

ANTI-TUMOR NECROSIS FACTOR RECEPTOR (TNFR2) ANTIBODIES AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

- 5 [0001] This application claims the benefit of United States Provisional Application Serial Number 63/047,490 filed July 2, 2020, which is hereby incorporated by reference in its entirety.

SEQUENCE LISTING STATEMENT

- 10 [0002] The instant application contains a Sequence Listing, which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on June 28, 2021, is named P-597451-PC_SL.txt and is 573,679 bytes in size.

FIELD OF THE INVENTION

- 15 [0003] The present disclosure relates in general to engineered antibodies. In one or more embodiments, the present disclosure describes the making and uses of antibodies against tumor necrosis factor receptor 2 (TNFR2).

BACKGROUND OF THE INVENTION

- 20 [0004] Tumor necrosis factor-alpha (TNF α) is an extremely pleiotropic cytokine. It is not only produced by immune cells which include activated macrophages, T cells, and natural killer (NK) cells, but can also be produced in endothelial cells, microglia, cardiac myocytes, and fibroblasts. Upon production, TNF α is presented as a membrane bound form (mTNF α), which is a 26 kDa transmembrane protein that can be cleaved by TNF-converting enzyme (TACE) to form a soluble form of TNF α (sTNF α) that is released from the membrane.
- 25 [0005] TNF α interacts with two receptors, tumor necrosis factor receptor superfamily member 1A (TNFR1) and tumor necrosis factor receptor superfamily member 1B (TNFR2). The extracellular domains of the two TNF receptors share a common structure composed of four cysteine-rich domains (CRDs) that are responsible for binding TNF α .
- 30 [0006] TNFR1 is constitutively expressed on virtually all nuclear cell types. It has been reported to bind sTNF α and mTNF α and is activated by both. One of TNFR1's main function is to mediate TNF-induced apoptosis via the NF-kB pathway; however, in certain situations, activation of the NF-kB pathway may result in production of anti-apoptotic proteins and pro-inflammatory cytokines such as IL-1 and IL-6.

- [0007] TNFR2, which was discovered and characterized more recently, is mostly expressed on activated T cells, myeloid cells and glial cells. It is a 75 kDa trans-membrane receptor with an extracellular domain consisting of four cysteine rich domains (CRD), a single transmembrane domain and a cytoplasmic domain that interacts with TNF receptor-associated factor 2 (TRAF2).
- 5 Recruitment of TRAF2 promotes a cascade of events resulting in activation of an alternative NF- κ B pathway. Although TNFR2 can bind tightly to both sTNF α and mTNF α , it is mainly activated through mTNF α and not sTNF α .
- [0008] It has been shown that TNFR2 is expressed at high levels on the surface of CD4⁺CD25⁺Foxp3⁺ regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs).
- 10 Activation of TNFR2 in these cells promotes their immunosuppressive activity and is crucial for Treg and MDSC proliferation and survival. Additionally, TNFR2 has a dual role on CD8⁺ effector T cells (Teffs): on one hand, TNFR2 mediates an activation signal to CD8⁺ Teffs during early immune response, while on the other hand, signals for apoptosis in these cells to terminate the immune response.
- 15 [0009] The key role of TNFR2 in regulation of the immune system is reflected in several pathogenic conditions. In cancer, TNFR2 is highly expressed in the tumor microenvironment (TME) on infiltrating Tregs, MDSC and on the tumor cells themselves. Activation of the TNFR2 receptor on Tregs and MDSC results in suppression of the immune system in the TME. Additionally, blocking of TNFR2 on cancer cell lines *in vitro* has been shown to have a dose
- 20 dependent killing effect.
- [0010] In autoimmune diseases, abnormalities in the TNF α signaling pathway have been reported in various autoimmune diseases including rheumatoid arthritis (RA), Crohn's disease (CD), multiple sclerosis (MS) and Type I diabetes. In many of these pathological conditions where TNFR1 is more dominant, blocking of TNF α mostly in its soluble form is sufficient to have an
- 25 efficacious effect. TNF α blockers like infliximab and adalimumab have been applied successfully in rheumatoid arthritis (RA), Crohn's disease (CD), and ulcerative colitis (UC). However, there are other conditions such as type 1 diabetes (T1D) and multiple sclerosis where the foregoing approach has been shown to be not particularly effective.
- [0011] In certain instances, specific inhibition or activation of the TNFR2 pathway may prove
- 30 beneficial, for example, in murine cancer models, specific inhibition of TNFR2 shows a robust effect both directly on cancer cells as well as on immune system activation REF?. TNFR2 has been strongly implicated as a driving force in some autoimmune diseases where Tregs and MDSC play a major role. In an experimental autoimmune encephalomyelitis (EAE) mice model of MS,

deletion of the TNFR2 gene was shown to have a deleterious effect. Agonizing TNFR2 in graft versus host disease (GvHD) models has been shown to reduce severity of GvHD. TNFR2 activation has also been implicated with Bacillus Calmette–Guerin (BCG) novel treatment of type 1 diabetes (T1D).

- 5 [0012] In view of the important roles of TNFR2 in the regulation of immune responses in various disease conditions, there is a need to develop improved targeting of TNFR2 by either antagonists or agonists as a strategy for improved therapies for treating diseases, such as, for example, cancer and autoimmune diseases.

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SUMMARY OF THE INVENTION

[0013] The present disclosure provides a number of anti-TNFR2 (tumor necrosis factor receptor 2) antibodies. In one embodiment, each of the anti-TNFR2 antibodies comprises a set of three complementarity determining regions (CDRs) on a heavy chain (HCDR1, HCDR2, and HCDR3) and a set of three CDRs on a light chain (LCDR1, LCDR2, and LCDR3). In one embodiment, the set of HCDR1, HCDR2, and HCDR3 comprises a combination of the amino acid sequences as shown in Table 7, and the set of LCDR1, LCDR2, and LCDR3 on a corresponding light chain comprises a combination of the amino acid sequences as shown in Table 8. In another embodiment, the set of HCDR1, HCDR2, HCDR3, and the set of LCDR1, LCDR2, LCDR3 on a corresponding light chain comprise the combination of amino acid sequences as shown in Table 9.

- 20 [0014] In one embodiment, each of the anti-TNFR2 antibodies comprises a heavy chain variable region and a light chain variable region, wherein the amino acid sequences for the heavy chain variable region and the light chain variable region can be one of the following pairs: SEQ ID NOs:289-290, SEQ ID NOs:3-4, SEQ ID NOs:7-8, SEQ ID NOs:11-12, SEQ ID NOs:15-16, SEQ ID NOs:19-20, SEQ ID NOs:23-24, SEQ ID NOs:27-28, SEQ ID NOs:31-32, SEQ ID NOs:35-36, SEQ ID NOs:39-40, SEQ ID NOs:43-44, SEQ ID NOs:47-48, SEQ ID NOs:51-52, SEQ ID NOs:55-56, SEQ ID NOs:59-60, SEQ ID NOs:63-64, SEQ ID NOs:67-68, SEQ ID NOs:71-72, SEQ ID NOs:75-76, SEQ ID NOs:79-80, SEQ ID NOs:83-84, SEQ ID NOs:87-88, SEQ ID NOs:91-92, SEQ ID NOs:95-96, SEQ ID NOs:99-100, SEQ ID NOs:103-104, SEQ ID NOs:107-108, SEQ ID NOs:111-112, SEQ ID NOs:115-116, SEQ ID NOs:119-120, SEQ ID NOs:123-124, SEQ ID NOs:127-128, SEQ ID NOs:131-132, SEQ ID NOs:135-136, SEQ ID NOs:139-140, SEQ ID NOs:143-144, SEQ ID NOs:147-148, SEQ ID NOs:151-152, SEQ ID NOs:157-158, SEQ ID NOs:163-164, SEQ ID NOs:169-170, SEQ ID NOs:175-176, SEQ ID NOs:181-182, SEQ ID NOs:193-194, SEQ ID NOs:199-200, SEQ ID NOs:205-206, SEQ ID NOs:211-212, SEQ ID

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NOs:217-218, SEQ ID NOs:223-224, SEQ ID NOs:229-230, SEQ ID NOs:233-234, SEQ ID NOs:237-238, SEQ ID NOs:241-242, SEQ ID NOs:245-246, SEQ ID NOs:249-250, SEQ ID NOs:253-254, SEQ ID NOs:257-258, SEQ ID NOs:261-262, SEQ ID NOs:265-266, SEQ ID NOs:269-270, SEQ ID NOs:273-274, SEQ ID NOs:277-278, SEQ ID NOs:281-282, SEQ ID NOs:285-286, SEQ ID NOs:293-294, SEQ ID NOs:297-298, SEQ ID NOs:301-302, SEQ ID NOs:305-306, SEQ ID NOs:309-310, SEQ ID NOs:313-314, SEQ ID NOs:317-318, SEQ ID NOs:321-322, SEQ ID NOs:325-326, or SEQ ID NOs:329-330.

[0015] In another embodiment, each of the anti-TNFR2 antibodies comprises a heavy chain and a light chain, wherein the amino acid sequences for the heavy chain and the light chain can be one of the following pairs: SEQ ID NOs:438 and 292, SEQ ID NO: 291-292, SEQ ID NOs:235-236, SEQ ID NOs:239-240, SEQ ID NOs:243-244, SEQ ID NOs:247-248, SEQ ID NOs:251-252, SEQ ID NOs:255-256, SEQ ID NOs:259-260, SEQ ID NOs:263-264, SEQ ID NOs:267-268, SEQ ID NOs:271-272, SEQ ID NOs:275-276, SEQ ID NOs:279-280, SEQ ID NOs:283-284, SEQ ID NOs:287-288, SEQ ID NOs:295-296, SEQ ID NOs:299-300, SEQ ID NOs:303-304, SEQ ID NOs:307-308, SEQ ID NOs:311-312, SEQ ID NOs:315-316, SEQ ID NOs:319-320, SEQ ID NOs:323-324, SEQ ID NOs:327-328, SEQ ID NOs:331-332, SEQ ID NOs: 432 and 260, SEQ ID NOs: 433 and 304, SEQ ID NOs: 434 and 308, SEQ ID NOs: 435 and 236, SEQ ID NOs: 436 and 244, SEQ ID NOs: 437 and 284, or SEQ ID NOs: 439 and 296.

[0016] In yet another embodiment, the present disclosure provides a composition comprising a pharmaceutically acceptable carrier and an anti-TNFR2 antibody disclosed herein.

[0017] The present disclosure also provides polynucleotide sequences encoding the anti-TNFR2 antibodies disclosed herein, as well as vectors and host cells comprising such polynucleotide sequences.

[0018] In a further embodiment, the anti-TNFR2 antibodies disclosed herein can be used to modulate the proliferation and/or functions of regulatory T cells. In another embodiment, the anti-TNFR2 antibodies disclosed herein can be used to modulate the proliferation and/or functions of myeloid-derived suppressor cells.

[0019] In one or more additional embodiments, the anti-TNFR2 antibodies disclosed herein can be used to treat diseases such as cancer, autoimmune diseases, GvHD, viral infections or bacterial infections.

[0020] These and other embodiments of the anti-TNFR2 antibodies will be appreciated from the ensuing descriptions of the figures and detailed description of the anti-TNFR2 antibodies which follow.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021] Some embodiments of the anti-TNFR2 antibodies and uses thereof are herein described, by way of example only, with reference to the accompanying drawings. With specific reference
 5 now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of embodiments of the anti-TNFR2 antibodies and uses thereof. In this regard, the description taken with the drawings makes apparent to those skilled in the art how embodiments of the anti-TNFR2 antibodies and uses thereof may be practiced.

[0022] **Figures 1A-1D** show yeast surface display (YSD) EC₅₀ (binding) of selected clones toward
 10 TNFR2-His. Key for **Figure 1A**: CID_327 (circle), CID_329 (square), CID_330 (upward triangle), CID_326 (downward triangle), CID_325 (diamond), CID_324 (star), CID_323 (asterisk), CID_328 (ring). **Figure 1B** shows the results for CID_251, **Figure 1C** shows the results for CID_436, and **Figure 1D** shows the results for CID_437.

[0023] **Figures 2A-2R** show size exclusion chromatography (SEC) analyses of IgG clones 30.086
 15 (**Figure 2A**), 30.095 (**Figure 2B**), 30.116 (**Figure 2C**), 30.111 (**Figure 2D**), 30.119 (**Figure 2E**), 30.123 (**Figure 2F**), 30.204 (**Figure 2G**), 30.116 (**Figure 2H**), 30.202 (**Figure 2I**), 30.202 (**Figure 2J**), 30.203 (**Figure 2K**), 30.115 (**Figure 2L**), 30.200 (**Figure 2M**), 30.201 (**Figure 2N**), 30.114 (**Figure 2O**), 30.117 (**Figure 2P**), 30.122 (**Figure 2Q**), and 30.118 (**Figure 2R**). The retention time of the indicated IgG was monitored at a wavelength of 280nm. Samples were run
 20 under the following conditions: a 100mg IgG sample was eluted at a rate of 0.8ml/min on a Superdex® 200 10/300pg column (**Figures 2A-2F**) or a 12mg IgG sample was eluted at a rate of 0.5ml/min on BioResolve SEC mAb column (**Figures 2G-2R**) with PBS as the mobile phase. Axes for **Figures 2A-2F** are Abs. 280 nm vs. vol. (ml) and for **Figures 2G-2R** are absorbance units (AU) vs. minutes.

[0024] **Figures 3A and 3B** show IgG binding specificity of selected clones to TNFR2 (**Figure 3A**: presents data for clones 30.092, 30.085, and 30.089; **Figure 3B** present data for clones 30.086, 30.116, 30.093, 30.117, 30.094, 30.118, 30.095, 30.119, 30.109, 30.111, 30.113, and 30.114). Wells were coated with 50ng/well IgG and tested for binding of 100nM TNFR1 or TNFR2 (**Figure 3A**). N.C.: negative control, human anti IL-2 IgG1 (50ng/well) does not bind either TNFR1 or
 30 TNFR2. LALA_N.C.: negative control, human anti IL-2 IgG1 (50ng/well) with a LALA mutation in the Fc region does not bind either TNFR1 or TNFR2. P.C.: TNFR1 and TNFR2 positive control, TNFα-Fc (50ng/well) were coated to the well and 100nM TNFR2 or TNFR1 were tested for binding to TNFα (**Figure 3B**).

[0025] **Figures 4A-4F** show ELISA EC₅₀ binding of selected IgG1 to TNFR2. IgG clones 30.080 (circle), 30.081 (square), and 30.084 (downward triangle) (**Figure 4A**), IgG clones 30.085 (circle), 30.087 (upward triangle), 30.088 (downward triangle), 30.089 (diamond), and 30.032 (square) (**Figure 4B**), IgG clones 30.092 (square) and 30.046 (circle) (**Figure 4C**), IgG 30.086 (square), 5 30.116 (triangle), and isotype control antibody (I.C.; circle) (**Figure 4D**), IgG clones 30.093 (square), 30.117 (circle), 30.094 (upward triangle), 30.118 (small hexagon), 30.095 (asterisk), and 30.119 (large hexagon) (**Figure 4E**), and IgG clones 30.109 (circle), 30.111 (square), 30.113 (upward triangle), and 30.114 (downward triangle) (**Figure 4F**).

[0026] **Figure 5** shows the expression of human TNFR2 by HEK-TNFR2 cells. 1x10⁶ cells were 10 harvested and lysed with either Tris Lysis Buffer (TLB) or with RIPA lysis buffer. Protein concentration was determined by the Bradford method, and 22ug protein cell lysate was subjected to western blot, detecting TNFR2 and GAPDH as control. Left 2 lanes: Untransfected HEK-Blue™ Null cells, right 2 lanes: HEK-Blue™ Null cells transfected with pCDNA3.1 plasmid encoding human TNFR2.

15 [0027] **Figures 6A-6C** show identification of TNFα responsive clones. **Figure 6A** shows a schematic representation of the selection process. Briefly, a parental plate of single clones was replicated and TNFα-Fc was added to the replica plate; soluble embryonic alkaline phosphatase (SEAP) activity was measured in both plates using QB reagent. Cells that exhibited a SEAP signal only in response to TNFα addition were selected. **Figure 6B** shows a photo of SEAP 20 activity colors of selected clones with or without TNFα. **Figure 6C** shows quantification of the colorimetric reaction of QB at OD 655 of selected clones.

[0028] **Figures 7A-7B** show TNFR2-TNF dependent activation of the NFκB pathway. **Figure 7A** shows the dose response of clone G6 to TNFα-Fc (circles). I.C is an isotype control antibody (upward triangles). **Figure 7B** shows that activation of TNFα (circles) is inhibited by the addition 25 of soluble TNFR2-Fc (asterisks). OD 620 values are presented.

[0029] **Figures 8A-8G** show the EC₅₀ of functional agonism of TNFR2 by the indicated antibodies incubated with the HEK293-TNFR2 cell line, which harbors soluble embryonic alkaline phosphatase (SEAP) under the control of a NFκB-controlled promoter. I.C. and N.C. are IgG and IgG-LALA isotype control antibodies, respectively. IgG clones 30.032 (circles), 30.085 (squares), 30 30.087 (downward triangles), and 30.088 (diamonds) (**Figure 8A**); IgG clones 30.046 (circles), 30.092 (squares), 30.093 (upward triangles), and 30.094 (downward triangles) (**Figure 8B**); I. C. (circles) and IgG clones 30.109 (squares), 30.111 (upward triangles), 30.113 (downward triangles), and 30.114 (diamonds) (**Figure 8C**); I.C. (circles) and IgG clones 30.086 (squares),

30.116 (upward triangles), 30.117 (downward triangles), 30.118 (diamonds), and 30.119 (hexagon) (**Figure 8D**); IgG clones 30.200 (squares), 30.201 (circles), and 30.122 (upward triangles), N.C. (downward triangles) (**Figure 8E**); IgG clones 30.115 (squares), 30.202 (circles), 30.203 (upward triangles), 30.204 (diamonds) and N.C. (downward triangles) (**Figure 8F**); and
5 IgG clone 30.123 (squares) and N.C. – negative control (downward triangles) (**Figure 8G**).

[0030] **Figures 9A-9C** show that antibody dependent activation of the NFkB controlled promoter is specific to TNFR2, and TNFR1 is not agonized by the TNFR2 specific antibodies. Representative flow cytometry labeling of HEK-TNFR2 and HEK-TNFR1 cell lines show that only HEK-TNFR2 is labeled by the 30.116 anti-TNFR2 antibody (**Figure 9A**). For the results
10 presented in **Figures 9B-9C**, 1mM of antibody clones 30.113, 30.114, 30.115, 30.116, 30.117, 30.118, 30.200, 30.201, 30.116, 30.119, 30.123, 30.202, 30.203, 30.204, or IgG-LALA isotype control N.C. 17069, did not activate the TNFR1 dependent NFkB pathway, although 11nM of human TNF α does activate the TNFR1 dependent NFkB pathway.

[0031] **Figures 10A-10D** show the effects of TNF α on antibody-dependent TNFR2 activation of
15 selected clones. HEK-TNFR2 reporter cells were incubated with 200nM soluble anti-TNFR2 antibodies (squares) for one hour, followed by subsequent addition of 0.05nM to 100nM TNF α (circles). Isotype control antibody (I.C.; upward triangle).

DETAILED DESCRIPTION OF THE INVENTION

20 [0032] The present disclosure presents a panel of high affinity antibody agonists that can agonize the TNFR2 receptor in an Fc independent manner, which could potentially be used for treatment of patients having a medical condition that requires (or could benefit from) activation of the TNFR2 pathway. As used herein, an “agonist antibody” of the TNFR2 receptor refers to an antibody that can essentially mimic the activity of the native ligand. The agonist activity can occur
25 when the antibody binds the TNF receptor in a manner that mimics the binding of the physiological ligand, thereby resulting in antibody-mediated agonism. As used herein, the terms “native ligand” and “normal ligand” may be used interchangeably, having all the same meanings and qualities.

[0033] In some embodiments, an agonist antibody described herein mimics the activity of the native ligand. In some embodiments, an agonist antibody described herein replaces the activity of
30 the normal ligand completely. In some embodiments, an agonist antibody described herein specifically activates the TNFR2 receptor. In some embodiments, an agonist antibody described herein replaces the activity of the normal ligand completely. In some embodiments, an agonist antibody described herein replaces the activity of the normal ligand and specifically activates the

TNFR2 receptor.

[0034] In some embodiments, 100% of the activity of the normal ligand is replaced, as compared with the activity in the presence of the native ligand. In some embodiments, 50%-100% of the activity of the normal ligand is replaced, as compared with the activity in the presence of the native
5 ligand. In some embodiments, 75%-100% of the activity of the normal ligand is replaced, as compared with the activity in the presence of the native ligand.

[0035] As used herein, the terms “comprise”, “comprises”, “comprising”, “includes”, “including”, “having” and their conjugates mean “including but not limited to”.

[0036] As used herein, the singular forms “a”, “an” and “the” include plural references unless the
10 context clearly dictates otherwise. For example, the term “an antibody” or “at least one antibody” may include a plurality of antibodies.

[0037] Throughout this application, various embodiments of the present disclosure may be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope
15 of the anti-TNFR2 antibodies and uses thereof. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for
20 example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

[0038] Whenever a numerical range is indicated herein, it is meant to include any cited numeral (fractional or integral) within the indicated range. The phrases “ranging/ranges between” a first indicate number and a second indicate number and “ranging/ranges from” a first indicate number
25 “to” a second indicate number are used herein interchangeably and are meant to include the first and second indicated numbers and all the fractional and integral numerals therebetween.

[0039] When values are expressed as approximations, by use of the antecedent “about,” it is understood that the particular value forms another embodiment. All ranges are inclusive and combinable. In one embodiment, the term “about” refers to a deviance of between 0.1-5% from the indicated number or range of numbers. In another embodiment, the term “about” refers to a
30 deviance of between 1-10% from the indicated number or range of numbers. In another embodiment, the term “about” refers to a deviance of up to 20% from the indicated number or range of numbers. In one embodiment, the term “about” refers to a deviance of $\pm 10\%$ from the indicated number or range of numbers. In another embodiment, the term “about” refers to a

deviance of $\pm 5\%$ from the indicated number or range of numbers.

