

ANTI-TNFR2 ANTIBODIES AND METHODS OF USE**Cross-Reference to Related Applications**

This application claims the benefit under 35 U.S.C. § 119(e) to U.S. Provisional Application No. 62/901,364, filed September 17, 2019; U.S. Provisional Application No. 62/985,509, filed March 5, 2020; U.S. Provisional Application No. 63/047,824, filed July 2, 2020; and U.S. Provisional Application No. 63/058,016, filed July 29, 2020; each of which is incorporated by reference in its entirety.

Statement Regarding the Sequence Listing

The Sequence Listing associated with this application is provided in text format in lieu of a paper copy, and is hereby incorporated by reference into the specification. The name of the text file containing the Sequence Listing is APEX-025/04WO_ST25.txt. The text file is about 265 KB, created on September 11, 2020, and is being submitted electronically via EFS-Web.

Background*Technical Field*

The present disclosure relates to anti-tumor necrosis factor receptor 2 (TNFR2) antibodies and related compositions, which may be used in any of a variety of therapeutic or diagnostic methods, including the treatment or diagnosis of oncological diseases, inflammatory and/or autoimmune diseases, and others.

Description of the Related Art

TNF- α is an essential inflammatory mediator that plays a critical role in both physiological and pathological conditions. TNF- α is primarily produced by macrophages and monocytes and can exist as both a membrane-bound trimer of 26 kDa (mTNF- α) and a soluble trimer of 17kDa (sTNF- α). TNF- α exerts its effects through two receptors, TNFR1 (TNFRSF1A; 55kDa) and TNFR2 (TNFRSF1B; 75kDa), which have similar extracellular domains containing 4 repeated cysteine-rich motifs, but which also have divergent intracellular domains that activate distinct signaling pathways.

TNFR1 is a ubiquitously expressed protein and can be engaged by both mTNF- α and sTNF- α , to signal cell survival/inflammation or apoptosis depending on context. In contrast, TNFR2 expression is regulated by activation status and is restricted mainly to T cells and immune suppressive myeloid cells, also known as myeloid-derived suppressor cells (MDSC) which include mononuclear and granulocytic myeloid cells. Mononuclear myeloid cells include terminally differentiated macrophages and dendritic cells (DCs), as well as monocytes, which under inflammatory conditions differentiate in tissues to macrophages and DCs. Granulocytic myeloid cells include populations of terminally differentiated polymorphonuclear neutrophils, eosinophils, basophils, and mast cells.

TNFR2 can only be fully engaged via mTNF- α . Unlike TNFR1, TNFR2 does not contain a death domain in its cytoplasm; instead, it can signal through TRAFs and the NF κ B pathway to regulate cell survival and immune suppression. mTNF also exhibits reverse signaling in the cell in which it is expressed upon receptor engagement. Both TNFRs can be cleaved by TACE enzymes and converted into soluble forms which may act to desensitize cells to TNF- α by removing receptor from the cell or acting as a decoy for sTNF- α .

TNFR2 plays a vital role in the modulation of the immune system, most likely through its effects on regulatory T cells (Tregs), which express high levels of TNFR2. In mice, deletion of TNFR2 exacerbates autoimmune disease and colitis by decreasing the function of Tregs. In human, known polymorphisms in TNFRSF1B result in decreased TNFR2 expression or decreased binding to TNF α and hamper TNFR2-mediated signaling in regulatory T cells. These polymorphisms are strongly correlated with autoimmune diseases including systemic lupus erythematosus (SLE), Crohn's disease, and ulcerative colitis. In both mice and human, TNFR2 is expressed on highly suppressive Tregs, including those found in tumors, but is not strongly expressed on effector T cells. TNFR2 expression is strongly correlated with a suppressive tumor microenvironment in numerous tumor types. Also, it was demonstrated in ovarian cancer that TNFR2+ Tregs could impair T effector responses within the TME (Govindaraj C). Mouse models have provided further evidence for the role of TNFR2 in hampering the immune response to cancer. Moreover, TNFR2 has been identified on more than 25 tumor types, including renal, colon, and ovarian cancers. Gain-of-function TNFR2 mutations occur in Sézary syndrome patients who have a rare form of CTCL that is refractory to treatment.

Thus, there remains a need in the art for therapeutic antibodies that effectively inhibit or otherwise antagonize TNFR2, and related methods of treating cancer and inflammatory diseases.

Brief Summary

The present disclosure relates to antibodies and antigen-binding fragments thereof that specifically bind to tumor necrosis factor receptor 2 (TNFR2) and methods of use thereof. One aspect provides an isolated antibody, or an antigen-binding fragment thereof, that binds to TNFR2, including human TNFR2, comprising:

a heavy chain variable (VH) region comprising VHCDR1, VHCDR2, and VHCDR3 regions set forth respectively in SEQ ID NOs: 1-3; and a light chain variable (VL) region comprising VLCDR1, VLCDR2, and VLCDR3 regions set forth respectively in SEQ ID NOs: 4-6;

a VH region comprising VHCDR1, a VHCDR2, and VHCDR3 regions set forth respectively in SEQ ID NOs: 7-9; and a VL region comprising VLCDR1, VLCDR2, and VLCDR3 regions set forth respectively in SEQ ID NOs: 10-12;

a VH region comprising VHCDR1, VHCDR2, and VHCDR3 regions set forth respectively in SEQ ID NOs: 13-15; and a VL region comprising VLCDR1, VLCDR2, and VLCDR3 regions set forth respectively in SEQ ID NOs: 16-18;

a VH region comprising VHCDR1, VHCDR2, and VHCDR3 regions set forth respectively in SEQ ID NOs: 19-21; and a VL region comprising VLCDR1, VLCDR2, and VLCDR3 regions set forth respectively in SEQ ID NOs: 22-24;

a VH region comprising VHCDR1, VHCDR2, and VHCDR3 regions set forth respectively in SEQ ID NOs: 25-27; and a VL region comprising VLCDR1, VLCDR2, and VLCDR3 regions set forth respectively in SEQ ID NOs: 28-30;

a VH region comprising VHCDR1, VHCDR2, and VHCDR3 regions set forth respectively in SEQ ID NOs: 31-33; and a VL region comprising VLCDR1, VLCDR2, and VLCDR3 regions set forth respectively in SEQ ID NOs: 34-36;

a VH region comprising VHCDR1, VHCDR2, and VHCDR3 regions set forth respectively in SEQ ID NOs: 37-39; and a VL region comprising VLCDR1, VLCDR2, and VLCDR3 regions set forth respectively in SEQ ID NOs: 40-42;

a VH region comprising VHCDR1, VHCDR2, and VHCDR3 regions set forth respectively in SEQ ID NOs: 43-45; and a VL region comprising VLCDR1, VLCDR2, and VLCDR3 regions set forth respectively in SEQ ID NOs: 46-48;

a VH region comprising VHCDR1, VHCDR2, and VHCDR3 regions set forth respectively in SEQ ID NOs: 49-51; and a VL region comprising VLCDR1, VLCDR2, and VLCDR3 regions set forth respectively in SEQ ID NOs: 52-54;

a VH region comprising VHCDR1, VHCDR2, and VHCDR3 regions set forth respectively in SEQ ID NOs: 55-57; and a VL region comprising VLCDR1, VLCDR2, and VLCDR3 regions set forth respectively in SEQ ID NOs: 58-60;

a VH region comprising VHCDR1, VHCDR2, and VHCDR3 regions set forth respectively in SEQ ID NOs: 61-63; and a VL region comprising VLCDR1, VLCDR2, and VLCDR3 regions set forth respectively in SEQ ID NOs: 64-66;

a VH region comprising VHCDR1, VHCDR2, and VHCDR3 regions set forth respectively in SEQ ID NOs: 67-69; and a VL region comprising VLCDR1, VLCDR2, and VLCDR3 regions set forth respectively in SEQ ID NOs: 70-72;

a VH region comprising VHCDR1, VHCDR2, and VHCDR3 regions set forth respectively in SEQ ID NOs: 73-75; and a VL region comprising VLCDR1, VLCDR2, and VLCDR3 regions set forth respectively in SEQ ID NOs: 76-78;

a VH region comprising VHCDR1, VHCDR2, and VHCDR3 regions set forth respectively in SEQ ID NOs: 79-81; and a VL region comprising VLCDR1, VLCDR2, and VLCDR3 regions set forth respectively in SEQ ID NOs: 82-84;

a VH region comprising VHCDR1, VHCDR2, and VHCDR3 regions set forth respectively in SEQ ID NOs: 85-87; and a VL region comprising VLCDR1, VLCDR2, and VLCDR3 regions set forth respectively in SEQ ID NOs: 88-90;

a VH region comprising VHCDR1, VHCDR2, and VHCDR3 regions set forth respectively in SEQ ID NOs: 91-93; and a VL region comprising VLCDR1, VLCDR2, and VLCDR3 regions set forth respectively in SEQ ID NOs: 94-96; or

a VH region comprising VHCDR1, VHCDR2, and VHCDR3 regions set forth respectively in SEQ ID NOs: 97-99; and a VL region comprising VLCDR1, VLCDR2, and VLCDR3 regions set forth respectively in SEQ ID NOs: 100-102;

or a variant of said antibody, or an antigen-binding fragment thereof, comprising heavy and light chain variable regions identical to the heavy and light chain variable regions of (i) and (ii) except for up to 1, 2, 3, 4, 5, 6, 7, or 8 total amino acid substitutions across said CDR regions.

In some embodiments, the VH region comprises an amino acid sequence having at least 90% identity to a sequence selected from SEQ ID NOs: 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, and 135. In some embodiments, the VL region comprises an amino acid sequence having at least 90% identity to a sequence selected from SEQ ID NOs: 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 134, and 136.

Certain antibodies, or antigen-binding fragments thereof, comprise:

the VH region set forth in SEQ ID NO: 103, and the VL region set forth in SEQ ID NO: 104;
the VH region set forth in SEQ ID NO: 105, and the VL region set forth in SEQ ID NO: 106;
the VH region set forth in SEQ ID NO: 107, and the VL region set forth in SEQ ID NO: 108;
the VH region set forth in SEQ ID NO: 109, and the VL region set forth in SEQ ID NO: 110;
the VH region set forth in SEQ ID NO: 111, and the VL region set forth in SEQ ID NO: 112;
the VH region set forth in SEQ ID NO: 113, and the VL region set forth in SEQ ID NO: 114;
the VH region set forth in SEQ ID NO: 115, and the VL region set forth in SEQ ID NO: 116;
the VH region set forth in SEQ ID NO: 117, and the VL region set forth in SEQ ID NO: 118;
the VH region set forth in SEQ ID NO: 119, and the VL region set forth in SEQ ID NO: 120;
the VH region set forth in SEQ ID NO: 121, and the VL region set forth in SEQ ID NO: 122;
the VH region set forth in SEQ ID NO: 123, and the VL region set forth in SEQ ID NO: 124;
the VH region set forth in SEQ ID NO: 125, and the VL region set forth in SEQ ID NO: 126;
the VH region set forth in SEQ ID NO: 127, and the VL region set forth in SEQ ID NO: 128;
the VH region set forth in SEQ ID NO: 129, and the VL region set forth in SEQ ID NO: 130;
the VH region set forth in SEQ ID NO: 131, and the VL region set forth in SEQ ID NO: 132;
the VH region set forth in SEQ ID NO: 133, and the VL region set forth in SEQ ID NO: 134;

or

the VH region set forth in SEQ ID NO: 135, and the VL region set forth in SEQ ID NO: 136.

Some embodiments include an isolated antibody, or an antigen-binding fragment thereof, that binds to tumor necrosis factor receptor 2 (TNFR2), comprises a heavy chain variable (VH) region which comprises an amino acid sequence having at least 90% identity to a sequence selected from SEQ ID NOs: 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, and 135, and, respectively, a light chain variable (VL) region which comprises an amino acid sequence having at least 90% identity to a sequence selected from SEQ ID NO: 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 134, and 136.

Some embodiments include an isolated antibody, or an antigen-binding fragment thereof, that binds to tumor necrosis factor receptor 2 (TNFR2), comprises a heavy chain variable (VH) region comprising VHCDR1, VHCDR2, and VHCDR3 regions selected from the underlined sequences in **Table R1**; and, respectively, a light chain variable (VL) region comprising VLCDR1, VLCDR2, and VLCDR3 regions selected from underlined sequences in **Table R2**.

Some embodiments include an isolated antibody, or an antigen-binding fragment thereof, of claim 6, comprising a VH region which comprises an amino acid sequence selected from **Table R1**, and, respectively, a VL region which comprises an amino acid sequence selected from **Table R2**.

Some embodiments include an isolated antibody, or an antigen-binding fragment thereof, that binds to human tumor necrosis factor receptor 2 (TNFR2) at an epitope that comprises, consists, or consists essentially of one or more residues selected from R21, Y23, T27, S33, K34, T51, and S55, as defined by the mature human TNFR2 sequence (residues 23-461 of FL human TNFR2), including, for example, wherein the epitope comprises, consists, or consists essentially of one or more residues selected from REY, TAQMCCSK (SEQ ID NO: 328), and TVCDS (SEQ ID NO: 329). In some embodiments, the isolated antibody, or antigen-binding fragment thereof, comprises a heavy chain variable (VH) region comprising VHCDR1, VHCDR2, and VHCDR3 regions set forth respectively in SEQ ID NOs: 37-39; and a light chain variable (VL) region comprising VLCDR1, VLCDR2, and VLCDR3 regions set forth respectively in SEQ ID NOs: 40-42. In some embodiments, the VH region comprises an amino acid sequence having at least 90% identity to SEQ ID NO: 115. In some embodiments, the VL region comprises an amino acid sequence having at least 90% identity to SEQ ID NO: 116. In specific embodiments, the isolated antibody, or antigen-binding fragment thereof, comprises the VH region set forth in SEQ ID NO: 115, and the VL region set forth in SEQ ID NO: 116.

In some embodiments, the isolated antibody, or an antigen-binding fragment thereof, binds to human TNFR2, for example, soluble and/or cell-expressed human TNFR2.

In some embodiments, the isolated antibody, or an antigen-binding fragment thereof, binds to at least one, two, three, four, or five human TNFR2 peptide epitopes selected from **Table T1**.

In some embodiments, the antibody is humanized. In some embodiments, the antibody is selected from the group consisting of a single chain antibody, a scFv, a univalent antibody lacking a hinge region, a minibody, and a probody. In some embodiments, the antibody is a Fab or a Fab'

fragment. In some embodiments, the antibody is a $F(ab')_2$ fragment. In some embodiments, the antibody is a whole antibody.

In some embodiments, the antibody comprises a human IgG constant domain. In some embodiments, the IgG constant domain comprises an IgG1 CH1 domain. In some embodiments, the IgG constant domain comprises an IgG1 Fc region, optionally a modified Fc region, optionally modified by one or more amino acid substitutions.

In some embodiments, the isolated antibody, or antigen-binding fragment thereof, binds to human TNFR2, for example, at least one peptide epitope from **Table T1**, with a K_D of about 2 nM or lower. In some embodiments, the isolated antibody, or antigen-binding fragment thereof, binds to human TNFR2 with a K_D of about 0.7 nM or lower, or binds to human TNFR2 on primary T cells, optionally T_{reg} s, with a K_D of about 50 pM or lower.

In some embodiments, the isolated antibody, or antigen-binding fragment thereof, has one or more of the following characteristics:

- (a) inhibits TNF- α binding to TNFR2;
- (b) inhibits TNFR2 signaling;
- (c) activates TNFR2 signaling;
- (d) inhibits TNFR2 trimerization;
- (e) cross-reactively binds to human TNFR2 and cynomolgus monkey TNFR2;
- (f) increases/induces cell-killing/depletion of tumor cells, T_{reg} s, and/or suppressive myeloid cells (optionally macrophages, neutrophils, and myeloid-derived suppressor cells (MDSCs)) by antibody-dependent cellular cytotoxicity (ADCC);
- (g) increases/induces cell-killing/depletion of tumor cells, T_{reg} s, and/or suppressive myeloid cells (optionally macrophages, neutrophils, and MDSCs) by macrophage-mediated antibody-dependent cellular phagocytosis (ADCP);
- (h) reduces immune suppression by myeloid cells (optionally macrophages, neutrophils, and MDSCs);
- (i) converts MDSCs and/or M2 macrophages into proinflammatory M1 macrophages;
- (j) converts T_{reg} s into effector T cells;
- (k) converts cold tumors into hot tumors;
- (l) reduces T_{reg} mediated immune suppression; or
- (m) a combination of any one or more of (a)-(k).

In some embodiments, the isolated antibody, or antigen-binding fragment thereof, does not substantially bind to TNFR1, herpesvirus entry mediator (HVEM), CD40, death receptor 6 (DR6), and/or osteoprotegerin (OPG). In some embodiments, the isolated antibody, or antigen-binding fragment thereof, is a TNFR2 antagonist. In some embodiments, the isolated antibody, or antigen-binding fragment thereof, is a TNFR2 agonist. In some embodiments, the isolated antibody, or antigen-binding fragment thereof, is a bi-specific or multi-specific antibody.

Certain embodiments include an isolated polynucleotide encoding the isolated antibody, or antigen-binding fragment thereof, as described herein, an expression vector comprising the isolated polynucleotide, or an isolated host cell comprising the vector.

Also included is a composition comprising a physiologically acceptable carrier and a therapeutically effective amount of the isolated antibody or antigen-binding fragment thereof described herein.

Also included are methods for treating a patient having a cancer, for instance, a cancer associated with aberrant TNFR2 expression, comprising administering to the patient a composition described herein, thereby treating the cancer.

Also included are methods for treating a patient having a cancer, for instance, a cancer associated with TNFR2 antagonist-mediated immune suppression, comprising administering to the patient a composition described herein, thereby treating the cancer. In some embodiments, the antibody, or antigen-binding fragment thereof, is a TNFR2 antagonist.

Also included are methods for treating a patient having an inflammatory and/or autoimmune disease, comprising administering to the patient a composition described herein, thereby treating the inflammation. In some embodiments, the inflammatory and/or autoimmune disease is associated with aberrant TNFR2 expression, for example, wherein the antibody, or antigen-binding fragment thereof, is a TNFR2 agonist. In some embodiments, the inflammatory and/or autoimmune disease is associated with TNFR2 agonist-mediated immune activation.

Brief Description of the Drawings

Figure 1 illustrates a proposed mechanism for the activity of anti-TNFR2 antibodies in cancer immunotherapy.

Figure 2 illustrates the antibody immunization and screening scheme described herein.

Figures 3A-3D show the results of the first set of human chimeric antibody characterization. In Figures 3A-3B, human (3A) or cynomolgus (3B) TNFR2-His tagged protein was coated on 96-well ELISA plates at 1 µg/mL and incubated overnight at 4°C. Plates were washed and blocked using 1% BSA. Antibodies were added for 1 hour at room temperature (RT), washed, and detected using anti-Human HRP. Assay was developed using TMB substrate for 3-5 minutes. In Figure 3C, FACS binding was performed on CHO cells that were engineered to express human TNFR2. Briefly, antibodies were incubated with 200,000 cells in MACS buffer for 1 hour followed by wash and detection using anti-human IgG BV421 for 20 minutes. Samples were analyzed using Cytotflex or MACSQuant flow cytometers. In Figure 3D, ligand blocking ELISA was performed by coating plates with 1 µg/mL TNFR-Fc overnight at 4°C. Plates were washed, blocked and antibodies were incubated for 1 hr at RT. Antibodies were washed and 100ng/mL TNF-α was added and incubated for 1 hour at RT. TNF-α was detected after washing with mouse anti-human TNF-α and anti-mouse IgG HRP. Assay was developed using TMB substrate for 5-7 minutes.

Figures 4A-4D show the results of the second set of human chimeric antibody characterization, including antibody binding to soluble (4A) and cell-based human TNFR2 (4C), Cyno TNFR2 (4B), and ELISA based TNF- α blocking (4D). Performed as described in Figures 3A-3D above.

Figure 5 shows TNFR signaling by human chimeric antibodies on the NF κ B HEK reporter line. HEK TNFR reporter cells from Promega were plated at 50,000 cells per well in a flat-bottom plate. 10 μ g/mL of the indicated antibodies or 0.2 ng/mL TNF- α were added and incubated with the cells at 37°C for 20 hrs. Reporter activity was detected using QuantiBlue reagent at a 4:1 ratio with supernatant for 10 minutes. Plates were read by SpectroMax at 655 nm. The data indicates that while the signal level is low, 55F6 has some agonist activity while the other antibodies do not have significant activity.

Figures 6A-6D show humanized antibody binding to soluble (6A) and cell-based human TNFR2 (6C), Cyno TNFR2 (6B), and ELISA based TNF- α blocking (6D).

Figure 7 shows cell-based TNF α blocking assay with humanized candidates. TNFR2-overexpressing CHO cells were plated at 100,000 cells per well. Cells were incubated with the indicated antibodies for 30 minutes on ice. Cells were washed and 14ng/mL biotinylated-TNF α was added for 30 minutes. TNF α was washed off and detected with SA-PE for 15 minutes. Cells were analyzed using Cytoflex or MACSQuant flow cytometer. Assay was repeated twice.

Figure 8 shows a soluble TNFR1 binding assay. TNFR1-His tagged protein was coated on ELISA plates at 1 μ g/mL at 4°C overnight. Plates were washed, blocked, and antibodies were added at indicated concentrations for 1 hour at RT. Antibodies were washed and detected with anti-human IgG HRP for 1 hour. Assay was developed using TMB substrate for 10 minutes.

Figures 9A-9B show ADCC of TNFR2-CHO cells by reporter assay. The ADCC Reporter Bioassay Core kit from Promega was used per manufacturer's protocol. Briefly, 25,000 TNFR2-CHO cells were added to flat-well plates in assay buffer. Antibodies were added at the indicated concentration. 75,000 effector cells were added per well (E:T = 3:1). Plates were incubated for 6 hours at 37°C. After 6 hours, plates were equilibrated to room temperature and reporter activity was detected using Bio-Glo Luciferase substrate measured after 5 minutes on the SpectroMAX plate reader. Figure 9A shows the fold change over isotype, which was calculated as RLU (induced-bkgd)/RLU (isotype control – bkgd). Figure 9B shows RLU rather than fold change.

Figures 10A-10F show high affinity monovalent binding of test antibodies by Octet [humanized clones 25-71 (A; K_{on} =3.58E+05 1/Ms; K_{off} =5.53E-04 1/s) and 25-108 (B; K_{on} =3.76E+05 1/Ms; K_{off} =2.33E-04 1/s)] to TNFR2 and binding to human TNFR2 protein by ELISA (10C), cynomolgus TNFR2 protein by ELISA (10D), cell-expressed human TNFR2 (10E), activated human T_{regs} (10F).

Figures 11A-11E show that 25-71 and 25-108 are specific to TNFR2, as shown by lack of binding to TNFR1 (11A), herpesvirus entry mediator (HVEM; 11B), CD40 (11C), death receptor 6 (DR6; 11D), and osteoprotegerin (OPG; 11E).

Figures 12A-12B show cell-killing/depletion of TNFR2-expressing cells (12A, transfectants) and TNFR2-expressing tumor cells (12B, K562, human AML cell line) by 25-71 and 25-108 via antibody-dependent cellular cytotoxicity (ADCC).

Figures 13A-13B demonstrate cell-killing/depletion of TNFR2-expressing human T_{regs} by test antibodies via ADCC.

Figures 14A-14B show cell-killing of TNFR2-expressing tumor cells by 5-71 and 25-108 via macrophage-mediated antibody-dependent cellular phagocytosis (ADCP).

Figures 15A-15C show that 25-71 and 25-108 can reverse myeloid-derived suppressor cell (MDSC)-mediated immune suppression from two different donors (15B and 15C). Percent suppression = T alone – [(T+MDSC)/T alone] X 100.

Figure 16 shows the epitope sites between antibody clone 25-71 and a region of full-length human TNFR2 (SEQ ID NO: 327), which epitope include residues 43, 45, 49, 55, 56, 73, and 77 of full-length TNFR2.

Figures 17A-17J show the interaction between antibody clone 25-71 and human TNFR2. The TNFR2 PDB structure 3ALQ is colored in gray on the epitope sites, corresponding to residues 43-45 (REY); residues 49-56 (TAQMCCSK; SEQ ID NO: 328); and residues 73-77 (TVCD; SEQ ID NO: 329) of the full-length human TNFR2 sequence. Shown are ribbon/surface representations of the front view (A), back view (B), side view 1 (C), side view 2 (D), and top view (E); and ribbon representations of the front view (F); back view (G), side view 1 (H), side view 2 (I), and top view (J).

Figures 18A-18E show that clone 25-71 reverses T_{reg} suppression of effector T cells. In **18A**, purified T_{regs} express TNFR2 when added to suppression assay. In **18B-18C**, the data is plotted as % proliferation of T responder cells (B) or percent Treg suppression (C). **Figures 18D-18E** show exemplary proliferation histograms from the 1:2 Tresp:Treg ratio condition (D) and IgG1 control (E).

Figures 19A-19C show the anti-tumor effects (48% TGI) of clone 25-71 in female nude mice injected with Colo205 cells. **19A** outlines the treatment protocol, **19B** shows the effect of test agents on tumor volume, and **19C** shows the effect on body weight.

Detailed Description

The present disclosure relates to antibodies, and antigen-binding fragments thereof, which specifically bind to tumor necrosis factor receptor 2 (TNFR2), in particular antibodies having specific epitopic specificity and functional properties. Some embodiments encompasses specific humanized antibodies and fragments thereof capable of binding to TNFR2, blocking TNFR2 binding with its ligand tumor necrosis factor- α (TNF- α), and inhibiting induced downstream cell signaling and biological effects. In certain embodiments, an anti-TNFR2 antibody, or antigen-binding fragment

thereof, is a TNFR2 antagonist or inhibitor. In some instances, an antagonist of TNFR2 enhances immune responses by blocking the immunosuppressive actions of TNFR2, for example, in the tumor microenvironment. TNFR2 antagonist antibodies described herein are useful in the treatment and prevention of, for example, cancer, including TNFR2-expressing cancers.

Some embodiments pertain to the use of anti-TNFR2 antibodies, or antigen-binding fragments thereof, for the diagnosis, assessment, and treatment of diseases and disorders associated with TNFR2 activity or aberrant expression thereof. The subject antibodies are used in the treatment or prevention of cancer among other diseases.

The practice of the present disclosure will employ, unless indicated specifically to the contrary, conventional methods of virology, immunology, microbiology, molecular biology and recombinant DNA techniques within the skill of the art, many of which are described below for the purpose of illustration. Such techniques are explained fully in the literature. See, *e.g.*, *Current Protocols in Molecular Biology* or *Current Protocols in Immunology*, John Wiley & Sons, New York, N.Y.(2009); Ausubel *et al.*, *Short Protocols in Molecular Biology*, 3rd ed., Wiley & Sons, 1995; Sambrook and Russell, *Molecular Cloning: A Laboratory Manual* (3rd Edition, 2001); Maniatis *et al.* *Molecular Cloning: A Laboratory Manual* (1982); *DNA Cloning: A Practical Approach*, vol. I & II (D. Glover, ed.); *Oligonucleotide Synthesis* (N. Gait, ed., 1984); *Nucleic Acid Hybridization* (B. Hames & S. Higgins, eds., 1985); *Transcription and Translation* (B. Hames & S. Higgins, eds., 1984); *Animal Cell Culture* (R. Freshney, ed., 1986); Perbal, *A Practical Guide to Molecular Cloning* (1984) and other like references.

As used in this specification and the appended claims, the singular forms “a,” “an” and “the” include plural references unless the content clearly dictates otherwise.

By “about” is meant a quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length that varies by as much as 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1% to a reference quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length.

An “antagonist” refers to an agent (*e.g.*, antibody) that interferes with or otherwise reduces the physiological action of another agent or molecule. In some instances, the antagonist specifically binds to the other agent or molecule. Included are full and partial antagonists.

An “agonist” refers to an agent (*e.g.*, antibody) that increases or enhances the physiological action of another agent or molecule. In some instances, the agonist specifically binds to the other agent or molecule. Included are full and partial agonists.

Throughout this specification, unless the context requires otherwise, the word “comprise”, or variations such as “comprises” or “comprising”, will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

By “consisting of” is meant including, and limited to, whatever follows the phrase “consisting of.” Thus, the phrase “consisting of” indicates that the listed elements are required or mandatory, and

that no other elements may be present. By “consisting essentially of” is meant including any elements listed after the phrase, and limited to other elements that do not interfere with or contribute to the activity or action specified in the disclosure for the listed elements. Thus, the phrase “consisting essentially of” indicates that the listed elements are required or mandatory, but that other elements are optional and may or may not be present depending upon whether or not they materially affect the activity or action of the listed elements.

Each embodiment in this specification is to be applied *mutatis mutandis* to every other embodiment unless expressly stated otherwise.

The terms “modulating” and “altering” include “increasing,” “enhancing” or “stimulating,” as well as “decreasing,” “reducing,” or “inhibiting,” typically in a statistically significant or a physiologically significant amount or degree relative to a control. An “increased,” “stimulated” or “enhanced” amount is typically a “statistically significant” amount, and may include an increase that is 1.1, 1.2, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100 or more times (e.g., 500, 1000 times) (including all integers and ranges in between e.g., 1.5, 1.6, 1.7, 1.8, etc.) the amount produced by no composition (e.g., the absence of agent) or a control composition. A “decreased” or “reduced” or “inhibited” amount is typically a “statistically significant” amount, and may include a 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% decrease (including all integers and ranges in between) in the amount produced by no composition (e.g., the absence of an agent) or a control composition. Examples of comparisons and “statistically significant” amounts are described herein.

“Substantially” or “essentially” means nearly totally or completely, for instance, 95%, 96%, 97%, 98%, 99% or greater of some given quantity.

By “statistically significant,” it is meant that the result was unlikely to have occurred by chance. Statistical significance can be determined by any method known in the art. Commonly used measures of significance include the p-value, which is the frequency or probability with which the observed event would occur, if the null hypothesis were true. If the obtained p-value is smaller than the significance level, then the null hypothesis is rejected. In simple cases, the significance level is defined at a p-value of 0.05 or less.

Standard techniques may be used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation (e.g., electroporation, lipofection). Enzymatic reactions and purification techniques may be performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein. These and related techniques and procedures may be 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 specific definitions are provided, the nomenclature utilized in connection with, and the laboratory procedures and techniques of, molecular biology, analytical chemistry, synthetic

organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well-known and commonly used in the art. Standard techniques may be used for recombinant technology, molecular biological, microbiological, chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

In certain embodiments, an antibody, or antigen-binding fragment thereof, is characterized by or comprises a heavy chain variable region (V_H) sequence that comprises complementary determining region V_HCDR1, V_HCDR2, and V_HCDR3 sequences, and a light chain variable region (V_L) sequence that comprises complementary determining region V_LCDR1, V_LCDR2, and V_LCDR3 sequences. Exemplary V_HCDR1, V_HCDR2, V_HCDR3, V_LCDR1, V_LCDR2, and V_LCDR3 sequences are provided in **Table H1** below.

Table H1: Humanized Antibody CDR Sequences		
CDR	Sequence	SEQ ID NO:
h600 23 4		
HCDR1	SYTMG	1
HCDR2	FISSSGHTYYANWAKG	2
HCDR3	EGGYGGYDYTGIFNL	3
LCDR1	QATESISSWLA	4
LCDR2	GASTLES	5
LCDR3	QQGYIYTNVDNT	6
h600 23 4 ED		
HCDR1	SYTMG	7
HCDR2	FISSSGHTYYANWAKG	8
HCDR3	DGGYGGYDYTGIFNL	9
LCDR1	QATESISSWLA	10
LCDR2	GASTLES	11
LCDR3	QQGYIYTNVDNT	12
h600 23 24 H		
HCDR1	SYGVN	13
HCDR2	GINTGGSTYYANWAKG	14
HCDR3	TSGNNVYNYFTL	15
LCDR1	QASQSIPSLA	16
LCDR2	APSTLAS	17
LCDR3	QSYYYGDNTYNNI	18
h600 25 9		
HCDR1	TYDIN	19
HCDR2	IIYTGGITNFANWAKG	20
HCDR3	GGYDSEGYVYPDAFDP	21
LCDR1	QASESISNLLA	22
LCDR2	RASILTS	23
LCDR3	QHGYTGTVQNV	24
h600 25 37		
HCDR1	NYAMG	25
HCDR2	SRRTDGITYYANWAEG	26
HCDR3	DVGEGGWYFNL	27
LCDR1	QASQSINIYLA	28
LCDR2	DASKLAS	29
LCDR3	QQGINNIG	30
h600 23 37 ED		
HCDR1	NYAMG	31
HCDR2	SRRTDGITYYANWAEG	32
HCDR3	DVGDDGGWYFNL	33
LCDR1	QASQSINIYLA	34

LCDR2	DASKLAS	35
LCDR3	QQGINNIG	36
h600 25 71		
HCDR1	SYAMG	37
HCDR2	DISTSGNAYYATWVKG	38
HCDR3	ADYGGETYAFDP	39
LCDR1	QASQSISSYLN	40
LCDR2	SASTLAS	41
LCDR3	QQGYSDSNIDNV	42
h600 25 92		
HCDR1	SHHMI	43
HCDR2	IIDAGSGSTYYASWAKG	44
HCDR3	GGLTESLGTYFDL	45
LCDR1	QASESIDSGLA	46
LCDR2	DSSTLAS	47
LCDR3	QSNYDTGSSVYDWGS	48
h600 25 108		
HCDR1	DYFMT	49
HCDR2	IINTGGDSYYATWAKG	50
HCDR3	DTGYGGYDYAGSFDP	51
LCDR1	QASENINSWLA	52
LCDR2	EASKLAS	53
LCDR3	QQGYIYIDVGNI	54
h600 24 2		
HCDR1	VSYWIC	55
HCDR2	CTDGGDGSSYYASWVNG	56
HCDR3	DRSDVFNL	57
LCDR1	QAGQSIDSNLA	58
LCDR2	RASTLAS	59
LCDR3	QSFYVTISAMVDYP	60
h600 24 10		
HCDR1	RYAMA	61
HCDR2	YIDTGDSTYYATWAKG	62
HCDR3	VGVRMYL	63
LCDR1	QASQSISSYLS	64
LCDR2	RASTLES	65
LCDR3	QCGYYGGSYIGA	66
h600 24 124		
HCDR1	SYGIS	67
HCDR2	YIYPDYGSTDYATWVNG	68
HCDR3	GYASSSGYYDPKYFGL	69
LCDR1	RASEDIESYLA	70
LCDR2	DASDLAS	71
LCDR3	QHGFYTSRSDSV	72
h600 24 31		
HCDR1	SYDMS	73
HCDR2	YIWSSGSAYYATWAEG	74
HCDR3	RYVGSSYDT	75
LCDR1	QSSQSVSSNNYLS	76
LCDR2	AASYLAS	77
LCDR3	LGDYDNDIDHA	78
h600 24 103		
HCDR1	SYAMG	79
HCDR2	FIDTGGSTYYANWAKG	80
HCDR3	VGARMYL	81
LCDR1	QASQSIISNLLA	82
LCDR2	RASTLES	83
LCDR3	QCSYYGGSYIGA	84

h600 HB 11D7.1		
HCDR1	RYYMS	85
HCDR2	YIDPIFGNTYYASWVNG	86
HCDR3	DGDAGYDGYGYGTDL	87
LCDR1	QASENIYSGLA	88
LCDR2	SAFTLAS	89
LCDR3	QTYYYGSVTYFNA	90
h600 HB 28B7.3		
HCDR1	SHYMI	91
HCDR2	IITSSDYIYYARWAKGR	92
HCDR3	YNYDDDGELFNL	93
LCDR1	QSSQSIDANNDLA	94
LCDR2	LASKLAS	95
LCDR3	LGGYDDADNT	96
h600 HB 55F6.6		
HCDR1	NNYYMC	97
HCDR2	CIYPSIVGPTYANWAKG	98
HCDR3	DRYDDYGDYFNL	99
LCDR1	QASQSIYNYLS	100
LCDR2	YASTLAS	101
LCDR3	QNSGVNGNRYGNA	102

Thus, in certain embodiments, an antibody or antigen-binding fragment thereof, binds to TNFR2 and comprises:

- a heavy chain variable region (V_H) sequence that comprises complementary determining region V_HCDR1, V_HCDR2, and V_HCDR3 sequences selected from **Table H1**; and
 - a light chain variable region (V_L) sequence that comprises complementary determining region V_LCDR1, V_LCDR2, and V_LCDR3 sequences selected from **Table H1**,
- including variants thereof which specifically bind to at least one TNFR2 polypeptide or epitope (selected, for example, from **Table T1**).

