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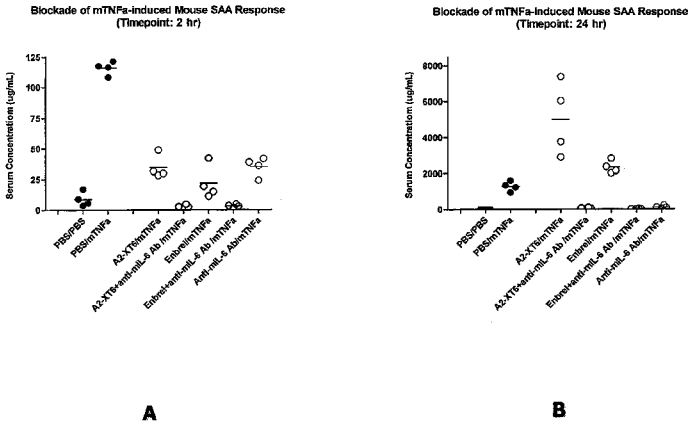
(54) **Title**: COMPOSITIONS COMPRISING TNF-ALPHA AND IL-6 ANTAGONISTS AND METHODS OF USE THEREOF

(57) **Abstract**: The present disclosure relates to compositions and methods for treating autoimmune diseases including rheumatoid arthritis. In particular, the present disclosure relates to compositions comprising IL-6 antagonists (e.g., anti-IL6 or anti-IL6R or anti-hyperIL6) and TNF-Alpha antagonists (e.g., (anti-TNF or etanercept) and methods of using same in the treatment of rheumatoid arthritis.

Figure 1



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COMPOSITIONS COMPRISING TNF-ALPHA AND IL-6 ANTAGONISTS AND METHODS OF USE THEREOF

CROSS-REFERENCE TO RELATED APPLICATION

This application claims the benefit under 35 U.S.C. § 119(e) of U.S. Provisional Patent Application No. 61/289,929, filed December 23, 2009, which provisional application is incorporated herein by reference in its entirety.

STATEMENT REGARDING SEQUENCE LISTING

The Sequence Listing associated with this application is provided in text format in lieu of a paper copy, and is hereby incorporated by reference into the specification. The name of the text file containing the Sequence Listing is 910180_423PC_SEQUENCE_LISTING.txt. The text file is 460 KB, was created on December 23, 2010, and is being submitted electronically via EFS-Web, concurrent with the filing of the specification.

TECHNICAL FIELD

The present disclosure relates generally to compositions and methods for treating autoimmune diseases including rheumatoid arthritis, systemic lupus erythematosus (SLE), psoriasis and inflammatory bowel disease (IBD). In particular, the present disclosure relates to compositions comprising IL-6 antagonists (*e.g.*, anti-IL6 or anti-IL6R or anti-hyperIL6) and TNF-α antagonists (*e.g.*, (anti-TNF or etanercept) and methods of using same in the treatment of rheumatoid arthritis.

BACKGROUND OF THE DISCLOSURE

Interleukin 6 ("IL6") is a pleiotropic cytokine that regulates host immune responses, inflammation, hematopoiesis, and oncogenesis. IL6 biology is mediated by a multicomponent molecular system with two distinct modes of signaling operative on overlapping but non-identical cell populations. These are referred to as cis-signaling (also known as “classical” signaling) and trans-signaling.

In cis-signaling, IL6 binds to cell surface IL6 receptor, the ligand binding part of IL6R that is referred to as IL6Rα or CD126 (previously called gp80). The cell-bound IL6|IL6Rα complex in turn binds to non-ligand binding but signal transducing membrane protein gp130 (also known as IL6ST, IL6Rβ, or CD130), which induces gp130 dimerization and initiation of signaling. Thus,

cis-signaling is restricted to the subset of cell types that express cell-surface IL6Rα, which is generally limited to, for example, mitogen-activated B cells, T cell subsets, peripheral monocytes, and certain tumors. The resultant ternary complex on the cell surface assembles into a very stable hexamer with a 2:2:2 ratio of IL6:IL6Rα:gp130 (Boulanger *et al.* (2003) Science 300:2101).

In trans-signaling, soluble IL6Rα ("sIL6Rα") complexes with IL6 and the resulting circulating sIL6xR complex can bind to and activate any gp130-expressing cell (but not cells also expressing IL6Rα, Taga *et al.* (1989) Cell 58:573). Many, perhaps all or nearly all, cells in the human body express gp130. Because gp130 is ubiquitous, trans-signaling can affect many cell types and thereby sometimes cause disease.

The membrane protein gp130 also exists in soluble form ("sgp130"), which can bind sIL6xR complex in circulation. But, the sIL6xR complex binds equally well to membrane and soluble gp130 (*see* Jones *et al.*, (2005) J. Interferon Cytokine Res. 25:241). Therefore, a molar excess of sgp130 can inhibit trans-signaling (by reducing the amount of available sIL6xR complex in circulation), which will not significantly affecting cis-signaling because the affinity of sgp130 is orders of magnitude less, as compared to cell surface gp130, for cell-bound IL6|IL6Rα complex (*see*, *e.g.*, Jostock *et al.* (2001) Eur. J. Biochem. 268:160). Thus, it has been suggested that spg130 may be useful in inhibiting IL6 activity (*see*, *e.g.*, Jostock *et al.* (2001) Eur. J. Biochem. 268:160). But, in addition to IL6, gp130 is a common signal-transducing protein for a family of gp130 cytokines. These include leukemia inhibitory factor (LIF), ciliary neurotrophic factor (CNTF), neuropoietin (NP), cardiotropin like cytokine (CLC), oncostatin M (OSM), IL-27, IL-31 and cardiotrophin-1 (CT-1). Hence, although sgp130 can inhibit trans-signaling, administering such a compound to patients may have some unintended adverse effects.

Increased production of IL6 has been implicated in various disease processes, including Alzheimer's disease, autoimmunity (*e.g.*, rheumatoid arthritis, SLE), inflammation, myocardial infarction, Paget's disease, osteoporosis, solid tumors (*e.g.*, colon cancer, RCC prostatic and bladder cancers), certain neurological cancers, B-cell malignancies, such as Castleman's disease, some lymphoma subtypes, CLL, and, in particular, multiple myeloma. In some instances, IL-6 is implicated in proliferation pathways because it acts with other factors, such as heparin-binding epithelial growth factor and hepatocyte growth factor.

Several IL6 and IL6Rα antibody antagonists are known. For example, for IL6, Way *et al.* (US Patent Application Publication No. 2007/0178098) disclose antibodies against IL6 to sterically block IL6 or sIL6xR complex from binding to gp130 (see also US Patent No. 7,291,721). For example, for IL6Rα, Kishimoto (US Patent No. 5,670,373) discloses antibodies against IL6Rα that inhibit IL6 activity.

Tumor Necrosis Factor Receptor (TNFR), also known as CD120 or cachectin, is a member of the tumor necrosis factor receptor superfamily and is the receptor for Tumor Necrosis Factor-α (TNF-α). There are two variants of this cytokine receptor, TNFR1 and TNFR2 (CD120a and CD120b receptors, respectively). TNFR1 has a molecular weight of about 55 KD and is therefore sometimes referred to as p55. TNFR2 has a molecular weight of about 75 KD and is therefore sometimes referred to as p75.

A majority of cell types and tissues appear to express both TNF receptors. Both exist on the cell surface as well as in soluble forms and both are active in signal transduction, although they can mediate distinct cellular responses. TNFR1 appears to be responsible for signaling most TNF responses. Among other activities, TNFR2 stimulates thymocyte proliferation, activates NF-κβ, and is an accessory to TNFR1 in the signaling of responses primarily mediated by TNFR1, such as cytotoxicity.

TNF antagonists, such as anti-TNF antibodies, can positively affect various inflammatory conditions. For example, infliximab is indicated in the United States for the treatment of rheumatoid arthritis, Crohn's disease, ankylosing spondylitis, psoriatic arthritis, plaque psoriasis, and ulcerative colitis. According to REMICADE® (infliximab) prescribing information, biological activities attributed to TNF include induction of pro-inflammatory cytokines such as interleukins (IL) 1 and 6, enhancement of leukocyte migration by increasing endothelial layer permeability and expression of adhesion molecules by endothelial cells and leukocytes, activation of neutrophil and eosinophil functional activity, induction of acute phase reactants and other liver proteins, as well as tissue degrading enzymes produced by synoviocytes and/or chondrocytes. Recently, perispinal delivery of the TNF-α inhibitor etanercept has been shown to reduce symptoms in patients with Alzheimer's disease (Tobinick and Gross (2008) BMC Neurol. 8:27-36; Griffin (2008) J. Neuroinflammation, 5:3-6).

One aspect of the disclosure provides a method for treating rheumatoid arthritis, SLE, psoriasis, or inflammatory bowel disease (including

Crohn's Disease and ulcerative colitis), comprising administering to a subject in need thereof a therapeutically effective amount of a combination of an IL6 antagonist and a TNF-α antagonist, which can be administered concurrently or sequentially or a combination thereof. In certain embodiments, the combination comprises an isolated IL6 antagonist comprising a binding domain specific for an IL6/IL6R (IL6xR) complex, wherein the binding domain specific for an IL6/IL6R (IL6xR) complex comprises an amino acid sequence that is at least 80% identical to one or more light chain variable regions as listed in SEQ ID NOs:373-434 and 799-804 and an amino acid sequence that is at least 80% identical to one or more of the heavy chain variable regions as listed in SEQ ID NOs:435-496 and 805-810, and an isolated TNF-α antagonist comprising a binding domain specific for TNF-α, wherein the binding domain specific for TNF-α comprises amino acids 23-257 of SEQ ID NO:846 or amino acids 31-211 of SEQ ID NO:847. In one embodiment, the isolated IL6 antagonist and the isolated TNF-α antagonist are both an antibody, or an antigen binding domain thereof, a Fab, or a scFv. In another embodiment, the isolated IL6 antagonist is an antibody and the isolated TNF-α antagonist is a small modular immunopharmaceutical (SMIP™). In a further embodiment, the isolated IL6 antagonist is a SMIP™ protein and the isolated TNF-α antagonist is an antibody. In yet another embodiment, the isolated isolated IL6 antagonist and the isolated TNF-α antagonist are both SMIP™ proteins. In a further embodiment, the binding domain of the isolated IL6 antagonist and the isolated TNF-α antagonist are an antibody or antigen binding domain thereof, a Fab, or a scFv. In this regard, the binding domain of the isolated IL6 antagonist may comprise a light chain variable region containing CDR1, CDR2, and CDR3 sequences that are each at least 80% identical to at least one light chain variable region CDR1, CDR2, and CDR3 as set forth in SEQ ID NOS:373-434 and 799-804, respectively. In one particular embodiment, the binding domain of the isolated IL6 antagonist comprises a heavy chain variable region containing CDR1, CDR2, and CDR3 sequences that are each at least 80% identical to at least one heavy chain variable region CDR1, CDR2, and CDR3 as set forth in SEQ ID NOS:435-496 and 805-810, respectively. In another embodiment, the binding domain of the isolated IL6 antagonist comprises a light chain variable region containing CDR1, CDR2, and CDR3 sequences that are each at least 80% identical to at least one light chain variable region CDR1, CDR2, and CDR3 as set forth in SEQ ID NOS:373-434 and 799-804, respectively, and comprises a heavy chain variable region containing CDR1,