[0040] Unless otherwise defined, all technical and/or scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the anti-TNFR2 antibodies and uses thereof pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the anti-TNFR2 antibodies and uses thereof, methods and/or materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be necessarily limiting. Each literature reference or other citation referred to herein is incorporated herein by reference in its entirety.

[0041] In the description presented herein, each of the steps of making and using the anti-TNFR2 antibodies and variations thereof are described. This description is not intended to be limiting and changes in the components, sequence of steps, and other variations would be understood to be within the scope of the present anti-TNFR2 antibodies, methods of making, and uses thereof.

[0042] It is appreciated that certain features of the anti-TNFR2 antibodies and uses thereof, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the anti-TNFR2 antibodies and uses thereof, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination or as suitable in any other described embodiment of the anti-TNFR2 antibodies and uses thereof. Certain features described in the context of various embodiments are not to be considered essential features of those embodiments, unless the embodiment is inoperative without those elements.

[0043] As used herein, the term "antibody" may be used interchangeably with the term "immunoglobulin", having all the same qualities and meanings. An antibody binding domain or an antigen binding site can be a fragment of an antibody or a genetically engineered product of one or more fragments of the antibody, which fragment is involved in specifically binding with a target antigen. By "specifically binding" is meant that the binding is selective for the antigen of interest and can be discriminated from unwanted or nonspecific interactions. For example, an antibody is said to specifically bind a TNFR2 epitope when the equilibrium dissociation constant is $\leq 10^{-5}$, 10^{-6} , or 10^{-7} M. In some embodiments, the equilibrium dissociation constant may be $\leq 10^{-8}$ M or 10^{-9} M. In some further embodiments, the equilibrium dissociation constant may be $\leq 10^{-10}$ M, 10^{-11} M, or 10^{-12} M. In some embodiments, the equilibrium dissociation constant may be in the range of $\leq 10^{-5}$ M to 10^{-12} M.

[0044] Half maximal effective concentration (EC_{50}) refers to the concentration of a drug, antibody or

toxicant which induces a response halfway between the baseline and maximum responses after a specified exposure time. In some embodiments, the response comprises a binding affinity. In some embodiments, the response comprises a functional response for example an agonistic response. A skilled artisan would appreciate that as used herein in certain embodiments, the EC₅₀ measurement of an anti-TNFR2 antibody disclosed herein provides a measure of a half-maximal binding of the anti-TNFR2 antibody to the TNFR2 antigen (EC₅₀ binding). Measure of EC₅₀ binding affinity comprises measuring the binding of an anti-TNFR2 antibody described herein to the TNFR2 antigen, as exemplified in Table 2 and Table 5 in the Examples. The skilled artisan would appreciate that as used herein in certain embodiments, the EC₅₀ measurement of an anti-TNFR2 antibody disclosed herein provides a measure of a half-maximal effective concentration of the anti-TNFR2 antibody to induce an agonist response (EC₅₀ functional agonism). Measure of EC₅₀ functional agonism comprises measuring the effects of the anti-TNFR2 antibodies described herein on cellular signaling of TNFR2, as exemplified in Table 6 in the Examples.

[0045] In some embodiments, EC₅₀ comprises the concentration of antibody required to obtain a 50% agonist response that would be observed upon binding of TNF α . In certain embodiments, a measure of EC₅₀ is commonly used as a measure of a drug's potency and may in some embodiments, reflect the binding of the antibody to the receptor. In some embodiments, anti-TNFR2 antibodies having nanomolar EC₅₀ binding concentration measurements, encompass tight binding anti-TNFR2 antibodies. In some embodiments, anti-TNFR2 antibodies having nanomolar EC₅₀ functional agonism concentration measurements encompass functionally effective agonistic antibodies. In certain embodiments, an anti-TNFR2 antibody disclosed herein comprises tight binding to the TNFR2 receptor. In certain embodiments, an anti-TNFR2 antibody disclosed herein comprises an agonist for the TNFR2 receptor. In certain embodiments, an anti-TNFR2 antibody disclosed herein comprises a tight binding agonist for the TNFR2 receptor.

[0046] In some embodiments, the binding EC₅₀ of an anti-TNFR2 antibody is in the nanomolar range. In some embodiments, the binding EC₅₀ of an anti-TNFR2 antibody is comprised within a range of about 0.05-100 nM. In some embodiments, the binding EC₅₀ of an anti-TNFR2 antibody is comprised within a range of about 0.05-50 nM. In some embodiments, the binding EC₅₀ of an anti-TNFR2 antibody is comprised within a range of about 0.05-20 nM. In some embodiments, the binding EC₅₀ of an anti-TNFR2 antibody is comprised within a range of about 0.05-10 nM. In some embodiments, the binding EC₅₀ of an anti-TNFR2 antibody is comprised within a range of about 0.1-100 nM. In some embodiments, the binding EC₅₀ of an anti-TNFR2 antibody is comprised within a range of about 0.1-50 nM. In some embodiments, the binding EC₅₀ of an anti-TNFR2 antibody is comprised within

a range of about 0.1-20 nM. In some embodiments, the binding EC_{50} of an anti-TNFR2 antibody is comprised within a range of about 0.1-10 nM. In some embodiments, the binding EC_{50} of an anti-TNFR2 antibody is comprised within a range of about 1-100 nM. In some embodiments, the binding EC_{50} of an anti-TNFR2 antibody is comprised within a range of about 1-20 nM. In some
5 embodiments, the binding EC_{50} of an anti-TNFR2 antibody is comprised within a range of about 20-40 nM. In some embodiments, the binding EC_{50} of an anti-TNFR2 antibody is comprised within a range of about 40-60 nM. In some embodiments, the binding EC_{50} of an anti-TNFR2 antibody is comprised within a range of about 60-80 nM. In some embodiments, the binding EC_{50} of an anti-TNFR2 antibody is comprised within a range of about 80-100 nM. In some embodiments, the binding
10 EC_{50} of an anti-TNFR2 antibody is comprised within a range of about 1-40 nM. In some embodiments, the binding EC_{50} of an anti-TNFR2 antibody is comprised within a range of about 1-60 nM. In some embodiments, the binding EC_{50} of an anti-TNFR2 antibody is comprised within a range of about 1-80 nM. In some embodiments, the binding EC_{50} of an anti-TNFR2 antibody is comprised within a range of about 1-50 nM. In some embodiments, the binding EC_{50} of an anti-TNFR2 antibody is comprised within a range of about 0.05-5 nM. In some embodiments, the binding
15 EC_{50} of an anti-TNFR2 antibody is comprised within a range of about 0.1-5 nM. In some embodiments, the binding EC_{50} of an anti-TNFR2 antibody is comprised within a range of about 0.05-20 nM.

[0047] In some embodiments, the binding EC_{50} of an anti-TNFR2 antibody is comprised within a
20 range of about 0.05-5 nM. In some embodiments, the binding EC_{50} of an anti-TNFR2 antibody is comprised within a range of about 0.1-5 nM. In some embodiments, the binding EC_{50} of an anti-TNFR2 antibody is comprised within a range of about 1-5 nM. In some embodiments, the binding EC_{50} of an anti-TNFR2 antibody is comprised within a range of about 0.05-10 nM. In some
25 embodiments, the binding EC_{50} of an anti-TNFR2 antibody is comprised within a range of about 0.1-10 nM. In some embodiments, the binding EC_{50} of an anti-TNFR2 antibody is comprised within a range of about 1-10 nM. In some embodiments, the binding EC_{50} of an anti-TNFR2 antibody is comprised within a range of about 5-10 nM. In some embodiments, the binding EC_{50} of an anti-TNFR2 antibody is comprised within a range of about 0.05-15 nM. In some
30 embodiments, the binding EC_{50} of an anti-TNFR2 antibody is comprised within a range of about 0.01-15 nM. In some embodiments, the binding EC_{50} of an anti-TNFR2 antibody is comprised within a range of about 1-15 nM.

[0048] In some embodiments, the EC_{50} measuring functional agonism is referred to herein as the functional EC_{50} , having all the same qualities. In some embodiments, the functional EC_{50} of an anti-

[illegible]

within a range of about 1-10 nM. In some embodiments, the functional EC₅₀ of an anti-TNFR2 antibody is comprised within a range of about 5-10 nM. In some embodiments, the functional EC₅₀ of an anti-TNFR2 antibody is comprised within a range of about 0.05-15 nM. In some embodiments, the functional EC₅₀ of an anti-TNFR2 antibody is comprised within a range of about 0.01-15 nM. In some embodiments, the functional EC₅₀ of an anti-TNFR2 antibody is comprised within a range of about 1-15 nM.

[0050] As used herein, the term “antibody” encompasses an antibody fragment or fragments that retain binding specificity including, but not limited to, IgG, heavy chain variable regions (VH), light chain variable regions (VL), Fab fragments, F(ab')₂ fragments, scFv fragments, Fv fragments, a nanobody, minibodies, diabodies, triabodies, tetrabodies, and single domain antibodies (see, e.g., Hudson and Souriau, Nature Med. 9: 129-134 (2003)). Also encompassed are humanized, primatized, and chimeric antibodies as these terms are generally understood in the art.

[0051] As used herein, the term “heavy chain variable region” may be used interchangeably with the term “VH domain” or the term “VH”, having all the same meanings and qualities. As used herein, the term “light chain variable region” may be used interchangeably with the term “VL domain” or the term “VL”, having all the same meanings and qualities. A skilled artisan would recognize that a “heavy chain variable region” or “VH” with regard to an antibody encompasses the fragment of the heavy chain that contains three complementarity determining regions (CDRs) interposed between flanking stretches known as framework regions. The framework regions are more highly conserved than the CDRs, and form a scaffold to support the CDRs. Similarly, a skilled artisan would also recognize that a “light chain variable region” or “VL” with regard to an antibody encompasses the fragment of the light chain that contains three CDRs interposed between framework regions.

[0052] As used herein, the term “complementarity determining region” or “CDR” refers to the hypervariable region(s) of a heavy or light chain variable region. Proceeding from the N-terminus, each of a heavy or light chain polypeptide has three CDRs denoted as “CDR1,” “CDR2,” and “CDR3”. Crystallographic analysis of a number of antigen-antibody complexes has demonstrated that the amino acid residues of CDRs form extensive contact with a bound antigen, wherein the most extensive antigen contact is with the heavy chain CDR3. Thus, the CDR regions are primarily responsible for the specificity of an antigen-binding site. In one embodiment, an antigen-binding site includes six CDRs, comprising the CDRs from each of a heavy and a light chain variable region.

[0053] As used herein, the term “framework region” or “FR” refers to the four flanking amino acid sequences which frame the CDRs of a heavy or light chain variable region. Some FR residues may contact bound antigen, however, FR residues are primarily responsible for folding the variable region

into the antigen-binding site. In some embodiments, the FR residues responsible for folding the variable regions comprise residues directly adjacent to the CDRs. Within FRs, certain amino residues and certain structural features are very highly conserved. In this regard, all variable region sequences contain an internal disulfide loop of around 90 amino acid residues. When a variable region folds

5 into an antigen binding site, the CDRs are displayed as projecting loop motifs that form an antigen-binding surface. It is generally recognized that there are conserved structural regions of FRs that influence the folded shape of the CDR loops into certain “canonical” structures regardless of the precise CDR amino acid sequence. Furthermore, certain FR residues are known to participate in non-covalent interdomain contacts which stabilize the interaction of the antibody heavy and light chains.

10 [0054] Wu and Kabat (Wu and Kabat, “An analysis of the sequences of the variable regions of Bence Jones proteins and myeloma light chains and their implications for antibody complementarity”, *Journal of Experimental Medicine*, 132, 2, 8 (1970); Kabat *et al.*, “Sequence of proteins of immunological interest”, Bethesda: National Institutes of Health; 323 (1983)) pioneered the alignment of antibody peptide sequences, and their contributions in this area were several-fold.

15 Firstly, through study of sequence similarities between variable domains, they identified correspondent residues that to a greater or lesser extent were homologous across all antibodies in all vertebrate species, inasmuch as they adopted similar three-dimensional structure, played similar functional roles, interacted similarly with neighboring residues, and existed in similar chemical environments. Secondly, they devised a peptide sequence numbering system in which homologous

20 immunoglobulin residues were assigned the same position number. One skilled in the art can unambiguously assign to any variable domain sequence what is now commonly called Kabat numbering without reliance on any experimental data beyond the sequence itself. Thirdly, Kabat and Wu calculated variability for each Kabat-numbered sequence position, which is the finding of few or many possible amino acids when variable domain sequences are aligned. They identified three

25 contiguous regions of high variability embedded within four less variable contiguous regions. Kabat and Wu formally demarcated residues constituting these variable tracts, and designated these “complementarity determining regions” (CDRs), referring to chemical complementarity between antibody and antigen. A role in three-dimensional folding of the variable domain, but not in antigen recognition, was ascribed to the remaining less-variable regions, which are now termed “framework

30 regions”. Fourthly, Kabat and Wu established a public database of antibody peptide and nucleic acid sequences, which continues to be maintained and is well known to those skilled in the art.

[0055] Chothia and coworkers (Cyrus Chothia, Arthur M. Lesk (1987), *Journal of Molecular Biology*. 196(4): 901-917) found that certain sub-portions within Kabat CDRs adopt nearly identical

peptide backbone conformations, despite having great diversity at the level of amino acid sequence. These sub-portions were designated as L1, L2 and L3 or H1, H2 and H3, where the “L” and the “H” designates the light chain and the heavy chains regions, respectively. These regions may be referred to as Chothia CDRs, which have boundaries that overlap with Kabat CDRs.

5 [0056] More recent studies have shown that virtually all antibody binding residues fall within regions of structural consensus. (Kunik, V., *et al.*, PloS Computational Biology 8(2):el002388 (February 2012)). In some embodiments, these regions are referred to as antibody binding regions. It was shown that these regions can be identified from the antibody sequence as well. "Paratome", an implementation of a structural approach for the identification of structural consensus in antibodies,
10 was used for this purpose. (Ofra, Y. *et al.*, J. Immunol. 757:6230-6235 (2008)). While residues identified by Paratome cover virtually all the antibody binding sites, the CDRs (as identified by the commonly used CDR identification tools) miss significant portions of them. Antibody binding residues which were identified by Paratome but were not identified by any of the common CDR identification methods are referred to as Paratome-unique residues. Similarly, antibody binding
15 residues that are identified by any of the common CDR identification methods but are not identified by Paratome are referred to as CDR-unique residues. Paratome-unique residues make crucial energetic contribution to antibody-antigen interactions, while CDRs-unique residues have a rather minor contribution. These results allow for better identification of antigen binding sites.

[0057] IMGT® is the international ImMunoGeneTics information system®, (See, Nucleic Acids
20 Res. 2015 Jan; 43 (Database issue):D413-22. doi: 10.1093/nar/gku1056. Epub 2014 Nov 5 Free article. PMID: 25378316 LIGM:441 and Dev Comp Immunol. 2003 Jan;27(1):55-77). IMGT is a unique numbering system for immunoglobulin and T cell receptor variable domains and Ig superfamily V-like domains (Lefranc *et al.*, Dev Comp Immunol. 27: 55-77 (2003)). IMGT® presents a uniform numbering system for these IG and TcR variable domain sequences, based on aligning 5
25 or more IG and TcR variable region sequences, taking into account and combining the Kabat definition of FRs and CDRs, structural data, and Chothia's characterization of the hypervariable loops. IMGT is considered well known in the art as a universal numbering scheme for antibodies.

[0058] In describing variant amino acid positions present in the VH and VL domains, in some embodiments any common CDR definition may be used to describe the CDR regions, for example
30 but not limited to the approaches used by IMGT®, KABAT, or Chothia and Paratome.

[0059] An antibody may exist in various forms or having various domains including, without limitation, a complementarity determining region (CDR), a variable region (Fv), a VH domain, a VL domain, a single chain variable region (scFv), and a Fab fragment.

- [0060] A person of ordinary skill in the art would appreciate that a scFv is a fusion polypeptide comprising the variable heavy chain (VH) and variable light chain (VL) regions of an immunoglobulin, connected by a short linker peptide. The linker may have, for example, 10 to about 25 amino acids.
- 5 [0061] A skilled artisan would also appreciate that the term “Fab” with regard to an antibody generally encompasses that portion of the antibody consisting of a single light chain (both variable and constant regions) bound to the variable region and first constant region of a single heavy chain by a disulfide bond, whereas F(ab')₂ comprises a fragment of a heavy chain comprising a VH domain and a light chain comprising a VL domain.
- 10 [0062] In some embodiments, an antibody encompasses whole antibody molecules, including monoclonal and polyclonal antibodies. In some embodiments, an antibody encompasses an antibody fragment or fragments that retain binding specificity including, but not limited to, variable heavy chain (VH) fragments, variable light chain (VL) fragments, Fab fragments, F(ab')₂ fragments, scFv fragments, Fv fragments, minibodies, diabodies, triabodies, and tetrabodies.
- 15 [0063] In one embodiment, the anti-TNFR2 antibodies disclosed herein can be incorporated as part of a bispecific antibody. As it is generally known in the art, a bispecific antibody is a recombinant protein that includes antigen-binding fragments of two different monoclonal antibodies, and is thereby capable of binding two different antigens. In some embodiments, bispecific antibodies are used for cancer immunotherapy by simultaneously targeting, for example, both CTLs (such as a CTL receptor
- 20 component such as CD3) or effector natural killer (NK) cells, and a tumor antigen. Similarly, a multi-specific antibody is a recombinant protein that includes antigen-binding fragments of at least two different monoclonal antibodies, such as two, three or four different monoclonal antibodies.

Anti-TNFR2 Antibodies

- [0064] The present disclosure provides a number of anti-TNFR2 (tumor necrosis factor receptor
- 25 2) antibodies. In one embodiment, each of the anti-TNFR2 antibodies comprises a set of three complementarity determining regions (CDRs) on a heavy chain (HCDR1, HCDR2, and HCDR3) and a set of three CDRs on a light chain (LCDR1, LCDR2, and LCDR3). In one embodiment, the set of HCDR1, HCDR2, and HCDR3 comprises a combination of the amino acid sequences as shown in Table 7, and the set of LCDR1, LCDR2, and LCDR3 on a corresponding light chain
- 30 comprises a combination of the amino acid sequences as shown in Table 8. For the purpose of illustration, take clone CID251 as an example (see Table 7), the set of HCDR1, HCDR2, and HCDR3 for this antibody comprises the amino acid sequences of SEQ ID NO:185, SEQ ID NO:190 and SEQ ID NO:347 respectively (see Table 7), whereas the set of LCDR1, LCDR2, and

LCDR3 for the same antibody comprises the amino acid sequences of SEQ ID NO:359, SEQ ID NO:372 and SEQ ID NO:378 respectively (see Table 8).

[0065] In some embodiments, the set of HCDR1, HCDR2, and HCDR3 comprises the amino acid sequences set forth in SEQ ID NOs: 185, 190, and 347, respectively, and the set of LCDR1, LCDR2, and LCDR3 for the same antibody comprises the amino acid sequences set forth in SEQ ID NOs: 359, 372, and 378, respectively. In some embodiments, the set of HCDR1, HCDR2, and HCDR3 comprises the amino acid sequences set forth in SEQ ID NOs: 185, 190, and 347, respectively, and the set of LCDR1, LCDR2, and LCDR3 for the same antibody comprises the amino acid sequences set forth in SEQ ID NOs: 359, 372, and 378, respectively. In some embodiments, the set of HCDR1, HCDR2, and HCDR3 comprises the amino acid sequences set forth in SEQ ID NOs: 185, 334, 347, respectively, and the set of LCDR1, LCDR2, and LCDR3 for the same antibody comprises the amino acid sequences set forth in SEQ ID NOs: 360, 372, and 379, respectively. In some embodiments, the set of HCDR1, HCDR2, and HCDR3 comprises the amino acid sequences set forth in SEQ ID NOs: 185, 190, and 347, respectively, and the set of LCDR1, LCDR2, and LCDR3 for the same antibody comprises the amino acid sequences set forth in SEQ ID NOs: 359, 372, and 380, respectively. In some embodiments, the set of HCDR1, HCDR2, and HCDR3 comprises the amino acid sequences set forth in SEQ ID NOs: 185, 190, and 347, respectively, and the set of LCDR1, LCDR2, and LCDR3 for the same antibody comprises the amino acid sequences set forth in SEQ ID NOs: 359, 372, and 381, respectively. In some embodiments, the set of HCDR1, HCDR2, and HCDR3 comprises the amino acid sequences set forth in SEQ ID NOs: 185, 190, and 347, respectively, and the set of LCDR1, LCDR2, and LCDR3 for the same antibody comprises the amino acid sequences set forth in SEQ ID NOs: 361, 372, and 381, respectively. In some embodiments, the set of HCDR1, HCDR2, and HCDR3 comprises the amino acid sequences set forth in SEQ ID NOs: 185, 190, and 347, respectively, and the set of LCDR1, LCDR2, and LCDR3 for the same antibody comprises the amino acid sequences set forth in SEQ ID NOs: 362, 372, and 382, respectively. In some embodiments, the set of HCDR1, HCDR2, and HCDR3 comprises the amino acid sequences set forth in SEQ ID NOs: 185, 335, and 347, respectively, and the set of LCDR1, LCDR2, and LCDR3 for the same antibody comprises the amino acid sequences set forth in SEQ ID NOs: 363, 372, and 383, respectively. In some embodiments, the set of HCDR1, HCDR2, and HCDR3 comprises the amino acid sequences set forth in SEQ ID NOs: 185, 190, 347, respectively, and the set of LCDR1, LCDR2, and LCDR3 for the same antibody comprises the amino acid sequences set forth in SEQ ID NOs: 364, 372, and 384, respectively. In some embodiments, the set of HCDR1, HCDR2, and