In certain embodiments, the CDR sequences are as follows:

- a heavy chain variable (V_H) region comprising V_HCDR1, V_HCDR2, and V_HCDR3 regions set forth respectively in SEQ ID NOs: 1-3; and a light chain variable (V_L) region comprising V_LCDR1, V_LCDR2, and V_LCDR3 regions set forth respectively in SEQ ID NOs: 4-6;

- a V_H region comprising V_HCDR1, a V_HCDR2, and V_HCDR3 regions set forth respectively in SEQ ID NOs: 7-9; and a V_L region comprising V_LCDR1, V_LCDR2, and V_LCDR3 regions set forth respectively in SEQ ID NOs: 10-12;

- a V_H region comprising V_HCDR1, V_HCDR2, and V_HCDR3 regions set forth respectively in SEQ ID NOs: 13-15; and a V_L region comprising V_LCDR1, V_LCDR2, and V_LCDR3 regions set forth respectively in SEQ ID NOs: 16-18;

- a V_H region comprising V_HCDR1, V_HCDR2, and V_HCDR3 regions set forth respectively in SEQ ID NOs: 19-21; and a V_L region comprising V_LCDR1, V_LCDR2, and V_LCDR3 regions set forth respectively in SEQ ID NOs: 22-24;

a VH region comprising VHCDR1, VHCDR2, and VHCDR3 regions set forth respectively in SEQ ID NOs: 25-27; and a VL region comprising VLCDR1, VLCDR2, and VLCDR3 regions set forth respectively in SEQ ID NOs: 28-30;

a VH region comprising VHCDR1, VHCDR2, and VHCDR3 regions set forth respectively in SEQ ID NOs: 31-33; and a VL region comprising VLCDR1, VLCDR2, and VLCDR3 regions set forth respectively in SEQ ID NOs: 34-36;

a VH region comprising VHCDR1, VHCDR2, and VHCDR3 regions set forth respectively in SEQ ID NOs: 37-39; and a VL region comprising VLCDR1, VLCDR2, and VLCDR3 regions set forth respectively in SEQ ID NOs: 40-42;

a VH region comprising VHCDR1, VHCDR2, and VHCDR3 regions set forth respectively in SEQ ID NOs: 43-45; and a VL region comprising VLCDR1, VLCDR2, and VLCDR3 regions set forth respectively in SEQ ID NOs: 46-48;

a VH region comprising VHCDR1, VHCDR2, and VHCDR3 regions set forth respectively in SEQ ID NOs: 49-51; and a VL region comprising VLCDR1, VLCDR2, and VLCDR3 regions set forth respectively in SEQ ID NOs: 52-54;

a VH region comprising VHCDR1, VHCDR2, and VHCDR3 regions set forth respectively in SEQ ID NOs: 55-57; and a VL region comprising VLCDR1, VLCDR2, and VLCDR3 regions set forth respectively in SEQ ID NOs: 58-60;

a VH region comprising VHCDR1, VHCDR2, and VHCDR3 regions set forth respectively in SEQ ID NOs: 61-63; and a VL region comprising VLCDR1, VLCDR2, and VLCDR3 regions set forth respectively in SEQ ID NOs: 64-66;

a VH region comprising VHCDR1, VHCDR2, and VHCDR3 regions set forth respectively in SEQ ID NOs: 67-69; and a VL region comprising VLCDR1, VLCDR2, and VLCDR3 regions set forth respectively in SEQ ID NOs: 70-72;

a VH region comprising VHCDR1, VHCDR2, and VHCDR3 regions set forth respectively in SEQ ID NOs: 73-75; and a VL region comprising VLCDR1, VLCDR2, and VLCDR3 regions set forth respectively in SEQ ID NOs: 76-78;

a VH region comprising VHCDR1, VHCDR2, and VHCDR3 regions set forth respectively in SEQ ID NOs: 79-81; and a VL region comprising VLCDR1, VLCDR2, and VLCDR3 regions set forth respectively in SEQ ID NOs: 82-84;

a VH region comprising VHCDR1, VHCDR2, and VHCDR3 regions set forth respectively in SEQ ID NOs: 85-87; and a VL region comprising VLCDR1, VLCDR2, and VLCDR3 regions set forth respectively in SEQ ID NOs: 88-90;

a VH region comprising VHCDR1, VHCDR2, and VHCDR3 regions set forth respectively in SEQ ID NOs: 91-93; and a VL region comprising VLCDR1, VLCDR2, and VLCDR3 regions set forth respectively in SEQ ID NOs: 94-96; or

a VH region comprising VHCDR1, VHCDR2, and VHCDR3 regions set forth respectively in SEQ ID NOs: 97-99; and a VL region comprising VLCDR1, VLCDR2, and VLCDR3 regions set forth respectively in SEQ ID NOs: 100-102.

Also included are variants thereof, including affinity matured variants, which bind to TNFR2, for example, variants having 1, 2, 3, 4, 5, 6, 7, or 8 total alterations across the CDR regions, for example, one or more the V_HCDR1, V_HCDR2, V_HCDR3, V_LCDR1, V_LCDR2, and/or V_LCDR3 sequences described herein. Exemplary “alterations” include amino acid substitutions, additions, and deletions.

In certain embodiments, an antibody, or antigen-binding fragment thereof, is characterized by or comprises a heavy chain variable region (V_H) sequence, and a light chain variable region (V_L) sequence. Exemplary humanized V_H and V_L sequences are provided in **Table H2** below, exemplary rabbit V_H sequences are provided in **Table R1** below (V_HCDR1, V_HCDR2, and V_HCDR3 regions are underlined), and exemplary rabbit V_L sequences are provided in **Table R2** below (V_LCDR1, V_LCDR2, and V_LCDR3 regions are underlined).

Table H2: Humanized Heavy and Light Chain Sequences		
Name	Sequence (CDRs underlined)	SEQ ID NO:
HC h600_23_4	EVQLVESGGGLVQPGGSLRLSCAASGIDLSSTMTGWVRQAPGKGLEW VGFISSSGHTYYANWAKGRFTISRDNKNTLYLQMNSLRAEDTAVYY CAREGGYGGYDYTGIFNLWGQGLTVTVSS	103
LC h600_23_4	DIQMTQSPSTLSASVGDRVTITCQATESISSWLAWYQQKPKGAPKLL IYGASTLESQVPSRFSGSGSGTEFTLTISLQPDDEFATYYCQQGYIY TNVDNTFGGGTKVEIK	104
HC h600_23_4_ED	EVQLVESGGGLVQPGGSLRLSCAASGIDLSSTMTGWVRQAPGKGLEW VGFISSSGHTYYANWAKGRFTISRDNKNTLYLQMNSLRAEDTAVYY CARDGGYGGYDYTGIFNLWGQGLTVTVSS	105
LC h600_23_4_ED	DIQMTQSPSTLSASVGDRVTITCQATESISSWLAWYQQKPKGAPKLL IYGASTLESQVPSRFSGSGSGTEFTLTISLQPDDEFATYYCQQGYIY TNVDNTFGGGTKVEIK	106
HC h600_23_24_H	EVQLVESGGGLVQPGGSLRLSCAASGFSLSYGVNWRQAPGKGLEW VGGINTGGSTYYANWAKGRFTISRDNKNTLYLQMNSLRAEDTAVYY CARTSGNNVYNYFTLWGQGLTVTVSS	107
LC h600_23_24_H	DIQMTQSPSSLSASVGDRVTITCQASQSIPLSLAWYQQKPKGAPKLL IYAPSTLASQVPSRFSGSGSGTDFTLTISLQPEDFATYYCQSYYYG DNTYNNIFGGGKVEIK	108
HC h600_25_9	EVQLVESGGGLVQPGGSLRLSCAASGFSLSYDINWRQAPGKGLEW VGIIYTGGITNFANWAKGRFTISRDNKNTLYLQMNSLRAEDTAVYY CARGGYDSEGYVYPDAFDPWGQGLTVTVSS	109
LC h600_25_9	DIQMTQSPSSLSASVGDRVTITCQASESISNLLAWYQQKPKGAPKLL IYRASILTSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQHGYTG TNVQNVFGGGTKVEIK	110
HC h600_25_37	EVQLVESGGGLVQPGGSLRLSCAASGIDLSNYAMGWVRQAPGKGLEW VGSRRTDGITYYANWAEGRFTISRDNKNTLYLQMNSLRAEDTAVYY CGRDVGGEGGWYFNLWGQGLTVTVSS	111
LC h600_25_37	DIQMTQSPSTLSASVGDRVTITCQASQSINIYLAWYQQKPKGAPKLL IYDASKLASQVPSRFSGSGSGTEFTLTISLQPDDEFATYYCQQGINN IGFGGGTKVEIK	112
HC h600_23_37_ED	EVQLVESGGGLVQPGGSLRLSCAASGIDLSNYAMGWVRQAPGKGLEW VGSRRTDGITYYANWAEGRFTISRDNKNTLYLQMNSLRAEDTAVYY CGRDVGGDGGWYFNLWGQGLTVTVSS	113

LC h600_23_37_ED	DIQMTQSPSTLSASVGDRTTITCQASQSINIYLAWYQQKPGKAPKLL IYDASKLASGVPSRFGSGSGTEFTLTISSLQPD ~~FATYYCQ~~ GINN IGFGGGTKVEIK	114
HC h600_25_71	EVQLVESGGGLVQPGGSLRLSCAASGIDLS ~~SYAMGWVRQAPGKGLEW~~ VGDI ~~STSGNAYYATWVKGRFTISRDN~~ SKNTLYLQMN ~~SLRAEDTAVYY~~ CARADYGGETYAFDPWGQGT ~~LVTVSS~~	115
LC h600_25_71	DIQMTQSPSSLSASVGDRTTITCQASQSISSYLNWYQQKPGKAPKLL IYSASTLASGVPSRFGSGSGTDFTLTISSLQPED ~~FATYYCQ~~ GYSD SNIDNVFGGGTKVEIK	116
HC h600_25_92	EVQLVESGGGLVQPGGSLRLSCAASGFSLS ~~SHHMIWVRQAPGKGLEW~~ VGIIDAGSGSTYYASWAKGRFTISRDN ~~SKNTLYLQMN~~ SLRAEDTAVY YCARGGLTESLGT ~~YFDLWGQGT~~ LVTVSS	117
LC h600_25_92	DIQMTQSPSSLSASVGDRTTITCQASESID ~~SGLAWYQQKPGKAPKLL~~ IYDSSTLASGVPSRFGSGSGTDFTLTISSLQPED ~~FATYYCQ~~ SNYDT GSSVYDWGSFGGGTKVEIK	118
HC h600_25_108	EVQLVESGGGLVQPGGSLRLSCAASGFSLS ~~DYFMTWVRQAPGKGLEW~~ VGIINTGGDSYYATWAKGRFTISRDN ~~SKNTLYLQMN~~ SLRAEDTAVYY CARDTGYGGYDYAGSFD ~~PWGQGT~~ LVTVSS	119
LC h600_25_108	DIQMTQSPSSVSASVGDRTTITCQASENIN ~~SWLAWYQQKPGKAPKLL~~ IYEASKLASGVPSRFGSGSGTDFTLTISSLQPED ~~FATYYCQ~~ GYIY IDVGNIFGGGTKVEIK	120
HC h600_24_2	EVQLVESGGGLVQPGGSLRLSCAASGFS ~~SFVSYWICWVRQAPGKGLE~~ WVACTDGGDGSSYYASWVNGRFTISRDN ~~SKNTLYLQMN~~ SLRAEDTAV YYCARD ~~RSDFNLWGQGT~~ LVTVSS	121
LC h600_24_2	DIQMTQSPSSLSASVGDRTTITCQAGQSID ~~SNLAWYQQKPGKAPKLL~~ IYRASTLASGVPSRFGSGSGTDFTLTISSLQPED ~~FATYYCQ~~ SFYVT ISAMVDY ~~PF~~ GGGTKVEIK	122
HC h600_24_10	EVQLLES ~~GGGLVQPGGSLRLS~~ CAASGIDLS ~~RYAMAWVRQAPGKGLEW~~ VG ~~YIDTGD~~ STYYATWAKGRFTISRDN ~~SKNTLYLQMN~~ SLRAEDTAVYY CTNVGVRMYLWGQGT ~~LVTVSS~~	123
LC h600_24_10	DIQMTQSPSSLSASVGDRTTITCQASQSISSYLSWYQQKPGKAPKLL IYRASTLES ~~GVPSRFGSGSGTDFTLTISSLQPEDFATYYCQ~~ CGYYG GSYIGAFGGGTKVEIK	124
HC h600_24_124	EVQLVESGGGLVQPGGSLRLSCAASGIDF ~~SSYGISWVRQAPGKGLEW~~ VAYIYPDYGSTDYATWVNGRFTISL ~~DN~~ SKNTLYLQMN ~~SLRAEDTAVY~~ YCASGYASSSGYYDPKYFGLWGQGT ~~LVTVSS~~	125
LC h600_24_124	DIQMTQSPSSLSASVGDRTTITCRASEDIE ~~SYLAWYQQKPGKAPKLL~~ IYDASDLASGVPSRFGSGSGTDFTLTISSLQPED ~~FATYYCQ~~ HGFYT SRSDSVFGGGTKVEIK	126
HC h600_24_31	EVQLVESGGGLVQPGGSLRLSCAASGFSLS ~~SYDMSWVRQAPGKGLEW~~ VG ~~YIWS~~ SGSAYYATWAEGRFTISRDN ~~SKNTLYLQMN~~ SLRAEDTAVYY CARRYVGSSYDTWGQGT ~~LVTVSS~~	127
LC h600_24_31	DIQMTQSPSSLSASVGDRTTITCQSSQSVSSNNYLSWYQQKPGKAPK LLIYAASYLASGVPSRFGSGSGTDFTLTISSLQPED ~~FATYYCQ~~ LDY DNDIDHAFGGGTKVEIK	128
HC h600_24_103	EVQLVESGGGLVQPGGSLRLSCAASGIDLS ~~SYAMGWVRQAPGKGLEW~~ VG ~~FIDTGG~~ STYYANWAKGRFTISRDN ~~SKNTLYLQMN~~ SLRAEDTAVYY CANVGARMYLWGQGT ~~LVTVSS~~	129
LC h600_24_103	DIQMTQSPSSLSASVGDRTTITCQASQISINLLAWYQQKPGKAPKLL IYRASTLES ~~GVPSRFGSGSGTDFTLTISSLQPEDFATYYCQ~~ CSYYG GSYIGAFGGGTKVEIK	130
HC h600_HB_11D7.1	EVQLVESGGGLVQPGGSLRLSCAASGFD ~~FSRYMSWVRQAPGKGLEW~~ VG ~~YIDPIFGNTYYASWVNGRFTISS~~ DNAKNSLYLQMN ~~SLRAEDTAVY~~ YCARDGDAGYDGYGTDLWGQGT ~~LVTVSS~~	131
LC h600_HB_11D7.1	DIQMTQSPSSLSASVGDRTTITCQASENIYSG ~~LAWYQQKPGKVPKLL~~ IVSAFTLASGVPSRFGSGSGTDFTLTISSLQPED ~~VATYYCQ~~ TYYYG SVTYFNAFGGGTKVEIK	132
HC h600_HB_28B7.3	EVQLVESGGGLVQPGGSLRLSCAASGFSLS ~~NHYMIWVRQAPGKGLEW~~ VG ~~IITSSDYIYYARWAKGRFTISRDN~~ SKNTLYLQMN ~~SLRAEDTAVYY~~ CARYNYDDDGELFNLWGQGT ~~LVTVSS~~	133

LC h600_HB_28B7.3	DIQMTQSPSSLSASVGDRVTITCQSSQSIDANNDLAWYQQKPGKAPK LLIYLASKLASGVPSRFSGSGSGTDFTLTITSSLPEDFATYYCLGGY DDDADNTFGGGTKVEIK	134
HC h600_HB_55F6.6	EVQLVESGGGLVQPGGSLRLSCAASGFSFTNNYYMCWVRQAPGKGLE WVGCIYPSIVGPTYANWAKGRFTISRDNKNTLYLQMNSLRAEDTA VYYCVRDRYDDYGDYFNLWGQGLTVTVSS	135
LC h600_HB_55F6.6	DIQMTQSPSSLSASVGDRVTITCQASQSIYNYLSWYQQKPGKAPKRL IYYASTLASGVPSRFSGSGSGTDFTLTITSSLPEDFATYYCQSNNGV NGNRYGNAFGGGTKVEIK	136

Table R1. Exemplary Rabbit Antibody Heavy Chain Sequences (HCDRs 1-3 are underlined)		
Name	Sequence	SEQ ID No:
rab600_24_2	QEQLVESGGGLVQPEGSLALTCKASGFSFVSYYWICWVRQAPGK GLEWIACTDGGDGSYYASWVNGRFTISKISSTTVTLQMTSLTA ADTAIFYCARDSDVFNLWGPGLTVTVSS	137
rab600_24_25	QSLEESGGGLVKPEGSLTLTCAVSGFDLSSYYWICWARQAPGK LEWIACTDGGSTGSAYYASWAKGRLSISKASSTTVTLQMTSLTA ADTATYFCARVQSYVGYANYGYPNYFNLWGPGLTVTVSS	138
rab600_24_62	QSLEESGGDLVVPGLTSLTLCTASGFDLSSYYWICWVRQAPGK LEWIACTDGGSTGSAYYASWAKGRFTISRDNKNTLYLQMTSLTA ADTATYFCARHQSYYETGYGVVYATYFSLWGPGLTVTVSS	139
rab600_24_97	QSLEESGGDLVKPGASLTTLCKASGFSFSSGHDMCWVRQAPGK LEWIACTDGGSTGSAYYASWVNGRFTISLDNAQNTVFLQMTSLTA ADTATYFCARSGYGGFRYGFNLWGPGLTVTVSS	140
rab600_24_124	QEQLVESGGGLVTLGGSLKLSCKASGIDFSSYGISWVRQAPGK LEWIACTDGGSTGSAYYASWVNGRFTISLDNAQNTVFLQMTSLTA ADTATYFCASGYASSSGYYPKYFGLWGPGLTVTVSS	141
rab600_25_10	QSVEESGGRLVAPGTPPLTLCTVSGIDLRLYAMAWVRQAPGKGL EYIGYIDTGDSTYYATWAKGRFTISRSTTVHLKIASPTTEDTA TYFCTNVGVRMYLWGPGLTVTVSS	142
rab600_25_20	QSVEESGGRLVTPGTPPLTLCTVSGIDLRLYAMGWVRQAPGKGL EYIGYIDTAGSTYYANWAKGRFTISRSTTVDLKIASPTTEDTA TYFCANVGARMYLVGPGLTVTVSS	143
rab600_25_31	QEQLKESGGGLVTPGTPPLTLCTASGFSLSYYDMSWVRQAPGK LEWIGYIWSGSAYYATWAEGRFTISRSTTVGLKITSPTTEDT ATYFCARRYVGSYDTWQGLTVTVSS	144
rab600_25_103	QEQLKESGGGLVTPGTPPLTLCTVSGIDLSSYAMGWVRQAPGK LEWIGYIDTAGSTYYANWAKGRFTISRSTTVDLKIASPTTEDA ATYFCANVGARMYLVGPGLTVTVSS	145
rab600_23_7H2.5	QSVEESGGRLVTPGTPPLTLCTASGFSLSNYMNVVRQAPGKGL EWIGIITDSGTTYASWVKGRTISRSTTVDLKMTSLTTEDTA TYFCAREPDYDGYAGYGYDLWGQGLTVTVSS	146
rab600_23_8G10.7	QQLEQSGGGAGGLVKPGGSLLELCKASGFTLINSHWICWVRQA PGKLEWIGICIFAGSAGSTYYATWVSGRFTLSRDIDQNTGCLQL NSLTAADTAMYYCARDQNTAYDPEFYLNWQGLTVTVSS	147
rab600_23_8G11.5	QSLEESGGRLVTPGTPPLTLCTVSGFSLNSNGMNVVRQAPGKGL EWIGGINAGGSAYYANWAKGRFTISRSTTVDLKITSPTTEDTA TYFCAKTSGINVYNYLNWQGLTVTVSS	148
rab600_23_9B11.2	QQQLVESGGGLVKPGASLTTLCKASGFSFNTYYMCWVRQAPGK GLEWIACTEAGDSESNYYASWAKGRFTISKASSTTVTLQMTSLT AADTATYFCARATYDTFGYGDYVYTPASFNLWGPGLTVTVSS	149
rab600_23_11D7.1	QEQLVESGGGLVQPGGSLKLSCKASGFDLSRYMSWVRQAPGK LEWIGYIDPIFGNTYYASWVNGRFTISRSHNAQNTLYLQNLNLT ADTATYFCARDGADGYGYGTDLWGPGLTVTVSS	150
rab600_23_11G12.1	QSLEESGGDLVQPGASLTTLCTASGFSVNVNSYMCWVRQAPGK LELIACIDTGGSGSTWYGSWAKGRFTISRSTNLNTVTLQMTSLT AADTATYFCARARNTYGYGDYVYGGAFDPWGPGLTVTVSS	151

rab600_23_28B7.3	QSVEESGGRLVTPGTPLTLTCTVSGFSLNSHYMIWVRQAPGKGL EYIGIITSSDIYYARWAKGRFTISKTSSTTVDLKITSPTTEDT ATYSCARYNYDDDGELFNLWGQGLVTVSS	152
rab600_23_37D1.4	QSLEESGGRLVTPGTPLTLTCTVSGFSLNSNGMNVVRQAPGKGL EWIGGINAGGSAYYANWAKGRFTISKSTMTVDLKITSPTTEDTA TYFCAKTSGINVYNLNLWGQGLVTVSS	153
rab600_23_37H4.1	QSVEESGGRLVTPGTPLTLTCTVSGFSLNSHYMIWVRQAPGKGL EYIGVITSSDIYYARWAKGRFTISKTSSTTVDLKITSPTTEDT ATYFCARYNYDDDGELFNLWGQGLVTVSS	154
rab600_23_55F6.6	QQLEESGGDLVKPGASLTVCTASGFSFTNNYMCWVRQAPGK GLEWIGCIYPSIVGPTYANWAKGRFTISKTSSTTVLEMTSLT AADTATYFCVRDRYDDYDYNLWGPGLVTVSS	155
rab600_23_4	QSVEESGGRLVTPGTPLTLTCTVSGIDLSSYTMGWVRQAPGKGL EYIGFISSSGHTYYANWAKGRFTISKTSSTTVDLKMTSLTTEDT ATYFCARDGGYGGYDYGIFNLWGQGLVTVSS	156
rab600_23_5	QSVEESGGRLVTPGTPLTLTCTVSGFSLSTYGVSWVRQAPGKGL DWIGIIDSSGSTWYTSWVKGRFTISKSTTVDLKVTSPPTEDTA TYFCARESYHSNFWGQGLVTVSS	157
rab600_23_6	QEQLKESGGRLVTPGTPLTLTCTASGFSLSYYVSWVRQAPGKGL LEWIGIIHSDGSIYYATWAKGLFTISRTSTTVDLKATSLTTEDT ATYFCVRGYPGYTSTFNRLDLWGQGLVTVSS	158
rab600_23_8	QSLEESGGDLVQPEGSLLTCTASGFSFSSYYICWVRQAPGKGL LEWIIACIYAGSSGSTYYASWAKGRFTISKTSSTTVTLQMTSLTA ADTATYFCARDVGSYYPDVFNFWGPGLVTVSS	159
rab600_23_21	QEQLKESRGLVVKPGGSLELCKASGFTLSSSHWICWVRQAPGK GLEWIGCIHAGSSGSAYYASWVNGRFTLSRDIDQSTGCLQLNSL TTADTAMYYCARDQTATTYPYLNWLWGQGLVTVSS	160
rab600_23_24	QSLEESGGRLVTPGTPLTLTCTASGFSLSYGVNWVRQAPGKGL EWIGGINGGSTYYANWAKGRFTISKTSAMVDLKVTSPPTEDTA TYVCARTSGNNVYNYFTLWGQGLVTVSS	161
rab600_23_33	QSVEVSGGRLVTPGTPLTLTCTVSGFSLTYMIWVRQAPGKGL EYIGIITSSGSTYYASWAKGRFTISKSTSVDLKVTSPPTEDTA TYFCARYTYDDDGELFNLWGQGLVTVSS	162
rab600_23_45	QSLEESGGDLVKPEGSLLTCTASGFDLSYYMCWVRQAPGKGL ELIACIYDGSSVSTYYASWAKGRFTMSKTSSTTVTLQMTSLTAA DTATYFCARDNLRHAGYGQPFNLWGPGLVTVSS	163
rab600_23_50	QSLEESGGDLVKPGASLTLTCTASGSSFSNSYYMCWVRQAPGKGL LEWIGCIYTGSGSTYYANWAKGRFTISETSTTVTLQMTSLTAA DTATYFCARYDAAYAGDGYTIGNAFDPWGPGLVTVSS	164
rab600_23_52	QEQLVESGGGLVQPEGSLSLTCTASGFTLNNYCMCWVRQAPGKA LEWIIACIAAGSSGTPYYASWAKGRFTISKTSSTTVTLQMTSLTA ADTATYFCARIYYSYGYGDVAYGAADPWGPGLVTVSS	165
rab600_23_53	QSVEESGGRLVTPGTPLTLTCTVSGFSLSSYYMIWVRQAPGKGL EWIGIITSSGSTYYASWAKGRFTISKTSSTTVDLKITSPTTEDT ATHFCARYSYNDDEFFNLWGQGLVTVSS	166
rab600_23_57	QSVEESGGRLVTPGTPLTLTCTVSGFSLSSYAMGWVRQAPGKGL EWIGIIGSGGNTYYATWAKGRFTISRTSTTVDLRITSPTTEDTA TYFCARDVGYGDYDALDLWGQGLVTVSS	167
rab600_23_62	QEQLKESGGGLVQPGGSLKLSCTASGFDFFSHYMSWVRQAPGKGL LEWIGYIDPVFGNTYYANWVNGRFTISSHNAQNTLYLQLNSLTV ADTATYFCARDGEAGYAGYGYGTDLWGPGLVTVSS	168
rab600_23_70	QSLEESGGDLVKPEGSLLTCTASGFSFSAWYIYWVRQAPGKGL LEWIIACIINGDDDTYYANWAKGRFTISKTSSTTVTLQMTSLTAA DTATYFCATDIHGGNSLDLWGPGLVTVSS	169
rab600_23_71	QSVEESGGRLVTPGTSLTLTCTVSGIDLSSYAMSWVRQAQGKGL EWIGIIGDSGSTWYASWAKGRFTISKSTTVDLKITSPTPEDTA TYFCAREPDYGGYAGYGDYDLWGQGLVTVSS	170
rab600_23_75	QEQLKESGGGLVQPGGSLKLSCKASGFDFFSHYMSWVRQAPGKGL LEWIGYIDPVFGNTYYANWVNGRFTISSHNAQNTLYLQLNSLTV ADTATYFCARDGEAGYAGYGYGTDLWGPGLVTVSS	171

rab600_23_79	QSVEESGGRLVTPGTPLTLTCTVSGFSLNSYYMIWVRQAPGKGL EWIGIITSSGYTYASWAKGRFTISKTSSTTVDLKITSPTTEDT ATYFCARYSYDDGELFNLWGQGTTLVTVSS	172
rab600_23_80	QSLEESGGDLVKPGASLTTLTCTASGFSSFNYYYMCWVRQAPGKG LEWIAICIYDGDGSTYYATWAKGRFTISKTSSTTVTLQMTSLTAA DTATYFCARTYTYGYNVDAALNLWGPGLTVTVSS	173
rab600_23_81	QSLEESGGRLVTPGTPLTLTCTASGFSLNSYYVSWVRQAPGKGL EWIGIIEETGGLYYASWAKGRFSLSKTSTTVDLKITSPTAEDTA TYFCVRGYPGYTHTFNRLDLWGQGTTLVTVSS	174
rab600_23_82	QEQLLEESGGDLVKPGGTTLTLTCTASGIDFSSYYMCWVRQAPGK GLEWIACIYSGSSNSTYYANWAKGRFTISKTSSTTVTLQMTSLT AADTATYFCARDHYAGYAGVAYGTEYNLWGPGLTVTVSS	175
rab600_23_85	QSLEESGGDLVKPGASLTTLTCKASGFSSFTYWMCWVRQAPGKGP EWIACIAAGSSDTPYYANWAQGRFTISKTSSTTVTLQMTSLTVA DTATYFCARIAYSYGYGDYGYGAFFDPWGPGLTVTVSS	176
rab600_23_90	QEQLQSGGGAGRLVKPGGSLELCCNASGFTLSNSYWICWVRQ APGKGLEWIGICIFAGSAGSAYATWVNGRFTLSRDIDQSTGCLQ LNSLTAADTAMYYCARDQSSTAYDPFFYFNSWGQGTTLVTVSS	177
rab600_23_102	QSVEESGGRLVTPGTPLTLTCTASGFSLTYDMIWVRQAPGKGL EWIGYIWSGDITDYASWAKGRFTISKTSSTTVDLKVTSPPTEDTA TYFCARDVGYAGYGYFDLWGQGTTLVTVSS	178
rab600_23_105	QSLEESGGDLVKPGASLTTLTCTASGFSSSYMCWVRQAPGKG LEWIAICIYVGSIGSTYYASWAKGRFTISKTSSTTVTLQMTSLTA ADTATYFCARDYTYDYGDYAYGTRLDLWGQGTTLVTVSS	179
rab600_23_106	QQQLVESGGDLVKPGASLTTLTCKASGIDFSSGYDMCWVRQAPGK GLEWIAICFDAASSDTPYYASWAKGRFTISRSTSTTVTLQATSLT VADTATYFCATIGYDAAGDWKYAFDPWGPGLTVTVSS	180
rab600_23_107	QSVEESGGRLVTPGTPLTLTCTVSGFSLSNAIWVRQAPGKGL EWIGINTYDNTAYATWAKGRFISRSTSTTVDLKITSPATKDTA TYFCARDVHNNVVPYYFDMWGQGTTLVTVSS	181
rab600_23_108	QSLEESGGDLVKPGASLTTLTCTASGFSSGSYYMYWVRQAPGKG LEWIACIYNGDGSTYYASWAKGRFTISKTSSTTVTLQMTSLTAA DTATYFCARTYTSYGYNVDAALNLWGPGLTVTVSS	182
rab600_23_110	QSVEESGGRLVTPGTPLTLTCTVSGFSLRSYNICWVRQAPGKGL EWVGLIGPAGNAYYASWAKGHFTLSKTSTTVDLIITSPTTEDTA TYFCSRDATIEGMSLWGPGLTVTVSS	183
rab600_23_114	QEQLLEESGGGLVQPGGSLKLCKASGFDFSGHYMSWVRQAPGKG LEWIGYFDPIFHSTYYASWVNGRFTISSHAQNTLYLQNLTLTA ADTATYFCARDGNAGYDGYGYGTDLWGPGLTVTVSS	184
rab600_23_119	QEQLLEESGGDLVKPEGSLLTLCTVSGFSSSYWICWVRQAPGK GLEWIAICIYAGSSGSTAYANWAKARFTISKSTTTVALQMTSLT VADTATYFCARGIYVGYGGNGYADLWGPGLTVTVSS	185
rab600_23_123	QSLEESGGDLVKPGASLTTLTCTASGFSSSGYDMCWVRQAPGKG LEWIAICIYTDGSTYYASWAKGRFTISKTSSTTVTLQMTSLTAA DTATYFCARDIGSDYYAFFNLWGPGLTVTVSS	186
rab600_23_127	QEQLLEESGGDLVKPEGSLLTLCTASGFDFSVNAMCWVRQAPGKG PEWIAIYISNADGSTHYASWVNGRFTISRSTSLNTVTLQMTRLTV SDTATYFCARAPYAGYTGYYLNLWGPGLTVTVSS	187
rab600_23_129	QSLEESGGGLVQPEGPLTLTCTASGFSSSTYYMCWVRQAPGKG LEWIAICIDAGSSNTYYASWAKGRFTISKTSSTTVTLQMTSLTV ADTATYFCARASYATYGYGDYIATAPQFFNLWGPGLTVTVSS	188
rab600_23_130	QSLEESGGDLVKPGASLTFTCTASGFSSGIDYMCWVRQAPGKG LEWIAICIYGGDGITYYASWAKGRFTISKASSTTVTLQMTSLTA ADTATYFCARVGSRYTGYPNYDDVPEHFKLWGPGLTVTVSS	189
rab600_23_132	QSLEESGGDLVKPGASLTTLTCTASGFSSSYWICWVRQAPGKG LEWIAICIYGGSGYNIYYASWAKGRFTISKSTPTTVTLQMTSLTG ADTATYFCARGIGVGYGGNGYADLWGPGLTVTVSS	190
rab600_23_133	QSLEESGGDLVKPGGTTLTLTCKASGIDFSSYYDMCWVRQAPGKG LELIACIYTSSGSTYYASWAKGRFTISKTSSTTVDLKMTSLTAA DTATYFCARDSGYAGYGYFSLWGPGLTVTVSS	191

rab600_23_135	QSLEESGGGLVQPEGSLLTLCKASGFSFSSGYDMCWVRQAPGKG LECIACIYTGDDSTTWYASWAKGRFTISRPSSTAVTLQMTSLTAA DTATYFCARDRDAGYYGYTYFNLWGPGLVTVSS	192
rab600_23_140	QSLEESGGDLVKPGASLLTLCKASGFSFSSGYVMCWVRQAPGKG LEWIACIDTSSGTTWYATWVNGRFTISRSTSLNTVTLQMTSLTA ADTATYFCARAGYINYSYTSDFDLWGPGLVTVSS	193
rab600_23_141	QEQLVESGGGLVTLGGSLKLCKASGIDFSSYGISWVRQAPGKG LEWIAIDIDPDYGNTDYASWVNGRFTISLDNAQNTVYLQMTSLTA ADTATYFCTRISFASSSGYSPYFNLWGPGLVTVSS	194
rab600_23_148	QEQLVESGGGLVTLGGSLKLCKASGDFPSSYSSWVRQAPGKG LEWIAIYIDPDYGITDYASWVNGRFTISLDKAQNTVFLQMTSLTA ADTATYFCASDVGYAGYAYDRGYFNLWGPGLVTVSS	195
rab600_23_152	QEQLVESGGGLVTLGGSLKLCKASGIDFSSNYGFSWVRQAPGKG LEWIAIYIDPDYGYTDYASWVNGRFTISLDNAQNTVFLQMTSLTA ADTATYFCTRDHYTYGDAGYADATSAFDPWGPGLVTVSS	196
rab600_23_153	QEQLVESGGDLVKPEGSLLTLCTASGFSFSSSYWICWVRQAPGK GLEWIGCIYTGSSGSTYYASWAKGRFTITKTSSTTVTLQMTSLT AADTATYFCARASGGSSVYMNFFTLWGPGLVTVSS	197
rab600_23_158	QSLEESGGDLVQPEGSLLTLCTASGFSFSSNYDMCWVRQAPGKG PEWIACIYTGDDSTYYASWAKGRFTISKTSSTTVTLQMTSLTAA DTATYFCARDIGSDYYAFFNLWGPGLVTVSS	198
rab600_23_163	LSLEESGGDLVKPGASLLTLCTASGFSYSSYWICWVRQAAGKG LEWVACIYAGSSGNPYASWAKGRFTISRASSTAVTLQMTSLTA ADTATYFCARDDYTTDGAGYAYGTRLDLWQGGLVTVSS	199
rab600_23_165	QQLEESGGGLVTLGGSLKLCKASGIDFSSFGITWVRQAPGKG LEWIAIYIDPDYGYTDYASWVNGRFTISLDNAQNTVFLQLTSLTA ADTATYFCARALYTSAGAGYADATGAFDPWGPGLVTVSS	200
rab600_23_167	QSLEESGGDLVKPGASLLTLCKASGFSFSSGYDMCWVRQAPGKG LEWIACIYTGDDSTYYASWAKGRFTISKTSSTTVTLQMTSLTAA DTATYFCARDIGSDYYAFFNLWGPGLVTVSS	201
rab600_23_177	QEQLVESGGDLVKPGASLLTLCTAAGFTISTTYWICWVRQAPGK GLEWIACIYNGGGTYYASWAKGRFTISKTSSTTVTLQMTSLTA ADTATYFCARLLNSYVDFNLWGPGLVTVSS	202
rab600_23_182	QSLEDSSGGDLVKPGASLLSCTASGDFDFSYYMCWVRQAPGKGL EWIACIGIGSGSAYYANWAKGRFTISEASSTTVTLQMTSLTAAD TATYFCGRDRDGGSMSYDLWGPGLVTVSS	203
rab600_25_9	QSVEESGGRLVTPGTPPLTLCTVSGFSLSTYDINWVRQAPGKGL EWIGIYITGGITNFANWAKGRFTISKTSSTTVDLKIASPTTEDTA TYFCARGGYDSGDYVYPDAFDPWGPGLVTVSS	204
rab600_25_14	QSVEESGGRLVTPGTPPLTLCTVSGFSLSSYDMIWVRQAPGGL EWIGSAAYDGGAYYASWAKGRFTISKTSSTTVDLKMTSPTTEDT ATYFCARGGYNDALSILWQGGLVTVSS	205
rab600_25_26	QSVEESGGRLVTPGTPPLTLCTVSGFSLNNYAMGWFRQAPGGL EWIGSMRTDGGTYANWAEGRFTISKTSSTTVDLKITSPTTEDTA TYFCGRDVGDDGGWYFNLWGPGLVTVSS	206
rab600_25_37	QSVEESGGRLVTPGTPPLTLCTVSGIDLNNYAMGWFRQAPGGL EWIGSRRTDGTYYANWAEGRFTISRTSTTVDLKITSPTTEDTA TYFCGRDVGDDGGWYFNLWGPGLVTVSS	207
rab600_25_38	QSVEESGGRLVTPGGSLLTLCTVSGFSLSSYNMQWVRQSPGKGL EWIGIMTIDAGPYAAWAKGRFTISKTSSTTVDLKMTGLTTEDT ATYFCARGFFGLWGPGLVTVSS	208
rab600_25_42	QSLEESGGRLVTPGTPPLTLCTVSGIDLSTYAMIWVRQAPGKGL EYIGFIRPGGSAWYASWAKGRFTISKTSSTTVDLKITSPTTEDTA TYFCATYDITYGYDTRLWGPGLVTVSS	209
rab600_25_43	QEQLKESGGGLVTPGTPPLTLCTASGFSLSYDMSWVRQAPGKG LEWIGYIWSSGSSYYASWAKGRFTISKTSSTTVGLKITSPTTED TATYFCARRYVGSSYVTWQGGLVTVSS	210
rab600_25_46	QSLEESGGRLVTPGGSLLTLCTVSGIDLSSYPMTWVRQAPGKGL EWIGMIYSGGAYYASWAKGRFTISKTSSTTVDLKMNSLTASDTA TYFCGRGSLWGPGLVTVSS	211

