFSVHCGVLGY SLISLVTVSCEGFVDAGRTKLAVLHPEFGTQQWAPARQAHWETPNSVSVTLTMGQSKARSSL ANTTFCCEFVTFPHGSRVACRDLHRSDPGLSAPTALNLQADLVRLGTSGVFLFGFIFILCLRW QQRHWCLSKSQPSLTSTQAQVETQPPHLASTHSSSFISMENGLYALA

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.

WT117-hPro1.ECD.His is the extracellular domain of human PVRIG (NP_076975.2) with a C-terminal polyhistidine tag; WT117-hPro1.ECD.hFc is the extracellular domain of human PVRIG (NP_076975.2) with the Fc region of human IgG1 at the C-terminus; WT117-

hPro1.ECD.mFc is the extracellular domain of human PVRIG (NP_076975.2) with the Fc region of mouse IgG2a at the C-terminus; WT117-mPro1.ECD.His is the extracellular domain of mouse PVRIG (XP_011239268.1) with a C-terminal polyhistidine tag; WT117-mPro1.ECD.hFc is the extracellular domain of mouse PVRIG (XP_011239268.1) with the Fc region of human IgG1 at the C-terminus; WT117-hPro1L1.ECD.mFc is the extracellular domain of human PVRL2 (NP_001036189.1) with the Fc region of mouse IgG2a at the C-terminus.

These antigens were purchased from vendors or prepared in house.

1.2 Preparation of benchmark antibodies (BMKs)

Anti-human TIGIT or PVRIG reference antibodies, named as WBP364-BMK1 and WBPT117-BMK1 herein, were used as controls in the experiments below. The sequences of the two antibodies were synthesized according to the disclosed sequences in respective patents, the information of which is summarized in Table 1. The human IgG1 isotype control antibody is an isotype control.

Table 1. Reference antibody information

Antibody Code	Company	Patent NO.	Clone ID
WBP364-BMK1 (Tiragolumab)	Roche (Genentech)	US20170088613	4.1D3
WBPT117-BMK1	Compugen	US20180244774	CHA.7.518.1

1.3 Cell Pool/Line Generation

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).

Human PVRIG-expressing cell line WT117-293F.hPro1.G11 was generated using 293F cells transfected with full-length human PVRIG (NP_076975.2). Cynomolgus monkey PVRIG-expressing cell pool WT117-Flpin293.cPro1.pool was generated using Flpin293 cells transfected with full-length cynomolgus monkey PVRIG (XP_005549281.1). Mouse PVRIG-expressing cell pool WT117-293F.mPro1.pool was generated using 293F cells transfected with full-length mouse PVRIG (XP_011239268.1).

EXAMPLE 2

Generation of Bispecific Antibodies

2.1 Plasmid construction

Monospecific anti-PVRIG antibodies and anti-TIGIT antibodies were developed respectively. Anti-TIGIT monoclonal antibody W3642 (or “T4”) was obtained by immunizing Sprague-Dawley (SD) rats followed by humanization, and anti-PVRIG monoclonal antibody WT1175 (or “U1”) was obtained by immunizing genetically engineered OmniRat. Their variable regions were extracted for constructing bispecific anti-PVRIG/TIGIT antibodies.

Construction of G15 format bispecific antibody: DNA sequence encoding scFv (VH-(G4S)4-VL) of anti-PVRIG antibody was linked to the C-terminus of anti-TIGIT antibody full length heavy chain with a (G4S)4 linker, i.e. in IgG(H)-scFv format. The sequence was cloned into pcDNA3.4 expression vector. The obtained bispecific antibody was named “W3XX104-T4U1.G15-2.uIgG1” or “T4U1.G15” or “G15”. The schematic graph of structure of W3XX104-T4U1.G15-2.uIgG1 is shown in Figure 1, left panel.

Construction of G17 format bispecific antibody: DNA sequence encoding scFv (VH-(G4S)4-VL) of anti-TIGIT antibody was linked to the N-terminus of anti-PVRIG antibody full length heavy chain with a (G4S)4 linker, i.e. in scFv-IgG(H) format. The sequence was cloned into pcDNA3.4 expression vector. The obtained bispecific antibody was named “W3XX104-T4U1.G17-2.uIgG1” or “T4U1.G17” or “G17”. The schematic graph of structure of W3XX104-T4U1.G17-2.uIgG1 is shown in Figure 1, right panel.

The sequences of the above antibodies are summarized in Tables A-C above.

2.2 Expression in Expi293 cells

Expi293 cells (Thermo fisher, cat#A14635) were used for protein expression. The plasmids of bi-specific antibody heavy chain and light chain were transfected into Expi293 cells. After transfection, the culture was incubated at 37°C, 8% CO₂ for 5 days until supernatant was harvest for protein purification.

2.3 Antibody purification

The supernatant of Expi293 cells culture was collected and filtered for purification using Protein A column (GE Healthcare, cat#175438). The concentration of purified antibodies was determined by UV absorbance at 280nm. The molecular weight and purity were tested by SDS-PAGE and SEC-HPLC, respectively.

EXAMPLE 3

In vitro Characterization

3.1 Human TIGIT or PVRIG binding assay

W364-CHOK1.hPro1.2A11 or WT117-293F.hPro1.G11 cells were incubated with various concentrations of antibodies at 4 °C for 1 hour. After washing with 1×PBS/1%BSA, the secondary antibody, PE-labeled goat anti-human IgG (Jackson ImmunoResearch, cat#109-115-098) was added and incubated with cells at 4 °C in dark for 1 hour. Anti-human TIGIT antibody WBP364-BMK1 and anti-PVRIG antibody WBPT117-BMK1 were used as respective positive control. Human IgG1 isotype control antibody was used as isotype control. The cells were then

washed and resuspended in 1×PBS/1%BSA. MFI of the cells was measured by a flow cytometry (BD) and analyzed by FlowJo.

The binding results of antibodies on W364-CHOK1.hPro1.2A11 or WT117-293F.hPro1.G11 are shown in Figure 2 and Figure 3. These results demonstrate that both G17 and G15 bind to human TIGIT-expressing cells W364-CHOK1.hPro1.2A11 with comparable potency to WBP364-BMK1, both G17 and G15 bind to PVRIG-expressing cells WT117-293F.hPro1.G11 with higher potency than WBPT117-BMK1. A summary of antibodies binding is shown in Table 4.

3.2 Cynomolgus monkey TIGIT or PVRIG binding assay

W364-FlpinCHO.cynoPro1.pool or WT117-Flpin293F.cPro1.pool cells were incubated with various concentrations of antibodies at 4 °C for 1 hour. After washing with 1×PBS/1%BSA, the secondary antibody, PE-labeled goat anti-human IgG (Jackson ImmunoResearch, cat#109-115-098) was added and incubated with cells at 4 °C in dark for 1 hour. Anti-human TIGIT antibody WBP364-BMK1 and anti-PVRIG antibody WBPT117-BMK1 were used as respective positive control. Human IgG1 isotype control antibody was used as isotype control. The cells were then washed and resuspended in 1×PBS/1%BSA. MFI of the cells was measured by a flow cytometry (BD) and analyzed by FlowJo.

The binding results of anti-TIGIT/PVRIG BsAbs on W364-FlpinCHO.cynoPro1.pool or WT117-Flpin293F.cPro1.pool are shown in Figure 4 and Figure 5. These results demonstrate that both G17 and G15 bind to cynomolgus monkey TIGIT-expressing cells W364-FlpinCHO.cynoPro1.pool with comparable potency to WBP364-BMK1, both G17 and G15 bind to PVRIG-expressing cells WT117-Flpin293F.cPro1.pool with higher potency than WBPT117-BMK1. A summary of antibodies binding is shown in Table 4.