HCDR3 comprises the amino acid sequences set forth in SEQ ID NOs: 185, 336, and 348, respectively, and the set of LCDR1, LCDR2, and LCDR3 for the same antibody comprises the amino acid sequences set forth in SEQ ID NOs: 365, 372, and 378, respectively. In some embodiments, the set of HCDR1, HCDR2, and HCDR3 comprises the amino acid sequences set forth in SEQ ID NOs: 185, 337, and 347, respectively, and the set of LCDR1, LCDR2, and LCDR3 for the same antibody comprises the amino acid sequences set forth in SEQ ID NOs: 359, 373, and 385, respectively. In some embodiments, the set of HCDR1, HCDR2, and HCDR3 comprises the amino acid sequences set forth in SEQ ID NOs: 185, 338, and 348, respectively, and the set of LCDR1, LCDR2, and LCDR3 for the same antibody comprises the amino acid sequences set forth in SEQ ID NOs: 359, 372, and 386, respectively. In some embodiments, the set of HCDR1, HCDR2, and HCDR3 comprises the amino acid sequences set forth in SEQ ID NOs: 185, 339, and 349, respectively, and the set of LCDR1, LCDR2, and LCDR3 for the same antibody comprises the amino acid sequences set forth in SEQ ID NOs: 359, 372, and 378, respectively. In some embodiments, the set of HCDR1, HCDR2, and HCDR3 comprises the amino acid sequences set forth in SEQ ID NOs: 185, 190, and 350, respectively, and the set of LCDR1, LCDR2, and LCDR3 for the same antibody comprises the amino acid sequences set forth in SEQ ID NOs: 366, 372, and 387, respectively. In some embodiments, the set of HCDR1, HCDR2, and HCDR3 comprises the amino acid sequences set forth in SEQ ID NOs: 185, 190, and 351, respectively, and the set of LCDR1, LCDR2, and LCDR3 for the same antibody comprises the amino acid sequences set forth in SEQ ID NOs: 359, 372, and 378, respectively. In some embodiments, the set of HCDR1, HCDR2, and HCDR3 comprises the amino acid sequences set forth in SEQ ID NOs: 185, 334, and 347, respectively, and the set of LCDR1, LCDR2, and LCDR3 for the same antibody comprises the amino acid sequences set forth in SEQ ID NOs: 360, 372, and 388, respectively. In some embodiments, the set of HCDR1, HCDR2, and HCDR3 comprises the amino acid sequences set forth in SEQ ID NOs: 185, 334, and 352, respectively, and the set of LCDR1, LCDR2, and LCDR3 for the same antibody comprises the amino acid sequences set forth in SEQ ID NOs: 359, 372, and 378, respectively. In some embodiments, the set of HCDR1, HCDR2, and HCDR3 comprises the amino acid sequences set forth in SEQ ID NOs: 185, 190, and 352, respectively, and the set of LCDR1, LCDR2, and LCDR3 for the same antibody comprises the amino acid sequences set forth in SEQ ID NOs: 359, 372, and 389, respectively. In some embodiments, the set of HCDR1, HCDR2, and HCDR3 comprises the amino acid sequences set forth in SEQ ID NOs: 185, 334, and 347, respectively, and the set of LCDR1, LCDR2, and LCDR3 for the same antibody comprises the amino acid sequences set forth in SEQ ID NOs: 359, 372, and

378, respectively. In some embodiments, the set of HCDR1, HCDR2, and HCDR3 comprises the amino acid sequences set forth in SEQ ID NOs: 185, 190, and 347, respectively, and the set of LCDR1, LCDR2, and LCDR3 for the same antibody comprises the amino acid sequences set forth in SEQ ID NOs: 365, 372, and 378, respectively. In some embodiments, the set of HCDR1, HCDR2, and HCDR3 comprises the amino acid sequences set forth in SEQ ID NOs: 185, 336, and 352, respectively, and the set of LCDR1, LCDR2, and LCDR3 for the same antibody comprises the amino acid sequences set forth in SEQ ID NOs: 365, 372, and 390, respectively. In some embodiments, the set of HCDR1, HCDR2, and HCDR3 comprises the amino acid sequences set forth in SEQ ID NOs: 185, 336, and 352, respectively, and the set of LCDR1, LCDR2, and LCDR3 for the same antibody comprises the amino acid sequences set forth in SEQ ID NOs: 365, 374, and 391, respectively. In some embodiments, the set of HCDR1, HCDR2, and HCDR3 comprises the amino acid sequences set forth in SEQ ID NOs: 186, 340, and 353, respectively, and the set of LCDR1, LCDR2, and LCDR3 for the same antibody comprises the amino acid sequences set forth in SEQ ID NOs: 367, 375, and 392, respectively. In some embodiments, the set of HCDR1, HCDR2, and HCDR3 comprises the amino acid sequences set forth in SEQ ID NOs: 186, 341, and 354, respectively, and the set of LCDR1, LCDR2, and LCDR3 for the same antibody comprises the amino acid sequences set forth in SEQ ID NOs: 367, 375, 392, respectively. In some embodiments, the set of HCDR1, HCDR2, and HCDR3 comprises the amino acid sequences set forth in SEQ ID NOs: 186, 341, and 354, respectively, and the set of LCDR1, LCDR2, and LCDR3 for the same antibody comprises the amino acid sequences set forth in SEQ ID NOs: 368, 375, and 392, respectively. In some embodiments, the set of HCDR1, HCDR2, and HCDR3 comprises the amino acid sequences set forth in SEQ ID NOs: 186, 342, and 354, respectively, and the set of LCDR1, LCDR2, and LCDR3 for the same antibody comprises the amino acid sequences set forth in SEQ ID NOs: 367, 375 and 392, respectively. In some embodiments, the set of HCDR1, HCDR2, and HCDR3 comprises the amino acid sequences set forth in SEQ ID NOs: 186, 343, and 354, respectively, and the set of LCDR1, LCDR2, and LCDR3 for the same antibody comprises the amino acid sequences set forth in SEQ ID NOs: 369, 375, and 393, respectively. In some embodiments, the set of HCDR1, HCDR2, and HCDR3 comprises the amino acid sequences set forth in SEQ ID NOs: 186, 343, and 355, respectively, and the set of LCDR1, LCDR2, and LCDR3 for the same antibody comprises the amino acid sequences set forth in SEQ ID NOs: 367, 375, and 392, respectively. In some embodiments, the set of HCDR1, HCDR2, and HCDR3 comprises the amino acid sequences set forth in SEQ ID NOs: 186, 341, and 354, respectively, and the set of LCDR1, LCDR2, and LCDR3 for the same antibody

comprises the amino acid sequences set forth in SEQ ID NOs: 367, 375, and 392, respectively. In some embodiments, the set of HCDR1, HCDR2, and HCDR3 comprises the amino acid sequences set forth in SEQ ID NOs: 186, 341, and 355, respectively, and the set of LCDR1, LCDR2, and LCDR3 for the same antibody comprises the amino acid sequences set forth in SEQ ID NOs: 367, 376, and 392, respectively. In some embodiments, the set of HCDR1, HCDR2, and HCDR3 comprises the amino acid sequences set forth in SEQ ID NOs: 186, 344, and 354, respectively, and the set of LCDR1, LCDR2, and LCDR3 for the same antibody comprises the amino acid sequences set forth in SEQ ID NOs: 370, 375, and 392, respectively. In some embodiments, the set of HCDR1, HCDR2, and HCDR3 comprises the amino acid sequences set forth in SEQ ID NOs: 186, 341, and 354, respectively, and the set of LCDR1, LCDR2, and LCDR3 for the same antibody comprises the amino acid sequences set forth in SEQ ID NOs: 367, 375, and 394, respectively. In some embodiments, the set of HCDR1, HCDR2, and HCDR3 comprises the amino acid sequences set forth in SEQ ID NOs: 186, 344, and 354, respectively, and the set of LCDR1, LCDR2, and LCDR3 for the same antibody comprises the amino acid sequences set forth in SEQ ID NOs: 367, 375, and 392, respectively. In some embodiments, the set of HCDR1, HCDR2, and HCDR3 comprises the amino acid sequences set forth in SEQ ID NOs: 186, 341, and 359, respectively, and the set of LCDR1, LCDR2, and LCDR3 for the same antibody comprises the amino acid sequences set forth in SEQ ID NOs: 367, 375, and 393, respectively. In some embodiments, the set of HCDR1, HCDR2, and HCDR3 comprises the amino acid sequences set forth in SEQ ID NOs: 187, 345, and 356, respectively, and the set of LCDR1, LCDR2, and LCDR3 for the same antibody comprises the amino acid sequences set forth in SEQ ID NOs: 371, 377, and 395, respectively. In some embodiments, the set of HCDR1, HCDR2, and HCDR3 comprises the amino acid sequences set forth in SEQ ID NOs: 188, 345, and 357, respectively, and the set of LCDR1, LCDR2, and LCDR3 for the same antibody comprises the amino acid sequences set forth in SEQ ID NOs: 371, 377, and 396, respectively. In some embodiments, the set of HCDR1, HCDR2, and HCDR3 comprises the amino acid sequences set forth in SEQ ID NOs: 189, 346, and 358, respectively, and the set of LCDR1, LCDR2, and LCDR3 for the same antibody comprises the amino acid sequences set forth in SEQ ID NOs: 371, 377, and 397, respectively.

[0066] In another embodiment, the anti-TNFR2 antibodies comprises heavy chain and light chain CDR sequences that are at least 80% (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, or 99%) identical to the amino acid sequences set forth above, for example but not limited to identity as determined using BlastP software of the National Center of Biotechnology Information (NCBI)

using default parameters.

[0067] A skilled artisan would appreciate that percent identity (% identity) provides a number that describes how similar the query sequence is to the target sequence (i.e., how many amino acids in each sequence are identical). The higher the percent identity is, the more significant the match.

5 [0068] When used in relation to polypeptide (or protein) sequences, the term “identity” refers to the degree of identity between two or more polypeptide (or protein) sequences or fragments thereof. Typically, the degree of similarity between two or more polypeptide (or protein) sequences refers to the degree of similarity of the composition, order, or arrangement of two or more amino acids of the two or more polypeptides (or proteins).

10 [0069] In yet some further embodiments, the set of HCDR1, HCDR2, HCDR3 on a heavy chain, and the set of LCDR1, LCDR2, LCDR3 on a corresponding light chain comprise the amino acid sequences as shown in Table 9. For the purpose of illustration, taking clone CID_264 as an example (see Table 9), the set of HCDR1, HCDR2, and HCDR3 for this antibody comprises the amino acid sequences of SEQ ID NO:402, SEQ ID NO:345, and SEQ ID NO:413, respectively
15 (see Table 9), whereas the set of LCDR1, LCDR2, and LCDR3 for the same antibody comprises the amino acid sequences of SEQ ID NO:371, SEQ ID NO:418, and SEQ ID NO:427, respectively (see Table 9).

[0070] In some embodiments, the set of HCDR1, HCDR2, and HCDR3 comprises the amino acid sequences set forth in SEQ ID NOs: 402, 345, and 413, respectively, and the set of LCDR1,
20 LCDR2, and LCDR3 for the same antibody comprises the amino acid sequences set forth in SEQ ID NOs: 371, 418, and 427, respectively. In some embodiments, the set of HCDR1, HCDR2, and HCDR3 comprises the amino acid sequences set forth in SEQ ID NOs: 398, 346, and 407, respectively, and the set of LCDR1, LCDR2, and LCDR3 for the same antibody comprises the amino acid sequences set forth in SEQ ID NOs: 371, 377, and 419, respectively. In some
25 embodiments, the set of HCDR1, HCDR2, and HCDR3 comprises the amino acid sequences set forth in SEQ ID NOs: 187, 346, and 408, respectively, and the set of LCDR1, LCDR2, and LCDR3 for the same antibody comprises the amino acid sequences set forth in SEQ ID NOs: 371, 377, and 420, respectively. In some embodiments, the set of HCDR1, HCDR2, and HCDR3 comprises the amino acid sequences set forth in SEQ ID NOs: 189, 406, and 409, respectively, and the set of
30 LCDR1, LCDR2, and LCDR3 for the same antibody comprises the amino acid sequences set forth in SEQ ID NOs: 371, 377, and 421, respectively. In some embodiments, the set of HCDR1, HCDR2, and HCDR3 comprises the amino acid sequences set forth in SEQ ID NOs: 187, 345, and 410, respectively, and the set of LCDR1, LCDR2, and LCDR3 for the same antibody

comprises the amino acid sequences set forth in SEQ ID NOs: 371, 418, and 422, respectively. In some embodiments, the set of HCDR1, HCDR2, and HCDR3 comprises the amino acid sequences set forth in SEQ ID NOs: 399, 346, and 411, respectively, and the set of LCDR1, LCDR2, and LCDR3 for the same antibody comprises the amino acid sequences set forth in SEQ ID NOs: 371, 418, and 423, respectively. In some embodiments, the set of HCDR1, HCDR2, and HCDR3 comprises the amino acid sequences set forth in SEQ ID NOs: 400, 346, and 412, respectively, and the set of LCDR1, LCDR2, and LCDR3 for the same antibody comprises the amino acid sequences set forth in SEQ ID NOs: 417, 377, and 424, respectively. In some embodiments, the set of HCDR1, HCDR2, and HCDR3 comprises the amino acid sequences set forth in SEQ ID NOs: 401, 346, and 413, respectively, and the set of LCDR1, LCDR2, and LCDR3 for the same antibody comprises the amino acid sequences set forth in SEQ ID NOs: 417, 418, and 425, respectively. In some embodiments, the set of HCDR1, HCDR2, and HCDR3 comprises the amino acid sequences set forth in SEQ ID NOs: 401, 346, and 414, respectively, and the set of LCDR1, LCDR2, and LCDR3 for the same antibody comprises the amino acid sequences set forth in SEQ ID NOs: 417, 418, and 426, respectively. In some embodiments, the set of HCDR1, HCDR2, and HCDR3 comprises the amino acid sequences set forth in SEQ ID NOs: 398, 346, and 415, respectively, and the set of LCDR1, LCDR2, and LCDR3 for the same antibody comprises the amino acid sequences set forth in SEQ ID NOs: 417, 377, and 428, respectively. In some embodiments, the set of HCDR1, HCDR2, and HCDR3 comprises the amino acid sequences set forth in SEQ ID NOs: 403, 346, and 412, respectively, and the set of LCDR1, LCDR2, and LCDR3 for the same antibody comprises the amino acid sequences set forth in SEQ ID NOs: 371, 418, and 429, respectively. In some embodiments, the set of HCDR1, HCDR2, and HCDR3 comprises the amino acid sequences set forth in SEQ ID NOs: 404, 346, and 412, respectively, and the set of LCDR1, LCDR2, and LCDR3 for the same antibody comprises the amino acid sequences set forth in SEQ ID NOs: 371, 418, and 425, respectively. In some embodiments, the set of HCDR1, HCDR2, and HCDR3 comprises the amino acid sequences set forth in SEQ ID NOs: 401, 346, and 414, respectively, and the set of LCDR1, LCDR2, and LCDR3 for the same antibody comprises the amino acid sequences set forth in SEQ ID NOs: 417, 418, and 430, respectively. In some embodiments, the set of HCDR1, HCDR2, and HCDR3 comprises the amino acid sequences set forth in SEQ ID NOs: 405, 406, and 416, respectively, and the set of LCDR1, LCDR2, and LCDR3 for the same antibody comprises the amino acid sequences set forth in SEQ ID NOs: 371, 377, and 431, respectively.

[0071] In another embodiment, the anti-TNFR2 antibodies comprises heavy chain and light chain

CDR sequences that are at least 80% (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, or 99%) identical to the amino acid sequences set forth above, for example but not limited to as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters.

- 5 [0072] In one embodiment, each of the anti-TNFR2 antibodies presented herein comprises a heavy chain variable region (VH) and a light chain variable region (VL), wherein the amino acid sequences for the heavy chain variable region and the light chain variable region can be one of the following pairs: SEQ ID NOs:289-290, SEQ ID NOs:3-4, SEQ ID NOs:7-8, SEQ ID NOs:11-12, SEQ ID NOs:15-16, SEQ ID NOs:19-20, SEQ ID NOs:23-24, SEQ ID NOs:27-28, SEQ ID
- 10 NOs:31-32, SEQ ID NOs:35-36, SEQ ID NOs:39-40, SEQ ID NOs:43-44, SEQ ID NOs:47-48, SEQ ID NOs:51-52, SEQ ID NOs:55-56, SEQ ID NOs:59-60, SEQ ID NOs:63-64, SEQ ID NOs:67-68, SEQ ID NOs:71-72, SEQ ID NOs:75-76, SEQ ID NOs:79-80, SEQ ID NOs:83-84, SEQ ID NOs:87-88, SEQ ID NOs:91-92, SEQ ID NOs:95-96, SEQ ID NOs:99-100, SEQ ID NOs:103-104, SEQ ID NOs:107-108, SEQ ID NOs:111-112, SEQ ID NOs:115-116, SEQ ID
- 15 NOs:119-120, SEQ ID NOs:123-124, SEQ ID NOs:127-128, SEQ ID NOs:131-132, SEQ ID NOs:135-136, SEQ ID NOs:139-140, SEQ ID NOs:143-144, SEQ ID NOs:147-148, SEQ ID NOs:151-152, SEQ ID NOs:157-158, SEQ ID NOs:163-164, SEQ ID NOs:169-170, SEQ ID NOs:175-176, SEQ ID NOs:181-182, SEQ ID NOs:193-194, SEQ ID NOs:199-200, SEQ ID NOs:205-206, SEQ ID NOs:211-212, SEQ ID NOs:217-218, SEQ ID NOs:223-224, SEQ ID
- 20 NOs:229-230, SEQ ID NOs:233-234, SEQ ID NOs:237-238, SEQ ID NOs:241-242, SEQ ID NOs:245-246, SEQ ID NOs:249-250, SEQ ID NOs:253-254, SEQ ID NOs:257-258, SEQ ID NOs:261-262, SEQ ID NOs:265-266, SEQ ID NOs:269-270, SEQ ID NOs:273-274, SEQ ID NOs:277-278, SEQ ID NOs:281-282, SEQ ID NOs:285-286, SEQ ID NOs:293-294, SEQ ID NOs:297-298, SEQ ID NOs:301-302, SEQ ID NOs:305-306, SEQ ID NOs:309-310, SEQ ID
- 25 NOs:313-314, SEQ ID NOs:317-318, SEQ ID NOs:321-322, SEQ ID NOs:325-326, or SEQ ID NOs:329-330.

- [0073] In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:289-290. In some embodiments, the amino acid sequences for the heavy chain variable region
- 30 and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:3-4. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:7-8. In some embodiments, the amino acid sequences for the heavy chain variable region

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antibody disclosed herein is set forth in SEQ ID NOs:75-76. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:79-80. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:83-84. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:87-88. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:91-92. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:95-96. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:99-100. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:103-104. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:107-108. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:111-112. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:115-116. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:119-120. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:123-124. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:127-128. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:131-132. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:135-136. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed

herein is set forth in SEQ ID NOs:139-140. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:143-144. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:147-148. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:151-152. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:157-158. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:163-164. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:169-170. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:175-176. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:181-182. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:193-194. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:199-200. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:205-206. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:211-212. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:217-218. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:223-224. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:229-230. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an

anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:233-234. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:237-238. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:241-242. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:245-246. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:249-250. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:253-254. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:257-258. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:261-262. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:265-266. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:269-270. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:273-274. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:277-278. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:281-282. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:285-286. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:293-294. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:297-298. In some embodiments, the amino acid sequences for the heavy chain variable region and the

light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:301-302. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:305-306. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:309-310. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:313-314. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:317-318. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:321-322. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in SEQ ID NOs:325-326. In some embodiments, the amino acid sequences for the heavy chain variable region and the light chain variable region of an anti-TNFR2 antibody disclosed herein is set forth in or SEQ ID NOs:329-330.

[0074] In another embodiment, the anti-TNFR2 antibodies comprise VH and VL sequences that are at least 80% (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, or 99%) identical to the amino acid sequences set forth above, for example but not limited to as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters.

[0075] In a further embodiment, provided herein is an anti-TNFR2 scFv. In view of the sequences for the heavy chain variable regions and light chain variable regions disclosed herein, one of ordinary skill in the art could readily construct an anti-TNFR2 scFv using standard techniques known in the art.

[0076] In certain embodiments, the present disclosure provides polypeptides comprising the VH and VL domains which can be dimerized under suitable conditions. For example, the VH and VL domains may be combined in a suitable buffer and dimerized through appropriate interactions such as hydrophobic interactions. Alternatively, the VH and VL domains may be combined in a suitable buffer containing an enzyme and/or a cofactor which can promote dimerization of the VH and VL domains. In yet a another approach, the VH and VL domains may be combined in a suitable vehicle that allows them to react with each other in the presence of a suitable reagent and/or catalyst.

[0077] In certain embodiments, the VH and VL domains may be contained within longer polypeptide sequences that may include for example, but are not limited to, constant regions, hinge regions, linker

regions, Fc regions, or disulfide binding regions, or any combination thereof. A constant domain is an immunoglobulin fold unit of the constant part of an immunoglobulin molecule, also referred to as a domain of the constant region (e.g. CH1, CH2, CH3, CH4, Ck, Cl). In some embodiments, the longer polypeptides may comprise multiple copies of one or both of the VH and VL domains
5 generated according to the methods disclosed herein; for example, when the polypeptides generated herein are used to form a diabody or a triabody.

[0078] In another embodiment, each of the anti-TNFR2 antibodies presented herein comprises a heavy chain and a light chain, wherein the amino acid sequences for the heavy chain and the light chain can be one of the following pairs: SEQ ID NOs: 438 and 292, SEQ ID NOs:291-292, SEQ
10 ID NOs:235-236, SEQ ID NOs:239-240, SEQ ID NOs:243-244, SEQ ID NOs:247-248, SEQ ID NOs:251-252, SEQ ID NOs:255-256, SEQ ID NOs:259-260, SEQ ID NOs:263-264, SEQ ID NOs:267-268, SEQ ID NOs:271-272, SEQ ID NOs:275-276, SEQ ID NOs:279-280, SEQ ID NOs:283-284, SEQ ID NOs:287-288, SEQ ID NOs:295-296, SEQ ID NOs:299-300, SEQ ID NOs:303-304, SEQ ID NOs:307-308, SEQ ID NOs:311-312, SEQ ID NOs:315-316, SEQ ID
15 NOs:319-320, SEQ ID NOs:323-324, SEQ ID NOs:327-328, SEQ ID NOs:331-332, SEQ ID NOs: 432 and 260, SEQ ID NOs: 433 and 304, SEQ ID NOs: 434 and 308, SEQ ID NOs: 435 and 236, SEQ ID NOs: 436 and 244, SEQ ID NOs: 437 and 284, or SEQ ID NOs: 439 and 296.

