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(54) Title: MONOVALENT TNF BINDING PROTEINS

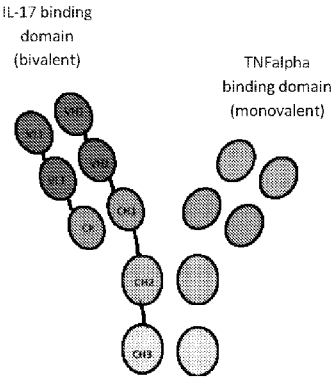

Fig. 1A

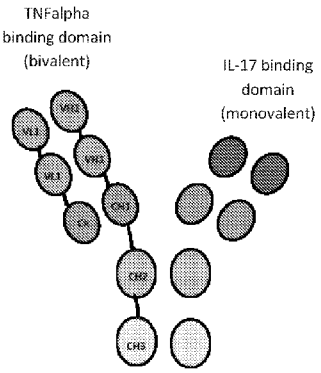

Fig. 1B

(57) Abstract: Provided are TNF binding proteins and methods of treatment using the same. Also provided are nucleic acids encoding the binding proteins and recombinant expression vectors and host cells for making such binding proteins.

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MONOVALENT TNF BINDING PROTEINS

Related Applications

This application claims priority to U.S. Provisional Application Serial No. 62/140,155, filed March 30, 2015, which is incorporated herein by reference in its entirety. This application is related to U.S. Application Serial No. 14/210,703 which claims priority to U.S. Provisional Application Serial No. 61/788,113, filed March 15, 2013, which is incorporated herein by reference in its entirety.

Background of the Invention

The use of therapeutic tissue necrosis factor alpha (TNFα) binding proteins, such as infliximab, adalimumab, etanercept, golimumab, and certolizumab pegol has revolutionized the treatment of many chronic inflammatory diseases, including inflammatory bowel disease (IBD), ankylosing spondylitis, multiple sclerosis, psoriasis and rheumatoid arthritis (RA). Despite their success in improving the quality of life of patients, long-term treatment with therapeutic TNFα binding proteins can elicit strong immunogenic responses that result in the development of anti-drug antibodies (ADA). Such ADA responses can impact both the safety and pharmacokinetics of therapeutic TNFα binding proteins, which, in turn, can affect the utility and efficacy of these drugs. Accordingly, there is a need in the art for novel TNFα binding proteins for use as therapeutics, which are less immunogenic in patients

Summary

The present disclosure provides novel TNFα binding proteins and methods of treatment using the same. Also provided are nucleic acids encoding the binding proteins and recombinant expression vectors and host cells for making such binding proteins. The present disclosure is based, at least in part, on the discovery that multivalent binding proteins which have a single monovalent binding specificity for TNFα (e.g., anti-TNFα monoclonal antibodies) (i.e., each binding protein is only able to bind to one TNFα molecule, e.g., on the surface of an antigen presenting cell) and bivalent for a second specificity), exhibit improved half-life over multivalent binding proteins having binding specificity for multiple TNFα molecules (see Figures 1 and 2).

In some aspects, a binding protein comprising first, second, third and fourth polypeptide chains is provided, wherein said first polypeptide chain comprises VD1-(X1)n-VD2-C-(X2)n, wherein VD1 is a first heavy chain variable domain, VD2 is a second heavy

chain variable domain, C is a CH1 domain, X1 is a linker with the proviso that it is not a constant domain, n is 0 or 1, and X2 is an Fc region; wherein said second polypeptide chain comprises VD1-(X1)n-VD2-C, wherein VD1 is a first light chain variable domain, VD2 is a second light chain variable domain, C is a CL domain, X1 is a linker with the proviso that it is not a constant domain, and n is 0 or 1; wherein the VD1 of the heavy chain and the VD1 of the light chain form a functional binding site and wherein the VD2 of the heavy chain and the VD2 of the light chain form a functional binding site; and wherein the VD1 and VD2 functional binding sites bind to the same non-TNFα antigen; and wherein said third polypeptide chain comprises VD3-C-(X1)n, wherein VD3 is a third heavy chain variable domain, C is a CH1 domain, X1 is an Fc region, and n is 0 or 1; wherein said fourth polypeptide chain comprises VD3-C, wherein VD3 is a first light chain variable domain; and C is a CL domain; wherein the VD3 of the heavy chain and the VD3 of the light chain form a functional binding site for TNFα. In some embodiments, the TNFα is human TNFα. In some embodiments, the VD3 heavy chain variable domain and the VD3 light chain variable domain are a heavy chain variable domain and a light chain variable domain from infliximab, adalimumab, certolizumab pegol, or golimumab.

In some embodiments, the Fc region of the first and third polypeptide chains each comprises a mutation, wherein said mutations on the two Fc regions enhance heterodimerization of the first and third polypeptide chains. In some embodiments, the Fc region of one of the first polypeptide and the second polypeptide comprises the sequence of SEQ ID NO: 121 and the Fc region of the other of the first polypeptide and the second polypeptide comprises the sequence of SEQ ID NO: 137. In some embodiments, the Fc region of the first and third polypeptide chains comprise one or more of the sequences provided in Table 4.

In some embodiments, monovalent binding of the binding protein to cell surface TNFα on antigen presenting cells provides a halflife greater than a molecule that is bivalent or tetravalent for TNFα. In some embodiments, monovalent binding of the binding protein to cell surface TNFα on antigen presenting cells generates less anti-drug antibodies (ADA) than a molecule that is bivalent or tetravalent for TNFα.

In some embodiments, the non-TNFα antigen is a soluble ligand. In some embodiments, the non-TNFα antigen is IL-17. In some embodiments, the non-TNFα antigen is human IL-17.

In some embodiments, the binding protein comprises one or more of the sequences provided in Tables 2, 5 or 6. In some embodiments, the binding protein comprises one or

more of the sequences of SEQ ID NOs: 112-143. In some embodiments, the VD3 heavy chain variable domain and light chain variable domain comprise one or more of the sequences provided in Tables 2, 5 or 6. In some embodiments, the VD1 and VD2 heavy chain variable domains and light chain variable domains comprise one or more of the sequences provided in Table 3.

In some embodiments, VD1 of the first polypeptide comprises CDR1, CDR2, and CDR3 sequences of SEQ ID NOs: 114-116, VD2 of the first polypeptide comprises the CDR1, CDR2, and CDR3 sequences of SEQ ID NOs: 118-120, VD1 of the second polypeptide comprises the CDR1, CDR2, and CDR3 sequences of SEQ ID NOs: 124-126, VD2 of the second polypeptide comprises the CDR1, CDR2, and CDR3 sequences of SEQ ID NOs: 128-130, VD3 of the third polypeptide comprises the CDR1, CDR2, and CDR3 sequences of SEQ ID NOs: 134-136, and VD3 of the fourth polypeptide comprises the CDR1, CDR2, and CDR3 sequences of SEQ ID NOs: 140-142. In some embodiments, VD1-(X1)n-VD2 of the first polypeptide comprise the sequence of SEQ ID NO: 113, VD1-(X1)n-VD2 of the second polypeptide comprise the sequence of SEQ ID NO: 123, VD3 of the third polypeptide comprises the sequence of SEQ ID NO: 133, and VD3 of the fourth polypeptide comprises the sequence of SEQ ID NO: 139. In some embodiments, the first polypeptide comprises the sequence of SEQ ID NO: 112, the second polypeptide comprises the sequence of SEQ ID NO: 122, the third polypeptide comprises the sequence of SEQ ID NO: 132 and the fourth polypeptide comprises the sequence of SEQ ID NO: 138.

In other aspects, a binding protein comprising first, second, third and fourth polypeptide chains is provided, wherein said first polypeptide chain comprises VD1-(X1)n-VD2-C-(X2)n, wherein VD1 is a first heavy chain variable domain, VD2 is a second heavy chain variable domain, C is a CH1 domain, X1 is a linker with the proviso that it is not a constant domain, n is 0 or 1, and X2 is an Fc region; wherein said second polypeptide chain comprises VD1-(X1)n-VD2-C, wherein VD1 is a first light chain variable domain, VD2 is a second light chain variable domain, C is a CL domain, X1 is a linker with the proviso that it is not a constant domain,

and n is 0 or 1; wherein the VD1 of the heavy chain and the VD1 of the light chain form a functional binding site and wherein the VD2 of the heavy chain and the VD2 of the light chain form a functional binding site; and wherein the VD1 and VD2 functional binding sites bind TNFα; and wherein said third polypeptide chain comprises VD3-C-(X1)n, wherein VD3 is a third heavy chain variable domain, C is a CH1 domain, X1 is an Fc region, and n is 0 or 1; wherein said fourth polypeptide chain comprises VD3-C, wherein VD3 is a first light chain

variable domain; and C is a CL domain; wherein the VD3 of the heavy chain and the VD3 of the light chain form a functional binding site for a non-TNFα antigen. In some embodiments, the TNFα is human TNFα.

In some embodiments, the VD1 and VD2 heavy chain variable domains and light chain variable domains are heavy chain variable domains and light chain variable domains from infliximab, adalimumab, certolizumab pegol, or golimumab.

In some embodiments, the Fc region of the first and third polypeptide chains each comprises a mutation, wherein said mutations on the two Fc regions enhance heterodimerization of the first and third polypeptide chains. In some embodiments, the Fc region of one of the first polypeptide and the second polypeptide comprises the sequence of SEQ ID NO: 153 and the Fc region of the other of the first polypeptide and the second polypeptide comprises the sequence of SEQ ID NO: 169. In some embodiments, the Fc region of the first and third polypeptide chains comprise one or more of the sequences provided in Table 4.

In some embodiments, the non-TNFα antigen is a soluble ligand. In some embodiments, the non-TNFα antigen is IL-17. In some embodiments, the non-TNFα antigen is human IL-17.

In some embodiments, the binding protein comprises one or more of the sequences provided in Tables 2, 5 or 6. In some embodiments, the binding protein comprises one or more of the sequences of SEQ ID NOs: 144-175. In some embodiments, the VD1 and VD2 heavy chain variable domains and light chain variable domains comprise one or more of the sequences provided in Table 2, 5 or 6. In some embodiments, the VD3 heavy chain variable domain and light chain variable domain comprise one or more of the sequences provided in Tables 3. In some embodiments, the first polypeptide comprises the sequence of SEQ ID NO: 144, the second polypeptide comprises the sequence of SEQ ID NO:154 the third polypeptide comprises the sequence of SEQ ID NO: 164 and the fourth polypeptide comprises the sequence of SEQ ID NO: 170.

In yet other aspects, a binding protein is provided comprising one or more of the sequences in Tables 5 or 6. In some embodiments, the binding protein comprises one or more of the sequences of SEQ ID NOs: 112-143. In some embodiments, the binding protein comprises one or more of the sequences of SEQ ID NOs: 144-175.

In other aspects, a method of treating a TNF-associated disorder in a subject in need thereof is provided, comprising administering to the subject an effective amount of any one of the binding proteins described herein.

In other aspects, a nucleic acid encoding any one of the binding proteins described herein is provided. In yet other aspects, a vector expressing the nucleic acid is provided. In another aspect, a host cell comprising the vector is provided. In other aspects, a method of producing a binding protein is provided, comprising culturing the host cell in culture medium under conditions sufficient to produce the binding protein. In another aspect, a protein produced according to the method is provided.

In yet other aspects, a pharmaceutical composition is provided comprising any one of the binding proteins described herein, and a pharmaceutically acceptable carrier.

Brief Description Of The Drawings

Figure 1A represents a MBMM2 (PR-1621611) molecule, which has monovalent binding specificity for TNFα and bivalent binding specificity for IL-17.

Figure 1B represents a MBMM1 (PR-1621615), which has bivalent binding specificity for TNFα and monovalent binding specificity for IL-17.

Figure 2A represents TV-GS or TV-LS molecules, which have tetravalent binding specificity for TNFα.

Figure 2B represents JMB-GS molecules, which have bivalent in-tandem binding specificity for TNFα.

Figure 2C represents Ambromab molecules, which have monovalent in-tandem binding specificity for TNFα and IL-17.

Figure 3A illustrates the serum concentration of MBMM2 after 5 mg/kg IV dose in CD-1 mice (Table 10).

Figure 3B illustrates the serum concentration of MBMM2 after 5 mg/kg IV dose in CD-1 mice (Table 12).

Figure 3C illustrates the superior pK characteristics of MBMM2 over MBMM1 after 5 mg/kg IV dose in CD-1 mice.

Figure 4 illustrates the serum concentration of TV-GS Molecule after 5 mg/kg IV dose in CD-1 mice (PR-1580725) (Table 15).

Figure 5 illustrates the serum concentration of TV-GS Molecule after 5 mg/kg IV dose in CD-1 mice (PR-1603912) (Table 17).

Figure 6 illustrates the serum concentration of TV-LS Molecule after 5 mg/kg IV dose in CD-1 mice (PR-1580724) (Table 19).

Figure 7 illustrates the serum concentration of JMB-GS Tandem Molecule after 5 mg/kg IV dose in CD-1 mice (PR-1603136) (Table 21).

Figure 8 illustrates the serum concentration of Ambromabs (PR-1603912 and PR-1603915) TV-GS (PR-1580725), TV-LS (PR-1580724), and JMB-GS Tandem (PR-1603136) Molecules after 5 mg/kg IV dose in CD-1 mice (PR-1603136) (Table 23).

Detailed Description

The present disclosure provides novel TNF binding proteins and methods of treatment using the same. Also provided are nucleic acids encoding the binding proteins and recombinant expression vectors and host cells for making such binding proteins. The present disclosure is based, at least in part, on the discovery that bivalent TNF binding proteins (e.g., anti-TNF monoclonal antibodies) can bind to TNF on the cell surface of antigen presenting cells and become internalized. The binding proteins disclosed herein are generally monovalent with regard to cell surface TNF binding (i.e., each binding protein is only able to bind to one TNF molecule on the surface of an antigen presenting cell). The monovalency with regard to TNF binding of these trivalent molecules resulted in enhanced pharmacokinetic characteristics.

I. Definitions

Unless otherwise defined herein, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. The meaning and scope of the terms should be clear, however, in the event of any latent ambiguity, definitions provided herein take precedent over any dictionary or extrinsic definition. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclature used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well known and commonly used in the art.

In order that the present invention may be more readily understood, certain terms are first defined.

The term "human TNFα" refers to a human cytokine that exists as a 17 kD secreted form and a 26 kD membrane associated form, the biologically active form of which is composed of a trimer of noncovalently bound 17 kD molecules. The structure of human TNFα is described further in, e.g., Pennica et al. (1984) Nature 312:724-729; Davis et al. (1987) Biochem. 26:1322-1326; and Jones et al. (1989) Nature 338:225-228. The term human TNFα includes recombinant human TNFα, which can be prepared by standard recombinant expression methods or purchased commercially (R & D Systems, Catalog No. 210-TA, Minneapolis, Minn.). TNF-α is a multifunctional pro-inflammatory cytokine secreted predominantly by monocytes/macrophages that also has effects on lipid metabolism, coagulation, insulin resistance, and endothelial function. TNF-α triggers pro-inflammatory pathways that result in tissue injury, such as degradation of cartilage and bone, induction of adhesion molecules, induction of pro-coagulant activity on vascular endothelial cells, an increase in the adherence of neutrophils and lymphocytes, and stimulation of the release of platelet activating factor from macrophages, neutrophils and vascular endothelial cells.

The term "infliximab" refers to the anti-TNF antibody marketed as REMICADE®, having Chemical Abstracts Service (CAS) designation 170277-31-3.

The term "golimumab" refers to the anti-TNF antibody marketed as SIMPONI®, having Chemical Abstracts Service (CAS) designation 476181-74-5.

The term "certolizumab pegol" refers to the anti-TNF antibody marketed as CIMZIA®, having Chemical Abstracts Service (CAS) designation 428863-50-7.

The terms "adalimumab" or "D2E7" refer to the anti-TNF antibody marketed as HUMIRA®, having Chemical Abstracts Service (CAS) designation 331731-18-1.

The term "etanercept" refers to the anti-TNF antibody marketed as ENBREL®, having Chemical Abstracts Service (CAS) designation 1094-08-2.

The terms "interleukin-17" or "IL-17" or "IL-17A" refer to an inflammatory cytokine produced by T_H17 T cells that contributes to the etiology of a number of inflammatory diseases. IL-17A may exist as either a homodimer or as a heterodimer complexed with its homolog IL-17F to form heterodimeric IL-17A/F. IL-17A and IL-17F share 55% amino acid identity and bind to the same receptor (IL-17R), which is expressed on a wide variety of cells including vascular endothelial cells, peripheral T cells, B cells, fibroblast, lung cells, myelomonocytic cells, and marrow stromal cells. IL-17A is involved in the induction of pro-inflammatory responses and induces or mediates expression of a variety of other cytokines, factors, and mediators including TNF-α, IL-6, IL-8, IL-1β, granulocyte colony-stimulating factor (G-CSF), prostaglandin E_2 (PGE_2), IL-10, IL-12, IL-1R antagonist, leukemia inhibitory

factor, and stromelysin. Through its role in T cell mediated autoimmunity, IL-17 is an important local orchestrator of neutrophil accumulation and plays a role in cartilage and bone destruction in a number of inflammatory diseases.

The term "antibody" refers to any immunoglobulin (Ig) molecule comprised of four polypeptide chains, two heavy (H) chains and two light (L) chains, or any functional fragment, mutant, variant, or derivation thereof, which retains the essential epitope binding features of an Ig molecule. Such mutant, variant, or derivative antibody formats are known in the art. Non-limiting embodiments of which are discussed below.

In a full-length antibody, each heavy chain is comprised of a heavy chain variable region (abbreviated herein as VH) and a heavy chain constant region (abbreviated herein as CH). The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as VL) and a light chain constant region (abbreviated herein as CL). The light chain constant region is comprised of one domain, CL. The VH and VL regions can be further subdivided into regions of hypervariability, termed “complementarity determining regions” or “CDRs”, interspersed with regions that are more conserved, termed “framework regions” or “FRs”. Each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. Immunoglobulin molecules can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG 1, IgG2, IgG 3, IgG4, IgA1 and IgA2) or subclass.

The terms "VH domain" and "VL domain" refer to single antibody variable heavy and light domains, respectively, comprising FR (Framework Regions) 1, 2, 3 and 4 and CDR (Complementary Determinant Regions) 1, 2 and 3.

The term "complementarity determining region" or “CDR” means the noncontiguous antigen combining sites found within the variable region of both heavy and light chain polypeptides. These regions have been described by Kabat et al. (1977) J. Biol. Chem. 252: 6609-6616 and by Chothia et al. (1987) J. Mol. Biol. 196: 901-917 and by MacCallum et al. (1996) J. Mol. Biol. 262: 732-745 where the definitions include overlapping or subsets of amino acid residues when compared against each other. Preferably, the term "CDR" is a CDR as defined by Kabat, based on sequence comparisons.

The term "framework region" or "FR region" refers to the amino acid residues that are part of the variable region, but are not part of the CDRs (e.g., using the Kabat definition of CDRs).