3.3 Mouse TIGIT binding assay

W364-FlpinCHO.mPro1.pool cells were incubated with various concentrations of antibodies at 4 °C for 1 hour. After washing with 1×PBS/1%BSA, the secondary antibody, PE-labeled goat anti-human IgG (Jackson ImmunoResearch, cat#109-115-098) was added and incubated with cells at 4 °C in dark for 1 hour. Human IgG1 isotype control antibody was used as isotype control. The cells were then washed and resuspended in 1×PBS/1%BSA. MFI of the cells was measured by a flow cytometry (BD) and analyzed by FlowJo.

The binding result of anti-TIGIT/PVRIG BsAbs on W364-FlpinCHO.mPro1.pool cells is shown in Figure 6. The result demonstrates that both G17 and G15 bind to mouse TIGIT-expressing cells, while WBP364-BMK1 does not. A summary of antibodies binding is shown in Table 4.

3.4 Mouse PVRIG binding assay

WT117-293F.mPro1.pool cells were incubated with various concentrations of antibodies at 4 °C for 1 hour. After washing with 1×PBS/1%BSA, the secondary antibody, PE-labeled goat anti-human IgG (Jackson ImmunoResearch, cat#109-115-098) was added and incubated with cells at 4 °C in dark for 1 hour. An anti-mouse PVRIG antibody fused to human IgG1 Fc was used as positive control. Human IgG1 isotype control antibody was used as isotype control. The cells were then washed and resuspended in 1×PBS/1%BSA. MFI of the cells was measured by a flow cytometry (BD) and analyzed by FlowJo.

The binding result of anti-TIGIT/PVRIG BsAbs on WT117-293F.mPro1.pool cells is shown in Figure 7. The result demonstrates that both G17 and G15, as well as WBPT117-BMK1 do not bind to mouse PVRIG.

3.5 Human TIGIT or PVRIG binding affinity assay

Affinity determination of anti-TIGIT/PVRIG BsAb against recombinant human TIGIT or PVRIG was performed by surface plasmon resonance (SPR) using a Biacore 8K instrument (Cytiva). Goat anti-human IgG Fc antibody (Jackson ImmunoResearch, cat#109-005-098) was immobilized on CM5 biosensor chips (GE, cat#29-1496-03), and the testing antibodies were captured by goat anti-human IgG Fc antibody. For kinetics measurements, a series concentration of W364-hPro1.ECD.His or WT1175-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 SPR binding affinity results of anti-TIGIT/PVRIG BsAbs to human TIGIT or PVRIG are shown in Table 2 and Table 3. G17 binds to human TIGIT and PVRIG proteins with an affinity of 1.17E-11 and 2.69E-09 M respectively. G15 binds to human TIGIT and PVRIG proteins with an affinity of 1.87E-11 and 5.42E-09 M respectively. A summary of antibodies binding affinity is shown in Table 4, and the sensorgrams are shown in Figure 8 and Figure 9.

Table 2. Affinity constant of antibodies to human TIGIT

Antibody	ka (1/Ms)	kd (1/s)	KD (M)
W3XX104-T4U1.G17-2.uIgG1	2.70E+06	3.17E-05	1.17E-11
W3XX104-T4U1.G15-2.uIgG1	2.41E+06	4.49E-05	1.87E-11
W3642	2.40E+06	4.46E-05	1.86E-11
WBP364-BMK1	9.70E+05	1.87E-05	1.93E-11

Table 3. Affinity constant of antibodies to human PVRIG

Antibody	ka (1/Ms)	kd (1/s)	KD (M)
W3XX104-T4U1.G17-2.uIgG1	3.14E+06	8.45E-03	2.69E-09

W3XX104-T4U1.G15-2.uIgG1	2.30E+06	1.25E-02	5.42E-09
WT1175	3.62E+06	5.66E-03	1.56E-09
WBPT117-BMK1	1.04E+06	3.12E-04	3.00E-10

3.6 Paralog proteins binding assay

Plate was pre-coated with 1 µg/mL of W364-hPro1.ECD.His, WT117-hPro1.ECD.His, recombinant human CD226, CD96 or PD-1 extracellular domain in 100 µL coating buffer (Na₂CO₃/NaHCO₃, pH9.2) per well at 4 °C overnight. After blocking with 200 µL of 1×PBS/2%BSA, 100 µL of testing antibodies were added to the plate at a concentration of 66.67 nM and 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. 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 and 540nm using M5e microplate reader (Molecule Devices).

The binding result of anti-TIGIT/PVRIG BsAbs to TIGIT and PVRIG paralog proteins are shown in Figure 10. The result demonstrates that both G17 and G15 specifically bind to TIGIT and PVRIG without cross-reactivity to human CD226, CD96 and PD-1.

3.7 Human TIGIT and PVRIG dual binding assay

Plate was pre-coated with 0.1 µg/mL of W364-hPro1.ECD.His in 100 µL coating buffer per well at 4 °C overnight. After blocking with 200 µL of 1×PBS/2%BSA, 100 µL of testing antibodies were added to the plate at various concentrations and incubated at ambient temperature for 2 hours. After incubation, the plate was washed using 1×PBST for 3 times. WT117-hPro1.ECD.mFc was added to the plate at the concentration of 0.1 µg/mL diluted in 1×PBS/2%BSA. After 1-hour incubation at ambient temperature, the plate was washed using 1×PBST for 3 times. HRP-labeled goat anti-mouse IgG antibody (Bethyl, cat#A90-231P) diluted in 1×PBS/2%BSA was added and incubated for 1 hour at ambient temperature. 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 450 nm and 540 nm using M5e microplate reader (Molecule Devices).

The dual binding result of anti-TIGIT/PVRIG BsAbs to immobilized human TIGIT and soluble human PVRIG is shown in Figure 11. The result demonstrates that both G17 and G15 simultaneously bind to human TIGIT and PVRIG, while the reference mAbs WBP364-BMK1 and WBPT117-BMK1 don't dual-bind to TIGIT and PVRIG. A summary of antibodies dual binding is shown in Table 4.

3.8 Human TIGIT/PVR blocking assay

The ability of anti-TIGIT/PVRIG BsAbs 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. Remove excess buffer in the wells by centrifugation. The serially diluted antibodies ($2 \times$ concentration) were pre-mixed with 4 $\mu\text{g/mL}$ ($2 \times$ 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 antibody WBP364-BMK1 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 human TIGIT/PVR blockade result is shown in Figure 12. The result demonstrates that both G17 and G15 block human PVR from binding to human TIGIT, and they showed equivalent blocking potency compared to the reference TIGIT mAb WBP364-BMK1. A summary of antibodies blocking activity is shown in Table 4. The maximum inhibition rate was calculated as $\text{inhibition\%} = (\text{MFI}_{\text{max}} - \text{MFI}_{\text{bottom}}) / \text{MFI}_{\text{max}} \times 100\%$, where MFI_{max} was defined as MFI in the absence of antibody.

3.9 Human PVRIG/PVRL2 blocking assay

The ability of anti-TIGIT/PVRIG BsAbs to block human PVRIG/PVRL2 interaction was tested by ELISA. Plates were pre-coated with 0.5 $\mu\text{g/mL}$ of rabbit anti-his antibody in 100 μL coating buffer per well overnight at 4 °C. After blocking with 200 μL of 1×PBS/2%BSA, 100 μL of WT117-hPro1.ECD.His was added to each well at the concentration of 0.8 $\mu\text{g/mL}$ and incubated at ambient temperature for 1 hours. The serially diluted antibodies ($2 \times$ concentration) were mixed with 0.6 $\mu\text{g/mL}$ ($2 \times$ concentration) of WT1175-hPro1L1.ECD.mFc at a volume ratio of 1:1. After incubation, 100 μL of antibody/ligand mixture were added to the plates and incubated at ambient temperature for 2 hours. After washing with 1×PBST for 3 times, HRP-labeled goat anti-mouse IgG (Bethyl, cat#A90-231P) was added to the plates and incubated for 1 hour at ambient temperature. After washing with 1×PBST for 6 times, TMB substrate was added and then the interaction was stopped by 2M HCl. Absorbance was read at 450 nm and 540 nm using M5e microplate reader (Molecule Devices).