CDR2, and CDR3 sequences that are each at least 80% identical to at least one heavy chain variable region CDR1, CDR2, and CDR3 as set forth in SEQ ID NOS:435-496 and 805-810, respectively. In a further embodiment, the IL6xR complex has an amino acid sequence as set forth in SEQ ID NO:606. In certain embodiments, this disclosure provides a method for treating rheumatoid arthritis, SLE, psoriasis, or inflammatory bowel disease (including Crohn's Disease and ulcerative colitis), comprising administering to a subject in need thereof a therapeutically effective amount of a composition comprising an IL6 antagonist and a TNF-α antagonist.

In further embodiments of this disclosure, the binding domains of the isolated IL6 antagonist and the isolated TNF-α antagonist are fused to (a) an immunoglobulin Fc domain or one or more CH domains of an immunoglobulin Fc domain, or (b) a serum protein binding protein. In this regard, the one or more CH domains of an immunoglobulin Fc domain may comprise a CH2 constant region and CH3 constant region, preferably IgG1 CH2 and CH3 domains. In one particular embodiment, I) the isolated IL6 antagonist comprises, from amino-terminus to carboxy-terminus, (a) the binding domain specific for IL6xR fused to a linker, (b) the linker fused to an immunoglobulin heavy chain CH2 constant region polypeptide, and (c) the CH2 constant region polypeptide fused to an immunoglobulin heavy chain CH3 constant region polypeptide; and II) the isolated TNF-α antagonist comprises, from amino-terminus to carboxy-terminus, (a) the binding domain specific for TNF-α fused to a linker, (b) the linker fused to an immunoglobulin heavy chain CH2 constant region polypeptide, and (c) the CH2 constant region polypeptide fused to an immunoglobulin heavy chain CH3 constant region polypeptide. In another embodiment, I) the isolated IL6 antagonist comprises, from carboxy-terminus to amino-terminus, (a) the polypeptide binding domain specific for IL6xR fused to a first linker, (b) the first linker fused to an immunoglobulin heavy chain CH3 constant region polypeptide, (c) the CH3 constant region polypeptide fused to an immunoglobulin heavy chain CH2 constant region polypeptide, and (d) the CH2 constant region polypeptide fused to a second linker; and II) the isolated TNF-α antagonist comprises, from carboxy-terminus to amino-terminus, (a) the binding domain specific for TNF-α fused to a first linker, (b) the first linker fused to an immunoglobulin heavy chain CH3 constant region polypeptide, (c) the CH3 constant region polypeptide fused to an immunoglobulin heavy chain CH2 constant region polypeptide, and (d) the CH2 constant region polypeptide fused to a second linker. In further embodiments,

the linker is an immunoglobulin hinge region polypeptide. In one particular embodiment, the linker is selected from the group consisting of SEQ ID NO:497-604, 823-828, 830-845, and 851-1106.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Zeu080a+anti-mIL6 Ab - Mediated Blockade of m TNF-α-Induced Biomarker Response. Serum concentration of mouse serum amyloid A (SAA) was measured in mice in each group as set forth in Table 1 at 2 hours (Panel A) and 24 hours (Panel B) post TNF-α injection.

Figure 2: Zeu080a+anti-mIL6 Ab - Mediated Blockade of mTNF-α-Induced Biomarker Response. Mouse serum concentration of TNF-α was measured at the 2-hour time point post TNF-α injection.

Figure 3: *In vivo* treatment with ENBREL® fusion protein (etanercept) in combination with anti-mIL6 antibody reduces severity of arthritis in a mouse model. The figure shows a graph of mean arthritis scores over time following various treatments. Note that curves for individual groups end when the first mouse was euthanized due to severe arthritis. Maximum score is 16.

Figure 4: *In vivo* treatment with ENBREL® in combination with anti-mIL6 antibody reduces weight loss in a mouse model of arthritis. The figure shows a graph of mean body weights over time through day 24 following various treatments. Primed mice were inoculated with 50μg G6PI and 25μg Mtb.

Figure 5: *In vivo* treatment with ENBREL® in combination with anti-mIL6 antibody reduces weight loss in a mouse model of arthritis. The figure shows a graph of mean body weights over time through day 6 (expanded from Figure 4) following various treatments. Primed mice were inoculated with 50μg G6PI and 25μg Mtb.

DETAILED DESCRIPTION

The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described. All documents, or portions of documents, cited herein, including but not limited to patents, patent applications, articles, books, and treatises, are hereby expressly incorporated by reference in their entirety for any purpose. In the event that one or more of the incorporated documents or portions of documents defines a term that contradicts the term's definition in the application, the definition that appears in this application controls.

The present disclosure relates generally to compositions comprising a combination of a TNF-α antagonist and an IL6 antagonist. In this regard, the present disclosure provides that administering a combination of the two antagonists is much more effective (synergistic) for reducing arthritis in a mouse model than administering either antagonist alone. Accordingly, the compositions of the present disclosure may be used in a variety of therapeutic settings for the treatment of SLE, psoriasis, or inflammatory bowel disease (including Crohn's Disease and ulcerative colitis), which are autoimmune diseases associated with TNF-α and IL6 activity.

Prior to setting forth this disclosure in more detail, it may be helpful to an understanding thereof to provide definitions of certain terms to be used herein. Additional definitions are set forth throughout this disclosure.

In the present description, any concentration range, percentage range, ratio range, or integer range is to be understood to include the value of any integer within the recited range and, when appropriate, fractions thereof (such as one tenth and one hundredth of an integer), unless otherwise indicated. Also, any number range recited herein relating to any physical feature, such as polymer subunits, size or thickness, are to be understood to include any integer within the recited range, unless otherwise indicated. As used herein, "about" or "consisting essentially of" mean ± 20% of the indicated range, value, or structure, unless otherwise indicated. It should be understood that the terms "a" and "an" as used herein refer to "one or more" of the enumerated components. The use of the alternative (*e.g.*, "or") should be understood to mean either one, both, or any combination thereof of the alternatives. As used herein, the terms "include" and "comprise" are used synonymously. In addition, it should be understood that the individual compounds, or groups of compounds, derived from the various combinations of the structures and substituents described herein, are disclosed by the present application to the same extent as if each compound or group of compounds was set forth individually. Thus, selection of particular structures or particular substituents is within the scope of the present disclosure.

A "binding domain" or "binding region" according to the present disclosure may be, for example, any protein, polypeptide, oligopeptide, or peptide that possesses the ability to specifically recognize and bind to a biological molecule (*e.g.*, TNF-α or IL6) or complex of more than one of the same or different molecule or assembly or aggregate, whether stable or transient (*e.g.*, IL6/IL6R complex). Such biological molecules include proteins,

polypeptides, oligopeptides, peptides, amino acids, or derivatives thereof, lipids, fatty acids, or derivatives thereof; carbohydrates, saccharides, or derivatives thereof; nucleotides, nucleosides, peptide nucleic acids, nucleic acid molecules, or derivatives thereof; glycoproteins, glycopeptides, glycolipids, lipoproteins, proteolipids, or derivatives thereof; other biological molecules that may be present in, for example, a biological sample; or any combination thereof. A binding region includes any naturally occurring, synthetic, semi-synthetic, or recombinantly produced binding partner for a biological molecule or other target of interest. A variety of assays are known for identifying binding domains of the present disclosure that specifically bind with a particular target, including Western blot, ELISA, or BIACORE® surface plasmon resonance analysis.

Binding domains and fusion proteins thereof of this disclosure are capable of binding to a desired degree, including "specifically or selectively binding" a target while not significantly binding other components present in a test sample, if they bind a target molecule with an affinity or K_a (*i.e.*, an equilibrium association constant of a particular binding interaction with units of 1/M) of, for example, greater than or equal to about 10^5 M^{-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^{-1}, or 10^{13} M^{-1}. "High affinity" binding domains refers to those binding domains with a K_a of at least 10^7 M^{-1}, at least 10^8 M^{-1}, at least 10^9 M^{-1}, at least 10^{10} M^{-1}, at least 10^{11} M^{-1}, at least 10^{12} M^{-1}, at least 10^{13} M^{-1}, or greater. Alternatively, affinity may be defined as an equilibrium dissociation constant (K_d) of a particular binding interaction with units of M (*e.g.*, 10^{-5} M to 10^{-13} M). Affinities of binding domain polypeptides and fusion proteins according to the present disclosure can be readily determined using conventional techniques (*see, e.g.*, Scatchard *et al.* (1949) Ann. N.Y. Acad. Sci. 51:660; U.S. Patent Nos. 5,283,173; 5,468,614; BIACORE® analysis; or the equivalent).