[0079] In some embodiments, an anti-TNFR2 antibody comprises a heavy chain and a light chain, wherein the amino acid sequences for the heavy chain and the light chain are set forth in SEQ ID
20 NOs: 438 and 292. In some embodiments, an anti-TNFR2 antibody comprises a heavy chain and a light chain, wherein the amino acid sequences for the heavy chain and the light chain are set forth in SEQ ID NOs:291-292. In some embodiments, an anti-TNFR2 antibody comprises a heavy chain and a light chain, wherein the amino acid sequences for the heavy chain and the light chain are set forth in SEQ ID NOs:235-236. In some embodiments, an anti-TNFR2 antibody comprises a heavy
25 chain and a light chain, wherein the amino acid sequences for the heavy chain and the light chain are set forth in SEQ ID NOs:239-240. In some embodiments, an anti-TNFR2 antibody comprises a heavy chain and a light chain, wherein the amino acid sequences for the heavy chain and the light chain are set forth in SEQ ID NOs:243-244. In some embodiments, an anti-TNFR2 antibody comprises a heavy chain and a light chain, wherein the amino acid sequences for the heavy chain
30 and the light chain are set forth in SEQ ID NOs:247-248. In some embodiments, an anti-TNFR2 antibody comprises a heavy chain and a light chain, wherein the amino acid sequences for the heavy chain and the light chain are set forth in SEQ ID NOs:251-252. In some embodiments, an anti-TNFR2 antibody comprises a heavy chain and a light chain, wherein the amino acid sequences

for the heavy chain and the light chain are set forth in SEQ ID NOs:255-256. In some embodiments, an anti-TNFR2 antibody comprises a heavy chain and a light chain, wherein the amino acid sequences for the heavy chain and the light chain are set forth in SEQ ID NOs:259-260. In some embodiments, an anti-TNFR2 antibody comprises a heavy chain and a light chain, wherein the amino acid sequences for the heavy chain and the light chain are set forth in SEQ ID NOs:263-264. In some embodiments, an anti-TNFR2 antibody comprises a heavy chain and a light chain, wherein the amino acid sequences for the heavy chain and the light chain are set forth in SEQ ID NOs:267-268. In some embodiments, an anti-TNFR2 antibody comprises a heavy chain and a light chain, wherein the amino acid sequences for the heavy chain and the light chain are set forth in SEQ ID NOs:271-272. In some embodiments, an anti-TNFR2 antibody comprises a heavy chain and a light chain, wherein the amino acid sequences for the heavy chain and the light chain are set forth in SEQ ID NOs:275-276. In some embodiments, an anti-TNFR2 antibody comprises a heavy chain and a light chain, wherein the amino acid sequences for the heavy chain and the light chain are set forth in SEQ ID NOs:279-280. In some embodiments, an anti-TNFR2 antibody comprises a heavy chain and a light chain, wherein the amino acid sequences for the heavy chain and the light chain are set forth in SEQ ID NOs:283-284. In some embodiments, an anti-TNFR2 antibody comprises a heavy chain and a light chain, wherein the amino acid sequences for the heavy chain and the light chain are set forth in SEQ ID NOs:287-288. In some embodiments, an anti-TNFR2 antibody comprises a heavy chain and a light chain, wherein the amino acid sequences for the heavy chain and the light chain are set forth in SEQ ID NOs:295-296. In some embodiments, an anti-TNFR2 antibody comprises a heavy chain and a light chain, wherein the amino acid sequences for the heavy chain and the light chain are set forth in SEQ ID NOs:299-300. In some embodiments, an anti-TNFR2 antibody comprises a heavy chain and a light chain, wherein the amino acid sequences for the heavy chain and the light chain are set forth in SEQ ID NOs:303-304. In some embodiments, an anti-TNFR2 antibody comprises a heavy chain and a light chain, wherein the amino acid sequences for the heavy chain and the light chain are set forth in SEQ ID NOs:307-308. In some embodiments, an anti-TNFR2 antibody comprises a heavy chain and a light chain, wherein the amino acid sequences for the heavy chain and the light chain are set forth in SEQ ID NOs:311-312. In some embodiments, an anti-TNFR2 antibody comprises a heavy chain and a light chain, wherein the amino acid sequences for the heavy chain and the light chain are set forth in SEQ ID NOs:315-316. In some embodiments, an anti-TNFR2 antibody comprises a heavy chain and a light chain, wherein the amino acid sequences for the heavy chain and the light chain are set forth in SEQ ID NOs:319-320. In some embodiments, an anti-TNFR2 antibody

comprises a heavy chain and a light chain, wherein the amino acid sequences for the heavy chain and the light chain are set forth in SEQ ID NOs:323-324. In some embodiments, an anti-TNFR2 antibody comprises a heavy chain and a light chain, wherein the amino acid sequences for the heavy chain and the light chain are set forth in SEQ ID NOs:327-328. In some embodiments, an anti-TNFR2 antibody comprises a heavy chain and a light chain, wherein the amino acid sequences for the heavy chain and the light chain are set forth in SEQ ID NOs:331-332. In some embodiments, an anti-TNFR2 antibody comprises a heavy chain and a light chain, wherein the amino acid sequences for the heavy chain and the light chain are set forth in SEQ ID NOs: 432 and 260. In some embodiments, an anti-TNFR2 antibody comprises a heavy chain and a light chain, wherein the amino acid sequences for the heavy chain and the light chain are set forth in SEQ ID NOs: 433 and 304. In some embodiments, an anti-TNFR2 antibody comprises a heavy chain and a light chain, wherein the amino acid sequences for the heavy chain and the light chain are set forth in SEQ ID NOs: 434 and 308. In some embodiments, an anti-TNFR2 antibody comprises a heavy chain and a light chain, wherein the amino acid sequences for the heavy chain and the light chain are set forth in SEQ ID NOs: 435 and 236. In some embodiments, an anti-TNFR2 antibody comprises a heavy chain and a light chain, wherein the amino acid sequences for the heavy chain and the light chain are set forth in SEQ ID NOs: 436 and 244. In some embodiments, an anti-TNFR2 antibody comprises a heavy chain and a light chain, wherein the amino acid sequences for the heavy chain and the light chain are set forth in SEQ ID NOs: 437 and 284. In some embodiments, an anti-TNFR2 antibody comprises a heavy chain and a light chain, wherein the amino acid sequences for the heavy chain and the light chain are set forth in or SEQ ID NOs: 439 and 296.

[0080] In another embodiment, the anti-TNFR2 antibodies comprises heavy chain and light chain amino acid sequences that are at least 80% (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, or 99%) identical to the amino acid sequences set forth above, for example but not limited to as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters.

[0081] In one embodiment, the anti-TNFR2 antibody presented herein can be an IgG, a Fv, a scFv, a Fab, a F(ab')₂, a minibody, a diabody, a triabody, a nanobody, a bispecific antibody, or a single domain antibody. For example, the anti-TNFR2 antibody can be an IgG antibody such as an IgG1, IgG2, IgG3, or IgG4 antibody. In some embodiments, the anti-TNFR2 antibody is an IgG1 antibody. In some other embodiments, the anti-TNFR2 antibody is an IgG2 antibody. In yet some other embodiments, the anti-TNFR2 antibody is an IgG3 antibody. In some further embodiments,

the anti-TNFR2 antibody is an IgG4 antibody.

[0082] In some embodiments, the anti-TNFR2 antibody presented herein can agonize TNFR2. In some embodiments, the anti-TNFR2 antibody presented herein can agonize TNFR2 in an Fc independent manner.

5 [0083] In one embodiment, the present disclosure provides antibodies that bind with high affinity to TNFR2. In one embodiment, binding affinity is calculated by a modification of the Scatchard method as described by Frankel et al. (Mol. Immunol., 16:101-106, 1979). In another embodiment, binding affinity is measured by an antigen/antibody dissociation rate. In another embodiment, binding affinity is measured by a competition radioimmunoassay. In another embodiment, binding
10 affinity is measured by ELISA. In another embodiment, antibody affinity is measured by flow cytometry.

[0084] In one embodiment, the anti-TNFR2 antibody presented herein activates signaling through TNFR2 at a level of at least 80% as compared to signaling activated by TNF. In another embodiment, the anti-TNFR2 antibody presented herein activates signaling through TNFR2 at a
15 level of at least 85%, at least 90%, at least 95%, at least 99%, or at least 100% as compared to signaling activated by TNF.

[0085] In one embodiment, the present disclosure also provides isolated polynucleotide sequences encoding the heavy chain and light chain CDRs as described herein. In another embodiment, the present disclosure provides a vector comprising such polynucleotide sequences. In view of the
20 amino acid sequences disclosed herein, one of ordinary skill in the art would be able to readily construct a vector or plasmid encoding the amino acid sequences. In another embodiment, the present disclosure also provides a host cell comprising such vector as provided herein. Depending on the uses and experimental conditions, one of skill in the art would readily employ a suitable host cell to carry and/or express the above-mentioned polynucleotide sequences. In some
25 embodiments, a host cell comprises a mammalian host cell, such as for example but not limited to ExpiCHO™ and Expi293F™ (ThermoFisher, USA)

[0086] In one embodiment, the present disclosure also provides isolated polynucleotide sequences encoding the heavy chain and light chain variable regions as described herein. In another embodiment, the present disclosure also provides a vector comprising such polynucleotide
30 sequences. In view of the amino acid sequences disclosed herein, one of ordinary skill in the art would readily construct a vector or plasmid to encode for the amino acid sequences. In another embodiment, the present disclosure also provides a host cell comprising the vector provided herein. Depending on the uses and experimental conditions, one of skill in the art would readily

employ a suitable host cell to carry and/or express the above-mentioned polynucleotide sequences. [0087] In one embodiment, the present disclosure also provides isolated polynucleotide sequence encoding the heavy chains and light chains as described herein. In another embodiment, the present disclosure also provides a vector comprising such polynucleotide sequences. In view of the amino acid sequences disclosed herein, one of ordinary skill in the art would readily construct a vector or plasmid to encode for the amino acid sequences. In another embodiment, the present disclosure also provides a host cell comprising the vector provided herein. Depending on the uses and experimental conditions, one of skill in the art would readily employ a suitable host cell to carry and/or express the above-mentioned polynucleotide sequences.

10 Compositions of Use

[0088] In one embodiment, the present disclosure also provides a composition comprising the anti-TNFR2 antibody disclosed herein and a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers of use are well-known in the art. For example, Remington's Pharmaceutical Sciences, by E.W. Martin, Mack Publishing Co., Easton, PA, 23rd Edition, 2020 describes compositions and formulations suitable for pharmaceutical delivery of the antibodies disclosed herein.

[0089] A composition comprising an anti-TNFR2 antibody or an antigen-binding fragment thereof, disclosed herein, can be administered to a subject (e.g. a human or an animal) alone, or in combination with a carrier, i.e., a pharmaceutically acceptable carrier. By pharmaceutically acceptable is meant a material that is not biologically or otherwise undesirable, i.e., the material can be administered to a subject without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the pharmaceutical composition in which it is contained. As would be well-known to one of ordinary skill in the art, the carrier is selected to minimize any degradation of the polypeptides disclosed herein and to minimize any adverse side effects in the subject. The pharmaceutical compositions may be prepared by methodologies well known in the pharmaceutical art.

[0090] In one embodiment, the composition comprises anti-TNFR2 antibodies that comprise a set of three complementarity determining regions (CDRs) on a heavy chain (HCDR1, HCDR2, and HCDR3) and a set of three CDRs on a light chain (LCDR1, LCDR2, and LCDR3). In one embodiment, the set of HCDR1, HCDR2, and HCDR3 comprises the amino acid sequences as shown in Table 7, and the set of LCDR1, LCDR2, and LCDR3 on a corresponding light chain comprises the amino acid sequences as shown in Table 8, as described above.

[0091] In some embodiments, the composition comprises an anti-TNFR2 antibody comprising

HCDR1, HCDR2, and HCDR3 amino acid sequences as set forth in SEQ ID NOs: 185, 190, and 347, respectively, and LCDR1, LCDR2, and LCDR3 amino acid sequences as set forth in SEQ ID NOs: 359, 372, and 378, respectively. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising HCDR1, HCDR2, and HCDR3 amino acid sequences as set forth in SEQ ID NOs: 185, 190, and 347, respectively, and LCDR1, LCDR2, and LCDR3 amino acid sequences as set forth in SEQ ID NOs: 359, 372, and 378, respectively. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising HCDR1, HCDR2, and HCDR3 amino acid sequences as set forth in SEQ ID NOs: 185, 334, 347, respectively, and LCDR1, LCDR2, and LCDR3 amino acid sequences as set forth in SEQ ID NOs: 360, 372, and 379, respectively. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising HCDR1, HCDR2, and HCDR3 amino acid sequences as set forth in SEQ ID NOs: 185, 190, and 347, respectively, and LCDR1, LCDR2, and LCDR3 amino acid sequences as set forth in SEQ ID NOs: 359, 372, and 380, respectively. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising HCDR1, HCDR2, and HCDR3 amino acid sequences as set forth in SEQ ID NOs: 185, 190, and 347, respectively, and LCDR1, LCDR2, and LCDR3 amino acid sequences as set forth in SEQ ID NOs: 359, 372, and 381, respectively. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising HCDR1, HCDR2, and HCDR3 amino acid sequences as set forth in SEQ ID NOs: 185, 190, and 347, respectively, and LCDR1, LCDR2, and LCDR3 amino acid sequences as set forth in SEQ ID NOs: 361, 372, and 381, respectively. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising HCDR1, HCDR2, and HCDR3 amino acid sequences as set forth in SEQ ID NOs: 185, 190, and 347, respectively, and LCDR1, LCDR2, and LCDR3 amino acid sequences as set forth in SEQ ID NOs: 362, 372, and 382, respectively. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising HCDR1, HCDR2, and HCDR3 amino acid sequences as set forth in SEQ ID NOs: 185, 335, and 347, respectively, and LCDR1, LCDR2, and LCDR3 amino acid sequences as set forth in SEQ ID NOs: 363, 372, and 383, respectively. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising HCDR1, HCDR2, and HCDR3 amino acid sequences as set forth in SEQ ID NOs: 185, 190, 347, respectively, and the set of LCDR1, LCDR2, and LCDR3 for the same antibody comprises the amino acid sequences set forth in SEQ ID NOs: 364, 372, and 384, respectively. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising HCDR1, HCDR2, and HCDR3 amino acid sequences as set forth in SEQ ID NOs: 185, 336, and 348, respectively, and LCDR1, LCDR2, and LCDR3 amino acid sequences as set forth in SEQ ID NOs: 365, 372,

and 378, respectively. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising HCDR1, HCDR2, and HCDR3 amino acid sequences as set forth in SEQ ID NOs: 185, 337, and 347, respectively, and LCDR1, LCDR2, and LCDR3 amino acid sequences as set forth in SEQ ID NOs: 359, 373, and 385, respectively. In some embodiments, the composition

5 comprises an anti-TNFR2 antibody comprising HCDR1, HCDR2, and HCDR3 amino acid sequences as set forth in SEQ ID NOs: 185, 338, and 348, respectively, and LCDR1, LCDR2, and LCDR3 amino acid sequences as set forth in SEQ ID NOs: 359, 372, and 386, respectively. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising HCDR1, HCDR2, and HCDR3 amino acid sequences as set forth in SEQ ID NOs: 185, 339, and 349,

10 respectively, and LCDR1, LCDR2, and LCDR3 amino acid sequences as set forth in SEQ ID NOs: 359, 372, and 378, respectively. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising HCDR1, HCDR2, and HCDR3 amino acid sequences as set forth in SEQ ID NOs: 185, 190, and 350, respectively, and LCDR1, LCDR2, and LCDR3 amino acid sequences as set forth in SEQ ID NOs: 366, 372, and 387, respectively. In some embodiments, the composition

15 comprises an anti-TNFR2 antibody comprising HCDR1, HCDR2, and HCDR3 amino acid sequences as set forth in SEQ ID NOs: 185, 190, and 351, respectively, and LCDR1, LCDR2, and LCDR3 amino acid sequences as set forth in SEQ ID NOs: 359, 372, and 378, respectively. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising HCDR1, HCDR2, and HCDR3 amino acid sequences as set forth in SEQ ID NOs: 185, 334, and 347,

20 respectively, and LCDR1, LCDR2, and LCDR3 amino acid sequences as set forth in SEQ ID NOs: 360, 372, and 388, respectively. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising HCDR1, HCDR2, and HCDR3 amino acid sequences as set forth in SEQ ID NOs: 185, 334, and 352, respectively, and LCDR1, LCDR2, and LCDR3 amino acid sequences as set forth in SEQ ID NOs: 359, 372, and 378, respectively. In some embodiments, the composition

25 comprises an anti-TNFR2 antibody comprising HCDR1, HCDR2, and HCDR3 amino acid sequences as set forth in SEQ ID NOs: 185, 190, and 352, respectively, and LCDR1, LCDR2, and LCDR3 amino acid sequences as set forth in SEQ ID NOs: 359, 372, and 389, respectively. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising HCDR1, HCDR2, and HCDR3 amino acid sequences as set forth in SEQ ID NOs: 185, 334, and 347,

30 respectively, and LCDR1, LCDR2, and LCDR3 amino acid sequences as set forth in SEQ ID NOs: 359, 372, and 378, respectively. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising HCDR1, HCDR2, and HCDR3 amino acid sequences as set forth in SEQ ID NOs: 185, 190, and 347, respectively, and LCDR1, LCDR2, and LCDR3 amino acid sequences as

set forth in SEQ ID NOs: 365, 372, and 378, respectively. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising HCDR1, HCDR2, and HCDR3 amino acid sequences as set forth in SEQ ID NOs: 185, 336, and 352, respectively, and LCDR1, LCDR2, and LCDR3 amino acid sequences as set forth in SEQ ID NOs: 365, 372, and 390, respectively. In

5 some embodiments, the composition comprises an anti-TNFR2 antibody comprising HCDR1, HCDR2, and HCDR3 amino acid sequences as set forth in SEQ ID NOs: 185, 336, and 352, respectively, and LCDR1, LCDR2, and LCDR3 amino acid sequences as set forth in SEQ ID NOs: 365, 374, and 391, respectively. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising HCDR1, HCDR2, and HCDR3 amino acid sequences as set forth in SEQ ID

10 NOs: 186, 340, and 353, respectively, and LCDR1, LCDR2, and LCDR3 amino acid sequences as set forth in SEQ ID NOs: 367, 375, and 392, respectively. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising HCDR1, HCDR2, and HCDR3 amino acid sequences as set forth in SEQ ID NOs: 186, 341, and 354, respectively, and LCDR1, LCDR2, and LCDR3 amino acid sequences as set forth in SEQ ID NOs: 367, 375, 392, respectively. In some

15 embodiments, the composition comprises an anti-TNFR2 antibody comprising HCDR1, HCDR2, and HCDR3 amino acid sequences as set forth in SEQ ID NOs: 186, 341, and 354, respectively, and LCDR1, LCDR2, and LCDR3 amino acid sequences as set forth in SEQ ID NOs: 368, 375, and 392, respectively. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising HCDR1, HCDR2, and HCDR3 amino acid sequences as set forth in SEQ ID NOs:

20 186, 342, and 354, respectively, and LCDR1, LCDR2, and LCDR3 amino acid sequences as set forth in SEQ ID NOs: 367, 375 and 392, respectively. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising HCDR1, HCDR2, and HCDR3 amino acid sequences as set forth in SEQ ID NOs: 186, 343, and 354, respectively, and LCDR1, LCDR2, and LCDR3 amino acid sequences as set forth in SEQ ID NOs: 369, 375, and 393, respectively. In

25 some embodiments, the composition comprises an anti-TNFR2 antibody comprising HCDR1, HCDR2, and HCDR3 amino acid sequences as set forth in SEQ ID NOs: 186, 343, and 355, respectively, and LCDR1, LCDR2, and LCDR3 amino acid sequences as set forth in SEQ ID NOs: 367, 375, and 392, respectively. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising HCDR1, HCDR2, and HCDR3 amino acid sequences as set forth in SEQ ID

30 NOs: 186, 341, and 354, respectively, and LCDR1, LCDR2, and LCDR3 amino acid sequences as set forth in SEQ ID NOs: 367, 375, and 392, respectively. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising HCDR1, HCDR2, and HCDR3 amino acid sequences as set forth in SEQ ID NOs: 186, 341, and 355, respectively, and LCDR1, LCDR2, and

LCDR3 amino acid sequences as set forth in SEQ ID NOs: 367, 376, and 392, respectively. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising HCDR1, HCDR2, and HCDR3 amino acid sequences as set forth in SEQ ID NOs: 186, 344, and 354, respectively, and LCDR1, LCDR2, and LCDR3 amino acid sequences as set forth in SEQ ID NOs: 370, 375, and 392, respectively. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising HCDR1, HCDR2, and HCDR3 amino acid sequences as set forth in SEQ ID NOs: 186, 341, and 354, respectively, and LCDR1, LCDR2, and LCDR3 amino acid sequences as set forth in SEQ ID NOs: 367, 375, and 394, respectively. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising HCDR1, HCDR2, and HCDR3 amino acid sequences as set forth in SEQ ID NOs: 186, 344, and 354, respectively, and LCDR1, LCDR2, and LCDR3 amino acid sequences as set forth in SEQ ID NOs: 367, 375, and 392, respectively. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising HCDR1, HCDR2, and HCDR3 amino acid sequences as set forth in SEQ ID NOs: 186, 341, and 359, respectively, and LCDR1, LCDR2, and LCDR3 amino acid sequences as set forth in SEQ ID NOs: 367, 375, and 393, respectively. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising HCDR1, HCDR2, and HCDR3 amino acid sequences as set forth in SEQ ID NOs: 187, 345, and 356, respectively, and LCDR1, LCDR2, and LCDR3 amino acid sequences as set forth in SEQ ID NOs: 371, 377, and 395, respectively. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising HCDR1, HCDR2, and HCDR3 amino acid sequences as set forth in SEQ ID NOs: 188, 345, and 357, respectively, and LCDR1, LCDR2, and LCDR3 amino acid sequences as set forth in SEQ ID NOs: 371, 377, and 396, respectively. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising HCDR1, HCDR2, and HCDR3 amino acid sequences as set forth in SEQ ID NOs: 189, 346, and 358, respectively, and LCDR1, LCDR2, and LCDR3 amino acid sequences as set forth in SEQ ID NOs: 371, 377, and 397, respectively.