rab600_25_48	QSLEESGGRLVTPGTPLTLTCTVSGIDLSSYAMSWVRQAPGKGL EWIGYIYNDSGSTFYATWARGRFTISGSSTTVDLKMTSLTTEDT ATYFCARWDSYGYGDFNLWGPGLTVTVSS	212
rab600_25_51	QSVEESGGRLVTPGTPLTLTCTVSGIDLSSYAMGWVRQAPVKGL KWIGFIDVDGSAYYATWAKGRFTISKSTTVDLKITSPTTEDSA TYFWTRYDNYGYGDFNLWGPGLTVTVSS	213
rab600_25_54	QEQLKESGGGLVTPGTPLTLTCTASGFSLSTYDMSWVRQAPGKGL LEWIGYIWSSGSAYYATWAQGRFTISKSTTVGLKIASPTTEDT ATYFCARRFVGSSTYDTWGQGLTVTVSS	214
rab600_25_63	QEQLKESGGGLVTPGTPLTLTCTASGFSLSSYDMSWVRQTPGKGL LEWIGYIWSSGSAYYASWAEGRFTISKSTTVGLKITSPTTEDT ATYFCARRFVGSSTYDTWGQGLTVTVSS	215
rab600_25_70	QSVEESGGRLVTPGTPLTLTCTVSGFSLSSHYMSWVRQAPGKGL EWIGIITSSGSTYYASWAKGRFTISKSTTVDLKITSPTTEDTA TYFCARDWYDDYGDYRSLWGPGLTVTVSS	216
rab600_25_71	QSVEESEGRVTPGTPLTLTCTVSGIDLSSYAMGWVRQAPGMGL EWIGDISTSGNAYYATWVKGRFTISRTSTTVDLKMASLTADTA TYFCARADYGGETYAFDPWGPGLTVTVSS	217
rab600_25_75	QSLEESGGRLVTPGGSPLTLTCTVSGIDLSSYPMTWVRQAPGKGL EWIGMIYGGGAYYATWAKGRFTISKSTTVDLKMNSLTASDTA TYFCGRGSLWGPGLTVTVSS	218
rab600_25_82	QSVEESGGRLVTPGTPLTLTCTASGFSLSSYDMSWVRQAPGKGL EWIGIYAGSGTNYATWAKGRFTISKSTTVDLKISSPTTEDT ATYFCARGGYDSDAYVYPDFDPWGPGLTVTVSS	219
rab600_25_84	QEQLKESGGGLVTPGTPLTLTCTASGFSLSSYDMSWVRQAPGKGL LEWIGYIWSSGSAYYASWAKGRFTISKSTTVGLKITSPTTEDT ATYFCARRFVGSSTYDTWGQGLTVTVSS	220
rab600_25_92	QSVEESGGRLVKPDETLTLTCTVSGFSLSSHMIWVRQAPGEGL EGIGIIDAGSGSTYYASWAKGRFTISRTSTTVDLKIASPTTEDT ATYFCARGGLTESLGTDFDLWGPGLTVTVSS	221
rab600_25_93	QSLEESGGRLVTPGTPLTLTCTVSGFFLSSYEMNWVRQAPGKGL EWIGVIYTDGSAYYASWAKGRFTISKASTTVDLKVTSPPTEDTA TYFCARGHPDYSSGMVFNWGPGLTVTVSS	222
rab600_25_97	QSLEESGGRLVKPDESLTLTCTASGIDLSSYYMIWVRQAPGKGL EWIGRIDANSNTYYASWAKGRFTISKSTTVDLKITSPTTADT ATYFCAGDFELWGPGLTVTVSS	223
rab600_25_100	QSVEESGGRLVTPGTPLTLTCTVSGFSLSSYALGWFRQAPGEGL EWIGSMRTDGVITYANWAEGRFTISKSTTVDLKITSPTTEDTA TYFCGRDVGDDGGWYFNWGPGLTVTVSS	224
rab600_25_108	QSVEESGGRLVTPGTPLTLTCKASGFSLSDYFMTWVRQAPGKGL EWIGINTGGDSYYATWAKGRFTISKSTTVDLKISSPTTEDTA TYFCARDTGYGGYDYAGSFDPWGPGLTVTVSS	225
rab600_25_110	QSVKESGGGLFKPTDTLTLTCTVSGFSLSDYYMSWVRQAPGKGL EYIGINTGGNTYYASWAKGRFTISKSTTVDLKISSPTTEDTA TYFCARDTGYGGYDYAGSFDPWGPGLTVTVSS	226
rab600_25_111	QSVEESGGRLVTPGTPLTLTCTVSGFSLNSHVMWVRQAPGKGL EWIGILTSSGYTYASWAKGRFTISKSTTVDLKITSPTTEDTA TYFCAREGYDYDDSGDYPPYFNIWGPGLTVTVSS	227
rab600_25_114	QSVAESGGRLVTPGTPLTLTCTVSGFSLSSYAMSWVRQAPGKGL EWIGIIGSRDNTHYASWAKGRFTISKSTTVDLKIASPTTEDTA TYFCARDIYGGYGDYTYDWLDLWGPGLTVTVSS	228

Table R2. Table R1. Exemplary Rabbit Antibody Light Chain Sequences (LCDRs 1-3 are underlined)

Name	Sequence	SEQ ID NO:
rab600_24_2	DDIVMTQTTPASVEAAVGGTVTIKQAGQSIDSNLAWYQQKPGQP PKLLIYRASTLASGVPSRFKSGSGTEFALTISDLECAADAATYF CQSFYVTISAMVDYFPFGGTEVVK	229

rab600_24_25	IEMTQTPSSVSAAVGGT ~~VT~~ INCQSS ~~ED~~ IDSYLAWYQQKPGQPPK LLIYHASYLTSGVPSR ~~F~~ SGSRSGTEFTLTISDLECD ~~DAATYYCQ~~ SAYSSSADNTFGGGTEVVVK	230
rab600_24_62	ALVMTQTPASVSAAVGGT ~~VT~~ INCQASEDIDSYLAWYQQKPGQPP KLLIYYASYLTSGVPSR ~~F~~ KSGSGTEYTLTISGVQCDDAATYYC QSAFYSNNTETAFGGGTEVVVK	231
rab600_24_97	ADIVMTQTPASVEAVGGT ~~VT~~ IKCQASEDIENYLAWYQQKPGQP PKLLIYDASDLTSGVPSR ~~F~~ KSGSGTQFTLTISDLECA ~~DAATYY~~ CQSVYYTSSDNYNNAFGGGTEVVVK	232
rab600_24_124	IKMTQTPASVSAAVGGT ~~VT~~ INCRASEDIESYLAWYQQKPGQPPK LLIYDASDLASGVPSR ~~V~~ KSGSGTEFTLTISGVRCD ~~DAATYYCQ~~ HGFYTSRSDSVFGGGTEVVVK	233
rab600_25_10	DVMTQTPASVEAAVGGT ~~VT~~ IKCQASQSISSYLSWYQQKPGQPP KLLIYRASTLES ~~GV~~ PSR ~~F~~ KSGSGTEFTLTISDLECA ~~DAATYYC~~ QCGYYGGSYIGAFGGGTEVVVK	234
rab600_25_20	DVMTQTPASVEAAVGGT ~~VT~~ IKCQASQSIGGVLSWYQQKPGQPP KLLIYRASTLES ~~GV~~ PSR ~~F~~ KSGSESGTEFTLTISDLECA ~~DAATYYC~~ QCNYYGGSYIGAFGGGTEVVVK	235
rab600_25_31	AVLTQTPSPVSAAVGGT ~~VT~~ ISCQSSQSVSNNNYLSWYQQKPGQP PKLLIYAASYLASGVPPR ~~F~~ SGSGSGTQFTLTISGVQCDDAATYY CLGDYDNDIDHAFGGGTEVVVK	236
rab600_25_103	DVMTQTPASVEAAVGGT ~~VT~~ INCQASQSI ~~SN~~ LLAWYQQKPGQPP KLLIYRASTLES ~~GV~~ PSR ~~F~~ KSGSGTEFTLTISDLECA ~~DAATYYC~~ QCSYYGGSYIGAFGGGTEVVVK	237
rab600_23_7H2.5	LDIKVTQTPAVSAAVGGT ~~VS~~ INCQASEDIKNYLAWYQQKPGQRP KLLIYDASKLASGVPSR ~~F~~ KSGSGTEYTLTISDLECD ~~DAATYYC~~ QHGYTSGXDNTFGGGTEVVVK	238
rab600_23_8G10.7	IEMTQTPFSVSAAVGGT ~~VT~~ INCQASENIYSS ~~L~~ AWYQQKPGQPPK LLIYAASDLASGVPSR ~~F~~ SGSGSGTEYTLTISGVQC ~~ADAATYYCQ~~ SAYSSGSDDNFGGGTEVVVK	239
rab600_23_8G11.5	ADIVMTQTPSPVSAAVGGT ~~VT~~ INCQASQSIY ~~T~~ ALAWYQQKSGQP PKLLIYAAS ~~T~~ LASGVPSR ~~F~~ KSGSGTQFTLTISDLECA ~~DAATYY~~ CQNYYYGDNTYNNTFGGGTEVVVK	240
rab600_23_9B11.2	ADIVMTQTPASVEAAVGGT ~~VT~~ IKCQASQ ~~T~~ ISNLLAWYQQKPGQP PKLLISRASILASGVPSR ~~F~~ KSGESGTXTFTLTITDLECA ~~DAATYY~~ CQSNYYSSSSSYGNTFGGGTEVVVK	241
rab600_23_11D7.1	QVLTQTPSSVSEPVGGT ~~VT~~ INCQASENIYSG ~~L~~ AWYQQKPGQPPK LLIVSAFTLASGVPSR ~~F~~ KSGSGTGTFTLTISGVQCDDAATYYC TYYG ~~S~~ VTYFNAFGGGTEVVVK	242
rab600_23_11G12.1	ADIVMTQTPASVEAAVGGT ~~VT~~ IKCQASQSISS ~~T~~ YLSWYQQKPGQ RPKLLIYQASTLASGVPSR ~~F~~ KSGSGTEFTLTISDLECA ~~DAATY~~ YCQGGYFSDNGCYNAFGGGTEVVVK	243
rab600_23_28B7.3	AVLTQTPSSVSAAVGGT ~~VT~~ LNCQSSQSIDANNDLAWYQQKPGQP PRLLIYLASKLASGVPSR ~~F~~ SGSGSGTQFTLTISGVQCDDAATYY CLGGYDDADNTFGGGTEVVVK	244
rab600_23_37D1.4	ADIVMTQTPSPVSAAVGGT ~~VT~~ INCQASQSIY ~~T~~ ALAWYQQKSGQP PKLLIYAAS ~~T~~ LASGVPSR ~~F~~ KSGSGTQFTLTISDLECA ~~DAATYY~~ CQNYYYGDNTYNNTFGGGTEVVVK	245
rab600_23_37H4.1	AVLTQTPSSVSAAVGGT ~~VT~~ LNCQSSQSIDANNDLAWYQQKPGQP PKLLIYLASTRASGVPSR ~~F~~ KSGSGTQFTLTISGVQCYDAATYY CLGGYDDADNTFGGGTEVVVK	246
rab600_23_55F6.6	ADIVMTQTPASVSPVGGT ~~VT~~ IKCQASQSIYNYLSWYQQKPGQP PKRLIYYASTLASGVPSR ~~F~~ SGSGSGTEFTLTISDLECA ~~DAATYY~~ CQNSGVNGNRYGNAFGGGTEVVVK	247
rab600_23_4	ADIVMTQTPASVSAAVGGT ~~VT~~ IKCQATESISSWLAWYQQKPGQP PKLLIYGASTLES ~~GV~~ PSR ~~F~~ SGSGSGTEFTLTISGVQCDDAATYY CQQGYIYTNVDNTFGGGTEVVVK	248
rab600_23_6	AYDMTQTPASVEAVGGT ~~VT~~ IKCQASQ ~~T~~ ISNELSWYQQKSGQPP KLLIYRASTLASGVPSR ~~F~~ SGSGSGTEFTLTISGVECD ~~DAATYYC~~ QQGYTTNNVDNLFGGGTEVVVK	249

rab600_23_8	ADIVMTQTPSSVSAAVGGTVTIRCQASESIGNALAWYQLKPGQR PKLLIYYTSTLASGVPSRFGSGSGTEFTLTISDLECDAAATYY CQSYDSVSSYGVFGGGTEVVVK	250
rab600_23_21	IEMTQTPFSVSAAVGGTVTINCQASENIYRSLAWYQQKPGQPPK LLIYDASDLASGVPSRFGSGSGTEYTLTISGVQCADAATYYCQ SAYTSSNTDNAFGGGTEVVVK	251
rab600_23_24	ADIVMTQTPSSVSAAVGGTVTIYCQASQSIPLSLAWYQQKSGQP PKLLIYAPSTLASGVPSRFGKASGSGTQFTLTISDLECDAAATYY CQSYYYGDNTYNNIFGGGTEVVVK	252
rab600_23_33	AVLTQTPSPVSAAVGGTVTISCQSSQDVKNNDLAWYQQKPGQP PKLLIYLASTLASGVPSRFGSGSGTQFSLTISGVQCDDAATYY CLGGYDDADNAFGGGTEVVVK	253
rab600_23_45	ALVMTQTPASVEAVGGTVTINCQASQISNLLAWYQQKPGQPP KLLIYYASTLASGVPSRFGSGSGTEYTLTIAGVQCADAAAYYC QGYDRSSTDMLAFGGGTEVVVK	254
rab600_23_50	IDMTQTPSSVSAAGVGTVTINCQASENIYSFLAWYQQKPGHSPK LLIYFASKLASGVSSRFGSGSGTQFTLTISDVQCDDAATYYCQ QYYSYSDADNTFGGGTEVVVK	255
rab600_23_52	QVLTQTPSSVSAAVGGTVTINCQSSQSVYRNNDLAWYQQKPGQP PKLLIYQASKLASGVPSRFGSGSGTQFTLTISDVQCDDAATYY CLGSYDCSSGDCFTFGGGTEVVVK	256
rab600_23_57	DVMTQTPSSVSEPVGGTVTIKCQASEEISSNLAWYQQKPGQPP KLLMYAASNLAGVSSRLKGRSGTDYTLTISGVQCDDAATYFC QCTYIGSGYVAFGGGTEVVVK	257
rab600_23_62	QVLTQTPSSVSEPVGGTVTINCQASENIYNALAWYQQKPGQPPK LLIYRASSLASGVPSRFGSGSGTEFTLTISAVQCDDAATYYCQ TCYYDSATYFNTFGGGTEVVVK	258
rab600_23_70	QVLTQTPASVSAAVGGTVTINCQASQSVYNKNYLAWFQQKPGQP PKRLIYQASKLASGVSSRFGSGSGTQFTLTISDVQCDDAATYY CLGTYACSSADCNVFGGGTEVVVK	259
rab600_23_71	IKMTQTLASVSAAVGGTGSISCQASEDIGNYVAWYQQKPGQPPK FLIYDTSHLASGVPSRFGKGRSGTEFTLTISGVQCDDAATYYCQ HGYTSDTDNTFGGGTEVVVK	260
rab600_23_75	QVLTQTPSSVSEPVGGTVTINCQASENIYNSLAWYQQKPGQPPK LLIYQASSLASGVPSRFGSGSGTEFTLTISGVQCDDAATYYCQ SYYSSVTYFNTFGGGTEVVVK	261
rab600_23_79	AVLTQTPSSVSAAVGGTVTINCQSSQSVNNNDLAWYQQKPGQPP KLLIYQASTLASGVPSRFGSGSGTQFTLTISGVQCDDAATYYC LGGYDDADNAFGGGTEVVVK	262
rab600_23_80	DVMTQTPASVSAAVGGTVTINCQASESIYNSLAWYQQKPGQPP KLLIYRASTLASGVPSRFGSGSGTEYTLTISDLECDAAATYYC QGYLYSSSVSYGNTFGGGTEVVVK	263
rab600_23_81	AYDMTQTPASVEAVGGTVTIKCQASESIANELSWYQRKSGQPP KLLIYRASTLASGVPSRFGSGSGTQFTLTISGVECDAAATYYC QQGYTTINIDNLFGGGTEVVVK	264
rab600_23_82	ANIKMTRTPFSVSAAVGGTVTINCQASESVYNSLAWFQQKPGQP PKLLIYAAASNPAAGVPSRFGSGSGTEYTLTISGVQCDDAATYY CQSAYYSGSGDVAFGGGTEVVVK	265
rab600_23_85	ADIVLTQTPASVGAAGGTVTIKCQASQTISTYLAWYQQKPGRP PKLLIYKASTLASGVPSRFGSGSGTEFTLTISDLECDAAATYY CQSYWGTSDIYAFGGGTEVVVK	266
rab600_23_90	IEMTQTPFSVSAAVGGTVTINCQASENIYSSLAWYQQKPGQPPK LLIYAASDLASGVPSRFGSGSGTEYTLTISGVQCADVATYYCQ HAYYSGIVDNGFGGGTEVVVK	267
rab600_23_102	ALVMTQTPASVEAVGGTVTIKCQASQISITNYLAWYRQKPGQPP KLLIYGASKLASGVPSRFGSGSGTEYTLTISGVQCDDAATYYC QQGYTSSNVDNPFGGGTEVVVK	268
rab600_23_105	ALVMTQTPSSVSAAVGGTVTINCQASQNIYNSLAWYQQKPGQRP KLLIYYTSLASGVSSRFGSGSGTEYTLTISDLECDAAATYYC QSAYYSSADNAFGGGTEVVVK	269

rab600_23_106	ADIVMT RTPVSVEAAVGGT VTIKQASESIDSNLAWYQQKPGQP PKLLIY RASTLASGVPSRFRKSGSGTEFTLTISDLECADAAATYY CQSNYYTTSTSYGNPFGGGTEVVVK	270
rab600_23_107	AYDMTQTPATVEVAVGGT VTI NCQASQSI SNLLAWYQQKPGQRP KLLIYDTS DLASGVPSRFRSGSGSGTEYTLTITGVECADAATYYC QQGYSSSNIDNVFGGGTEVVVK	271
rab600_23_108	ADIVMTQTPFSVSAAVGGT VTI NCQASESIY SNLAWYQQKPGQP PKLLIY RASTLASGVPSRFRKSGSGTEYTLTISDLECADATYY CQGYSSSSSSSYGNTFGGGTEVVVK	272
rab600_23_110	QVLTQTPSPVSVAVGGT VTI NCQATQSVYDNNALSWYQQKPGQP PKLLIYAAS TLASGVPSQFKGSGSGTQFTLTISDVQCDDAATYH CLGSYSGGIRAFGGGTEVVVK	273
rab600_23_114	QVLTQTPSSVSEPVGAT VTI NCCHASENIYASLAWYQQKPGQPPK LLIYSAFTLASGVPSRFRKSGSGTEFTLTISGVQCDDAATYYCQ SYYSSTVTFNVFGGGTEVVVK	274
rab600_23_119	AFEMTQTPSSVEAAVGGT VTIKQASQSIYNALAWYQQKPGQPP KLLIYFAATLTSGVPSRFRKSGSGTEYTLTISDLECADAGTYYY QSYDGVPGFWPFGGGTEVVVK	275
rab600_23_123	IVMTQTPSSKSVPGDT VTI NCQASESVYGNLAWYQQKAGQP PKLLIYQASTLASGVPSRFRKSGSGTQFTLTISDVVCDDAATYY CTGWKDEIDGIFGGGTEVVVK	276
rab600_23_127	DVMTQTPASVSGPVGGT VTIKQASQNI DSDLAWYQQKPGQRP KLLIYDASKLASGVPSRFRSGSGYGTFTLTISGVQCEDAATYYC QYTYIINTYGGAFGGGTEVVVK	277
rab600_23_129	ADIVMTQTPASVEAAVGGT VTI NCQASQSSNLLAWYQQKPGQP PKLLIY RASTLASGVPSRFRKSGSGIEFTLTISDLECADAAATYY CQTNYYRSSSSTYEGAFGGGTEVVVK	278
rab600_23_130	AYDMTQTPASVEVAVGGT VTIKQASQSI SNLLAWYQQKPGQPP KLLIYRASDLASGVPSRFRKSGSGTEFTLTISGVQCADAATYYC QQGYSYSNVDNAFGGGTEVVVK	279
rab600_23_132	AFELTQTPSSVEAAVGGT VTIKQASQSI SNALAWYQQKPGQPP KLLIYSASTLASGVPSRFRKSGSGTEYTLTISDLECADAAATYYC QGYDGSIGFWPFGGGTEVVVK	280
rab600_23_133	AVLTQTPSPVSEPVGGT VTI NCQSSQSIYNNYLSWYQQKPGQP PKLLIYKASTLASGVPSRFRKSGSGTQFTLTISDVQCDDAATYY CAGDYDITTDIVFGGGTEVVVK	281
rab600_23_135	ADIVMTQTPASVEAAVGGT VTIKQASEDIESYLAWYQQKPGQP PKLLIYGASTLESVPDRFRKSGSGTQFTLTISDLECADAAATYF CQSYYYTDSNDYGANNVFGGGTEVVVK	282
rab600_23_140	DVMTQTPASVSEPVGGT VTI NCQASEDIESYLAWYQQKPGQRP KLLIYGASNLASGVSSRFRKSGSGTQFTLTISDLECADAAATYYC QCTYYATIYANVFGGGTEVVVK	283
rab600_23_141	QGPTQTPSSVSAAVGGT VTI NCQTSSESVNSNNILSWYQQKPGQP PKLLIYDTS TLASGVPSRFRKSGSGTQFTLTISDVQCDDAATYY CQGSYASSGWYVAFGGGTEVVVK	284
rab600_23_148	QGPTQTPSSVSAAVGGT VTI NCQTSSEFSGGNILSWYQQKPGQP PKLLIYDSSTLTSGVPSRFRSGSGTQFTLTISGVQCDDAATYY CQGSDHSGAWYAFGGGTEVVVK	285
rab600_23_152	AVLTQTPSPVSVVGGT VTIKQSSQTIYSNYLSWYQQRPGQPP KLLIWSASSLASGVPSRFRSGSGTQFTLTISGVQCDDAATYYC LGGYDDADPNAFGGGTEVVVK	286
rab600_23_153	AQVMTQTPAVSAAVGGT VTIKQASQNIY SNLAWYQQKPGQPP KLLIYGTSTLASGVPSRFRSGSGTDFTLTISGVQCEDAATYYC QGYYSRSADTAFGGGTEVVVK	287
rab600_23_158	IVMTQTPSSKSVPGDT VTI NCQASESVYGNLAWYQQKPGQP PKLLIYLASTLASGVPSRFRSGSGTQFTLTISDVVCDDAATYY CTGFKDEIAGTAFGGGTEVVVK	288
rab600_23_163	ANIVLTQTASPVSGAVGGT VTIKQASQNIY SNLAWYQQKPGQP PNLLIYYTSTLASGVPSRFRKSGSGAEYTLTISGVQCDDAATYY CQSAYYSGSGNCAFGGGTEVVVK	289

rab600_23_165	QVLTPSPSTSEPVGGT ¹ VTINCQASQSISSYLSWYQQKPGPPK LLIYSASTLASWVPKRFSGSRSGTQFTLTISGVQCDDAATYYCL GAYGYTSDDAFAGGGTEVVVK	290
rab600_23_167	IVMTQTPSSKSPVPGDTVTINCQASESVYGNWLA ² WYQQKTGQP PKLLIYQASTLASGVPSRFGSGSGTQFTLTISDVVCDDAATYY CTGWKDEIDGIAFGGGTEVVVK	291
rab600_23_177	ALVMTQTPSPVSAAVGGT ³ VTINCQASQSVYDSNYLAWFQQKPGQ PPKLLIYWVSTLASGV ⁴ PD ⁵ RFSGSGSGTQFTLTISGVQCDDAATY YCLGLYGDDSTWAFAGGGTEVVVK	292
rab600_23_182	AYDMTQTPASVEAVGGT ⁶ VTIKQASESIYNFLAWYQQKPGQPP KLLIYSASTLASGVPSRFGSGSGTEYTLTISDLECADAATYYC QQGYDYSVDNAFGGGTEVVVK	293
rab600_25_9	ALVMTQTPASVEADVGGT ⁷ VTINCQASESISNLLAWYQQKPGQRP KLLIYRASILTSGVSSRFGSGSGTEYTLTINGVQCADAATYYC QHGYTGTVNQNVFGGGTEVVVK	294
rab600_25_14	QVLTPSPSTSAAVGGT ⁸ VTINCQSSQSVYKSDWLGWYQQKPGQP PKLLIYKASTLASGVPSRFGSGSGTQFTLTISDLECDDAATYY CQGGYSPASYPFGGGTEVVVK	295
rab600_25_26	AYDMTQTPASVEVPVGGT ⁹ VTIKQASQSI ¹⁰ SYLAWYQQKPGQPP KLLIRDASDLASGVPSRFTGSGSGAQFTLTISGVECADAATYYC QQGLNSIGFGGGTEVVVK	296
rab600_25_37	AGDMTQTPASVEAVGGT ¹¹ VTIKQASQSI ¹² NYLAWYQQKPGQPP KLLIYDASKLASGVPSRFGSGSGTEFTLTISDLECADAATYYC QQGINNIGFGGGTEVVVK	297
rab600_25_38	QVLTPASPVSAAVGGT ¹³ VTSISCQSSENVYKNNYLAWFQHKPGQP PKRLIDSASTLES ¹⁴ GVPSRFGSGSGTQFTLTISGVQCDDAATYY CVALYSGNIYIVGGGGTEVVVK	298
rab600_25_42	AYDMTQTPASVEAVGGT ¹⁵ VTIKQASESIFS ¹⁶ YLAWYQQKPGQRP KLLIYYASTLASGVPSRFGSGSGTQFTLTISGVECADAATYYC QQGYDFS ¹⁷ AVDNVFGGGTEVVVK	299
rab600_25_43	AVLTQTPSPVSAAVGGT ¹⁸ V ¹⁹ TISCQSSQSVSNNNYLSWYQQKPGQP PKLLIYAASYLETGVPSRFGSGSGTQFTLTISGVQCDDAATYY CLGDYDNDVDHAFGGGGTEVVVK	300
rab600_25_46	QVLTPASPVSAAVGST ²⁰ VTINCQASRSVYNNNYLSWFQQKSGQP PKLLIYSASTLPSGVSSRFGSGSGTQFTLTISDVQCDDAATYY CLGNYDCGSADCYAFGGGGTEVVVK	301
rab600_25_48	AYDMTQTPASVEAVGGT ²¹ VTINCQASQSI ²² NLLAWYQQKPGQRP KLLIYYASTLASGVSSRFGSGSGTEFTLTISDVECADAATYYC QQGYSSGNLDNFGGGTEVVVK	302
rab600_25_51	AYDMTQTPASVEAVGGT ²³ VTIKQASQSI ²⁴ SYLAWYQQKPGQPP KQLIYYTSTLASGVPSRFGSGSGTEFTLTISGVECADAATYYC QQGYSKTDLDNAFGGGTEVVVK	303
rab600_25_54	AVLTQTPSPVSAAVGGT ²⁵ V ²⁶ TISCQSSQSVSNDNYLSWYQQRPEQP PKLLIYAASYLASGVPSRFGSGSGTQFTLTISGVQCDDAATYY CLGDYDNDVDHAFGGGGTEVVVK	304
rab600_25_63	AVLTQTPSPVSAAVGGT ²⁷ V ²⁸ TISCQSSQSVSNNNYLSWYQQKPGQP PKLLIYAASYLASGVPSRFGSGSGTQFTLTISGVQCDDAATYY CLGDYDNDVDHAFGGGGTEVVVK	305
rab600_25_70	AVLTQTPSPVSAAVGGT ²⁹ V ³⁰ TISCQSSQSVDSNNDLAWYQRKPGQP PKLLIYQASKLASGVPSRFGSGSGTQFTLTISGVQCDDAATYY CLGGYDDDADNAFGGGTEVVVK	306
rab600_25_71	ADIVMTQTPASVEAVGGT ³¹ VTIKQASQSISSYLNWYQQKPGQP PKLLIYSASTLASGVPSRFGSGSGTQFTLTISGVECADAATYY CQQGYSDSNIDNVFGGGTEVVVK	307
rab600_25_75	QVLTPASPVSAAVGNT ³² VTINCQASQSVYNNNYLSWFQQKPGQP PKLLIYSASTLPSGVSSRFGSGSGTQFTLTIRDVQCDDAATYY CLGNYDCGSADCYAFGGGGTEVVVK	308
rab600_25_82	ALVMTQTPASVEAVGGT ³³ VTINCQASQSI ³⁴ NLLAWYQQKPGQRP KLLIYRASTLASGVPSRFGSGAGTEYTLTISGVQCDDAATYHC QHGYTGSNVHNVFGGGTEVVVK	309

rab600_25_84	AVLTQTPSPVSAAVGGT ~~VT~~ ISCQSSQSVSN ~~NN~~ YLSWYQQKPGQP PKLLIYAASYLASGVPSR ~~F~~ SGSGSGTQFTLIISGVQCDDAATYY CLGDYDNDVDHAFGGGTEVVVK	310
rab600_25_92	ADIVMTQTPASVEAAVGGT ~~VT~~ IKCQASESID ~~S~~ GLAWYQQKPGQR PKLLIYDSSTLASGVPSR ~~F~~ KSGSGTDFTLTISDLECA ~~D~~ AATYY CQSNYDTGSSVYDWGSFGGGTEVVVK	311
rab600_25_93	LVMTQTPSPVSAAVGGT ~~VT~~ ISCQASQSLYN ~~K~~ DACSWYQQKPGQP PKLLIYYAFTLASGVPSR ~~F~~ KSGSGTQFTLTISDVQCDDAATYY CAGDFISSDNGFGGGTEVVVK	312
rab600_25_97	QVLTQTASSVSAAVGGT ~~VT~~ ISCQSSQSVYNN ~~N~~ WLAWYQQKPGQR PKLLIYDASKLASGVPSR ~~F~~ KSGSGTRFTLTISDVQCDDAATYY CLGGYPGGSDVHAFGGGTEVVVK	313
rab600_25_100	AYDMTQTPASVEAVGGT ~~VT~~ IKCQASQSI ~~V~~ TYLAWYQQKPGQPP KLLIYDASDLASGVPSR ~~F~~ KSGSGTQFTLTISGVECA ~~D~~ AATYYC QQGINNIAFGGGTEVVVK	314
rab600_25_108	AIKMTQTPASVSEPVGGT ~~VT~~ IKCQASENINS ~~W~~ LAWYQQKPGQPP KLLIYEASKLASGVPSR ~~F~~ KSGSGTQFTLTISDLECA ~~D~~ AATYYC QQGYIYIDVGNIFGGGTEVVVK	315
rab600_25_110	AIKMTQTPSSVSAAVGGT ~~VT~~ INCQASESI ~~S~~ SWLSWYQQKPGQRP KLLIYEASKLASGVPSR ~~F~~ KSGSGTQFTLTISDLECA ~~D~~ AATYYC QQGYIYIDVGNIFGGGTEVVVK	316
rab600_25_111	AALTQTPSPVSAAVGGT ~~VT~~ IKCQSSQSV ~~D~~ NNNELSWYQQKPGRP PMLLIYAASNLASGVPSR ~~F~~ SGSGSGTQFSLTISGVQCDDAATYY CLGGYDDDAENAFGGGTEVVVK	317
rab600_25_114	DVMTQTPASVSEPVGGT ~~VT~~ IKCQASESI ~~G~~ NNLAWYQQKPGQPP KLLIYGTSTLASGVPSR ~~F~~ KSGSRGTEFTLTISDLECA ~~D~~ AATYYC QCTYYGSSYVESSFGGGTEVVVK	318

Thus, in certain embodiments, an antibody, or antigen-binding fragment thereof, binds to TNFR2 and comprises a VH and a corresponding VL region selected from **Table H2**. In particular embodiments, the VH region comprises an amino acid sequence having at least 90%, 95%, 98%, 99%, or 100% identity to a sequence selected from SEQ ID NOs: 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, and 135. In some embodiments, the VL region comprises an amino acid sequence having at least 90%, 95%, 98%, 99%, or 100% identity to a sequence selected from SEQ ID NOs: 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 134, and 136. In specific embodiments, an antibody, or antigen-binding fragment thereof, comprises:

- the VH region set forth in SEQ ID NO: 103, and the VL region set forth in SEQ ID NO: 104;
- the VH region set forth in SEQ ID NO: 105, and the VL region set forth in SEQ ID NO: 106;
- the VH region set forth in SEQ ID NO: 107, and the VL region set forth in SEQ ID NO: 108;
- the VH region set forth in SEQ ID NO: 109, and the VL region set forth in SEQ ID NO: 110;
- the VH region set forth in SEQ ID NO: 111, and the VL region set forth in SEQ ID NO: 112;
- the VH region set forth in SEQ ID NO: 113, and the VL region set forth in SEQ ID NO: 114;
- the VH region set forth in SEQ ID NO: 115, and the VL region set forth in SEQ ID NO: 116;
- the VH region set forth in SEQ ID NO: 117, and the VL region set forth in SEQ ID NO: 118;
- the VH region set forth in SEQ ID NO: 119, and the VL region set forth in SEQ ID NO: 120;
- the VH region set forth in SEQ ID NO: 121, and the VL region set forth in SEQ ID NO: 122;
- the VH region set forth in SEQ ID NO: 123, and the VL region set forth in SEQ ID NO: 124;

the VH region set forth in SEQ ID NO: 125, and the VL region set forth in SEQ ID NO: 126;
 the VH region set forth in SEQ ID NO: 127, and the VL region set forth in SEQ ID NO: 128;
 the VH region set forth in SEQ ID NO: 129, and the VL region set forth in SEQ ID NO: 130;
 the VH region set forth in SEQ ID NO: 131, and the VL region set forth in SEQ ID NO: 132;
 the VH region set forth in SEQ ID NO: 133, and the VL region set forth in SEQ ID NO: 134;

or

the VH region set forth in SEQ ID NO: 135, and the VL region set forth in SEQ ID NO: 136.