Binding domains of this disclosure can be generated as described herein or by a variety of methods known in the art (*see, e.g.*, US Patent Nos. 6,291,161; 6,291,158). Sources include antibody gene sequences from various species (which can be formatted as antibodies, sFvs, scFvs or Fabs, such as in a phage library), including human, camelid (from camels, dromedaries, or llamas; Hamers-Casterman *et al.* (1993) Nature, 363:446 and Nguyen *et al.* (1998) J. Mol. Biol., 275:413), shark (Roux *et al.* (1998) Proc. Nat'l. Acad. Sci. (USA) 95:11804), fish (Nguyen *et al.* (2002) Immunogenetics, 54:39), rodent, avian, ovine, sequences that encode random peptide libraries or sequences that encode an engineered diversity of amino acids in loop regions of

alternative non-antibody scaffolds, such as fibrinogen domains (*see, e.g.*, Weisel *et al.* (1985) Science 230:1388), Kunitz domains (*see, e.g.*, US Patent No. 6,423,498), lipocalin domains (*see, e.g.*, WO 2006/095164), V-like domains (*see, e.g.*, US Patent Application Publication No. 2007/0065431), C-type lectin domains (Zelensky and Gready (2005) FEBS J. 272:6179), mAb^2 or FCAB™ (*see, e.g.*, PCT Patent Application Publication Nos. WO 2007/098934; WO 2006/072620), or the like. Additionally, traditional strategies for hybridoma development using a synthetic single chain IL6/IL6R complex, such as a human IL6/IL6R complex or Hyper-IL6 (IL6 joined by a peptide linker IL6R), as an immunogen in convenient systems (*e.g.*, mice, HUMAB MOUSE®, TC MOUSE™, KM-MOUSE®, llamas, chicken, rats, hamsters, rabbits, *etc.*) can be used to develop binding domains of this disclosure.

Terms understood by those in the art as referring to antibody technology are each given the meaning acquired in the art, unless expressly defined herein. For example, the terms "V_L" and "V_H" refer to the variable binding region derived from an antibody light and heavy chain, respectively. The variable binding regions are made up of discrete, well-defined sub-regions known as "complementarity determining regions" (CDRs) and "framework regions" (FRs). The terms "C_L" and "C_H" refer to an "immunoglobulin constant region," *i.e.*, a constant region derived from an antibody light or heavy chain, respectively, with the latter region understood to be further divisible into C_{H1}, C_{H2}, C_{H3} and C_{H4} constant region domains, depending on the antibody isotype (IgA, IgD, IgE, IgG, IgM) from which the region was derived. A portion of the constant region domains makes up the Fc region (the "fragment crystallizable" region), which contains domains responsible for the effector functions of an immunoglobulin, such as ADCC (antibody-dependent cell-mediated cytotoxicity), ADCP (antibody-dependent cell-mediated phagocytosis), CDC (complement-dependent cytotoxicity) and complement fixation, binding to Fc receptors, greater half-life in vivo relative to a polypeptide lacking an Fc region, protein A binding, and perhaps even placental transfer (*see* Capon *et al.* (1989) Nature, 337:525). Further, a polypeptide containing an Fc region allows for dimerization or multimerization of the polypeptide. A "hinge region," also referred to herein as a "linker," is an amino acid sequence interposed between and connecting the variable binding and constant regions of a single chain of an antibody, which is known in the art as providing flexibility in the form of a hinge to antibodies or antibody-like molecules.

The domain structure of immunoglobulins is amenable to engineering, in that the antigen binding domains and the domains conferring effector functions may be exchanged between immunoglobulin classes and subclasses. Immunoglobulin structure and function are reviewed, for example, in Harlow *et al.*, Eds., *Antibodies: A Laboratory Manual*, Chapter 14 (Cold Spring Harbor Laboratory, Cold Spring Harbor, 1988). An extensive introduction as well as detailed information about all aspects of recombinant antibody technology can be found in the textbook *Recombinant Antibodies* (John Wiley & Sons, NY, 1999). A comprehensive collection of detailed antibody engineering lab Protocols can be found in R. Kontermann and S. Dübel, Eds., *The Antibody Engineering Lab Manual* (Springer Verlag, Heidelberg/New York, 2000).

"Derivative" as used herein refers to a chemically or biologically modified version of an antagonist that is structurally similar to the parent antagonist and (actually or theoretically) derivable from that parent antagonist. Generally, a "derivative" differs from an "analogue" in that a parent antagonist may be the starting material to generate a "derivative," whereas the parent antagonist may not necessarily be used as the starting material to generate an "analogue." An analogue may have different chemical, biological or physical properties of the parent antagonist. For example, a derivative may be more hydrophilic or it may have altered reactivity (*e.g.*, a CDR having an amino acid change that alters its affinity for a target) as compared to the parent antagonist.

The term “biological sample” includes a blood sample, biopsy specimen, tissue explant, organ culture, biological fluid or any other tissue or cell or other preparation from a subject or a biological source. A subject or biological source may, for example, be a human or non-human animal, a primary cell culture or culture adapted cell line including genetically engineered cell lines that may contain chromosomally integrated or episomal recombinant nucleic acid sequences, somatic cell hybrid cell lines, immortalized or immortalizable cell lines, differentiated or differentiatable cell lines, transformed cell lines, or the like. In further embodiments of this disclosure, a subject or biological source may be suspected of having or being at risk for having a disease, disorder or condition, including a malignant disease, disorder or condition or a B cell disorder. In certain embodiments, a subject or biological source may be a hyperproliferative, inflammatory, or autoimmune disease, and in certain other embodiments of this disclosure the subject or biological source

may be known to be free of a risk or presence of such disease, disorder, or condition.

In certain embodiments, the present disclosure makes possible the depletion or modulation of cells associated with aberrant TNF-α and/or IL6 activity by providing antagonist proteins that bind to TNF-α and antagonists that bind to IL6, IL6R, an IL6/IL6R complex, or any combination thereof.

The complex of IL6 with membrane or soluble IL6 receptor (IL6Rα) is referred to herein as IL6xR when referring to IL6 with either membrane IL6Rα or soluble IL6Rα (sIL6Rα), and as sIL6xR when referring only to the complex of IL6 with sIL6Rα. In some embodiments, antagonist proteins containing a binding domain specific for IL6xR have one or more of the following properties: (1) have greater or equal affinity for an IL6xR complex than for IL6 alone or IL6Rα alone, or have greater affinity for IL6Rα alone or an IL6xR complex than for IL6 alone; (2) compete with membrane gp130 for binding with a sIL6xR complex or enhance soluble gp130 binding with a sIL6xR complex; (3) preferentially inhibit IL6 trans-signaling over IL6 cis-signaling; and (4) do not inhibit signaling of gp130 family cytokines other than IL6.

As used herein, a “hinge region” or a “hinge” refers to (a) an immunoglobulin hinge region (made up of, for example, upper and core regions) or a functional variant thereof, including wild type and altered immunoglobulin hinges, (b) a lectin interdomain region or a functional variant thereof, (c) a cluster of differentiation (CD) molecule stalk region or a functional variant thereof, or (d) a portion of a cell surface receptor (interdomain region) that connects immunoglobulin V-like or immunoglobulin C-like domains.

“Junction amino acids” or “junction amino acid residues” refer to one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10, or more) amino acid residues between two adjacent regions or domains of a single chain polypeptide, such as between a hinge and an adjacent Fc region portion or between a hinge and an adjacent binding domain or between a peptide linker that links two immunoglobulin variable domains and an adjacent immunoglobulin variable domain. Junction amino acids may result from the construct design of a single chain polypeptide (*e.g.*, amino acid residues resulting from the use of a restriction enzyme site during the construction of a nucleic acid molecule encoding a single chain polypeptide).

"Sequence identity," as used herein, refers to the percentage of amino acid residues in one sequence that are identical with the amino acid residues in another reference polypeptide sequence after aligning the

sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. The percentage sequence identity values are generated by the NCBI BLAST2.0 software as defined by Altschul *et al.* (1997) "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs," Nucleic Acids Res. 25:3389-3402, with the parameters set to default values.

In certain embodiments, an altered immunoglobulin domain only contains conservative amino acid substitutions of a wild type immunoglobulin domain. In certain other embodiments, an altered immunoglobulin domain only contains non-conservative amino acid substitutions of a wild type immunoglobulin domain. In yet other embodiments, an altered immunoglobulin domain contains both conservative and non-conservative amino acid substitutions.

A "conservative substitution" is recognized in the art as a substitution of one amino acid for another amino acid that has similar properties. Exemplary conservative substitutions are well known in the art (*see, e.g.*, WO 97/09433, page 10, published March 13, 1997; Lehninger, Biochemistry, Second Edition; Worth Publishers, Inc. NY:NY (1975), pp.71-77; Lewin, Genes IV, Oxford University Press, NY and Cell Press, Cambridge, MA (1990), p. 8). In certain embodiments, a conservative substitution includes a leucine to serine substitution.

As used herein, unless otherwise provided, a position of an amino acid residue in a variable region of an immunoglobulin molecule is numbered according to the Kabat numbering convention (Kabat, *Sequences of Proteins of Immunological Interest*, 5th ed. Bethesda, MD: Public Health Service, National Institutes of Health (1991)), and a position of an amino acid residue in a constant region of an immunoglobulin molecule is numbered according to EU nomenclature (Ward *et al.*, 1995 *Therap. Immunol.* 2:77-94).

"Treatment," "treating" or "ameliorating" refers to either a therapeutic treatment or prophylactic/preventative treatment. A treatment is therapeutic if at least one symptom of disease in an individual receiving treatment improves or a treatment may delay worsening of a progressive disease in an individual, or prevent onset of additional associated diseases.

A "therapeutically effective amount (or dose)" or "effective amount (or dose)" of a specific binding molecule or compound refers to that amount of the compound sufficient to result in amelioration of one or more symptoms of

the disease being treated in a statistically significant manner. When referring to an individual active ingredient, administered alone, a therapeutically effective dose refers to that ingredient alone. When referring to a combination, a therapeutically effective dose refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered serially or simultaneously.

The term "pharmaceutically acceptable" refers to molecular entities and compositions that do not produce allergic or other serious adverse reactions when administered using routes well known in the art.

A "patient in need" refers to a patient at risk of, or suffering from, a disease, disorder or condition that is amenable to treatment or amelioration with a polypeptide heterodimer or a composition thereof provided herein.