[0092] In another embodiment, the set of HCDR1, HCDR2, HCDR3, and the set of LCDR1, LCDR2, LCDR3 on a corresponding light chain comprise the amino acid sequences as shown in Table 9, as described above.

[0093] In some embodiments, the composition comprises an anti-TNFR2 antibody comprising HCDR1, HCDR2, and HCDR3 amino acid sequences as set forth in SEQ ID NOs: 402, 345, and 413, respectively, and LCDR1, LCDR2, and LCDR3 amino acid sequences as set forth in SEQ ID NOs: 371, 418, and 427, respectively. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising HCDR1, HCDR2, and HCDR3 amino acid sequences as set forth in

SEQ ID NOs: 398, 346, and 407, respectively, and LCDR1, LCDR2, and LCDR3 amino acid sequences as set forth in SEQ ID NOs: 371, 377, and 419, respectively. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising HCDR1, HCDR2, and HCDR3 amino acid sequences as set forth in SEQ ID NOs: 187, 346, and 408, respectively, and LCDR1, LCDR2, and LCDR3 amino acid sequences as set forth in SEQ ID NOs: 371, 377, and 420, respectively. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising HCDR1, HCDR2, and HCDR3 amino acid sequences as set forth in SEQ ID NOs: 189, 406, and 409, respectively, and LCDR1, LCDR2, and LCDR3 amino acid sequences as set forth in SEQ ID NOs: 371, 377, and 421, respectively. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising HCDR1, HCDR2, and HCDR3 amino acid sequences as set forth in SEQ ID NOs: 187, 345, and 410, respectively, and LCDR1, LCDR2, and LCDR3 amino acid sequences as set forth in SEQ ID NOs: 371, 418, and 422, respectively. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising HCDR1, HCDR2, and HCDR3 amino acid sequences as set forth in SEQ ID NOs: 399, 346, and 411, respectively, and LCDR1, LCDR2, and LCDR3 amino acid sequences as set forth in SEQ ID NOs: 371, 418, and 423, respectively. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising HCDR1, HCDR2, and HCDR3 amino acid sequences as set forth in SEQ ID NOs: 400, 346, and 412, respectively, and LCDR1, LCDR2, and LCDR3 amino acid sequences as set forth in SEQ ID NOs: 417, 377, and 424, respectively. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising HCDR1, HCDR2, and HCDR3 amino acid sequences as set forth in SEQ ID NOs: 401, 346, and 413, respectively, and LCDR1, LCDR2, and LCDR3 amino acid sequences as set forth in SEQ ID NOs: 417, 418, and 425, respectively. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising HCDR1, HCDR2, and HCDR3 amino acid sequences as set forth in SEQ ID NOs: 401, 346, and 414, respectively, and LCDR1, LCDR2, and LCDR3 amino acid sequences as set forth in SEQ ID NOs: 417, 418, and 426, respectively. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising HCDR1, HCDR2, and HCDR3 amino acid sequences as set forth in SEQ ID NOs: 398, 346, and 415, respectively, and LCDR1, LCDR2, and LCDR3 amino acid sequences as set forth in SEQ ID NOs: 417, 377, and 428, respectively. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising HCDR1, HCDR2, and HCDR3 amino acid sequences as set forth in SEQ ID NOs: 403, 346, and 412, respectively, and LCDR1, LCDR2, and LCDR3 amino acid sequences as set forth in SEQ ID NOs: 371, 418, and 429, respectively. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising HCDR1,

- HCDR2, and HCDR3 amino acid sequences as set forth in SEQ ID NOs: 404, 346, and 412, respectively, and LCDR1, LCDR2, and LCDR3 amino acid sequences as set forth in SEQ ID NOs: 371, 418, and 425, respectively. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising HCDR1, HCDR2, and HCDR3 amino acid sequences as set forth in SEQ ID NOs: 401, 346, and 414, respectively, and LCDR1, LCDR2, and LCDR3 amino acid sequences as set forth in SEQ ID NOs: 417, 418, and 430, respectively. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising HCDR1, HCDR2, and HCDR3 amino acid sequences as set forth in SEQ ID NOs: 405, 406, and 416, respectively, and LCDR1, LCDR2, and LCDR3 amino acid sequences as set forth in SEQ ID NOs: 371, 377, and 431, respectively.
- 5 [0094] In another embodiment, the composition comprises anti-TNFR2 antibodies having heavy chain and light chain CDR sequences that are at least 80% (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, or 99%) identical to the amino acid sequences set forth above, for example but not limited to identity as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters.
- 10 [0095] In another embodiment, the composition comprises anti-TNFR2 antibodies having one of the following pairs of heavy chain variable region and light chain variable region: SEQ ID NOs:289-290, SEQ ID NOs:3-4, SEQ ID NOs:7-8, SEQ ID NOs:11-12, SEQ ID NOs:15-16, SEQ ID NOs:19-20, SEQ ID NOs:23-24, SEQ ID NOs:27-28, SEQ ID NOs:31-32, SEQ ID NOs:35-36, SEQ ID NOs:39-40, SEQ ID NOs:43-44, SEQ ID NOs:47-48, SEQ ID NOs:51-52, SEQ ID NOs:55-56, SEQ ID NOs:59-60, SEQ ID NOs:63-64, SEQ ID NOs:67-68, SEQ ID NOs:71-72, SEQ ID NOs:75-76, SEQ ID NOs:79-80, SEQ ID NOs:83-84, SEQ ID NOs:87-88, SEQ ID NOs:91-92, SEQ ID NOs:95-96, SEQ ID NOs:99-100, SEQ ID NOs:103-104, SEQ ID NOs:107-108, SEQ ID NOs:111-112, SEQ ID NOs:115-116, SEQ ID NOs:119-120, SEQ ID NOs:123-124, SEQ ID NOs:127-128, SEQ ID NOs:131-132, SEQ ID NOs:135-136, SEQ ID NOs:139-140, SEQ ID NOs:143-144, SEQ ID NOs:147-148, SEQ ID NOs:151-152, SEQ ID NOs:157-158, SEQ ID NOs:163-164, SEQ ID NOs:169-170, SEQ ID NOs:175-176, SEQ ID NOs:181-182, SEQ ID NOs:193-194, SEQ ID NOs:199-200, SEQ ID NOs:205-206, SEQ ID NOs:211-212, SEQ ID NOs:217-218, SEQ ID NOs:223-224, SEQ ID NOs:229-230, SEQ ID NOs:233-234, SEQ ID NOs:237-238, SEQ ID NOs:241-242, SEQ ID NOs:245-246, SEQ ID NOs:249-250, SEQ ID NOs:253-254, SEQ ID NOs:257-258, SEQ ID NOs:261-262, SEQ ID NOs:265-266, SEQ ID NOs:269-270, SEQ ID NOs:273-274, SEQ ID NOs:277-278, SEQ ID NOs:281-282, SEQ ID NOs:285-286, SEQ ID NOs:293-294, SEQ ID NOs:297-298, SEQ ID NOs:301-302, SEQ ID NOs:305-306, SEQ ID NOs:309-310, SEQ ID NOs:313-314, SEQ ID NOs:317-318, SEQ ID
- 20
- 25
- 30

NOs:321-322, SEQ ID NOs:325-326, or SEQ ID NOs:329-330.

[0096] In certain embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:289-290. In certain embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:3-4. In certain embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:7-8. In certain embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:11-12. In certain embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:15-16. In certain embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:19-20. In certain embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:23-24. In certain embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:27-28. In certain embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:31-32. In certain embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:35-36. In certain embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:39-40. In certain embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:43-44. In certain embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:47-48. In certain embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:51-52. In certain embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:55-56. In certain embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:59-60. In certain

embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:63-64. In certain embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:67-68. In certain
5 embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:71-72. In certain embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:75-76. In certain
10 embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:79-80. In certain embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:83-84. In certain
embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:87-88. In certain
15 embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:91-92. In certain embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:95-96. In certain
embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:99-100. In certain
20 embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:103-104. In certain
embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:107-108. In certain
25 embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:111-112. In certain
embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:115-116. In certain
embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain
30 variable region and light chain variable region as set forth in SEQ ID NOs:119-120. In certain
embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:123-124. In certain
embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain

variable region and light chain variable region as set forth in SEQ ID NOs:127-128. In certain embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:131-132. In certain embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:135-136. In certain
5 embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:139-140. In certain embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:143-144. In certain
10 embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:147-148. In certain embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:151-152. In certain
15 embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:157-158. In certain embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:163-164. In certain
20 embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:169-170. In certain embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:175-176. In certain
25 embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:181-182. In certain embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:193-194. In certain
30 embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:199-200. In certain embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:205-206. In certain
embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:211-212. In certain
embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:217-218. In certain

embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:223-224. In certain embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:229-230. In certain
5 embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:233-234. In certain embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:237-238. In certain
10 embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:241-242. In certain embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:245-246. In certain
embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:249-250. In certain
15 embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:253-254. In certain embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:257-258. In certain
embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:261-262. In certain
20 embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:265-266. In certain embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:269-270. In certain
25 embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:273-274. In certain embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:277-278. In certain
embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:281-282. In certain
30 embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:285-286. In certain
embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain

variable region and light chain variable region as set forth in SEQ ID NOs:293-294. In certain embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:297-298. In certain embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:301-302. In certain embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:305-306. In certain embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:309-310. In certain embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:313-314. In certain embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:317-318. In certain embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:321-322. In certain embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in SEQ ID NOs:325-326. In certain embodiments, the composition comprises an anti-TNFR2 antibody comprising the heavy chain variable region and light chain variable region as set forth in or SEQ ID NOs:329-330.

[0097] In another embodiment, the composition comprises anti-TNFR2 antibodies having VH and VL sequences that are at least 80% (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, or 99%) identical to the amino acid sequences set forth above, for example but not limited to identity as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters.

[0098] In yet another embodiment, the composition comprises anti-TNFR2 antibodies having one of the following pairs of heavy chain and light chain: SEQ ID NOs: 438 and 292, SEQ ID NOs:291-292, SEQ ID NOs:235-236, SEQ ID NOs:239-240, SEQ ID NOs:243-244, SEQ ID NOs:247-248, SEQ ID NOs:251-252, SEQ ID NOs:255-256, SEQ ID NOs:259-260, SEQ ID NOs:263-264, SEQ ID NOs:267-268, SEQ ID NOs:271-272, SEQ ID NOs:275-276, SEQ ID NOs:279-280, SEQ ID NOs:283-284, SEQ ID NOs:287-288, SEQ ID NOs:295-296, SEQ ID NOs:299-300, SEQ ID NOs:303-304, SEQ ID NOs:307-308, SEQ ID NOs:311-312, SEQ ID NOs:315-316, SEQ ID NOs:319-320, SEQ ID NOs:323-324, SEQ ID NOs:327-328, SEQ ID NOs:331-332, SEQ ID NOs: 432 and 260, SEQ ID NOs: 433 and 304, SEQ ID NOs: 434 and 308,

SEQ ID NOs: 435 and 236, SEQ ID NOs: 436 and 244, SEQ ID NOs: 437 and 284, or SEQ ID NOs: 439 and 296.

[0099] In some embodiments, the composition comprises an anti-TNFR2 antibody comprising a heavy chain and a light chain as set forth in SEQ ID NOs: 438 and 292. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising a heavy chain and a light chain as set forth in SEQ ID NOs:291-292. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising a heavy chain and a light chain as set forth in SEQ ID NOs:235-236. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising a heavy chain and a light chain as set forth in SEQ ID NOs:239-240. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising a heavy chain and a light chain as set forth in SEQ ID NOs:243-244. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising a heavy chain and a light chain as set forth in SEQ ID NOs:247-248. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising a heavy chain and a light chain as set forth in SEQ ID NOs:251-252. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising a heavy chain and a light chain as set forth in SEQ ID NOs:255-256. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising a heavy chain and a light chain as set forth in SEQ ID NOs:259-260. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising a heavy chain and a light chain as set forth in SEQ ID NOs:263-264. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising a heavy chain and a light chain as set forth in SEQ ID NOs:267-268. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising a heavy chain and a light chain as set forth in SEQ ID NOs:271-272. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising a heavy chain and a light chain as set forth in SEQ ID NOs:275-276. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising a heavy chain and a light chain as set forth in SEQ ID NOs:279-280. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising a heavy chain and a light chain as set forth in SEQ ID NOs:283-284. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising a heavy chain and a light chain as set forth in SEQ ID NOs:287-288. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising a heavy chain and a light chain as set forth in SEQ ID NOs:295-296. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising a heavy chain and a light chain as set forth in SEQ ID NOs:299-300. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising a heavy chain and

a light chain as set forth in SEQ ID NOs:303-304. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising a heavy chain and a light chain as set forth in SEQ ID NOs:307-308. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising a heavy chain and a light chain as set forth in SEQ ID NOs:311-312. In some
5 embodiments, the composition comprises an anti-TNFR2 antibody comprising a heavy chain and a light chain as set forth in SEQ ID NOs:315-316. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising a heavy chain and a light chain as set forth in SEQ ID NOs:319-320. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising a heavy chain and a light chain as set forth in SEQ ID NOs:323-324. In some
10 embodiments, the composition comprises an anti-TNFR2 antibody comprising a heavy chain and a light chain as set forth in SEQ ID NOs:327-328. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising a heavy chain and a light chain as set forth in SEQ ID NOs:331-332. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising a heavy chain and a light chain as set forth in SEQ ID NOs: 432 and 260. In some
15 embodiments, the composition comprises an anti-TNFR2 antibody comprising a heavy chain and a light chain as set forth in SEQ ID NOs: 433 and 304. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising a heavy chain and a light chain as set forth in SEQ ID NOs: 434 and 308. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising a heavy chain and a light chain as set forth in SEQ ID NOs: 435 and 236. In some
20 embodiments, the composition comprises an anti-TNFR2 antibody comprising a heavy chain and a light chain as set forth in SEQ ID NOs: 436 and 244. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising a heavy chain and a light chain as set forth in SEQ ID NOs: 437 and 284. In some embodiments, the composition comprises an anti-TNFR2 antibody comprising a heavy chain and a light chain as set forth in or SEQ ID NOs: 439 and 296.

25 [0100] . In another embodiment, the composition comprises anti-TNFR2 antibodies having heavy chains and light chains that are at least 80% (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, or 99%) identical to the amino acid sequences set forth above, for example but not limited to identity as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters.

30 [0101] In one embodiment, the antibodies disclosed herein can be in the form of a conjugate. As used herein, a "conjugate" is an antibody or antibody fragment (such as an antigen-binding fragment) covalently linked to an effector molecule or a second protein (such as a second antibody). The effector molecule can be, for example, a drug, toxin, therapeutic agent, detectable

label, protein, nucleic acid, lipid, nanoparticle, carbohydrate or recombinant virus. An antibody conjugate can also be referred to as an "immunoconjugate." When the conjugate comprises an antibody linked to a drug (e.g., a cytotoxic agent), the conjugate can be referred to as an "antibody-drug conjugate". Other antibody conjugates include, for example, multi-specific (such as bispecific or trispecific) antibodies and chimeric antigen receptors (CARs).

[0102] The pharmaceutical compositions comprising the antibodies or antigen-binding fragments thereof disclosed herein can be administered (e.g., to a mammal, a cell, or a tissue) in any suitable manner depending on whether local or systemic treatment is desired. For example, the composition can be administered topically (e.g. ophthalmically, vaginally, rectally, intranasally, transdermally, and the like), orally, by inhalation, or parenterally (including by intravenous drip or subcutaneous, intracavity, intraperitoneal, intradermal, or intramuscular injection). Topical intranasal administration refers to delivery of the compositions into the nose and nasal passages through one or both of the nares. The composition can be delivered by a spraying mechanism or droplet mechanism, or through aerosolization. Alternatively, administration can be intratumoral, e.g. local or intravenous injection.

[0103] If the composition is to be administered parenterally, the administration is generally by injection. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for suspension in liquid prior to injection, or as emulsions. Additionally, parental administration can involve preparation of a slow-release or sustained-release system so as to maintain a constant dosage.

Methods of Use

[0104] In one embodiment, the anti-TNFR2 antibodies disclosed herein can be used to modulate the proliferation and/or functions of regulatory T cells (T-reg cells) or myeloid-derived suppressor cells.

[0105] T-reg cells represent a heterogeneous class of T cells that can be distinguished based on their unique surface protein presentation. The most studied T-reg cells include CD4⁺, CD25⁺, FoxP3⁺ T-reg cells and CD17⁺ T-reg cells. It has been shown that certain classes of T-reg cells inhibit production of the proliferation-inducing cytokine, interleukin-2 (IL-2), in target T cells and may additionally sequester IL-2 from autoreactive cells by virtue of the affinity of CD25 (a subdomain of the IL-2 receptor) for IL-2. Moreover, it has been shown that CD4⁺, CD25⁺, FoxP3⁺ T-reg cells are also present in B cell-rich areas and are capable of directly suppressing immunoglobulin production independent of their ability to attenuate TH2-cell activity.

[0106] As used herein, the term "myeloid-derived suppressor cell" or "MDSC" refers to a cell of

the immune system that can modulate the activity of a variety of effector cells and antigen-presenting cells, such as T cells, NK cells, dendritic cells, and macrophages, among others. Myeloid derived suppressor cells are distinguished by their gene expression profile.

[0107] In another embodiment, the anti-TNFR2 antibodies disclosed herein can be used to treat diseases such as cancer, autoimmune diseases, GvHd, viral infection or bacterial infection.

[0108] As used herein, the term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known manners, means, techniques and procedures by practitioners of the chemical, pharmacological, biological, biochemical and medical arts.

[0109] As used herein, the terms "treat", "treatment", or "therapy" (as well as different forms thereof) refer to therapeutic treatment, including prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) an undesired physiological change associated with a disease or condition. Beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of the extent of a disease or condition, stabilization of a disease or condition (i.e., where the disease or condition does not worsen), delay or slowing of the progression of a disease or condition, amelioration or palliation of the disease or condition, and remission (whether partial or total) of the disease or condition, whether detectable or undetectable. Those in need of treatment include those already with the disease or condition as well as those prone to having the disease or condition or those in which the disease or condition is to be prevented.

[0110] The terms "subject," "individual," and "patient" are used interchangeably herein, and refer to human or non-human animals to whom treatment with a composition or formulation in accordance with the present anti-TNFR2 antibodies is provided. The terms "non-human animals" and "non-human mammals" are used interchangeably herein and include all vertebrates, e.g., mammals, such as non-human primates (e.g. higher primates), sheep, dog, rodent (e.g. mouse or rat), guinea pig, goat, pig, cat, rabbits, cows, horses, or non-mammals such as reptiles, amphibians, chickens, and turkeys. The compositions described herein can be used to treat any suitable mammal, including primates, such as monkeys and humans, horses, cows, cats, dogs, rabbits, and rodents such as rats and mice. In one embodiment, the mammal to be treated is human. The human can be any human of any age. In one embodiment, the human is an adult. In another embodiment, the human is a child. The human can be male, female, pregnant, middle-aged, adolescent, or elderly.

- [0111] Pharmaceutical compositions suitable for use in the methods disclosed herein include compositions wherein the active ingredients are contained in an amount effective to achieve the intended purpose. In one embodiment, a therapeutically effective amount means an amount of one or more active ingredients (e.g., an anti-TNFR2 antibody) effective to prevent, alleviate or ameliorate symptoms of disease or prolong the survival of the subject being treated. Determination of a therapeutically effective amount is well within the capability of those skilled in the art.
- [0112] In one embodiment, the present disclosure provides a method of modulating activities or functions of regulatory T cells in a subject. As used herein, “modulating” refers to “stimulating” or “inhibiting” an activity of a molecular target or pathway. For example, a composition modulates the activity of a molecular target or pathway if it stimulates or inhibits the activity of the molecular target or pathway by at least 10%, by at least about 20%, by at least about 25%, by at least about 30%, by at least about 40%, by at least about 50%, by at least about 60%, by at least about 70%, by at least about 75%, by at least about 80%, by at least about 90%, by at least about 95%, by at least about 98%, or by about 99% or more relative to the activity of the molecular target or pathway under the same conditions but lacking only the presence of the composition. In another example, a composition modulates the activity of a molecular target or pathway if it stimulates or inhibits the activity of the molecular target or pathway by at least 2-fold, at least 5 -fold, at least 10-fold, at least 20-fold, at least 50-fold, at least 100-fold relative to the activity of the molecular target or pathway under the same conditions but lacking only the presence of the composition. The activity of a molecular target or pathway may be measured by any reproducible means. The activity of a molecular target or pathway may be measured *in vitro* or *in vivo*. For example, the activity of a molecular target or pathway may be measured *in vitro* or *in vivo* by an appropriate assay known in the art measuring the activity. Control samples (untreated with the composition) can be assigned a relative activity value of 100%.
- [0113] In one embodiment, the method comprises the step of administering to the subject a composition comprising an effective amount of the anti-TNFR2 antibody disclosed herein. In one embodiment, the composition comprises anti-TNFR2 antibodies having the heavy chain and light chain CDR sequences as described herein. In another embodiment, the composition comprises anti-TNFR2 antibodies having the VH and VL sequences as described herein. In yet another embodiment, the composition comprises anti-TNFR2 antibodies having the heavy chain and light chain sequences as described herein. In one embodiment, the regulatory T cells are CD4+CD25+Foxp3+ or alternatively CD4+CD25+C127_{low}. In one embodiment, the method stimulates proliferation of the regulatory T cells. In another embodiment, the method can activate

the regulatory T cells. In another embodiment, the method can modulate immune responses mediated by said regulatory T cells.

[0114] One skilled in the art would appreciate that in some embodiments, activation of regulatory T cells suppresses proliferation of immune system effector cells including Teff and NK cells, or
5 reduces cytotoxic activity of effector cells including Teff and NK cells, or encourages immune system effector cells including Teff and NK cells to produce less proinflammatory cytokines, or any combination thereof. In some embodiments, administration of an anti-TNFR2 antibody to a subject in a method of use as described herein, suppresses proliferation of immune system effector cells including Teff and NK cells. In some embodiments, administration of an anti-TNFR2
10 antibody to a subject in a method as described herein, reduces cytotoxic activity of immune system effector cells including Teff and NK cells. In some embodiments, use of an anti-TNFR2 antibody for administration to a subject in a method as described herein, encourages immune system effector cells including Teff and NK cells to produce fewer proinflammatory cytokines.