The term "Fc region" is used to define the C-terminal region of an immunoglobulin heavy chain, which may be generated by papain digestion of an intact antibody. The Fc region may be a native sequence Fc region or a variant Fc region. The Fc region of an immunoglobulin generally comprises two constant domains, a CH2 domain and a CH3 domain, and optionally comprises a CH4 domain. Replacements of amino acid residues in the Fc portion to alter antibody effector function are known in the art (Winter et al. U.S. Patent Nos. 5,648,260; 5,624,821). The Fc portion of an antibody mediates several important effector functions, e.g., cytokine induction, antibody dependent cell-mediated cytotoxicity (ADCC), phagocytosis, complement dependent cytotoxicity (CDC) and half-life/clearance rate of antibody and antigen-antibody complexes. In some cases, these effector functions are desirable for a therapeutic antibody but in other cases they might be unnecessary or even deleterious, depending on the therapeutic objectives. Certain human IgG isotypes, particularly IgG1 and IgG3, mediate ADCC and CDC via binding to Fcγ receptors (FcγR) and complement C1q, respectively. Neonatal Fc receptors (FcRn) are the critical components determining the circulating half-life of antibodies. In still another embodiment at least one amino acid residue is replaced in the constant region of the antibody, e.g., the Fc region of the antibody, such that effector functions of the antibody are altered. The dimerization of two identical heavy chains of an immunoglobulin is mediated by the dimerization of CH3 domains and is stabilized by the disulfide bonds within the hinge region (Huber et al. (1976) Nature 264: 415-20; Thies et al. (1999) J. Mol. Biol. 293: 67-79).

The term “knobs into holes” refers to heterodimerization technology in which complementary mutations are made in the constant region, e.g., CH3 domain, of each heavy chain such that non-covalent interactions drive assembly toward heterodimer formation. For example, a 'knob' variant is obtained by replacement of a small amino acid with a larger one in the CH3 domain of an IgG, such as T366Y. The knob is designed to insert into a 'hole' in the CH3 domain of a different antibody created by judicious replacement of a large residue with a smaller one, such as Y407T.

The term "antigen-binding portion" of an antibody refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen. Such antibody embodiments may also be bispecific, dual specific, or multi-specific, specifically binding to two or more different antigens. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody with regard to the trivalent molecules of the present disclosure include fragments that comprise (i) a trivalent fragment consisting of one VH-VH-CH1, one VL-VL-CL, one VH-CH1, and one VL-CL where the anti TNF binder is on the

monovalent arm; (ii) a trivalent fragment consisting of one VH-VH-CH1, one VL-VL-CL, one VH-CH1, and one VL-CL where the anti TNF binder is on the bivalent arm; (iii) a $F(ab')_2$ fragment of the above, a trivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iv) a triabody where two of the variable domains are the same and the third is different; (v) a tribi-minibody; (vi) a tribody; (vii) a Fab3 DNL; and (viii) a barnase-barnstar trimer. Furthermore, although the two domains of the Fv fragment, VH and VL, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VH and VL regions pair to form monovalent molecules (known as single chain Fv (scFv). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody. Other forms of single chain antibodies, such as diabodies are also encompassed. Diabodies are bivalent, bispecific antibodies in which VH and VL domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites (see e.g., Holliger et al. (1993) Proc. Natl. Acad. Sci. USA 90: 6444-6448; Poljak et al. (1994) Structure 2: 1121-1123). Such antibody binding portions are known in the art (Kontermann and Dubel eds., Antibody Engineering (2001) Springer-Verlag. New York. 790 pp. (ISBN 3-540-41354-5).

The term “bivalent” refers to a binding molecule that binds two antigens. The antigens may be the same or different.

The term “bispecific” refers to a binding molecule that binds two different antigens.

The term “trivalent” refers to a binding molecule that binds three antigens. The antigens may be the same or different.

The term “trispecific” refers to a binding molecule that binds three different antigens.

The term “tetravalent” refers to a binding molecule that binds four antigens. The antigens may be the same or different.

The term “tetraspecific” refers to a binding molecule that binds four different antigens.

As used herein, the term "specifically binds to" refers to the ability of a binding polypeptide to bind to an antigen with a Kd of at least about 1×10^{-6} M, 1×10^{-7} M, 1×10^{-8} M, 1×10^{-9} M, 1×10^{-10} M, 1×10^{-11} M, 1×10^{-12} M, or more, and/or to bind to an antigen with an affinity that is at least two-fold greater than its affinity for a nonspecific antigen. It shall be understood, however, that the binding polypeptides of the invention are capable of

specifically binding to two or more antigens which are related in sequence. For example, the binding polypeptides of the invention can specifically bind to both human and a non-human (e.g., mouse or non-human primate) ortholog of an antigen.

The term "polypeptide" refers to any polymeric chain of amino acids. The terms "peptide" and "protein" are used interchangeably with the term polypeptide and also refer to a polymeric chain of amino acids. The term "polypeptide" encompasses native or artificial proteins, protein fragments and polypeptide analogs of a protein sequence. A polypeptide may be monomeric or polymeric.

The term "linker" refers to polypeptides comprising two or more amino acid residues joined by peptide bonds and are used to link one or more antigen binding portions. Such linker polypeptides are well known in the art. Preferred linkers include, but are not limited to, the amino acid linkers set forth in Table 1 herein.

The term "K_{on}" refers to the on rate constant for association of an antibody to the antigen to form the antibody/antigen complex.

The term "K_{off}" refers to the off rate constant for dissociation of an antibody from the antibody/antigen complex.

The term "Kd" refers to the dissociation constant of a particular antibody-antigen interaction.

The term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply, "expression vectors"). In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" may be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g.,

replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The term "recombinant host cell" (or simply "host cell") refers to a cell into which exogenous DNA has been introduced. Such terms are intended to refer not only to the particular subject cell, but, to the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term "host cell" as used herein. Preferably host cells include prokaryotic and eukaryotic cells selected from any of the Kingdoms of life. Preferred eukaryotic cells include protist, fungal, plant and animal cells. Most preferably host cells include but are not limited to the prokaryotic cell line E. Coli; mammalian cell lines CHO, HEK 293 and COS; the insect cell line Sf9; and the fungal cell *Saccharomyces cerevisiae*.

II. Improved TNF Binding Proteins

In one aspect the invention provides novel TNF binding proteins. These binding proteins exhibit monovalent binding to TNF alpha on the surface of a cell (e.g., an antigen presenting cell), i.e., each binding protein is only able to bind to one TNF molecule on the surface of an antigen presenting). In certain embodiments, the binding proteins disclosed herein binds to human TNF, wherein the binding protein exhibits a reduced of cellular internalization upon binding to cell surface TNF compared to the cellular internalization exhibited by a reference antibody (e.g., infliximab, adalimumab, certolizumab pegol, or golimumab).

In certain embodiments, the TNF binding domains of known TNF binding agents are reformatted to produce the novel TNF binding proteins disclosed herein. The TNF binding domains of any TNF binding agents can be employed. In certain embodiments, the variable domains (or CDRs thereof) of the anti-TNF antibodies infliximab, adalimumab, certolizumab pegol, and/or golimumab are employed. In certain embodiments, the TNF binding domain of etanercept is employed. In certain embodiments, one or more of the variable domain amino an amino acid set forth in Tables 2, 3, 5, and 6 are employed.

In certain embodiments, a binding protein comprising first, second, third and fourth polypeptide chains is provided, wherein said first polypeptide chain comprises VD1-(X1)n-VD2-C-(X2)n, wherein VD1 is a first heavy chain variable domain, VD2 is a second heavy chain variable domain, C is a CH1 domain, X1 is a linker with the proviso that it is not a constant domain, n is 0 or 1, and X2 is an Fc region; wherein said second polypeptide chain

comprises VD1-(X1)n-VD2-C, wherein VD1 is a first light chain variable domain, VD2 is a second light chain variable domain, C is a CL domain, X1 is a linker with the proviso that it is not a constant domain, and n is 0 or 1; wherein the VD1 of the heavy chain and the VD1 of the light chain form a functional binding site and wherein the VD2 of the heavy chain and the VD2 of the light chain form a functional binding site; and wherein the VD1 and VD2 functional binding sites bind a non-TNFα antigen; and wherein said third polypeptide chain comprises VD3-C-(X1)n, wherein VD3 is a third heavy chain variable domain, C is a CH1 domain, X1 is an Fc region, and n is 0 or 1; wherein said fourth polypeptide chain comprises VD3-C, wherein VD3 is a first light chain variable domain; and C is a CL domain; wherein the VD3 of the heavy chain and the VD3 of the light chain form a functional binding site for TNFα.

In certain embodiments, a binding protein comprising first, second, third and fourth polypeptide chains is provided, wherein said first polypeptide chain comprises VD1-CH-(X2)n, wherein VD1 is a first heavy chain variable domain, CH is a heavy chain constant domain, and X2 is an Fc region; wherein said second polypeptide chain comprises VD1 -CL-(X2)n, wherein VD1 is a first light chain variable domain, VD2 is a second light chain variable domain, CL is a light chain constant domain, X2 does not comprise an Fc region; wherein said third polypeptide chain comprises VD2-(X3)n-VD3-CL-(X4)n, wherein VD2 is a second heavy chain variable domain, VD3 is a third heavy chain variable domain, CL is a light chain constant domain, X3 is a linker with the proviso that it is not a constant domain, and X4 is an Fc region; wherein said fourth polypeptide chain comprises VD2-(X3)n-VD3-CH-(X4)n, wherein VD2 is a second light chain variable domain, VD3 is a third light chain variable domain, CH is a heavy chain constant domain, X3 is a linker with the proviso that it is not a constant domain, and X4 does not comprise an Fc region; wherein n is 0 or 1, and wherein the VD1 domains on the first and second polypeptide chains form one functional binding site for human TNFα, the VD2 domains on the first and second polypeptide chains form one functional binding site for a non-TNFα antigen, the VD3 domains on the third and fourth polypeptide chains form one functional binding site for a second non-TNFα antigen. In some embodiments, the Fc region of the first and third polypeptide chains each comprises a mutation, wherein said mutations on the two Fc regions enhance heterodimerization of the first and third polypeptide chains. In some embodiments, the VD1 domains that form the functional binding site for human TNFα are from infliximab, adalimumab, certolizumab pegol, or golimumab. In some embodiments, the binding protein binds monovalently to cell surface human TNF on antigen presenting cells. In some embodiments, the non-TNFα

antigen is a soluble ligand. In some embodiments, the non-TNFα antigen is IL17. In some embodiments, a method of treating a TNF-associated disorder in a subject in need thereof is provided, comprising administering to the subject an effective amount of the binding protein.

In certain embodiments, the TNF binding proteins are receptor DVD (rDVD) molecules comprising first, second, third and fourth polypeptide chains, wherein said first polypeptide chain comprises RD1-(X)n-VD1-C-Y or VD1-(X)n-RD1-C-Y, wherein RD1 comprises a ligand-binding domain of a receptor; VD1 is a heavy chain variable domain; C is a CH1 domain; X is a linker with the proviso that it is not CH1; n is 0 or 1; and Y is an Fc region; and wherein said second polypeptide chain comprises RD1-(X)n-VD1-C or VD1-(X)n-RD1-C, wherein RD1 comprises a ligand-binding domain of a receptor; VD1 is a light chain variable domain; C is a CL domain; X is a linker with the proviso that it is not CH1; n is 0 or 1; wherein the RD1 of the heavy chain and the RD1 of the light chain form a functional binding site and wherein the VD1 of the heavy chain and the VD1 of the light chain form a functional binding site and wherein the RD1 and VD1 functional binding sites binds to the same antigen; and wherein said third polypeptide chain comprises VD3-C-(X1)n, wherein VD3 is a third heavy chain variable domain, C is a CH1 domain, X1 is an Fc region, and n is 0 or 1; wherein said fourth polypeptide chain comprises VD3-C, wherein VD3 is a first light chain variable domain, and C is a CL domain; wherein the VD3 of the heavy chain and the VD3 of the light chain form a functional binding site for TNFα.

Any amino acid linker can be used in the TNF binding proteins disclosed herein. In certain embodiments, the linker comprises amino an amino acid sequence selected from those set forth in Table 1.

In certain embodiments, the TNF binding protein or domains comprise one or more amino acid sequences selected from those set forth in Tables 2, 5, and 6.

In certain embodiments, the IL-17 binding protein or domains comprise one or more amino acid sequences selected from those set forth in Table 3 or any of those disclosed in US Patent No: 8,835,610, which is incorporated by reference herein for any purpose.

Any Fc mutants can be used to achieve the half-molecules disclosed herein. In certain embodiments, the Fc mutants are selected from those set forth in Table 4.

Table 1: List of Linkers Used in Construction of Monovalent TNF Binding Molecules

Name	SEQ ID NO	Sequence 12345678901234567890123456789012345678901234567890
HG-short	1	ASTKGP
LK-short	2	TVAAP
LK-long	3	TVAAPSVFIFPP
HG-long	4	ASTKGPSVFPLAP
GS-H5	5	GGGGSG
GS-L5	6	GGSGG
QH	7	QEPKSSDKTHTSP
N/A	8	AKTTPKLEEGEFSEAR
N/A	9	AKTTPKLEEGEFSEARV
N/A	10	AKTTPKLGG
N/A	11	SAKTTPKLGG
N/A	12	SAKTTP
N/A	13	RADAAP
N/A	14	RADAAPTVS
N/A	15	RADAAAAGGPGS
N/A	16	RADAAAA(G4S)4
N/A	17	SAKTTPKLEEGEFSEARV
N/A	18	ADAAP
N/A	19	ADAAPTVSIFPP
N/A	20	TVAAP
N/A	21	TVAAPSVFIFPP
N/A	22	QPKAAP
N/A	23	QPKAAPSVTLFPP
N/A	24	AKTTPP
N/A	25	AKTTPPSVTPLAP
N/A	26	AKTTAP
N/A	27	AKTTAPSVYPLAP
N/A	28	ASTKGP
N/A	29	ASTKGPSVFPLAP
N/A	30	GGGGSGGGGSGGGGS
N/A	31	GENKVEYAPALMALS
N/A	32	GPAKELTPLKEAKVS
N/A	33	GHEAAAVMQVQYPAS
N/A	34	TVAAPSVFIFPPTVAAPSVFIFPP
N/A	35	ASTKGPSVFPLAPASTKGPSVFPLAP
G4S repeats	36	(GGGGS)N
GS-H7	37	GGGGSGG
GS-H10	38	GGGGSGGGGS
GS-H13	39	GGGGSGGGGSGGG
HEH-7	40	TPAPLPT
HEH-13	41	TPAPLPAPLPAPT
HNG-9	42	TSPPSPAPE
HNG-12	43	TSPPSPAPELLG

Table 2: Examples of Anti-TNF Binding Molecules

Name	SEQ ID NO	Sequence 12345678901234567890123456789012345678901234567890
MAK199.4VH	44	EVQLVQSGAEVKKPGASVKVSCKASGYTFNNYGIIWVRQAPGQGLEWMGW INTYTGKPTYAQKFQGRVTMTTDTSTSTAYMELSSLRSEDTAVYYCARKL FNTVAVTDNAMDYWGQGTTVTVSS
MAK199.4VL	45	DIQMTQSPSSLSASVGDRVTITCRASQDIENYLNWYQQKPGKAPKLLIYY TSRLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYFCQQGNTQPPTFGQ GTKLEIKR
MAK195.24 VH	46	EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYGVEWVRQAPGKGLEWVSG IWADGSTHYADTVKSRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAREWQ HGPVAYWGQGTLVTVSS
MAK195.24 VL	47	DIQMTQSPSSLSASVGDRVTITCKASQLVSSAVAWYQQKPGKAPKLLIYW ASTLHTGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQHYRTPFTFGQ GTKLEIKR
MAK199.21 VH	48	EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYGVTWVRQAPGKGLEWVSM IWADSTHYASSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAREWQH GPVAYWGQGTLVTVSS
MAK199.21 VL	49	DIQMTQSPSSLSASVGDRVTITCRASQLVSSAVAWYQQKPGKAPKLLIYW ASARHTGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQHYKTPFTFGQ GTKLEIKR
VHTNF D2E7	50	EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSA ITWNSGHIDYADSVEGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAKVS YLSTASSLDYWGQGTLVTVSS
VLTNF D2E7	51	DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLAWYQQKPGKAPKLLIYA ASTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYCQRYNRAPYTFGQ GTKVEIKR
VHTNF MAK195	52	QVQLKESGPGLVAPSQSLSITCTVSGFSLTDYGVNWVRQPPGKGLEWLGM IWGDGSTDYDSTLKSRLSISKDNSKSQIFLKMNSLQTDDTARYYCAREWH HGPVAYWGQGTLVTVSA
VLTNF MAK195	53	DIVMTQSHKFMSTTVGDRVSITCKASQAVSSAVAWYQQKPGQSPKLLIYW ASTRHTGVPDRFTGSGSVTDFTLTIHNLQAEDLALYYCQQHYSTPFTFGS GTKLEIKR
VHTNF Inf.	54	EVKLEESGGGLVQPGGSMKLSCVASGFIFSNHWMNWVRQSPEKGLEWVAE IRSKSINSATHYAESVKGRFTISRDDSKSAVYLQMTDLRTEDTGVYYCSR NYYGSTYDYWGQGTTLTVSS
VLTNF Inf.	55	DILLTQSPAILSVSPGERVSFSCRASQFVGSSIHWYQQRTNGSPRLLIKY ASESMSGIPSRFSGSGSGTDFTLSINTVESEDIADYYCQESHSWPFTFGS GTNLEVKR
VHTNF Gol.	56	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGNGLEWVAF MSYDGSNKYAKDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDR GIAAGGNYYYYGMDVWGQGTTVTVSS
VLTNF Gol.	57	EIVLTQSPATLSLSPGERATLSCRASQSVYSYLAWYQQKPGQAPRLLIYD ASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPPFTFG PGTKVDIKR
VHTNF ABX299	58	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYDMHWVRQAPGKGLEWVAV IWSDGSIKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAREV ESAMGGFYYNGMDVWGQGTTVTVSS
VLTNF ABX299	59	DIQMTQSPSSLSASVGDRVTITCRASQGIRIDLGWYQQKPGKAPKRLIYA ASTLQSGVPSRFSGSGSGTEFIFTISSLQPEDFASYYCLQHKSYPLTFGG GTKVEIKR
VHTNF ABX263	60	EVQLVESGGGLIQPGGSLRLSCAASGFTVSRNYMSWVRQAPGKGLEWVSV IYSGDRTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGEG

		GFDYWGQGTLVTVSS
VLTNF ABX263	61	EIVMTQSPATLSVSPGERATLSCRASQSVSSNLAWYQQKPGQAPRLLIHG ASIRATGLPARFSGSGSGTEFTLTISSLQSEDFAVYYCQQYNYWWTFGQG TKVEIKR