In some embodiments, an antibody, or an antigen-binding fragment thereof, binds to tumor necrosis factor receptor 2 (TNFR2), and comprises a heavy chain variable (VH) region comprising VHCDR1, VHCDR2, and VHCDR3 regions selected from the underlined sequences in **Table R1**; and a corresponding (by clone name) light chain variable (VL) region comprising VLCDR1, VLCDR2, and VLCDR3 regions selected from underlined sequences in **Table R2**. In some embodiments, the VH region comprises an amino acid sequence selected from **Table R1**, and the VL region comprises a corresponding (by clone name) amino acid sequence selected from **Table R2**.

In some embodiments, as noted above, an antibody, or an antigen-binding fragment thereof, binds to tumor necrosis factor receptor 2 (TNFR2), for example, soluble or cell-expressed TNFR2. In particular embodiments, the TNFR2 is human TNFR2, or a peptide epitope thereof. Exemplary peptide epitopes of human TNFR2 are provided in **Table T1** below.

Table T1. Exemplary TNFR2 peptide epitopes		
Domain and Residues	Sequence	SEQ ID NO:
PLAD or CRD1 a.a. 17-54 of mature TNFR2	TCRLREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCD	319
PLAD or CRD1 a.a. 18-58 of mature TNFR2	CRL <u>REYYDQ</u> TAQMCC <u>SK</u> CSPGQHAKVFCTKTS <u>D</u> TVCD <u>SC</u> ED	327
Epitope a.a. 21-23 of mature TNFR2	REY	N/A
Epitope a.a. 27-34 of mature TNFR2	TAQMCCSK	328
Epitope a.a. 51-55 of mature TNFR2	TVCD	329
CRD2 a.a. 58-93 of mature TNFR2	DSTYTQLWNWVPECLSCGSRSSDQVETQACTREQN	320
CRD3 a.a. 106-133 of mature TNFR2	LSKQEGCRLCAPLRKCRPGFGVARPGTE	321
CRD3 a.a. 114-133 of mature TNFR2	LCAPLRKCRPGFGVARPGTE	322

CRD4 a.a. 146-174 of mature TNFR2	TFSNTTSSTDICRPHQICNVVAIPGNAS	323
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In certain embodiments, an antibody, or an antigen-binding fragment thereof, specifically binds to human TNFR2, for example, at least one human TNFR2 peptide epitope selected from **Table T1**, for example, at least one, two, three, four, or five peptide epitopes selected from **Table T1**. In some embodiments, an antibody, or an antigen-binding fragment thereof, specifically binds to human TNFR2 at a peptide epitope that comprises, consists, or consists essentially of one or more residues selected from R21, Y23, T27, S33, K34, T51, and S55, as defined by the mature human TNFR2 sequence (residues 23-461 of FL human TNFR2). In some embodiments, an antibody, or an antigen-binding fragment thereof, specifically binds to human TNFR2 at a peptide epitope that comprises, consists, or consists essentially of one or more residues selected from REY, TAQMCCSK (SEQ ID NO: 328), and TVCDS (SEQ ID NO: 329). In specific embodiments, an antibody, or antigen-binding fragment thereof, binds to human TNFR2 with a K_D of about 2 nM or lower, or with a K_D of about 0.7 nM or lower. In some embodiments, an antibody, or an antigen-binding fragment thereof, binds to cynomolgus TNFR2, for example, it cross-reactively binds to human TNFR2 and cynomolgus TNFR2.

In some embodiments, an antibody, or antigen-binding fragment thereof, is a TNFR2 antagonist. For instance, in some embodiments, an antibody, or antigen-binding fragment thereof, inhibits or otherwise reduces TNF- α binding to TNFR2. In some embodiments, an antibody, or antigen-binding fragment thereof, inhibits or otherwise reduces TNFR2 multimerization or trimerization. In some embodiments, an antibody, or antigen-binding fragment thereof, inhibits or otherwise reduces TNFR2-mediated activation of T regulatory cells (Tregs), for example, systemically or in the tumor microenvironment. In particular embodiments, an antibody, or antigen-binding fragment thereof, binds to TNFR2, is a TNFR2 antagonist, and does not substantially bind to tumor necrosis factor receptor 1 (TNFR1), for example, human TNFR1. In some embodiments, an antibody, or antigen-binding fragment thereof, does not substantially bind to herpesvirus entry mediator (HVEM), CD40, death receptor 6 (DR6), and/or osteoprotegerin (OPG).

In some embodiments, for example, in vitro or in vivo, an anti TNFR2 antibody, or antigen-binding fragment thereof, increases cell-killing/depletion of tumor cells (for example, TNFR2-expressing tumor cells), T_{regs} , and/or myeloid-derived suppressor cells (MDSCs) by antibody-dependent cellular cytotoxicity (ADCC), for example, by about or at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 2000% or more, relative to a control or reference. In some embodiments, an anti TNFR2 antibody, or antigen-binding fragment thereof, increases cell-killing/depletion of tumor cells (for example, TNFR2-expressing tumor cells), T_{regs} , and/or MDSCs by macrophage-mediated antibody-dependent cellular phagocytosis (ADCP), for

example, by about or at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 2000% or more, relative to a control or reference. In some embodiments, an anti TNFR2 antibody, or antigen-binding fragment thereof, reduces MDSC-mediated immune suppression, for example, by about or at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 2000% or more, relative to a control or reference. In some embodiments, an anti TNFR2 antibody, or antigen-binding fragment thereof, converts MDSCs and/or M2 macrophages into proinflammatory M1 macrophages, and/or converts T_{reg} s into effector T cells, for example, by increasing said conversion by about or at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 2000% or more, relative to a control or reference. In some embodiments, anti TNFR2 antibody, or antigen-binding fragment thereof, converts “cold” tumors into “hot” tumors. A “cold” tumor is a tumor that has not been recognized or has not provoked a strong response by the immune system. For instance, the microenvironment of a cold tumor typically has low or insignificant levels of CD4+ or CD8+ T cells; instead, it often has relatively high levels of myeloid-derived suppressor cells and/or Tregs, which secrete immunosuppressive cytokines to impede the movement of CD4+ or CD8+ T cells into the tumor microenvironment. By contrast, a “hot” tumor is a tumor that has high levels of CD4+ or CD8+ T cells, resulting in an inflamed tumor microenvironment. In particular embodiments, an anti TNFR2 antibody, or antigen-binding fragment thereof, has a combination of any one or more of the foregoing characteristics.

Merely for illustrative purposes, the binding interactions between an antibody, or antigen-binding fragment thereof, and a TNFR2 polypeptide can be detected and quantified using a variety of routine methods, including biacore assays (for example, with appropriately tagged soluble reagents, bound to a sensor chip), FACS analyses with cells expressing a TNFR2 polypeptide on the cell surface (either native, or recombinant), immunoassays, fluorescence staining assays, ELISA assays, and microcalorimetry approaches such as ITC (Isothermal Titration Calorimetry).

As is well known in the art, an antibody is an immunoglobulin molecule capable of specific binding to a target, such as a carbohydrate, polynucleotide, lipid, polypeptide, etc., through at least one epitope recognition site, located in the variable region of the immunoglobulin molecule. As used herein, the term encompasses not only intact polyclonal or monoclonal antibodies, but also fragments thereof (such as dAb, Fab, Fab', F(ab')₂, Fv), single chain (scFv), synthetic variants thereof, naturally occurring variants, fusion proteins comprising an antibody portion with an antigen-binding fragment of the required specificity, humanized antibodies, chimeric antibodies, and any other modified configuration of the immunoglobulin molecule that comprises an antigen-binding site or fragment (epitope recognition site) of the required specificity. “Diabodies”, multivalent or multispecific fragments constructed by gene fusion (WO94/13804; P. Holliger et al., Proc. Natl. Acad. Sci. USA 90 6444-6448, 1993) are also a particular form of antibody contemplated herein. Minibodies comprising a scFv joined to a CH3 domain are also included herein (S. Hu et al., Cancer Res., 56, 3055-3061,

1996). See *e.g.*, Ward, E. S. *et al.*, Nature 341, 544-546 (1989); Bird *et al.*, Science, 242, 423-426, 1988; Huston *et al.*, PNAS USA, 85, 5879-5883, 1988); PCT/US92/09965; WO94/13804; P. Holliger *et al.*, Proc. Natl. Acad. Sci. USA 90 6444-6448, 1993; Y. Reiter *et al.*, Nature Biotech, 14, 1239-1245, 1996; S. Hu *et al.*, Cancer Res., 56, 3055-3061, 1996.

The term “antigen-binding fragment” as used herein refers to a polypeptide fragment that contains at least one CDR of an immunoglobulin heavy and/or light chains that binds to the antigen of interest, in particular to TNFR2. In this regard, an antigen-binding fragment of the herein described antibodies may comprise 1, 2, 3, 4, 5, or all 6 CDRs of a VH and VL sequence set forth herein from antibodies that bind TNFR2.

The term “antigen” refers to a molecule or a portion of a molecule capable of being bound by a selective binding agent, such as an antibody, and additionally capable of being used in an animal to produce antibodies capable of binding to an epitope of that antigen. An antigen may have one or more epitopes.

The term “epitope” includes any determinant, preferably a polypeptide determinant, capable of specific binding to an immunoglobulin or T-cell receptor. An epitope is a region of an antigen that is bound by an antibody. In certain embodiments, epitope determinants include chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl or sulfonyl, and may in certain embodiments have specific three-dimensional structural characteristics, and/or specific charge characteristics. In certain embodiments, an antibody is said to specifically bind an antigen when it preferentially recognizes its target antigen in a complex mixture of proteins and/or macromolecules. An antibody is said to specifically bind an antigen when the equilibrium dissociation constant is $\leq 10^{-7}$ or 10^{-8} M. In some embodiments, the equilibrium dissociation constant may be $\leq 10^{-9}$ M or $\leq 10^{-10}$ M.

In certain embodiments, antibodies and antigen-binding fragments thereof as described herein include a heavy chain and a light chain CDR set, respectively interposed between a heavy chain and a light chain framework region (FR) set which provide support to the CDRs and define the spatial relationship of the CDRs relative to each other. As used herein, the term “CDR set” refers to the three hypervariable regions of a heavy or light chain V region. Proceeding from the N-terminus of a heavy or light chain, these regions are denoted as “CDR1,” “CDR2,” and “CDR3” respectively. An antigen-binding site, therefore, includes six CDRs, comprising the CDR set from each of a heavy and a light chain V region. A polypeptide comprising a single CDR, (*e.g.*, a CDR1, CDR2 or CDR3) is referred to herein as a “molecular recognition unit.” Crystallographic analysis of a number of antigen-antibody complexes has demonstrated that the amino acid residues of CDRs form extensive contact with bound antigen, wherein the most extensive antigen contact is with the heavy chain CDR3. Thus, the molecular recognition units are primarily responsible for the specificity of an antigen-binding site.

As used herein, the term “FR set” refers to the four flanking amino acid sequences which frame the CDRs of a CDR set of a heavy or light chain V region. Some FR residues may contact

bound antigen; however, FRs are primarily responsible for folding the V region into the antigen-binding site, particularly the FR residues directly adjacent to the CDRs. Within FRs, certain amino residues and certain structural features are very highly conserved. In this regard, all V region sequences contain an internal disulfide loop of around 90 amino acid residues. When the V regions fold into a binding-site, the CDRs are displayed as projecting loop motifs which form an antigen-binding surface. It is generally recognized that there are conserved structural regions of FRs which influence the folded shape of the CDR loops into certain “canonical” structures, regardless of the precise CDR amino acid sequence. Further, certain FR residues are known to participate in non-covalent interdomain contacts which stabilize the interaction of the antibody heavy and light chains.

The structures and locations of immunoglobulin variable domains may be determined by reference to Kabat, E. A. et al., *Sequences of Proteins of Immunological Interest*. 4th Edition. US Department of Health and Human Services. 1987, and updates thereof, now available on the Internet (immuno.bme.nwu.edu).

A “monoclonal antibody” refers to a homogeneous antibody population wherein the monoclonal antibody is comprised of amino acids (naturally occurring and non-naturally occurring) that are involved in the selective binding of an epitope. Monoclonal antibodies are highly specific, being directed against a single epitope. The term “monoclonal antibody” encompasses not only intact monoclonal antibodies and full-length monoclonal antibodies, but also fragments thereof (such as Fab, Fab', F(ab')₂, Fv), single chain (scFv), variants thereof, fusion proteins comprising an antigen-binding portion, humanized monoclonal antibodies, chimeric monoclonal antibodies, and any other modified configuration of the immunoglobulin molecule that comprises an antigen-binding fragment (epitope recognition site) of the required specificity and the ability to bind to an epitope. It is not intended to be limited as regards the source of the antibody or the manner in which it is made (*e.g.*, by hybridoma, phage selection, recombinant expression, transgenic animals, etc.). The term includes whole immunoglobulins as well as the fragments, etc., described herein under the definition of “antibody”.

The proteolytic enzyme papain preferentially cleaves IgG molecules to yield several fragments, two of which (the F(ab) fragments) each comprise a covalent heterodimer that includes an intact antigen-binding site. The enzyme pepsin is able to cleave IgG molecules to provide several fragments, including the F(ab')₂ fragment which comprises both antigen-binding sites. An Fv fragment for use according to certain embodiments of the present disclosure can be produced by preferential proteolytic cleavage of an IgM, and on rare occasions of an IgG or IgA immunoglobulin molecule. Fv fragments are, however, more commonly derived using recombinant techniques known in the art. The Fv fragment includes a non-covalent V_H:V_L heterodimer including an antigen-binding site which retains much of the antigen recognition and binding capabilities of the native antibody molecule. Inbar *et al.* (1972) *Proc. Nat. Acad. Sci. USA* 69:2659-2662; Hochman *et al.* (1976) *Biochem* 15:2706-2710; and Ehrlich *et al.* (1980) *Biochem* 19:4091-4096.

In certain embodiments, single chain Fv or scFv antibodies are contemplated. For example, Kappa bodies (Ill *et al.*, *Prot. Eng.* 10: 949-57 (1997); minibodies (Martin *et al.*, *EMBO J* 13: 5305-9 (1994); diabodies (Holliger *et al.*, *PNAS* 90: 6444-8 (1993); or Janusins (Traunecker *et al.*, *EMBO J* 10: 3655-59 (1991) and Traunecker *et al.*, *Int. J. Cancer Suppl.* 7: 51-52 (1992), may be prepared using standard molecular biology techniques following the teachings of the present application with regard to selecting antibodies having the desired specificity. In some embodiments, bispecific or chimeric antibodies may be made that encompass the ligands of the present disclosure. For example, a chimeric antibody may comprise CDRs and framework regions from different antibodies, while bispecific antibodies may be generated that bind specifically to TNFR2 through one binding domain and to a second molecule through a second binding domain. These antibodies may be produced through recombinant molecular biological techniques or may be physically conjugated together.

A single chain Fv (scFv) polypeptide is a covalently linked V_H::V_L heterodimer which is expressed from a gene fusion including V_H- and V_L-encoding genes linked by a peptide-encoding linker. Huston *et al.* (1988) *Proc. Nat. Acad. Sci. USA* 85(16):5879-5883. A number of methods have been described to discern chemical structures for converting the naturally aggregated, but chemically separated, light and heavy polypeptide chains from an antibody V region into an scFv molecule which will fold into a three dimensional structure substantially similar to the structure of an antigen-binding site. See, *e.g.*, U.S. Pat. Nos. 5,091,513 and 5,132,405, to Huston *et al.*; and U.S. Pat. No. 4,946,778, to Ladner *et al.*

Certain embodiments include “probodies”, or antibodies where the binding site(s) are masked or otherwise inert until activated by proteolytic cleavage in target or disease tissue. Certain of these and related embodiments comprise one or more masking moieties that sterically hinder the antigen binding site(s) of the antibody, and which are fused to the antibody via one or more proteolytically-cleavable linkers (see, for example, Polu and Lowman, *Expert Opin. Biol. Ther.* 14:1049-1053, 2014).

In certain embodiments, a TNFR2 binding antibody as described herein is in the form of a diabody. Diabodies are multimers of polypeptides, each polypeptide comprising a first domain comprising a binding region of an immunoglobulin light chain and a second domain comprising a binding region of an immunoglobulin heavy chain, the two domains being linked (*e.g.*, by a peptide linker) but unable to associate with each other to form an antigen binding site: antigen binding sites are formed by the association of the first domain of one polypeptide within the multimer with the second domain of another polypeptide within the multimer (WO94/13804).

A dAb fragment of an antibody consists of a V_H domain (Ward, E. S. *et al.*, *Nature* 341, 544-546 (1989)).

Where bispecific or multi-specific antibodies are to be used, these may be conventional bispecific antibodies, which can be manufactured in a variety of ways (Holliger, P. and Winter G. *Current Opinion Biotechnol.* 4, 446-449 (1993)), *e.g.*, prepared chemically or from hybrid hybridomas, or may be any of the bispecific antibody fragments mentioned above. Diabodies and

scFv can be constructed without an Fc region, using only variable domains, potentially reducing the effects of anti-idiotypic reaction.

Bispecific diabodies, as opposed to bispecific whole antibodies, may also be particularly useful because they can be readily constructed and expressed in *E. coli*. Diabodies (and many other polypeptides such as antibody fragments) of appropriate binding specificities can be readily selected using phage display (WO94/13804) from libraries. If one arm of the diabody is to be kept constant, for instance, with a specificity directed against antigen X, then a library can be made where the other arm is varied and an antibody of appropriate specificity selected. Bispecific whole antibodies may be made by knobs-into-holes engineering (J. B. B. Ridgeway et al., *Protein Eng.*, 9, 616-621, 1996).

In certain embodiments, the antibodies described herein may be provided in the form of a UniBody®. A UniBody® is an IgG4 antibody with the hinge region removed (see GenMab Utrecht, The Netherlands; see also, *e.g.*, US20090226421). This proprietary antibody technology creates a stable, smaller antibody format with an anticipated longer therapeutic window than current small antibody formats. IgG4 antibodies are considered inert and thus do not interact with the immune system. Fully human IgG4 antibodies may be modified by eliminating the hinge region of the antibody to obtain half-molecule fragments having distinct stability properties relative to the corresponding intact IgG4 (GenMab, Utrecht). Halving the IgG4 molecule leaves only one area on the UniBody® that can bind to cognate antigens (*e.g.*, disease targets) and the UniBody® therefore binds univalently to only one site on target cells. For certain cancer cell surface antigens, this univalent binding may not stimulate the cancer cells to grow as may be seen using bivalent antibodies having the same antigen specificity, and hence UniBody® technology may afford treatment options for some types of cancer that may be refractory to treatment with conventional antibodies. The small size of the UniBody® can be a great benefit when treating some forms of cancer, allowing for better distribution of the molecule over larger solid tumors and potentially increasing efficacy.

In certain embodiments, the antibodies of the present disclosure may take the form of a Nanobody®. Nanobodies® are encoded by single genes and are efficiently produced in almost all prokaryotic and eukaryotic hosts *e.g.* *E. coli* (see *e.g.* U.S. Pat. No. 6,765,087), molds (for example *Aspergillus* or *Trichoderma*) and yeast (for example *Saccharomyces*, *Kluyvermyces*, *Hansenula* or *Pichia* (see *e.g.* U.S. Pat. No. 6,838,254). The production process is scalable and multi-kilogram quantities of Nanobodies® have been produced. Nanobodies may be formulated as a ready-to-use solution having a long shelf life. The Nanoclone® method (see, *e.g.*, WO 06/079372) is a proprietary method for generating Nanobodies against a desired target, based on automated high-throughput selection of B-cells.

In certain embodiments, the anti- TNFR2 antibodies or antigen-binding fragments thereof as disclosed herein are humanized. This refers to a chimeric molecule, generally prepared using recombinant techniques, having an antigen-binding site derived from an immunoglobulin from a non-human species and the remaining immunoglobulin structure of the molecule based upon the structure

and/or sequence of a human immunoglobulin. The antigen-binding site may comprise either complete variable domains fused onto constant domains or only the CDRs grafted onto appropriate framework regions in the variable domains. Epitope binding sites may be wild type or modified by one or more amino acid substitutions. This eliminates the constant region as an immunogen in human individuals, but the possibility of an immune response to the foreign variable region remains (LoBuglio, A. F. *et al.*, (1989) *Proc Natl Acad Sci USA* 86:4220-4224; Queen *et al.*, *PNAS* (1988) 86:10029-10033; Riechmann *et al.*, *Nature* (1988) 332:323-327). Illustrative methods for humanization of the anti-TNFR2 antibodies disclosed herein include the methods described in U.S. Patent No. 7,462,697. Illustrative humanized antibodies according to certain embodiments comprise the humanized sequences provided in **Table H1** and **Table H2**.

Another approach focuses not only on providing human-derived constant regions, but modifying the variable regions as well so as to reshape them as closely as possible to human form. It is known that the variable regions of both heavy and light chains contain three complementarity-determining regions (CDRs) which vary in response to the epitopes in question and determine binding capability, flanked by four framework regions (FRs) which are relatively conserved in a given species and which putatively provide a scaffolding for the CDRs. When nonhuman antibodies are prepared with respect to a particular epitope, the variable regions can be “reshaped” or “humanized” by grafting CDRs derived from nonhuman antibody on the FRs present in the human antibody to be modified. Application of this approach to various antibodies has been reported by Sato, K., *et al.*, (1993) *Cancer Res* 53:851-856. Riechmann, L., *et al.*, (1988) *Nature* 332:323-327; Verhoeyen, M., *et al.*, (1988) *Science* 239:1534-1536; Kettleborough, C. A., *et al.*, (1991) *Protein Engineering* 4:773-3783; Maeda, H., *et al.*, (1991) *Human Antibodies Hybridoma* 2:124-134; Gorman, S. D., *et al.*, (1991) *Proc Natl Acad Sci USA* 88:4181-4185; Tempest, P. R., *et al.*, (1991) *Bio/Technology* 9:266-271; Co, M. S., *et al.*, (1991) *Proc Natl Acad Sci USA* 88:2869-2873; Carter, P., *et al.*, (1992) *Proc Natl Acad Sci USA* 89:4285-4289; and Co, M. S. *et al.*, (1992) *J Immunol* 148:1149-1154. In some embodiments, humanized antibodies preserve all CDR sequences (for example, a humanized rabbit antibody which contains all six CDRs from the rabbit antibody). In some embodiments, humanized antibodies have one or more CDRs (one, two, three, four, five, six) which are altered with respect to the original antibody, which are also termed one or more CDRs “derived from” one or more CDRs from the original antibody.

In certain embodiments, the antibodies of the present disclosure may be chimeric antibodies. In this regard, a chimeric antibody is comprised of an antigen-binding fragment of an anti-TNFR2 antibody operably linked or otherwise fused to a heterologous Fc portion of a different antibody. In certain embodiments, the heterologous Fc domain is of human origin. In some embodiments, the heterologous Fc domain may be from a different Ig class from the parent antibody, including IgA (including subclasses IgA1 and IgA2), IgD, IgE, IgG (including subclasses IgG1, IgG2, IgG3, and IgG4), and IgM. In further embodiments, the heterologous Fc domain may be comprised of CH2 and

CH3 domains from one or more of the different Ig classes. As noted above with regard to humanized antibodies, the anti- TNFR2 antigen-binding fragment of a humanized antibody may comprise only one or more of the CDRs of the antibodies described herein (e.g., 1, 2, 3, 4, 5, or 6 CDRs of the antibodies described herein), or may comprise an entire variable domain (VL, VH or both).

In certain embodiments, a TNFR2-binding antibody comprises one or more of the CDRs of the antibodies described herein. In this regard, it has been shown in some cases that the transfer of only the VHCDR3 of an antibody can be performed while still retaining desired specific binding (Barbas *et al.*, *PNAS* (1995) 92: 2529-2533). See also, McLane *et al.*, *PNAS* (1995) 92:5214-5218, Barbas *et al.*, *J. Am. Chem. Soc.* (1994) 116:2161-2162.

Marks *et al.* (*Bio/Technology*, 1992, 10:779-783) describe methods of producing repertoires of antibody variable domains in which consensus primers directed at or adjacent to the 5' end of the variable domain area are used in conjunction with consensus primers to the third framework region of human VH genes to provide a repertoire of VH variable domains lacking a CDR3. Marks *et al.* further describe how this repertoire may be combined with a CDR3 of a particular antibody. Using analogous techniques, the CDR3-derived sequences of the presently described antibodies may be shuffled with repertoires of VH or VL domains lacking a CDR3, and the shuffled complete VH or VL domains combined with a cognate VL or VH domain to provide an antibody or antigen-binding fragment thereof that binds TNFR2. The repertoire may then be displayed in a suitable host system such as the phage display system of WO 92/01047 so that suitable antibodies or antigen-binding fragments thereof may be selected. A repertoire may consist of at least from about 10^4 individual members and upwards by several orders of magnitude, for example, to about from 10^6 to 10^8 or 10^{10} or more members. Analogous shuffling or combinatorial techniques are also disclosed by Stemmer (*Nature*, 1994, 370:389-391), who describes the technique in relation to a β -lactamase gene but observes that the approach may be used for the generation of antibodies.

A further alternative is to generate novel VH or VL regions carrying one or more CDR-derived sequences described herein using random mutagenesis of one or more selected VH and/or VL genes to generate mutations within the entire variable domain. Such a technique is described by Gram *et al.* (1992, *Proc. Natl. Acad. Sci., USA*, 89:3576-3580), who used error-prone PCR. Another method which may be used is to direct mutagenesis to CDR regions of VH or VL genes. Such techniques are disclosed by Barbas *et al.*, (1994, *Proc. Natl. Acad. Sci., USA*, 91:3809-3813) and Schier *et al.* (1996, *J. Mol. Biol.* 263:551-567).

In certain embodiments, a specific VH and/or VL of the antibodies described herein may be used to screen a library of the complementary variable domain to identify antibodies with desirable properties, such as increased affinity for TNFR2. Such methods are described, for example, in Portolano *et al.*, *J. Immunol.* (1993) 150:880-887; Clarkson *et al.*, *Nature* (1991) 352:624-628.

Other methods may also be used to mix and match CDRs to identify antibodies having desired binding activity, such as binding to TNFR2. For example: Klimka *et al.*, *British Journal of*

Cancer (2000) 83: 252-260, describe a screening process using a mouse VL and a human VH library with CDR3 and FR4 retained from the mouse VH. After obtaining antibodies, the VH was screened against a human VL library to obtain antibodies that bound antigen. Beiboer *et al.*, *J. Mol. Biol.* (2000) 296:833-849 describe a screening process using an entire mouse heavy chain and a human light chain library. After obtaining antibodies, one VL was combined with a human VH library with the CDR3 of the mouse retained. Antibodies capable of binding antigen were obtained. Rader *et al.*, *PNAS* (1998) 95:8910-8915 describe a process similar to Beiboer et al above.

These just-described techniques are, in and of themselves, known as such in the art. The skilled person will, however, be able to use such techniques to obtain antibodies or antigen-binding fragments thereof according to several embodiments described herein, using routine methodology in the art.

Also disclosed herein is a method for obtaining an antibody antigen binding domain specific for a TNFR2 antigen, the method comprising providing by way of addition, deletion, substitution or insertion of one or more amino acids in the amino acid sequence of a VH domain set out herein a VH domain which is an amino acid sequence variant of the VH domain, optionally combining the VH domain thus provided with one or more VL domains, and testing the VH domain or VH/VL combination or combinations to identify a specific binding member or an antibody antigen binding domain specific for TNFR2 and optionally with one or more desired properties. The VL domains may have an amino acid sequence which is substantially as set out herein. An analogous method may be employed in which one or more sequence variants of a VL domain disclosed herein are combined with one or more VH domains.

An epitope that “specifically binds” or “preferentially binds” (used interchangeably herein) to an antibody or a polypeptide is a term well understood in the art, and methods to determine such specific or preferential binding are also well known in the art. A molecule is said to exhibit “specific binding” or “preferential binding” if it reacts or associates more frequently, more rapidly, with greater duration and/or with greater affinity with a particular cell or substance than it does with alternative cells or substances. An antibody “specifically binds” or “preferentially binds” to a target if it binds with greater affinity, avidity, more readily, and/or with greater duration than it binds to other substances. For example, an antibody that specifically or preferentially binds to a TNFR2 epitope is an antibody that binds one TNFR2 epitope with greater affinity, avidity, more readily, and/or with greater duration than it binds to other TNFR2 epitopes or non- TNFR2 epitopes. It is also understood by reading this definition that, for example, an antibody (or moiety or epitope) that specifically or preferentially binds to a first target may or may not specifically or preferentially bind to a second target. As such, “specific binding” or “preferential binding” does not necessarily require (although it can include) exclusive binding. Generally, but not necessarily, reference to binding means preferential binding.

Immunological binding generally refers to the non-covalent interactions of the type which occur between an immunoglobulin molecule and an antigen for which the immunoglobulin is specific, for example by way of illustration and not limitation, as a result of electrostatic, ionic, hydrophilic and/or hydrophobic attractions or repulsion, steric forces, hydrogen bonding, van der Waals forces, and other interactions. The strength, or affinity of immunological binding interactions can be expressed in terms of the dissociation constant (K_d) of the interaction, wherein a smaller K_d represents a greater affinity. Immunological binding properties of selected polypeptides can be quantified using methods well known in the art. One such method entails measuring the rates of antigen-binding site/antigen complex formation and dissociation, wherein those rates depend on the concentrations of the complex partners, the affinity of the interaction, and on geometric parameters that equally influence the rate in both directions. Thus, both the “on rate constant” (K_{on}) and the “off rate constant” (K_{off}) can be determined by calculation of the concentrations and the actual rates of association and dissociation. The ratio of K_{off}/K_{on} enables cancellation of all parameters not related to affinity, and is thus equal to the dissociation constant K_d . See, generally, Davies *et al.* (1990) *Annual Rev. Biochem.* 59:439-473.

The term “immunologically active”, with reference to an epitope being or “remaining immunologically active”, refers to the ability of an anti-TNFR2 antibody to bind to the epitope under different conditions, for example, after the epitope has been subjected to reducing and denaturing conditions.

An antibody or antigen-binding fragment thereof according to some embodiments may be one that competes for binding to TNFR2 with any antibody described herein which both (i) specifically binds to the antigen and (ii) comprises a VH and/or VL domain disclosed herein, or comprises a VH CDR3 disclosed herein, or a variant of any of these. Competition between antibodies may be assayed easily *in vitro*, for example using ELISA and/or by tagging a specific reporter molecule to one antibody which can be detected in the presence of other untagged antibodies, to enable identification of specific antibodies which bind the same epitope or an overlapping epitope. Thus, there is provided herein a specific antibody or antigen-binding fragment thereof, comprising a human antibody antigen-binding site which competes with an antibody described herein that binds to TNFR2.

In this regard, as used herein, the terms “competes with”, “inhibits binding” and “blocks binding” (*e.g.*, referring to inhibition/blocking of binding of a ligand (*e.g.*, TNF- α) and/or counter-receptor to TNFR2, or referring to inhibition/blocking of binding of an anti-TNFR2 antibody to TNFR2) are used interchangeably and encompass both partial and complete inhibition/blocking. The inhibition/blocking of a ligand and/or counter-receptor to TNFR2 preferably reduces or alters the normal level or type of cell signaling that occurs when a ligand and/or counter-receptor binds to TNFR2 without inhibition or blocking. Inhibition and blocking are also intended to include any measurable decrease in the binding of a ligand and/or counter-receptor to TNFR2 when in contact with an anti-TNFR2 antibody as disclosed herein as compared to the ligand not in contact with an

anti-TNFR2 antibody, *e.g.*, the blocking of a ligand (*e.g.*, TNF- α) and/or counter-receptor to TNFR2 by at least about 10%, 20%, 30%, 40%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%.

The constant regions of immunoglobulins show less sequence diversity than the variable regions, and are responsible for binding a number of natural proteins to elicit important biochemical events. In humans there are five different classes of antibodies including IgA (which includes subclasses IgA1 and IgA2), IgD, IgE, IgG (which includes subclasses IgG1, IgG2, IgG3, and IgG4), and IgM. The distinguishing features between these antibody classes are their constant regions, although subtler differences may exist in the V region.

The Fc region of an antibody interacts with a number of Fc receptors and ligands, imparting an array of important functional capabilities referred to as effector functions. In one embodiment, an anti-TNFR2 antibody comprises an Fc region. For IgG the Fc region comprises Ig domains CH2 and CH3 and the N-terminal hinge leading into CH2. An important family of Fc receptors for the IgG class are the Fc gamma receptors (Fc γ Rs). These receptors mediate communication between antibodies and the cellular arm of the immune system (Raghavan *et al.*, 1996, *Annu Rev Cell Dev Biol* 12:181-220; Ravetch *et al.*, 2001, *Annu Rev Immunol* 19:275-290). In humans this protein family includes Fc γ RI (CD64), including isoforms Fc γ RIa, Fc γ RIb, and Fc γ RIc; Fc γ RII (CD32), including isoforms Fc γ RIIa (including allotypes H131 and R131), Fc γ RIIb (including Fc γ RIIb-1 and Fc γ RIIb-2), and Fc γ RIIc; and Fc γ RIII (CD16), including isoforms Fc γ RIIIa (including allotypes V158 and F158) and Fc γ RIIIb (including allotypes Fc γ RIIIb-NA1 and Fc γ RIIIb-NA2) (Jefferis *et al.*, 2002, *Immunol Lett* 82:57-65). These receptors typically have an extracellular domain that mediates binding to Fc, a membrane spanning region, and an intracellular domain that may mediate some signaling event within the cell. These receptors are expressed in a variety of immune cells including monocytes, macrophages, neutrophils, dendritic cells, eosinophils, mast cells, platelets, B cells, large granular lymphocytes, Langerhans' cells, natural killer (NK) cells, and T cells. Formation of the Fc/Fc γ R complex recruits these effector cells to sites of bound antigen, typically resulting in signaling events within the cells and important subsequent immune responses such as release of inflammation mediators, B cell activation, endocytosis, phagocytosis, and cytotoxic attack.

The ability to mediate cytotoxic and phagocytic effector functions is a potential mechanism by which antibodies destroy targeted cells. The cell-mediated reaction wherein nonspecific cytotoxic cells that express Fc γ Rs recognize bound antibody on a target cell and subsequently cause lysis of the target cell is referred to as antibody dependent cell-mediated cytotoxicity (ADCC) (Raghavan *et al.*, 1996, *Annu Rev Cell Dev Biol* 12:181-220; Ghetie *et al.*, 2000, *Annu Rev Immunol* 18:739-766; Ravetch *et al.*, 2001, *Annu Rev Immunol* 19:275-290). The cell-mediated reaction wherein nonspecific cytotoxic cells that express Fc γ Rs recognize bound antibody on a target cell and subsequently cause phagocytosis of the target cell is referred to as antibody dependent cell-mediated phagocytosis (ADCP). All Fc γ Rs bind the same region on Fc, at the N-terminal end of the C γ 2 (CH2)

domain and the preceding hinge. This interaction is well characterized structurally (Sondermann *et al.*, 2001, J Mol Biol 309:737-749), and several structures of the human Fc bound to the extracellular domain of human FcγRIIIb have been solved (pdb accession code 1E4K) (Sondermann *et al.*, 2000, Nature 406:267-273.) (pdb accession codes 1IIS and 1IIX) (Radaev *et al.*, 2001, J Biol Chem 276:16469-16477.)