[0115] One skilled in the art would appreciate that in some embodiments, modulation of an
15 immune response encompasses a reduction of inflammation or elimination of inflammation in a situation wherein the expected outcome without the use of an anti-TNFR2 antibody described herein, would have been inflammation.

[0116] In one embodiment, the present disclosure also provides uses of a composition comprising the subject anti-TNFR2 antibodies for modulating activities or functions of regulatory T cells in a
20 subject. In one embodiment, the composition comprises anti-TNFR2 antibodies having the heavy chain and light chain CDR sequences as described herein. In another embodiment, the composition comprises anti-TNFR2 antibodies having the VH and VL sequences as described herein. In yet another embodiment, the composition comprises anti-TNFR2 antibodies having the heavy chain and light chain sequences as described herein.

[0117] In one embodiment, the present disclosure provides a method of modulating activities or
25 functions of myeloid-derived suppressor cells (MDSCs) in a subject. In one embodiment, the method comprises the step of administering to the subject a composition comprising an effective amount of the anti-TNFR2 antibody disclosed herein. In one embodiment, the composition comprises anti-TNFR2 antibodies having the heavy chain and light chain CDR sequences as
30 described herein. In another embodiment, the composition comprises anti-TNFR2 antibodies having the VH and VL sequences as described herein. In yet another embodiment, the composition comprises anti-TNFR2 antibodies having the heavy chain and light chain sequences as described herein. In one embodiment, the method stimulates proliferation of the MDSCs. In another

embodiment, the method can activate the MDSCs.

[0118] One skilled in the art would appreciate that in some embodiments, activation of MDSCs suppresses proliferation of immune system effector cells including Teff and NK cells, or reduces cytotoxic activity of immune system effector cells including Teff and NK cells, or down-regulates the production of proinflammatory cytokines, or any combination thereof by immune system effector cells including Teff and NK cells. In some embodiments, administration of an anti-TNFR2 antibody to subject in need, in a method described herein, suppresses proliferation of Immune system effector cells including Teff and NK cells. In some embodiments, use of an anti-TNFR2 antibody for administration to subject in need in a method described herein, suppresses proliferation of Immune system effector cells including Teff and NK cells. In some embodiments, use of an anti-TNFR2 antibody to a subject in a method described herein, reduces cytotoxic activity of Immune system effector cells including Teff and NK cells. In some embodiments, use of an anti-TNFR2 antibody to a subject in a method described herein, encourages Immune system effector cells including Teff and NK cells to produce less proinflammatory cytokines.

[0119] In one embodiment, the present disclosure also provides uses of a composition comprising anti-TNFR2 antibodies for modulating activities or functions of MDSCs in a subject. In one embodiment, the composition comprises anti-TNFR2 antibodies having the heavy chain and light chain CDR sequences as described herein. In another embodiment, the composition comprises anti-TNFR2 antibodies having the VH and VL sequences as described herein. In yet another embodiment, the composition comprises anti-TNFR2 antibodies having the heavy chain and light chain sequences as described herein.

[0120] In another embodiment, the present disclosure provides a method of treating a disease in a subject, comprising the step of administering to the subject a composition comprising an effective amount of the anti-TNFR2 antibody disclosed herein. In one embodiment, the composition comprises anti-TNFR2 antibodies having the heavy chain and light chain CDR sequences as described herein. In another embodiment, the composition comprises anti-TNFR2 antibodies having the VH and VL sequences as described herein. In yet another embodiment, the composition comprises anti-TNFR2 antibodies having the heavy chain and light chain sequences as described herein.

[0121] In one embodiment, the present disclosure also provides uses of a composition comprising anti-TNFR2 antibodies for treating a disease in a subject. In one embodiment, the composition comprises anti-TNFR2 antibodies having the heavy chain and light chain CDR sequences as described herein. In another embodiment, the composition comprises anti-TNFR2 antibodies

having the VH and VL sequences as described herein. In yet another embodiment, the composition comprises anti-TNFR2 antibodies having the heavy chain and light chain sequences as described herein.

[0122] In one embodiment, the exact amount of the present polypeptides or compositions thereof required to elicit the desired effects will vary from subject to subject, depending on the species, age, gender, weight, and general condition of the subject, the particular polypeptides, the route of administration, and whether other drugs are included in the regimen. Thus, it is not possible to specify an exact amount for every composition. However, an appropriate amount can be determined by one of ordinary skill in the art using routine experimentation. Dosages can vary, and the polypeptides can be administered in one or more (e.g., two or more, three or more, four or more, or five or more) doses daily, for one or more days. Guidance in selecting appropriate doses for antibodies can be readily found in the literature.

[0123] In one embodiment, the disease can be viral infection, bacterial infection, cancer, autoimmune disease or immune disorder. In one embodiment, the disease can be an upper respiratory viral infection, an early stage lung infection, or a late stage lung infection. A number of diseases and cancers are known to be caused by viruses. Examples of disease-causing viruses include, but are not limited to, norovirus; rotavirus; hepatitis virus A, B, C, D, or E; rabies virus, West Nile virus, enterovirus, echovirus, coxsackievirus, herpes simplex virus (HSV), HSV-2, varicella-zoster virus, mosquito-borne viruses, arbovirus, St. Louis encephalitis virus, California encephalitis virus, lymphocytic choriomeningitis virus, human immunodeficiency virus (HIV), poliovirus, zika virus, rubella virus, cytomegalovirus, human papillomavirus (HPV), enterovirus D68, severe acute respiratory syndrome (SARS) coronavirus, Middle East respiratory syndrome coronavirus, SARS coronavirus 2, Epstein-Barr virus, influenza virus, respiratory syncytial virus, polyoma viruses (such as JC virus, BK virus), Ebola virus, Dengue virus, or any combination thereof.

[0124] In another embodiment, the disease is a cancer that can be, but is not limited to, carcinoma, sarcoma, lymphoma, leukemia, germ cell tumor, blastoma, chondrosarcoma, Ewing's sarcoma, malignant fibrous histiocytoma of bone, osteosarcoma, rhabdomyosarcoma, heart cancer, brain cancer, astrocytoma, glioma, medulloblastoma, neuroblastoma, breast cancer, medullary carcinoma, adrenocortical carcinoma, thyroid cancer, Merkel cell carcinoma, eye cancer, gastrointestinal cancer, colon cancer, gallbladder cancer, gastric (stomach) cancer, gastrointestinal carcinoid tumor, hepatocellular cancer, pancreatic cancer, rectal cancer, bladder cancer, cervical cancer, endometrial cancer, ovarian cancer, renal cell carcinoma, prostate cancer, testicular cancer,

urethral cancer, uterine sarcoma, vaginal cancer, head cancer, neck cancer, nasopharyngeal carcinoma, hematopoietic cancer, Non-hodgkin lymphoma, skin cancer, basal-cell carcinoma, melanoma, small cell lung cancer, non-small cell lung cancer, or any combination thereof.

[0125] In another embodiment, the disease is an autoimmune disease that can be, but is not limited to, achalasia, amyloidosis, ankylosing spondylitis, anti-gbm/anti-tbm nephritis, antiphospholipid syndrome, arthritis, autoimmune angioedema, autoimmune encephalomyelitis, autoimmune hepatitis, autoimmune myocarditis, autoimmune oophoritis, autoimmune orchitis, autoimmune pancreatitis, autoimmune retinopathy, autoimmune urticaria, Behcet's disease, celiac disease, chagas disease, chronic inflammatory demyelinating polyneuropathy, Cogan's syndrome, congenital heart block, Crohn's disease, dermatitis, dermatomyositis, discoid lupus, Dressler's syndrome, endometriosis, fibromyalgia, fibrosing alveolitis, granulomatosis with polyangiitis, Graves' disease, Guillain-Barre syndrome, herpes gestationis, immune thrombocytopenic purpura, interstitial cystitis, juvenile arthritis, juvenile diabetes (type 1 diabetes), juvenile myositis, Kawasaki disease, Lambert-Eaton syndrome, lichen planus, lupus, Lyme disease, multiple sclerosis, myasthenia gravis, myositis, neonatal lupus, neutropenia, palindromic rheumatism, peripheral neuropathy, polyarteritis nodosa, polymyalgia rheumatica, polymyositis, postmyocardial infarction syndrome, postpericardiotomy syndrome, primary biliary cirrhosis, primary sclerosing cholangitis, progesterone dermatitis, psoriasis, psoriatic arthritis, reactive arthritis, retroperitoneal fibrosis, rheumatic fever, rheumatoid arthritis, sarcoidosis, Schmidt syndrome, scleritis, scleroderma, Sjögren's syndrome, thrombocytopenic purpura, type 1 diabetes, ulcerative colitis, uveitis, vasculitis, and vitiligo.

[0126] In some embodiments, the disease is a transplantation-related disease such as graft-versus-host disease (GvHD). According to one embodiment, the GVHD is acute GVHD. According to another embodiment, the GVHD is chronic GVHD.

[0127] Agonistic anti-TNFR2 antibodies (or antigen-binding fragments thereof) as described herein can additionally be used to treat patients in need of organ repair or regeneration. For instance, agonistic TNFR2 antibodies or antigen-binding fragments thereof may be used to stimulate organ repair or regeneration, e.g., by binding TNFR2 on the surface of cells within damaged tissue so as to induce TRAF2/3- and/or NFκB-mediated cell proliferation. Examples of tissues and organs that may be induced to regenerate by administration of an agonistic TNFR2 antibody or antigen-binding fragment thereof include, but are not limited to, pancreas, salivary gland, pituitary gland, kidney, heart, lung, hematopoietic system, cranial nerves, heart, blood vessels including the aorta, olfactory gland, ear, nerves, structures of the head, eye, thymus,

tongue, bone, liver, small intestine, large intestine, gut, lung, brain, skin, peripheral nervous system, central nervous system, spinal cord, breast, embryonic structures, embryos, and testes.

[0128] Agonistic anti-TNFR2 antibodies (or antigen-binding fragments thereof) as described herein can also be administered to a subject (e.g., a human) in order to treat a neurological disease or condition, such as brain tumor, brain metastasis, spinal cord injury, schizophrenia, epilepsy, Amyotrophic lateral sclerosis (ALS), Parkinson's disease, Alzheimer's disease, Huntington's disease, or stroke.

[0129] The agonistic TNFR2 antibodies (or antigen-binding fragments thereof) described herein may also be admixed, conjugated, or administered with, or administered separately from, another agent that promotes regulatory T cells proliferation. Additional agents that can be used to promote regulatory T cell expansion include, but are not limited to, IL-2 and TNF α , the cognate ligand for TNFR2.

[0130] In another embodiment, the present disclosure provides a method of using a polynucleotide to treat a disease or condition as described above, wherein the polynucleotide encodes an anti-TNFR2 antibody as described herein.

[0131] Various embodiments and embodiments of the present anti-TNFR2 antibodies as delineated hereinabove and as claimed in the claims section below find experimental support in the following examples. Exemplified herein is the generation of anti-TNFR2 agonist antibodies that present as non-aggregated species and migrated on the SEC columns as a typically folded IgG1. The anti-TNFR2 IgG1 agonist antibodies generated bound soluble TNFR2-His at about an EC₅₀ range of 1.8nM to 66nM, indicating that these antibodies are tight binders. Further, the antibodies specifically bound TNFR2 and did not bind TNFR1. Anti-TNFR2 antibodies generated include high affinity functional agonists, which may agonize the TNFR2 receptor without the addition of other molecules with an EC₅₀ range of 0.5nM to 277nM. Moreover, TNFR2 activation by anti-TNFR2 antibodies generated appears to be independent of IgG-Fc clustering. Examination of the effects of TNF α on the activation properties of the anti-TNFR2 antibodies generated, indicated that these antibodies activate the TNFR2 receptor to almost its full capacity, and most likely at an epitope that is not blocking the TNF α binding site.

EXAMPLES

EXAMPLE 1

Generation of TNFR2 Agonists

EXPERIMENTAL PROCEDURES

Library Generation

Library Construction

[0132] Libraries were constructed based on three template antibodies (PDB: 2I5Y, 4IOI and 3E8U) by overlapping extension PCR with degenerate oligonucleotides. PCR used to introduce diversity was carried out using Phusion high fidelity DNA polymerase (New England Biolabs USA, Cat: M0530) according to manufacturer instructions in a 3-step reaction (98°C for 30 sec, 5 65°C for 20 sec, 72°C for 30 sec, 30 cycles). The PCR products were gel purified using a gel purification kit and assembled in equimolar ratios in a 3-step PCR reaction, as above, but in the absence of primers. The assembled PCR product was reused as template for PCR amplifying the full scFv library, as above, using forward and reverse primers adding the yeast surface display 10 (YSD) expression vector homology sequences at the 5' and 3' to the scFv library to efficiently perform homologous recombination in yeast cells.

[0133] scFv libraries were constructed with three repeats of flexible linkers of Gly-Gly-Gly-Gly-Ser (SEQ ID NO:333) between the VH and VL.

[0134] Fab display libraries were constructed in a similar fashion to the scFv libraries with the following modifications: the VL and VH were constructed separately and were cloned under two 15 promoters. The VH was cloned in-frame under the Gal 10 promoter, between the *aga2* gene and the constant heavy chain domain 1 (CH1). The VL was cloned in-frame under the Gal1 promoter between a signal peptide and a constant light domain (CL). The Fab fragments were combined using PCR into one fragment and cloned into the pFAB1 expression vector in a similar fashion to 20 the scFv libraries.

Library Transformation

[0135] Library transformation was carried out as published (Benatuil *et al.*, An improved yeast transformation method for the generation of very large human antibody libraries. *Protein Eng. Des. Sel.* 23, 155–159 (2010)). Four hundred µl of a yeast suspension (EBY100, ATCC, USA) per 25 0.2cm cuvette (cell projects) was electroporated (BioRad, USA, GenePulser) with 4µg linearized vector (pCTcon3 or pFAB1) and 12µg DNA insert (either scFv fragment or assemble Fab) in a 1:3 vector to insert ratio (Chao, G. *et al.*, Isolating and engineering human antibodies using yeast surface display. *Nat. Protoc.* 1, 755–768 (2006)). The average number of transformants in a library was determined to be $\sim 1 \times 10^8$ by serial dilutions of transformed cells.

Library Screening

Screening and Selection Using Yeast Surface Display

[0136] Yeast display libraries were grown in a SDCAA selective medium and induced for expression with 2% w/v galactose at 30°C overnight according to established protocols (Chao, G.

et al., (2006)). Briefly, the library was incubated with 1000 to 0.1nM of recombinant human TNFR2 with 6xhis tag or TNFR2-Fc fusion (Reprokine, Israel) in PBS 0.1% BSA for 1 hour, then washed three times with PBS 0.1% BSA and labeled with either: mouse anti c-Myc FITC (Miltenyi Biotec, cat #130-116-485), mouse anti c-Myc (Santa Cruze, USA cat# sc-40), and also
 5 fluorescently labeled goat anti-mouse IgG-FITC (Sigma-Aldrich, cat # F4143-1ml), monoclonal anti His APC (Miltenyi Biotec, Germany. cat 0020130-119-782), or anti Fc APC (Jackson ImmunoResearch, USA. cat. 109-135-098).

[0137] When it was necessary to avoid non-specific binding, the fluorescent-labeled antibodies were alternated with rabbit anti c-Myc (Abcam, cat# ab9106), goat anti-rabbit APC (Abcam, Cat#
 10 ab130805), and anti-His Alexa488 (Qiagen, cat# 20-35310).

[0138] Post labeling the library was selected on MACS beads, until the library size was reduced to 1×10^6 and then sorted on BioRad S3e or BD ARIA III Fluorescence Activated Cell Sorter (FACS) for high affinity binders of recombinant human TNFR2. Clones isolated from the final sort were sequenced by extraction of plasmid DNA from the yeast clones using a Zymoprep kit
 15 (Zymo Research, USA) and the DNA was sequenced.

[0139] When applicable yeast displaying Fab were labeled and selected under the same conditions mentioned for scFv selection with the addition of anti-FLAG-PE (Miltenyi Biotec, cat #130-101-576) for the detection of Fab light chain display.

Koff Selection

[0140] To select for binders with improved off rate, the yeast were incubated for 30 min with 10nM to 1nM TNFR2-His. Subsequently the yeast were washed three times with 1ml PBS 0.1% BSA and incubated for 4h, 6h, and 24h with 100nM TNFR2-Fc. Alternatively, post wash, the cells were diluted 10-fold in PBS 0.1% BSA and incubated for the indicated time points. The yeast were then washed three times and labeled with anti-Myc-FITC (Santa Cruze, USA, Cat# 9E10) and
 25 monoclonal anti-His/APC (Miltenyi Biotec, Germany. cat 0020130-119-782), and sorted on Se3 FACS as described above.

[0141] Furthermore, two rounds of Koff selections were done at the most suitable time point in which approximately 50%-70% of initial binding was lost.

Analysis of Clones by Yeast Surface Display EC₅₀

[0142] To determine the effective concentration₅₀ (EC₅₀) of TNFR2 binding to yeast scFv or Fab clones (EC₅₀ – TNFR2 binding), specific clones were labeled at TNFR concentrations ranging from 0.1nM to 1000nM and analyzed on an FACS. Median fluorescence intensity (MFI) was determined for each TNFR2 concentration and EC₅₀ (TNFR2 binding) was calculated using Prisma

8 GraphPad (GraphPad, San Diego, USA) software.

IgG Production

Reformatting

[0143] Selected scFv and Fab clones were reformatted to human IgG1 format. The sequences of
5 the light chain (LC) and heavy chain (HC) variable regions were optimized to mammalian codon
usage and ordered as genblocks (GB) from IDT (Integrated DNA Technologies, Coralville, Iowa
USA). The GB were cloned using standard cloning techniques into pSF-CMV-HuIgG1_HC (HC
plasmid) and pSF-CMV-HuLambda_LC (LC plasmid) (Oxford genetics, Oxford UK). When
indicated, a pSF-CMV-HuIgG1_HC with L234A/L235A (LALA) mutation was used.

IgG Expression

[0144] Expi-CHO cells (Thermo Fisher Scientific, USA) were transfected with LC and HC
plasmids at a ratio of 2:1 and expression was carried out according to the manufacturer's
instructions. Briefly: 50ml Expi-CHO cells were cultured at 37°C, 120rpm, CO₂ 8% to a density
of 6x10⁶ cells/ml. Then, 50µg of expression heavy chain and light chain plasmids at a ratio of 1:2
15 were transfected into the CHO cells. Post transfections, a booster and feed were added to the
culture, and growth conditions were changed to 32°C, 120rpm, 5% CO₂. The cells were harvested
10 days after transfection. The IgGs were purified from the supernatant using protein A beads
(Tosoh Bioscience GmbH, Germany), followed by size exclusion chromatography (SEC)
purification on SUPERDEX 200™ 10/300 increase column, with PBS as mobile phase (GE
20 healthcare, USA).

Size Exclusion Chromatography

[0145] To analyze and purify the IgGs, samples were loaded on a SUPERDEX 200™ 10/300
increase column (GE healthcare, USA) at a flow rate of 0.8ml/min on a GE AKTA Explorer
chromatography system (GE healthcare, USA). Alternatively, where indicated, up to 12mg IgGs
25 were loaded on a Waters ACQUITY arc HPLC, equipped with BioResolve™ SEC mAb column
(Waters, USA), with a flow rate of 0.5ml/min and run time of 20 minutes. PBS served as mobile
phase and retention was monitored at 280nm for both columns.

Ligand Binding ELISA

[0146] IgG binding affinity to TNFR2 was examined by ELISA. 96 well plates (Greiner Bio-One
30 high binding) were coated with (50ng/well) of the analyzed antibody and incubated overnight at
4°C. The plates were then washed three times with 300µl PBS buffer containing 0.05% Tween 20
(PBS-T), blocked with 300µl PBS-T supplemented with 1% to 2% BSA, and incubated for 1 hour
at room temperature. Antibodies-coated plates were washed three times with 300µl PBS-T and

incubated with serial dilutions of test ligand hTNFR2-His (Reprokine, Israel) in a final volume of 50ml for 1-2 hours. Plates were then washed three times with 300µl PBS-T and incubated with 50µl anti-HIS-HRP (Santa Cruz Biotechnology, USA, SC-8036HRP) conjugate that was diluted 1:250 in PBS. After an additional wash step of six washes, the reaction was developed with 50ml
5 tetramethylbenzidine (TMB) reagent (Southern biotech, USA), and stopped with 50ml 0.5N H₂SO₄. Detection was done on a Synergy LX BioTek (BioTek, USA) plate reader with an absorbance filter set to 450nm. The binding affinity was determined by fitting the data to a specific binding non-linear regression model on Prisma 8 GraphPad software.

[0147] TNFR2/TNFR1 specificity ELISA was done in a similar manner to the EC₅₀ ELISA assay
10 (EC₅₀ -TNFR2 binding) but with a concentration of 100-1000nM for both TNFR1 and TNFR2.

FACS analysis

[0148] LALA mutated Fc format anti-TNFR2 antibody (30.116), was incubated with HEK-TNFR1(HEK-Blue-TNFα (InvivoGen, Cat: hkb-tnfdmyd) and HEK-TNFR2 (described herein) cell lines for 15 min on ice (200nM of Ab, 1,000,000 cells per well). Subsequently, the cells were
15 stained with goat anti-human Fc-APC conjugated, for 30 min on ice according to manufacture instructions (Jackson immune research, cat. # 109-135-098). Cell were analyzed on CytoFLEX flow cytometer (Beckman, USA). Gates were determined separately for each cell population, based on secondary only control.