The human PVRIG/PVRL2 blockade result is shown in Figure 13. The result demonstrates that both G17 and G15 block human PVRL2 from binding to human PVRIG, and they showed equivalent blocking potency compared to the reference PVRIG mAb WBPT117-BMK1. A summary of antibodies blocking activity is shown in Table 4. The maximum inhibition rate was calculated as $\text{inhibition\%} = (\text{OD}_{\text{max}} - \text{OD}_{\text{bottom}}) / \text{OD}_{\text{max}} \times 100\%$, where OD_{max} was defined as the value of OD₄₅₀ minus OD₅₄₀ in the absence of antibody.

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

Jurkat cells over-expressing human TIGIT, PVRIG and NFAT-luciferase reporter were stimulated by engagement of T cell receptor by co-culturing with artificial APC. The artificial APC was engineered HT1080 cells which constitutively express PVR and PVRL2, and was transfected to express human TCR activator. HT1080/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 antibodies and Jurkat/TIGIT/PVRIG/NFAT-luciferase cells (4×10^4 cells/well) were added to the plate, and were co-cultured at 37 °C, 5% CO₂ for 5.5 hours. After incubation, reconstituted luciferase substrate (Promega, cat#E605B) was added to each well and mixed well. The luciferase intensity was read using Envision microplate reader (PerkinElmer).

The result of Jurkat TIGIT/PVRIG/NFAT-luciferase reporter gene assay is shown in Figure 14. The result demonstrates that both G17 and G15 dramatically enhanced TCR/NFAT signaling via blocking TIGIT/PVR and PVRIG/PVRL2 interactions. Importantly, the potency and efficacy of BsAbs were higher than that of the reference mAb WBP364-BMK1 and WBPT117-BMK1 alone, and their combo. A summary of antibodies activity of reversing TCR/NFAT suppression in shown in Table 4. The fold change was defined as the ratio between luciferase of sample and luciferase in the absence of antibody.

3.11 Human primary NK cell killing assay

HT1080 is a human fibrosarcoma cell line, which expresses human PVR, PVRL2 and PVRL3. HT1080 expressing human PVR and PVRL2 was used as target cell, and human primary NK cell was used as effector cell. Human primary NK cells were isolated from human peripheral blood mononuclear cells (PBMCs) by magnetic selection using human CD56 MicroBeads (Milenyi Biotec, cat#130-050-401) according to the manufacturer's protocol. The isolated human NK cells (4×10^4 cells/well) were co-cultured with HT1080 cells (2×10^4 cells/well) in the presence of serially diluted antibodies at 37 °C, 5% CO₂ for 8 hours. Target cell lysis was determined by LDH-based cytotoxicity detection kit (Roche, cat#04744934001). Absorbance was read at 492nm using M5e microplate reader (Molecule Devices).

The result of NK killing assay is shown in Figure 15. The result demonstrates that both G17 and G15 enhanced the cytotoxicity of primary NK cells in a dose-dependent manner, with a potency higher than WBP364-BMK1 and WBPT117-BMK1 alone and their combo. A summary of antibodies enhancing NK cell activity is shown in Table 4.

3.12 Human primary T cell activation assay

Human primary CD8⁺ T cell was stimulated by engagement of T cell receptor by co-culturing with artificial APC. The artificial APC was engineered HT1080 cells which

constitutively express PVR and PVRL2, and was transfected to express human TCR activator. Human CD8⁺ T cells were isolated from human PBMCs by magnetic selection using human CD8 MicroBeads (Miltenyi Biotec, cat#130-045-201) according to the manufacturer's protocol. The isolated human CD8⁺ T cells were co-cultured with Human T-activator CD3/CD28 Dynabeads (Gibco, cat#11132D) for 7 days to induce exhaustion. After the magnetic beads were removed by magnet, these exhausted T cells (1×10^5 per well) were co-cultured with mitomycin C-treated HT1080/TCR-activator cells (1×10^4 per well) in the presence of serially diluted antibodies. After 5 days' incubation, the supernatants were collected for IFN- γ measurement by ELISA (capture antibody Thermo cat#M700A, detection antibody Thermo cat#M701B). The absorbance was detected using M5e microplate reader (Molecule Devices).

The result of T cell activation assay is shown in Figure 16. The result demonstrates that both G17 and G15 enhanced activation of exhausted human CD8⁺ T cells in a dose-dependent manner, with a potency higher than WBP364-BMK1 and WBPT117-BMK1 alone and their combo. A summary of antibodies enhancing T cell activity is shown in Table 4.

Table 4. Summary of antibody characterizations

Assay	W3XX104-T4U1.G17-2.μIgG1	W3XX104-T4U1.G15-2.μIgG1	WBP364-BMK1	WBPT117-BMK1	WBP364-BMK1 + WBPT117-BMK1
Antibodies binding to human TIGIT, EC50 (nM)	0.12	0.084	0.20	ND	ND
Antibodies binding to human PVRIG, EC50 (nM)	0.044	0.081	ND	0.19	ND
Antibodies binding to cynomolgus monkey TIGIT, EC50 (nM)	0.41	0.30	0.72	ND	ND
Antibodies binding to cynomolgus monkey PVRIG, EC50 (nM)	0.11	0.13	ND	0.49	ND
Antibodies binding to mouse TIGIT, EC50 (nM)	0.38	0.26	NA	ND	ND
Antibodies binding to mouse PVRIG	NA	NA	ND	NA	ND
SPR affinity to human TIGIT, KD (M)	1.17E-11	1.87E-11	1.93E-11	ND	ND
SPR affinity to human PVRIG, KD (M)	2.69E-09	5.42E-09	ND	3.00E-10	ND

Dual binding of antibodies to human TIGIT and PVRIG, EC50 (nM)	0.44	0.20	NA	NA	NA
Antibody blocking PVR binding to TIGIT, IC50 (nM)	0.47	0.25	0.53	ND	ND
Antibody blocking PVRL2 binding to PVRIG, IC50 (nM)	0.76	0.97	ND	0.91	ND
Antibody reversing TCR/NFAT signaling suppression, EC50 (nM)	0.34	0.27	4.4	0.69	2.4
NK cell killing assay, EC50 (nM)	~0.14	~0.16	~0.24	NA	~0.50
T cell activation assay, EC50 (nM)	0.13	~0.12	0.45	~0.92	0.34

ND: not determined; NA: not available

3.13 Antibody developability

(1) Thermal stability assay

The conformational stability is a very important property for a successful antibody candidate. Conformational stability can be assessed by measuring thermal stability using differential scanning fluorimetry (DSF) method, which is sensitive to changes in protein folding. DSF measures the temperature of the protein unfolding transition (T_m) 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 antibodies and transfer the mixture to a 96-well plate. Then the plate was transferred to a Quant Studio[®] 7 Flex Real-Time PCR system with the temperature ranging from 26 °C to 95 °C at a heating rate of 0.9 °C/min. The first two temperatures of protein unfolding transitions were recorded as T_{m1} and T_{m2} . The two values were calculated according to the melt curve using QuantStudio[®] Real Time PCR software (v1.3).

The result of DSF is shown in Table 5 and Figure 17. DSF thermogram for the BsAbs display two transitions: T_{m1} reflecting the dissolving of the CH2 domain, and T_{m2} reflecting the temperature of CH3 and Fab melting. T_{m1} for G17 and G15 were 57.1 °C and 55.6 °C, while T_{m2} were 64.7 °C and 63.6 °C, respectively. The results demonstrate good thermal stability of both G17 and G15.