Table 3: Examples of Anti-IL-17 Binding Molecules

Name	SEQ ID NO	Sequence 12345678901234567890123456789012345678901234567890
10F7M11VH	62	EVQLVQSGAEVKKPGSSVKVSCKASGYTFTDYEIHWVRQAPGQGLEWMGV NDPESGGTFYNQKFDGRVTLTADESTSTAYMELSSLRSEDTAVYYCTRYS KWDSFDGMDYWGQGTTVTVSS
10F7M11VL	63	DIQMTQSPSSLSASVGDRVTITCRASSGIISYIDWFQQKPGKAPKRLIYA TFDLASGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCRQVGSYPETFGQ GTKLEIKR
Secukinumab VH	64	EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYWMNWVRQAPGKGLEWVAA INQDGSEKYYVGSVKGRFTISRDNAKNSLYLQMNSLRVEDTAVYYCVRDY YDILTDYYIHYWYFDLWGRGTLVTVSSA
Secukinumab VL	65	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIY GASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPCTFG QGTRLEIKR
Ixekizumab VH	66	QVQLVQSGAEVKKPGSSVKVSCKASGYSFTDYHIHWVRQAPGQGLEWMGV INPMYGTTDYNQRFKGRVTITADESTSTAYMELSSLRSEDTAVYYCARYD YFTGTGVYWGQGTLVTVSSA
Ixekizumab VL	67	DIVMTQTPLSLSVTPGQPASISCRSSRSLVHSRGNTYLHWYLQKPGQSPQ LLIYKVSNRFIGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCSQSTHLP FTFGQGTKLEIKR
Bimekizumab VH	68	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYNMAWVRQAPGKGLEWVAT ITYEGRNTYYRDSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCASPP QYYEGSIYRLWFAHWGQGTLVTVSS
Bimekizumab VL	69	AIQLTQSPSSLSASVGDRVTITCRADESVRTLMHWYQQKPGKAPKLLIYL VSNSEIGVPDRFSGSGSGTDFRLTISSLQPEDFATYYCQQTWSDPWTFGQ GTKVEIKR
Perakizumab VH	70	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYTMLWVRQAPGKGLEWVAI IKSGGSYSYYPDSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARDG DYGSSYGAMDYWGQGTLVTVSSA
Perakizumab VL	71	DIQMTQSPSSLSASVGDRVTITCRASQDINSYLSWFQQKPGKAPKSLIVR ANRLVDGVPSRFSGSGSGQDYSLTISSLQPEDFATYYCLQYDAFPPYTFG QGTKLEIKR
Afasevikumab VH	72	EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSG INWSSGGIGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTALYYCARDI GGFGEFYWNFGLWGRGTLVTVSSA
Afasevikumab VL	73	EIVLTQSPATLSLSPGERATLSCRASQSVRSYLAWYQQKPGQAPRLLIYD ASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPPATFG GGTKVEIKR
US8,329,431 VH	74	QVQLKESGPGLVAPSQSLSITCTVSGFSLTTYGVHWIRQPPGKGLEWLVV IWSDGYTTYNSALKSRLSITKDNSKSQVFLKMNSLQTDDTAMYYCARNDG DYFYSMDYWGQGTSVTVSS
US8,329,431 VL	75	DVVMTQTPLSLPVSLGDQASFSCRSSQSLVHSNGNTYLHWYLQKPGQSPK LLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYFCSQSTHVP TFGGGTKLEIKR
CAT-2200 VH	76	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSA ISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDL

		IHGVTRNWGQGTLVTVSSA
CAT-2200 VL	77	NFMLTQPHSVSESPGKTVTISCTRSSGSLANYYVQWYQQRPGSSPTIVIF ANNQRPSGVPDRFSGSIDSSSNSASLTISGLKTEDEADYYCQTYDPYSVV FGGGTKLTVLG
gH11gL3 VH	78	EVQLVESGGGVVQPGGSLRLSCAVSGFSLTTYGVHWVRQAPGKGLEWVAV IWSDGYTTYNSALKSRFTISRDNSKNTVYLQMNSLRAEDTAVYYCARNDG DYFYSMDYWGQGTLVTVSS
gH11gL3 VL	79	DVQMTQSPSSLSASVGDRVTITCRSSQSLVHSNGNTYLHWYQQKPGKAPK LLIYKVSNRFSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCSQSTHVP TFGQGTKVEIKR
gH4gL2 (CA048_497. g2) VH	80	EVQLVESGGGLVKPGGSLRLSCAASGVIFSDYYMAWVRQAPGKGLEWVAS INFNADISYYRESVKGRFTISRDDSKNTLYLQMNSLKTEDTAVYYCTTDA NRQNYDWFAYWGQGTLVTVSS
gH4gL2 (CA048_497. g2) VL	81	AIQLTQSPSSLSASVGDRVTITCKASESVSSSMYSYMHWYQQKPGKAPKL LIYRASNLESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSWTAPR TFGQGTKVEIKR
3C1 Mab106 VH	82	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYTMLWVRQAPGKGLEWVAI IKSGGSYSYYPDSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARDG DYGSSYGAMDYWGQGTLVTVSS
3C1 Mab106 VL	83	DIQMTQSPSSLSASVGDRVTITCRASQDINSYLSWFQQKPGKAPKSLIYR ANRLVDGVPSRFSGSGSGQDYSLTISSLQPEDFATYYCLQYDAFPPYTFG QGTKLEIK
3C1 Mab107 VH	84	QVQLVESGGGLVKPGGSLRLSCAASGFTFSDYTMLWIRQAPGKGLEWVSI IKSGGSYSYYPDSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARDG DYGSSYGAMDYWGQGTLVTVSS
3C1 Mab107 VL	85	DIQMTQSPSSLSASVGDRVTITCRASQDINSYLSWFQQKPGKAPKSLIYR ANRLVDGVPSRFSGSGSGQDYSLTISSLQPEDFATYYCLQYDAFPPYTFG QGTKLEIK
Jan-86-79 VH	86	QVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVST ISLTSGFTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARQL TLDVWGQGTLVTVSS
Jan-86-79 VL	87	QSVLTQPPSVSVAPGQTARISCSGDNLGDKYANWYQQKPGQAPVLVIYDD IDRPSGIPERFSGSNSGNTATLTISGTQAEDEADYYCGSYDFFLGMIVFG GGTKLTVL
Y108 VH	88	QVQLVQSGAEVKKPGASVKVSCKASGYTFNEYTMHWVKQAPGQRLEWMGG INPNSGGVSYNQNFKGKATLTVDTSASTAYMELSSLRSEDTAVYYCARGG DGYYTNYFDIDYWGQGTTVTVSS
Y108 VL	89	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIY GASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSYTFGQ GTKLEIK
OREG-210 VH	90	MAVLGLLLCLVTFPSCVLSQVQLKESGPDLVAPSQSLSITCTVSGFSLTS YGIHWVRQPPGKGLEWLVVIWSDGTTTYNSALKSRLSISKDNSKSQVFLK MNSLQTDDTAMYYCASSYDYLYHYTMDYWGQGTSVTVSS
OREG-210 VL	91	MKLPVRLLVLMFWIPASSSDVVMTQTPLSLPVSLGDQASISCRSSQSLVH SNGNTYFHWYLQKPGQSPKLLIYKVSNRFSGVPDRFSGSGSGTDFTLKIS RVEAEDLGVYFCSQSTHVPLTFGAGTNLELK
OREG-203 VH	92	MAWISIILFLVATAIGVHSQAQLQQSGAELVKPGASVKMSCKAFGYTFTT FPIEWMKQNHGKSLEWIGNFHPYNDYTKYNEKFKGKAKLTVEKSSRTVYL ELSRLTSDDSAVYYCARGAYYGDYVSHTMDFWGQGTSVTVSS
OREG-203 VL	93	METDTLLLWVLLLWVPGSTGNIVLTQSPASLAVSLGQRATISCRASESVD SYGNSFMHWYQQKPGQPPKLLIYLASNLESGVPARFSGSGSRTDFTLTID PVEADDAAIYYCQQNNEDPLTFGAGTKLELK
OREG-207 VH	94	MAWISIILFLVATAIGVHSQAQLQQSGAELVKPGASVKMSCKAFGYTFTT FPIEWMKQNHGKSLEWIGNFHPYNDYTKYNEKFKGKAKLTVEKSSSTVYL ELSRLTSDDSAVYYCARGAYYGDYVSHTMDFWGQGTSVTVSS

OREG-207 VL	95	METDTLLLWVLLLWVPGSTGNIVLTQSPASLAVSLGQRATISCRASESVD SYGNSFMHWYQQKPGQPPKLLIYLASNLESGVPARFSGSGSRTDFTLTID PVEADDAAIYYCQQNNEDPLTFGAGTKLELK
XAB4 VH	96	EVQLVESGGDLVQPGGSLRLSCAASGFTFSSYWMSWVRQAPGKGLEWVAN IKQDGSEKYYVDSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARDR GSLYYWGQGTLVTVSS
XAB4 VL	97	AIQLTQSPSSLSASVGDRVTITCRPSQGINWELAWYQQKPGKAPKLLIYD ASSLEQGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQFNSYPLTFGG GTKVEIK
16C10 (humanized) 4C3 (humanized) VH	98	QVQLQESGPGLVKPSETLSLTCTVSGFSLPSHSVSWIRQPPGKGLEWIGI IWNQGGTDYNSAFKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARNAY ITDYYYENYFMDAWGQGTLVTVSS
16C10 (humanized) 4C3 (humanized) VL	99	DIVMTQSPLSLPVTPGEPASISCKSSQSLLFSENQKNYLAWYLQKPGQSP QLLIYWTSTRQSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCQQSYYT PYTFGQGTKVEIKR
30C10 (humanized) VH	100	QVQLVESGGGVVQPGRSLRLSCAASGFTFNNYWMTWVRQAPGKGLEWVAS VSNTGSSTYYPASVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAREG AYYLDYWGQGTLVTVSS
30C10 (humanized) VL	101	DIVMTQSPLSLPVTPGEPASISCKSSQSLFWSESHMNYLAWYLQKPGQSP QLLIYYASTRQSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCHHHYDS HTFGQGTKVEIKR

Table 4: Sequence of Fc Variants for Producing Monovalent Binding Proteins

Name	SEQ ID NO	Sequence 1234567890123456789012345678901234567890123456789 0
Half body constant region	102	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV EPKSCDKTHT**S**PP**S**PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF**R**LYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
AvvMab CH2-CH3 (Knob) Figure 2B truncated Fc	103	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL**W**C LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
pCH123Kn constant region (Knob) Figures 1A and 1B	104	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSL**W**C**L**VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

pCH123h constant region (Hole) Figures 1A, 1B, 2B	105	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSL**S**C**A**VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL**V**SKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
hCk Figures 1A, 1B, 2A, 2B	106	TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSG NSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVT KSFNRGEC
Tetravalent Fc	107	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
JMB-GS Fc (Knob)	108	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLWC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
JMB-GS Fc (Hole)	109	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
Ambromab Knob	110	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV VECPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLWCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK
Ambromab Hole	111	TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSG NSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVT KSFNRGECVECPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

III. Engineered TNF Binding Proteins

In certain preferred embodiments, the TNF binding proteins produced using the methods and compositions disclosed herein exhibit improved properties (e.g., affinity or stability) with respect to a corresponding parental reference binding protein. For example, the engineered binding protein may dissociate from its target antigen with a k_{off} rate constant of about $0.1s^{-1}$ or less, as determined by surface plasmon resonance, or inhibit the activity of

the target antigen with an IC_{50} of about 1 x 10^{-6}M or less. Alternatively, the binding protein may dissociate from the target antigen with a k_{off} rate constant of about 1 x $10^{-2}s^{-1}$ or less, as determined by surface plasmon resonance, or may inhibit activity of the target antigen with an IC_{50} of about 1 x 10^{-7}M or less. Alternatively, the binding protein may dissociate from the target with a k_{off} rate constant of about 1 x $10^{-3}s^{-1}$ or less, as determined by surface plasmon resonance, or may inhibit the target with an IC_{50} of about 1 x 10^{-8}M or less. Alternatively, binding protein may dissociate from the target with a k_{off} rate constant of about 1 x $10^{-4}s^{-1}$ or less, as determined by surface plasmon resonance, or may inhibit its activity with an IC_{50} of about 1 x 10^{-9}M or less. Alternatively, binding protein may dissociate from the target with a k_{off} rate constant of about 1 x $10^{-5}s^{-1}$ or less, as determined by surface plasmon resonance, or inhibit its activity with an IC_{50} of about 1 x 10^{-10}M or less. Alternatively, binding protein may dissociate from the target with a k_{off} rate constant of about 1 x $10^{-5}s^{-1}$ or less, as determined by surface plasmon resonance, or may inhibit its activity with an IC_{50} of about 1 x 10^{-11}M or less.

In certain embodiments, the engineered binding protein comprises a heavy chain constant region, such as an IgG1, IgG2, IgG3, IgG4, IgA, IgE, IgM or IgD constant region. Preferably, the heavy chain constant region is an IgG1 heavy chain constant region or an IgG4 heavy chain constant region. Furthermore, the binding protein can comprise a light chain constant region, either a kappa light chain constant region or a lambda light chain constant region. The binding protein comprises a kappa light chain constant region. Alternatively, the binding protein portion can be, for example, a Fab fragment or a single chain Fv fragment.

In certain embodiments, the engineered binding protein comprises an engineered effector function known in the art. The Fc portion of a binding protein mediates several important effector functions, e.g., cytokine induction, ADCC, phagocytosis, CDC, and half-life/ clearance rate of binding protein and antigen-binding protein complexes. In some cases these effector functions are desirable for therapeutic binding protein but in other cases might be unnecessary or even deleterious, depending on the therapeutic objectives. Certain human IgG isotypes, particularly IgG1 and IgG3, mediate ADCC and CDC via binding to FcγRs and complement C1q, respectively. Neonatal Fc receptors (FcRn) are the critical components determining the circulating half-life of binding proteins. In still another embodiment at least one amino acid residue is replaced in the constant region of the binding protein, for example

the Fc region of the binding protein, such that effector functions of the binding protein are altered.

In certain embodiments, the engineered binding protein is derivatized or linked to another functional molecule (*e.g.*, another peptide or protein). For example, a labeled binding protein of the invention can be derived by functionally linking a binding protein or binding protein portion of the invention (by chemical coupling, genetic fusion, non-covalent association or otherwise) to one or more other molecular entities, such as another binding protein (*e.g.*, a bispecific binding protein or a diabody), a detectable agent, a cytotoxic agent, a pharmaceutical agent, and/or a protein or peptide that can mediate associate of the binding protein with another molecule (such as a streptavidin core region or a polyhistidine tag).

Useful detectable agents with which a binding protein or binding protein portion of the invention may be derivatized include fluorescent compounds. Exemplary fluorescent detectable agents include fluorescein, fluorescein isothiocyanate, rhodamine, 5-dimethylamine-1-napthalenesulfonyl chloride, phycoerythrin and the like. A binding protein may also be derivatized with detectable enzymes, such as alkaline phosphatase, horseradish peroxidase, glucose oxidase and the like. When a binding protein is derivatized with a detectable enzyme, it is detected by adding additional reagents that the enzyme uses to produce a detectable reaction product. For example, when the detectable agent horseradish peroxidase is present, the addition of hydrogen peroxide and diaminobenzidine leads to a colored reaction product, which is detectable. A binding protein may also be derivatized with biotin, and detected through indirect measurement of avidin or streptavidin binding.

In other embodiment, the engineered binding protein is further modified to generate glycosylation site mutants in which the O- or N-linked glycosylation site of the binding protein has been mutated. One skilled in the art can generate such mutants using standard well-known technologies. Glycosylation site mutants that retain the biological activity, but have increased or decreased binding activity, are another object of the present invention.

In still another embodiment, the glycosylation of the engineered binding protein or antigen-binding portion of the invention is modified. For example, an aglycoslated binding protein can be made (*i.e.*, the binding protein lacks glycosylation). Glycosylation can be altered to, for example, increase the affinity of the binding protein for antigen. Such carbohydrate modifications can be accomplished by, for example, altering one or more sites of glycosylation within the binding protein sequence. For example, one or more amino acid substitutions can be made that result in elimination of one or more variable region glycosylation sites to thereby eliminate glycosylation at that site. Such aglycosylation may

increase the affinity of the binding protein for antigen. Such an approach is described in further detail in PCT Publication WO2003016466A2, and U.S. Pat. Nos. 5,714,350 and 6,350,861, each of which is incorporated herein by reference in its entirety.

Additionally or alternatively, an engineered binding protein of the invention can be further modified with an altered type of glycosylation, such as a hypofucosylated binding protein having reduced amounts of fucosyl residues or a binding protein having increased bisecting GlcNAc structures. Such altered glycosylation patterns have been demonstrated to increase the ADCC ability of binding proteins. Such carbohydrate modifications can be accomplished by, for example, expressing the binding protein in a host cell with altered glycosylation machinery. Cells with altered glycosylation machinery have been described in the art and can be used as host cells in which to express recombinant binding proteins of the invention to thereby produce a binding protein with altered glycosylation. See, for example, Shields et al. (2002) J. Biol. Chem. 277: 26733-26740; Umana et al. (1999) Nat. Biotech. 17: 176-1, as well as, European Patent No: EP 1,176,195; and PCT Publications Nos WO 03/035835 and WO 99/54342 80, each of which is incorporated herein by reference in its entirety. Using techniques known in the art a practitioner may generate binding proteins exhibiting human protein glycosylation. For example, yeast strains have been genetically modified to express non-naturally occurring glycosylation enzymes such that glycosylated proteins (glycoproteins) produced in these yeast strains exhibit protein glycosylation identical to that of animal cells, especially human cells (U.S. Patent Nos. 7,449,308 and 7,029,872 and PCT Publication No. WO2005100584 A2).

IV. Production of TNF Binding Proteins

TNF Binding proteins of the present invention may be produced by any of a number of techniques known in the art. For example, expression from host cells, wherein expression vector(s) encoding the heavy and light chains is (are) transfected into a host cell by standard techniques. The various forms of the term "transfection" are intended to encompass a wide variety of techniques commonly used for the introduction of exogenous DNA into a prokaryotic or eukaryotic host cell, *e.g.*, electroporation, calcium-phosphate precipitation, DEAE-dextran transfection and the like. Although it is possible to express the binding proteins of the invention in either prokaryotic or eukaryotic host cells, expression of binding proteins in eukaryotic cells is preferable, and most preferable in mammalian host cells, because such eukaryotic cells (and in particular mammalian cells) are more likely than

prokaryotic cells to assemble and secrete a properly folded and immunologically active binding protein.

Preferred mammalian host cells for expressing the recombinant binding proteins of the invention include Chinese Hamster Ovary (CHO cells) (including dhfr- CHO cells, described in Urlaub and Chasin (*1980) Proc. Natl. Acad. Sci. USA* 77:4216-4220, used with a DHFR selectable marker, *e.g.*, as described in Kaufman and Sharp (1982) Mol. Biol. 159:601-621), NS0 myeloma cells, COS cells and SP2 cells. When recombinant expression vectors encoding binding protein genes are introduced into mammalian host cells, the binding proteins are produced by culturing the host cells for a period of time sufficient to allow for expression of the binding protein in the host cells or, more preferably, secretion of the binding protein into the culture medium in which the host cells are grown. Binding proteins can be recovered from the culture medium using standard protein purification methods.