The different IgG subclasses have different affinities for the FcγRs, with IgG1 and IgG3 typically binding substantially better to the receptors than IgG2 and IgG4 (Jefferis *et al.*, 2002, Immunol Lett 82:57-65). All FcγRs bind the same region on IgG Fc, yet with different affinities: the high affinity binder FcγRI has a K_d for IgG1 of 10^{-8} M^{-1} , whereas the low affinity receptors FcγRII and FcγRIII generally bind at 10^{-6} and 10^{-5} respectively. The extracellular domains of FcγRIIIa and FcγRIIIb are 96% identical; however, FcγRIIIb does not have an intracellular signaling domain. Furthermore, whereas FcγRI, FcγRIIa/c, and FcγRIIIa are positive regulators of immune complex-triggered activation, characterized by having an intracellular domain that has an immunoreceptor tyrosine-based activation motif (ITAM), FcγRIIb has an immunoreceptor tyrosine-based inhibition motif (ITIM) and is therefore inhibitory. Thus the former are referred to as activation receptors, and FcγRIIb is referred to as an inhibitory receptor. The receptors also differ in expression pattern and levels on different immune cells. Yet another level of complexity is the existence of a number of FcγR polymorphisms in the human proteome. A particularly relevant polymorphism with clinical significance is V158/F158 FcγRIIIa. Human IgG1 binds with greater affinity to the V158 allotype than to the F158 allotype. This difference in affinity, and presumably its effect on ADCC and/or ADCP, has been shown to be a significant determinant of the efficacy of the anti-CD20 antibody rituximab (Rituxan®, a registered trademark of IDEC Pharmaceuticals Corporation). Patients with the V158 allotype respond favorably to rituximab treatment; however, patients with the lower affinity F158 allotype respond poorly (Cartron *et al.*, 2002, Blood 99:754-758). Approximately 10-20% of humans are V158/V158 homozygous, 45% are V158/F158 heterozygous, and 35-45% of humans are F158/F158 homozygous (Lehrnbecher *et al.*, 1999, Blood 94:4220-4232; Cartron *et al.*, 2002, Blood 99:754-758). Thus 80-90% of humans are poor responders, that is, they have at least one allele of the F158 FcγRIIIa.

The Fc region is also involved in activation of the complement cascade. In the classical complement pathway, C1 binds with its C1q subunits to Fc fragments of IgG or IgM, which has formed a complex with antigen(s). In certain embodiments, modifications to the Fc region comprise modifications that alter (either enhance or decrease) the ability of a TNFR2-specific antibody as described herein to activate the complement system (see *e.g.*, U.S. Patent 7,740,847). To assess complement activation, a complement-dependent cytotoxicity (CDC) assay may be performed (See, *e.g.*, Gazzano-Santoro *et al.*, J. Immunol. Methods, 202:163 (1996)).

Thus in certain embodiments, the present disclosure provides anti-TNFR2 antibodies having a modified Fc region with altered functional properties, such as reduced or enhanced CDC, ADCC, or

ADCP activity, or enhanced binding affinity for a specific FcγR or increased serum half-life. Other modified Fc regions contemplated herein are described, for example, in issued U.S. Patents 7,317,091; 7,657,380; 7,662,925; 6,538,124; 6,528,624; 7,297,775; 7,364,731; Published U.S. Applications US2009092599; US20080131435; US20080138344; and published International Applications WO2006/105338; WO2004/063351; WO2006/088494; WO2007/024249.

Thus, in certain embodiments, antibody variable domains with the desired binding specificities are fused to immunoglobulin constant domain sequences. In certain embodiments, the fusion is with an Ig heavy chain constant domain, comprising at least part of the hinge, C_H2, and C_H3 regions. In some instances, it is preferred to have the first heavy-chain constant region (C_H1) containing the site necessary for light chain bonding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host cell. This provides for greater flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yield of the desired bispecific antibody. It is, however, possible to insert the coding sequences for two or all three polypeptide chains into a single expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios have no significant effect on the yield of the desired chain combination.

Antibodies of the present disclosure (and antigen-binding fragments and variants thereof) may also be modified to include an epitope tag or label, *e.g.*, for use in purification or diagnostic applications. There are many linking groups known in the art for making antibody conjugates, including, for example, those disclosed in U.S. Pat. No. 5,208,020 or EP Patent 0 425 235 B1, and Chari *et al.*, Cancer Research 52: 127-131 (1992). The linking groups include disulfide groups, thioether groups, acid labile groups, photolabile groups, peptidase labile groups, or esterase labile groups, as disclosed in the above-identified patents, disulfide and thioether groups being preferred.

In some embodiments, a TNFR2-specific antibody as described herein may be conjugated or operably linked to another agent or therapeutic compound, referred to herein as a conjugate. The agent or therapeutic compound may be a polypeptide agent, a polynucleotide agent, cytotoxic agent, a chemotherapeutic agent, a cytokine, an anti-angiogenic agent, a tyrosine kinase inhibitor, a toxin, a radioisotope, or other therapeutically active agent. Chemotherapeutic agents, cytokines, anti-angiogenic agents, tyrosine kinase inhibitors, and other therapeutic agents have been described herein, and all of these aforementioned therapeutic agents may find use as antibody conjugates. Such conjugates can be used, for example, to target the agent or compound to a site of action, for example, a tumor or tumor microenvironment characterized by the expression of TNFR2.

In some embodiments, the antibody is conjugated or operably linked to a toxin, including but not limited to small molecule toxins, polypeptides, nucleic acids, and enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof. Small molecule

toxins include but are not limited to saporin (Kuroda K, *et al.*, The Prostate 70:1286-1294 (2010); Lip, WL. *et al.*, 2007 Molecular Pharmaceutics 4:241-251; Quadros EV., *et al.*, 2010 Mol Cancer Ther; 9(11); 3033-40; Polito L., *et al.* 2009 British Journal of Haematology, 147, 710-718), calicheamicin, maytansine (U.S. Pat. No. 5,208,020), trichothene, and CC1065. Toxins include but are not limited to RNase, gelonin, enediynes, ricin, abrin, diphtheria toxin, cholera toxin, gelonin, *Pseudomonas* exotoxin (PE40), *Shigella* toxin, *Clostridium perfringens* toxin, and pokeweed antiviral protein.

In some embodiments, an antibody or antigen-binding fragment thereof is conjugated to one or more maytansinoid molecules. Maytansinoids are mitotic inhibitors that act by inhibiting tubulin polymerization. Maytansine was first isolated from the east African shrub *Maytenus serrata* (U.S. Pat. No. 3,896,111). Subsequently, it was discovered that certain microbes also produce maytansinoids, such as maytansinol and C-3 maytansinol esters (U.S. Pat. No. 4,151,042). Synthetic maytansinol and derivatives and analogues thereof are disclosed, for example, in U.S. Pat. Nos. 4,137,230; 4,248,870; 4,256,746; 4,260,608; 4,265,814; 4,294,757; 4,307,016; 4,308,268; 4,308,269; 4,309,428; 4,313,946; 4,315,929; 4,317,821; 4,322,348; 4,331,598; 4,361,650; 4,364,866; 4,424,219; 4,450,254; 4,362,663; and 4,371,533. Immunoconjugates containing maytansinoids and their therapeutic use are disclosed, for example, in U.S. Pat. Nos. 5,208,020, 5,416,064 and European Patent EP 0 425 235 B1. Liu *et al.*, Proc. Natl. Acad. Sci. USA 93:8618-8623 (1996) described immunoconjugates comprising a maytansinoid designated DM1 linked to the monoclonal antibody C242 directed against human colorectal cancer. The conjugate was found to be highly cytotoxic towards cultured colon cancer cells, and showed antitumor activity in an in vivo tumor growth assay.

Antibody-maytansinoid conjugates are prepared by chemically linking an antibody to a maytansinoid molecule without significantly diminishing the biological activity of either the antibody or the maytansinoid molecule. An average of 3-4 maytansinoid molecules conjugated per antibody molecule has shown efficacy in enhancing cytotoxicity of target cells without negatively affecting the function or solubility of the antibody, although even one molecule of toxin/antibody would be expected to enhance cytotoxicity over the use of naked antibody. Maytansinoids are well known in the art and can be synthesized by known techniques or isolated from natural sources. Suitable maytansinoids are disclosed, for example, in U.S. Pat. No. 5,208,020 and in the other patents and non-patent publications referred to hereinabove. Preferred maytansinoids are maytansinol and maytansinol analogues modified in the aromatic ring or at other positions of the maytansinol molecule, such as various maytansinol esters.

Another conjugate of interest comprises an antibody conjugated to one or more calicheamicin molecules. The calicheamicin family of antibiotics is capable of producing double-stranded DNA breaks at sub-picomolar concentrations. Structural analogues of calicheamicin that may also be used (Hinman *et al.*, 1993, Cancer Research 53:3336-3342; Lode *et al.*, 1998, Cancer Research 58:2925-2928) (U.S. Pat. No. 5,714,586; U.S. Pat. No. 5,712,374; U.S. Pat. No. 5,264,586; U.S. Pat. No. 5,773,001). Dolastatin 10 analogs such as auristatin E (AE) and monomethylauristatin E (MMAE)

may find use as conjugates for the presently disclosed antibodies, or variants thereof (Doronina *et al.*, 2003, Nat Biotechnol 21(7):778-84; Francisco *et al.*, 2003 Blood 102(4):1458-65). Useful enzymatically active toxins include but are not limited to diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, *Phytolacca americana* proteins (PAPI, PAPII, and PAP-S), *momordica charantia* inhibitor, curcumin, crotonin, *sapaonaria officinalis* inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin and the tricothecenes. See, for example, PCT WO 93/21232. The present disclosure further contemplates embodiments in which a conjugate or fusion is formed between a TNFR2-specific antibody as described herein and a compound with nucleolytic activity, for example a ribonuclease or DNA endonuclease such as a deoxyribonuclease (DNase).

In some embodiments, a herein-disclosed antibody may be conjugated or operably linked to a radioisotope to form a radioconjugate. A variety of radioactive isotopes are available for the production of radioconjugate antibodies. Examples include, but are not limited to ^{90}Y , ^{123}I , ^{125}I , ^{131}I , ^{186}Re , ^{188}Re , ^{211}At , and ^{212}Bi .

Antibodies described herein may in certain embodiments be conjugated to a therapeutic moiety such as a cytotoxin (*e.g.*, a cytostatic or cytotoxic agent), a therapeutic agent or a radioactive element (*e.g.*, alpha-emitters, gamma-emitters, etc.). Cytotoxins or cytotoxic agents include any agent that is detrimental to cells. Examples include paclitaxel/paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, teniposide, vincristine, vinblastine, colchicine, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. One exemplary cytotoxin is saporin (available from Advanced Targeting Systems, San Diego, CA). Therapeutic agents include, but are not limited to, antimetabolites (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.*, mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.*, daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.*, dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC), and anti-mitotic agents (*e.g.*, vincristine and vinblastine).

Moreover, a TNFR2-specific antibody (including a functional fragment thereof as provided herein such as an antigen-binding fragment) may in certain embodiments be conjugated to therapeutic moieties such as a radioactive material or macrocyclic chelators useful for conjugating radiometal ions. In certain embodiments, the macrocyclic chelator is 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA) which can be attached to the antibody via a linker molecule. Such linker molecules are commonly known in the art and described in Denardo *et al.*, 1998, Clin

Cancer Res. 4:2483-90; Peterson *et al.*, 1999, Bioconjug. Chem. 10:553; and Zimmerman *et al.*, 1999, Nucl. Med. Biol. 26:943-50.

In some embodiments, an antibody may be conjugated to a “receptor” (such as streptavidin) for utilization in tumor pretargeting wherein the antibody-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a “ligand” (*e.g.* avidin) which is conjugated to a cytotoxic agent (*e.g.* a radionucleotide). In some embodiments, the antibody is conjugated or operably linked to an enzyme in order to employ Antibody Dependent Enzyme Mediated Prodrug Therapy (ADEPT). ADEPT may be used by conjugating or operably linking the antibody to a prodrug-activating enzyme that converts a prodrug (*e.g.* a peptidyl chemotherapeutic agent, see PCT WO 81/01145) to an active anti-cancer drug. See, for example, PCT WO 88/07378 and U.S. Pat. No. 4,975,278. The enzyme component of the immunoconjugate useful for ADEPT includes any enzyme capable of acting on a prodrug in such a way so as to convert it into its more active, cytotoxic form. Enzymes that are useful in the method of these and related embodiments include but are not limited to alkaline phosphatase useful for converting phosphate-containing prodrugs into free drugs; arylsulfatase useful for converting sulfate-containing prodrugs into free drugs; cytosine deaminase useful for converting non-toxic 5-fluorocytosine into the anti-cancer drug, 5-fluorouracil; proteases, such as serratia protease, thermolysin, subtilisin, carboxypeptidases and cathepsins (such as cathepsins B and L), that are useful for converting peptide-containing prodrugs into free drugs; D-alanylcarboxypeptidases, useful for converting prodrugs that contain D-amino acid substituents; carbohydrate-cleaving enzymes such as beta-galactosidase and neuraminidase useful for converting glycosylated prodrugs into free drugs; beta-lactamase useful for converting drugs derivatized with beta-lactams into free drugs; and penicillin amidases, such as penicillin V amidase or penicillin G amidase, useful for converting drugs derivatized at their amine nitrogens with phenoxyacetyl or phenylacetyl groups, respectively, into free drugs. Alternatively, antibodies with enzymatic activity, also known in the art as “abzymes”, may be used to convert prodrugs into free active drugs (see, for example, Massey, 1987, Nature 328: 457-458). Antibody-abzyme conjugates can be prepared for delivery of the abzyme to a tumor cell population.

Immunoconjugates may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP), succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate, iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl)hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). Particular coupling agents include N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP) (Carlsson *et al.*, Biochem. J. 173:723-737 [1978])

and N-succinimidyl-4-(2-pyridylthio)pentanoate (SPP) to provide for a disulfide linkage. The linker may be a “cleavable linker” facilitating release of one or more cleavable components. For example, an acid-labile linker may be used (Cancer Research 52: 127-131 (1992); U.S. Pat. No. 5,208,020).

Other modifications of the antibodies (and polypeptides) of the disclosure are also contemplated herein. For example, the antibody may be linked to one of a variety of nonproteinaceous polymers, *e.g.*, polyethylene glycol, polypropylene glycol, polyoxyalkylenes, or copolymers of polyethylene glycol and polypropylene glycol. The antibody also may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization (for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate)microcapsules, respectively), in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules), or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences, 16th edition, Oslo, A., Ed., (1980).

“Carriers” as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers that are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as polysorbate 20 (TWEEN™), polyethylene glycol (PEG), and poloxamers (PLURONICS™), and the like.

The desired functional properties of anti-TNFR2 antibodies may be assessed using a variety of methods known to the skilled person affinity/binding assays (for example, surface plasmon resonance, competitive inhibition assays); cytotoxicity assays, cell viability assays, cell proliferation or differentiation assays, cancer cell and/or tumor growth inhibition using *in vitro* or *in vivo* models. Other assays may test the ability of antibodies described herein to block normal TNFR2-mediated responses. The antibodies described herein may also be tested for *in vitro* and *in vivo* efficacy. Such assays may be performed using well-established protocols known to the skilled person (see *e.g.*, Current Protocols in Molecular Biology (Greene Publ. Assoc. Inc. & John Wiley & Sons, Inc., NY, NY); Current Protocols in Immunology (Edited by: John E. Coligan, Ada M. Kruisbeek, David H. Margulies, Ethan M. Shevach, Warren Strober 2001 John Wiley & Sons, NY, NY); or commercially available kits.

The present disclosure further provides in certain embodiments an isolated nucleic acid encoding an antibody or antigen-binding fragment thereof as described herein, for instance, a nucleic acid which codes for a CDR or VH or VL domain as described herein. Nucleic acids include DNA

and RNA. These and related embodiments may include polynucleotides encoding antibodies that bind TNFR2 as described herein. The term “isolated polynucleotide” as used herein shall mean a polynucleotide of genomic, cDNA, or synthetic origin or some combination thereof, which by virtue of its origin the isolated polynucleotide (1) is not associated with all or a portion of a polynucleotide in which the isolated polynucleotide is found in nature, (2) is linked to a polynucleotide to which it is not linked in nature, or (3) does not occur in nature as part of a larger sequence.

The term “operably linked” means that the components to which the term is applied are in a relationship that allows them to carry out their inherent functions under suitable conditions. For example, a transcription control sequence “operably linked” to a protein coding sequence is ligated thereto so that expression of the protein coding sequence is achieved under conditions compatible with the transcriptional activity of the control sequences.

The term “control sequence” as used herein refers to polynucleotide sequences that can affect expression, processing or intracellular localization of coding sequences to which they are ligated or operably linked. The nature of such control sequences may depend upon the host organism. In particular embodiments, transcription control sequences for prokaryotes may include a promoter, ribosomal binding site, and transcription termination sequence. In other particular embodiments, transcription control sequences for eukaryotes may include promoters comprising one or a plurality of recognition sites for transcription factors, transcription enhancer sequences, transcription termination sequences and polyadenylation sequences. In certain embodiments, “control sequences” can include leader sequences and/or fusion partner sequences.

The term “polynucleotide” as referred to herein means single-stranded or double-stranded nucleic acid polymers. In certain embodiments, the nucleotides comprising the polynucleotide can be ribonucleotides or deoxyribonucleotides or a modified form of either type of nucleotide. Said modifications include base modifications such as bromouridine, ribose modifications such as arabinoside and 2',3'-dideoxyribose and internucleotide linkage modifications such as phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoraniladate and phosphoroamidate. The term “polynucleotide” specifically includes single and double stranded forms of DNA.

The term “naturally occurring nucleotides” includes deoxyribonucleotides and ribonucleotides. The term “modified nucleotides” includes nucleotides with modified or substituted sugar groups and the like. The term “oligonucleotide linkages” includes oligonucleotide linkages such as phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoraniladate, phosphoroamidate, and the like. See, *e.g.*, LaPlanche *et al.*, 1986, *Nucl. Acids Res.*, 14:9081; Stec *et al.*, 1984, *J. Am. Chem. Soc.*, 106:6077; Stein *et al.*, 1988, *Nucl. Acids Res.*, 16:3209; Zon *et al.*, 1991, *Anti-Cancer Drug Design*, 6:539; Zon *et al.*, 1991, *OLIGONUCLEOTIDES AND ANALOGUES: A PRACTICAL APPROACH*, pp. 87-108 (F. Eckstein, Ed.), Oxford University Press, Oxford England; Stec *et al.*, U.S. Pat. No. 5,151,510;

Uhlmann and Peyman, 1990, Chemical Reviews, 90:543, the disclosures of which are hereby incorporated by reference for any purpose. An oligonucleotide can include a detectable label to enable detection of the oligonucleotide or hybridization thereof.

The term “vector” is used to refer to any molecule (*e.g.*, nucleic acid, plasmid, or virus) used to transfer coding information to a host cell. The term “expression vector” refers to a vector that is suitable for transformation of a host cell and contains nucleic acid sequences that direct and/or control expression of inserted heterologous nucleic acid sequences. Expression includes, but is not limited to, processes such as transcription, translation, and RNA splicing, if introns are present.

As will be understood by those skilled in the art, polynucleotides may include genomic sequences, extra-genomic and plasmid-encoded sequences and smaller engineered gene segments that express, or may be adapted to express, proteins, polypeptides, peptides and the like. Such segments may be naturally isolated, or modified synthetically by the skilled person.

As will be also recognized by the skilled artisan, polynucleotides may be single-stranded (coding or antisense) or double-stranded, and may be DNA (genomic, cDNA or synthetic) or RNA molecules. RNA molecules may include HnRNA molecules, which contain introns and correspond to a DNA molecule in a one-to-one manner, and mRNA molecules, which do not contain introns. Additional coding or non-coding sequences may, but need not, be present within a polynucleotide according to the present disclosure, and a polynucleotide may, but need not, be linked to other molecules and/or support materials. Polynucleotides may comprise a native sequence or may comprise a sequence that encodes a variant or derivative of such a sequence.

Therefore, according to these and related embodiments, the present disclosure also provides polynucleotides encoding the anti- TNFR2 antibodies described herein. In certain embodiments, polynucleotides are provided that comprise some or all of a polynucleotide sequence encoding an antibody as described herein and complements of such polynucleotides.

In other related embodiments, polynucleotide variants may have substantial identity to a polynucleotide sequence encoding an anti- TNFR2 antibody described herein. For example, a polynucleotide may be a polynucleotide comprising at least 70% sequence identity, preferably at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% or higher, sequence identity compared to a reference polynucleotide sequence such as a sequence encoding an antibody described herein, using the methods described herein, (*e.g.*, BLAST analysis using standard parameters, as described below). One skilled in this art will recognize that these values can be appropriately adjusted to determine corresponding identity of proteins encoded by two nucleotide sequences by taking into account codon degeneracy, amino acid similarity, reading frame positioning and the like.

Typically, polynucleotide variants will contain one or more substitutions, additions, deletions and/or insertions, preferably such that the binding affinity of the antibody encoded by the variant polynucleotide is not substantially diminished relative to an antibody encoded by a polynucleotide sequence specifically set forth herein.

In certain embodiments, polynucleotide fragments may comprise or consist essentially of various lengths of contiguous stretches of sequence identical to or complementary to a sequence encoding an antibody as described herein. For example, polynucleotides are provided that comprise or consist essentially of at least about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 200, 300, 400, 500 or 1000 or more contiguous nucleotides of a sequence that encodes an antibody, or antigen-binding fragment thereof, disclosed herein as well as all intermediate lengths there between. It will be readily understood that “intermediate lengths”, in this context, means any length between the quoted values, such as 50, 51, 52, 53, *etc.*; 100, 101, 102, 103, *etc.*; 150, 151, 152, 153, *etc.*; including all integers through 200-500; 500-1,000, and the like. A polynucleotide sequence as described here may be extended at one or both ends by additional nucleotides not found in the native sequence. This additional sequence may consist of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides at either end of a polynucleotide encoding an antibody described herein or at both ends of a polynucleotide encoding an antibody described herein.

In some embodiments, polynucleotides are provided that are capable of hybridizing under moderate to high stringency conditions to a polynucleotide sequence encoding an antibody, or antigen-binding fragment thereof, provided herein, or a fragment thereof, or a complementary sequence thereof. Hybridization techniques are well known in the art of molecular biology. For purposes of illustration, suitable moderately stringent conditions for testing the hybridization of a polynucleotide as provided herein with other polynucleotides include prewashing in a solution of 5 X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0); hybridizing at 50°C-60°C, 5 X SSC, overnight; followed by washing twice at 65°C for 20 minutes with each of 2X, 0.5X and 0.2X SSC containing 0.1% SDS. One skilled in the art will understand that the stringency of hybridization can be readily manipulated, such as by altering the salt content of the hybridization solution and/or the temperature at which the hybridization is performed. For example, in some embodiments, suitable highly stringent hybridization conditions include those described above, with the exception that the temperature of hybridization is increased, *e.g.*, to 60°C-65°C or 65°C-70°C.

In certain embodiments, the polynucleotides described above, *e.g.*, polynucleotide variants, fragments and hybridizing sequences, encode antibodies that bind TNFR2, or antigen-binding fragments thereof. In certain embodiments, such polynucleotides encode antibodies or antigen-binding fragments, or CDRs thereof, that bind to TNFR2 at least about 50%, at least about 70%, and in certain embodiments, at least about 90% as well as an antibody sequence specifically set forth herein. In further embodiments, such polynucleotides encode antibodies or antigen-binding fragments, or CDRs thereof, that bind to TNFR2 with greater affinity than the antibodies set forth herein, for example, that bind quantitatively at least about 105%, 106%, 107%, 108%, 109%, or 110% as well as an antibody sequence specifically set forth herein.

As described elsewhere herein, determination of the three-dimensional structures of representative polypeptides (*e.g.*, variant TNFR2-specific antibodies as provided herein, for instance, an antibody protein having an antigen-binding fragment as provided herein) may be made through routine methodologies such that substitution, addition, deletion or insertion of one or more amino acids with selected natural or non-natural amino acids can be virtually modeled for purposes of determining whether a so derived structural variant retains the space-filling properties of presently disclosed species. A variety of computer programs are known to the skilled artisan for determining appropriate amino acid substitutions (or appropriate polynucleotides encoding the amino acid sequence) within an antibody such that, for example, affinity is maintained or better affinity is achieved.

The polynucleotides described herein, or fragments thereof, regardless of the length of the coding sequence itself, may be combined with other DNA sequences, such as promoters, polyadenylation signals, additional restriction enzyme sites, multiple cloning sites, other coding segments, and the like, such that their overall length may vary considerably. It is therefore contemplated that a nucleic acid fragment of almost any length may be employed, with the total length preferably being limited by the ease of preparation and use in the intended recombinant DNA protocol. For example, illustrative polynucleotide segments with total lengths of about 10,000, about 5000, about 3000, about 2,000, about 1,000, about 500, about 200, about 100, about 50 base pairs in length, and the like, (including all intermediate lengths) are contemplated to be useful.

When comparing polynucleotide sequences, two sequences are said to be “identical” if the sequence of nucleotides in the two sequences is the same when aligned for maximum correspondence, as described below. Comparisons between two sequences are typically performed by comparing the sequences over a comparison window to identify and compare local regions of sequence similarity. A “comparison window” as used herein, refers to a segment of at least about 20 contiguous positions, usually 30 to about 75, 40 to about 50, in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned.

Optimal alignment of sequences for comparison may be conducted using the Megalign™ program in the Lasergene® suite of bioinformatics software (DNASTAR, Inc., Madison, WI), using default parameters. This program embodies several alignment schemes described in the following references: Dayhoff, M.O. (1978) A model of evolutionary change in proteins – Matrices for detecting distant relationships. In Dayhoff, M.O. (ed.) *Atlas of Protein Sequence and Structure*, National Biomedical Research Foundation, Washington DC Vol. 5, Suppl. 3, pp. 345-358; Hein J., *Unified Approach to Alignment and Phylogenies*, pp. 626-645 (1990); *Methods in Enzymology* vol. 183, Academic Press, Inc., San Diego, CA; Higgins, D.G. and Sharp, P.M., *CABIOS* 5:151-153 (1989); Myers, E.W. and Muller W., *CABIOS* 4:11-17 (1988); Robinson, E.D., *Comb. Theor* 11:105 (1971); Santou, N. Nes, M., *Mol. Biol. Evol.* 4:406-425 (1987); Sneath, P.H.A. and Sokal, R.R., *Numerical*

Taxonomy – the Principles and Practice of Numerical Taxonomy, Freeman Press, San Francisco, CA (1973); Wilbur, W.J. and Lipman, D.J., *Proc. Natl. Acad. Sci. USA* 80:726-730 (1983).

Alternatively, optimal alignment of sequences for comparison may be conducted by the local identity algorithm of Smith and Waterman, *Add. APL. Math* 2:482 (1981), by the identity alignment algorithm of Needleman and Wunsch, *J. Mol. Biol.* 48:443 (1970), by the search for similarity methods of Pearson and Lipman, *Proc. Natl. Acad. Sci. USA* 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, BLAST, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, WI), or by inspection.

One example of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul *et al.*, *Nucl. Acids Res.* 25:3389-3402 (1977), and Altschul *et al.*, *J. Mol. Biol.* 215:403-410 (1990), respectively. BLAST and BLAST 2.0 can be used, for example with the parameters described herein, to determine percent sequence identity among two or more the polynucleotides. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. In one illustrative example, cumulative scores can be calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always >0) and N (penalty score for mismatching residues; always <0). Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a word length (W) of 11, and expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff and Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915 (1989)) alignments, (B) of 50, expectation (E) of 10, M=5, N=-4 and a comparison of both strands.

In certain embodiments, the “percentage of sequence identity” is determined by comparing two optimally aligned sequences over a window of comparison of at least 20 positions, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (*i.e.*, gaps) of 20 percent or less, usually 5 to 15 percent, or 10 to 12 percent, as compared to the reference sequences (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid bases occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the reference sequence (*i.e.*, the window size) and multiplying the results by 100 to yield the percentage of sequence identity.

It will be appreciated by those of ordinary skill in the art that, as a result of the degeneracy of the genetic code, there are many nucleotide sequences that encodes an antibody as described herein. Some of these polynucleotides bear minimal sequence identity to the nucleotide sequence of the

native or original polynucleotide sequence that encode antibodies that bind to TNFR2. Nonetheless, polynucleotides that vary due to differences in codon usage are expressly contemplated by the present disclosure. In certain embodiments, sequences that have been codon-optimized for mammalian expression are specifically contemplated.

In some embodiments, a mutagenesis approach, such as site-specific mutagenesis, may be employed for the preparation of variants and/or derivatives of the antibodies described herein. By this approach, specific modifications in a polypeptide sequence can be made through mutagenesis of the underlying polynucleotides that encode them. These techniques provides a straightforward approach to prepare and test sequence variants, for example, incorporating one or more of the foregoing considerations, by introducing one or more nucleotide sequence changes into the polynucleotide.

Site-specific mutagenesis allows the production of mutants through the use of specific oligonucleotide sequences which encode the DNA sequence of the desired mutation, as well as a sufficient number of adjacent nucleotides, to provide a primer sequence of sufficient size and sequence complexity to form a stable duplex on both sides of the deletion junction being traversed. Mutations may be employed in a selected polynucleotide sequence to improve, alter, decrease, modify, or otherwise change the properties of the polynucleotide itself, and/or alter the properties, activity, composition, stability, or primary sequence of the encoded polypeptide.

In certain embodiments, the inventors contemplate the mutagenesis of the polynucleotide sequences that encode an antibody disclosed herein, or an antigen-binding fragment thereof, to alter one or more properties of the encoded polypeptide, such as the binding affinity of the antibody or the antigen-binding fragment thereof, or the function of a particular Fc region, or the affinity of the Fc region for a particular FcγR. The techniques of site-specific mutagenesis are well-known in the art, and are widely used to create variants of both polypeptides and polynucleotides. For example, site-specific mutagenesis is often used to alter a specific portion of a DNA molecule. In such embodiments, a primer comprising typically about 14 to about 25 nucleotides or so in length is employed, with about 5 to about 10 residues on both sides of the junction of the sequence being altered.

As will be appreciated by those of skill in the art, site-specific mutagenesis techniques have often employed a phage vector that exists in both a single stranded and double stranded form. Typical vectors useful in site-directed mutagenesis include vectors such as the M13 phage. These phage are readily commercially-available and their use is generally well-known to those skilled in the art. Double-stranded plasmids are also routinely employed in site directed mutagenesis that eliminates the step of transferring the gene of interest from a plasmid to a phage.

In general, site-directed mutagenesis in accordance herewith is performed by first obtaining a single-stranded vector or melting apart of two strands of a double-stranded vector that includes within its sequence a DNA sequence that encodes the desired peptide. An oligonucleotide primer bearing the desired mutated sequence is prepared, generally synthetically. This primer is then annealed with the

single-stranded vector, and subjected to DNA polymerizing enzymes such as *E. coli* polymerase I Klenow fragment, in order to complete the synthesis of the mutation-bearing strand. Thus, a heteroduplex is formed wherein one strand encodes the original non-mutated sequence and the second strand bears the desired mutation. This heteroduplex vector is then used to transform appropriate cells, such as *E. coli* cells, and clones are selected which include recombinant vectors bearing the mutated sequence arrangement.

The preparation of sequence variants of the selected peptide-encoding DNA segments using site-directed mutagenesis provides a means of producing potentially useful species and is not meant to be limiting as there are other ways in which sequence variants of peptides and the DNA sequences encoding them may be obtained. For example, recombinant vectors encoding the desired peptide sequence may be treated with mutagenic agents, such as hydroxylamine, to obtain sequence variants. Specific details regarding these methods and protocols are found in the teachings of Maloy *et al.*, 1994; Segal, 1976; Prokop and Bajpai, 1991; Kuby, 1994; and Maniatis *et al.*, 1982, each incorporated herein by reference, for that purpose.

As used herein, the term “oligonucleotide directed mutagenesis procedure” refers to template-dependent processes and vector-mediated propagation which result in an increase in the concentration of a specific nucleic acid molecule relative to its initial concentration, or in an increase in the concentration of a detectable signal, such as amplification. As used herein, the term “oligonucleotide directed mutagenesis procedure” is intended to refer to a process that involves the template-dependent extension of a primer molecule. The term template dependent process refers to nucleic acid synthesis of an RNA or a DNA molecule wherein the sequence of the newly synthesized strand of nucleic acid is dictated by the well-known rules of complementary base pairing (see, for example, Watson, 1987). Typically, vector mediated methodologies involve the introduction of the nucleic acid fragment into a DNA or RNA vector, the clonal amplification of the vector, and the recovery of the amplified nucleic acid fragment. Examples of such methodologies are provided by U. S. Patent No. 4,237,224, specifically incorporated herein by reference in its entirety.

In another approach for the production of polypeptide variants, recursive sequence recombination, as described in U.S. Patent No. 5,837,458, may be employed. In this approach, iterative cycles of recombination and screening or selection are performed to “evolve” individual polynucleotide variants having, for example, increased binding affinity. Certain embodiments also provide constructs in the form of plasmids, vectors, transcription or expression cassettes which comprise at least one polynucleotide as described herein.

In many embodiments, the nucleic acids encoding a subject monoclonal antibody are introduced directly into a host cell, and the cell incubated under conditions sufficient to induce expression of the encoded antibody. The antibodies of this disclosure are prepared using standard techniques well known to those of skill in the art in combination with the polypeptide and nucleic acid sequences provided herein. The polypeptide sequences may be used to determine appropriate nucleic

acid sequences encoding the particular antibody disclosed thereby. The nucleic acid sequence may be optimized to reflect particular codon “preferences” for various expression systems according to standard methods well known to those of skill in the art.

According to certain related embodiments there is provided a recombinant host cell which comprises one or more constructs as described herein; a nucleic acid encoding any antibody, CDR, VH or VL domain, or antigen-binding fragment thereof; and a method of production of the encoded product, which method comprises expression from encoding nucleic acid therefor. Expression may conveniently be achieved by culturing under appropriate conditions recombinant host cells containing the nucleic acid. Following production by expression, an antibody or antigen-binding fragment thereof, may be isolated and/or purified using any suitable technique, and then used as desired.

Antibodies or antigen-binding fragments thereof as provided herein, and encoding nucleic acid molecules and vectors, may be isolated and/or purified, *e.g.*, from their natural environment, in substantially pure or homogeneous form, or, in the case of nucleic acid, free or substantially free of nucleic acid or genes of origin other than the sequence encoding a polypeptide with the desired function. Nucleic acid may comprise DNA or RNA and may be wholly or partially synthetic. Reference to a nucleotide sequence as set out herein encompasses a DNA molecule with the specified sequence, and encompasses a RNA molecule with the specified sequence in which U is substituted for T, unless context requires otherwise.

Systems for cloning and expression of a polypeptide in a variety of different host cells are well known. Suitable host cells include bacteria, mammalian cells, yeast and baculovirus systems. Mammalian cell lines available in the art for expression of a heterologous polypeptide include Chinese hamster ovary cells, HeLa cells, baby hamster kidney cells, NSO mouse melanoma cells and many others. A common, preferred bacterial host is *E. coli*.

The expression of antibodies and antigen-binding fragments in prokaryotic cells such as *E. coli* is well established in the art. See, for example, Pluckthun (Bio/Technology 9: 545-551, 1991). Expression in eukaryotic cells in culture is also available to those skilled in the art as an option for production of antibodies or antigen-binding fragments thereof, see recent reviews, for example Ref, M. E. (1993) Curr. Opin. Biotech. 4: 573-576; Trill J. J. *et al.* (1995) Curr. Opin. Biotech 6: 553-560.

Suitable vectors can be chosen or constructed, containing appropriate regulatory sequences, including promoter sequences, terminator sequences, polyadenylation sequences, enhancer sequences, marker genes and other sequences as appropriate. Vectors may be plasmids, viral *e.g.* phage, or phagemid, as appropriate. For further details see, for example, Molecular Cloning: a Laboratory Manual: 2nd edition, Sambrook *et al.*, 1989, Cold Spring Harbor Laboratory Press. Many known techniques and protocols for manipulation of nucleic acid, for example in preparation of nucleic acid constructs, mutagenesis, sequencing, introduction of DNA into cells and gene expression, and

analysis of proteins, are described in detail in Current Protocols in Molecular Biology, Second Edition, Ausubel *et al.* eds., John Wiley & Sons, 1992, or subsequent updates thereto.