TNF-α Antagonists

TNFRs are type I transmembrane proteins having an extracellular domain that contains three well ordered cysteine rich domains (CRD1, CRD2, CRD3) characteristic of the TNFR superfamily, and a fourth less well conserved, membrane proximal CRD (Banner *et al*. (1993) Cell 73:431). A TNF-α antagonist of this disclosure inhibits the inflammatory or hyperproliferative activity of TNF-α. The antagonist domains may block TNFR multimerization or TNF-α binding, or the domains may bind to components of the receptor system and block activity either by preventing ligand activity or by preventing the assembly of the receptor complex. Various TNF-α antagonists are known in the art, including anti-TNF antibodies, such as infliximab and adalimumab, and soluble TNF receptor (sTNFR). Such antibody antagonists bind and inhibit TNF-α, but do not significantly inhibit TNF-β. Anti-TNF antibodies, including monoclonal antibodies, can be prepared using known techniques and are known in the art (*see*, *e.g.*, US Patent No. 6,509,015). A TNF-α antagonist of this disclosure can also comprise one or more TNF-α binding domains present in a TNFR ectodomain.

TNF-α antagonists contemplated include a TNFR extracellular domain or sub-domain, one or more TNFR CRD domains (such as CRD2 and CRD3), or TNF-α-specific antibody-derived binding domains (analogous to the IL6 or IL6xR complex-specific antibody-derived binding domain described herein). In some embodiments, a TNF-α antagonist may be an extracellular domain ("ectodomain") of a TNFR, such as an ectodomain of TNFR1 or TNFR2. As used herein, a TNFR ectodomain refers to a sTNFR, one or more

CRDs, or any combination thereof of the TNFR domains. In certain embodiments, a TNF-α antagonist comprises an amino-terminal portion of TNFR2 (also known as p75, TNFRSF1B), such as the first 257 amino acids of TNFR2 as set forth in GenBank Accession No. NP_001057.1 (SEQ ID NO:846). In other embodiments, a TNF-α antagonist comprises amino acids 23-257 of SEQ ID NO:846 (*i.e.*, without the native leader sequence). In preferred embodiments, a TNF-α antagonist comprises a fragment of TNFR2 (*e.g.*, an ectodomain), such amino acids 23-163 of SEQ ID NO:846 or amino acids 23-185 of SEQ ID NO:846 or amino acids 23-235 of SEQ ID NO:846. In other preferred embodiments, a TNF-α antagonist comprises a derivative of a TNFR2 fragment, such amino acids 23-163 of SEQ ID NO:846 with a deletion of amino acid glutamine at position 109 or amino acids 23-185 of SEQ ID NO:846 with a deletion of amino acid glutamine at position 109 and a deletion of amino acid proline at position 109 or amino acids 23-235 of SEQ ID NO:846 with a deletion of amino acid glutamine at position 109, a deletion of amino acid proline at position 109, and an substitution of amino acid aspartate at position 235 (to, for example, a threonine, alanine, serine, or glutamate). In further embodiments, a TNF-α antagonist comprises an amino-terminal portion of TNFR1 (also known as p55, TNFRSF1A), such as the first 211 amino acids of TNFR1 as set forth in GenBank Accession No. NP_001056.1 (SEQ ID NO:847). In other embodiments, a TNF-α antagonist comprises amino acids 31-211 of SEQ ID NO:847 (*i.e.*, without the native leader sequence).

Thus, a TNF-α antagonist can comprise an ectodomain of TNFRSF1A as set forth in SEQ ID NO:696 (with or without the native leader peptide sequence included in this sequence) or an ectodomain of TNFRSF1B as set forth in SEQ ID NO:695 (with or without the native leader peptide sequence included in this sequence).

In one aspect, a TNF-α antagonist or fusion protein thereof of this disclosure is specific for TNF-α wherein it has an affinity with a dissociation constant (K_d) of about 10^{-5} M to 10^{-13} M, or less. In certain embodiments, the TNF-α antagonist or fusion protein thereof binds TNF-α with an affinity that is less than about 300 pM. Another measure, the kinetic dissociation (k_d), also referred to herein as k_{OFF}, is a measure of the rate of complex dissociation and, thus, the 'dwell time' of the target molecule bound by a polypeptide binding domain of this disclosure. The k_d (k_{OFF}) has units of 1/sec. Exemplary TNF-α antagonists of this disclosure can have a k_{OFF} of about 10^{-4}/sec (*e.g.*, about a day) to about 10^{-8}/sec or less. In certain embodiments, the k_{OFF} can range from

about 10^{-1}/sec, about 10^{-2}/sec, about 10^{-3}/sec, about 10^{-4}/sec, about 10^{-5}/sec, about 10^{-6}/sec, about 10^{-7}/sec, about 10^{-8}/sec, about 10^{-9}/sec, about 10^{-10}/sec, or less (*see* Graff *et al.* (2004) Protein Eng. Des. Sel. 17:293). In some embodiments, a TNF-α antagonist or fusion protein thereof of this disclosure will bind TNF-α with higher affinity and have a lower k_{OFF} rate as compared to the cognate TNFR binding to TNF-α. In further embodiments, a TNF-α antagonist or fusion protein thereof of this disclosure that blocks or alters TNF-α dimerization or other cell surface activity may have a more moderate affinity (*i.e.*, a K_d of about 10^{-8} M to about 10^{-9} M) and a more moderate off rate (*i.e.*, a k_{OFF} closer to about 10^{-4}/sec) as compared to the affinity and dimerization rate of cognate TNFR.

Exemplary binding domains that function as TNF-α antagonists of this disclosure can be generated as described herein or by a variety of methods known in the art (*see*, *e.g.*, US Patent Nos. 6,291,161, 6,291,158). Sources include antibody gene sequences from various species (which can be formatted as scFvs or Fabs, such as in a phage library), including human, camelid (from camels, dromedaries, or llamas; Hamers-Casterman *et al.* (1993) Nature, 363:446 and Nguyen *et al.* (1998) J. Mol. Biol., 275:413), shark (Roux *et al.* (1998) Proc. Nat'l. Acad. Sci. (USA) 95:11804), fish (Nguyen *et al.* (2002) Immunogenetics, 54:39), rodent, avian, ovine, sequences that encode random peptide libraries or sequences that encode an engineered diversity of amino acids in loop regions of alternative non-antibody scaffolds, such as fibrinogen domains (*see, e.g.*, Weisel *et al.* (1985) Science 230:1388), Kunitz domains (*see, e.g.*, US Patent No. 6,423,498), lipocalin domains (*see, e.g.*, WO 2006/095164), V-like domains (*see, e.g.*, US Patent Publication No. 2007/0065431), C-type lectin domains (Zelensky and Gready (2005) FEBS J. 272:6179), or the like. Additionally, traditional strategies for hybridoma development using a synthetic TNF-α or single chain TNFR ectodomain as an immunogen in convenient systems (*e.g.*, mice, HUMAB MOUSE®, TC mouse™, KM-MOUSE®, llamas, chicken, rats, hamsters, rabbits, *etc.*) can be used to develop binding domains of this disclosure.

In an illustrative example, TNF-α antagonists of this disclosure specific for an TNF-α or single chain TNFR ectodomain can be identified using a Fab phage library of fragments (*see*, *e.g.*, Hoet *et al.* (2005) Nature Biotechnol. 23:344) by screening for binding to a synthetic or recombinant TNF-α (using an amino acid sequence or fragment thereof as set forth in GenBank Accession No. NP_000585.2) or single chain TNFR ectodomain. A TNF-α or a

single chain TNFR ectodomain, as described herein or known in the art, can be used for such a screening. In certain embodiments, a TNF-α or single chain TNFR ectodomain used to generate a TNF-α antagonist can further comprise an intervening domain or a dimerization domain, as described herein, such as an immunoglobulin Fc domain or fragment thereof.

In some embodiments, TNF-α antagonist domains of this disclosure comprise V_H and V_L domains as described herein, such as those from infliximab or adalimumab. In certain embodiments, the V_H and V_L domains are rodent (*e.g.*, mouse, rat), humanized, or human. In further embodiments, there are provided TNF-α antagonist domains of this disclosure that have a sequence that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or at least 100% identical to the amino acid sequence of one or more light chain variable regions (V_L) or to one or more heavy chain variable regions (V_H), or both, wherein each CDR has at most three amino acid substitutions (for instance, substitutions may be in the framework regions).

The terms "identical" or "percent identity," in the context of two or more polypeptide or nucleic acid molecule sequences, means two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same over a specified region (*e.g.*, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity or 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical), when compared and aligned for maximum correspondence over a comparison window, or designated region, as measured using methods known in the art, such as a sequence comparison algorithm, by manual alignment, or by visual inspection. For example, preferred algorithms suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul *et al.* (1977) Nucleic Acids Res. 25:3389 and Altschul *et al.* (1990) J. Mol. Biol. 215:403, respectively.

In any of these or other embodiments described herein, the V_L and V_H domains may be arranged in either orientation and may be separated by about a five to about a thirty amino acid linker as disclosed herein or any other amino acid sequence capable of providing a spacer function compatible with interaction of the two sub-binding domains. In certain embodiments, a linker joining the V_H and V_L domains comprises an amino acid sequence as set forth

in SEQ ID NO:497-604, 823-828, 830-845, and 851-1106, such as Linker 46, (SEQ ID NO:542), Linker 47 (SEQ ID NO:543), or Linker 80 (SEQ ID NO:576). Multi-specific binding domains will have at least two specific sub-binding domains, by analogy to camelid antibody organization, or at least four specific sub-binding domains, by analogy to the more conventional mammalian antibody organization of paired V_H and V_L chains.

In further embodiments, TNF-α antagonist domains and fusion proteins may comprise a binding domain including one or more complementarity determining region ("CDR"), or multiple copies of one or more such CDRs, which have been obtained, derived, or designed from variable regions of an anti-TNF-α (such as infliximab or adalimumab) or anti-TNFR scFv or Fab fragment or from heavy or light chain variable regions thereof.