Host cells can also be used to produce functional binding protein fragments, such as Fab fragments or scFv molecules. It will be understood that variations on the above procedure are within the scope of the present invention. For example, it may be desirable to transfect a host cell with DNA encoding functional fragments of either the light chain and/or the heavy chain of a binding protein of this invention. Recombinant DNA technology may also be used to remove some, or all, of the DNA encoding either or both of the light and heavy chains that is not necessary for binding to the antigens of interest. The molecules expressed from such truncated DNA molecules are also encompassed by the binding proteins of the invention. In addition, bifunctional binding proteins may be produced in which one heavy and one light chain are a binding protein of the invention and the other heavy and light chain are specific for an antigen other than the antigens of interest by crosslinking a binding protein of the invention to a second binding protein by standard chemical crosslinking methods.

In a preferred system for recombinant expression of a binding protein, or antigen-binding portion thereof, of the invention, a recombinant expression vector encoding both the binding protein heavy chain and the binding protein light chain is introduced into dhfr- CHO cells by calcium phosphate-mediated transfection. Within the recombinant expression vector, the binding protein heavy and light chain genes are each operatively linked to CMV enhancer/AdMLP promoter regulatory elements to drive high levels of transcription of the genes. The recombinant expression vector also carries a DHFR gene, which allows for selection of CHO cells that have been transfected with the vector using methotrexate selection/amplification. The selected transformant host cells are cultured to allow for

expression of the binding protein heavy and light chains and intact binding protein is recovered from the culture medium. Standard molecular biology techniques are used to prepare the recombinant expression vector, transfect the host cells, select for transformants, culture the host cells and recover the binding protein from the culture medium. Still further the invention provides a method of synthesizing a recombinant binding protein of the invention by culturing a host cell of the invention in a suitable culture medium until a recombinant binding protein of the invention is synthesized. The method can further comprise isolating the recombinant binding protein from the culture medium.

V. Pharmaceutical Compositions

In one aspect, pharmaceutical compositions comprising one or more binding proteins, either alone or in combination with prophylactic agents, therapeutic agents, and/or pharmaceutically acceptable carriers are provided. The pharmaceutical compositions comprising binding proteins provided herein are for use in, but not limited to, diagnosing, detecting, or monitoring a disorder, in preventing, treating, managing, or ameliorating a disorder or one or more symptoms thereof, and/or in research. The formulation of pharmaceutical compositions, either alone or in combination with prophylactic agents, therapeutic agents, and/or pharmaceutically acceptable carriers, are known to one skilled in the art (see e.g., US Patent No. 9,035,027).

Methods of administering a prophylactic or therapeutic agent provided herein include, but are not limited to, parenteral administration (e.g., intradermal, intramuscular, intraperitoneal, intravenous and subcutaneous), epidural administration, intratumoral administration, mucosal administration (e.g., intranasal and oral routes) and pulmonary administration (e.g., aerosolized compounds administered with an inhaler or nebulizer). The formulation of pharmaceutical compositions for specific routes of administration, and the materials and techniques necessary for the various methods of administration are available and known to one skilled in the art (see e.g., US Patent No. 9,035,027).

Dosage regimens may be adjusted to provide the optimum desired response (e.g., a therapeutic or prophylactic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. The term "dosage unit form" refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit

containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms provided herein are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic or prophylactic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

An exemplary, non-limiting range for a therapeutically or prophylactically effective amount of a binding protein provided herein is 0.1-20 mg/kg, for example, 1-10 mg/kg. It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens may be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

VI. Methods of Treatment Using TNF Binding Molecules

In one aspect, provided herein are methods of treating a TNF-associated disorder in a subject by administering to the individual in need of such treatment a therapeutically effective amount a TNF binding molecule disclosed herein. Such methods can be used to treat any TNF-associated disorder including, without limitation:

A. Sepsis

Tumor necrosis factor has an established role in the pathophysiology of sepsis, with biological effects that include hypotension, myocardial suppression, vascular leakage syndrome, organ necrosis, stimulation of the release of toxic secondary mediators and activation of the clotting cascade. Accordingly, a TNF binding protein of the invention can be used to treat sepsis in any of its clinical settings, including septic shock, endotoxic shock, gram negative sepsis and toxic shock syndrome.

Furthermore, to treat sepsis, a combination of the invention can be coadministered with one or more additional therapeutic agents that may further alleviate sepsis, such as an interleukin-1 inhibitor (such as those described in PCT Publication Nos. WO 92/16221 and WO 92/17583), the cytokine interleukin-6 (see e.g., PCT Publication No. WO 93/11793) or an antagonist of platelet activating factor (see e.g., European Patent Application Publication

No. EP 374 510). Other combination therapies for the treatment of sepsis are discussed further in herein.

Additionally, in certain embodiments, a TNF binding protein of the invention is administered to a human subject within a subgroup of sepsis patients having a serum or plasma concentration of IL-6 above 500 pg/ml (e.g., above 1000 pg/ml) at the time of treatment (see PCT Publication No. WO 95/20978).

B. Autoimmune Diseases

Tumor necrosis factor has been implicated in playing a role in the pathophysiology of a variety of autoimmune diseases. For example, TNFα has been implicated in activating tissue inflammation and causing joint destruction in rheumatoid arthritis. TNFα also has been implicated in promoting the death of islet cells and in mediating insulin resistance in diabetes. TNFα has been implicated in mediating cytotoxicity to oligodendrocytes and induction of inflammatory plaques in multiple sclerosis. Chimeric, humanized murine, and fully human anti-hTNFα antibodies have undergone clinical testing for treatment of rheumatoid arthritis.

Anti-TNF/IL-17 combinations of the invention can be used to treat autoimmune diseases, in particular those associated with inflammation, including rheumatoid arthritis, rheumatoid spondylitis, osteoarthritis and gouty arthritis, allergy, multiple sclerosis, autoimmune diabetes, autoimmune uveitis and nephrotic syndrome. Typically, the combination is administered systemically, although for certain disorders, local administration of the anti-TNF and/or IL-17 at a site of inflammation may be beneficial (e.g., local administration in the joints in rheumatoid arthritis or topical application to diabetic ulcers, alone or in combination with a cyclohexane-ylidene derivative as described in PCT Publication No. WO 93/19751). Anti-TNF/IL-17 combinations of the invention also can be administered with one or more additional therapeutic agents useful in the treatment of autoimmune diseases, as discussed further herein.

C. Infectious Diseases

Tumor necrosis factor has been implicated in mediating biological effects observed in a variety of infectious diseases. For example, TNFα has been implicated in mediating brain inflammation and capillary thrombosis and infarction in malaria. TNFα also has been implicated in mediating brain inflammation, inducing breakdown of the blood-brain bather, triggering septic shock syndrome and activating venous infarction in meningitis. TNFα also

has been implicated in inducing cachexia, stimulating viral proliferation and mediating central nervous system injury in acquired immune deficiency syndrome (AIDS). Accordingly, the anti-TNF/IL-17 combinations of the invention, can be used in the treatment of infectious diseases, including bacterial meningitis (see e.g., European Patent Application Publication No. EP 585 705), cerebral malaria, AIDS and AIDS-related complex (ARC) (see e.g., European Patent Application Publication No. EP 230 574), as well as cytomegalovirus infection secondary to transplantation (see e.g., Fietze et al. (1994) Transplantation 58: 675-680). Anti-TNF/IL-17 combinations of the invention, also can be used to alleviate symptoms associated with infectious diseases, including fever and myalgias due to infection (such as influenza) and cachexia secondary to infection (e.g., secondary to AIDS or ARC).

D. Transplantation

Tumor necrosis factor has been implicated as a key mediator of allograft rejection and graft versus host disease (GVHD) and in mediating an adverse reaction that has been observed when the rat antibody OKT3, directed against the T cell receptor CD3 complex, is used to inhibit rejection of renal transplants. Accordingly, anti-TNF/IL-17 combinations of the invention, can be used to inhibit transplant rejection, including rejections of allografts and xenografts and to inhibit GVHD. Although the combination may be used alone, it can be used in combination with one or more other agents that inhibit the immune response against the allograft or inhibit GVHD. For example, in one embodiment, a TNF binding protein is used in combination with OKT3 to inhibit OKT3-induced reactions. In another embodiment, a TNF binding protein is used in combination with one or more antibodies directed at other targets involved in regulating immune responses, such as the cell surface molecules CD25 (interleukin-2 receptor-.alpha.), CD11a (LFA-1), CD54 (ICAM-1), CD4, CD45, CD28/CTLA4, CD80 (B7-1) and/or CD86 (B7-2). In yet another embodiment, a TNF binding protein of the invention is used in combination with one or more general immunosuppressive agents, such as cyclosporin A or FK506.

E. Malignancy

Tumor necrosis factor has been implicated in inducing cachexia, stimulating tumor growth, enhancing metastatic potential and mediating cytotoxicity in malignancies. Accordingly, a TNF binding protein of the invention can be used in the treatment of malignancies, to inhibit tumor growth or metastasis and/or to alleviate cachexia secondary to malignancy. The anti-TNF/ IL-17 combination may be administered systemically or locally

to the tumor site.

F. Pulmonary Disorders

Tumor necrosis factor has been implicated in the pathophysiology of adult respiratory distress syndrome (ARDS), including stimulating leukocyte-endothelial activation, directing cytotoxicity to pneumocytes and inducing vascular leakage syndrome. Accordingly, a TNF binding protein of the invention, can be used to treat various pulmonary disorders, including adult respiratory distress syndrome (see e.g., PCT Publication No. WO 91/04054), shock lung, chronic pulmonary inflammatory disease, pulmonary sarcoidosis, pulmonary fibrosis and silicosis. The anti-TNF/ IL-17 combination may be administered systemically or locally to the lung surface, for example as an aerosol. An anti-TNF/ IL-17 combination of the invention also can be administered with one or more additional therapeutic agents useful in the treatment of pulmonary disorders, as discussed further in herein.

G. Intestinal Disorders

Tumor necrosis factor has been implicated in the pathophysiology of inflammatory bowel disorders. Chimeric murine anti-hTNFα antibodies have undergone clinical testing for treatment of Crohn's disease. The anti-TNF/ IL-17 combinations of the invention, also can be used to treat intestinal disorders, such as idiopathic inflammatory bowel disease, which includes two syndromes, Crohn's disease and ulcerative colitis. An anti-TNF/ IL-17 combination of the invention also can be administered with one or more additional therapeutic agents useful in the treatment of intestinal disorders, as discussed further in herein.

H. Cardiac Disorders

The anti-TNF/ IL-17 combinations of the invention, also can be used to treat various cardiac disorders, including ischemia of the heart (see e.g., European Patent Application Publication No. EP 453 898) and heart insufficiency (weakness of the heart muscle)(see e.g., PCT Publication No. WO 94/20139).

I. Others Disorders

The anti-TNF/ IL-17 combination of the invention, also can be used to treat various other disorders in which TNF-alpha activity is detrimental. Examples of other diseases and disorders in which TNF-alpha activity has been implicated in the pathophysiology, and thus

which can be treated using a TNF binding protein of the invention, include inflammatory bone disorders and bone resorption disease; hepatitis, including alcoholic hepatitis, viral hepatitis, and fulminant hepatitis; coagulation disturbances, burns, reperfusion injury, keloid formation, scar tissue formation; pyrexia; periodontal disease; obesity and radiation toxicity.

In certain embodiments, an anti-TNF/ IL-17 combinations of the invention is used for the treatment of a TNF-associated disorder selected from the group consisting of osteoarthritis, rheumatoid arthritis, juvenile chronic arthritis, septic arthritis, Lyme arthritis, psoriatic arthritis, reactive arthritis, spondyloarthropathy, systemic lupus erythematosus, Crohn's disease, ulcerative colitis, inflammatory bowel disease, insulin dependent diabetes mellitus, thyroiditis, asthma, allergic diseases, psoriasis, dermatitis, scleroderma, graft versus host disease, organ transplant rejection, acute or chronic immune disease associated with organ transplantation, sarcoidosis, atherosclerosis, disseminated intravascular coagulation, Kawasaki's disease, Grave's disease, nephrotic syndrome, chronic fatigue syndrome, Wegener's granulomatosis, Henoch-Schoenlein purpurea, microscopic vasculitis of the kidneys, chronic active hepatitis, uveitis, septic shock, toxic shock syndrome, sepsis syndrome, cachexia, infectious diseases, parasitic diseases, acute transverse myelitis, Huntington's chorea, Parkinson's disease, Alzheimer's disease, stroke, primary biliary cirrhosis, hemolytic anemia, malignancies, heart failure, myocardial infarction, Addison's disease, sporadic polyglandular deficiency type I, polyglandular deficiency type II (Schmidt's syndrome), adult (acute) respiratory distress syndrome, alopecia, alopecia greata, seronegative arthropathy, arthropathy, Reiter's disease, psoriatic arthropathy, ulcerative colitic arthropathy, enteropathic synovitis, Chlamydia-associated arthropathy, Yersinia-associated arthropathy, Salmonella-associated arthropathy, spondyloarthropathy, atheromatous disease/arteriosclerosis, atopic allergy, autoimmune bullous disease, pemphigus vulgaris, pemphigus foliaceus, pemphigoid, linear IgA disease, autoimmune haemolytic anaemia, Coombs positive haemolytic anaemia, acquired pernicious anaemia, juvenile pernicious anaemia, myalgic encephalitis/Royal Free disease, chronic mucocutaneous candidiasis, giant cell arteritis, primary sclerosing hepatitis, cryptogenic autoimmune hepatitis, acquired immunodeficiency syndrome, acquired immunodeficiency related diseases, hepatitis B, hepatitis C, common varied immunodeficiency (common variable hypogammaglobulinaemia), dilated cardiomyopathy, female infertility, ovarian failure, premature ovarian failure, fibrotic lung disease, cryptogenic fibrosing alveolitis, post-inflammatory interstitial lung disease, interstitial pneumonitis, connective tissue disease associated interstitial lung disease, mixed connective tissue disease associated lung disease,

systemic sclerosis associated interstitial lung disease, rheumatoid arthritis associated interstitial lung disease, systemic lupus erythematosus associated lung disease, dermatomyositis/polymyositis associated lung disease, Sjogren's disease associated lung disease, ankylosing spondylitis associated lung disease, vasculitic diffuse lung disease, haemosiderosis associated lung disease, drug-induced interstitial lung disease, fibrosis, radiation fibrosis, bronchiolitis obliterans, chronic eosinophilic pneumonia, lymphocytic infiltrative lung disease, postinfectious interstitial lung disease, gouty arthritis, autoimmune hepatitis, type-1 autoimmune hepatitis (classical autoimmune or lupoid hepatitis), type-2 autoimmune hepatitis (anti-LKM antibody hepatitis), autoimmune mediated hypoglycemia, type B insulin resistance with acanthosis nigricans, hypoparathyroidism, acute immune disease associated with organ transplantation, chronic immune disease associated with organ transplantation, osteoarthrosis, primary sclerosing cholangitis, psoriasis type 1, psoriasis type 2, idiopathic leucopaenia, autoimmune neutropaenia, renal disease NOS, glomerulonephritides, microscopic vasculitis of the kidneys, Lyme disease, discoid lupus erythematosus, male infertility idiopathic or NOS, sperm autoimmunity, multiple sclerosis (all subtypes), sympathetic ophthalmia, pulmonary hypertension secondary to connective tissue disease, Goodpasture's syndrome, pulmonary manifestation of polyarteritis nodosa, acute rheumatic fever, rheumatoid spondylitis, Still's disease, systemic sclerosis, Sjorgren's syndrome, Takayasu's disease/arteritis, autoimmune thrombocytopaenia, idiopathic thrombocytopaenia, autoimmune thyroid disease, hyperthyroidism, goitrous autoimmune hypothyroidism (Hashimoto's disease), atrophic autoimmune hypothyroidism, primary myxoedema, phacogenic uveitis, primary vasculitis, vitiligo, acute liver disease, chronic liver diseases, alcoholic cirrhosis, alcohol-induced liver injury, cholestasis, idiosyncratic liver disease, drug-induced hepatitis, non-alcoholic steatohepatitis, allergy, group B streptococci (GBS) infection, mental disorders (e.g., depression and schizophrenia), Th2 Type and Th1 Type mediated diseases, acute and chronic pain (different forms of pain), cancers such as lung, breast, stomach, bladder, colon, pancreas, ovarian, prostate and rectal cancer and hematopoietic malignancies (leukemia and lymphoma), abetalipoproteinemia, acrocyanosis, acute and chronic parasitic or infectious processes, acute leukemia, acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), acute or chronic bacterial infection, acute pancreatitis, acute renal failure, adenocarcinomas, atrial ectopic beats, AIDS dementia complex, alcohol-induced hepatitis, allergic conjunctivitis, allergic contact dermatitis, allergic rhinitis, allograft rejection, alpha-1-antitrypsin deficiency, amyotrophic lateral sclerosis, anemia, angina pectoris, anterior horn cell degeneration, antiphospholipid syndrome, anti-

receptor hypersensitivity reactions, aortic and peripheral aneurysms, aortic dissection, arterial hypertension, arteriosclerosis, arteriovenous fistula, ataxia, atrial fibrillation (sustained or paroxysmal), atrial flutter, atrioventricular block, B cell lymphoma, bone graft rejection, bone marrow transplant (BMT) rejection, bundle branch block, Burkitt's lymphoma, burns, cardiac arrhythmias, cardiac stun syndrome, cardiac tumors, cardiomyopathy, cardiopulmonary bypass inflammation response, cartilage transplant rejection, cerebellar cortical degenerations, cerebellar disorders, chaotic or multifocal atrial tachycardia, chemotherapy associated disorders, chronic myelocytic leukemia (CML), chronic alcoholism, chronic inflammatory pathologies, chronic lymphocytic leukemia (CLL), chronic obstructive pulmonary disease (COPD), chronic salicylate intoxication, colorectal carcinoma, congestive heart failure, conjunctivitis, contact dermatitis, cor pulmonale, coronary artery disease, Creutzfeldt-Jakob disease, culture negative sepsis, cystic fibrosis, cytokine therapy associated disorders, dementia pugilistica, demyelinating diseases, dengue hemorrhagic fever, dermatitis, dermatologic conditions, diabetes, diabetic arteriosclerotic disease, diffuse Lewy body disease, dilated congestive cardiomyopathy, disorders of the basal ganglia, Down's syndrome in middle age, drug-induced movement disorders induced by drugs which block CNS dopamine receptors, drug sensitivity, eczema, encephalomyelitis, endocarditis, endocrinopathy, epiglottitis, Epstein-Barr virus infection, erythromelalgia, extrapyramidal and cerebellar disorders, familial hemophagocytic lymphohistiocytosis, fetal thymus implant rejection, Friedreich's ataxia, functional peripheral arterial disorders, fungal sepsis, gas gangrene, gastric ulcer, glomerular nephritis, graft rejection of any organ or tissue, gram negative sepsis, gram positive sepsis, granulomas due to intracellular organisms, hairy cell leukemia, Hallervorden-Spatz disease, Hashimoto's thyroiditis, hay fever, heart transplant rejection, hemochromatosis, hemodialysis, hemolytic uremic syndrome/thrombolytic thrombocytopenic purpura, hemorrhage, hepatitis A, His bundle arrhythmias, HIV infection/HIV neuropathy, Hodgkin's disease, hyperkinetic movement disorders, hypersensitivity reactions, hypersensitivity pneumonitis, hypertension, hypokinetic movement disorders, hypothalamic-pituitary-adrenal axis evaluation, idiopathic Addison's disease, idiopathic pulmonary fibrosis, antibody mediated cytotoxicity, asthenia, infantile spinal muscular atrophy, inflammation of the aorta, influenza A, ionizing radiation exposure, iridocyclitis/uveitis/optic neuritis, ischemia-reperfusion injury, ischemic stroke, juvenile rheumatoid arthritis, juvenile spinal muscular atrophy, Kaposi's sarcoma, kidney transplant rejection, legionella, leishmaniasis, leprosy, lesions of the corticospinal system, lipedema, liver transplant rejection, lymphedema, malaria, malignant lymphoma, malignant