The term “host cell” is used to refer to a cell into which has been introduced, or which is capable of having introduced into it, a nucleic acid sequence encoding one or more of the herein described antibodies, and which further expresses or is capable of expressing a selected gene of interest, such as a gene encoding any herein described antibody. The term includes the progeny of the parent cell, whether or not the progeny are identical in morphology or in genetic make-up to the original parent, so long as the selected gene is present. Accordingly there is also contemplated a method comprising introducing such nucleic acid into a host cell. The introduction may employ any available technique. For eukaryotic cells, suitable techniques may include calcium phosphate transfection, DEAE-Dextran, electroporation, liposome-mediated transfection and transduction using retrovirus or other virus, *e.g.* vaccinia or, for insect cells, baculovirus. For bacterial cells, suitable techniques may include calcium chloride transformation, electroporation and transfection using bacteriophage. The introduction may be followed by causing or allowing expression from the nucleic acid, *e.g.*, by culturing host cells under conditions for expression of the gene. In some embodiments, the nucleic acid is integrated into the genome (*e.g.*, chromosome) of the host cell. Integration may be promoted by inclusion of sequences which promote recombination with the genome, in accordance with standard techniques.

The present disclosure also provides, in certain embodiments, a method which comprises using a construct as stated above in an expression system in order to express a particular polypeptide such as a TNFR2-specific antibody as described herein. The term “transduction” is used to refer to the transfer of genes from one bacterium to another, usually by a phage. “Transduction” also refers to the acquisition and transfer of eukaryotic cellular sequences by retroviruses. The term “transfection” is used to refer to the uptake of foreign or exogenous DNA by a cell, and a cell has been “transfected” when the exogenous DNA has been introduced inside the cell membrane. 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, Cold Spring Harbor Laboratories; Davis *et al.*, 1986, BASIC METHODS IN MOLECULAR BIOLOGY, Elsevier; and Chu *et al.*, 1981, Gene 13:197. Such techniques can be used to introduce one or more exogenous DNA moieties into suitable host cells.

The term “transformation” as used herein refers to a change in a cell’s genetic characteristics, and a cell has been transformed when it has been modified to contain a new DNA. For example, a cell is transformed where it is genetically modified from its native state. Following transfection or transduction, the transforming DNA may recombine with that of the cell by physically integrating into a chromosome of the cell, or may be maintained transiently as an episomal element without being replicated, or may replicate independently as a plasmid. A cell is considered to have been stably transformed when the DNA is replicated with the division of the cell. The term “naturally occurring”

or “native” when used in connection with biological materials such as nucleic acid molecules, polypeptides, host cells, and the like, refers to materials which are found in nature and are not manipulated by a human. Similarly, “non-naturally occurring” or “non-native” as used herein refers to a material that is not found in nature or that has been structurally modified or synthesized by a human.

The terms “polypeptide” “protein” and “peptide” and “glycoprotein” are used interchangeably and mean a polymer of amino acids not limited to any particular length. The term does not exclude modifications such as myristoylation, sulfation, glycosylation, phosphorylation and addition or deletion of signal sequences. The terms “polypeptide” or “protein” means one or more chains of amino acids, wherein each chain comprises amino acids covalently linked by peptide bonds, and wherein said polypeptide or protein can comprise a plurality of chains non-covalently and/or covalently linked together by peptide bonds, having the sequence of native proteins, that is, proteins produced by naturally-occurring and specifically non-recombinant cells, or genetically-engineered or recombinant cells, and comprise molecules having the amino acid sequence of the native protein, or molecules having deletions from, additions to, and/or substitutions of one or more amino acids of the native sequence. The terms “polypeptide” and “protein” specifically encompass the antibodies that bind to TNFR2 of the present disclosure, or sequences that have deletions from, additions to, and/or substitutions of one or more amino acid of an anti-TNFR2 antibody. Thus, a “polypeptide” or a “protein” can comprise one (termed “a monomer”) or a plurality (termed “a multimer”) of amino acid chains.

The term “isolated protein” referred to herein means that a subject protein (1) is free of at least some other proteins with which it would typically be found in nature, (2) is essentially free of other proteins from the same source, *e.g.*, from the same species, (3) is expressed by a cell from a different species, (4) has been separated from at least about 50 percent of polynucleotides, lipids, carbohydrates, or other materials with which it is associated in nature, (5) is not associated (by covalent or noncovalent interaction) with portions of a protein with which the “isolated protein” is associated in nature, (6) is operably associated (by covalent or noncovalent interaction) with a polypeptide with which it is not associated in nature, or (7) does not occur in nature. Such an isolated protein can be encoded by genomic DNA, cDNA, mRNA or other RNA, or may be of synthetic origin, or any combination thereof. In certain embodiments, the isolated protein is substantially free from proteins or polypeptides or other contaminants that are found in its natural environment that would interfere with its use (therapeutic, diagnostic, prophylactic, research or otherwise).

The term “polypeptide fragment” refers to a polypeptide, which can be monomeric or multimeric, that has an amino-terminal deletion, a carboxyl-terminal deletion, and/or an internal deletion or substitution of a naturally-occurring or recombinantly-produced polypeptide. In certain embodiments, a polypeptide fragment can comprise an amino acid chain at least 5 to about 500 amino acids long. It will be appreciated that in certain embodiments, fragments are at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37,

38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 150, 200, 250, 300, 350, 400, or 450 amino acids long. Particularly useful polypeptide fragments include functional domains, including antigen-binding domains or fragments of antibodies. In the case of an anti-TNFR2 antibody, useful fragments include, but are not limited to: a CDR region, especially a CDR3 region of the heavy or light chain; a variable region of a heavy or light chain; a portion of an antibody chain or just its variable region including two CDRs; and the like.

Polypeptides may comprise a signal (or leader) sequence at the N-terminal end of the protein, which co-translationally or post-translationally directs transfer of the protein. Any polypeptide amino acid sequences provided herein that include a signal peptide are also contemplated for any use described herein without such a signal or leader peptide. As would be recognized by the skilled person, the signal peptide is usually cleaved during processing and is not included in the active antibody protein. The polypeptide may also be fused in-frame or conjugated to a linker or other sequence for ease of synthesis, purification or identification of the polypeptide (*e.g.*, poly-His), or to enhance binding of the polypeptide to a solid support.

A peptide linker/spacer sequence may also be employed to separate multiple polypeptide components by a distance sufficient to ensure that each polypeptide folds into its secondary and/or tertiary structures, if desired. Such a peptide linker sequence can be incorporated into a fusion polypeptide using standard techniques well known in the art.

Certain peptide spacer sequences may be chosen, for example, based on: (1) their ability to adopt a flexible extended conformation; (2) their inability to adopt a secondary structure that could interact with functional epitopes on the first and second polypeptides; and/or (3) the lack of hydrophobic or charged residues that might react with the polypeptide functional epitopes.

In one illustrative embodiment, peptide spacer sequences contain, for example, Gly, Asn and Ser residues. Other near neutral amino acids, such as Thr and Ala, may also be included in the spacer sequence.

Other amino acid sequences which may be usefully employed as spacers include those disclosed in Maratea *et al.*, *Gene* 40:39-46 (1985); Murphy *et al.*, *Proc. Natl. Acad. Sci. USA* 83:8258-8262 (1986); U.S. Pat. No. 4,935,233 and U.S. Pat. No. 4,751,180.

Other illustrative spacers may include, for example, Glu-Gly-Lys-Ser-Ser-Gly-Ser-Gly-Ser-Glu-Ser-Lys-Val-Asp (SEQ ID NO: 324) (Chaudhary *et al.*, 1990, *Proc. Natl. Acad. Sci. U.S.A.* 87:1066-1070) and Lys-Glu-Ser-Gly-Ser-Val-Ser-Ser-Glu-Gln-Leu-Ala-Gln-Phe-Arg-Ser-Leu-Asp (SEQ ID NO: 325) (Bird *et al.*, 1988, *Science* 242:423-426).

In some embodiments, spacer sequences are not required when the first and second polypeptides have non-essential N-terminal amino acid regions that can be used to separate the functional domains and prevent steric interference. Two coding sequences can be fused directly without any spacer or by using a flexible polylinker composed, for example, of the pentamer Gly-Gly-Gly-Gly-Ser (SEQ ID NO: 326) repeated 1 to 3 times. Such a spacer has been used in constructing

single chain antibodies (scFv) by being inserted between VH and VL (Bird et al., 1988, *Science* 242:423-426; Huston et al., 1988, *Proc. Natl. Acad. Sci. U.S.A.* 85:5979-5883).

A peptide spacer, in certain embodiments, is designed to enable the correct interaction between two beta-sheets forming the variable region of the single chain antibody.

In certain embodiments, a peptide spacer is between 1 to 5 amino acids, between 5 to 10 amino acids, between 5 to 25 amino acids, between 5 to 50 amino acids, between 10 to 25 amino acids, between 10 to 50 amino acids, between 10 to 100 amino acids, or any intervening range of amino acids. In some embodiments, a peptide spacer comprises about 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50 or more amino acids in length.

Amino acid sequence modification(s) of the antibodies described herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. For example, amino acid sequence variants of an antibody may be prepared by introducing appropriate nucleotide changes into a polynucleotide that encodes the antibody, or a chain thereof, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of, residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution may be made to arrive at the final antibody, provided that the final construct possesses the desired characteristics (*e.g.*, high affinity binding to TNFR2). The amino acid changes also may alter post-translational processes of the antibody, such as changing the number or position of glycosylation sites. Any of the variations and modifications described above for polypeptides of the present disclosure may be included in antibodies of the present disclosure.

The present disclosure provides variants of the antibodies disclosed herein. In certain embodiments, such variant antibodies or antigen-binding fragments, or CDRs thereof, bind to TNFR2 at least about 50%, at least about 70%, and in certain embodiments, at least about 90% as well as an antibody sequence specifically set forth herein. In further embodiments, such variant antibodies or antigen-binding fragments, or CDRs thereof, bind to TNFR2 with greater affinity than the antibodies set forth herein, for example, that bind quantitatively at least about 105%, 106%, 107%, 108%, 109%, or 110% as well as an antibody sequence specifically set forth herein.

Determination of the three-dimensional structures of representative polypeptides (*e.g.*, variant TNFR2-specific antibodies as provided herein, for instance, an antibody protein having an antigen-binding fragment as provided herein) may be made through routine methodologies such that substitution, addition, deletion or insertion of one or more amino acids with selected natural or non-natural amino acids can be virtually modeled for purposes of determining whether a so derived structural variant retains the space-filling properties of presently disclosed species. *See*, for instance, Donate et al., 1994 *Prot. Sci.* 3:2378; Bradley et al., *Science* 309: 1868-1871 (2005); Schueler-Furman et al., *Science* 310:638 (2005); Dietz et al., *Proc. Nat. Acad. Sci. USA* 103:1244 (2006); Dodson et al., *Nature* 450:176 (2007); Qian et al., *Nature* 450:259 (2007); Raman *et al.* *Science*

327:1014-1018 (2010). Some additional non-limiting examples of computer algorithms that may be used for these and related embodiments, such as for rational design of TNFR2-specific antibodies antigen-binding domains thereof as provided herein, include VMD which is a molecular visualization program for displaying, animating, and analyzing large biomolecular systems using 3-D graphics and built-in scripting (see the website for the Theoretical and Computational Biophysics Group, University of Illinois at Urbana-Champaign, at ks.uiuc.edu/Research/vmd/). Many other computer programs are known in the art and available to the skilled person and which allow for determining atomic dimensions from space-filling models (van der Waals radii) of energy-minimized conformations; GRID, which seeks to determine regions of high affinity for different chemical groups, thereby enhancing binding, Monte Carlo searches, which calculate mathematical alignment, and CHARMM (Brooks et al. (1983) *J. Comput. Chem.* 4:187-217) and AMBER (Weiner et al (1981) *J. Comput. Chem.* 106: 765), which assess force field calculations, and analysis (see also, Eisenfield et al. (1991) *Am. J. Physiol.* 261:C376-386; Lybrand (1991) *J. Pharm. Belg.* 46:49-54; Froimowitz (1990) *Biotechniques* 8:640-644; Burbam et al. (1990) *Proteins* 7:99-111; Pedersen (1985) *Environ. Health Perspect.* 61:185-190; and Kini et al. (1991) *J. Biomol. Struct. Dyn.* 9:475-488). A variety of appropriate computational computer programs are also commercially available, such as from Schrödinger (Munich, Germany).

In some embodiments, the anti-TNFR2 antibodies and humanized versions thereof are derived from rabbit monoclonal antibodies, and in particular are generated using APXiMAB™ technology. These antibodies are advantageous as they require minimal sequence modifications, thereby facilitating retention of functional properties after humanization using mutational lineage guided (MLG) humanization technology (see, e.g., U.S. Patent No. 7,462,697). Thus, illustrative methods for making the anti-TNFR2 antibodies of the present disclosure include the APXiMAB™ rabbit monoclonal antibody technology described, for example, in U.S. Patents 5,675,063 and 7,429,487. In this regard, in certain embodiments, the anti-TNFR2 antibodies of the disclosure are produced in rabbits. In particular embodiments, a rabbit-derived immortal B-lymphocyte capable of fusion with a rabbit splenocyte or peripheral B lymphocyte is used to produce a hybrid cell that produces an antibody. The immortal B-lymphocyte does not detectably express endogenous immunoglobulin heavy chain and may contain, in certain embodiments, an altered immunoglobulin heavy chain-encoding gene.

Compositions and Methods of Use

The present disclosure provides compositions comprising the TNFR2-specific antibodies, or antigen-binding fragments thereof, and administration of such composition in a variety of therapeutic settings, including the treatment of cancers, inflammatory and autoimmune diseases, and other diseases.

Administration of the TNFR2-specific antibodies described herein, in pure form or in an appropriate pharmaceutical composition, can be carried out via any of the accepted modes of administration of agents for serving similar utilities. The pharmaceutical compositions can be prepared by combining an antibody or antibody-containing composition with an appropriate physiologically acceptable carrier, diluent or excipient, and may be formulated into preparations in solid, semi-solid, liquid or gaseous forms, such as tablets, capsules, powders, granules, ointments, solutions, suppositories, injections, inhalants, gels, microspheres, and aerosols. In addition, other pharmaceutically active ingredients (including other anti-cancer agents as described elsewhere herein) and/or suitable excipients such as salts, buffers and stabilizers may, but need not, be present within the composition. Administration may be achieved by a variety of different routes, including oral, parenteral, nasal, intravenous, intradermal, subcutaneous or topical. Preferred modes of administration depend upon the nature of the condition to be treated or prevented. An amount that, following administration, reduces, inhibits, prevents or delays the progression and/or metastasis of a cancer is considered effective.

In certain embodiments, the amount administered is sufficient to result in tumor regression, as indicated by a statistically significant decrease in the amount of viable tumor, for example, at least a 50% decrease in tumor mass, or by altered (*e.g.*, decreased with statistical significance) scan dimensions.

The precise dosage and duration of treatment is a function of the disease being treated and may be determined empirically using known testing protocols or by testing the compositions in model systems known in the art and extrapolating therefrom. Controlled clinical trials may also be performed. Dosages may also vary with the severity of the condition to be alleviated. A pharmaceutical composition is generally formulated and administered to exert a therapeutically useful effect while minimizing undesirable side effects. The composition may be administered one time, or may be divided into a number of smaller doses to be administered at intervals of time. For any particular subject, specific dosage regimens may be adjusted over time according to the individual need.

The TNFR2-specific antibody-containing compositions may be administered alone or in combination with other known cancer treatments, such as radiation therapy, chemotherapy, transplantation, immunotherapy, hormone therapy, photodynamic therapy, *etc.* The compositions may also be administered in combination with antibiotics.

Typical routes of administering these and related pharmaceutical compositions thus include, without limitation, oral, topical, transdermal, inhalation, parenteral, sublingual, buccal, rectal, vaginal, intravitreal, and intranasal. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intrasternal injection or infusion techniques. Pharmaceutical compositions according to certain embodiments are formulated so as to allow the active ingredients contained therein to be bioavailable upon administration of the composition to a patient. Compositions that will

be administered to a subject or patient may take the form of one or more dosage units, where for example, a tablet may be a single dosage unit, and a container of a herein described TNFR2-specific antibody in aerosol form may hold a plurality of dosage units. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in this art; for example, see *Remington: The Science and Practice of Pharmacy*, 20th Edition (Philadelphia College of Pharmacy and Science, 2000). The composition to be administered will, in any event, contain a therapeutically effective amount of an antibody of the present disclosure, for treatment of a disease or condition of interest in accordance with teachings herein.

A pharmaceutical composition may be in the form of a solid or liquid. In one embodiment, the carrier(s) are particulate, so that the compositions are, for example, in tablet or powder form. The carrier(s) may be liquid, with the compositions being, for example, an oral oil, injectable liquid or an aerosol, which is useful in, for example, inhalatory administration. When intended for oral administration, the pharmaceutical composition is preferably in either solid or liquid form, where semi-solid, semi-liquid, suspension and gel forms are included within the forms considered herein as either solid or liquid.

As a solid composition for oral administration, the pharmaceutical composition may be formulated into a powder, granule, compressed tablet, pill, capsule, chewing gum, wafer or the like. Such a solid composition will typically contain one or more inert diluents or edible carriers. In addition, one or more of the following may be present: binders such as carboxymethylcellulose, ethyl cellulose, microcrystalline cellulose, gum tragacanth or gelatin; excipients such as starch, lactose or dextrans, disintegrating agents such as alginic acid, sodium alginate, Primogel, corn starch and the like; lubricants such as magnesium stearate or Sterotex; glidants such as colloidal silicon dioxide; sweetening agents such as sucrose or saccharin; a flavoring agent such as peppermint, methyl salicylate or orange flavoring; and a coloring agent. When the pharmaceutical composition is in the form of a capsule, for example, a gelatin capsule, it may contain, in addition to materials of the above type, a liquid carrier such as polyethylene glycol or oil.

The pharmaceutical composition may be in the form of a liquid, for example, an elixir, syrup, solution, emulsion or suspension. The liquid may be for oral administration or for delivery by injection, as two examples. When intended for oral administration, certain compositions contain, in addition to the present compounds, one or more of a sweetening agent, preservatives, dye/colorant and flavor enhancer. In a composition intended to be administered by injection, one or more of a surfactant, preservative, wetting agent, dispersing agent, suspending agent, buffer, stabilizer and isotonic agent may be included.

The liquid pharmaceutical compositions, whether they be solutions, suspensions or other like form, may include one or more of the following adjuvants: sterile diluents such as water for injection, saline solution, preferably physiological saline, Ringer's solution, isotonic sodium chloride, fixed oils such as synthetic mono or diglycerides which may serve as the solvent or suspending medium,

polyethylene glycols, glycerin, propylene glycol or other solvents; antibacterial agents such as benzyl alcohol or methyl paraben; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic. Physiological saline is an exemplary adjuvant. An injectable pharmaceutical composition is preferably sterile.

A liquid pharmaceutical composition intended for either parenteral or oral administration should contain an amount of a TNFR2-specific antibody as herein disclosed such that a suitable dosage will be obtained. Typically, this amount is at least 0.01% of the antibody in the composition. When intended for oral administration, this amount may be varied to be between 0.1 and about 70% of the weight of the composition. Certain oral pharmaceutical compositions contain between about 4% and about 75% of the antibody. In certain embodiments, pharmaceutical compositions and preparations are prepared so that a parenteral dosage unit contains between 0.01 to 10% by weight of the antibody prior to dilution.

The pharmaceutical composition may be intended for topical administration, in which case the carrier may suitably comprise a solution, emulsion, ointment or gel base. The base, for example, may comprise one or more of the following: petrolatum, lanolin, polyethylene glycols, bee wax, mineral oil, diluents such as water and alcohol, and emulsifiers and stabilizers. Thickening agents may be present in a pharmaceutical composition for topical administration. If intended for transdermal administration, the composition may include a transdermal patch or iontophoresis device. The pharmaceutical composition may be intended for rectal administration, in the form, for example, of a suppository, which will melt in the rectum and release the drug. The composition for rectal administration may contain an oleaginous base as a suitable nonirritating excipient. Such bases include, without limitation, lanolin, cocoa butter and polyethylene glycol.

The pharmaceutical composition may include various materials, which modify the physical form of a solid or liquid dosage unit. For example, the composition may include materials that form a coating shell around the active ingredients. The materials that form the coating shell are typically inert, and may be selected from, for example, sugar, shellac, and other enteric coating agents. Alternatively, the active ingredients may be encased in a gelatin capsule. The pharmaceutical composition in solid or liquid form may include an agent that binds to the antibody and thereby assists in the delivery of the compound. Suitable agents that may act in this capacity include other monoclonal or polyclonal antibodies, one or more proteins or a liposome. The pharmaceutical composition may consist essentially of dosage units that can be administered as an aerosol. The term aerosol is used to denote a variety of systems ranging from those of colloidal nature to systems consisting of pressurized packages. Delivery may be by a liquefied or compressed gas or by a suitable pump system that dispenses the active ingredients. Aerosols may be delivered in single phase,

bi-phasic, or tri-phasic systems in order to deliver the active ingredient(s). Delivery of the aerosol includes the necessary container, activators, valves, subcontainers, and the like, which together may form a kit. One of ordinary skill in the art, without undue experimentation may determine preferred aerosols.

The pharmaceutical compositions may be prepared by methodology well known in the pharmaceutical art. For example, a pharmaceutical composition intended to be administered by injection can be prepared by combining a composition that comprises a TNFR2-specific antibody as described herein and optionally, one or more of salts, buffers and/or stabilizers, with sterile, distilled water so as to form a solution. A surfactant may be added to facilitate the formation of a homogeneous solution or suspension. Surfactants are compounds that non-covalently interact with the antibody composition so as to facilitate dissolution or homogeneous suspension of the antibody in the aqueous delivery system.

The compositions may be administered in a therapeutically effective amount, which will vary depending upon a variety of factors including the activity of the specific compound (*e.g.*, TNFR2-specific antibody) employed; the metabolic stability and length of action of the compound; the age, body weight, general health, sex, and diet of the patient; the mode and time of administration; the rate of excretion; the drug combination; the severity of the particular disorder or condition; and the subject undergoing therapy. Generally, a therapeutically effective daily dose is (for a 70 kg mammal) from about 0.001 mg/kg (*i.e.*, 0.07 mg) to about 100 mg/kg (*i.e.*, 7.0 g); preferably a therapeutically effective dose is (for a 70 kg mammal) from about 0.01 mg/kg (*i.e.*, 0.7 mg) to about 50 mg/kg (*i.e.*, 3.5 g); more preferably a therapeutically effective dose is (for a 70 kg mammal) from about 1 mg/kg (*i.e.*, 70 mg) to about 25 mg/kg (*i.e.*, 1.75 g).

Compositions comprising the TNFR2-specific antibodies of the present disclosure may also be administered simultaneously with, prior to, or after administration of one or more other therapeutic agents. Such combination therapy may include administration of a single pharmaceutical dosage formulation which contains an antibody and one or more additional active agents, as well as administration of compositions comprising antibodies of the disclosure and each active agent in its own separate pharmaceutical dosage formulation. For example, an antibody as described herein and the other active agent can be administered to the patient together in a single oral dosage composition such as a tablet or capsule, or each agent administered in separate oral dosage formulations. Similarly, an antibody as described herein and the other active agent can be administered to the patient together in a single parenteral dosage composition such as in a saline solution or other physiologically acceptable solution, or each agent administered in separate parenteral dosage formulations. Where separate dosage formulations are used, the compositions comprising antibodies and one or more additional active agents can be administered at essentially the same time, *i.e.*, concurrently, or at separately staggered times, *i.e.*, sequentially and in any order; combination therapy is understood to include all these regimens.

Thus, in certain embodiments, also contemplated is the administration of anti-TNFR2 antibody compositions of this disclosure in combination with one or more other therapeutic agents. Such therapeutic agents may be accepted in the art as a standard treatment for a particular disease state as described herein, such as rheumatoid arthritis, inflammation or cancer. Exemplary therapeutic agents contemplated include cytokines, growth factors, steroids, NSAIDs, DMARDs, anti-inflammatories, chemotherapeutics, radiotherapeutics, or other active and ancillary agents.

In certain embodiments, the anti-TNFR2 antibodies disclosed herein are administered in combination with one or more cancer immunotherapy agents. In certain instances, an immunotherapy agent modulates the immune response of a subject, for example, to increase or maintain a cancer-related or cancer-specific immune response, and thereby results in increased immune cell inhibition or reduction of cancer cells. Exemplary immunotherapy agents include polypeptides, for example, antibodies and antigen-binding fragments thereof, ligands, and small peptides, and mixtures thereof. Also include as immunotherapy agents are small molecules, cells (e.g., immune cells such as T-cells), various cancer vaccines, gene therapy or other polynucleotide-based agents, including viral agents such as oncolytic viruses, and others known in the art. Thus, in certain embodiments, the cancer immunotherapy agent is selected from one or more of immune checkpoint modulatory agents, cancer vaccines, oncolytic viruses, cytokines, and cell-based immunotherapies.

In certain embodiments, the cancer immunotherapy agent is an immune checkpoint modulatory agent. Particular examples include “antagonists” of one or more inhibitory immune checkpoint molecules, and “agonists” of one or more stimulatory immune checkpoint molecules. Generally, immune checkpoint molecules are components of the immune system that either turn up a signal (co-stimulatory molecules) or turn down a signal, the targeting of which has therapeutic potential in cancer because cancer cells can perturb the natural function of immune checkpoint molecules (see, e.g., Sharma and Allison, *Science*. 348:56-61, 2015; Topalian et al., *Cancer Cell*. 27:450-461, 2015; Pardoll, *Nature Reviews Cancer*. 12:252-264, 2012). In some embodiments, the immune checkpoint modulatory agent (e.g., antagonist, agonist) “binds” or “specifically binds” to the one or more immune checkpoint molecules, as described herein.

In some embodiments, the immune checkpoint modulatory agent is an antagonist or inhibitor of one or more inhibitory immune checkpoint molecules. Exemplary inhibitory immune checkpoint molecules include Programmed Death-Ligand 1 (PD-L1), Programmed Death-Ligand 2 (PD-L2), Programmed Death 1 (PD-1), V-domain Ig suppressor of T cell activation (VISTA), Cytotoxic T-Lymphocyte-Associated protein 4 (CTLA-4), Indoleamine 2,3-dioxygenase (IDO), tryptophan 2,3-dioxygenase (TDO), T-cell Immunoglobulin domain and Mucin domain 3 (TIM-3), Lymphocyte Activation Gene-3 (LAG-3), B and T Lymphocyte Attenuator (BTLA), CD160, T-cell immunoreceptor with Ig and ITIM domains (TIGIT), and signal regulatory protein α (SIRP α).

In certain embodiments, the agent is a PD-1 (receptor) antagonist or inhibitor, the targeting of which has been shown to restore immune function in the tumor environment (see, e.g., Phillips et al.,

Int Immunol. 27:39-46, 2015). PD-1 is a cell surface receptor that belongs to the immunoglobulin superfamily and is expressed on T cells and pro-B cells. PD-1 interacts with two ligands, PD-L1 and PD-L2. PD-1 functions as an inhibitory immune checkpoint molecule, for example, by reducing or preventing the activation of T-cells, which in turn reduces autoimmunity and promotes self-tolerance. The inhibitory effect of PD-1 is accomplished at least in part through a dual mechanism of promoting apoptosis in antigen specific T-cells in lymph nodes while also reducing apoptosis in regulatory T cells (suppressor T cells). Some examples of PD-1 antagonists or inhibitors include an antibody or antigen-binding fragment or small molecule that specifically binds to PD-1 and reduces one or more of its immune-suppressive activities, for example, its downstream signaling or its interaction with PD-L1. Specific examples of PD-1 antagonists or inhibitors include the antibodies nivolumab, pembrolizumab, PDR001, MK-3475, AMP-224, AMP-514, and pidilizumab, and antigen-binding fragments thereof (see, e.g., U.S. Patent Nos. 8,008,449; 8,993,731; 9,073,994; 9,084,776; 9,102,727; 9,102,728; 9,181,342; 9,217,034; 9,387,247; 9,492,539; 9,492,540; and U.S. Application Nos. 2012/0039906; 2015/0203579).

In some embodiments, the agent is a PD-L1 antagonist or inhibitor. As noted above, PD-L1 is one of the natural ligands for the PD-1 receptor. General examples of PD-L1 antagonists or inhibitors include an antibody or antigen-binding fragment or small molecule that specifically binds to PD-L1 and reduces one or more of its immune-suppressive activities, for example, its binding to the PD-1 receptor. Specific examples of PD-L1 antagonists include the antibodies atezolizumab (MPDL3280A), avelumab (MSB0010718C), and durvalumab (MEDI4736), and antigen-binding fragments thereof (see, e.g., U.S. Patent Nos. 9,102,725; 9,393,301; 9,402,899; 9,439,962).

In some embodiments, the agent is a PD-L2 antagonist or inhibitor. As noted above, PD-L2 is one of the natural ligands for the PD-1 receptor. General examples of PD-L2 antagonists or inhibitors include an antibody or antigen-binding fragment or small molecule that specifically binds to PD-L2 and reduces one or more of its immune-suppressive activities, for example, its binding to the PD-1 receptor.

In certain embodiments, the agent is a VISTA antagonist or inhibitor. VISTA is approximately 50 kDa in size and belongs to the immunoglobulin superfamily (it has one IgV domain) and the B7 family. It is primarily expressed in white blood cells, and its transcription is partially controlled by p53. There is evidence that VISTA can act as both a ligand and a receptor on T cells to inhibit T cell effector function and maintain peripheral tolerance. VISTA is produced at high levels in tumor-infiltrating lymphocytes, such as myeloid-derived suppressor cells and regulatory T cells, and its blockade with an antibody results in delayed tumor growth in mouse models of melanoma and squamous cell carcinoma. Exemplary anti-VISTA antagonist antibodies include, for example, the antibodies described in WO 2018/237287, which is incorporated by reference in its entirety.

In some embodiments, the agent is a CTLA-4 antagonist or inhibitor. CTLA4 or CTLA-4 (cytotoxic T-lymphocyte-associated protein 4), also known as CD152 (cluster of differentiation 152), is a protein receptor that functions as an inhibitory immune checkpoint molecule, for example, by transmitting inhibitory signals to T-cells when it is bound to CD80 or CD86 on the surface of antigen-presenting cells. General examples CTLA-4 antagonists or inhibitors include an antibody or antigen-binding fragment or small molecule that specifically binds to CTLA-4. Particular examples include the antibodies ipilimumab and tremelimumab, and antigen-binding fragments thereof. At least some of the activity of ipilimumab is believed to be mediated by antibody-dependent cell-mediated cytotoxicity (ADCC) killing of suppressor Tregs that express CTLA-4.

In some embodiments, the agent is an IDO antagonist or inhibitor, or a TDO antagonist or inhibitor. IDO and TDO are tryptophan catabolic enzymes with immune-inhibitory properties. For example, IDO is known to suppress T-cells and NK cells, generate and activate Tregs and myeloid-derived suppressor cells, and promote tumor angiogenesis. General examples of IDO and TDO antagonists or inhibitors include an antibody or antigen-binding fragment or small molecule that specifically binds to IDO or TDO (see, e.g., Platten et al., *Front Immunol.* 5: 673, 2014) and reduces or inhibits one or more immune-suppressive activities. Specific examples of IDO antagonists or inhibitors include indoximod (NLG-8189), 1-methyl-tryptophan (1MT), β -Carboline (norharmane; 9H-pyrido[3,4-b]indole), rosmarinic acid, and epacadostat (see, e.g., Sheridan, *Nature Biotechnology*. 33:321-322, 2015). Specific examples of TDO antagonists or inhibitors include 680C91 and LM10 (see, e.g., Pilotte et al., *PNAS USA*. 109:2497-2502, 2012).

In some embodiments, the agent is a TIM-3 antagonist or inhibitor. T-cell Immunoglobulin domain and Mucin domain 3 (TIM-3) is expressed on activated human CD4⁺ T-cells and regulates Th1 and Th17 cytokines. TIM-3 also acts as a negative regulator of Th1/Tc1 function by triggering cell death upon interaction with its ligand, galectin-9. TIM-3 contributes to the suppressive tumor microenvironment and its overexpression is associated with poor prognosis in a variety of cancers (see, e.g., Li et al., *Acta Oncol.* 54:1706-13, 2015). General examples of TIM-3 antagonists or inhibitors include an antibody or antigen-binding fragment or small molecule that specifically binds to TIM-3 and reduces or inhibits one or more of its immune-suppressive activities.

In some embodiments, the agent is a LAG-3 antagonist or inhibitor. Lymphocyte Activation Gene-3 (LAG-3) is expressed on activated T-cells, natural killer cells, B-cells and plasmacytoid dendritic cells. It negatively regulates cellular proliferation, activation, and homeostasis of T-cells, in a similar fashion to CTLA-4 and PD-1 (see, e.g., Workman and Vignali, *European Journal of Immun.* 33: 970-9, 2003; and Workman et al., *Journal of Immun.* 172: 5450-5, 2004), and has been reported to play a role in Treg suppressive function (see, e.g., Huang et al., *Immunity*. 21: 503-13, 2004). LAG3 also maintains CD8⁺ T-cells in a tolerogenic state and combines with PD-1 to maintain CD8 T-cell exhaustion. General examples of LAG-3 antagonists or inhibitors include an antibody or antigen-binding fragment or small molecule that specifically binds to LAG-3 and inhibits one or more

of its immune-suppressive activities. Specific examples include the antibody BMS-986016, and antigen-binding fragments thereof.

In some embodiments, the agent is a BTLA antagonist or inhibitor. B- and T-lymphocyte attenuator (BTLA; CD272) expression is induced during activation of T-cells, and it inhibits T-cells via interaction with tumor necrosis family receptors (TNF-R) and B7 family of cell surface receptors. BTLA is a ligand for tumor necrosis factor (receptor) superfamily, member 14 (TNFRSF14), also known as herpes virus entry mediator (HVEM). BTLA-HVEM complexes negatively regulate T-cell immune responses, for example, by inhibiting the function of human CD8⁺ cancer-specific T-cells (see, e.g., Derré et al., J Clin Invest 120:157–67, 2009). General examples of BTLA antagonists or inhibitors include an antibody or antigen-binding fragment or small molecule that specifically binds to BTLA-4 and reduce one or more of its immune-suppressive activities.

In some embodiments, the agent is an HVEM antagonist or inhibitor, for example, an antagonist or inhibitor that specifically binds to HVEM and interferes with its interaction with BTLA or CD160. General examples of HVEM antagonists or inhibitors include an antibody or antigen-binding fragment or small molecule that specifically binds to HVEM, optionally reduces the HVEM/BTLA and/or HVEM/CD160 interaction, and thereby reduces one or more of the immune-suppressive activities of HVEM.

In some embodiments, the agent is a CD160 antagonist or inhibitor, for example, an antagonist or inhibitor that specifically binds to CD160 and interferes with its interaction with HVEM. General examples of CD160 antagonists or inhibitors include an antibody or antigen-binding fragment or small molecule that specifically binds to CD160, optionally reduces the CD160/HVEM interaction, and thereby reduces or inhibits one or more of its immune-suppressive activities.

In some embodiments, the agent is a TIGIT antagonist or inhibitor. T cell Ig and ITIM domain (TIGIT) is a co-inhibitory receptor that is found on the surface of a variety of lymphoid cells, and suppresses antitumor immunity, for example, via Tregs (Kurtulus et al., J Clin Invest. 125:4053-4062, 2015). General examples of TIGIT antagonists or inhibitors include an antibody or antigen-binding fragment or small molecule that specifically binds to TIGIT and reduce one or more of its immune-suppressive activities (see, e.g., Johnston et al., Cancer Cell. 26:923-37, 2014).

In certain embodiments, the agent is a SIRP α antagonist or inhibitor. SIRP α is a regulatory membrane glycoprotein expressed mainly by myeloid cells, which interacts with broadly expressed transmembrane protein CD47 to negatively control effector function of innate immune cells such as host cell phagocytosis. Certain cancer cells activate the inhibitory SIRP α -CD47 signaling pathway, for example, by overexpressing CD47, and thereby inhibit macrophage-mediated phagocytosis. SIRP α inhibitors have been shown to reduce cancer growth and metastasis, alone and in synergy with other cancer treatments (see, for example, Yanagita, JCI Insight. 2017 Jan 12; 2(1): e89140). General examples of SIRP α antagonist or inhibitors include an antibody or antigen-binding fragment or small molecule that specifically binds to SIRP α and interferes with SIRP α -CD47 signaling (see Id.).