CDRs are defined in various ways in the art, including the Kabat, Chothia, AbM, and contact definitions. The Kabat definition is based on sequence variability and is the most commonly used definition to predict CDR regions (Johnson *et al.* (2000) Nucleic Acids Res. 28:214). The Chothia definition is based on the location of the structural loop regions (Chothia *et al.* (1986) J. Mol. Biol. 196:901; Chothia *et al.* (1989) Nature 342:877). The AbM definition, a compromise between the Kabat and Chothia definitions, is an integral suite of programs for antibody structure modeling produced by the Oxford Molecular Group (Martin *et al.* (1989) Proc. Nat'l. Acad. Sci. (USA) 86:9268; Rees *et al.*, ABMTM, a computer program for modeling variable regions of antibodies, Oxford, UK; Oxford Molecular, Ltd.). An additional definition, known as the contact definition, has been introduced more recently (*see* MacCallum *et al.* (1996) J. Mol. Biol. 5:732), which is based on an analysis of available complex crystal structures.

By convention, the CDR domains in the heavy chain are referred to as H1, H2, and H3, which are numbered sequentially in order moving from the amino terminus to the carboxy terminus. The CDR-H1 is about ten to 12 residues in length and starts four residues after a Cys according to the Chothia and AbM definitions, or five residues later according to the Kabat definition. The H1 can be followed by a Trp, Trp-Val, Trp-Ile, or Trp-Ala. The length of H1 is approximately ten to 12 residues according to the AbM definition, while the Chothia definition excludes the last four residues. The CDR-H2 starts 15 residues after the end of H1 according to the Kabat and AbM definitions, which is generally preceded by sequence Leu-Glu-Trp-Ile-Gly (but a number of variations are known) and is generally followed by sequence Lys/Arg-

Leu/Ile/Val/Phe/Thr/Ala-Thr/Ser/Ile/Ala. According to the Kabat definition, the length of H2 is about 16 to 19 residues, while the AbM definition predicts the length to be nine to 12 residues. The CDR-H3 usually starts 33 residues after the end of H2, is generally preceded by the amino acid sequence Cys-Ala-Arg and followed by the amino acid Gly, and has a length that ranges from three to about 25 residues.

By convention, the CDR regions in the light chain are referred to as L1, L2, and L3, which are numbered sequentially in order moving from the amino terminus to the carboxy terminus. The CDR-L1 generally starts at about residue 24 and generally follows a Cys. The residue after the CDR-L1 is always Trp, which begins one of the following sequences: Trp-Tyr-Gln, Trp-Leu-Gln, Trp-Phe-Gln, or Trp-Tyr-Leu. The length of CDR-L1 is approximately ten to 17 residues. The CDR-L2 starts about 16 residues after the end of L1 and will generally follow residues Ile-Tyr, Val-Tyr, Ile-Lys, or Ile-Phe. The CDR-L2 is about seven residues in length. The CDR-L3 usually starts 33 residues after the end of L2 and generally follows a Cys, which is generally followed by the sequence Phe-Gly-XXX-Gly and has a length of about seven to 11 residues.

Thus, a binding domain of this disclosure can comprise a single CDR from a variable region of an anti-TNF-α or anti-TNFR, or it can comprise multiple CDRs that can be the same or different. In certain embodiments, binding domains of this disclosure comprise V_H and V_L domains specific for a TNF-α or TNFR comprising framework regions and CDR1, CDR2 and CDR3 regions, wherein (a) the V_H domain comprises an amino acid sequence of a heavy chain CDR3; or (b) the V_L domain comprises an amino acid sequence of a light chain CDR3; or (c) the binding domain comprises a V_H amino acid sequence of (a) and a V_L amino acid sequence of (b); or the binding domain comprises a V_H amino acid sequence of (a) and a V_L amino acid sequence of (b) and wherein the V_H and V_L are found in the same reference sequence. In further embodiments, binding domains of this disclosure comprise V_H and V_L domains specific for an TNF-α or TNFR comprising framework regions and CDR1, CDR2 and CDR3 regions, wherein (a) the V_H domain comprises an amino acid sequence of a heavy chain CDR1, CDR2, and CDR3; or (b) the V_L domain comprises an amino acid sequence of a light chain CDR1, CDR2, and CDR3; or (c) the binding domain comprises a V_H amino acid sequence of (a) and a V_L amino acid sequence of (b); or the binding domain comprises a V_H amino acid sequence of (a) and a V_L amino acid sequence of (b), wherein the V_H and V_L amino acid sequences are from the same reference sequence.

In any of the embodiments described herein comprising specific CDRs, a binding domain can comprise (i) a V_H domain having an amino acid sequence that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of a V_H domain, wherein each CDR has at most three amino acid changes (*i.e.*, many of the changes will be in the framework regions); or (ii) a V_L domain having an amino acid sequence that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of a V_L domain, wherein each CDR has at most three amino acid changes (*i.e.*, many of the changes will be in the framework regions); or (iii) both a V_H domain of (i) and a V_L domain of (ii); or both a V_H domain of (i) and a V_L domain of (ii) wherein the V_H and V_L are from the same reference sequence.

A TNF-α binding domain antagonist of this disclosure may be an immunoglobulin-like domain such as an immunoglobulin scaffold. In certain embodiments, the TNF-α binding domain antagonist, such as an immunoglobulin-like domain, may be part of a fusion protein. Immunoglobulin scaffolds contemplated by this disclosure include, but are not limited to, a scFv, a domain antibody or a heavy chain-only antibody. In a scFv, this disclosure contemplates the heavy and light chain variable regions are joined by any linker peptide known in the art to be compatible with domain or region joinder in a binding molecule. Exemplary linkers are linkers based on the Gly_4Ser linker motif, such as $(Gly_4Ser)_n$, wherein n = 1-6. If a binding domain of a fusion protein of this disclosure is based on a non-human immunoglobulin or includes non-human immunoglobulin CDRs, the binding domain may be "humanized" according to methods known in the art.

Alternatively, a TNF-α antagonist domain of fusion proteins of this disclosure may be a scaffold other than an immunoglobulin scaffold. Other scaffolds contemplated by this disclosure present the TNF-α-specific CDR(s) in a functional conformation. Other scaffolds contemplated include, but are not limited to, an A domain molecule, a fibronectin III domain, an anticalin, an ankyrin-repeat engineered binding molecule, an adnectin, a Kunitz domain or a protein AZ domain affibody.

In certain embodiments, a TNF-α or IL6 binding domain or protein of this disclosure is comprised in a SMIP™ or a reverse SMIP (PIMS) fusion protein. In this regard, the term SMIP refers to a highly modular compound class having enhanced drug properties over monoclonal and recombinant antibodies. SMIP proteins comprise a single polypeptide chain including a

target-specific binding domain, based, for example, upon an antibody variable domain, in combination with a variable Fc region that permits the specific recruitment of a desired class of effector cells (such as, *e.g.*, macrophages and natural killer (NK) cells) or recruitment of complement-mediated killing. Depending upon the choice of target and hinge regions, SMIP proteins can signal or block signaling via cell surface receptors. As used herein, engineered fusion proteins, termed "small modular immunopharmaceutical" or "SMIP products or proteins", are as described in US Patent Publication Nos. 2003/133939, 2003/0118592, and 2005/0136049, and International Patent Publications WO02/056910, WO2005/037989, and WO2005/017148. In certain preferred embodiments, a SMIP protein comprises an scFv (linked VH and VL domains) from infliximab or adalimumab.

A PIMS molecule is a SMIP molecule in reverse orientation. In some embodiments, a TNF-α or IL6 binding domain may be comprised within a PIMS molecule such as those described in US Patent Publication No. 2009/0148447 and International Patent Publication WO2009/023386.

IL6 Antagonists

As noted above, in certain embodiments the present disclosure provides polypeptides containing a binding region or domain that is an IL6 antagonist (*e.g.*, preferentially inhibits IL6 trans-signaling or inhibits both IL6 cis- and trans-signaling). In certain embodiments, the present disclosure provides multi-specific fusion proteins containing a binding region or domain specific for an IL6/IL6R complex that has one or more of the following properties: (1) greater or equal affinity for an IL6xR complex than for IL6 or IL6Rα alone or has greater affinity for IL6Rα alone or an IL6/IL6R complex than for IL6 alone, (2) competes with membrane gp130 for binding with a sIL6/IL6R complex or augments soluble gp130 binding to sIL6/IL6R complex, (3) preferentially inhibits IL6 trans-signaling over IL6 cis-signaling, or (4) does not inhibit signaling of gp130 family cytokines other than IL6. In certain preferred embodiments, a binding domain specific for an IL6/IL6R complex according to this disclosure has the following properties: (1) greater affinity for IL6Rα alone or an IL6/IL6R complex than for IL6 alone, (2) augments soluble gp130 binding to sIL6xR complex, (3) preferentially inhibits IL6 trans-signaling over IL6 cis-signaling, and (4) does not inhibit signaling of gp130 family cytokines other than IL6. For example, a binding region or domain specific for an IL6/IL6R complex may be an immunoglobulin variable binding domain or derivative thereof, such as an

antibody, Fab, scFv, or the like. In the context of this disclosure, it should be understood that a binding region or domain specific for an IL6/IL6R complex is not gp130 as described herein.

As used herein, "IL6xR complex" or "IL6xR" refers to a complex of an IL6 with an IL6 receptor, wherein the IL6 receptor (also known as, for example, IL6Rα, IL6RA, IL6R1, and CD126) is either a membrane protein (referred to herein as mIL6R or mIL6Rα) or a soluble form (referred to herein as sIL6R or sIL6Rα). The term "IL6R" encompasses both mIL6Rα and sIL6Rα. In one embodiment, IL6xR comprises a complex of IL6 and mIL6Rα. In certain embodiments, the IL6xR complex is held together via one or more covalent bonds. For example, the carboxy terminus of an IL6R can be fused to the amino-terminus of an IL6 via a peptide linker, which is known in the art as a Hyper-IL6 (*see, e.g.*, Fischer *et al.* (1997) Nat. Biotechnol. 15:142). A Hyper-IL6 linker can be comprised of a cross-linking compound, a one to 50 amino acid sequence, or a combination thereof. A Hyper-IL6 may further include an additional peptide tag or tags (*e.g.*, AviFlagHis), or further include a dimerization domain, such as an immunoglobulin Fc domain or an immunoglobulin constant domain sub-region. In certain embodiments, the IL6xR complex is held together via non-covalent interactions, such as by hydrogen bonding, electrostatic interactions, Van der Waal's forces, salt bridges, hydrophobic interactions, or the like, or any combination thereof. For example, an IL6 and IL6R can naturally associate non-covalently (*e.g.*, as found in nature, or as synthetic or recombinant proteins) or each can be fused to a domain that promotes multimerization, such as an immunoglobulin Fc domain, to further enhance complex stability.