Table 5. T_m values of antibody

Antibody	Tm1 (°C)	Tm2 (°C)
W3XX104-T4U1.G17-2.uIgG1	57.1	64.7
W3XX104-T4U1.G15-2.uIgG1	55.6	63.6

(2) Solubility test by DLS-kD

Dynamic light scattering (DLS) is widely used to assess the aggregation and stability of proteins. The diffusion interaction parameter (kD) describes how the diffusion rate of the protein is impacted by protein concentration as a result of intermolecular forces.

To determine the kD value of the antibodies, DLS was carried out using DynaPro Plate Reader III (Wyatt Technology). A concentration series (20, 15, 10, 5 and 2.5 mg/mL) of antibodies were prepared, and 7.5 μ L of sample dilution was added to a 1536-well microplate. The plate was sealed with ClearSeal Film (Hampton Research, cat#HR4-521), and centrifuged at 3,000 rpm for 5 minutes to remove aggregates. Each sample was tested in duplicate. The plate was read using DynaPro1 Plate Reader III (Wyatt Technology), and the data was collected by the DYNAMICS operation software (v7.8.1.3). To get the kD value, the diffusion coefficient was determined and plotted against sample concentrations. The slope of the plotted line is the kD value of the sample.

The result of DLS-kD is shown in Table 6 and Figure 18. The kD values for G17 and G15 were -7.29 and -7.24 mL/g, respectively. No aggregation or particle was observed during the experiment. The result demonstrates good solubility of both G17 and G15.

Table 6. Solubility of antibody

Antibody	kD (mL/g)	R ²	Size distribution	Appearance
W3XX104-T4U1.G17-2.uIgG1	-7.29	0.9422	Monodisperse size	4.8 mg/mL, colorless, particle-free
W3XX104-T4U1.G15-2.uIgG1	-7.24	0.9203	Monodisperse size	6.6 mg/mL, colorless, particle-free

(3) Hydrophobicity test by HIC-HPLC

Hydrophobic interaction chromatography-high performance liquid chromatography (HIC-HPLC) is a powerful analytical method that has been employed for prediction of relative hydrophobicity of therapeutic proteins. The retention time of a protein of interest reflects its overall hydrophobicity.

To predict the hydrophobicity of antibodies, HPLC 1260 Infinity II system (Agilent Technologies™) with TSKgel butyl-NPR column (Tosoh, cat#0042168) was used to calculate their retention time. Each sample was diluted to 0.5 mg/mL in PBS and inject 20 µL into the column, with a separated flow rate of 0.5 mL/min for 61 mins. The running buffer was prepared by mixing Buffer A (25 mM sodium phosphate, pH 7.0) and Buffer D (25 mM sodium phosphate, 1.5 M (NH₄)₂SO₄, pH 7.0). The separation was performed from 3 to 53 min using a running buffer gradient (Buffer D from 0% to 100%). The UV absorbance was detected at 280nm and 230nm to determine the peak retention. The retention time was calculated by integrating all peak areas from 20 min to 40 min using software OpenLab CDS Workstation (v2.6.0.691).

The result of HIC-HPLC is shown in Table 7 and Figure 19. The retention time for G17 and G15 were 30.31 and 28.62 min, respectively, which reflect normal hydrophobicity.

Table 7. Hydrophobicity of antibody

Antibody	Concentration (mg/mL)	Retention time (min)
W3XX104-T4U1.G17-2.uIgG1	0.5	30.31
W3XX104-T4U1.G15-2.uIgG1	0.5	28.62

(4) Stress test at 4 °C and 40 °C

To investigate the thermal stability of BsAbs, T4U1.G15 and T4U1.G17 were incubated at 4 °C and 40 °C in formulation buffer (20 mM histidine, 200 mM arginine, 70 mM sucrose and 0.01% PS80, pH7.0) for up to 28 days. Samples were collected on day 0, day 3, day 7, day 14 and day 28, respectively, and stored at a freezer set to -40 °C until analysis. The samples were analyzed for physical appearance by observation, concentration by UV absorbance at 280nm, as well as changes in the high molecular weight (HMW), monomer (Mono), and low molecular weight (LMW) species present in solution by SEC-HPLC.

The results are shown in Table 8 and Table 9. Under 40 °C thermal stress, for G17, the percentage of LMW was slightly increased while the percentage of Mono being slightly decreased, and for G15, the percentage of HMW was slightly increased while the percentage of Mono being slightly decreased. There was no significant change with protein concentrations.

Table 8. Stability of T4U1.G17 under thermal stress

Antibody	W3XX104-T4U1.G17-2.uIgG1
Buffer	20 mM histidine, 200 mM arginine, 70 mM sucrose, 0.01% (w/v) PS80, pH7.0

	Day 0	Concentration (mg/mL)	Appearance	HMW %	Mono %	LMW %
4 °C	Day 3	2.01	Colorless, particle-free	2.21	97.46	0.33
	Day 7	2.00	Colorless, particle-free	2.48	97.18	0.34
	Day 14	1.95	Colorless, particle-free	2.31	97.31	0.38
	Day 28	1.96	Colorless, particle-free	2.51	97.09	0.4
40 °C	Day 0	1.95	Colorless, particle-free	2.81	96.73	0.46
	Day 3	2.00	Colorless, particle-free	2.14	97.51	0.35
	Day 7	1.99	Colorless, particle-free	2.20	95.12	2.68
	Day 14	2.01	Colorless, particle-free	2.16	92.92	4.92
	Day 28	2.03	Colorless, particle-free	2.31	89.73	7.96

Table 9. Stability of T4U1.G15 under thermal stress

Antibody		W3XX104-T4U1.G15-2.uIgG1				
Buffer		20 mM histidine, 200 mM arginine, 70 mM sucrose, 0.01% (w/v) PS80, pH7.0				
	Day 0	Concentration (mg/mL)	Appearance	HMW %	Mono %	LMW %
4 °C	Day 3	1.99	Colorless, particle-free	2.91	96.99	0.10
	Day 7	1.97	Colorless, particle-free	2.80	97.10	0.10
	Day 14	1.92	Colorless, particle-free	2.83	96.94	0.23
	Day 28	1.99	Colorless,	2.70	97.10	0.20

			particle-free			
40 °C	Day 0	1.95	Colorless, particle-free	2.60	97.04	0.36
	Day 3	2.02	Colorless, particle-free	2.95	96.96	0.09
	Day 7	1.93	Colorless, particle-free	3.73	95.72	0.55
	Day 14	2.00	Colorless, particle-free	4.38	94.81	0.81
	Day 28	2.05	Colorless, particle-free	5.58	92.31	2.11

(5) Stress test after rounds of freeze-thaw

To investigate the stability of BsAbs after multiple freeze-thaw (F/T) cycles, T4U1.G15 and T4U1.G17 stored at -80 °C were thawed at ambient temperature, and refrozen at -80 °C, and rethawed again. After a total of 5 F/T cycles, the samples were collected for evaluating physical appearance by observation, concentration by UV absorbance at 280nm, as well as changes in the HMW, Mono, and LMW species present in solution by SEC-HPLC.

The results are shown in Table 10 and Table 11. After 5 cycles of F/T, there was no discernible effect on G17 and G15 stability, detection items including appearance, concentration, as well as relative proportion of the HMW, Mono and LWM species.