In certain embodiments, the immune checkpoint modulatory agent is an agonist of one or more stimulatory immune checkpoint molecules. Exemplary stimulatory immune checkpoint molecules include CD40, OX40, Glucocorticoid-Induced TNFR Family Related Gene (GITR), CD137 (4-1BB), CD27, CD28, CD226, and Herpes Virus Entry Mediator (HVEM).

In some embodiments, the agent is a CD40 agonist. CD40 is expressed on antigen-presenting cells (APC) and some malignancies. Its ligand is CD40L (CD154). On APC, ligation results in upregulation of costimulatory molecules, potentially bypassing the need for T-cell assistance in an antitumor immune response. CD40 agonist therapy plays an important role in APC maturation and their migration from the tumor to the lymph nodes, resulting in elevated antigen presentation and T cell activation. Anti-CD40 agonist antibodies produce substantial responses and durable anticancer immunity in animal models, an effect mediated at least in part by cytotoxic T-cells (see, e.g., Johnson et al. Clin Cancer Res. 21: 1321-1328, 2015; and Vonderheide and Glennie, Clin Cancer Res. 19:1035-43, 2013). General examples of CD40 agonists include an antibody or antigen-binding fragment or small molecule or ligand that specifically binds to CD40 and increases one or more of its immunostimulatory activities. Specific examples include CP-870,893, dacetuzumab, Chi Lob 7/4, ADC-1013, CD40L, rhCD40L, and antigen-binding fragments thereof. Specific examples of CD40 agonists include, but are not limited to, APX005 (see, e.g., US 2012/0301488) and APX005M (see, e.g., US 2014/0120103).

In some embodiments, the agent is an OX40 agonist. OX40 (CD134) promotes the expansion of effector and memory T cells, and suppresses the differentiation and activity of T-regulatory cells (see, e.g., Croft et al., Immunol Rev. 229:173–91, 2009). Its ligand is OX40L (CD252). Since OX40 signaling influences both T-cell activation and survival, it plays a key role in the initiation of an anti-tumor immune response in the lymph node and in the maintenance of the anti-tumor immune response in the tumor microenvironment. General examples of OX40 agonists include an antibody or antigen-binding fragment or small molecule or ligand that specifically binds to OX40 and increases one or more of its immunostimulatory activities. Specific examples include OX86, OX-40L, Fc-OX40L, GSK3174998, MEDI0562 (a humanized OX40 agonist), MEDI6469 (murine OX40 agonist), and MEDI6383 (an OX40 agonist), and antigen-binding fragments thereof.

In some embodiments, the agent is a GITR agonist. Glucocorticoid-Induced TNFR family Related gene (GITR) increases T cell expansion, inhibits the suppressive activity of Tregs, and extends the survival of T-effector cells. GITR agonists have been shown to promote an anti-tumor response through loss of Treg lineage stability (see, e.g., Schaer et al., Cancer Immunol Res. 1:320–31, 2013). These diverse mechanisms show that GITR plays an important role in initiating the immune response in the lymph nodes and in maintaining the immune response in the tumor tissue. Its ligand is GITRL. General examples of GITR agonists include an antibody or antigen-binding fragment or small molecule or ligand that specifically binds to GITR and increases one or more of its

immunostimulatory activities. Specific examples include GITRL, INCAGN01876, DTA-1, MEDI1873, and antigen-binding fragments thereof.

In some embodiments, the agent is a CD137 agonist. CD137 (4-1BB) is a member of the tumor necrosis factor (TNF) receptor family, and crosslinking of CD137 enhances T-cell proliferation, IL-2 secretion, survival, and cytolytic activity. CD137-mediated signaling also protects T-cells such as CD8⁺ T-cells from activation-induced cell death. General examples of CD137 agonists include an antibody or antigen-binding fragment or small molecule or ligand that specifically binds to CD137 and increases one or more of its immunostimulatory activities. Specific examples include the CD137 (or 4-1BB) ligand (see, e.g., Shao and Schwarz, *J Leukoc Biol.* 89:21-9, 2011) and the antibody utomilumab, including antigen-binding fragments thereof.

In some embodiments, the agent is a CD27 agonist. Stimulation of CD27 increases antigen-specific expansion of naïve T cells and contributes to T-cell memory and long-term maintenance of T-cell immunity. Its ligand is CD70. The targeting of human CD27 with an agonist antibody stimulates T-cell activation and antitumor immunity (see, e.g., Thomas et al., *Oncoimmunology.* 2014;3:e27255. doi:10.4161/onci.27255; and He et al., *J Immunol.* 191:4174-83, 2013). General examples of CD27 agonists include an antibody or antigen-binding fragment or small molecule or ligand that specifically binds to CD27 and increases one or more of its immunostimulatory activities. Specific examples include CD70 and the antibodies varlilumab and CDX-1127 (1F5), including antigen-binding fragments thereof.

In some embodiments, the agent is a CD28 agonist. CD28 is constitutively expressed CD4⁺ T cells some CD8⁺ T cells. Its ligands include CD80 and CD86, and its stimulation increases T-cell expansion. General examples of CD28 agonists include an antibody or antigen-binding fragment or small molecule or ligand that specifically binds to CD28 and increases one or more of its immunostimulatory activities. Specific examples include CD80, CD86, the antibody TAB08, and antigen-binding fragments thereof.

In some embodiments, the agent is CD226 agonist. CD226 is a stimulating receptor that shares ligands with TIGIT, and opposite to TIGIT, engagement of CD226 enhances T-cell activation (see, e.g., Kurtulus et al., *J Clin Invest.* 125:4053-4062, 2015; Bottino et al., *J Exp Med.* 194:557-567, 2003; and Tahara-Hanaoka et al., *Int Immunol.* 16:533-538, 2004). General examples of CD226 agonists include an antibody or antigen-binding fragment or small molecule or ligand (e.g., CD112, CD155) that specifically binds to CD226 and increases one or more of its immunostimulatory activities.

In some embodiments, the agent is an HVEM agonist. Herpesvirus entry mediator (HVEM), also known as tumor necrosis factor receptor superfamily member 14 (TNFRSF14), is a human cell surface receptor of the TNF-receptor superfamily. HVEM is found on a variety of cells including T-cells, APCs, and other immune cells. Unlike other receptors, HVEM is expressed at high levels on resting T-cells and down-regulated upon activation. It has been shown that HVEM signaling plays a

crucial role in the early phases of T-cell activation and during the expansion of tumor-specific lymphocyte populations in the lymph nodes. General examples of HVEM agonists include an antibody or antigen-binding fragment or small molecule or ligand that specifically binds to HVEM and increases one or more of its immunostimulatory activities.

In certain embodiments, the anti-TNFR2 antibodies disclosed herein are administered in combination with one or more bi-specific or multi-specific antibodies. For instance, certain bi-specific or multi-specific antibodies are able to (i) bind to and inhibit one or more inhibitory immune checkpoint molecules, and also (ii) bind to and agonize one or more stimulatory immune checkpoint molecules. In certain embodiments, a bi-specific or multi-specific antibody (i) binds to and inhibits one or more of PD-L1, PD-L2, PD-1, CTLA-4, IDO, TDO, TIM-3, LAG-3, BTLA, CD160, and/or TIGIT, and also (ii) binds to and agonizes one or more of CD40, OX40 Glucocorticoid-Induced TNFR Family Related Gene (GITR), CD137 (4-1BB), CD27, CD28, CD226, and/or Herpes Virus Entry Mediator (HVEM).

In some embodiments, the anti-TNFR2 antibodies disclosed herein are administered in combination with one or more cancer vaccines. In certain embodiments, the cancer vaccine is selected from one or more of Oncophage, a human papillomavirus HPV vaccine optionally Gardasil or Cervarix, a hepatitis B vaccine optionally Engerix-B, Recombivax HB, or Twinrix, and sipuleucel-T (Provenge), or comprises a cancer antigen selected from one or more of human Her2/neu, Her1/EGF receptor (EGFR), Her3, A33 antigen, B7H3, CD5, CD19, CD20, CD22, CD23 (IgE Receptor), MAGE-3, C242 antigen, 5T4, IL-6, IL-13, vascular endothelial growth factor VEGF (e.g., VEGF-A) VEGFR-1, VEGFR-2, CD30, CD33, CD37, CD40, CD44, CD51, CD52, CD56, CD74, CD80, CD152, CD200, CD221, CCR4, HLA-DR, CTLA-4, NPC-1C, tenascin, vimentin, insulin-like growth factor 1 receptor (IGF-1R), alpha-fetoprotein, insulin-like growth factor 1 (IGF-1), carbonic anhydrase 9 (CA-IX), carcinoembryonic antigen (CEA), guanylyl cyclase C, NY-ESO-1, p53, survivin, integrin $\alpha\beta 3$, integrin $\alpha 5\beta 1$, folate receptor 1, transmembrane glycoprotein NMB, fibroblast activation protein alpha (FAP), glycoprotein 75, TAG-72, MUC1, MUC16 (or CA-125), phosphatidylserine, prostate-specific membrane antigen (PMSA), NR-LU-13 antigen, TRAIL-R1, tumor necrosis factor receptor superfamily member 10b (TNFRSF10B or TRAIL-R2), SLAM family member 7 (SLAMF7), EGP40 pancarcinoma antigen, B-cell activating factor (BAFF), platelet-derived growth factor receptor, glycoprotein EpCAM (17-1A), Programmed Death-1, protein disulfide isomerase (PDI), Phosphatase of Regenerating Liver 3 (PRL-3), prostatic acid phosphatase, Lewis-Y antigen, GD2 (a disialoganglioside expressed on tumors of neuroectodermal origin), glypican-3 (GPC3), and mesothelin.

In some embodiments, the anti-TNFR2 antibodies disclosed herein are administered in combination with one or more oncolytic viruses. In some embodiments, the oncolytic virus selected from one or more of talimogene laherparepvec (T-VEC), coxsackievirus A21 (CAVATAK™), Oncorine (H101), pelareorep (REOLYSIN®), Seneca Valley virus (NTX-010), *Senecavirus* SVV-

001, ColoAd1, SEPREHVIR (HSV-1716), CGTG-102 (Ad5/3-D24-GMCSF), GL-ONC1, MV-NIS, and DNX-2401.

In certain embodiments, the cancer immunotherapy agent is a cytokine. Exemplary cytokines include interferon (IFN)- α , IL-2, IL-12, IL-7, IL-21, and Granulocyte-macrophage colony-stimulating factor (GM-CSF).

In certain embodiments, the cancer immunotherapy agent is cell-based immunotherapy, for example, a T-cell based adoptive immunotherapy. In some embodiments, the cell-based immunotherapy comprises cancer antigen-specific T-cells, optionally *ex vivo*-derived T-cells. In some embodiments, the cancer antigen-specific T-cells are selected from one or more of chimeric antigen receptor (CAR)-modified T-cells, and T-cell Receptor (TCR)-modified T-cells, tumor infiltrating lymphocytes (TILs), and peptide-induced T-cells. In specific embodiments, the CAR-modified T-cell is targeted against CD-19 (see, e.g., Maude et al., Blood. 125:4017-4023, 2015).

In some embodiments, the anti-TNFR2 antibodies disclosed herein are used as part of adoptive immunotherapies, for example, autologous immunotherapies. Certain embodiments thus include methods of treating a cancer in a patient in need thereof, comprising:

- (a) incubating *ex vivo*-derived immune cells with an anti-TNFR2 antibody, or antigen-binding fragment thereof, described herein; and
- (b) administering the autologous immune cells to the patient.

In some instances, the *ex vivo*-derived immune cells are autologous cells, which are obtained from the patient to be treated. In some embodiments, the autologous immune cells comprise lymphocytes, natural killer (NK) cells, macrophages, and/or dendritic cells (DCs). In some embodiments, the lymphocytes comprise T-cells, optionally cytotoxic T-lymphocytes (CTLs). See, for example, June, J Clin Invest. 117: 1466-1476, 2007; Rosenberg and Restifo, Science. 348:62-68, 2015; Cooley et al., Biol. of Blood and Marrow Transplant. 13:33-42, 2007; and Li and Sun, Chin J Cancer Res. 30:173-196, 2018, for descriptions of adoptive T-cell and NK cell immunotherapies. In some embodiments, the T-cells comprise cancer antigen-specific T-cells, which are directed against at least one "cancer antigen", as described herein. In certain embodiments, the anti-TNFR2 antibody, or antigen-binding fragment thereof, enhances the efficacy of the adoptively transferred immune cells.

In certain embodiments, the anti-TNFR2 antibodies disclosed herein may be administered in conjunction with any number of chemotherapeutic agents. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and cyclophosphamide (CYTOXANTM); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramidate, triethylenethiophosphoramidate and trimethylolomelamine; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine,

prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; antibiotics such as aclacinomysins, actinomycin, anthramycin, azaserine, bleomycins, cactinomycin, calicheamicin, carabycin, carminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine, 5-FU; androgens such as calusterone, dromostanolone propionate, epitostanol, mepitiostane, testolactone; anti-adrenals such as aminogluthethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK®; razoxane; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2, 2', 2''-trichlorotriethylamine; urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, e.g. paclitaxel (TAXOL®, Bristol-Myers Squibb Oncology, Princeton, N.J.) and doxetaxel (TAXOTERE®, Rhne-Poulenc Rorer, Antony, France); chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitomycin C; mitoxantrone; vincristine; vinorelbine; navelbine; novantrone; teniposide; daunomycin; aminopterin; xeloda; ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylomithine (DMFO); retinoic acid derivatives such as Targretin™ (bexarotene), Panretin™ (alitretinoin) ; ONTAK™ (denileukin diftitox); esperamicins; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any of the above. Also included in this definition are anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens including for example tamoxifen, raloxifene, aromatase inhibiting 4(5)-imidazoles, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and toremifene (Fareston); and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

A variety of other therapeutic agents may be used in conjunction with the anti-TNFR2 antibodies described herein. In some embodiments, the antibody is administered with an anti-inflammatory agent. Anti-inflammatory agents or drugs include, but are not limited to, steroids and glucocorticoids (including betamethasone, budesonide, dexamethasone, hydrocortisone acetate,

hydrocortisone, hydrocortisone, methylprednisolone, prednisolone, prednisone, triamcinolone), nonsteroidal anti-inflammatory drugs (NSAIDs) including aspirin, ibuprofen, naproxen, methotrexate, sulfasalazine, leflunomide, anti-TNF medications, cyclophosphamide and mycophenolate.

Exemplary NSAIDs are chosen from the group consisting of ibuprofen, naproxen, naproxen sodium, Cox-2 inhibitors such as VIOXX® (rofecoxib) and CELEBREX® (celecoxib), and sialylates. Exemplary analgesics are chosen from the group consisting of acetaminophen, oxycodone, tramadol or propoxyphene hydrochloride. Exemplary glucocorticoids are chosen from the group consisting of cortisone, dexamethasone, hydrocortisone, methylprednisolone, prednisolone, or prednisone. Exemplary biological response modifiers include molecules directed against cell surface markers (*e.g.*, CD4, CD5, etc.), cytokine inhibitors, such as the TNF antagonists (*e.g.*, etanercept (ENBREL®), adalimumab (HUMIRA®) and infliximab (REMICADE®)), chemokine inhibitors and adhesion molecule inhibitors. The biological response modifiers include monoclonal antibodies as well as recombinant forms of molecules. Exemplary DMARDs include azathioprine, cyclophosphamide, cyclosporine, methotrexate, penicillamine, leflunomide, sulfasalazine, hydroxychloroquine, Gold (oral (auranofin) and intramuscular) and minocycline.

In certain embodiments, the antibodies described herein are administered in conjunction with a cytokine. By “cytokine” as used herein is meant a generic term for proteins released by one cell population that act on another cell as intercellular mediators. Examples of such cytokines are lymphokines, monokines, and traditional polypeptide hormones. Included among the cytokines are growth hormones such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prorelaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); hepatic growth factor; fibroblast growth factor; prolactin; placental lactogen; tumor necrosis factor-alpha and -beta; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin; thrombopoietin (TPO); nerve growth factors such as NGF-beta; platelet-growth factor; transforming growth factors (TGFs) such as TGF-alpha and TGF-beta; insulin-like growth factor-I and -II; erythropoietin (EPO); osteoinductive factors; interferons such as interferon-alpha, beta, and -gamma; colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as IL-1, IL-1alpha, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12; IL-15, a tumor necrosis factor such as TNF-alpha or TNF-beta; and other polypeptide factors including LIF and kit ligand (KL). As used herein, the term cytokine includes proteins from natural sources or from recombinant cell culture, and biologically active equivalents of the native sequence cytokines.

In some embodiments, the compositions comprising herein described TNFR2-specific antibodies are administered to an individual afflicted with a disease as described herein, including, but

not limited to cancers, inflammatory diseases, and autoimmune diseases. Cancers include, but are not limited to, non-Hodgkin's lymphomas, Hodgkin's lymphoma, cutaneous T cell lymphomas, chronic lymphocytic leukemias, acute myeloid leukemias, hairy cell leukemias, acute lymphoblastic leukemias, multiple myeloma, carcinomas of the pancreas, colon, gastric intestine, prostate, bladder, kidney, ovary, cervix, breast, lung, nasopharynx, and malignant melanoma, among others. Thus, certain embodiments include methods for treating a patient having a cancer, comprising administering to the patient a composition described herein, thereby treating the cancer. In some embodiments, the cancer is associated with aberrant TNFR2 expression and/or TNFR2 antagonist-mediated immune suppression. In certain embodiments, the antibody for use in treating cancer is a TNFR2 antagonist.

In some embodiments, the inflammatory or autoimmune disease is associated with aberrant TNFR2 expression. In some embodiments, the inflammatory or autoimmune disease is associated with TNFR2 agonist-mediated immune activation. Exemplary autoimmune diseases include, but are not limited to, arthritis (including rheumatoid arthritis, reactive arthritis), systemic lupus erythematosus (SLE), psoriasis and inflammatory bowel disease (IBD), encephalomyelitis, uveitis, myasthenia gravis, multiple sclerosis, insulin dependent diabetes, Addison's disease, celiac disease, chronic fatigue syndrome, autoimmune hepatitis, autoimmune alopecia, ankylosing spondylitis, ulcerative colitis, Crohn's disease, fibromyalgia, pemphigus vulgaris, Sjogren's syndrome, Kawasaki's Disease, hyperthyroidism/Graves' disease, hypothyroidism/Hashimoto's disease, endometriosis, scleroderma, pernicious anemia, Goodpasture syndrome, Guillain-Barré syndrome, Wegener's disease, glomerulonephritis, aplastic anemia (including multiply transfused aplastic anemia patients), paroxysmal nocturnal hemoglobinuria, myelodysplastic syndrome, idiopathic thrombocytopenic purpura, autoimmune hemolytic anemia, Evan's syndrome, Factor VIII inhibitor syndrome, systemic vasculitis, dermatomyositis, polymyositis and rheumatic fever, autoimmune lymphoproliferative syndrome (ALPS), autoimmune bullous pemphigoid, Parkinson's disease, sarcoidosis, vitiligo, primary biliary cirrhosis, and autoimmune myocarditis.

Exemplary inflammatory diseases include, but are not limited to, Crohn's disease, colitis, dermatitis, psoriasis, diverticulitis, hepatitis, irritable bowel syndrome (IBS), lupus erythematosus, nephritis, Parkinson's disease, ulcerative colitis, multiple sclerosis (MS), Alzheimer's disease, arthritis, rheumatoid arthritis, asthma, and various cardiovascular diseases such as atherosclerosis and vasculitis. In certain embodiments, the inflammatory disease is selected from the group consisting of rheumatoid arthritis, diabetes, gout, cryopyrin-associated periodic syndrome, and chronic obstructive pulmonary disorder. In certain embodiments, the antibody for use in treating an inflammatory or autoimmune disease is a TNFR2 agonist.

For instance, certain embodiments provide a method of treating or reducing the severity of an inflammatory disease, by administering to a patient in need thereof a therapeutically effective amount of a herein disclosed composition comprising agonistic anti-TNFR2 antibodies. Some embodiments provide a method of treating, reducing the severity of, or preventing graft-versus-host disease, by

administering to a transplant patient in need thereof a therapeutically effective amount of a herein disclosed composition comprising agonistic anti-TNFR2 antibodies. Some embodiments provide a method of treating, reducing the severity of, or preventing graft rejection, by administering to a transplant patient in need thereof a therapeutically effective amount of a herein disclosed composition comprising agonistic anti-TNFR2 antibodies.

Certain embodiments provide a method of treating, reducing the severity of or preventing an infectious disease, by administering to a patient in need thereof a therapeutically effective amount of a herein disclosed composition comprising agonistic anti-TNFR2 antibodies. Infectious diseases include, but are not limited to, viral, bacterial, fungal optionally yeast, and protozoal infections.

For *in vivo* use for the treatment of human disease, the antibodies described herein are generally incorporated into a pharmaceutical composition prior to administration. A pharmaceutical composition comprises one or more of the antibodies described herein in combination with a physiologically acceptable carrier or excipient as described elsewhere herein. To prepare a pharmaceutical composition, an effective amount of one or more of the compounds is mixed with any pharmaceutical carrier(s) or excipient known to those skilled in the art to be suitable for the particular mode of administration. A pharmaceutical carrier may be liquid, semi-liquid or solid. Solutions or suspensions used for parenteral, intradermal, subcutaneous or topical application may include, for example, a sterile diluent (such as water), saline solution, fixed oil, polyethylene glycol, glycerin, propylene glycol or other synthetic solvent; antimicrobial agents (such as benzyl alcohol and methyl parabens); antioxidants (such as ascorbic acid and sodium bisulfite) and chelating agents (such as ethylenediaminetetraacetic acid (EDTA)); buffers (such as acetates, citrates and phosphates). If administered intravenously, suitable carriers include physiological saline or phosphate buffered saline (PBS), and solutions containing thickening and solubilizing agents, such as glucose, polyethylene glycol, polypropylene glycol and mixtures thereof.

The compositions comprising TNFR2-specific antibodies as described herein may be prepared with carriers that protect the antibody against rapid elimination from the body, such as time release formulations or coatings. Such carriers include controlled release formulations, such as, but not limited to, implants and microencapsulated delivery systems, and biodegradable, biocompatible polymers, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, polyorthoesters, polylactic acid and others known to those of ordinary skill in the art.

Provided herein are methods of treatment using the antibodies that bind TNFR2. In one embodiment, an antibody of the present disclosure is administered to a patient having a disease involving inappropriate expression of TNFR2, which is meant in the context of the present disclosure to include diseases and disorders characterized by aberrant TNFR2 expression or activity, due for example to alterations (*e.g.*, statistically significant increases or decreases) in the amount of a protein present, or the presence of a mutant protein, or both. An overabundance may be due to any cause, including but not limited to overexpression at the molecular level, prolonged or accumulated

appearance at the site of action, or increased (*e.g.*, in a statistically significant manner) activity of TNFR2 relative to that which is normally detectable. Such an overabundance of TNFR2 can be measured relative to normal expression, appearance, or activity of TNFR2 signaling events, and said measurement may play an important role in the development and/or clinical testing of the antibodies described herein.

In particular, the present antibodies are useful for the treatment of a variety of cancers, including cancers associated with the expression or overexpression of TNFR2. For example, certain embodiments provide a method for the treatment of a cancer including, but not limited to, non-Hodgkin's lymphomas, Hodgkin's lymphoma, cutaneous T cell lymphomas, chronic lymphocytic leukemias, hairy cell leukemias, acute lymphoblastic leukemias, multiple myeloma, carcinomas of the pancreas, colon, gastric intestine, prostate, bladder, kidney, ovary, cervix, breast, lung, nasopharynx, and malignant melanoma, by administering to a cancer patient a therapeutically effective amount of a herein disclosed TNFR2-specific antibody. An amount that, following administration, inhibits, prevents, or delays the progression and/or metastasis of a cancer in a statistically significant manner (*i.e.*, relative to an appropriate control as will be known to those skilled in the art) is considered effective.

Some embodiments provide a method for reducing or preventing metastasis of a cancer including, but not limited to, non-Hodgkin's lymphomas, Hodgkin's lymphoma, cutaneous T cell lymphomas, chronic lymphocytic leukemias, hairy cell leukemias, acute lymphoblastic leukemias, multiple myeloma, carcinomas of the pancreas, colon, gastric intestine, prostate, bladder, kidney, ovary, cervix, breast, lung, nasopharynx, and malignant melanoma, by administering to a cancer patient a therapeutically effective amount of a herein disclosed TNFR2-specific antibody (*e.g.*, an amount that, following administration, inhibits, prevents or delays metastasis of a cancer in a statistically significant manner, *i.e.*, relative to an appropriate control as will be known to those skilled in the art).

Some embodiments provide a method for preventing a cancer including, but not limited to, non-Hodgkin's lymphomas, Hodgkin's lymphoma, cutaneous T cell lymphomas, chronic lymphocytic leukemias, hairy cell leukemias, acute lymphoblastic leukemias, multiple myeloma, carcinomas of the pancreas, colon, gastric intestine, prostate, bladder, kidney, ovary, cervix, breast, lung, nasopharynx, and malignant melanoma, by administering to a cancer patient a therapeutically effective amount of a herein disclosed TNFR2-specific antibody.

Some embodiments provide a method for treating, inhibiting the progression of, or prevention of non-Hodgkin's lymphomas, Hodgkin's lymphoma, cutaneous T cell lymphomas, chronic lymphocytic leukemias, hairy cell leukemias, acute lymphoblastic leukemias, multiple myeloma, carcinomas of the pancreas, colon, gastric intestine, prostate, bladder, kidney, ovary, cervix, breast, lung, nasopharynx, or malignant melanoma by administering to a patient afflicted by one or more of these diseases a therapeutically effective amount of a herein disclosed TNFR2-specific antibody.

In some embodiments, anti-TNFR2 antibodies are used to determine the structure of bound antigen, *e.g.*, conformational epitopes, which structure may then be used to develop compounds having or mimicking this structure, *e.g.*, through chemical modeling and SAR methods.

Some embodiments relate, in part, to diagnostic applications for detecting the presence of cells or tissues expressing TNFR2. Thus, the present disclosure provides methods of detecting TNFR2 in a sample, such as detection of cells or tissues expressing TNFR2. Such methods can be applied in a variety of known detection formats, including, but not limited to immunohistochemistry (IHC), immunocytochemistry (ICC), *in situ* hybridization (ISH), whole-mount *in situ* hybridization (WISH), fluorescent DNA *in situ* hybridization (FISH), flow cytometry, enzyme immuno-assay (EIA), and enzyme linked immuno-assay (ELISA).

ISH is a type of hybridization that uses a labeled complementary DNA or RNA strand (*i.e.*, primary binding agent) to localize a specific DNA or RNA sequence in a portion or section of a cell or tissue (*in situ*), or if the tissue is small enough, the entire tissue (whole mount ISH). One having ordinary skill in the art would appreciate that this is distinct from immunohistochemistry, which localizes proteins in tissue sections using an antibody as a primary binding agent. DNA ISH can be used on genomic DNA to determine the structure of chromosomes. Fluorescent DNA ISH (FISH) can, for example, be used in medical diagnostics to assess chromosomal integrity. RNA ISH (hybridization histochemistry) is used to measure and localize mRNAs and other transcripts within tissue sections or whole mounts.

In various embodiments, the antibodies described herein are conjugated to a detectable label that may be detected directly or indirectly. In this regard, an antibody “conjugate” refers to an anti-TNFR2 antibody that is covalently linked to a detectable label. In the present disclosure, DNA probes, RNA probes, monoclonal antibodies, antigen-binding fragments thereof, and antibody derivatives thereof, such as a single-chain-variable-fragment antibody or an epitope tagged antibody, may all be covalently linked to a detectable label. In “direct detection”, only one detectable antibody is used, *i.e.*, a primary detectable antibody. Thus, direct detection means that the antibody that is conjugated to a detectable label may be detected, *per se*, without the need for the addition of a second antibody (secondary antibody).

A “detectable label” is a molecule or material that can produce a detectable (such as visually, electronically or otherwise) signal that indicates the presence and/or concentration of the label in a sample. When conjugated to an antibody, the detectable label can be used to locate and/or quantify the target to which the specific antibody is directed. Thereby, the presence and/or concentration of the target in a sample can be detected by detecting the signal produced by the detectable label. A detectable label can be detected directly or indirectly, and several different detectable labels conjugated to different specific-antibodies can be used in combination to detect one or more targets.

Examples of detectable labels, which may be detected directly, include fluorescent dyes and radioactive substances and metal particles. In contrast, indirect detection requires the application of

one or more additional antibodies, *i.e.*, secondary antibodies, after application of the primary antibody. Thus, the detection is performed by the detection of the binding of the secondary antibody or binding agent to the primary detectable antibody. Examples of primary detectable binding agents or antibodies requiring addition of a secondary binding agent or antibody include enzymatic detectable binding agents and hapten detectable binding agents or antibodies.

In some embodiments, the detectable label is conjugated to a nucleic acid polymer which comprises the first binding agent (*e.g.*, in an ISH, WISH, or FISH process). In certain embodiments, the detectable label is conjugated to an antibody which comprises the first binding agent (*e.g.*, in an IHC process).

Examples of detectable labels which may be conjugated to antibodies used in the methods of the present disclosure include fluorescent labels, enzyme labels, radioisotopes, chemiluminescent labels, electrochemiluminescent labels, bioluminescent labels, polymers, polymer particles, metal particles, haptens, and dyes.

Examples of fluorescent labels include 5-(and 6)-carboxyfluorescein, 5- or 6-carboxyfluorescein, 6-(fluorescein)-5-(and 6)-carboxamido hexanoic acid, fluorescein isothiocyanate, rhodamine, tetramethylrhodamine, and dyes such as Cy2, Cy3, and Cy5, optionally substituted coumarin including AMCA, PerCP, phycobiliproteins including R-phycoerythrin (RPE) and allophycoerythrin (APC), Texas Red, Princeton Red, green fluorescent protein (GFP) and analogues thereof, and conjugates of R-phycoerythrin or allophycoerythrin, inorganic fluorescent labels such as particles based on semiconductor material like coated CdSe nanocrystallites.

Examples of polymer particle labels include micro particles or latex particles of polystyrene, PMMA or silica, which can be embedded with fluorescent dyes, or polymer micelles or capsules which contain dyes, enzymes or substrates.

Examples of metal particle labels include gold particles and coated gold particles, which can be converted by silver stains. Examples of haptens include DNP, fluorescein isothiocyanate (FITC), biotin, and digoxigenin. Examples of enzymatic labels include horseradish peroxidase (HRP), alkaline phosphatase (ALP or AP), β -galactosidase (GAL), glucose-6-phosphate dehydrogenase, β -N-acetylglucosaminidase, β -glucuronidase, invertase, Xanthine Oxidase, firefly luciferase and glucose oxidase (GO). Examples of commonly used substrates for horseradish peroxidase include 3,3'-diaminobenzidine (DAB), diaminobenzidine with nickel enhancement, 3-amino-9-ethylcarbazole (AEC), Benzidine dihydrochloride (BDHC), Hanker-Yates reagent (HYR), Indophane blue (IB), tetramethylbenzidine (TMB), 4-chloro-1-naphthol (CN), alpha-naphthol pyronin (.alpha.-NP), o-dianisidine (OD), 5-bromo-4-chloro-3-indolylphosphate (BCIP), Nitro blue tetrazolium (NBT), 2-(p-iodophenyl)-3-p-nitrophenyl-1-5-phenyl tetrazolium chloride (INT), tetranitro blue tetrazolium (TNBT), 5-bromo-4-chloro-3-indoxyl-beta-D-galactoside/ferro-ferricyanide (BCIG/FF).

Examples of commonly used substrates for Alkaline Phosphatase include Naphthol-AS-B 1-phosphate/fast red TR (NABP/FR), Naphthol-AS-MX-phosphate/fast red TR (NAMP/FR), Naphthol-

AS-B1-phosphate/- fast red TR (NABP/FR), Naphthol-AS-MX-phosphate/fast red TR (NAMP/FR), Naphthol-AS-B1-phosphate/new fuschin (NABP/NF), bromochloroindolyl phosphate/nitroblue tetrazolium (BCIP/NBT), 5-Bromo-4-chloro-3-indolyl-b-- d-galactopyranoside (BCIG).

Examples of luminescent labels include luminol, isoluminol, acridinium esters, 1,2-dioxetanes and pyridopyridazines. Examples of electrochemiluminescent labels include ruthenium derivatives. Examples of radioactive labels include radioactive isotopes of iodide, cobalt, selenium, tritium, carbon, sulfur and phosphorous.

Detectable labels may be linked to the antibodies described herein or to any other molecule that specifically binds to a biological marker of interest, *e.g.*, an antibody, a nucleic acid probe, or a polymer. Furthermore, one of ordinary skill in the art would appreciate that detectable labels can also be conjugated to second, and/or third, and/or fourth, and/or fifth binding agents or antibodies, etc. Moreover, the skilled artisan would appreciate that each additional binding agent or antibody used to characterize a biological marker of interest may serve as a signal amplification step. The biological marker may be detected visually using, *e.g.*, light microscopy, fluorescent microscopy, electron microscopy where the detectable substance is for example a dye, a colloidal gold particle, a luminescent reagent. Visually detectable substances bound to a biological marker may also be detected using a spectrophotometer. Where the detectable substance is a radioactive isotope detection can be visually by autoradiography, or non-visually using a scintillation counter. See, *e.g.*, Larsson, 1988, *Immunocytochemistry: Theory and Practice*, (CRC Press, Boca Raton, Fla.); *Methods in Molecular Biology*, vol. 80 1998, John D. Pound (ed.) (Humana Press, Totowa, N.J.).

The disclosure further provides kits for detecting TNFR2 or cells or tissues expressing TNFR2 in a sample, wherein the kits contain at least one antibody, polypeptide, polynucleotide, vector or host cell as described herein. In certain embodiments, a kit may comprise buffers, enzymes, labels, substrates, beads or other surfaces to which the antibodies of the disclosure are attached, and the like, and instructions for use.

Examples

Example 1

Immunization and Primary Screening

To prepare antibodies for screening, four New Zealand white rabbits were immunized and subsequently boosted with either human 293-TNFR2 overexpressing cells or human TNFR2-Fc fusion protein. All rabbits had serum titers specific against human TNFR2 and were used to generate hybridoma using APXiMAB™ technology and antibodies by B cell culture method (RevMAb). Upon primary screening, 460 antibodies were identified that bound to CHO-TNFR2 overexpressing cells. The screening process to identify potential lead candidates is shown in **Figure 2**.

Supernatants from hybridoma and B cell cultures were then used to screen for antibodies that could block the binding of TNF- α to soluble TNFR2-His (Sino Biological; 10417-H08H) in an

ELISA-based receptor-ligand binding assay. Of the 460 antibodies identified, 173 showed inhibition of TNF- α binding to TNFR2 at greater than 75% of maximum binding.

110 antibodies were selected to move to the human IgG chimeric stage. Of the 110 antibodies advanced from B cell cloning, 28 clones failed to amplify. The heavy and light chains of the 82 remaining clones were amplified and directly cloned onto the human IgG1 backbone. The 82 chimeric antibodies were expressed and supernatants tested positive for binding to cell-based TNFR2 and were sequenced. All 10 antibodies identified from hybridoma that passed the initial screening funnel were chimerized, sequenced, and moved forward.

Sequence analysis (uniqueness and limited potential development liabilities such as glycosylation, deamination, etc., and CDR3 length) was employed to select 25 antibodies from B cell culture for further study. All 10 hybridoma clones were advanced independently of sequence analysis. Two sets of clones from hybridoma had identical sequences as indicated in summary table (**Table E1**) with asterisks or hashtags (8G11.5 = 37D1.4 and 28B7.3=37H4.1). To express the recombinant chimeric antibodies, Heavy (H) and Light (L) chain plasmids were co-transfected into 293 cells and supernatants were harvested after 7-10 days. Antibodies were purified via Protein A column and dialyzed against PBS.