As used herein, "gp130" refers to a signal transduction protein that binds to an IL6xR complex. The gp130 protein can be in a membrane (mgp130), soluble (sgp130), or any other functional form thereof. Exemplary gp130 proteins have a sequence as set forth in GenBank Accession No. NP_002175.2 or any soluble or derivative form thereof (*see*, *e.g.*, Narazaki *et al.* (1993) Blood 82:1120 or Diamant *et al.* (1997) FEBS Lett. 412:379). By way of illustration and not wishing to be bound by theory, an mgp130 protein can bind to either an IL6/mILR or an IL6/sILR complex, whereas a sgp130 primarily binds with an IL6/sILR complex (*see* Scheller *et al.* (2006) Scand. J. Immunol. 63:321). Thus, certain embodiments of binding domains, or fusion proteins thereof, of the instant disclosure can inhibit IL6xR complex trans-signaling by binding with higher affinity to IL6xR than to either IL6 or IL6Rα alone and

preferably by competing with sIL6xR complex binding to mgp130. A binding domain of the instant disclosure "competes" with gp130 binding to a sIL6xR when (1) a binding domain or fusion protein thereof prevents gp130 from binding a sIL6xR and the binding domain binds sIL6xR with equal or higher affinity as compared to the binding of gp130 with sIL6xR, or (2) a binding domain or fusion protein thereof enhances or promotes sgp130 binding to sIL6xR and therby reduces the amount of time sIL6xR complex is available for binding to mgp130.

In one aspect, an IL6 antagonist binding domain of this disclosure has an affinity for IL6 or IL6xR complex that is at least 2-fold to 1000-fold greater than for IL6Rα alone or has an affinity for IL6Rα or IL6xR complex that is at least 2-fold to 1000-fold greater than for IL6 alone. By binding to IL6, IL6R, or IL6xR complex, an IL6 antagonist binding domain of this disclosure preferentially inhibits IL6 cis- and trans-signaling. In certain embodiments, the affinity of a binding domain for IL6, IL6R, or sIL6xR complex is about the same as the affinity of gp130 for IL6xR complex – with "about the same" meaning equal or up to about 2-fold higher affinity. In certain embodiments, the affinity of the binding domain for IL6, IL6R, or IL6xR complex is higher than the affinity of gp130 for IL6xR complex by at least 2-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 6-fold, at least 7-fold, at least 8-fold, at least 9-fold, at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, at least 100-fold, 1000-fold, or greater. For example, if the affinity of gp130 for a IL6xR complex is about 2 nM (*see, e.g.*, Gaillard *et al.* (1999) Eur. Cytokine Netw. 10:337), then a binding domain having at least a 10-fold higher affinity for the IL6xR complex would have a dissociation constant (K_d) of about 0.2 nM or less.

In further embodiments, an IL6 antagonist binding domain of this disclosure comprises a polypeptide sequence that (a) binds to a sIL6xR complex with an affinity at least 2-fold, 10-fold, 25-fold, 50-fold, 75-fold to 100-fold, 100-fold to 1000-fold higher than for either IL6 or IL6Rα alone and (b) competes with membrane gp130 for binding to sIL6xR complex or augments soluble gp130 binding to sIL6xR complex. In further embodiments, a polypeptide binding domain of this disclosure that binds to a sIL6xR complex with an affinity at least 2-fold, 10-fold, 25-fold, 50-fold, 75-fold to 100-fold, 100-fold to 1000-fold higher than for either IL6 or IL6Rα alone may also (i) more significantly or preferentially inhibit IL6 trans-signaling over IL6 cis-signaling, (ii) not inhibit signaling of gp130 cytokine family members other than IL6, (iii) preferentially inhibit IL6 trans-signaling over IL6 cis-signaling and not detectably

inhibit signaling of gp130 family cytokines other than IL6, (iv) may have two or more of these properties, or (v) may have all of these properties.

In certain embodiments, a polypeptide IL6 antagonist binding domain of this disclosure binds to a sIL6xR complex with an affinity at least 2-fold to 1000-fold higher than for either IL6 or IL6Rα alone and more significantly or preferentially inhibits IL6 trans-signaling over IL6 cis-signaling. To "preferentially inhibit IL6 trans-signaling over IL6 cis-signaling" refers to altering trans-signaling to an extent that sIL6xR activity is measurably decreased while the decrease in IL6 cis-signaling is not substantially altered (*i.e.*, meaning inhibition is minimal, non-existent, or not measurable). For example, a biomarker for sIL6xR activity (*e.g.*, acute phase expression of antichymotrypsin (ACT) in HepG2 cells) can be measured to detect trans-signaling inhibition. A representative assay is described by Jostock *et al.* (Eur. J. Biochem., 2001) – briefly, HepG2 cells can be stimulated to overexpress ACT in the presence of sIL6xR (trans-signaling) or IL6 (cis-signaling), but adding spg130 will inhibit the overexpression of ACT induced by sIL6xR while not substantially affecting IL6 induced expression. Similarly, a polypeptide binding domain of this disclosure that preferentially inhibits IL6 trans-signaling over IL6 cis-signaling will inhibit the overexpression of ACT induced by sIL6xR (*i.e.*, inhibit trans-signaling) while not substantially affecting IL6 induced expression (*i.e.*, not measurably decrease cis-signaling). This and other assays known in the art can be used to measure preferential inhibition of IL6 trans-signaling over IL6 cis-signaling (*see*, *e.g.*, other biomarkers described in Sporri *et al.* (1999) Int. Immunol. 11:1053; Mihara *et al.* (1995) Br. J. Rheum. 34:321; Chen *et al.* (2004) Immun. 20:59).

In further embodiments, signaling by gp130 family cytokines other than IL6 is not substantially inhibited by binding domain polypeptides or multi-specific fusion proteins thereof of this disclosure. For example, cis- and trans-signaling by an IL6xR complex via gp130 will be inhibited, but signaling by one or more other gp130 family cytokines will be minimally affected or unaffected, such as signaling via leukemia inhibitory factor (LIF), ciliary neurotropic factor (CNTF), neuropoietin (NPN), cardiotropin like cytokine (CLC), oncostatin M (OSM), IL-11, IL-27, IL-31, cardiotrophin-1 (CT-1), or any combination thereof.

It will be appreciated by those skilled in the art that the preferred *in vivo* half-life of a binding domain of this disclosure is on the order of days or weeks, but while the binding domain concentration may be low, the target may be plentiful as both IL6 and sIL6 production can be quite elevated in disease states (*see*, *e.g.*, Lu *et al.* (1993) Cytokine 5:578). Thus, in certain

embodiments, a binding domain of this disclosure has a k_{OFF} of about 10^{-5}/sec (*e.g.*, about a day) or less. In certain embodiments, the k_{OFF} can range from about 10^{-1}/sec, about 10^{-2}/sec, about 10^{-3}/sec, about 10^{-4}/sec, about 10^{-5}/sec, about 10^{-6}/sec, about 10^{-7}/sec, about 10^{-8}/sec, about 10^{-9}/sec, about 10^{-10}/sec, or less.

In an illustrative example, binding domains of this disclosure specific for an IL6 or IL6xR complex were identified in a Fab phage library of fragments (*see* Hoet *et al*. (2005) Nature Biotechnol. 23:344) by screening for binding to a synthetic IL6xR complex. The synthetic IL6xR complex used for this screening comprises a structure of N-IL6Rα(frag)-L1-IL6(frag)-L2-ID-C, wherein N is the amino-terminus and C is the carboxy-terminus, IL6Rα(frag) is a fragment of full length IL6Rα, IL6(frag) is a fragment of IL6, L1 and L2 are linkers, and ID is an intervening or dimerization domain, such as an immunoglobulin Fc domain.

More specifically, an IL6xR (which is a form of Hyper IL6) used to identify the binding domains specific for IL6xR complex has a structure, from amino-terminus to carboxy-terminus, as follows: (a) a central fragment of 212 amino acids from IL6Rα that is missing the first 110 amino acids of the full length protein and a carboxy-terminal portion that will depend on the isoform used (*see* GenBank Accession No. NP_000556.1, isoform 1 or NP_852004.1, isoform 2) fused to (2) a linker of G_3S that is in turn fused to (3) a 175 amino acid carboxy-terminal fragment of IL6 (*i.e.*, missing the first 27 amino acids of the full length protein; GenBank Accession No. NP_000591.1) that is in turn fused to (4) a linker that is an IgG2A hinge as set forth in SEQ ID NO:589, which is finally fused to a dimerization domain comprised of an immunoglobulin G1 (IgG1) Fc domain. In certain embodiments, the dimerization domain comprised of an IgG1 Fc domain has one or more of the following amino acids mutated (*i.e.*, have a different amino acid at that position): leucine at position 234 (L234), leucine at position 235 (L235), glycine at position 237 (G237), glutamate at position 318 (E318), lysine at position 320 (K320), lysine at position 322 (K322), or any combination thereof (numbering according to EU). For example, any one of these amino acids can be substituted with alanine. In a further embodiment, an IgG1 Fc domain has each of L234, L235, G237, E318, K320, and K322 (according to EU numbering) mutated to an alanine (*i.e.*, L234A, L235A, G237A, E318A, K320A, and K322A, respectively).

In one embodiment, an IL6xR complex used to identify the IL6 antagonist binding domains of this disclosure has an amino acid sequence as

set forth in SEQ ID NO:606. In certain embodiments, the IL6xR is a sIL6xR and has the amino acid sequence as set forth in SEQ ID NO:606. In further embodiments, IL6 antagonists containing a binding domain specific for an IL6xR complex (1) have greater or equal affinity for an IL6xR complex than for IL6 or IL6Rα alone, or have greater affinity for IL6Rα alone or an IL6xR complex than for IL6 alone, (2) compete with membrane gp130 for binding with a sIL6xR complex or augment soluble gp130 binding to sIL6xR complex, (3) preferentially inhibit IL6 trans-signaling over IL6 cis-signaling, or (4) do not inhibit signaling of gp130 family cytokines other than IL6, (5) have any combination thereof of properties (1) – (4), or (6) have all of the properties of (1) – (4). Other exemplary IL6xR complexes that may be used to identify binding domains of the instant disclosure or used as a reference complex to measure any of the aforementioned binding properties are described, for example, in US Patent Publication Nos. 2007/0172458; 2007/0031376; and US Patent Nos. 7,198,781; 5,919,763.