histiocytosis, malignant melanoma, meningitis, meningococcemia, metabolic migraine headache, idiopathic migraine headache, mitochondrial multisystem disorder, mixed connective tissue disease, monoclonal gammopathy, multiple myeloma, multiple systems degenerations (Menzel, Dejerine-Thomas, Shy-Drager, and Machado-Joseph), myasthenia gravis, mycobacterium avium intracellulare, mycobacterium tuberculosis, myelodysplastic syndrome, myocardial infarction, myocardial ischemic disorders, nasopharyngeal carcinoma, neonatal chronic lung disease, nephritis, nephrosis, neurodegenerative diseases, neurogenic muscular atrophies, neutropenic fever, non-Hodgkin's lymphoma, occlusion of the abdominal aorta and its branches, occlusive arterial disorders, orchitis/epididymitis, orchitis/vasectomy reversal procedures, organomegaly, osteoporosis, pancreas transplant rejection, pancreatic carcinoma, paraneoplastic syndrome/hypercalcemia of malignancy, parathyroid transplant rejection, pelvic inflammatory disease, perennial rhinitis, pericardial disease, peripheral atherosclerotic disease, peripheral vascular disorders, peritonitis, pernicious anemia, pneumocystis carinii pneumonia, pneumonia, POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes syndrome), post perfusion syndrome, post pump syndrome, post-MI cardiotomy syndrome, preeclampsia, progressive supranucleo palsy, primary pulmonary hypertension, radiation therapy, Raynaud's phenomenon, Raynaud's disease, Refsum's disease, regular narrow QRS tachycardia, renovascular hypertension, reperfusion injury, restrictive cardiomyopathy, sarcomas, senile chorea, senile dementia of Lewy body type, seronegative arthropathies, shock, sickle cell anemia, skin allograft rejection, skin changes syndrome, small bowel transplant rejection, solid tumors, specific arrhythmias, spinal ataxia, spinocerebellar degenerations, streptococcal myositis, structural lesions of the cerebellum, subacute sclerosing panencephalitis, syncope, syphilis of the cardiovascular system, systemic anaphylaxis, systemic inflammatory response syndrome, systemic onset juvenile rheumatoid arthritis, telangiectasia, thromboangiitis obliterans, thrombocytopenia, toxicity, transplants, trauma/hemorrhage, type III hypersensitivity reactions, type IV hypersensitivity, unstable angina, uremia, urosepsis, urticaria, valvular heart diseases, varicose veins, vasculitis, venous diseases, venous thrombosis, ventricular fibrillation, viral and fungal infections, viral encephalitis/aseptic meningitis, viral-associated hemophagocytic syndrome, Wernicke-Korsakoff syndrome, Wilson's disease, xenograft rejection of any organ or tissue, acute coronary syndromes, acute idiopathic polyneuritis, acute inflammatory demyelinating polyradiculoneuropathy, acute ischemia, adult Still's disease, alopecia greata, anaphylaxis, anti-phospholipid antibody syndrome, aplastic anemia, arteriosclerosis, atopic eczema, atopic

dermatitis, autoimmune dermatitis, autoimmune disorder associated with streptococcus infection, autoimmune enteropathy, autoimmune hearing loss, autoimmune lymphoproliferative syndrome (ALPS), autoimmune myocarditis, autoimmune premature ovarian failure, blepharitis, bronchiectasis, bullous pemphigoid, cardiovascular disease, catastrophic antiphospholipid syndrome, celiac disease, cervical spondylosis, chronic ischemia, cicatricial pemphigoid, clinically isolated syndrome (CIS) with risk for multiple sclerosis, childhood onset psychiatric disorder, chronic obstructive pulmonary disease (COPD), dacryocystitis, dermatomyositis, diabetic retinopathy, disk herniation, disk prolapse, drug induced immune hemolytic anemia, endocarditis, endometriosis, endophthalmitis, episcleritis, erythema multiforme, erythema multiforme major, gestational pemphigoid, Guillain-Barre syndrome (GBS), hay fever, Hughes syndrome, idiopathic Parkinson's disease, idiopathic interstitial pneumonia, IgE-mediated allergy, immune hemolytic anemia, inclusion body myositis, infectious ocular inflammatory disease, inflammatory demyelinating disease, inflammatory heart disease, inflammatory kidney disease, IPF/UIP, iritis, keratitis, keratojunctivitis sicca, Kussmaul disease or Kussmaul-Meier disease, Landry's paralysis, Langerhan's cell histiocytosis, livedo reticularis, macular degeneration, microscopic polyangiitis, Morbus Bechterev, motor neuron disorders, mucous membrane pemphigoid, multiple organ failure, myasthenia gravis, myelodysplastic syndrome, myocarditis, nerve root disorders, neuropathy, non-A non-B hepatitis, optic neuritis, osteolysis, ovarian cancer, pauciarticular JRA, peripheral artery occlusive disease (PAOD), peripheral vascular disease (PVD), peripheral artery disease (PAD), phlebitis, polyarteritis nodosa (or periarteritis nodosa), polychondritis, polymyalgia rheumatica, poliosis, polyarticular JRA, polyendocrine deficiency syndrome, polymyositis, polymyalgia rheumatica (PMR), post-pump syndrome, primary Parkinsonism, prostate and rectal cancer and hematopoietic malignancies (leukemia and lymphoma), prostatitis, pure red cell aplasia, primary adrenal insufficiency, recurrent neuromyelitis optica, restenosis, rheumatic heart disease, SAPHO (synovitis, acne, pustulosis, hyperostosis, and osteitis), secondary amyloidosis, shock lung, scleritis, sciatica, secondary adrenal insufficiency, silicone associated connective tissue disease, Sneddon-Wilkinson dermatosis, spondylitis ankylosans, Stevens-Johnson syndrome (SJS), systemic inflammatory response syndrome, temporal arteritis, toxoplasmic retinitis, toxic epidermal necrolysis, transverse myelitis, TRAPS (tumor-necrosis factor receptor type 1 (TNFR)-associated periodic syndrome), type 1 allergic reaction, type II diabetes, urticaria, usual interstitial pneumonia (UIP), vasculitis, vernal conjunctivitis, viral retinitis, Vogt-Koyanagi-Harada

syndrome (VKH syndrome), and wet macular degeneration. In a particular embodiment, the TNF-associated disease or disorder is rheumatoid arthritis.

VII. Diagnostics

The disclosure herein also provides diagnostic applications including, but not limited to, diagnostic assay methods, diagnostic kits containing one or more TNF binding proteins, and adaptation of the methods and kits for use in automated and/or semi-automated systems. The methods, kits, and adaptations provided may be employed in the detection, monitoring, and/or treatment of a disease or disorder in an individual. This is further elucidated below.

Method of assay

The present disclosure also provides a method for determining the presence, amount or concentration of an analyte, or fragment thereof, in a test sample using at least one binding protein as described herein. Any suitable assay as is known in the art can be used in the method. Examples include, but are not limited to, immunoassays and/or methods employing mass spectrometry.

Immunoassays provided by the present disclosure may include sandwich immunoassays, radioimmunoassay (RIA), enzyme immunoassay (EIA), enzyme-linked immunosorbent assay (ELISA), competitive-inhibition immunoassays, fluorescence polarization immunoassay (FPIA), enzyme multiplied immunoassay technique (EMIT), bioluminescence resonance energy transfer (BRET), and homogenous chemiluminescent assays, among others.

A chemiluminescent microparticle immunoassay, in particular one employing the ARCHITECT® automated analyzer (Abbott Laboratories, Abbott Park, IL), is an example of an immunoassay.

Methods employing mass spectrometry are provided by the present disclosure and include, but are not limited to MALDI (matrix-assisted laser desorption/ionization) or by SELDI (surface-enhanced laser desorption/ionization).

Methods for collecting, handling, processing, and analyzing biological test samples using immunoassays and mass spectrometry would be well-known to one skilled in the art, are provided for in the practice of the present disclosure (US 2009-0311253 A1).

Kit

A kit for assaying a test sample for the presence, amount or concentration of an analyte, or fragment thereof, in a test sample is also provided. The kit comprises at least one component for assaying the test sample for the analyte, or fragment thereof, and instructions for assaying the test sample for the analyte, or fragment thereof. The at least one component for assaying the test sample for the analyte, or fragment thereof, can include a composition comprising a binding protein, as disclosed herein, and/or an anti-analyte binding protein (or a fragment, a variant, or a fragment of a variant thereof), which is optionally immobilized on a solid phase.

Optionally, the kit may comprise a calibrator or control, which may comprise isolated or purified analyte. The kit can comprise at least one component for assaying the test sample for an analyte by immunoassay and/or mass spectrometry. The kit components, including the analyte, binding protein, and/or anti-analyte binding protein, or fragments thereof, may be optionally labeled using any art-known detectable label. The materials and methods for the creation provided for in the practice of the present disclosure would be known to one skilled in the art (US Patent No. 9,035,027).

Adaptation of kit and method

The kit (or components thereof), as well as the method of determining the presence, amount or concentration of an analyte in a test sample by an assay, such as an immunoassay as described herein, can be adapted for use in a variety of automated and semi-automated systems (including those wherein the solid phase comprises a microparticle), as described, for example, in US Patent Nos. 5,089,424 and 5,006,309, and as commercially marketed, for example, by Abbott Laboratories (Abbott Park, IL) as ARCHITECT®.

Other platforms available from Abbott Laboratories include, but are not limited to, AxSYM®, IMx® (see, for example, US Patent No. 5,294,404, PRISM®, EIA (bead), and Quantum™ II, as well as other platforms. Additionally, the assays, kits and kit components can be employed in other formats, for example, on electrochemical or other hand-held or point-of-care assay systems. The present disclosure is, for example, applicable to the commercial Abbott Point of Care (i-STAT®, Abbott Laboratories) electrochemical immunoassay system that performs sandwich immunoassays. Immunosensors and their methods of manufacture and operation in single-use test devices are described, for example in, US Patent No. 5,063,081; 7,419,821; 7,682,833; 7,723,099; and US 9,035,027; and US Publication Nos. 20040018577

Exemplification

Example 1: Amino Acid Sequences of TNF and/or IL-17 Binding Molecules

Binding molecule structures were synthesized by standard recombinant methods. Figure 1A shows a molecule that is bivalent for IL-17 and monovalent for TNF named MBMM2. The amino acid sequence for MBMM2 is provided in Table 5. Figure 1B shows a molecule that is bivalent for TNF and monovalent for IL-17 named MBMM1. The amino acid sequence for MBMM1 is provided in Table 6. Figure 2A shows a molecule that is tetravalent for TNF named TV-GS (PR-1580725 or PR-1603912) or TV-LS (PR-1580724), depending upon which linker is used. The amino acid sequences for the TV-GS and TV-LS molecules is provided in Table 7. Figure 2B shows a molecule that is bivalent for TNF named JMB-GS (PR-1603136). The sequence for JMB-GS is provided in Table 8. Figure 2C shows a molecule that is monovalent for both for TNF and IL-17 named PR-1603912 or PR-1603915. The sequences for 1603912 or 1603915 are provided in Table 9. Not shown are a molecule that is tetravalent for IL-17 named PR-1611416 (with GS linker) or PR-1611418 (with LS linker). The sequences for PR-1611416 or PR-1611418 are provided in Table 10.

Table 5: Amino Acid Sequences of MBMM2 (PR-1621611) Anti-TNF/IL-17 Bispecific

Name	SEQ ID NO	Amino Acid Sequence 1234567890123456789012345678901234567890
MBMM2 17-L-17 Kn Fc Full Heavy	112	EVQLVQSGAEVKKPGSSVKVSCKASGGSFGGYGIGWVRQA PGQGLEWMGGITPFFGFADYAQKFQGRVTITADESTTTAY MELSGLTSDDTAVYYCARDPNEFWGGYYSTHDFDSWGQGT TVTVSSASTKGPSVFPLAPEVQLVQSGAEVKKPGSSVKVS CKASGGSFGGYGIGWVRQAPGQGLEWMGGITPFFGFADYA QKFQGRVTITADESTTTAYMELSGLTSDDTAVYYCARDPN EFWGGYYSTHDFDSWGQGTTVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA VLQSSGLYSLSRVVTVPSSSLGTQTYICNVNHKPSNTKVD KKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLM ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSREEMTKNQVSLWCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
17-L-17 Variable Heavy	113	EVQLVQSGAEVKKPGSSVKVSCKASGGSFGGYGIGWVRQA PGQGLEWMGGITPFFGFADYAQKFQGRVTITADESTTTAY MELSGLTSDDTAVYYCARDPNEFWGGYYSTHDFDSWGQGT TVTVSSASTKGPSVFPLAPEVQLVQSGAEVKKPGSSVKVS CKASGGSFGGYGIGWVRQAPGQGLEWMGGITPFFGFADYA

		QKFQGRVTITADESTTTAYMELSGLTSDDTAVYYCARDPN EFWGGYYSTHDFDSWGQGTTVTVSS
17 CDRH1	114	GGSFGGYGIG
17 CDRH2	115	GITPFFGFADYAQ
17 CDRH3	116	DPNEFWGGYYSTHDFDS
Linker	117	ASTKGPSVFPLAP
17 CDRH1	118	GGSFGGYGIG
17 CDRH2	119	GITPFFGFADYAQ
17 CDRH3	120	DPNEFWGGYYSTHDFDS
CH	121	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSRVVTVPSSSLGTQT YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGG PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE MTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGK
MBMM2 17-S-17 hCk Full Light	122	EIVLTQSPDFQSVTPKEKVTITCRASQDIGSELHWYQQKP DQPPKLLIKYASHSTSGVPSRFSGSGSGTDFTLTINGLEA EDAGTYYCHQTDSLPYTFGPGTKVDIKRTVAAPEIVLTQS PDFQSVTPKEKVTITCRASQDIGSELHWYQQKPDQPPKLL IKYASHSTSGVPSRFSGSGSGTDFTLTINGLEAEDAGTYY CHQTDSLPYTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKS GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK SFNRGEC
17-S-17 Variable Light	123	EIVLTQSPDFQSVTPKEKVTITCRASQDIGSELHWYQQKP DQPPKLLIKYASHSTSGVPSRFSGSGSGTDFTLTINGLEA EDAGTYYCHQTDSLPYTFGPGTKVDIKRTVAAPEIVLTQS PDFQSVTPKEKVTITCRASQDIGSELHWYQQKPDQPPKLL IKYASHSTSGVPSRFSGSGSGTDFTLTINGLEAEDAGTYY CHQTDSLPYTFGPGTKVDIKR
17 CDRL1	124	RASQDIGSELH
17 CDRL2	125	YASHSTS
17 CDRL3	126	HQTDSLPYT
Linker	127	TVAAP
17 CDRL1	128	RASQDIGSELH
17 CDRL2	129	YASHSTS
17 CDRL3	130	HQTDSLPY
CL	131	TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQW KVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEK HKVYACEVTHQGLSSPVTKSFNRGEC

MBMM2 D2E7 hole Fc Full Heavy	132	EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQA PGKGLEWVSAITWNSGHIDYADSVEGRFTISRDNAKNSLY LQMNSLRAEDTAVYYCAKVSYLSTASSLDYWGQGTLVTVS SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSRVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAG GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRE EMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPGK
D2E7 Variable Heavy	133	EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQA PGKGLEWVSAITWNSGHIDYADSVEGRFTISRDNAKNSLY LQMNSLRAEDTAVYYCAKVSYLSTASSLDYWGQGTLVTVS S
D2E7 CDRH1	134	GFTFDDYAMH
D2E7 CDRH2	135	AITWNSGHIDYADSVEG
D2E7 CDRH3	136	VSYLSTASSLDY
CH	137	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSRVVTVPSSSLGTQT YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGG PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE MTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGK
MBMM2 D2E7 hCk Full Light	138	DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLAWYQQKP GKAPKLLIYAASTLQSGVPSRFSGSGSGTDFTLTISSLQP EDVATYYCQRYNRAPYTFGQGTKVEIKRTVAAPSVFIFPP SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ ESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQG LSSPVTKSFNRGEC
D2E7 Variable Light	139	DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLAWYQQKP GKAPKLLIYAASTLQSGVPSRFSGSGSGTDFTLTISSLQP EDVATYYCQRYNRAPYTFGQGTKVEIKR
D2E7 CDRL1	140	RASQGIRNYLA
D2E7 CDRL2	141	AASTLQS
D2E7 CDRL3	142	QRYNRAPYT
CL	143	TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQW KVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEK HKVYACEVTHQGLSSPVTKSFNRGEC

Table 6: Amino Acid Sequences of MBMM1 (PR-1621615) Anti-TNF/IL-17 Bispecific

Name	SEQ ID NO	Amino Acid Sequence 12345678901234567890123456789012345678901234567890
MBMM1 D2E7-L-D2E7 hole Fc Full Heavy	144	EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQA PGKGLEWVSAITWNSGHIDYADSVEGRFTISRDNAKNSLY LQMNSLRAEDTAVYYCAKVSYLSTASSLDYWGQGTLVTVS SASTKGPSVFPLAPEVQLVESGGGLVQPGRSLRLSCAASG FTFDDYAMHWVRQAPGKGLEWVSAITWNSGHIDYADSVEG RFTISRDNAKNSLYLQMNSLRAEDTAVYYCAKVSYLSTAS SLDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAAL GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL SRVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDK THTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPGK
D2E7-L-D2E7 hole Fc Variable Heavy	145	EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQA PGKGLEWVSAITWNSGHIDYADSVEGRFTISRDNAKNSLY LQMNSLRAEDTAVYYCAKVSYLSTASSLDYWGQGTLVTVS SASTKGPSVFPLAPEVQLVESGGGLVQPGRSLRLSCAASG FTFDDYAMHWVRQAPGKGLEWVSAITWNSGHIDYADSVEG RFTISRDNAKNSLYLQMNSLRAEDTAVYYCAKVSYLSTAS SLDYWGQGTLVTVSS
D2E7 CDRH1	146	GFTFDDYAMH
D2E7 CDRH2	147	AITWNSGHIDYADSVEG
D2E7 CDRH3	148	VSYLSTASSLDY
Linker	149	ASTKGPSVFPLAP
D2E7 CDRH1	150	GFTFDDYAMH
D2E7 CDRH2	151	AITWNSGHIDYADSVEG
D2E7 CDRH3	152	VSYLSTASSLDY
CH	153	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSRVVTVPSSSLGTQT YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGG PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE MTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGK
MBMM1 D2E7-S-D2E7 hCk Full Light	154	DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLAWYQQKP GKAPKLLIYAASTLQSGVPSRFSGSGSGTDFTLTISSLQP EDVATYYCQRYNRAPYTFGQGTKVEIKRTVAAPDIQMTQS