Table 10. Stability of T4U1.G17 under freeze-thaw stress

Antibody		W3XX104-T4U1.G17-2.µIgG1		
Buffer		20 mM histidine, 200 mM arginine, 70 mM sucrose, 0.01% (w/v) PS80, pH7.0		
Appearance	0 F/T	Colorless, particle-free		
	5× F/T	Colorless, particle-free		
Concentration (mg/mL)	0 F/T	2.0		
	5× F/T	2.0		
SEC-HPLC	-	HMW %	Mono %	LMW %
	0 F/T	9.3	90.7	0
	5× F/T	9.5	90.5	0

Table 11. Stability of T4U1.G15 under freeze-thaw stress

Antibody	W3XX104-T4U1.G15-2.µIgG1
----------	--------------------------

Buffer		20 mM histidine, 200 mM arginine, 70 mM sucrose, 0.01% (w/v) PS80, pH7.0		
Appearance	0 F/T	Colorless, particle-free		
	5× F/T	Colorless, particle-free		
Concentration (mg/mL)	0 F/T	2.0		
	5× F/T	2.0		
SEC-HPLC	-	HMW %	Mono %	LMW %
	0 F/T	3.1	96.9	0
	5× F/T	3.2	96.7	0.1

EXAMPLE 4

In-vivo Characterization

4.1 Pilot 4-week pharmacokinetics study in cynomolgus monkey

5 The objective of this study was to evaluate the pharmacokinetics profile of anti-TIGIT/PVRIG BsAbs following repeated intravenous infusion administrations in naïve male and female cynomolgus monkeys.

 Eight healthy cynomolgus monkeys (4 male and 4 female) were used in this study. The basic information is shown in Table 12.

10

Table 12. Basic information of cynomolgus monkey

Animal species and strain:	Cynomolgus Monkey (<i>M fascicularis</i>)
History of treatment:	Naïve
Sex and age:	4 male and 4 female, 3-4 years old, <2.8 kg
Breeder/supplier:	Suzhou Xishan Laboratory Animal Co. Ltd.
Study Facility:	Prisys Biotechnologies Co., Ltd.
Animal vivarium:	Suzhou Xishan Laboratory Animal Co. Ltd.
Adaptation:	Not less than 14 days
Room:	Conventional Room
Room temperature:	16 - 28°C
Room relative humidity:	40 - 70%
Light cycle:	12-hour light/dark cycle (08:00 - 20:00)
Animal hosting:	1 monkey/cage
Food:	Free access to food (Shanghai Shilin Biotechnology Co., Ltd.)
Water:	Free access to drinking water (municipal tap water purified with filter system)

The bi-specific antibody W3XX104-T4U1.G15-2.uIgG1 or W3XX104-T4U1.G17-2.uIgG1 was administered intravenously at 50 or 125 mg/kg/dose in one male and one female cynomolgus monkey. Dosing regimen was once per week for four doses (i.v. infusion administered on Days 0, 7, 14 and 21). This study was a non-terminal study, and no scheduled
5 necropsy has been planned. Animals were returned to relaxation rooms for wash-out and relax. Study endpoints included: daily observations, body weight, serum cytokines concentration and pharmacokinetics.

Animals were recorded for body weight twice a week. Serum samples were collected for pharmacokinetics analysis at the indicated timepoints (Day-7, -2, 0 (1h, and 8h post-dose), 1, 2, 3,
10 5, 7 (pre-dose), 11, 14 (pre-dose), 18, 21 (pre-dose, 1h and 8h post-dose), 22, 23, 24, 26 and 28 post first dose). After centrifugation, serum was collected and stored at -80 °C until shipping for analysis. The concentration of testing BsAbs in monkey serum was analyzed by ELISA method. Briefly, goat anti-human IgG (Southern Biotech, cat#2049-01) was used as the capture reagent, and biotinylated goat anti-human IgG (Southern Biotech, cat#2049-08) was used as the detecting
15 reagent. HRP-labeled streptavidin (Thermo, cat#SNN1004) and TMB substrate were used for color development, and the reaction was stopped by 2M HCl. Absorbance was read at 450nm and 540nm using M5e microplate reader (Molecule Devices). The serum concentrations of testing BsAbs were determined from four-parameter fits of the standard curve. The concentrations were subjected to a non-compartmental pharmacokinetic analysis by suing
20 Phoenix WinNonlin software (version 8.1, Pharsight). The linear/log trapezoidal rule was applied in calculating the PK parameters.

Intravenous infusion administration of W3XX104-T4U1.G17-2.uIgG1 and W3XX104-T4U1.G15-2.uIgG1 were well tolerated in cynomolgus monkey at the dose of 50 and 125 mg/kg once per week for 4 weeks, and all animals survived until the end of the study.

25 The bi-specific antibodies T4U1.G17 and T4U1.G15 didn't result in any significant body weight change (Figure 20) during the course of the study.

Exposure was confirmed in all animals by measurement of serum PK. The PK profile is shown in Figures 21-24, and the PK parameters are summarized in Table 13. For T4U1.G17, the mean C_{max} values were 1890 µg/mL and 4980 µg/mL for 50 mg/kg and 125 mg/kg, respectively, while the mean AUC_{0-t} values were 155,645 h*µg/mL and 539,834 h*µg/mL for 50
30 mg/kg and 125 mg/kg, respectively. For T4U1.G15, the mean C_{max} values were 1335 µg/mL and 3305 µg/mL for 50 mg/kg and 125 mg/kg, respectively, while the mean AUC_{0-t} values were 266,105 h*µg/mL and 945,971 h*µg/mL for 50 mg/kg and 125 mg/kg, respectively. Following 4-week repeated weekly dosing to monkeys, the C_{max} values of both T4U1.G17 and T4U1.G15
35 increased in a dose-dependent manner and the AUC_{0-t} values increased in a greater than dose-proportional manner. Based on the serum concentration of 4 doses (day 1 to 28), for the 50 mg/kg and 125 mg/kg group, the mean half-life of T4U1.G17 was 54.5 and 56.3 hours respectively, while T4U1.G15 was 63.5 and 72.5 hours, respectively. Based on the serum

concentration of 1 dose (day 1 to 7), for the 50 mg/kg and 125 mg/kg group, the mean half-life of T4U1.G17 was 55.1 and 73.9 hours respectively, while T4U1.G15 was 90.8 and 94.5 hours, respectively. No sex differences were observed over the study doses of 50 and 125 mg/kg.

Table 13. Summary of PK parameters

PK parameters (Day 0~28)	W3XX104-T4U1.G17- 2.µIgG1		W3XX104-T4U1.G15- 2.µIgG1	
Dose	50 mg/kg	125 mg/kg	50 mg/kg	125 mg/kg
t _½ (h)	54.5	56.3	63.5	72.5
C _{max} (µg/ml)	1890	4980	1335	3305
AUC _{0-t} (h*µg/ml)	155645	539834	266105	945971
C _{min} (µg/ml)	141	426	185	481
C _{avg} (µg/ml)	423	1053	417	1216
Fluctuation (%)	412	429	275	235
V _{ss_obs} (mL/kg)	173	304	409	494
Accumulation_Index	1.09	1.14	1.19	1.26
AUC_TAU(h*µg/ml)	71003	176982	70058	204233
PK parameters (Day 0~7)	W3XX104-T4U1.G17- 2.µIgG1		W3XX104-T4U1.G15- 2.µIgG1	
t _½ (h)	55.1	73.9	90.8	94.5
C _{max} (µg/mL)	1890	4980	1335	3305
AUC _{0-t} (h*µg/ml)	70933	176769	69965	203992
Cl _{obs} (ml/day/kg)	14.8	13.3	12.8	11.2
MRTINF _{obs} (h)	75.3	102	119	121
V _{ss_obs} (mL/kg)	45.7	55.1	63.3	57

5

4.2 *In-vivo* efficacy of anti-TIGIT/PVRIG BsAbs in CT26 colon cancer cell model

This study was performed to evaluate the *in-vivo* efficacy of test articles in the syngeneic CT26 colon cancer cell model in human TIGIT and PVRIG transgenic BALB/c mice (BALB/c-hTIGIT/hPVRIG mice). CT26 cells (1×10⁶ cells/100 µL/mouse) were inoculated subcutaneously into BALB/c-hTIGIT/hPVRIG mice aged 6-8 weeks (provided by GemPharmatech). When the average tumor volume reached 65.85 mm³, 40 tumor-bearing mice were randomly divided into 5 groups (n=8/group) according to tumor volume. The mice in group G1 were administered with PBS. The mice in groups G2-G4 were administered with W3XX104-T4U1.G15-2.µIgG1 at 1.3 mpk, 4 mpk and 13.2 mpk. The mice in G5 were administered with 10 mpk of WBPT117-BMK1 (COM701) and 10 mpk of anti-TIGIT mAb WBP364-BMK1 (Tiragolumab). COM701 is

10

15

produced according to the sequence in patent US 2019/0382477 A1 (Table 1, clone ID: CHA.7.518.1). Tiragolumab is produced according to the sequences in INN PROP. LIST 117.