RESULTS

20 Library Design

[0149] In order to generate an antibody that binds TNFR2, a “re-epitoping” approach was applied to an existing antibody. The re-epitoping process allows for the introduction of new specificity to an existing antibody, and can allow for choosing a known antibody with favorable biophysical and biochemical properties as a template. Therefore, the re-epitoped antibody can have both a new
25 specificity and desirable developability profile. The computational process of re-epitoping requires two steps: (i) using any computational analysis that identifies putative complementarity between existing antibody and a new epitope, and (ii) application of any computational analysis or tool that can suggest introduction of specific mutations predicted to enhance antibody binding to the new, desired epitope. Examples of such computational processes are presented in published U.S. Patent
30 Application No. US20180068055, and Nimrod et al., *Cell Rep.* 25(8):2121-2131 (2018). In one embodiment, three libraries were designed using the sequences of the variable domains of re-epitoping template antibodies 2I5Y, 4IOI and 3E8U, which are predicted to be good candidates for introducing new specificity towards TNFR2. The sequences of some of these templates were

further modified in later generation libraries to account for better developability or for humanization. The libraries for re-epitoping were constructed and introduced to yeast as described above.

YSD Screen for TNFR2 Binders

- 5 [0150] Following introduction of variations, the library was screened in a yeast surface display (YSD) format to identify clones that specifically bind to TNFR2. Initially, the libraries underwent MACS (magnetic beads) selection followed by FCAS selection. Clones from all three re-epitoping templates showed relative binding.
- [0151] To further enhance affinity, affinity maturation libraries were constructed in a similar
- 10 fashion to the construction of libraries described above. The affinity maturation libraries were passed through regular and specific Koff improvement selection as described above. Best binders were gated, yeast clones were isolated and sequenced. These clones are listed in Table 1.

TABLE 1
Sequences of TNFR2 Binders in scFv and Fab Format

Clone_ID	Sequences	SEQ ID NO:
CID251	DNA sequence	1
	Protein sequence, VH+VL	2
	protein sequence - VH	3
	protein sequence - VL	4
CID_252	DNA sequence	5
	Protein sequence, VH+VL	6
	protein sequence - VH	7
	protein sequence - VL	8
CID_253	DNA sequence	9
	Protein sequence, VH+VL	10
	protein sequence - VH	11
	protein sequence - VL	12
CID_254	DNA sequence	13
	Protein sequence, VH+VL	14
	protein sequence - VH	15
	protein sequence - VL	16
CID_255	DNA sequence	17

Clone_ID	Sequences	SEQ ID NO:
	Protein sequence, VH+VL	18
	protein sequence - VH	19
	protein sequence - VL	20
CID_256	DNA sequence	21
	Protein sequence, VH+VL	22
	protein sequence - VH	23
	protein sequence - VL	24
CID_257	DNA sequence	25
	Protein sequence, VH+VL	26
	protein sequence - VH	27
	protein sequence - VL	28
CID_258	DNA sequence	29
	Protein sequence, VH+VL	30
	protein sequence - VH	31
	protein sequence - VL	32
CID_259	DNA sequence	33
	Protein sequence, VH+VL	34
	protein sequence - VH	35
	protein sequence - VL	36
CID_272	DNA sequence	37
	Protein sequence, VH+VL	38
	protein sequence - VH	39
	protein sequence - VL	40
CID_274	DNA sequence	41
	Protein sequence, VH+VL	42
	protein sequence - VH	43
	protein sequence - VL	44
CID_275	DNA sequence	45
	Protein sequence, VH+VL	46
	protein sequence - VH	47
	protein sequence - VL	48

Clone_ID	Sequences	SEQ ID NO:
CID_276	DNA sequence	49
	Protein sequence, VH+VL	50
	protein sequence - VH	51
	protein sequence - VL	52
CID_277	DNA sequence	53
	Protein sequence, VH+VL	54
	protein sequence - VH	55
	protein sequence - VL	56
CID_278	DNA sequence	57
	Protein sequence, VH+VL	58
	protein sequence - VH	59
	protein sequence - VL	60
CID_280	DNA sequence	61
	Protein sequence, VH+VL	62
	protein sequence - VH	63
	protein sequence - VL	64
CID_281	DNA sequence	65
	Protein sequence, VH+VL	66
	protein sequence - VH	67
	protein sequence - VL	68
CID_282	DNA sequence	69
	Protein sequence, VH+VL	70
	protein sequence - VH	71
	protein sequence - VL	72
CID_283	DNA sequence	73
	Protein sequence, VH+VL	74
	protein sequence - VH	75
	protein sequence - VL	76
CID_284	DNA sequence	77
	Protein sequence, VH+VL	78
	protein sequence - VH	79

Clone_ID	Sequences	SEQ ID NO:
	protein sequence - VL	80
CID_285	DNA sequence	81
	Protein sequence, VH+VL	82
	protein sequence - VH	83
	protein sequence - VL	84
CID_436	DNA sequence	85
	Protein sequence, VH+VL	86
	protein sequence - VH	87
	protein sequence - VL	88
CID_437	DNA sequence	89
	Protein sequence, VH+VL	90
	protein sequence - VH	91
	protein sequence - VL	92
CID_331	DNA sequence	93
	Protein sequence, VH+VL	94
	protein sequence - VH	95
	protein sequence - VL	96
CID_332	DNA sequence	97
	Protein sequence, VH+VL	98
	protein sequence - VH	99
	protein sequence - VL	100
CID_333	DNA sequence	101
	Protein sequence, VH+VL	102
	protein sequence - VH	103
	protein sequence - VL	104
CID_323	DNA sequence	105
	Protein sequence, VH+VL	106
	protein sequence - VH	107
	protein sequence - VL	108
CID_324	DNA sequence	109
	Protein sequence, VH+VL	110

Clone_ID	Sequences	SEQ ID NO:
	protein sequence - VH	111
	protein sequence - VL	112
CID_325	DNA sequence	113
	Protein sequence, VH+VL	114
	protein sequence - VH	115
	protein sequence - VL	116
CID_326	DNA sequence	117
	Protein sequence, VH+VL	118
	protein sequence - VH	119
	protein sequence - VL	120
CID_327	DNA sequence	121
	Protein sequence, VH+VL	122
	protein sequence - VH	123
	protein sequence - VL	124
CID_328	DNA sequence	125
	Protein sequence, VH+VL	126
	protein sequence - VH	127
	protein sequence - VL	128
CID_329	DNA sequence	129
	Protein sequence, VH+VL	130
	protein sequence - VH	131
	protein sequence - VL	132
CID_330	DNA sequence	133
	Protein sequence, VH+VL	134
	protein sequence - VH	135
	protein sequence - VL	136
CID_350	DNA sequence	137
	Protein sequence, VH+VL	138
	protein sequence - VH	139
	protein sequence - VL	140
CID_279	DNA sequence	141

Clone_ID	Sequences	SEQ ID NO:
	Protein sequence, VH+VL	142
	protein sequence - VH	143
	protein sequence - VL	144
CID_438	DNA sequence	145
	Protein sequence, VH+VL	146
	protein sequence - VH	147
	protein sequence - VL	148
CID_229	DNA - VH+CH1	149
	DNA - VL+CL	150
	Amino acid sequence - VH	151
	Amino acid sequence - VL	152
	Amino acid sequence - CH1	153
	Amino acid sequence - CL	154
CID_231	DNA - VH+CH1	155
	DNA - VL+CL	156
	Amino acid sequence - VH	157
	Amino acid sequence - VL	158
	Amino acid sequence - CH1	159
	Amino acid sequence - CL	160
CID_232	DNA - VH+CH1	161
	DNA - VL+CL	162
	Amino acid sequence - VH	163
	Amino acid sequence - VL	164
	Amino acid sequence - CH1	165
	Amino acid sequence - CL	166
CID_234	DNA - VH+CH1	167
	DNA - VL+CL	168
	Amino acid sequence - VH	169
	Amino acid sequence - VL	170
	Amino acid sequence - CH1	171
	Amino acid sequence - CL	172

Clone_ID	Sequences	SEQ ID NO:
CID_261	DNA - VH+CH1	173
	DNA - VL+CL	174
	Amino acid sequence - VH	175
	Amino acid sequence - VL	176
	Amino acid sequence - CH1	177
	Amino acid sequence - CL	178
CID_262	DNA - VH+CH1	179
	DNA - VL+CL	180
	Amino acid sequence - VH	181
	Amino acid sequence - VL	182
	Amino acid sequence - CH1	183
	Amino acid sequence - CL	184
CID_264	DNA - VH+CH1	191
	DNA - VL+CL	192
	Amino acid sequence - VH	193
	Amino acid sequence - VL	194
	Amino acid sequence - CH1	195
	Amino acid sequence - CL	196
CID_266	DNA - VH+CH1	197
	DNA - VL+CL	198
	Amino acid sequence - VH	199
	Amino acid sequence - VL	200
	Amino acid sequence - CH1	201
	Amino acid sequence - CL	202
CID_267	DNA - VH+CH1	203
	DNA - VL+CL	204
	Amino acid sequence - VH	205
	Amino acid sequence - VL	206
	Amino acid sequence - CH1	207
	Amino acid sequence - CL	208
CID_268	DNA - VH+CH1	209

Clone_ID	Sequences	SEQ ID NO:
	DNA - VL+CL	210
	Amino acid sequence - VH	211
	Amino acid sequence - VL	212
	Amino acid sequence - CH1	213
	Amino acid sequence - CL	214
CID_271	DNA - VH+CH1	215
	DNA - VL+CL	216
	Amino acid sequence - VH	217
	Amino acid sequence - VL	218
	Amino acid sequence - CH1	219
	Amino acid sequence - CL	220
CID_230	DNA - VH+CH1	221
	DNA - VL+CL	222
	Amino acid sequence - VH	223
	Amino acid sequence - VL	224
	Amino acid sequence - CH1	225
	Amino acid sequence - CL	226
CID_265	DNA - VH+CH1	227
	DNA - VL+CL	228
	Amino acid sequence - VH	229
	Amino acid sequence - VL	230
	Amino acid sequence - CH1	231
	Amino acid sequence - CL	232

Yeast Surface Display EC₅₀ Binding to TNFR2

[0152] To verify that the TNFR2 binding clones exhibit at least single to double digit nanomolar affinity to TNFR2, a limited set of clones were subjected to a yeast surface display (YSD) EC₅₀ binding assay as described above. As can be seen in **Figures 1A-1D**, these clones show affinity to TNFR2-His in the range of 1nM to 20nM, indicating that the yeast-displayed scFv and Fabs are tight binders of human TNFR2. A summary of the YSD-EC₅₀ values of these clones is presented in

5 Table 2.

TABLE 2**YSD EC₅₀ Binding Value for Yeast Clones Towards Recombinant TNFR2-His**

scFv (YSD clone ID)	EC ₅₀ Binding value (nM)
CID_327	4.5
CID_329	8.6
CID_330	17.7
CID_326	6
CID_325	2.9
CID_324	5.6
CID_323	5.2
CID_328	5.7
CID_436	3.4
CID_437	1.2
CID_251	1

Analysis of Clones That Bind TNFR2 in IgG Format**5 SEC analysis**

[0153] To further characterize the clones that demonstrated binding to TNFR2, clones listed in Table 1 and Table 2 that showed the most promising binding in scFv and Fab were reformatted to human IgG1, transiently expressed in expi-CHO cells according to the manufacturer's instructions and purified as described herein. Additionally, DNA for clones 30.032, 30.046, 30.033 was synthesized and IgG was produced in HEK cells by Genscript antibody services (Genscript, USA).

[0154] Clones 30.113, 30.114, 30.116, 30.117, 30.118, 30.119 were reformatted to IgG1 with a heavy chain L234A/L235A mutation (LALA mutation) designed to reduce binding of the IgG's Fc to Fc-gamma receptor. Further, additional IgG1 clones comprising the heavy chain L234A/L235A mutation (LALA mutation) were produced. The list of IgG1 anti-TNFR2 antibody clones comprising a heavy chain L234A/L235A mutation includes: BDG30.113, BDG30.114,

BDG30.115, BDG30.116, BDG30.117, BDG30.118, BDG30.119, BDG30.122, BDG30.123, BDG30.200, BDG30.201, BDG30.202, BDG30.203, and BDG30.204.

[0155] A summary of the scFv and Fabs that were reformatted to an IgG form is listed in Table 3; where noted, the heavy chain includes the LALA mutation.

5

TABLE 3
Sequences of TNFR2 Binders in Human IgG Format

Clone_ID	IgG_ID	Human IgG	Sequences	SEQ ID NO:
CID_251	BDG30.046	Human IgG1	VH	233
			VL	234
			H chain	235
			L chain	236
CID_272	BDG30.090	Human IgG1	VH	237
			VL	238
			H chain	239
			L chain	240
CID_276	BDG30.092	Human IgG1	VH	241
			VL	242
			H chain	243
			L chain	244
CID_277	BDG30.093	Human IgG1	VH	245
			VL	246
			H chain	247
			L chain	248
CID_280	BDG30.094	Human IgG1	VH	249
			VL	250
			H chain	251
			L chain	252
CID_284	BDG30.095	Human IgG1	VH	253
			VL	254
			H chain	255
			L chain	256

Clone_ID	IgG_ID	Human IgG	Sequences	SEQ ID NO:
CID_436	BDG30.032	Human IgG1	VH	257
			VL	258
			H chain	259
			L chain	260
CID_437	BDG30.033	Human IgG1	VH	261
			VL	262
			H chain	263
			L chain	264
CID_229	BDG30.080	Human IgG1	VH	265
			VL	266
			H chain	267
			L chain	268
CID_231	BDG30.081	Human IgG1	VH	269
			VL	270
			H chain	271
			L chain	272
CID_232	BDG30.082	Human IgG1	VH	273
			VL	274
			H chain	275
			L chain	276
CID_234	BDG30.084	Human IgG1	VH	277
			VL	278
			H chain	279
			L chain	280
CID_261	BDG30.085	Human IgG1	VH	281
			VL	282
			H chain	283
			L chain	284
CID_262	BDG30.086	Human IgG1	VH	285
			VL	286
			H chain	287

Clone_ID	IgG_ID	Human IgG	Sequences	SEQ ID NO:
			L chain	288
CID_264	BDG30.087	Human IgG1	VH	289
			VL	290
			H chain	291
			L chain	292
CID_266	BDG30.088	Human IgG1	VH	293
			VL	294
			H chain	295
			L chain	296
CID_267	BDG30.089	Human IgG1	VH	297
			VL	298
			H chain	299
			L chain	300
CID_332	BDG30.109	Human IgG1	VH	301
			VL	302
			H chain	303
			L chain	304
CID_350	BDG30.111	Human IgG1	VH	305
			VL	306
			H chain	307
			L chain	308
CID_327	BDG30.113	Human IgG1(L234A/L235A)	VH	309
			VL	310
			H chain	311
			L chain	312
CID_328	BDG30.114	Human IgG1(L234A/L235A)	VH	313
			VL	314
			H chain	315
			L chain	316

Clone_ID	IgG_ID	Human IgG	Sequences	SEQ ID NO:
CID_262	BDG30.116	Human IgG1(L234A/L235A)	VH	317
			VL	318
			H chain	319
			L chain	320
CID_436	BGD30.115	Human IgG1(L234A/L235A)	VH	257
			VL	258
			H chain	432
			L chain	260
CID_277	BDG30.117	Human IgG1(L234A/L235A)	VH	321
			VL	322
			H chain	323
			L chain	324
CID_280	BDG30.118	Human IgG1(L234A/L235A)	VH	325
			VL	326
			H chain	327
			L chain	328
CID_284	BDG30.119	Human IgG1(L234A/L235A)	VH	329
			VL	330
			H chain	331
			L chain	332
CID_332	BDG30.122	Human IgG1(L234A/L235A)	VH	301
			VL	302
			H chain	433
			L chain	304
CID_350	BDG30.123	Human	VH	305

Clone_ID	IgG_ID	Human IgG	Sequences	SEQ ID NO:
		IgG1(L234A/L235A)		
			VL	306
			H chain	434
			L chain	308
CID_251	BDG30.200	Human IgG1(L234A/L235A)	VH	233
			VL	234
			H chain	435
			L chain	236
CID_276	BDG30.201	Human IgG1(L234A/L235A)	VH	241
			VL	242
			H chain	436
			L chain	244
CID_261	BDG30.202	Human IgG1(L234A/L235A)	VH	281
			VL	282
			H chain	437
			L chain	284
CID_264	BDG30.203	Human IgG1(L234A/L235A)	VH	289
			VL	290
			H chain	438
			L chain	292
CID_266	BDG30.204	Human IgG1(L234A/L235A)	VH	293
			VL	294
			H chain	439
			L chain	296

[0156] Examples of amino acid sequences for the complementarity determining regions (CDRs)

are shown in Tables 7-9. Table 7 shows examples of sets of three CDRs on a heavy chain (HCDR1, HCDR2, and HCDR3), and Table 8 shows sets of three CDRs on the corresponding light chain (LCDR1, LCDR2, and LCDR3). Table 9 shows yet other examples of sets of HCDR1, HCDR2, HCDR3 and sets of LCDR1, LCDR2, and LCDR3 for the anti-TNFR2 antibodies disclosed herein.

5 [0157] To test these antibodies' tendency to aggregate, the IgGs went through size exclusion chromatography (SEC) on an Akta, Superdex 10/300 increase column or on Waters ACQUITY arc HPLC, BioResolve SEC mAb column with PBS as mobile phase as described above. Examples of SEC analyses of selected antibodies are shown in **Figures 2A-2R** and a summary of the complete SEC analysis of the clones is presented in Table 4. These results indicate that IgGs

10 30.086-30.113, 30.115, 30.117-119, 30.122-30.123, 30.202 and 30.204 were eluted from protein A mostly as non-aggregated species, and migrated on the SEC columns as a typically folded IgG1.

TABLE 4
SEC Profile of produced IgGs

IgG I.D.	Superdex 10/300 retention Volume (ml)	% Aggregation
30.086	13.16	1.1
30.095	13.00	0.2
30.116	13.57	2.3
30.111	13.2	1.02
30.119	13.08	0
30.123	13.24	0.7
IgG I.D.	BioResolve SEC retention Volume (time)	% Aggregation
30.113	4.72	0.6
30.114	4.70	5.8
30.115	4.74	2.8

IgG I.D.	Superdex 10/300 retention Volume (ml)	% Aggregation
30.116	4.73	8.4
30.117	4.66	2.5
30.118	4.62	1.5
30.122	4.69	2.6
30.200	4.66	3.4
30.201	4.65	1.4
30.202	4.74	4.6
30.203	4.74	7.7
30.204	4.77	4.4

Specificity ELISA

[0158] The extracellular domains of TNFR2 and TNFR1 share 27% homology. To test specificity to TNFR2, the antibodies were analyzed by an ELISA assay against TNFR1 and TNFR2 as described herein. Briefly, antibodies were coated directly on the ELISA plate wells, the wells were blocked, and 100nM of TNFR1-His-Fc or TNFR2-His-Fc were added to the wells, washed and detected using anti His-HRP. As can be seen in **Figures 3A-3B**, the IgG 30.092, 30.093, 30.094, 30.095, 30.085, 30.089, 30.086, 30.116, 30.118, 30.119, 30.111, 30.113 and 30.114 showed specific binding to TNFR2 and did not bind TNFR1.

10

Binding Affinity of IgGs Towards TNFR2

IgG ELISA EC₅₀

[0159] To test binding affinity of clones reformatted as IgG1 and IgG1_{LALA} to TNFR2, an ELISA EC₅₀ binding experiment was conducted for each antibody. As can be seen in **Figures 4A-4F**, the antibodies bind soluble TNFR2-His at an EC₅₀ range of 1.8nM to 66nM, indicating that these antibodies are tight binders. Table 5 lists the TNFR2 EC₅₀ value for the indicated antibodies, the

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values are an average of at least two biological repeats.

TABLE 5
TNFR2 EC₅₀ Value for Indicated Antibodies

Abs	EC₅₀ – Binding (nM)
30.032	21.9
30.080	66.3
30.081	50.7
30.084	58.9
30.085	4.3
30.086	8.1
30.087	18.4
30.088	13.1
30.089	50.4
30.046	13.4
30.092	15.4
30.093	6.8
30.094	3.7
30.095	4.6
30.109	5.6
30.111	3.55
30.113	2.5
30.114	2.2

Abs	EC ₅₀ – Binding (nM)
30.116	5.1
30.117	2.9
30.118	1.7
30.119	1.8

Establishment of TNFR2 Dependent NFκB Cell Based Assay

[0160] To characterize whether the antibodies affect the TNFR2 cellular signaling functionally, and whether they have an agonistic, antagonistic or no effect on the TNFR2, a reporter cell line was created.

[0161] HEK-Blue™ Null cells were purchased from InvivoGen (Toulouse France). These cells contain TNFR1 null mutation and a plasmid encoding soluble embryonic alkaline phosphatase (SEAP) under the control IFN-β minimal promoter fused to five NF-κB and AP-1 binding sites. A pCDNA3.1 plasmid encoding human TNFR2 (residues 1-461) under the CMV promoter was transfected into HEK-Blue™ Null cells. The cells were kept for 14 days under the selection of 50ug/ml hygromycin B. After the selection period, TNFR2 expression was validated by Western blot analysis (**Figure 5**).

[0162] Subsequently, the cells were diluted to a limiting dilution of 0.5 cells per well in 96 well plate and propagated in a growth medium containing DMEM supplemented with 10% FBS, L-Glutamin, pen/strep and 50ug/ml hygromycin B. To identify TNFα reactive clones, a replica plate was made of the single clones and tested for TNFα dependent activation of NFκB using Quanti-Blue (QB, InvivoGen) substrate as instructed by the manufacturer (**Figures 6A-6C**).

[0163] **Figures 7A-7B** show the results of TNFα-dependent NFκB response of a specific clone, with dynamic range of 20pM to 1000pM. The activation was inhibited in a dose dependent manner by soluble TNFR2 (**Figure 7B**), but was not affected by isotype control antibody (**Figure 7A**). Clone G6 was selected for evaluating the target antibodies for agonizing or antagonizing the TNFR2 receptor.

Functional Testing of the IgGs In HEK-TNFR2 Reporter Cell Line

[0164] To test the effects of the anti-TNFR2 antibodies on cellular signaling of TNFR2, IgGs were incubated overnight with the HEK-TNFR2 reporter cell line up to a concentration of 600nM and

tested for antibody dependent NF κ B activation as detailed herein. (**Figures 8A-8D**)

[0165] The IgGs listed in Table 6 except 30.088 and 30.117 showed a dose dependent NF κ B activation with an EC₅₀ functional agonism value ranging between 0.5nM and 25.7nM, demonstrating that these antibodies are high affinity functional agonists and can agonize the receptor without the addition of other molecules.