		PSSLSASVGDRVTITCRASQGIRNYLAWYQQKPGKAPKLL IYAASTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYY CQRYNRAPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKS GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK SFNRGEC
D2E7-S-D2E7 hCk Variable Light	155	DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLAWYQQKP GKAPKLLIYAASTLQSGVPSRFSGSGSGTDFTLTISSLQP EDVATYYCQRYNRAPYTFGQGTKVEIKRTVAAPDIQMTQS PSSLSASVGDRVTITCRASQGIRNYLAWYQQKPGKAPKLL IYAASTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYY CQRYNRAPYTFGQGTKVEIKR
D2E7 CDRL1	156	RASQGIRNYLA
D2E7 CDRL2	157	AASTLQS
D2E7 CDRL3	158	QRYNRAPYT
Linker	159	TVAAP
D2E7 CDRL1	160	RASQGIRNYLA
D2E7 CDRL2	161	AASTLQS
D2E7 CDRL3	162	QRYNRAPY
CL	163	TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQW KVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEK HKVYACEVTHQGLSSPVTKSFNRGEC
MBMM1 17 Knob Fc Full Heavy	164	EVQLVQSGAEVKKPGSSVKVSCKASGGSFGGYGIGWVRQA PGQGLEWMGGITPFFGFADYAQKFQGRVTITADESTTTAY MELSGLTSDDTAVYYCARDPNEFWGGYYSTHDFDSWGQGT TVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFP EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSRVVTVPSS SLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPA PEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPGK
17 Variable Heavy	165	EVQLVQSGAEVKKPGSSVKVSCKASGGSFGGYGIGWVRQA PGQGLEWMGGITPFFGFADYAQKFQGRVTITADESTTTAY MELSGLTSDDTAVYYCARDPNEFWGGYYSTHDFDSWGQGT TVTVSS
17 CDRH1	166	GGSFGGYGIG
17 CDRH2	167	GITPFFGFADYAQ
17 CDRH3	168	DPNEFWGGYYSTHDFDS
CH	169	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSRVVTVPSSSLGTQT

		YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGG PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE MTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGK
MBMM1 17 hCk Full Light	170	EIVLTQSPDFQSVTPKEKVTITCRASQDIGSELHWYQQKP DQPPKLLIKYASHSTSGVPSRFSGSGSGTDFTLTINGLEA EDAGTYYCHQTDSLPYTFGPGTKVDIKRTVAAPSVFIFPP SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ ESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQG LSSPVTKSFNRGEC
17 Variable Light	171	EIVLTQSPDFQSVTPKEKVTITCRASQDIGSELHWYQQKP DQPPKLLIKYASHSTSGVPSRFSGSGSGTDFTLTINGLEA EDAGTYYCHQTDSLPYTFGPGTKVDIKR
17 CDRL1	172	RASQDIGSELH
17 CDRL2	173	YASHSTS
17 CDRL3	174	HQTDSLPYT
CL	175	TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQW KVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEK HKVYACEVTHQGLSSPVTKSFNRGEC

Table 7: Amino Acid Sequences Anti-TNF Tetravalent Constructs

Clone Name	**Structure**	**SEQ ID NOs.**
PR-1580725	m-TV-D2E7 (GS)	133, 139, 176
PR-1603912	m-TV-D2E7 (GS)	133, 139, 176
PR-1580724	m-TV-D2E7 (LS)	133, 139, 149, 154
Name	**Sequence**	**SEQ ID**
GS linker	GGGGSGGGGS	176
Fc knob	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLM ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSREEMTKNQVSLWCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS PGK	177

Table 8: Amino Acid Sequences Anti-TNF Bivalent Constructs

Clone Name	**Structure**	**SEQ ID NOs.**
PR-1603136	mB-D2E7-Intact Fc	145, 155, 177

Table 9: Amino Acid Sequences Ambromab Constructs

Clone Name	Structure/Sequence	SEQ ID NOs.
PR-1603912	D2E7-GS10-IL-17 (QL, 234/235)	
PR-1603912 VH	EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMH WVRQAPGKGLEWVSAITWNSGHIDYADSVEGRFTIS RDNAKNSLYLQMNSLRAEDTAVYYCAKVSYLSTAS SLDYWGQGTLVTVSSGGGGSGGGGSEVQLVQSGAE VKKPGSSVKVSCKASGGSFGGYGIGWVRQAPGQGL EWMGGITPFFGFADYAQKFQGRVTITADESTTTAYM ELSGLTSDDTAVYYCARDPNEFWGGYYSTHDFDSW GQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP KSCVECPPCPAPEAAGGPSVFLFPPKPKDQLMISRTP EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVLHEALH NHYTQKSLSLSPGK	178
PR-1603912 VL	DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLAWY QQKPGKAPKLLIYAASTLQSGVPSRFSGSGSGTDFTL TISSLQPEDVATYYCQRYNRAPYTFGQGTKVEIKRG GSGGGGSGEIVLTQSPDFQSVTPKEKVTITCRASQDI GSELHWYQQKPDQPPKLLIKYASHSTSGVPSRFSGS GSGTDFTLTINGLEAEDAGTYYCHQTDSLPYTFGPG TKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNN FYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF NRGECVECPPCPAPEAAGGPSVFLFPPKPKDQLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKN	179

	QVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLVSKLTVDKSRWQQGNVFSCSVLHEALH NHYTQKSLSLSPGK	
PR-1603915	D2E7SS22-GS10-IL-17 (234/235)	
PR-1603915 VH	EVQLVESGGGLVQPGRSLRLSCAASGFTFDHYAMH WVRQAPGKGLEWVSAITWNSGHIDYADSVEGRFTIS RDNAKNSLYLQMNSLRAEDTAVYYCAKVSYLSTAS SLDYWGQGTLVTVSSGGGGSGGGGSEVQLVQSGAE VKKPGSSVKVSCKASGGSFGGYGIGWVRQAPGQGL EWMGGITPFFGFADYAQKFQGRVTITADESTTTAYM ELSGLTSDDTAVYYCARDPNEFWGGYYSTHDFDSW GQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVV ECPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL WCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDS DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK	180
PR-1603915 VL	DIQMTQSPSSLSASVGDRVTITCRASHSIRNYLSWYQ QKPGKAPKLLIYAASTLQSGVPSRFSGSGSGTDFTLT ISSLQPEDVATYYCQRYNRAPYTFGQGTKVEIKRGG SGGGGSGEIVLTQSPDFQSVTPKEKVTITCRASQDIG SELHWYQQKPDQPPKLLIKYASHSTSGVPSRFSGSGS GTDFTLTINGLEAEDAGTYYCHQTDSLPYTFGPGTK VDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFY PREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GECVECPPCPAPEAAGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN	181

	KALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGKGRSRPARPDPPTSTSG	

Example 2: Pharmacokinetic Methods and Materials

Pharmacokinetic Study

Male CD-1 mice (n=5 per group) received a single, 5 mg/kg intravenous dose of PR-study binding protein. Whole blood samples were collected over a period of 21 days from the tail vein, immediately diluted in assay buffer (1% MSD Blocker A in Tris-buffered saline with 0.02% Tween-20) and stored frozen at -80 until analysis.

Bioanalysis of PK Samples

Binding protein concentrations were measured by an electrochemiluminescent (MSD) method using biotinylated IL-17 capture (PR-1264676) and sulfo tagged (goat) anti-human IgG (MSD Cat# R32AJ-1) for detection. The samples were analyzed at a 1% final matrix concentration. MSD standard curve fitting and data evaluation was performed using XLfit4 software (Version 4.2.1 Build 16). A calibration curve was plotted from MSD luminescence units versus theoretical standard concentrations. A four-parameter logistic model was used for curve fitting. The regression equation for the calibration curve was then used to back calculate the measured concentrations. The lower limit of quantitation (LLOQ) was 0.02 µg.mL. The linear range was 0.02-15 µg/mL. Values that were below the quantitation limit were omitted from calculation. Using the rationale that biologics do not generally partition into circulating blood cells, whole blood concentrations were multiplied by two to estimate serum concentrations. Pharmacokinetic parameters were calculated with Non-compartmental analysis using WinNonlin Professional (version 5.0.1, Pharsight, Mountain View, California, USA).

Table 10 and Figure 3A illustrate the results of the MBMM2 study over 21 days.

Table 10: Serum Concentration (µg/mL) of MBMM2 After 5 mg/kg IV Dose in CD-1 Mice

Time (hr)	Animal 1*	Animal 2	Animal 3*	Animal 4*	Animal 5	Average	SD
1	147.80	132.59	117.57	126.83	136.61	134.60	2.84
24	97.77	71.57	71.35	59.26	87.79	79.68	11.47
96	65.92	52.00	66.52	59.06	48.89	50.44	2.2
168	56.18	55.75	60.83	44.46	71.75	63.75	11.31
240	55.98	40.69	40.89	35.32	51.42	46.06	7.58
336	42.65	28.96	20.90	16.73	42.15	35,56	9.33
504	BQL	22.27	BQL	BQL	32.41	27.34	7.17

*Eliminated from $t_{1/2}$ and AUC_{0-t} calculations due to probable ADA.
BQL: below quantifiable levels
SD: standard deviation

Table 11 shows the serum concentrations of MBMM2.

Table 11: Serum Concentration (µg/mL) of MBMM2 After 5 mg/kg IV Dose in CD-1 Mice

Mouse #	$T_{1/2}$ (hr)	V_{ss} (L/kg)	AUC (µg·hr/mL)	AUC_{0-t} (µg·hr/mL)	CL_P (L/hr/kg)	MRT (hr)
1	n.f.	n.f.	n.f.	22030	n.f.	n.f.
2	315	0.0675	32040	21930	0.0001561	432
3	n.f.	n.f.	n.f.	18470	n.f.	n.f.
4	n.f.	n.f.	n.f.	15630	n.f.	n.f.
5	401	0.0614	45930	27170	0.0001089	564
Mean	353*	0.0644	38990	21050	0.0001325	498
SEM		0.0030	6949	1940	0.00002361	65.9

$T_{1/2}$: halflife
V_{ss}: volume of distribution at steady state [(dose/AUC)(MRT) = ((µg/kg)/(µg*h/l))/h = l/kg]
AUC: area under curve
AUC_{0-t}: area under the plasma concentration-time curve from time zero to the last measurable concentration
CL_P: clearance from plasma
MRT: mean residence time; represents the average time a molecule stays in the body
n.f.: no fit
*harmonic mean

Table 12 and Figure 3B illustrate the results of the MBMM1 study over 21 days.

Table 12: Serum Concentration (µg/mL) of MBMM1 After 5 mg/kg IV Dose in CD-1 Mice

Time (hr)	Animal 1*	Animal 2*	Animal 3**	Animal 4*	Animal 5*	Average	SD
1	144.04	146.13	125.71	101.11	131.67	129.73	18.12
24	60.01	50.31	33.29	47.26	65.44	51.26	12.43
96	52.80	47.72	NS	38.65	56.26	48.86	7.66
168	41.16	2.45	NS	35.02	37.07	28.93	17.83
240	2.43	BQL	NS	3.80	BQL	3.11	0.97
336	BQL	BQL	NS	BQL	BQL	N/A	N/A
504	BQL	BQL	NS	BQL	BQL	N/A	N/A

*Eliminated from PK calculations due to probable ADA.
**Not enough samples for calculations.
NS: no sample
N/A: not applicable
BQL: below quantifiable levels

Table 13 shows the serum concentration of MBMM1.

Table 13: Serum Concentration (µg/mL) of MBMM1 After 5 mg/kg IV Dose in CD-1 Mice

Mouse #	AUC_{0-t} (µg·hr/mL)
1	11510
2	7744
3	1958
4	8951
5	10140
Mean	8060*
SEM	1648

*harmonic mean
Four animals displayed probable ADA. Animal #3 died prior to the 96hr sampling.

Tables 10-13 and Figures 3A and 3B demonstrate that the trivalent binding molecules that were bivalent for IL-17 and monovalent for TNF had a significantly longer half-life than the trivalent molecules that were bivalent for TNF and monovalent for IL-17. The averaged data are presented in Figure 3C and Table 14.

Table 14: PK Parameters of Anti-TNFα/IL-17 MBMM Molecules After 5 mg/kg IV Dosing in CD-1 Mice

PR#	ID	$T_{1/2}$ (day)	CL (mL/h/kg)	V_{ss} (mL/kg)	Probable ADA (N=5)
PR-1621611	MBMM2	14.7	0.13	64	3
PR-1621615	MBMM1	NC	NC	NC	5

*One animal in PR-1621615 dose group died before 96h; other 4 developed probable ADA
ADA: antidrug antibodies
NC: not calculable

Anti-TNF/IL-17 molecule PR-1621611 (MBMM2) displayed a long half-life (14.7 days), low clearance (0.13 mL/h/kg), and small volume of distribution (64 mL/kg). PK calculations were based on 2 out of 5 mice. Animals in the PR-1621615 (MBMM1) group displayed short half-life and probable ADA, interfering in PK calculations.

The MBMM molecules were designed to test the impacts of binding geometries and molecular valency on the effects of ADA as seen in mouse PK models with regard to anti-TNF biologics. TNFα antigen is a trimer, consisting of 3 potential binding sites, which poses interesting dynamics when it is targeted and bound by a bivalent antibody. In the presence of a high concentration of TNFα, such as is seen in inflammation, a bivalent therapeutic can and will cross link TNFα antigen, and if in a high enough concentration, form a lattice of mAB-antigen complex that is easily recognizable by immune cells. It is postulated that this complex can contribute to and exacerbate the immunogenicity of a biologic.

The molecules described herein contain either a bivalent anti-TNFα in-tandem paired with a monovalent anti-IL17 (MBMM1, PR-1621615), or a bivalent in-tandem anti-IL17 paired with a monovalent D2E7 (MBMM2, PR-1621611). The PK results of these molecules show a striking difference in molecular half-life (Figure 3C), whereby the MBMM1

molecules are exhibiting a significant reduction in half-life and a probable ADA response by mice; MBMM2 exhibits a significant increase in PK and no probable ADA response.

Example 3: Pharmacokinetic Parameters of Monospecific Tetravalent and Monospecific Bivalent Anti-TNFα Molecules After 5 mg/kg IV Dosing in CD-1 Mice

Three tetravalent anti-TNF molecules named TV-GS (PR-1580725 or PR-1603912) or TV-LS (PR-1580724) (Figure 2a) and one bivalent anti-TNF molecule named JMB-GS (PR-1603136) (Figure 2b) were prepared by standard methods known in the art and their pharmacokinetic parameters tested.

Table 15 and Figure 4 illustrate the results of the PR-1580725 study over 21 days

Table 15: Serum Concentration (µg/mL) of TV-GS Molecule (PR-1580725) After 5 mg/kg IV Dose in CD-1 Mice

Time (hr)	Animal 1	Animal 2	Animal 3	Animal 4	Animal 5	Average	SD
1	139.1	120.3	141.9	142.7	108.2	130.4	15.5
24	70.4	65.3	78.7	66.2	73.0	70.7	5.4
96	47.2	48.0	44.1	45.8	51.8	47.4	2.9
168	BQL	BQL	1.7	0.4	BQL	1.0	N/A
240	BQL	BQL	BQL	BQL	BQL	BQL	N/A
336	BQL	BQL	BQL	BQL	BQL	BQL	N/A
504	BQL	BQL	BQL	BQL	BQL	BQL	N/A

BQL: below quantifiable levels

Table 16 shows the serum concentrations of PR-1580725.

Table 16: Serum Concentration (µg/mL) of TV-GS Molecule (PR-1580725) After 5 mg/kg IV Dose in CD-1 Mice

Mouse #	$T_{1/2}$ (hr)	C_{max} (µg/mL)	V_{ss} (L/kg)	AUC (µg·hr/mL)	AUC_{0-t} (µg·hr/mL)	CL_P (L/hr/kg)	MRT (hr)
1	125	139	0.0555	15300	6780	0.00033	169.37384
2	161	120	0.0639	17500	6340	0.00029	223.52454

3	26.1	142	0.027	8820	8750	0.00057	47.65766
4	19.1	143	0.0292	8250	8240	0.00061	48.20739
5	145	108	0.0581	17500	6690	0.00029	203.58212
Mean	44.7*	130	0.0467	13500	7360	0.00041	138.46912
SEM		6.92	0.00773	2060	477	0.00007	37.96257

C_{max}: maximum observed plasma concentration of a drug after administration
*harmonic mean

Table 17 and Figure 5 illustrate the results of the PR-1603912 study over 21 days.

Table 17: Serum Concentration (µg/mL) of Ambromab Molecule (PR-1603912) After 5 mg/kg IV Dose in CD-1 Mice

Time (hr)	Animal 1	Animal 2	Animal 3	Animal 4	Animal 5	Average	SD
1	135.8	128.7	132.2	143.8	149.3	137.9	8.5
24	56.8	54.6	60.4	70.1	86.8	65.7	13.2
96	47.3	53.9	41.2	50.4	60.7	50.7	7.3
168	36.4	50.4	25.1	48.5	59.1	48.7	11.4
240	BQL	43.8	BQL	4.8	4.0	17.5	17.5
336	BQL	26.9	BQL	BQL	BQL	26.9	N/A
504	BQL	20.3	BQL	BQL	BQL	20.3	N/A

BQL: below quantifiable levels

Table 18 shows the serum concentrations of PR-1603912.

Table 18: Serum Concentration (µg/mL) of Ambromab Molecule (PR-1603912) After 5 mg/kg IV Dose in CD-1 Mice

Mouse #	$T_{1/2}$ (hr)	C_{max} (µg/mL)	V_{ss} (L/kg)	AUC (µg·hr/mL)	AUC_{0-t} (µg·hr/mL)	CL_P (L/hr/kg)	MRT (hr)
1	191	136	0.069	19100	9120	0.00026	263.91745
3	114	132	0.0599	12500	8400	0.00040	150.12775
4	42.6	144	0.0355	12700	12400	0.00039	90.21921
5	36.6	149	0.0297	15000	14800	0.00033	88.91838
Mean	61.7*	140	0.0485	14800	11200	0.00035	148.29570

SEM		3.86	0.00946	1530	1480	0.00003	41.09977

*harmonic mean

Table 19 and Figure 6 illustrate the results of the PR-1580724 study over 21 days.