***In Vitro* Characterization of Human Chimeric Antibodies.** Chimeric antibodies were screened for binding to soluble and cell expressed human TNFR2, soluble cynomolgus and mouse TNFR2, and ELISA-based TNF- α blocking. The data set in Figures 3A-3D shows the first set of antibodies, and the data set in Figures 4A-4D shows the second set of antibodies. Antibodies were also screened in a peptide-binding ELISA for preliminary epitope binning using peptides from the PLAD or CRD1 (aa 17-54; TCRLREYYDQTAQMCCSKCSPGQHAKVFCTKTSDTVCD), CRD2 (aa 58-93; DSTYTQLWNWVPECLSCGSRCSDDQVETQACTREQN), CRD3 (aa 106-133; LSKQEGCRLCAPLRKCRPGFGVARPGTE and aa 114-133; LCAPLRKCRPGFGVARPGTE) and CRD4 (peptide 5; aa 146-174; and TFSNTTSSTDICRPHQICNVVAIPGNAS) domains (see data summary in **Table E1** below).

Clone	EC50 (nM) hTNFR2 (soluble)	EC50 (nM) hTNFR2 (cell)	hTNFR2 cell binding rank	EC50 (nM) Cyno TNFR2	IC50 (nM) TNF α Blocking	TNF α Blocking Rank	Potential peptide bin	mouse cross- reactivity
4	0.094	0.147	10	0.099	2.743	4	ND	N
6	0.147	0.277	17	0.104	5.634	18	PLAD	N
9	0.144	0.147	11	0.151	2.783	5	ND	N
23,71	0.206	0.260	16	0.243	5.41	16	3/4	N
24	0.098	0.100	6	0.093	3.156	9	2/3	N
25,71	0.147	0.091	4	0.197	2.98	7	3	N
37	0.126	0.100	7	0.148	3.253	10	ND	N
42	0.242	0.091	5	0.184	11.17	21	ND	N
57	0.067	0.073	1	0.057	0.7407	1	2/3	N
75	0.1927	0.1261	9	0.2154	4.247	13	3/4	N
81	7.387	9.443	24	4.036	NO	-	PLAD	N
82	0.238	0.172	13	0.192	3.542	11	3	N
92	0.131	0.086	2	0.191	3.056	8	3	N

102	0.2068	0.09014	3	0.3268	100	25	ND	N
107	0.325	0.485	19	0.315	2.221	2	ND	N
108	0.256	0.180	15	0.327	4.555	15	4	N
127	0.3134	0.1097	8	60.04	6.616	20	ND	N
7H2.5	0.067	ND	ND	0.1774	5.542	17	3	N
8G10.7	0.2431	ND	ND	0.2686	NO	-	ND	N
8G11.5*	0.157	ND	ND	0.09275	24.57	23	ND	N
9B11.2	0.193	ND	ND	0.1382	WEAK	-	ND	N
11D7.1	0.1398	0.17	12	0.03963	6.095	19	3	N
11G12.1	0.2612	ND	ND	0.1908	WEAK	-	ND	N
28B7.3#	0.1486	0.321	18	0.07626	2.97	7	3	N
37D1.4*	0.1559	ND	ND	0.08096	27.79	24	ND	N
37H4.1#	0.1839	ND	ND	0.1947	2.923	6	3	N
55F6.1	0.1238	1.32	22	0.08616	4.353	14	ND	N
24-2	0.0981	ND	ND	0.07422	NO	-	5	N
25-10	0.08616	0.513	20	0.02353	ENHANCE	-	5	Y
24-25	0.2101	ND	ND	0.1893	ENHANCE	-	5	Y
24-62	0.1374	ND	ND	0.09579	ENHANCE	-	5	Y
24-97	0.06322	ND	ND	0.08597	NO	-	5	Y
24-124	0.1372	0.179	14	0.08818	2.537	3	5	N
25-31	0.07043	3.145	23	0.03804	4.129	12	5	N
25-103	0.04426	0.946	21	0.0003477	ENHANCE	-	5	Y

Fifteen of the 35 antibodies were selected for humanization based on binding affinity, cyno cross-reactivity, blocking ability, and ability to capture a range of epitope bins (highlighted in bold in Table E1). Of the 15 clones humanized, three clones did not block TNF- α binding by ELISA but bound to peptide five and were hypothesized to block TNF- α on the cell surface by blocking TNFR2 trimerization. Clones were deselected that had identical sequences, and which did not potently bind cynomolgus or human TNFR2, or block TNF- α binding.

Antibodies were humanized using a proprietary Mutational Lineage Guided (MLG) technology on the human IgG₁ framework. One of the 15 antibodies lost binding to antigen on ELISA and could not be recovered. The other 14 antibodies were screened for soluble human TNFR1 and TNFR2 binding, cell-based TNFR2 binding, cyno TNFR2 binding, and TNF- α blocking by both ELISA and FACS (see Figures 5-8). The antibodies that bound peptide 5 and did not block TNF- α at the chimeric stage did not block TNF- α binding by cell-based ELISA, and thus were deselected. Antibodies that retained strong cell-based TNFR2 binding were tested for ADCC of TNFR2 overexpressing CHO cell lines using the Promega ADCC Jurkat NFAT reporter kit (Figures 9A-9B). The characteristics of the humanized candidates are shown in Table E2 below. Five lead candidates (highlighted in bold) met the desired criteria.

Table E2								
Clone	EC50 (nM) hTNFR2 (soluble)	EC50 (nM) hTNFR2 (cell)	KD (nM) hTNFR2 (OE cell)	EC50 (nM) Cyno TNFR2	IC50 (nM) TNF α Blocking (soluble)	IC50 (nM) TNF α Blocking (cell)	ADCC	human TNFR1 cross- reactivity
108	0.105	0.31	0.0994	0.046	1.333	0.198	+++	
25-71	0.159	0.242	0.12	0.123	1.964	0.149	+++	
11D7.1	0.091	0.239	0.2013	0.054	2.955	0.3225	++	+
28B7.3	0.24	0.28	0.1298	0.211	2.465	0.3788	+	
24-2	0.149	0.377	0.2449	0.131	3.517	0.447	+	
55F6.1	0.131	0.672	0.539	0.109	3.81	0.566		
24-124	0.098	0.408	0.2906	0.058	2.295	0.5669	++	
9	0.051	0.587	0.3868	0.046	0.465	7.02		
4	0.111	1.129		0.34	3.572	NO		
25-10	0.053	1.38	1.053	0.113	NO	NO	+	
25-31	0.72	12.08	12.08	1.858	NO	NO	+	+
25-103	0.097	0.57	0.468	0.107	NO	NO	+++	
24	0.142	1.986	1.496	0.091	WEAK	NO		
37	0.072	109.1	n/a	0.056	NO	ND		
92	-	-	-		ND	ND		

Selection of Lead Candidate and Backup. ADCC assay on overexpressing cell line, along with TNFR family member specificity, cytokine release assays, and developability analysis were completed to aid in selection of the 2 lead candidates advanced to CLD. **Table E3** below shows the percent humanization analysis.

Table E3				
Humanized Leads	HC Germline framework	HC % identity to IMGT framework	LC Germline framework	LC % identity to IMGT framework
h600_23_28B7	VH3-66 5//87	94.3	VK02 4/87	95.4
h600_25_108	VH3-66 4//87	95.4	VK02 3/87	96.5
h600_25_71	VH3-66 5//87	94.3	VKL5 3/87	96.5
h600_24_2	VH3-66 4//87	95.4	VK02 2/87	97.7
h600_24_124	VH3-66 6//87	93.1	VK02 2/87	97.7

Example 2

Binding and Activity Characteristics of Clones h600-25-71 and h600-25-108

Humanized clones h600-25-71 and h600-25-108 were tested for binding and activity characteristics. To test binding to TNFR family members, target proteins were coated on ELISA plates at 1 µg/mL at 4°C overnight. Plates were washed, blocked, and antibodies were added at indicated concentrations for 1 hour at RT. Positive control antibodies for each protein were used at ~1 µg/mL. Antibodies were washed and detected with anti-human IgG HRP for 1 hour. Assay was developed using TMB substrate for 10 minutes. For cell-binding, human CD4⁺ T cells purified from buffy coats were cultured in flat-bottom plates with anti-CD3/CD28 for 24 hours. Cells were harvested and stained for viability, CD4, CD25 and FOXP3. Test antibody staining was detected using anti-human IgG APC. Binding titration of test antibodies was evaluated on CD4⁺CD25^{hi}FOXP3⁺ regulatory T cells and plotted as percent binding.

The results in **Figures 10A-10F** show that 25-71 and 25-108 bind with high affinity to TNFR2, including human TNFR2 protein (10C), cynomolgus TNFR2 protein (10D), cell-expressed human TNFR2 (10E), and activated human T_{regs} (10F). The results in **Figures 11A-11E** show that 25-71 and 25-108 are specific to TNFR2, and do not bind to TNFR1, HVEM, CD40, DR6, or OPG. IgG isotype control is shown in triangles and positive controls are shown as an asterisk.

To test the effect of antibodies against TNFR2-expressing cells via antibody-dependent cellular cytotoxicity (ADCC), ADCC assays were performed as described above. As shown in **Figures 12A-12B**, 25-71 and 25-108 induced ADCC against TNFR2-expressing cells, including tumor cells (12B).

To test the effect of antibodies on T_{regs}, PBMCs were isolated from leukocyte reduction chambers. 2x10⁵ PBMCs were stimulated with 0.1 µg/mL anti-CD3 (clone: OKT3) and treated with anti-TNFR2 antibodies for 16-24 hr in a humidified, 5% CO₂ incubator at 37°C in a 96-well flat-bottom plate. The cells were cultured in complete RPMI (10% heat-inactivated FBS, 1X penicillin-

streptomycin, 1mM Sodium Pyruvate, 1X MEM non-essential amino acid, 50mM β -Mercaptoethanol). Cells were harvested and CD4⁺ Treg were identified as CD4⁺ CD25^{hi} FoxP3⁺ by flow cytometry. As shown in Figures 13A-13B, 25-71 and 25-108 depleted T_{regs} from human PBMCs. Both graphs show an average of 5 donors wherein each condition was performed in duplicate. % depletion = $100 * (\text{No Depletion} - \text{Experimental}) / (\text{No Depletion})$ where no depletion refers to PBMCs stimulated with anti-CD3 only.

To test the effect of antibodies on macrophage-mediated antibody-dependent cellular phagocytosis (ADCP) of TNFR2-expressing tumor cells (see Figure 14A), macrophages were produced by culturing human CD14⁺ monocytes with 10 μ g/mL M-CSF for 6 days in a 37°C incubator with 5% CO₂. Resulting macrophages, along with TNFR2-overexpressing CHO cells (target), were labeled with CellTrace CFSE and Violet, respectively. Target cells were incubated with anti-TNFR2 antibody or isotype control on ice for 30 minutes. Labeled cells were co-cultured at a ratio of 100,000 macrophages to 50,000 target cells in RPMI with low human IgG serum for 3 hours. Cells were analyzed using a Cytoflex LX flow cytometer. As shown in Figure 14B, 25-71 and 25-108 induced macrophage-mediated ADCP of TNFR2⁺ cells. Percent phagocytosis was calculated as follows: $(\% \text{ dual positive cells}) / (\text{Total } \% \text{ target cells}) * 100$.

To test the effect of antibodies on myeloid-derived suppressor cell (MDSC) suppression of CD8 T cells, MDSC were generated by co-culture of human PBMC with 786-O tumor cell line for 7 days. CD33⁺ MDSC were isolated by positive selection using microbeads (Miltenyi). Autologous CD8 T cells were labeled with cell trace violet and co-cultured with MDSC at a 4:1 ratio for 3 days with CD3/CD28 stimulation. After 3 days, cells were labeled with viability dye and CD8 T cell proliferation was measured using violet dye dilution. See experimental outline in Figure 15A.

As shown in Figures 15B-15C, 25-71 and 25-108 significantly reduced MDSC suppression of CD8 T cells. Percent suppression was calculated as follows: $[(\text{T cells alone} - (\text{T} + \text{MDSC})) / \text{T alone}] * 100$. Data is plotted as mean $\pm$ SEM. IgG1 isotype is plotted using black bars, 25-71 is in gray bars and 25-108 is in open bars. Statistics were generated using ANOVA and * = $P < 0.01$ and ** = $P < 0.001$.

To further test the effect of antibodies on regulatory T cells, CD4 T cells were isolated from PBMCs derived from healthy human buffy coats by negative selection using magnetic beads (Miltenyi). CD25⁺ T_{regs} were depleted using CD25 magnetic beads according to manufacturers protocol (Miltenyi), and the resulting CD4 cells (T responder cells) were cryopreserved until assay set up. T_{regs} from autologous donors were isolated according manufacturers protocol and expanded for 15 days using Treg expander beads (DynaBeads) and 100nM rapamycin. On day before assay set up, T responder cells were thawed and rested overnight. The next day, T responder cells were labeled with Cell Trace Violet and added to T_{regs} at the indicated ratios in round-bottom plates with 10 μ g/mL of 25-71 or isotype control. T_{reg} inspector beads (Miltenyi) were added and assays was incubated for 5 days. Cells were harvested on day 5, stained with viability dye, and analyzed on a MACSQuant Analyzer.

Percent T cell suppression was calculated as (proliferation of stimulated T responder only – proliferation of test value)/(stimulated T responder only). Experiment was performed on at least four donors. As shown in **Figures 18A-18E**, clone 25-71 reverses T_{reg} suppression of effector T cells.

Binding affinity (KD) and EC₅₀ values from the foregoing experiments are summarized in **Table E4** below.

Table E4		
	Ab 25-71	Ab 25-108
Primary Cell Affinity (KD)	47 pM	50 pM
ADCC EC ₅₀ (reporter line)	1.14 nM	1.135 nM
ADCP EC ₅₀	0.71 nM	0.92 nM

To test the effects of antibodies in mice, female nude mice were injected SQ with Colo205 tumors cells. At tumor volume of 100mm³, mice were treated as indicated in **Figure 19A**. Significant anti-tumor effect (48% TGI) was seen with antibody 25-71 relative to control (see **Figure 19B**). No change in body weight was seen in treatment groups relative to control (see **Figure 19C**).

Example 3

Identification of Epitope Sites for Clone 25-71

Experiments were performed to determine the epitope of the TNFR2/25-71 complex with high resolution.

First, high-mass MALDI analysis was performed on samples of TNFR2 alone and clone 25-71 alone to verify integrity and aggregation level. The measurements were performed using an Autoflex II MALDI ToF mass spectrometer (Bruker) equipped with an HM4 interaction module (CovalX), which contains a detecting system designed to optimize detection up to 2Mda with nano-molar sensitivity. The TNFR2 sample powder was dissolved with distilled water to reach a concentration of 1 mg/ml, and 20 µl of each protein sample of TNFR2 and clone 25-71 was pipetted to prepare 8 dilutions with a final volume 10µl. Then, 1 µl of each dilution was mixed with 1 µl of a matrix composed of a re-crystallized sinapinic acid matrix (10 mg/ml) in acetonitrile/water (1:1, v/v), TFA 0.1% (K200 MALDI Kit). After mixing, 1 µl of each sample was spotted on the MALDI plate (SCOUT 384). After crystallization at room temperature, the plate was introduced in the MALDI mass spectrometer and analyzed immediately in High-Mass MALDI mode.

Cross-link Experiments. The cross-linking experiments allow the direct analysis of non-covalent interaction by High-Mass MALDI mass spectrometry. By mixing a protein sample containing non covalent interactions with a cross-linking mixture (Bich et al., Anal. Chem. 82 (1), pp 172–179, 2010), it is possible to specifically detect non covalent complex with high-sensitivity. The covalent binding generated allows the interacting species to survive the sample preparation process and the MALDI ionization. A High-Mass detection system allows characterizing the interaction in the High-Mass range.

Each mixture prepared for the control experiment (9 μ l left) was submitted to cross-linking using the K200 MALDI MS analysis kit (CovalX). Nine μ l of the mixtures (from 1 to 1/128) were mixed with 1 μ l of K200 Stabilizer reagent (2 mg/ml) and incubated at room temperature. After the incubation time (180 minutes) the samples were prepared for MALDI analysis as for Control experiments. The samples were analyzed by High-Mass MALDI analysis immediately after crystallization, using the HM4 interaction module (CovalX) with a standard nitrogen laser and focusing on different mass ranges from 0 to 1500 kDa.

Results. High-Mass MALDI mass spectrometry and chemical cross-linking analysis did not detect any non-covalent aggregates of clone 25-71 or multimers of TNFR2.

Complexes of TNFR2/25-71 were then characterized using an Autoflex II MALDI ToF mass spectrometer (Bruker) equipped with HM4 interaction module (CovalX). A 10 μ l mixture of TNFR2/25-71 was prepared with the respective concentrations of 1.25 μ M/0.5 μ M. One μ l of the mixture was mixed with 1 μ l of a matrix composed of a re-crystallized sinapinic acid matrix (10 mg/ml) in acetonitrile/water (1:1, v/v), TFA 0.1% (K200 MALDI Kit). After mixing, 1 μ l of each sample was spotted on the MALDI plate (SCOUT 384). After crystallization at room temperature, the plate was introduced in the MALDI mass spectrometer and analyzed immediately.

Cross-link Experiments. The mixture prepared for the control experiment (9 μ l left) was submitted to cross-linking using a K200 MALDI MS analysis kit (CovalX). Nine μ l of the mixture was mixed with 1 μ l of K200 Stabilizer reagent (2 mg/ml) and incubated at room temperature. After the incubation time (180 minutes) the samples were prepared for MALDI analysis as for Control experiments. The samples were analyzed by High-Mass MALDI analysis immediately after crystallization.

The MALDI ToF MS analysis has been performed using the HM4 interaction module (CovalX) with a standard nitrogen laser and focusing on different mass ranges from 0 to 1500 kDa.

Results. For the control experiment, TNFR2 and clone 25-71 were detected with $MH^+=37.179$ kDa and $MH^+=147.708$ kDa. After cross-linking, two additional peaks were detected with $MH^+=189.401$ kDa and $MH^+=228.341$ kDa. The control and cross-link spectra were overlaid using Complex Tracker software, which detected two non-covalent protein complexes with $MH^+=186.113$ kDa and $MH^+=224.384$ kDa.

For epitope determination, TNFR2 was first subject to trypsin, chymotrypsin, Asp-N, elastase, and thermolysin proteolysis followed by nLC-LTQ-Orbitrap MS/MS analysis. Combined mapping of the peptides confirmed coverage of about 94.57% of the TNFR2 sequence (data not shown).

To determine the epitope of TNFR2/25-71 complexes with high resolution, the complexes were incubated with deuterated cross-linkers and subjected to multi-enzymatic cleavage by trypsin, chymotrypsin, Asp-N, elastase, and thermolysin. After enrichment of the cross-linked peptides, the

samples were analyzed by high resolution mass spectrometry (nLC-LTQ-Orbitrap MS) and the data generated were analyzed using XQuest and Stavrox software.

Reduction Alkylation. Twenty μL of the TNFR2/clone 25-71 mixture was mixed with 2 μL of DSS d0/d12 (2 mg/mL; DMF) for 180 minutes incubation time at room temperature. After incubation, the reaction was stopped by adding 1 μL of Ammonium Bicarbonate (20 mM final concentration) before 1 hour incubation time at room temperature. Then, the solution was dried using a speedvac before H_2O 8M urea suspension (20 μL). After mixing, 2 μL of DTT (500 mM) was added to the solution. The mixture was then incubated for 1 hour at 37°C. After incubation, 2 μL of iodoacetamide (1M) was added before 1 hour incubation time at room temperature, in a dark room. After incubation, 80 μL of the proteolytic buffer was added. The trypsin buffer contained 50mM Ambic pH 8.5, 5% acetonitrile, the chymotrypsin buffer contained Tris HCl 100mM, CaCl_2 10mM pH 7.8, the ASP-N buffer contained phosphate buffer 50MM pH 7.8, the elastase buffer contained Tris HCl 50mM pH 8.0, and the thermolysin buffer contained Tris HCl 50mM, CaCl_2 0.5mM pH 9.0.

For trypsin proteolysis, 100 μL of the reduced/alkyld TNFR2/25-71 mixture was mixed with 0.5 μL of trypsin (Promega) with the ratio 1/100, and the proteolytic mixtures were incubated overnight at 37°C. For chymotrypsin proteolysis, 100 μL of the reduced/alkyld TNFR2/25-71 mixture was mixed with 0.25 μL of chymotrypsin (Promega) with the ratio 1/200, and the proteolytic mixtures were incubated overnight at 25°C. For ASP-N proteolysis, 100 μL of the reduced/alkyld TNFR2/25-71 mixture was mixed with 0.25 μL of ASP-N (Promega) with the ratio 1/200, and the proteolytic mixtures were incubated overnight at 37°C. For elastase proteolysis, 100 μL of the reduced/alkyld TNFR2/25-71 mixture was mixed with 0.5 μL of elastase (Promega) with the ratio 1/100, and the proteolytic mixtures were incubated overnight at 37°C. For thermolysin proteolysis, 100 μL of the reduced/alkyld TNFR2/25-71 mixture was mixed with 1 μL of thermolysin (Promega) with a ratio 1/50, and the proteolytic mixtures were incubated overnight at 70°C. After digestion, formic acid 1% final was added to the solution. The cross-linked peptides were analyzed using Xquest version 2.0 and Stavrox 3.6 software.

Results. After trypsin, chymotrypsin, ASP-N, elastase, and thermolysin proteolysis of the TNFR2/25-71 complexes with deuterated d0d12, the nLC-orbitrap MS/MS analysis detected 12 cross-linked peptides between TNFR2 and clone 25-71. Using chemical cross-linking, High-Mass MALDI mass spectrometry, and nLC-Orbitrap mass spectrometry, the molecular interface between TNFR2 and the antibody 25-71 was characterized. The results of this analysis are illustrated in **Figure 16** and **Figures 17A-17J**, which indicate that the TNFR2/25-71 interaction includes the following residues of **full-length** human TNFR2; R43, Y45, T49, S55, K56, T73, and S77; or the following residues of **mature** human TNFR2; R21, Y23, T27, S33, K34, T51, and S55.

Claims

1. An isolated antibody, or an antigen-binding fragment thereof, that binds to tumor necrosis factor receptor 2 (TNFR2), comprising:
 - a heavy chain variable (VH) region comprising VHCDR1, VHCDR2, and VHCDR3 regions set forth respectively in SEQ ID NOs: 1-3; and a light chain variable (VL) region comprising VLCDR1, VLCDR2, and VLCDR3 regions set forth respectively in SEQ ID NOs: 4-6;
 - a VH region comprising VHCDR1, a VHCDR2, and VHCDR3 regions set forth respectively in SEQ ID NOs: 7-9; and a VL region comprising VLCDR1, VLCDR2, and VLCDR3 regions set forth respectively in SEQ ID NOs: 10-12;
 - a VH region comprising VHCDR1, VHCDR2, and VHCDR3 regions set forth respectively in SEQ ID NOs: 13-15; and a VL region comprising VLCDR1, VLCDR2, and VLCDR3 regions set forth respectively in SEQ ID NOs: 16-18;
 - a VH region comprising VHCDR1, VHCDR2, and VHCDR3 regions set forth respectively in SEQ ID NOs: 19-21; and a VL region comprising VLCDR1, VLCDR2, and VLCDR3 regions set forth respectively in SEQ ID NOs: 22-24;
 - a VH region comprising VHCDR1, VHCDR2, and VHCDR3 regions set forth respectively in SEQ ID NOs: 25-27; and a VL region comprising VLCDR1, VLCDR2, and VLCDR3 regions set forth respectively in SEQ ID NOs: 28-30;
 - a VH region comprising VHCDR1, VHCDR2, and VHCDR3 regions set forth respectively in SEQ ID NOs: 31-33; and a VL region comprising VLCDR1, VLCDR2, and VLCDR3 regions set forth respectively in SEQ ID NOs: 34-36;
 - a VH region comprising VHCDR1, VHCDR2, and VHCDR3 regions set forth respectively in SEQ ID NOs: 37-39; and a VL region comprising VLCDR1, VLCDR2, and VLCDR3 regions set forth respectively in SEQ ID NOs: 40-42;
 - a VH region comprising VHCDR1, VHCDR2, and VHCDR3 regions set forth respectively in SEQ ID NOs: 43-45; and a VL region comprising VLCDR1, VLCDR2, and VLCDR3 regions set forth respectively in SEQ ID NOs: 46-48;
 - a VH region comprising VHCDR1, VHCDR2, and VHCDR3 regions set forth respectively in SEQ ID NOs: 49-51; and a VL region comprising VLCDR1, VLCDR2, and VLCDR3 regions set forth respectively in SEQ ID NOs: 52-54;
 - a VH region comprising VHCDR1, VHCDR2, and VHCDR3 regions set forth respectively in SEQ ID NOs: 55-57; and a VL region comprising VLCDR1, VLCDR2, and VLCDR3 regions set forth respectively in SEQ ID NOs: 58-60;
 - a VH region comprising VHCDR1, VHCDR2, and VHCDR3 regions set forth respectively in SEQ ID NOs: 61-63; and a VL region comprising VLCDR1, VLCDR2, and VLCDR3 regions set forth respectively in SEQ ID NOs: 64-66;

a VH region comprising VHCDR1, VHCDR2, and VHCDR3 regions set forth respectively in SEQ ID NOs: 67-69; and a VL region comprising VLCDR1, VLCDR2, and VLCDR3 regions set forth respectively in SEQ ID NOs: 70-72;

a VH region comprising VHCDR1, VHCDR2, and VHCDR3 regions set forth respectively in SEQ ID NOs: 73-75; and a VL region comprising VLCDR1, VLCDR2, and VLCDR3 regions set forth respectively in SEQ ID NOs: 76-78;

a VH region comprising VHCDR1, VHCDR2, and VHCDR3 regions set forth respectively in SEQ ID NOs: 79-81; and a VL region comprising VLCDR1, VLCDR2, and VLCDR3 regions set forth respectively in SEQ ID NOs: 82-84;

a VH region comprising VHCDR1, VHCDR2, and VHCDR3 regions set forth respectively in SEQ ID NOs: 85-87; and a VL region comprising VLCDR1, VLCDR2, and VLCDR3 regions set forth respectively in SEQ ID NOs: 88-90;

a VH region comprising VHCDR1, VHCDR2, and VHCDR3 regions set forth respectively in SEQ ID NOs: 91-93; and a VL region comprising VLCDR1, VLCDR2, and VLCDR3 regions set forth respectively in SEQ ID NOs: 94-96; or

a VH region comprising VHCDR1, VHCDR2, and VHCDR3 regions set forth respectively in SEQ ID NOs: 97-99; and a VL region comprising VLCDR1, VLCDR2, and VLCDR3 regions set forth respectively in SEQ ID NOs: 100-102;

or a variant of said antibody, or an antigen-binding fragment thereof, comprising heavy and light chain variable regions identical to the heavy and light chain variable regions of (i) and (ii) except for up to 1, 2, 3, 4, 5, 6, 7, or 8 total amino acid substitutions across said CDR regions.

2. The isolated antibody, or antigen-binding fragment thereof, of claim 1, wherein the VH region comprises an amino acid sequence having at least 90% identity to a sequence selected from SEQ ID NOs: 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, and 135.

3. The isolated antibody, or antigen-binding fragment thereof, of claim 1 or 2, wherein the VL region comprises an amino acid sequence having at least 90% identity to a sequence selected from SEQ ID NOs: 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 134, and 136.

4. The isolated antibody, or antigen-binding fragment thereof, of claim 2 or 3, comprising:

the VH region set forth in SEQ ID NO: 103, and the VL region set forth in SEQ ID NO: 104;
the VH region set forth in SEQ ID NO: 105, and the VL region set forth in SEQ ID NO: 106;
the VH region set forth in SEQ ID NO: 107, and the VL region set forth in SEQ ID NO: 108;

the VH region set forth in SEQ ID NO: 109, and the VL region set forth in SEQ ID NO: 110;
the VH region set forth in SEQ ID NO: 111, and the VL region set forth in SEQ ID NO: 112;
the VH region set forth in SEQ ID NO: 113, and the VL region set forth in SEQ ID NO: 114;
the VH region set forth in SEQ ID NO: 115, and the VL region set forth in SEQ ID NO: 116;
the VH region set forth in SEQ ID NO: 117, and the VL region set forth in SEQ ID NO: 118;
the VH region set forth in SEQ ID NO: 119, and the VL region set forth in SEQ ID NO: 120;
the VH region set forth in SEQ ID NO: 121, and the VL region set forth in SEQ ID NO: 122;
the VH region set forth in SEQ ID NO: 123, and the VL region set forth in SEQ ID NO: 124;
the VH region set forth in SEQ ID NO: 125, and the VL region set forth in SEQ ID NO: 126;
the VH region set forth in SEQ ID NO: 127, and the VL region set forth in SEQ ID NO: 128;
the VH region set forth in SEQ ID NO: 129, and the VL region set forth in SEQ ID NO: 130;
the VH region set forth in SEQ ID NO: 131, and the VL region set forth in SEQ ID NO: 132;
the VH region set forth in SEQ ID NO: 133, and the VL region set forth in SEQ ID NO: 134;

or

the VH region set forth in SEQ ID NO: 135, and the VL region set forth in SEQ ID NO: 136.

5. An isolated antibody, or an antigen-binding fragment thereof, that binds to tumor necrosis factor receptor 2 (TNFR2), comprising a heavy chain variable (VH) region which comprises an amino acid sequence having at least 90% identity to a sequence selected from SEQ ID NOs: 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, and 135, and, respectively, a light chain variable (VL) region which comprises an amino acid sequence having at least 90% identity to a sequence selected from SEQ ID NO: 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 134, and 136.

6. An isolated antibody, or an antigen-binding fragment thereof, that binds to tumor necrosis factor receptor 2 (TNFR2), comprising a heavy chain variable (VH) region comprising VHCDR1, VHCDR2, and VHCDR3 regions selected from the underlined sequences in **Table R1**; and, respectively, a light chain variable (VL) region comprising VLCDR1, VLCDR2, and VLCDR3 regions selected from underlined sequences in **Table R2**.

7. The isolated antibody, or an antigen-binding fragment thereof, of claim 6, comprising a VH region which comprises an amino acid sequence selected from **Table R1**, and, respectively, a VL region which comprises an amino acid sequence selected from **Table R2**.

8. An isolated antibody, or an antigen-binding fragment thereof, that binds to human tumor necrosis factor receptor 2 (TNFR2) at an epitope that comprises, consists, or consists essentially of one or more residues selected from R21, Y23, T27, S33, K34, T51, and S55, as defined

by the mature human TNFR2 sequence (residues 23-461 of FL human TNFR2), optionally wherein the epitope comprises, consists, or consists essentially of one or more residues selected from REY, TAQMCCSK (SEQ ID NO: 328), and TVCDS (SEQ ID NO: 329).

9. The isolated antibody, or antigen-binding fragment thereof, of claim 8, comprising a heavy chain variable (VH) region comprising VHCDR1, VHCDR2, and VHCDR3 regions set forth respectively in SEQ ID NOs: 37-39; and a light chain variable (VL) region comprising VLCDR1, VLCDR2, and VLCDR3 regions set forth respectively in SEQ ID NOs: 40-42.

10. The isolated antibody, or antigen-binding fragment thereof, of claim 8 or 9, wherein the VH region comprises an amino acid sequence having at least 90% identity to SEQ ID NO: 115.

11. The isolated antibody, or antigen-binding fragment thereof, of any one of claims 8-10, wherein the VL region comprises an amino acid sequence having at least 90% identity to SEQ ID NO: 116.

12. The isolated antibody, or antigen-binding fragment thereof, of claim 10 or 11, comprising the VH region set forth in SEQ ID NO: 115, and the VL region set forth in SEQ ID NO: 116.

13. The isolated antibody, or an antigen-binding fragment thereof, of any one of claims 1-12, which binds to human TNFR2, optionally soluble and cell-expressed human TNFR2.

14. The isolated antibody, or an antigen-binding fragment thereof, of claim 13, which binds to at least one, two, three, four, or five human TNFR2 peptide epitopes selected from **Table T1**.

15. The isolated antibody of any one of claims 1-14, wherein the antibody is humanized.

16. The isolated antibody of any one of claims 1-15, wherein the antibody is selected from the group consisting of a single chain antibody, a scFv, a univalent antibody lacking a hinge region, a minibody, and a probody.

17. The isolated antibody of any one of claims 1-15, wherein the antibody is a Fab or a Fab' fragment.

18. The isolated antibody of any one of claims 1-15, wherein the antibody is a F(ab')₂ fragment.

19. The isolated antibody of any one of claims 1-15, wherein the antibody is a whole antibody.
20. The isolated antibody of any one of claims 1-19, comprising a human IgG constant domain.
21. The isolated antibody of claim 20, wherein the IgG constant domain comprises an IgG1 CH1 domain.
22. The isolated antibody of claim 20, wherein the IgG constant domain comprises an IgG1 Fc region, optionally a modified Fc region, optionally modified by one or more amino acid substitutions.
23. The isolated antibody, or antigen-binding fragment thereof, of any one of claims 1-22 that binds to human TNFR2, optionally at least one peptide epitope from **Table T1**, with a K_D of about 2 nM or lower.
24. The isolated antibody, or antigen-binding fragment thereof, of any one of claims 1-23 that binds to human TNFR2 with a K_D of about 0.7 nM or lower, or binds to human TNFR2 on primary T cells, optionally T_{reg} s, with a K_D of about 50 pM or lower.
25. The isolated antibody, or antigen-binding fragment thereof, of any one of claims 1-24, wherein the isolated antibody, or antigen-binding fragment thereof:
- (a) inhibits TNF- α binding to TNFR2;
 - (b) inhibits TNFR2 signaling;
 - (c) activates TNFR2 signaling;
 - (d) inhibits TNFR2 dimerization/trimerization;
 - (e) cross-reactively binds to human TNFR2 and cynomolgus monkey TNFR2;
 - (f) increases/induces cell-killing/depletion of tumor cells, T_{reg} s, and/or suppressive myeloid cells (optionally macrophages, neutrophils, and myeloid-derived suppressor cells (MDSCs)) by antibody-dependent cellular cytotoxicity (ADCC);
 - (g) increases/induces cell-killing/depletion of tumor cells, T_{reg} s, and/or suppressive myeloid cells (optionally macrophages, neutrophils, and MDSCs) by macrophage-mediated antibody-dependent cellular phagocytosis (ADCP);
 - (h) reduces immune suppression by myeloid cells (optionally macrophages, neutrophils, and MDSCs);

- (i) converts MDSCs and/or M2 macrophages into proinflammatory M1 macrophages;
- (j) converts T_{regs} into effector T cells;
- (k) converts cold tumors into hot tumors;
- (l) reduces T_{reg} mediated immune suppression; or
- (m) a combination of any one or more of (a)-(k).

26. The isolated antibody, or antigen-binding fragment thereof, of any one of claims 1-25, which does not substantially bind to TNFR1, herpesvirus entry mediator (HVEM, CD40, death receptor 6 (DR6), and/or osteoprotegerin (OPG).

27. The isolated antibody, or antigen-binding fragment thereof, of any one of claims 1-26, which is a TNFR2 antagonist.

28. The isolated antibody, or antigen-binding fragment thereof, of any one of claims 1-26, which is a TNFR2 agonist.

29. The isolated antibody, or antigen-binding fragment thereof, of any one of claims 1-28, which is a bi-specific or multi-specific antibody.

30. An isolated polynucleotide encoding the isolated antibody, or antigen-binding fragment thereof, according to any one of claims 1-29, an expression vector comprising the isolated polynucleotide, or an isolated host cell comprising the vector.

31. A composition comprising a physiologically acceptable carrier and a therapeutically effective amount of the isolated antibody or antigen-binding fragment thereof according to any one of claims 1-29.

32. A method for treating a patient having a cancer, optionally a cancer associated with aberrant TNFR2 expression, comprising administering to the patient the composition of claim 31, thereby treating the cancer.

33. A method for treating a patient having a cancer, optionally a cancer associated with TNFR2 antagonist-mediated immune suppression, comprising administering to the patient the composition of claim 31, thereby treating the cancer.

34. The method of claim 32 or 33, wherein the antibody, or antigen-binding fragment thereof, is a TNFR2 antagonist.

35. A method for treating a patient having an inflammatory and/or autoimmune disease, comprising administering to the patient the composition of claim 231, thereby treating the inflammation.

36. The method of claim 35, wherein the disease is associated with aberrant TNFR2 expression, optionally wherein the antibody, or antigen-binding fragment thereof, is a TNFR2 agonist.

37. The method of claim 35, wherein the disease is associated with TNFR2 agonist-mediated immune activation.