[0166] To validate that the NF κ B activation is dependent on direct activation of TNFR2 receptor by an antibody, and could not be activated by a TNFR1 alternative route, antibodies 30.113, 30.114, 30.115, 30.116, 30.117, 30.118, 30.119, 30.123, 30.200, 30.201, 30.202, 30.203, and 30.204 were tested using HEK-Blue-TNF α cells (InvivoGen, Cat: hkb-tnfdmyd). HEK-Blue-TNF α cells do not express TNFR2, but natively express TNFR1 and contain a plasmid encoding soluble embryonic alkaline phosphatase (SEAP) under the control IFN- β minimal promoter fused to five NF- κ B and AP-1 binding sites.

[0167] As can be seen in **Figure 9A** HEK-Blue-TNF α cells do not express TNFR2, as evident by FACS analysis of these cells labeled with 200nM 30.116 anti-TNFR2 Ab. On the other hand, NF κ B is readily activated by the addition of 11nM TNF α (**Figure 9B**), suggesting that NF κ B activation is by the TNFR1 route. While the indicated antibodies were shown to activate NF κ B in a HEK-TNFR2 cell line, addition of 1mM of 30.113, 30.114, 30.115, 30.116, 30.117, 30.118, 30.119, 30.123, 30.200, 30.201, 30.202, 30.203, or 30.204 antibodies did not activate NF κ B in a HEK-TNFR1 cell line, suggesting that the activation is specific to TNFR2 (**Figures 9B-9C**).

[0168] The TNFR2 activation is independent of IgG-Fc clustering. This is supported by the fact that HEK293 reporter cell line does not express the Fc gamma receptors, and the IgGs in the above experiments were added to the cells in soluble form and were not plate bound. Moreover, 30.113 to 30.204 IgGs were expressed with a LALA mutation that is designed to reduce IgG binding to Fc-gamma receptor. Taken together, these data suggest that the TNFR2 activation is independent of IgG-Fc clustering. Table 6 presents a summary of EC₅₀ of functional agonism of TNFR2 in HEK293-NF κ B reporter cell line. The values shown are an average of at least two biological repeats.

TABLE 6
EC₅₀ of Functional Agonism of TNFR2 in HEK293-NF κ B Reporter Cell Line

Abs	Functional Agonism EC ₅₀ (nM)
30.032	0.85

Abs	Functional Agonism EC₅₀ (nM)
30.085	24.7
30.086	1.4
30.087	3.9
30.088	49
30.046	22
30.092	17.6
30.093	20.6
30.094	3.9
30.109	4.2
30.111	7.4
30.113	3.8
30.114	6.4
30.116	3
30.115	1
30.117	277
30.118	5.4
30.119	2.6
30.122	2.3
30.123	2.6
30.200	24.4

Abs	Functional Agonism EC ₅₀ (nM)
30.201	20
30.202	2.9
30.203	0.5
30.204	3.1

[0169] To examine the effects of TNF α on antibody dependent NF κ B activation, the HEK-TNFR2 cells were incubated with 200nM anti-TNFR2 antibodies and TNF α was added up to a concentration of 100nM. As shown in **Figures 10A-10D**, TNF α had a minor effect on maximal
5 activation induced by 30.116, 30.111, 30.086 and 30.119 antibodies, indicating that these antibodies activate the TNFR2 receptor to almost its full capacity, likely at an epitope that is not blocking the TNF α binding site.

[0170] Taking together, the results shown above demonstrate that the antibodies presented in this disclosure are tight binders to TNFR2 in the sub-nanomolar to low double digit nanomolar range,
10 are specific to TNFR2 and agonize TNFR2 in an Fc independent manner.

[0171] While certain features of the anti-TNFR2 antibodies have been illustrated and described herein, many modifications, substitutions, changes, and equivalents will now occur to those of ordinary skill in the art. It is, therefore, to be understood that the appended claims are intended to cover all such modifications and changes as fall within the true spirit of the anti-TNFR2 antibodies

TABLE 7
Embodiments of Heavy Chain CDR Sequences

CID	HCDR1	SEQ ID NO	HCDR2	SEQ ID NO	HCDR3	SEQ ID NO
CID_251	GDTFTSYS	185	IIILDDT	190	AGVYEGPNSEGSYDDYGFLKH	347
CID_252	GDTFTSYS	185	IIILDDT	190	AGVYEGPNSEGSYDDYGFLKH	347
CID_253	GDTFTSYS	185	IIDLDDT	334	AGVYEGPNSEGSYDDYGFLKH	347
CID_254	GDTFTSYS	185	IIILDDT	190	AGVYEGPNSEGSYDDYGFLKH	347
CID_255	GDTFTSYS	185	IIILDDT	190	AGVYEGPNSEGSYDDYGFLKH	347
CID_256	GDTFTSYS	185	IIILDDT	190	AGVYEGPNSEGSYDDYGFLKH	347
CID_257	GDTFTSYS	185	IIILDDT	190	AGVYEGPNSEGSYDDYGFLKH	347
CID_258	GDTFTSYS	185	IIIPDDT	335	AGVYEGPNSEGSYDDYGFLKH	347

CID	HCDR1	SEQ ID NO	HCDR2	SEQ ID NO	HCDR3	SEQ ID NO
CID_259	GDTFTSYS	185	IITILDDT	190	AGVYEGPNSEGSYDDYGFLKH	347
CID_272	GDTFTSYS	185	IITIDDDT	336	AGVYEGPNSEGSYDDYGFKH	348
CID_274	GDTFTSYS	185	IITILDDS	337	AGVYEGPNSEGSYDDYGFLKH	347
CID_275	GDTFTSYS	185	IITIDDDT	338	AGVYEGPNSEGSYDDYGFKH	348
CID_276	GDTFTSYS	185	IITIYDDT	339	AGVYEGPNSEGSYDDYGFFKH	349
CID_277	GDTFTSYS	185	IITILDDT	190	AGVYEGPNSEGSYDDYGFLKY	350
CID_278	GDTFTSYS	185	IITILDDT	190	AGVYEGPNSEGSYDDYGALKH	351
CID_279	GDTFTSYS	185	IITDLDDT	334	AGVYEGPNSEGSYDDYGFLKH	347
CID_280	GDTFTSYS	185	IITDLDDT	334	AGVYEGPNSEGSYDDYGQLKH	352
CID_281	GDTFTSYS	185	IITILDDT	190	AGVYEGPNSEGSYDDYGQLKH	352

CID	HCDR1	SEQ ID NO	HCDR2	SEQ ID NO	HCDR3	SEQ ID NO
CID_282	GDTFTSYS	185	IITDLDDT	334	AGVYEGPNSEGSYDDYGFLKH	347
CID_283	GDTFTSYS	185	IITILDDT	190	AGVYEGPNSEGSYDDYGFLKH	347
CID_284	GDTFTSYS	185	IITIDDDT	336	AGVYEGPNSEGSYDDYGQLKH	352
CID_285	GDTFTSYS	185	IITIDDDT	336	AGVYEGPNSEGSYDDYGQLKH	352
CID_323	GYTFTDLG	186	INTHTGMP	340	ARDHPLGLDY	353
CID_324	GYTFTDLG	186	INTHTGEP	341	TRDHPLGLDY	354
CID_325	GYTFTDLG	186	INTHTGEP	341	TRDHPLGLDY	354
CID_326	GYTFTDLG	186	INTHTGKP	342	TRDHPLGLDY	354
CID_327	GYTFTDLG	186	INTQTGEP	343	TRDHPLGLDY	354
CID_328	GYTFTDLG	186	INTQTGEP	343	ARDHPLGLDY	355
CID_329	GYTFTDLG	186	INTHTGEP	341	TRDHPLGLDY	354
CID_330	GYTFTDLG	186	INTHTGEP	341	ARDHPLGLDY	355
CID_331	GYTFTDLG	186	INTITGEP	344	TRDHPLGLDY	354
CID_332	GYTFTDLG	186	INTHTGEP	341	TRDHPLGLDY	354
CID_333	GYTFTDLG	186	INTITGEP	344	TRDHPLGLDY	354

CID	HCDR1	SEQ ID NO	HCDR2	SEQ ID NO	HCDR3	SEQ ID NO
CID_350	GYTFTDLG	186	INTHTGEP	341	TRDHPLGLDY	354
CID_436	GFNIRDY	187	IDPDNGDT	345	SRDLADGYNAFDR	356
CID_437	GFNIRDTF	188	IDPDNGDT	345	SREGEDGYNAWDL	357
CID_438	GFNIRATY	189	IDPTNGDT	346	TREGDDGYNAWDK	358

TABLE 8
Embodiments of Light Chain CDR Sequences

CID	LCDR1	SEQ ID NO	LCDR2	SEQ ID NO	LCDR3	SEQ ID NO
CID_251	ESVAND	359	GAS	372	QQYANWPPRET	378
CID_252	ESVAND	359	GAS	372	QQYANWPPRET	378
CID_253	ESGAND	360	GAS	372	QQYANWPPRGT	379
CID_254	ESVAND	359	GAS	372	QQYTNWPPRET	380
CID_255	ESVAND	359	GAS	372	QQYAIWPPRET	381
CID_256	RSVAND	361	GAS	372	QQYAIWPPRET	381
CID_257	ESVANN	362	GAS	372	QQYARWPPRET	382
CID_258	ESVATD	363	GAS	372	QQYDNWPPRET	383
CID_259	ESVRND	364	GAS	372	QQYANWPPHET	384
CID_272	ESVGND	365	GAS	372	QQYANWPPRET	378
CID_274	ESVAND	359	DAS	373	QQYANWPPWRET	385

CID	LCDR1	SEQ ID NO	LCDR2	SEQ ID NO	LCDR3	SEQ ID NO
CID_275	ESVAND	359	GAS	372	QQGANWPPRET	386
CID_276	ESVAND	359	GAS	372	QQYANWPPRET	378
CID_277	ESVAVD	366	GAS	372	QQYANKPPRET	387
CID_278	ESVAND	359	GAS	372	QQYANWPPRET	378
CID_279	ESGAND	360	GAS	372	QQYANWPPRGT	388
CID_280	ESVAND	359	GAS	372	QQYANWPPRET	378
CID_281	ESVAND	359	GAS	372	QQYANTPPRET	389
CID_282	ESVAND	359	GAS	372	QQYANWPPRET	378
CID_283	ESVGND	365	GAS	372	QQYANWPPRET	378
CID_284	ESVGND	365	GAS	372	QQYANVPPRET	390
CID_285	ESVGND	365	GIS	374	QQYANDPPRET	391
CID_323	QSVDVNGVSY	367	KAS	375	QCSREDPYT	392
CID_324	QSVDVNGVSY	367	KAS	375	QCSREDPYT	392
CID_325	QSVDVNGLSY	368	KAS	375	QCSREDPYT	392
CID_326	QSVDVNGVSY	367	KAS	375	QCSREDPYT	392
CID_327	QSVDVAGVSY	369	KAS	375	QQAREDPYT	393
CID_328	QSVDVNGVSY	367	KAS	375	QCSREDPYT	392
CID_329	QSVDVNGVSY	367	KAS	375	QCSREDPYT	392
CID_330	QSVDVNGVSY	367	KAV	376	QCSREDPYT	392

CID	LCDR1	SEQ ID NO	LCDR2	SEQ ID NO	LCDR3	SEQ ID NO
CID_331	QSLDVNGVSY	370	KAS	375	QCSREDPYT	392
CID_332	QSV DVNGVSY	367	KAS	375	QQNREDPYT	394
CID_333	QSV DVNGVSY	367	KAS	375	QCSREDPYT	392
CID_350	QSV DVNGVSY	367	KAS	375	QQAREDPYT	393
CID_436	QDVSTA	371	SAS	377	QQPTYTPPT	395
CID_437	QDVSTA	371	SAS	377	QQPPYTPPT	396
CID_438	QDVSTA	371	SAS	377	QQPSYTPPT	397

TABLE 9

Embodiments of Heavy And Light Chain CDR Sequences

CID	HCDR1	SEQ ID NO	HCDR2	SEQ ID NO	HCDR3	SEQ ID NO	LCDR1	SEQ ID NO	LCDR2	SEQ ID NO	LCDR3	SEQ ID NO
CID_264	GFNIKAYY	402	IDPDNGDT	345	SREGQDGYNAWDQ	413	QDVSTA	371	PAS	418	QQPTLTPPT	427
CID_229	GFNIRAMY	398	IDPTNGDT	346	TREGQDGYNAFDK	407	QDVSTA	371	SAS	377	QQHRYTPPT	419
CID_230	GFNIRDTY	187	IDPTNGDT	346	SREMADGYNAFDL	408	QDVSTA	371	SAS	377	QQPNYTPPT	420
CID_231	GFNIRATY	189	ISPDNGDT	406	SRDLADGYNAFDL	409	QDVSTA	371	SAS	377	QQHGYTPPT	421
CID_232	GFNIRDTY	187	IDPDNGDT	345	TREGADGYNAFDL	410	QDVSTA	371	PAS	418	QQPDYTPPT	422
CID_234	GFNIRAFY	399	IDPTNGDT	346	TREGEDGYNAFDQ	411	QDVSTA	371	PAS	418	QQPRYTPPT	423
CID_261	GFNIKDFF	400	IDPTNGDT	346	SREGQDGYNAWDL	412	QDSTA	417	SAS	377	QQPSLTPPT	424

CID	HCDR1	SEQ ID NO	HCDR2	SEQ ID NO	HCDR3	SEQ ID NO	LCDR1	SEQ ID NO	LCDR2	SEQ ID NO	LCDR3	SEQ ID NO
CID_262	GFIKDYF	401	IDPTNGDT	346	SREGQDGYNAWDQ	413	QDISTA	417	PAS	418	QQHPYTPPT	425
CID_263	GFIKDYF	401	IDPTNGDT	346	SREGQDGYNAWDM	414	QDISTA	417	PAS	418	QQHGYTPPT	426
CID_265	GFIKAMY	398	IDPTNGDT	346	TREGQDGYNAFDM	415	QDISTA	417	SAS	377	QQPNLTPPT	428
CID_266	GFIKAYY	403	IDPTNGDT	346	SREGQDGYNAWDL	412	QDVSTA	371	PAS	418	QQPGYTPPT	429
CID_267	GFIKDYF	404	IDPTNGDT	346	SREGQDGYNAWDL	412	QDVSTA	371	PAS	418	QQHPYTPPT	425
CID_268	GFIKDYF	401	IDPTNGDT	346	SREGQDGYNAWDM	414	QDISTA	417	PAS	418	QQPLYTPPT	430
CID_271	GFIKDMF	405	ISPDNGDT	406	TREGEDGYNAFDM	416	QDVSTA	371	SAS	377	QQHAYTPPT	431

CLAIMS

What is claimed is:

1. An isolated anti-TNFR2 (tumor necrosis factor receptor 2) antibody comprising a set of three complementarity determining regions (CDRs) on a heavy chain (HCDR1, HCDR2, and HCDR3) and a set of three CDRs on a light chain (LCDR1, LCDR2, and LCDR3), wherein
 - (i) the set of HCDR1, HCDR2, HCDR3, and the set of LCDR1, LCDR2, LCDR3 on a corresponding light chain comprise the amino acid sequences as shown in Table 9; or
 - (ii) the set of HCDR1, HCDR2, and HCDR3 comprise the amino acid sequences as shown in Table 7, and the set of LCDR1, LCDR2, and LCDR3 on a corresponding light chain comprise the amino acid sequences as shown in Table 8.
2. The anti-TNFR2 antibody of claim 1, wherein the antibody comprises a heavy chain variable region and a light chain variable region, said heavy chain variable region and light chain variable region comprise the amino acid sequences selected from the group consisting of SEQ ID NOs:289-290, SEQ ID NOs:3-4, SEQ ID NOs:7-8, SEQ ID NOs:11-12, SEQ ID NOs:15-16, SEQ ID NOs:19-20, SEQ ID NOs:23-24, SEQ ID NOs:27-28, SEQ ID NOs:31-32, SEQ ID NOs:35-36, SEQ ID NOs:39-40, SEQ ID NOs:43-44, SEQ ID NOs:47-48, SEQ ID NOs:51-52, SEQ ID NOs:55-56, SEQ ID NOs:59-60, SEQ ID NOs:63-64, SEQ ID NOs:67-68, SEQ ID NOs:71-72, SEQ ID NOs:75-76, SEQ ID NOs:79-80, SEQ ID NOs:83-84, SEQ ID NOs:87-88, SEQ ID NOs:91-92, SEQ ID NOs:95-96, SEQ ID NOs:99-100, SEQ ID NOs:103-104, SEQ ID NOs:107-108, SEQ ID NOs:111-112, SEQ ID NOs:115-116, SEQ ID NOs:119-120, SEQ ID NOs:123-124, SEQ ID NOs:127-128, SEQ ID NOs:131-132, SEQ ID NOs:135-136, SEQ ID NOs:139-140, SEQ ID NOs:143-144, SEQ ID NOs:147-148, SEQ ID NOs:151-152, SEQ ID NOs:157-158, SEQ ID NOs:163-164, SEQ ID NOs:169-170, SEQ ID NOs:175-176, SEQ ID NOs:181-182, SEQ ID NOs:193-194, SEQ ID NOs:199-200, SEQ ID NOs:205-206, SEQ ID NOs:211-212, SEQ ID NOs:217-218, SEQ ID NOs:223-224, SEQ ID NOs:229-230, SEQ ID NOs:233-234, SEQ ID NOs:237-238, SEQ ID NOs:241-242, SEQ ID NOs:245-246, SEQ ID NOs:249-250, SEQ ID NOs:253-254, SEQ ID NOs:257-258, SEQ ID NOs:261-262, SEQ ID NOs:265-266, SEQ ID NOs:269-270, SEQ ID NOs:273-274, SEQ

ID NOs:277-278, SEQ ID NOs:281-282, SEQ ID NOs:285-286, SEQ ID NOs:293-294, SEQ ID NOs:297-298, SEQ ID NOs:301-302, SEQ ID NOs:305-306, SEQ ID NOs:309-310, SEQ ID NOs:313-314, SEQ ID NOs:317-318, SEQ ID NOs:321-322, SEQ ID NOs:325-326, and SEQ ID NOs:329-330.

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3. The anti-TNFR2 antibody of claim 1, wherein the antibody comprises a heavy chain and a light chain, said heavy chain and a light chain comprise the amino acid sequences selected from the group consisting of SEQ ID NOs: 438 and 292, SEQ ID NOs: 291-292, SEQ ID NOs:235-236, SEQ ID NOs:239-240, SEQ ID NOs:243-244, SEQ ID NOs:247-248, SEQ ID NOs:251-252, SEQ ID NOs:255-256, SEQ ID NOs:259-260, SEQ ID NOs:263-264, SEQ ID NOs:267-268, SEQ ID NOs:271-272, SEQ ID NOs:275-276, SEQ ID NOs:279-280, SEQ ID NOs:283-284, SEQ ID NOs:287-288, SEQ ID NOs:295-296, SEQ ID NOs:299-300, SEQ ID NOs:303-304, SEQ ID NOs:307-308, SEQ ID NOs:311-312, SEQ ID NOs:315-316, SEQ ID NOs:319-320, SEQ ID NOs:323-324, SEQ ID NOs:327-328, SEQ ID NOs:331-332, SEQ ID NOs 432 and 260, SEQ ID NOs: 433 and 304, SEQ ID NOs: 434 and 308, SEQ ID NOs: 434 and 308, SEQ ID NOs: 435 and 236, SEQ ID NOs: 436 and 244, SEQ ID NOs: 437 and 284, and SEQ ID NOs: 439 and 296.
4. The anti-TNFR2 antibody of any of claims 1, 2, or 3, wherein the antibody is an IgG, a Fv, a scFv, a Fab, a F(ab')₂, a minibody, a diabody, a triabody, a nanobody, a bispecific antibody, or a single domain antibody.
5. The anti-TNFR2 antibody of claim 4, wherein said IgG is IgG1, IgG2, IgG3, or IgG4.
6. The anti-TNFR2 antibody of claim 1, wherein the antibody agonizes TNFR2.
7. The anti-TNFR2 antibody of claim 6, wherein the antibody agonizes TNFR2 in an Fc independent manner.
8. The anti-TNFR2 antibody of claim 1, wherein the antibody has an EC₅₀ binding measurement of about 0.05-100 nM.
9. The anti-TNFR2 antibody of claim 1, wherein the antibody has an EC₅₀ functional agonism

measurement of about 0.05-100 nM.

10. The anti-TNFR2 antibody of claim 1, wherein the antibody activates signaling through TNFR2 at a level of at least 80% as compared to signaling activated by TNF.
- 5 11. The anti-TNFR2 antibody of claim 1, wherein the antibody activates signaling through TNFR2 at a level of at least 95% as compared to signaling activated by TNF.
- 10 12. A composition comprising the anti-TNFR2 antibody of any one of claims 1-3 and a pharmaceutically acceptable carrier.
13. An isolated polynucleotide sequence encoding the anti-TNFR2 antibody of any of claims 1-3.
- 15 14. A vector comprising the polynucleotide sequence of claim 13.
15. A host cell comprising the vector of claim 14.
- 20 16. A method of modulating activities or functions of regulatory T cells in a subject, comprising the step of administering to the subject a composition comprising an effective amount of the anti-TNFR2 antibody of any one of claims 1-3.
17. The method of claim 16, wherein the regulatory T cells are CD4⁺CD25⁺Foxp3⁺.
- 25 18. The method of claim 16, wherein the method stimulates proliferation of the regulatory T cells.
19. The method of claim 16, wherein the method activates said regulatory T cells.
- 30 20. The method of claim 16, wherein the method modulates immune responses mediated by said regulatory T cells.
21. A method of modulating activities or functions of myeloid-derived suppressor cells

(MDSCs) in a subject, comprising the step of administering to the subject a composition comprising an effective amount of the anti-TNFR2 antibody of claim 1-3.

- 5
22. The method of claim 21, wherein the method stimulates proliferation of the MDSCs.
23. The method of claim 21, wherein the method activates said MDSCs.
- 10
24. A method of treating a disease in a subject, comprising the step of administering to the subject a composition comprising an effective amount of the anti-TNFR2 antibody of any of claims 1-3.
25. The method of claim 24, wherein the disease is a cancer, an autoimmune disease, GvHD, a viral infection, or a bacterial infection.