Table 19: Serum Concentration (µg/mL) of TV-LS Molecule (PR-1580724) After 5 mg/kg IV Dose in CD-1 Mice

Time (hr)	Animal 1	Animal 2	Animal 3	Animal 4	Animal 5	Average	SD
1	135.4	187.3	174.6	156.6	168.6	164.5	19.7
24	72.3	86.7	78.3	81.5	79.8	79.7	5.2
96	56.5	62.3	50.1	40.0	48.3	51.5	8.5
168	28.1	1.4	BQL	BQL	4.5	11.3	14.6
240	1.8	BQL	BQL	BQL	BQL	1.8	N/A
336	BQL	BQL	BQL	BQL	BQL	BQL	N/A
504	BQL	BQL	BQL	BQL	BQL	BQL	N/A

BQL: below quantifiable levels

Table 20 shows the serum concentrations of PR-1580724.

Table 20: Serum Concentration (µg/mL) of TV-LS (PR-1580724) Molecule After 5 mg/kg IV Dose in CD-1 Mice

Mouse #	$T_{1/2}$ (hr)	C_{max} (µg/mL)	V_{ss} (L/kg)	AUC (µg·hr/mL)	AUC_{0-t} (µg·hr/mL)	CL_P (L/hr/kg)	MRT (hr)
1	29.1	135	0.033	11400	11300	0.00044	74.98274
2	24.0	187	0.0225	11000	11000	0.00045	49.76372
3	112	175	0.047	15800	7710	0.00032	148.47389
4	70.3	157	0.0403	11300	7270	0.00044	91.29062
5	34.7	169	0.0264	9760	9540	0.00051	51.53255
Mean	39.1*	164	0.0338	11900	9360	0.00043	83.20870
SEM		8.79	0.00447	1030	822	0.00003	18.04782

*harmonic mean

Table 21 and Figure 7 illustrate the results of the PR-1603136 study over 21 days.

Table 21: Serum Concentration (µg/mL) of JMB-GS Tandem (PR-1603136) Molecule After 5 mg/kg IV Dose in CD-1 Mice

Time (hr)	Animal 1	Animal 2	Animal 3	Animal 4	Animal 5	Average	SD
1	82.7	82.5	80.3	75.5	78.2	79.8	3.0
24	30.0	25.1	29.0	30.3	31.0	29.1	2.3
96	19.4	16.8	17.7	20.1	23.1	19.4	2.4
168	2.8	13.3	0.2	BQL	4.0	5.1	5.7
240	BQL	BQL	BQL	BQL	BQL	BQL	N/A
336	BQL	BQL	BQL	BQL	BQL	BQL	N/A
504	BQL	BQL	BQL	BQL	BQL	BQL	N/A

BQL: below quantifiable levels

Table 22 shows the serum concentrations of PR-1603136.

Table 22: Serum Concentration (µg/mL) of JMB-GS Tandem (PR-1603136) Molecule After 5 mg/kg IV Dose in CD-1 Mice

Mouse #	$T_{1/2}$ (hr)	C_{max} (µg/mL)	V_{ss} (L/kg)	AUC (µg·hr/mL)	AUC_{0-t} (µg·hr/mL)	CL_P (L/hr/kg)	MRT (hr)
1	42.2	82.7	0.0661	4130	3960	0.00121	54.61869
2	157	82.5	0.148	6910	3910	0.00072	204.02878
3	19.9	80.3	0.0588	3670	3670	0.00136	43.18717
4	122	75.5	0.121	6630	3110	0.00075	160.08524
5	48.9	78.2	0.0698	4540	4260	0.00110	63.36590
Mean	45.8*	79.8	0.0926	5180	3780	0.00103	105.05715
SEM		1.36	0.0176	667	193	0.00013	32.35241

*harmonic mean

Table 23: PK Parameters of Monospecific Tetravalent and Monospecific Bivalent Anti-TNFα Molecules After 5 mg/kg IV Dosing in CD-1 Mice

PR#	ID	$T_{1/2}$ (day)	CL (mL/h/kg)	V_{ss} (mL/kg)	Probable ADA (N=5)
PR-1603912	Ambromab	10.2	0.18	65	4

PR-1603915	Ambromab	4.3	0.34	59	5
PR-1580725	TV-GS	1.9	0.41	47	5
PR-1580724	TV-LS	1.6	0.43	34	5
PR-1603136	JMB-GS Tandem	1.9	1.03	93	5

*One animal in PR-1621615 dose group died before 96h; other 4 developed probable ADA

Anti-TNF monovalent molecule PR-1603912 had 1 of 5 animals with measurable antibody levels out to 21 days. This animal displayed a long half-life and low CL (10.2 days and 0.18 mL/h/kg). All other animals within this group and all other dose groups displayed probable ADA at 168 hours or 240 hours. The short half-lives for PR-1603136, PR-1580725, and PR-1580724 prior to the onset of ADA could be due to a number or mechanisms including renal clearance and in vivo stability.

When comparing the MBMM2 PK results of Example 2 to the above PK results of in-tandem, bivalent, monospecific anti-TNFα molecule (PR-1603136) and tetravalent, anti-TNFα molecules (PR-1580725, PR-1580724), the contrasts in valency again reveal an improved PK for MBMM2 which features a monovalent anti-TNFα binder. Figure 8 features PK results from a monovalent anti-TNFα molecule (PR-1603912) versus tetravalent (PR-1580725, PR-1580724) and in-tandem, bivalent anti-TNFα (PR–1603136) molecules; and the same extended PK is seen with the monovalent format when compared to the bivalent, in-tandem and tetravalent molecules, which again exhibit low half-lives and probable ADA responses in mice.

The molecules tested herein provide evidence of an improved half-life and mitigated ADA response in mice, while remaining bivalent and exhibiting full potency in its binding to a second antigen. MBMM2 is advantageous in that it can modify potential ADA responses from an anti-TNFα targeting molecule while retaining full affinity and activity for an additional target and allow for the production of a disease-specific, fit-for-purpose bispecific molecule.

Equivalents

The disclosure may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting of the disclosure. Scope of the

disclosure is thus indicated by the appended claims rather than by the foregoing description, and all changes that come within the meaning and range of equivalency of the claims are therefore intended to be embraced herein.

We claim:

1. A binding protein comprising first, second, third and fourth polypeptide chains,
wherein said first polypeptide chain comprises VD1-(X1)n-VD2-C-(X2)n,
wherein VD1 is a first heavy chain variable domain,
VD2 is a second heavy chain variable domain,
C is a CH1 domain,
X1 is a linker with the proviso that it is not a constant domain,
n is 0 or 1, and
X2 is an Fc region;
wherein said second polypeptide chain comprises VD1-(X1)n-VD2-C,
wherein VD1 is a first light chain variable domain,
VD2 is a second light chain variable domain,
C is a CL domain,
X1 is a linker with the proviso that it is not a constant domain,
and n is 0 or 1;
wherein the VD1 of the heavy chain and the VD1 of the light chain form a functional binding site and wherein the VD2 of the heavy chain and the VD2 of the light chain form a functional binding site; and
wherein the VD1 and VD2 functional binding sites bind to the same non-TNFα antigen; and
wherein said third polypeptide chain comprises VD3-C-(X1)n,
wherein VD3 is a third heavy chain variable domain,
C is a CH1 domain,
X1 is an Fc region, and
n is 0 or 1;
wherein said fourth polypeptide chain comprises VD3-C,
wherein VD3 is a first light chain variable domain; and
C is a CL domain;
wherein the VD3 of the heavy chain and the VD3 of the light chain form a functional binding site for TNFα.

2. The binding protein of claim 1, wherein the TNFα is human TNFα.

3. The binding protein of claim 2, wherein the VD3 heavy chain variable domain and the VD3 light chain variable domain are a heavy chain variable domain and a light chain variable domain from infliximab, adalimumab, certolizumab pegol, or golimumab.

4. The binding protein of any one of claims 1 to 3, wherein the Fc region of the first and third polypeptide chains each comprises a mutation, wherein said mutations on the two Fc regions enhance heterodimerization of the first and third polypeptide chains.

5. The binding protein of claim 4, wherein the Fc region of one of the first polypeptide and the second polypeptide comprises the sequence of SEQ ID NO: 121 and the Fc region of the other of the first polypeptide and the second polypeptide comprises the sequence of SEQ ID NO: 137.

6. The binding protein of any one of claims 1 to 5, wherein monovalent binding of the binding protein to cell surface TNFα on antigen presenting cells provides a halflife greater than a molecule that is bivalent or tetravalent for TNFα.

7. The binding protein of any one of claims 1 to 6, wherein monovalent binding of the binding protein to cell surface TNFα on antigen presenting cells generates less anti-drug antibodies (ADA) than a molecule that is bivalent or tetravalent for TNFα.

8. The binding protein of any one of claims 1 to 7, wherein the non-TNFα antigen is a soluble ligand.

9. The binding protein of claim 8, wherein the non-TNFα antigen is IL-17.

10. The binding protein of claim 9, wherein the non-TNFα antigen is human IL-17.

11. The binding protein of any one of claims 1 to 10, wherein VD1 of the first polypeptide comprises CDR1, CDR2, and CDR3 sequences of SEQ ID NOs: 114-116, VD2 of the first polypeptide comprises the CDR1, CDR2, and CDR3 sequences of SEQ ID NOs: 118-120, VD1 of the second polypeptide comprises the CDR1, CDR2, and CDR3 sequences of SEQ ID NOs: 124-126, VD2 of the second polypeptide comprises the CDR1, CDR2, and CDR3 sequences of SEQ ID NOs: 128-130, VD3 of the third polypeptide comprises the CDR1, CDR2, and CDR3 sequences of SEQ ID NOs: 134-136, and VD3 of the fourth polypeptide comprises the CDR1, CDR2, and CDR3 sequences of SEQ ID NOs: 140-142.

12. The binding protein of any one of claims 1 to 11, wherein VD1-(X1)n-VD2 of the first polypeptide comprise the sequence of SEQ ID NO: 113, VD1-(X1)n-VD2 of the second polypeptide comprise the sequence of SEQ ID NO: 123, VD3 of the third polypeptide comprises the sequence of SEQ ID NO: 133, and VD3 of the fourth polypeptide comprises the sequence of SEQ ID NO: 139.

13. The binding protein of any one of claims 1 to 12, wherein the first polypeptide comprises the sequence of SEQ ID NO: 112, the second polypeptide comprises the sequence of SEQ ID NO: 122, the third polypeptide comprises the sequence of SEQ ID NO: 132 and the fourth polypeptide comprises the sequence of SEQ ID NO: 138.

14. A method of treating a TNF-associated disorder in a subject in need thereof, comprising administering to the subject an effective amount of the binding protein of any one of claims 1 to 13.

15. A nucleic acid encoding the binding protein of any one of claims 1 to 13.

16. A vector expressing the nucleic acid of claim 15.

17. A host cell comprising the vector of claim 16.

18. A method of producing a binding protein, comprising culturing a host cell of claim 17 in culture medium under conditions sufficient to produce the binding protein.

19. A protein produced according to the method of claim 18.

20. A pharmaceutical composition comprising the binding protein any one of claims 1 to 13, and a pharmaceutically acceptable carrier.

21. A binding protein comprising first, second, third and fourth polypeptide chains, wherein said first polypeptide chain comprises VD1-(X1)n-VD2-C-(X2)n,
wherein VD1 is a first heavy chain variable domain,
VD2 is a second heavy chain variable domain,
C is a CH1 domain,
X1 is a linker with the proviso that it is not a constant domain,
n is 0 or 1, and
X2 is an Fc region;

wherein said second polypeptide chain comprises VD1-(X1)n-VD2-C,
wherein VD1 is a first light chain variable domain,
VD2 is a second light chain variable domain,
C is a CL domain,
X1 is a linker with the proviso that it is not a constant domain,
and n is 0 or 1;
wherein the VD1 of the heavy chain and the VD1 of the light chain form a functional binding site and wherein the VD2 of the heavy chain and the VD2 of the light chain form a functional binding site; and
wherein the VD1 and VD2 functional binding sites bind TNFα; and
wherein said third polypeptide chain comprises VD3-C-(X1)n,
wherein VD3 is a third heavy chain variable domain,
C is a CH1 domain,
X1 is an Fc region, and
n is 0 or 1;
wherein said fourth polypeptide chain comprises VD3-C,
wherein VD3 is a first light chain variable domain; and
C is a CL domain;
wherein the VD3 of the heavy chain and the VD3 of the light chain form a functional binding site for a non-TNFα antigen.

22. The binding protein of claim 21, wherein the TNFα.is human TNFα.

23. The binding protein of claim 22, wherein the VD1 and VD2 heavy chain variable domains and light chain variable domains are heavy chain variable domains and light chain variable domains from infliximab, adalimumab, certolizumab pegol, or golimumab.

24. The binding protein of any one of claims 21 to 23, wherein the Fc region of the first and third polypeptide chains each comprises a mutation, wherein said mutations on the two Fc regions enhance heterodimerization of the first and third polypeptide chains.

25. The binding protein of claim 24, wherein the Fc region of one of the first polypeptide and the second polypeptide comprises the sequence of SEQ ID NO: 153 and the Fc region of the other of the first polypeptide and the second polypeptide comprises the sequence of SEQ ID NO: 169.

26. The binding protein of any one of claims 21 to 25, wherein the non-TNFα antigen is a soluble ligand.

27. The binding protein of claim 26, wherein the non-TNFα antigen is IL-17.

28. The binding protein of claim 27, wherein the non-TNFα antigen is human IL-17.

29. The binding protein of claim 21, wherein binding protein comprises one or more of the sequences in Table 6.

30. The binding protein of claim 21, wherein the first polypeptide comprises the sequence of SEQ ID NO: 144, the second polypeptide comprises the sequence of SEQ ID NO:154 the third polypeptide comprises the sequence of SEQ ID NO: 164 and the fourth polypeptide comprises the sequence of SEQ ID NO: 170.

31. A method of treating a TNF-associated disorder in a subject in need thereof, comprising administering to the subject an effective amount of the binding protein of any one of claims 21 to 30.

32. A nucleic acid encoding the binding protein of any one of claims 21 to 30.

33. A vector expressing the nucleic acid of claim 32.

34. A host cell comprising the vector of claim 33.

35. A method of producing a binding protein, comprising culturing a host cell of claim 34 in culture medium under conditions sufficient to produce the binding protein.

36. A protein produced according to the method of claim 35.

37. A pharmaceutical composition comprising the binding protein any one of claims 21 to 30, and a pharmaceutically acceptable carrier.

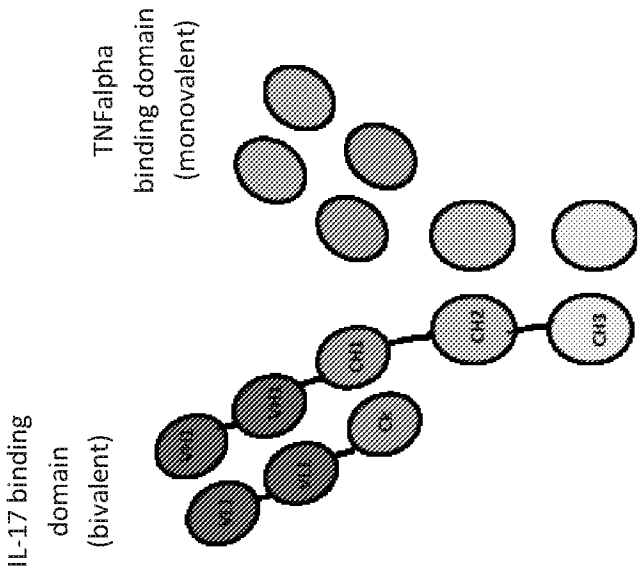
IL-17 binding domain (bivalent)
TNFalpha binding domain (monovalent)
CH2
CH3

Fig. 1A

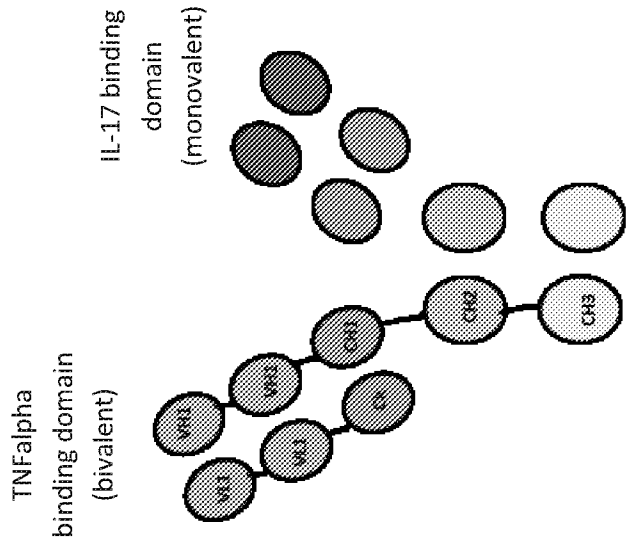
TNFalpha binding domain (bivalent)
IL-17 binding domain (monovalent)
CH2
CH3