In certain embodiments, anti-IL6 binding domains comprise or are derived from the mouse monoclonal antibodies from hybridomas AH64, AH65, BSF2-77, CLB-8, CLB-12, CLB-16, HH61-08 and HH61-10, or binding fragments, or V_H and V_L domains thereof or CDRs thereof, which are all IL6 antagonist binding domains. (See *e.g.*,: SEQ ID NOs:787-792: AH65 & BSF2-77 light chain CDRs; SEQ ID NOs:793-798: AH65 & BSF2-77 heavy chain CDRs; SEQ ID NO: 799: AH65 light chain variable region; SEQ ID NO: 800: BSF2-77 light chain variable region; SEQ ID NO: 801: humanized AH 65-1 light chain variable region; SEQ ID NO: 802: humanized AH65-3 light chain variable region; SEQ ID NO: 803: humanized BSF2-77-1 light chain variable region; SEQ ID NO: 804: humanized BSF2-77-2 light chain variable region; SEQ ID NO: 805: AH65 heavy chain variable region; SEQ ID NO: 806: BSF2-77 heavy chain variable region; SEQ ID NO: 807: humanized AH 65-1 heavy chain variable region; SEQ ID NO: 808: humanized AH65-3 heavy chain variable region; SEQ ID NO: 809: humanized BSF2-77-1 heavy chain variable region; SEQ ID NO: 810: humanized BSF2-77-2 heavy chain variable region; SEQ ID NO: 811-822: DNAs encoding for SEQ ID NO: 799-810).

In some embodiments, IL6 antagonist binding domains of this disclosure comprise V_H and V_L domains specific for an IL6, IL6R, or IL6xR complex as described herein, and preferably human IL6, human IL6R, or human IL6xR complex. In certain embodiments, the V_H and V_L domains are rodent (*e.g.*, mouse, rat), humanized, or human. Examples of binding domains

containing such V_H and V_L domains specific for IL6, IL6R, or IL6xR are set forth in SEQ ID NOS:435-496 and 373-434, respectively. In further embodiments, there are provided polypeptide binding domains specific for an IL6xR complex that bind to the IL6xR with a higher or equal affinity than either IL6 or IL6Rα alone, and either compete with membrane gp130 for binding to the sIL6xR complex or augment soluble gp130 binding to sIL6xR complex, wherein the binding domain comprises a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5% , or at least 100% identical to an amino acid sequence of one or more light chain variable regions (V_L) or to one or more heavy chain variable regions (V_H), or both, as set forth in SEQ ID NOS:373-434 and 435-496, respectively, wherein each CDR has up to three amino acid substitutions (for instance, the substitutions may be in the framework regions).

In further embodiments, binding domains of this disclosure comprise V_H and V_L domains specific for an IL6xR as set forth in SEQ ID NOS:435-496 and 373-434, respectively, which are at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to the amino acid sequence of such V_H domain, V_L domain, or both, wherein each CDR has at most from zero to three amino acid substitutions. For example, the amino acid sequence of a V_H domain, V_L domain, or both of this disclosure can be at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to the amino acid sequence of V_H domain (*e.g.*, amino acids 512 to 631), V_L domain (*e.g.*, amino acids 649 to 758), or both, respectively, from a multispecific fusion protein containing binding domain TRU(XT6)-1002 (SEQ ID NO:608), wherein each CDR has at most from zero to three amino acid substitutions.

In any of these or other embodiments described herein, the V_L and V_H domains may be arranged in either orientation and may be separated by five up to about a thirty-five amino acid linker as disclosed herein or any other amino acid sequence capable of providing a spacer function such that the two sub-binding domains can interact to form a functional binding domain. In

certain embodiments, a linker joining the V_H and V_L domains (also referred to herein as a "variable domain linker") includes those belonging to the (Gly_nSer) family, such as $(Gly_3Ser)_n(Gly_4Ser)_1$, $(Gly_3Ser)_1(Gly_4Ser)_n$, $(Gly_3Ser)_n(Gly_4Ser)_n$, or $(Gly_4Ser)_n$, wherein n is an integer of 1 to 6. In certain embodiments, the linker is GGGGSGGGGSGGGGS (SEQ ID NO:542). In preferred embodiments, these (Gly_nSer)-based linkers are used to link the VH and VL domains in a binding domain, but are not used to link a binding domain to any other domain, *e.g.*, a heterodimerization domain or to an Fc region portion. In some embodiments, the linker joining the V_H and V_L domains comprises an amino acid sequence as set forth in SEQ ID NO:497-604, 823-828, 830-845, and 851-1106, such as Linker 46 (SEQ ID NO:542), Linker 47 (SEQ ID NO:543) or Linker 80 (SEQ ID NO:576).

In further embodiments, IL6 antagonist binding domains of this disclosure may comprise one or more complementarity determining region ("CDR"), or multiple copies of one or more such CDRs, which have been obtained, derived, or designed from variable regions of an anti-IL6, anti-IL6R, or anti-IL6xR complex scFv or Fab fragment or from heavy or light chain variable regions thereof. Thus, a binding domain of this disclosure can comprise a single CDR3 from a variable region of an anti-IL6, anti-IL6R, or anti-IL6xR, or it can comprise multiple CDRs that can be the same or different. In certain embodiments, IL6 antagonist binding domains of this disclosure comprise V_H and V_L domains comprising framework regions and CDR1, CDR2 and CDR3 regions, wherein (a) the V_H domain comprises the amino acid sequence of a heavy chain CDR3 found in any one of SEQ ID NOS:435-496; or (b) the V_L domain comprises the amino acid sequence of a light chain CDR3 found in any one of SEQ ID NOS:373-434; or (c) the binding domain comprises a V_H amino acid sequence of (a) and a V_L amino acid sequence of (b); or the binding domain comprises a V_H amino acid sequence of (a) and a V_L amino acid sequence of (b) and wherein the V_H and V_L are found in the same reference sequence. In further embodiments, binding domains of this disclosure comprise V_H and V_L domains specific for an IL6xR complex comprising framework regions and CDR1, CDR2 and CDR3 regions, wherein (a) the V_H domain comprises the amino acid sequence of a heavy chain CDR1, CDR2, and CDR3 found in any one of SEQ ID NOS:435-496; or (b) the V_L domain comprises the amino acid sequence of a light chain CDR1, CDR2, and CDR3 found in any one of SEQ ID NOS:373-434; or (c) the binding domain comprises a V_H amino acid sequence of (a) and a V_L amino acid sequence of (b); or the

binding domain comprises a V_H amino acid sequence of (a) and a V_L amino acid sequence of (b), wherein the V_H and V_L amino acid sequences are from the same reference sequence. Exemplary light and heavy chain variable domain CDRs directed against IL6, IL6R, or IL6xR are provided in SEQ ID NOS:1-187 and 787-792, and SEQ ID NOS:187-372 and 793-798, respectively.

Amino acid sequences of IL6 antagonist light chain variable regions are provided in SEQ ID NOS:373-434 and 799-804 and IL6 antagonist heavy chain variable regions are provided in SEQ ID NO:435-496 and 805-810.

In any of the embodiments described herein comprising specific CDRs against IL6, IL6R, or IL6xR, a binding domain can comprise (i) a V_H domain having an amino acid sequence that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of a V_H domain found in any one of SEQ ID NOS:435-496 and 805-810; or (ii) a V_L domain having an amino acid sequence that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of a V_L domain found in any one of SEQ ID NOS:373-434 and 799-804; or (iii) both a V_H domain of (i) and a V_L domain of (ii); or both a V_H domain of (i) and a V_L domain of (ii) wherein the V_H and V_L are from the same reference sequence.

In certain embodiments, a binding domain of this disclosure may be an immunoglobulin-like domain, such as an immunoglobulin scaffold. Immunoglobulin scaffolds contemplated in this disclosure include a scFv, Fab, a domain antibody, or a heavy chain-only antibody. In further embodiments, there are provided anti-IL6 or anti-IL6xR antibodies (*e.g.*, non-human such as mouse or rat, chimeric, humanized, human) or Fab fragments or scFv fragments that have an amino acid sequence that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of a V_H and V_L domain set in any one of SEQ ID NOS:435-496 and 805-801, and 373-434 and 799-804, respectively, which may also have one or more of the following properties: (1) have greater or equal affinity for an IL6xR complex than for IL6 or IL6Rα alone, or have greater affinity for IL6Rα alone or an IL6xR complex than for IL6 alone, (2) compete with membrane gp130 for binding with a sIL6xR complex or augment soluble gp130 binding to sIL6xR complex, (3) preferentially inhibit IL6 trans-signaling over IL6 cis-signaling, or (4) do not inhibit signaling of gp130 family cytokines other than IL6. Such antibodies, Fabs, or scFvs can be used in any of the methods described herein. In certain embodiments, the present disclosure provides

polypeptides containing a binding domain that is an IL6 antagonist (*i.e.*, can inhibit IL6 cis- and trans-signaling). In further embodiments, an IL6 antagonist according to this disclosure does not inhibit signaling of gp130 family cytokines other than IL6. Exemplary IL6 antagonists include binding domains specific for an IL6 or IL6xR, such as an immunoglobulin variable binding domain or derivative thereof (*e.g.*, an antibody, Fab, scFv, or the like).

Alternatively, binding domains of this disclosure may be part of a scaffold other than an immunoglobulin. Other scaffolds contemplated include an A domain molecule, a fibronectin III domain, an anticalin, an ankyrin-repeat engineered binding molecule, an adnectin, a Kunitz domain, or a protein AZ domain affibody.