All mice were administered intraperitoneally (*i.p.*) twice a week (BIW) for 5 doses. Tumor volume and body weight were measured twice a week (D0, D4, D7, D11, D14, D17). The tumor volume was expressed in mm³ using the formula: $TV = 0.5 a \times b^2$, where a and b were the long and short diameters of the tumors, respectively. Inhibition rate of tumor growth based on tumor volume (TGI_{TV}) and inhibition rate of tumor growth based on tumor weight (TGI_{TW}) were measured to evaluate the in-vivo efficacy of test articles. The data were expressed as mean $\pm$ standard error (Mean $\pm$ SEM). For comparison between two groups, Independent Samples T-test was performed. $P < 0.05$ was considered to be statistically significant. GraphPad Prism 9 was used for data visualization.

Table 14. Experimental design

Group	Number of animals	Treatment	Dosage (mpk)	ROA	Frequency
G1	8	PBS	-	<i>i.p.</i>	BIW×5 doses
G2	8	W3XX104-T4U1.G15-2.uIgG1	1.3	<i>i.p.</i>	BIW×5 doses
G3	8	W3XX104-T4U1.G15-2.uIgG1	4	<i>i.p.</i>	BIW×5 doses
G4	8	W3XX104-T4U1.G15-2.uIgG1	13.2	<i>i.p.</i>	BIW×5 doses
G5	8	COM701	10	<i>i.p.</i>	BIW×5 doses
		Tiragolumab	10	<i>i.p.</i>	BIW×5 doses

The body weight changes in different groups are shown in Figure 28. No significant weight loss was observed in any of the groups. The tumor volume changes of mice in different groups are shown in Figure 25, Figure 26, and Table 15. The tumor weight changes of mice in different groups are shown in Figure 27. The statistical analysis of tumor growth inhibition and the P value of different groups are shown in Table 16.

Table 15. TGI_{TV} Changes

Group	D 0	D 4	D 7	D 11	D 14	D 17
G1	-	-	-	-	-	-
G2	0.00%	39.99%	59.37%	66.09%	67.27%	62.08%

G3	0.00%	34.52%	58.82%	69.87%	75.92%	76.75%
G4	0.00%	38.82%	65.85%	79.40%	86.27%	87.30%
G5	0.00%	37.62%	63.32%	74.07%	81.36%	86.69%

Based on the statistical analysis of the tumor volume at endpoint (D 17), W3XX104-T4U1.G15-2.uIgG1 showed dose dependent tumor growth inhibition at dosing levels of 1.3, 4 and 13.2 mg/kg with TGI_{TV} of 62.08%, 76.75% and 86.69% respectively (W3XX104-T4U1.G15-2.uIgG1 treatment groups vs vehicle control, $P<0.05$). Moreover, tumor suppression in W3XX104-T4U1.G15-2.uIgG1 monotherapy group (13.2 mg/kg) was comparable to COM701 and Tiragolumab combination group (10+10 mg/kg) (TGI: 87.30% vs 86.69%, $P>0.05$).

Table 16. Statistical Analysis of Tumor Weight

Group	Tumor weight (g)	TGI_{TW}	<i>P</i> value (vs G1)
G1	1.9012±0.2267	-	-
G2	0.6599±0.1715	65.29%	<0.001***
G3	0.4288±0.1815	77.44%	<0.001***
G4	0.2083±0.0586	89.04%	<0.001***
G5	0.2271±0.0817	88.05%	<0.001***

Note: Data shown as Mean ± SEM; Statistics performed using Independent samples T-test, *: $P<0.05$; **: $P<0.01$; ***: $P<0.001$.

Based on the statistical analysis of tumor weight at endpoint (D 17), W3XX104-T4U1.G15-2.uIgG1 showed dose dependent tumor growth inhibition at dosing levels of 1.3, 4 and 13.2 mg/kg with TGI_{TW} of 65.29%, 77.44% and 89.04% respectively (W3XX104-T4U1.G15-2.uIgG1 treatment groups vs vehicle control, $P<0.05$). Moreover, tumor suppression in W3XX104-T4U1.G15-2.uIgG1 monotherapy group (13.2 mg/kg) was comparable to COM701 and Tiragolumab combination group (10+10 mg/kg) (TGI_{TW}: 89.04% vs 88.05%, $P>0.05$).

In summary, the study showed that the W3XX104-T4U1.G15-2.uIgG1 could dose-dependently (1.3 mpk, 4 mpk and 13.2 mpk) inhibit tumour volume ($P<0.01$) and tumour weight ($P<0.001$) compared with the vehicle control. In addition, single-agent treatment of W3XX104-T4U1.G15-2.uIgG1 (G4, 13.2 mpk of W3XX104-T4U1.G15-2.uIgG1, equal in molecular number to 10 mpk of COM701 or Tiragolumab) could lead to comparable tumor suppression effect compared with the combination treatment of COM701 and Tiragolumab (G5, COM701+Tiragolumab, 10 mpk+10 mpk).

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
5 embodiments that 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.

References

- 10 [1] Manieri NA, Chiang EY, Grogan JL. TIGIT: A Key Inhibitor of the Cancer Immunity Cycle. Trends Immunol. 2017 Jan;38(1):20-28.
- [2] Rotte A, Jin JY, Lemaire V. Mechanistic overview of immune checkpoints to support the rational design of their combinations in cancer immunotherapy. Ann Oncol. 2018 Jan 1;29(1):71-83.
- 15 [3] Yu X, Harden K, et al. The surface protein TIGIT suppresses T cell activation by promoting the generation of mature immunoregulatory dendritic cells. Nat Immunol. 2009 Jan;10(1):48-57.
- [4] Joller N, et al. Treg cells expressing the coinhibitory molecule TIGIT selectively inhibit proinflammatory Th1 and Th17 cell responses. Immunity. 2014 Apr 17;40(4):569-81.
- [5] Stanietsky N, et al. The interaction of TIGIT with PVR and PVRL2 inhibits human NK cell cytotoxicity. Proc Natl Acad Sci U S A. 2009 Oct 20;106(42):17858-63.
- 20 [6] Stengel KF, et al. Structure of TIGIT immunoreceptor bound to poliovirus receptor reveals a cell-cell adhesion and signaling mechanism that requires cis-trans receptor clustering. Proc Natl Acad Sci U S A. 2012 Apr 3;109(14):5399-404.
- [7] Stanietsky N, et al. Mouse TIGIT inhibits NK-cell cytotoxicity upon interaction with PVR. Eur J Immunol. 2013 Aug;43(8):2138-50.
- 25 [8] Reches A, et al. Nectin4 is a novel TIGIT ligand which combines checkpoint inhibition and tumor specificity. J Immunother Cancer. 2020 Jun;8(1):e000266.
- [9] Lozano E, Dominguez-Villar M, Kuchroo V, Hafler DA. The TIGIT/CD226 axis regulates human T cell function. J Immunol. 2012 Apr 15;188(8):3869-75.
- 30 [10] Pauken KE, Wherry EJ. TIGIT and CD226: tipping the balance between costimulatory and coinhibitory molecules to augment the cancer immunotherapy toolkit. Cancer Cell. 2014 Dec 8;26(6):785-787.
- [11] Stamm H, Wellbrock J, Fiedler W. Interaction of PVR/PVRL2 with TIGIT/DNAM-1 as a novel immune checkpoint axis and therapeutic target in cancer. Mamm Genome. 2018 Dec;29(11-12):694-702.
- 35 [12] Chauvin JM, et al. TIGIT and PD-1 impair tumor antigen-specific CD8⁺ T cells in melanoma patients. J Clin Invest. 2015 May;125(5):2046-58.