Fig. 1B

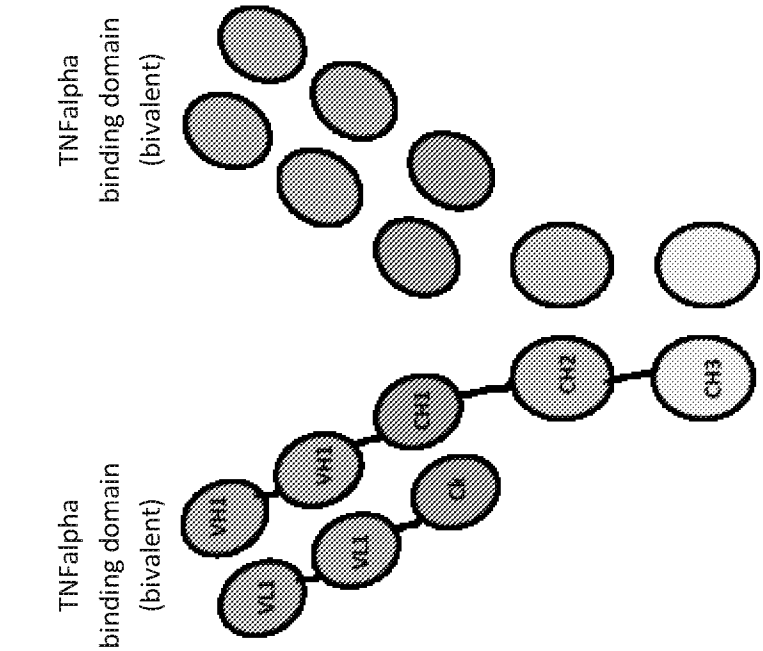

Fig. 2A

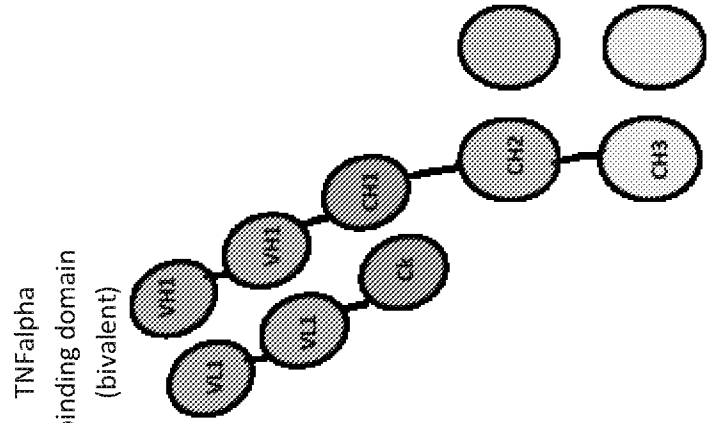

Fig. 2B

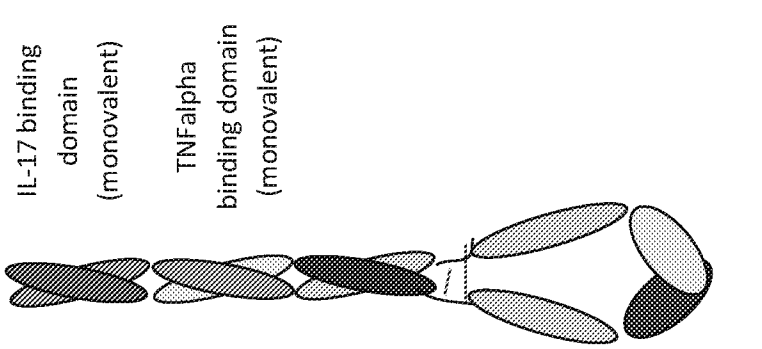

Fig. 2C

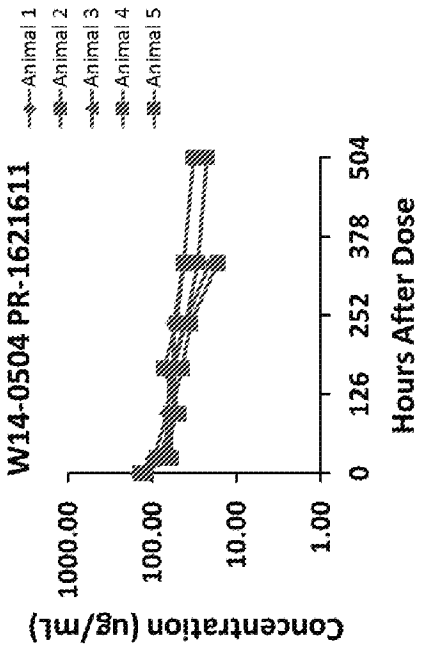

Fig. 3A

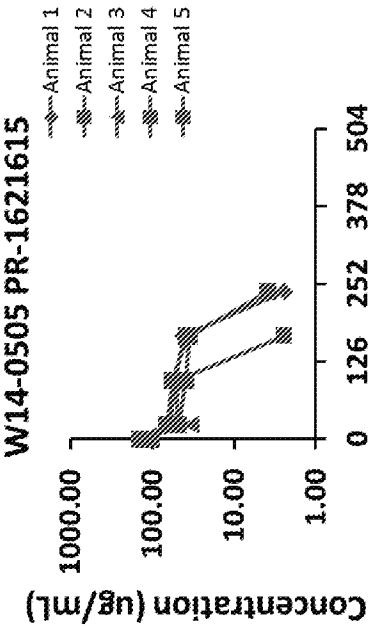

Fig. 3B

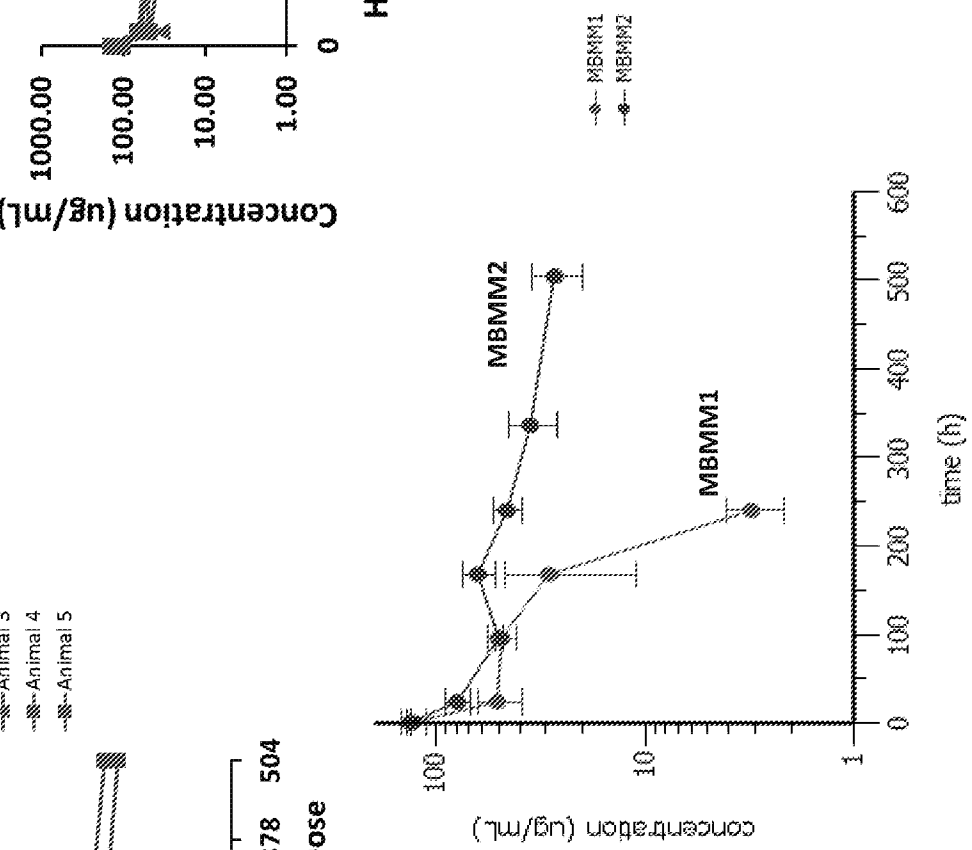

Fig. 3C

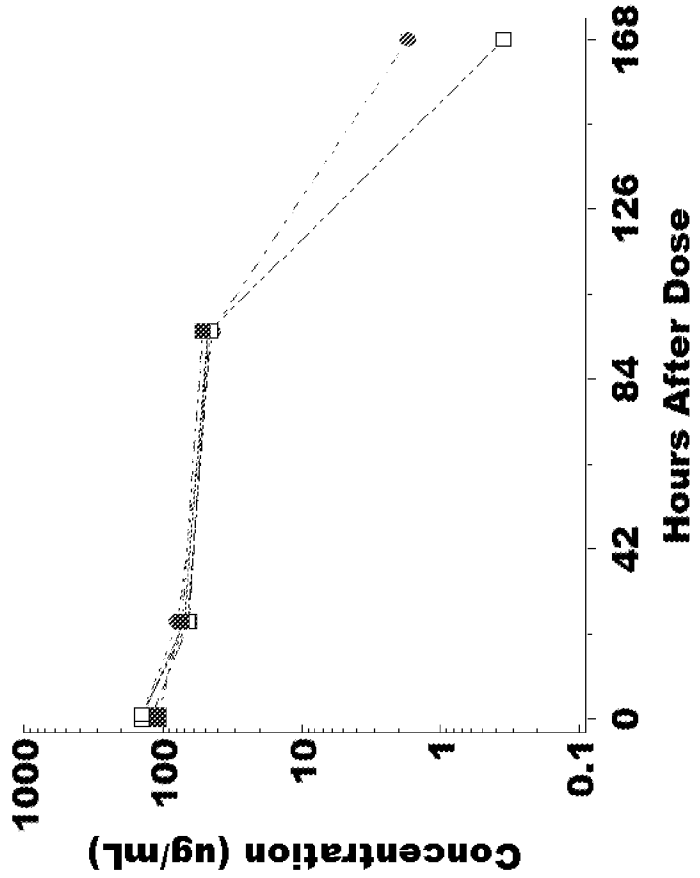
Concentration (ug/mL)
1000
100
10
1
0.1
0
42
84
126
168
Hours After Dose

Fig. 4

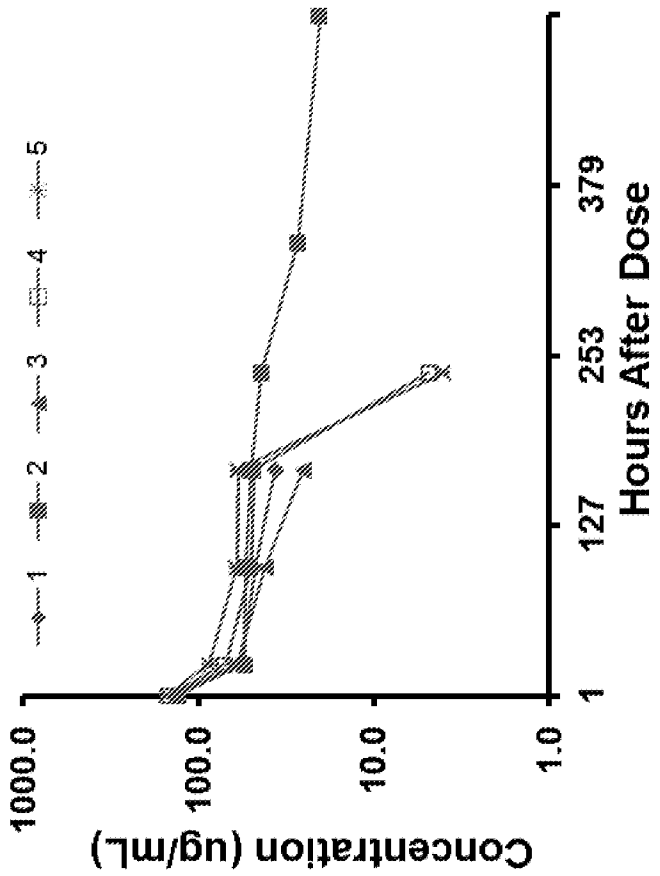
1
2
3
4
5
1000.0
100.0
10.0
1.0
Concentration (ug/mL)
1
127
253
379
Hours After Dose

Fig. 5

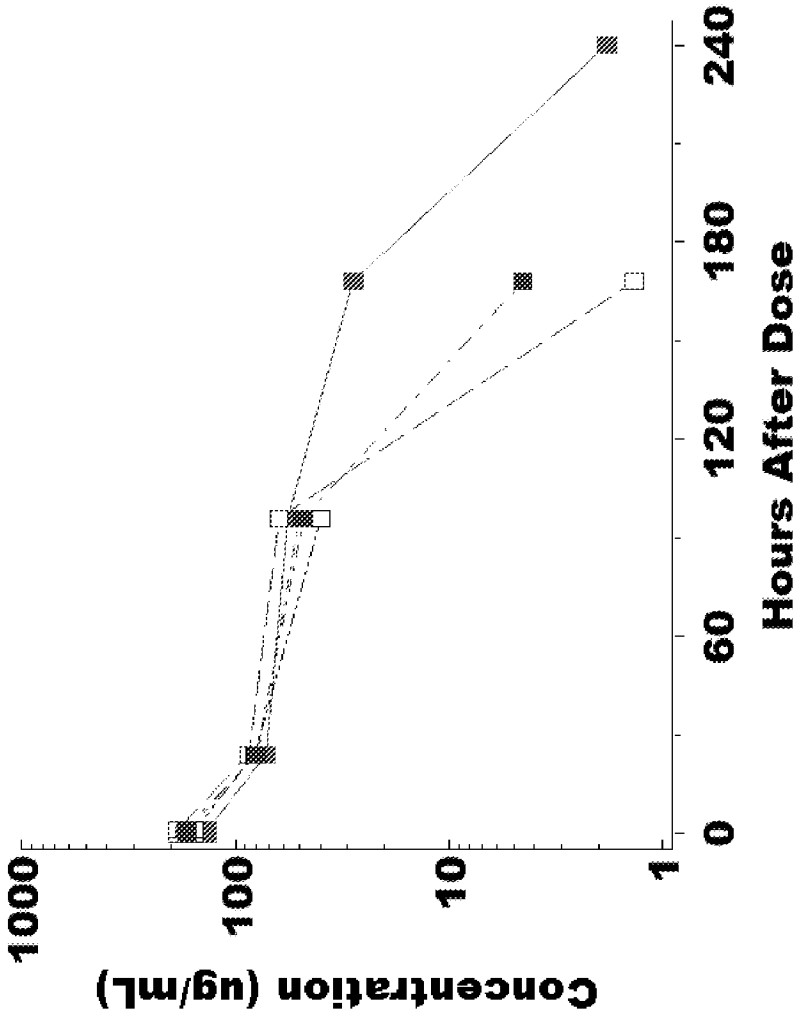
Concentration (ug/mL)
1000
100
10
1
0
60
120
180
240
Hours After Dose

Fig. 6

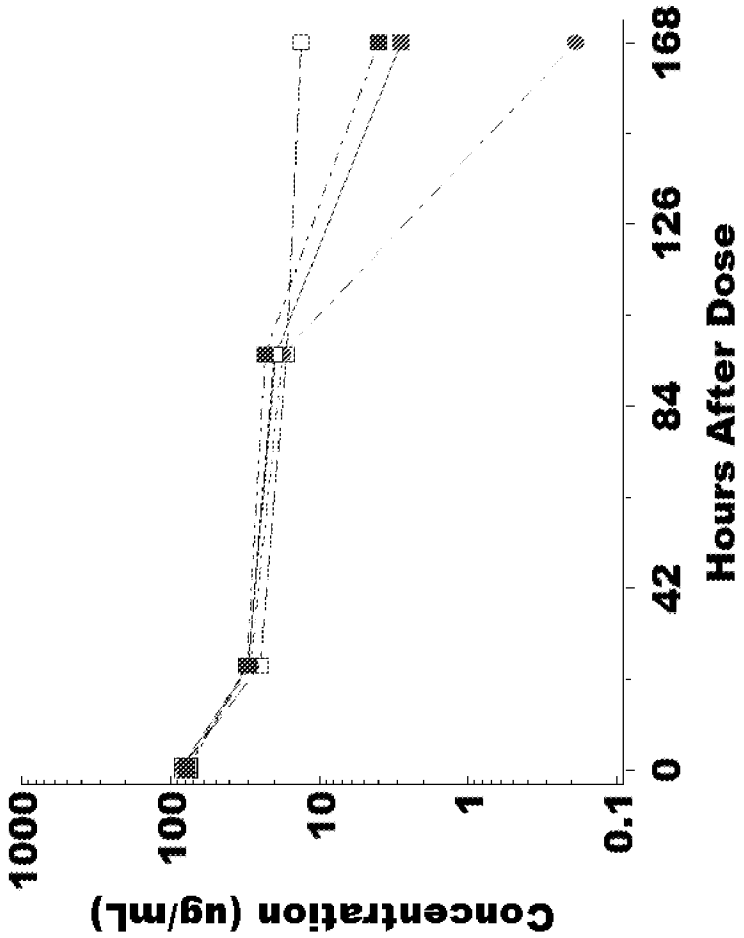
Concentration (ug/mL)
1000
100
10
1
0.1
0
42
84
126
168
Hours After Dose

Fig. 7

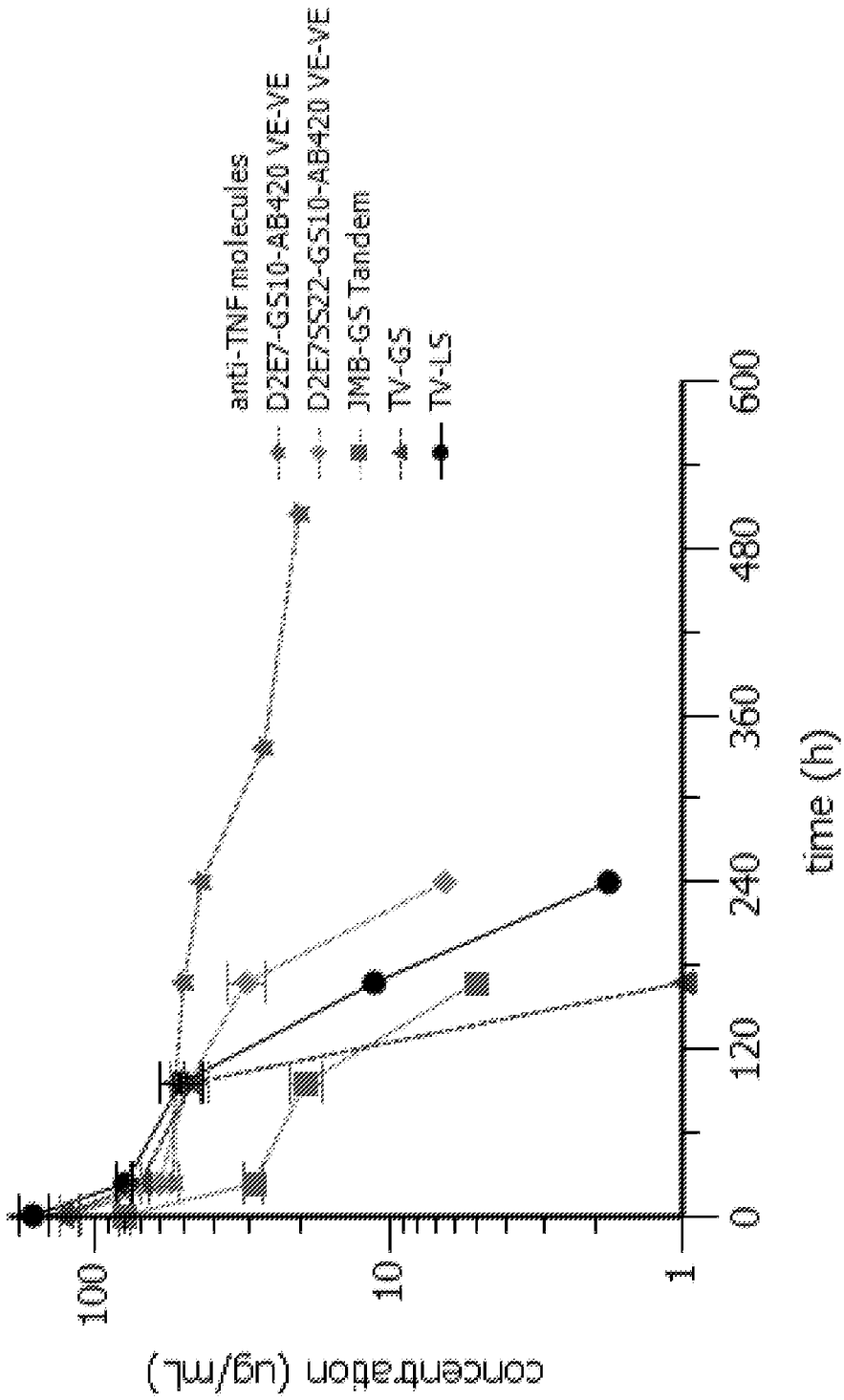
anti-TNF molecules
D2E7-GS10-AB420 VE-VE
D2E7SS22-GS10-AB420 VE-VE
JMB-GS Tandem
TV-GS
TV-LS
concentration (ug/mL)
100
10
1
0
120
240
360
480
600
time (h)

Fig. 8