- [13] Kong Y, et al. T-Cell Immunoglobulin and ITIM Domain (TIGIT) Associates with CD8+ T-Cell Exhaustion and Poor Clinical Outcome in AML Patients. *Clin Cancer Res.* 2016 Jun 15;22(12):3057-66.
- [14] Johnston RJ, et al. The immunoreceptor TIGIT regulates antitumor and antiviral CD8(+) T cell effector function. *Cancer Cell.* 2014 Dec 8;26(6):923-937.
- [15] Fleury M, et al. Increased Expression and Modulated Regulatory Activity of Coinhibitory Receptors PD-1, TIGIT, and TIM-3 in Lymphocytes From Patients With Systemic Sclerosis. *Arthritis Rheumatol.* 2018 Apr;70(4):566-577.
- [16] Fromentin R, et al. CD4+ T Cells Expressing PD-1, TIGIT and LAG-3 Contribute to HIV Persistence during ART. *PLoS Pathog.* 2016 Jul 14;12(7):e1005761.
- [17] Galletti G, et al. Two subsets of stem-like CD8+ memory T cell progenitors with distinct fate commitments in humans. *Nat Immunol.* 2020 Dec;21(12):1552-1562.
- [18] Banta KL, et al. Mechanistic convergence of the TIGIT and PD-1 inhibitory pathways necessitates co-blockade to optimize anti-tumor CD8+ T cell responses. *Immunity.* 2022 Mar 8;55(3):512-526.e9.
- [19] Zhu Y, et al. Identification of CD112R as a novel checkpoint for human T cells. *J Exp Med.* 2016 Feb 8;213(2):167-76.
- [20] Xu F, Sunderland A, Zhou Y, Schulick RD, Edil BH, Zhu Y. Blockade of CD112R and TIGIT signaling sensitizes human natural killer cell functions. *Cancer Immunol Immunother.* 2017 Oct;66(10):1367-1375.
- [21] Murter B, et al. Mouse PVRIG Has CD8+ T Cell-Specific Coinhibitory Functions and Dampens Antitumor Immunity. *Cancer Immunol Res.* 2019 Feb;7(2):244-256.
- [22] Whelan S, et al. PVRIG and PVRL2 Are Induced in Cancer and Inhibit CD8+ T-cell Function. *Cancer Immunol Res.* 2019 Feb;7(2):257-268.
- [23] Sanchez-Correa B, et al. DNAM-1 and the TIGIT/PVRIG/TACTILE Axis: Novel Immune Checkpoints for Natural Killer Cell-Based Cancer Immunotherapy. *Cancers (Basel).* 2019 Jun 23;11(6):877.
- [24] Zhang Z, Wu N, Lu Y, Davidson D, Colonna M, Veillette A. DNAM-1 controls NK cell activation via an ITT-like motif. *J Exp Med.* 2015 Nov 16;212(12):2165-82.
- [25] Alteber Z, Kotturi MF, Whelan S, Ganguly S, Weyl E, Pardoll DM, Hunter J, Ophir E. Therapeutic Targeting of Checkpoint Receptors within the DNAM1 Axis. *Cancer Discov.* 2021 May;11(5):1040-1051.

CLAIMS

1. A polypeptide complex, comprising a TIGIT-binding moiety and a PVRIG-binding moiety, wherein:

the TIGIT-binding moiety comprises a heavy chain variable region (VH) comprising a heavy chain CDR (HCDR) 1, a HCDR2, and a HCDR3, and a light chain variable region (VL) comprising a light chain CDR (LCDR) 1, a LCDR2 and a LCDR3, wherein:

the HCDR1 comprises the amino acid sequence of SEQ ID NO: 1 or an amino acid sequence with substitution of no more than 1, 2 or 3 amino acids compared with SEQ ID NO: 1, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 2 or an amino acid sequence with substitution of no more than 1, 2 or 3 amino acids compared with SEQ ID NO: 2, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 3 or an amino acid sequence with substitution of no more than 1, 2 or 3 amino acids compared with SEQ ID NO: 3, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 4 or an amino acid sequence with substitution of no more than 1, 2 or 3 amino acids compared with SEQ ID NO: 4, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 5 or an amino acid sequence with substitution of no more than 1, 2 or 3 amino acids compared with SEQ ID NO: 5, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 6 or an amino acid sequence with substitution of no more than 1, 2 or 3 amino acids compared with SEQ ID NO: 6;

wherein the substitution is a conservative substitution.

2. The polypeptide complex of claim 1, wherein the PVRIG-binding moiety comprises a VH comprising a HCDR1, a HCDR2, and a HCDR3, and a VL comprising a LCDR1, a LCDR2 and a LCDR3, wherein:

the HCDR1 comprises the amino acid sequence of SEQ ID NO: 7 or an amino acid sequence with substitution of no more than 1, 2 or 3 amino acids compared with SEQ ID NO: 7, the HCDR2 comprises the amino acid sequence of SEQ ID NO: 8 or an amino acid sequence with substitution of no more than 1, 2 or 3 amino acids compared with SEQ ID NO: 8, the HCDR3 comprises the amino acid sequence of SEQ ID NO: 9 or an amino acid sequence with substitution of no more than 1, 2 or 3 amino acids compared with SEQ ID NO: 9, the LCDR1 comprises the amino acid sequence of SEQ ID NO: 10 or an amino acid sequence with substitution of no more than 1, 2 or 3 amino acids compared with SEQ ID NO: 10, the LCDR2 comprises the amino acid sequence of SEQ ID NO: 11 or an amino acid sequence with substitution of no more than 1, 2 or 3 amino acids compared with SEQ ID NO: 11, and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 12 or an amino acid sequence with substitution of no more than 1, 2 or 3 amino acids compared with SEQ ID NO: 12, wherein the substitution is a conservative substitution.

3. The polypeptide complex of claim 1 or 2, wherein the TIGIT-binding moiety and PVRIG-binding moiety are in any of a Fab and a scFv format.
4. The polypeptide complex of claim 1 or 2, wherein the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 of the TIGIT-binding moiety comprise the amino acid sequences of SEQ ID NOs: 1-6, respectively; and the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 of the PVRIG-binding moiety comprise the amino acid sequences of SEQ ID NOs: 7-12, respectively.
5. The polypeptide complex of any of claims 1-4, wherein the VH of the TIGIT-binding moiety comprises the amino acid sequence of SEQ ID NO: 13 or an amino acid sequence at least 85%, 90%, or 95% identical to SEQ ID NO: 13, and/or
the VL of the TIGIT-binding moiety comprises the amino acid sequence of SEQ ID NO: 14 or an amino acid sequence at least 85%, 90%, or 95% identical to SEQ ID NO: 14.
6. The polypeptide complex of any of claims 1-5, wherein the VH of the PVRIG-binding moiety comprises the amino acid sequence of SEQ ID NO: 15 or an amino acid sequence at least 85%, 90%, or 95% identical to SEQ ID NO: 15, and/or
the VL of the PVRIG-binding moiety comprises the amino acid sequence of SEQ ID NO: 16 or an amino acid sequence at least 85%, 90%, or 95% identical to SEQ ID NO: 16.
7. The polypeptide complex of any of the preceding claims, wherein:
the TIGIT-binding moiety is in Fab format and the PVRIG-binding moiety is in scFv format;
the TIGIT-binding moiety is in scFv format and the PVRIG-binding moiety is in Fab format; or
both the TIGIT-binding moiety and the PVRIG-binding moiety are in Fab format.
8. The polypeptide complex of any of the preceding claims, wherein the polypeptide complex further comprises an immunoglobulin constant region, such as an IgG constant region, such as a human IgG1, IgG4, IgG2, IgG3 Fc region or a variant thereof.
9. The polypeptide complex of claim 8, wherein the human IgG Fc region is a human IgG1 Fc region or a variant thereof.
10. The polypeptide complex of claim 9, wherein the variant comprises one or more substitutions to modulate receptor binding or effector function, to promote dimerization, to prevent glycosylation, and/or to extend its half-life.

11. The polypeptide complex of any of claims 8-10, wherein the polypeptide complex comprises:
- (a) the TIGIT-binding moiety operably linked to the Fc region and the Fc region operably linked to the PVRIG-binding moiety;
 - (b) the TIGIT-binding moiety operably linked to the PVRIG-binding moiety and the PVRIG-binding moiety operably linked to the Fc region; or
 - (c) the PVRIG-binding moiety operably linked to the TIGIT-binding moiety and the TIGIT-binding moiety operably linked to the Fc region.
12. The polypeptide complex of any of the preceding claims, comprising one, two or more TIGIT-binding moieties, as well as one, two or more PVRIG-binding moieties.
13. The polypeptide complex of claim 12, wherein the TIGIT-binding moieties are identical or different, and/or the PVRIG-binding moieties are identical or different.
14. The polypeptide complex of any of the preceding claims, comprising two heavy chains and two light chains, wherein the TIGIT-binding moiety is in Fab format and the PVRIG-binding moiety is in scFv format, and wherein from N-terminus to C-terminus:
- the first and second heavy chains each comprises domains that are operably linked into the following format: VH1-CH1-Fc-scFv or scFv-VH1-CH1-Fc,
- the first and second light chains each comprises domains that are operably linked into the following format: VL1-CL,
- wherein the VH1-CH1 and VL1-CL are from the TIGIT-binding moiety and the scFv is from the PVRIG-binding moiety.
15. The polypeptide complex of any of claims 1-13, comprising two heavy chains and two light chains, wherein the TIGIT-binding moiety is in scFv format and the PVRIG-binding moiety is in Fab format, and wherein from N-terminus to C-terminus:
- the first and second heavy chains each comprises domains that are operably linked into the following format: scFv-VH2-CH1-Fc or VH2-CH1-Fc-scFv,
- the first and second light chains each comprises domains that are operably linked into the following format: VL2-CL,
- wherein the scFv is from the TIGIT-binding moiety and the VH2-CH1 and VL2-CL are from the PVRIG-binding moiety.
16. The polypeptide complex of any of claims 1-13, comprising two heavy chains and four light chains, wherein the TIGIT-binding moiety and the PVRIG-binding moiety are in Fab format, and

wherein from N-terminus to C-terminus:

the first and second heavy chains each comprises domains that are operably linked into the following format: VH1-CH1-Fc-VH2-CH1, VH2-CH1-Fc-VH1-CH1, VH1-CH1-VH2-CH1-Fc or VH2-CH1-VH1-CH1-Fc,

the first and second light chains each comprises domains that are operably linked into the following format: VL1-CL,

the third and fourth light chains each comprises domains that are operably linked into the following format: VL2-CL,

wherein the VH1-CH1 and VL1-CL are from the TIGIT-binding moiety and the VH2-CH1 and VL2-CL are from the PVRIG-binding moiety.

17. The polypeptide complex of any of claims 1-13, comprising two heavy chains and four light chains, wherein the TIGIT-binding moiety and the PVRIG-binding moiety are in Fab format, and wherein from N-terminus to C-terminus:

the first and second heavy chains each comprises domains that are operably linked into the following format: VH1-C1-Fc-VH2-CH1, VH2-CH1-Fc-VH1-C1, VH1-C1-VH2-CH1-Fc or VH2-CH1-VH1-C1-Fc,

the first and second light chains each comprises domains that are operably linked into the following format: VL1-C2,

the third and fourth light chains each comprises domains that are operably linked into the following format: VL2-CL,

wherein the VH1-C1 and VL1-C2 are from the TIGIT-binding moiety and the VH2-CH1 and VL2-CL are from the PVRIG-binding moiety.

18. The polypeptide complex of any of claims 1-13, comprising two heavy chains and four light chains, wherein the TIGIT-binding moiety and the PVRIG-binding moiety are in Fab format, and wherein from N-terminus to C-terminus:

the first and second heavy chains each comprises domains that are operably linked into the following format: VH1-CH1-Fc-VH2-C1, VH2-C1-Fc-VH1-CH1, VH1-CH1-VH2-C1-Fc or VH2-C1-VH1-CH1-Fc,

the first and second light chains each comprises domains that are operably linked into the following format: VL1-CL,

the third and fourth light chains each comprises domains that are operably linked into the following format: VL2-C2,

wherein the VH1-CH1 and VL1-CL are from the TIGIT-binding moiety and the VH2-C1 and

VL2-C2 are from the PVRIG-binding moiety.

19. The polypeptide complex of any of claims 7-18, wherein the scFv comprises a VH region operably linked to a VL region, and the VH region is at the N terminal of the VL region or the VL region is at the N terminal of the VH region.

20. The polypeptide complex of any of claims 11-19, wherein operably linked is via a direct linkage or via a peptide linker.

21. The polypeptide complex of claim 20, wherein the peptide linker is a hinge region or a GS linker, such as (GS)_n, (GGS)_n, (GGGS)_n, (GGGGS)_n, (GGSG)_n, (GGGSS)_n, wherein n is an integer of 1-9.

22. The polypeptide complex of any of claims 14-15, comprising:

(i) a first and a second heavy chain that comprise SEQ ID NO: 17, and a first and a second light chain that comprise SEQ ID NO: 18; or

(ii) a first and a second heavy chain that comprise SEQ ID NO: 19, and a first and a second light chain that comprise SEQ ID NO: 20.

23. 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 polypeptide complex of any of claims 1-22.

24. A vector comprising the isolated nucleic acid molecule of claim 23.

25. A host cell comprising the vector of claim 24 or the isolated nucleic acid molecule of claim 23.

26. A pharmaceutical composition comprising the polypeptide complex of any of claims 1-22 and a pharmaceutically acceptable carrier.

27. A method for producing the polypeptide complex of any of claims 1-22 comprising the steps of:

- culturing a host cell comprising an expression vector(s) encoding the polypeptide complex under suitable conditions; and

- harvesting the polypeptide complex from the cell culture.

28. A method for treating or preventing a cancer or an immune related disorder in a subject (such as human and non-human mammals such as mouse), comprising administering an effective amount of the polypeptide complex of any of claims 1-22 or the pharmaceutical composition of claim 26 to the subject.

29. The method of claim 28, 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, for example the cancer is colon cancer.

30. The method of claim 28, wherein the immune related disorder is a T cell dysfunctional disorder or an infection.

31. The method of any of claims 29-30, wherein the method further comprises administering an additional therapeutic agent to the subject.

32. The polypeptide complex of any of claims 1-22 for use in treating or preventing cancer or a T cell dysfunctional disorder or an infection.

33. Use of the polypeptide complex of any of claims 1-22 in the manufacture of a medicament for treating or preventing cancer or a T cell dysfunctional disorder or an infection.

34. The polypeptide complex for use according to claim 32 or the use of claim 33, 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, for example the cancer is colon cancer.

35. A kit comprising a container comprising the polypeptide complex of any of claims 1-22.