Making TNF and IL6 Antagonists

A leader peptide may be used to facilitate secretion of expressed IL6 antagonist and TNF-α antagonist polypeptides and fusion proteins. Using any of the conventional leader peptides (signal sequences) can be expected to direct nascently expressed polypeptides or fusion proteins into a secretory pathway and to result in cleavage of the leader peptide from the mature polypeptide or fusion protein at or near the junction between the leader peptide and the polypeptide or fusion protein. A particular leader peptide will be chosen based on considerations known in the art, such as using sequences encoded by polynucleotides that allow the easy inclusion of restriction endonuclease cleavage sites at the beginning or end of the coding sequence for the leader peptide to facilitate molecular engineering, provided that such introduced sequences specify amino acids that either do not interfere unacceptably with any desired processing of the leader peptide from the nascently expressed protein or do not interfere unacceptably with any desired function of a polypeptide or fusion protein molecule if the leader peptide is not cleaved during maturation of the polypeptides or fusion proteins. Exemplary leader peptides of this disclosure include natural leader sequences or others, such as H_3N-MDFQVQIFSFLLISASVIMSRG$(X)_n$-CO_2H, wherein X is any amino acid and n is zero to three (SEQ ID NOS:785, 848-850) or H_3N-MEAPAQLLFLLLLWLPDTTG-CO_2H (SEQ ID NO:786).

In certain embodiments of the invention, the IL6 antagonist and/or TNF-α antagonist is glycosylated, the pattern of glycosylation being dependent upon a variety of factors including the host cell in which the protein is expressed (if prepared in recombinant host cells) and the culture conditions. Glycosylation

patterns may be modified using the methods disclosed in US 2009/0041765 and US 2010/0150948, each of which is herein incorporated by reference in its entirety.

This disclosure also provides derivatives of the binding domain of this disclosure or of fusion proteins comprising such binding domain. Derivatives include specific binding agent polypeptides bearing modifications other than insertion, deletion, or substitution of amino acid residues. Preferably, the modifications are covalent in nature, and include for example, chemical bonding with polymers, lipids, other organic, and inorganic moieties. Derivatives of this disclosure may be prepared to increase circulating half-life of a specific binding agent polypeptide, or may be designed to improve targeting capacity for the polypeptide to desired cells, tissues, or organs.

In certain embodiments, the *in vivo* half-life of the binding domain polypeptide or fusion protein thereof of this disclosure can be increased using methods known in the art for increasing the half-life of large molecules. For example, this disclosure embraces fusion proteins that are covalently modified or derivatized to include one or more water-soluble polymer attachments, such as polyethylene glycol, polyoxyethylene glycol, or polypropylene glycol (*see, e.g.*, U.S. Patent Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192 ; 4,179,337). Still other useful polymers known in the art include monomethoxy-polyethylene glycol, dextran, cellulose, and other carbohydrate-based polymers, poly-(N-vinyl pyrrolidone)-polyethylene glycol, propylene glycol homopolymers, a polypropylene oxide/ethylene oxide co-polymer, polyoxyethylated polyols (*e.g.*, glycerol) and polyvinyl alcohol, as well as mixtures of these polymers. Particularly preferred are polyethylene glycol (PEG)–derivatized proteins. Water-soluble polymers may be bonded at specific positions, for example at the amino terminus of the proteins and polypeptides according to this disclosure, or randomly attached to one or more side chains of the polypeptide. The use of PEG for improving therapeutic capacities is described in US Patent No. 6,133,426.

Such methods also include creating fusion proteins wherein the binding domain is fused to a protein that conveys a longer half life to the binding domain fusion protein than that of the binding domain alone. Such fusion proteins can include proteins that themselves bind to proteins that have a long half life, *e.g.*, immunoglobulin, immunoglobulin Fc domains, transferrin, streptococcal G protein, or albumin. Such fusions of binding domains to stable plasma proteins are disclosed, *e.g.*, in US Patent Nos. 5,428,130; 5,116,964.

A particular embodiment of this disclosure is an immunoglobulin or an Fc fusion protein. Such a fusion protein can have a long half-life, *e.g.*, several hours, a day or more, or even a week or more, especially if the Fc domain is capable of interacting with FcRn, the neonatal Fc receptor. The binding site for FcRn in an Fc domain is also the site at which the bacterial proteins A and G bind. The tight binding between these proteins can be used as a means to purify antibodies or fusion proteins of this disclosure by, for example, employing protein A or protein G affinity chromatography during protein purification.

As indicated herein, antagonist fusion polypeptides of the present disclosure may comprise an Fc region constant domain portion (also referred to as an Fc region portion). The inclusion of an Fc region portion slows clearance of the heterodimers from circulation after administration to a subject. By mutations or other alterations, the Fc region portion further enables relatively easy modulation of heterodimer polypeptide effector functions (*e.g.*, ADCC, ADCP, CDC, complement fixation and binding to Fc receptors), which can either be increased or decreased depending on the disease being treated, as known in the art and described herein. In certain embodiments, an Fc region portion will be capable of mediating one or more of these effector functions.

An Fc region portion for use in the fusion proteins of the present disclosure may comprise a CH2 domain, a CH3 domain, a CH4 domain or any combination thereof. For example, an Fc region portion may comprise a CH2 domain, a CH3 domain, both CH2 and CH3 domains, both CH3 and CH4 domains, two CH3 domains, a CH4 domain, or two CH4 domains. In certain embodiments, a CH2, CH3 and/or CH4 domain that may form an Fc region portion of an antagonist binding protein of the present disclosure may be a wild type immunoglobulin CH2, CH3, or CH4 domain or an altered immunoglobulin CH2, CH3, or CH4 domain thereof from certain immunoglobulin classes or subclasses (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, or IgD) and from various species (including human, mouse, rat, and other mammals). Illustrative altered domains with desired functional attributes are described, for example, in PCT WO2010/042904, incorporated herein in its entirety.

Protein purification techniques are well known to those of skill in the art. These techniques involve, at one level, the crude fractionation of the polypeptide and non-polypeptide fractions. Further purification using chromatographic and electrophoretic techniques to achieve partial or complete purification (or purification to homogeneity) is frequently desired. Analytical

methods particularly suited to the preparation of a pure fusion protein are ion-exchange chromatography, exclusion chromatography; polyacrylamide gel electrophoresis; and isoelectric focusing. Particularly efficient methods of purifying peptides are fast protein liquid chromatography and HPLC.

Certain aspects of the present disclosure concern the purification, and in particular embodiments, the substantial purification, of a polypeptide of this disclosure. The term "purified" as used herein, is intended to refer to a composition, isolatable from other components, wherein the fusion protein is purified to any degree relative to its naturally obtainable state. A purified protein therefore also refers to such protein, isolated from the environment in which it naturally occurs.

Generally, "purified" will refer to a polypeptide composition that has been subjected to fractionation to remove various other components, and which composition substantially retains its expressed biological activity. Where the term "substantially purified" is used, this designation refers to a binding domain protein composition in which the protein forms the major component of the composition, such as constituting about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 99% or more of the protein, by weight, in the composition.

Various methods for quantifying the degree of purification are known to those of skill in the art in light of the present disclosure. These include, for example, determining the specific binding activity of an active fraction, or assessing the amount of protein in a fraction by SDS/PAGE analysis. A preferred method for assessing the purity of a protein fraction is to calculate the binding activity of the fraction, to compare it to the binding activity of the initial extract, and to thus calculate the degree of purification, herein assessed by a "-fold purification number." The actual units used to represent the amount of binding activity will, of course, be dependent upon the particular assay technique chosen to follow the purification and whether or not the expressed protein exhibits a detectable binding activity.

Various techniques suitable for use in protein purification are well known to those of skill in the art. These include, for example, precipitation with ammonium sulfate, PEG, antibodies and the like, or by heat denaturation, followed by centrifugation; chromatography steps such as ion exchange, gel filtration, reverse phase, hydroxylapatite and affinity chromatography; isoelectric focusing; gel electrophoresis; and combinations of these and other techniques. As is generally known in the art, the order of conducting various purification

steps may be changed, or certain steps may be omitted and still result in a suitable method for the preparation of a substantially purified protein.

Linkers

The fusion proteins of this disclosure may comprise one or more peptide linkers between the various domains as described herein. In addition to providing a spacing function, a linker can provide flexibility or rigidity suitable for properly orienting the one or more binding domains of a fusion protein, both within the fusion protein and between or among the fusion proteins and their target(s). Further, a linker can support expression of a full-length fusion protein and stability of the purified protein both in vitro and in vivo following administration to a subject in need thereof, such as a human, and is preferably non-immunogenic or poorly immunogenic in those same subjects. In certain embodiments, a linker of a dimerization domain of fusion proteins of this disclosure may comprise part or all of a human immunoglobulin hinge.

Exemplary linkers that can be used to join an intervening domain (*e.g.*, an immunoglobulin-derived constant sub-region) to a binding domain or to join two variable regions of a binding domain are set forth in SEQ ID NOS:497-604, 823-828 and 830-845. Illustrative linkers that may be used in constructing the antagonists described herein also include SEQ ID NOs:851-1106.

Linkers contemplated in this disclosure include, for example, peptides derived from any interdomain region of an immunoglobulin superfamily member (*e.g.*, an antibody hinge region) or a stalk region of C-type lectins, a family of type II membrane proteins. These linkers range in length from about two to about 150 amino acids, or about two to about 40 amino acids, or about eight to about 20 amino acids preferably about ten to about 60 amino acids, more preferably about 10 to about 30 amino acids, and most preferably about 15 to about 25 amino acids. For example, Linker 1 (SEQ ID NO:497) is two amino acids in length and Linker 116 (SEQ ID NO:603) is 36 amino acids in length.

Beyond general length considerations, a linker suitable for use in the fusion proteins of this disclosure includes an antibody hinge region selected from an IgG hinge, IgA hinge, IgD hinge, IgE hinge, or variants thereof. In certain embodiments, a linker may be an antibody hinge region (upper and core region) selected from human IgG1, human IgG2, human IgG3, human IgG4, or fragments or variants thereof. As used herein, a linker that is an "immunoglobulin hinge region" refers to the amino acids found between the

carboxyl end of CH1 and the amino terminal end of CH2 (for IgG, IgA, and IgD) or the amino terminal end of CH3 (for IgE and IgM). A "wild type immunoglobulin hinge region," as used herein, refers to a naturally occurring amino acid sequence interposed between and connecting the CH1 and CH2 regions (for IgG, IgA, and IgD) or interposed between and connecting the CH2 and CH3 regions (for IgE and IgM) found in the heavy chain of an antibody. In preferred embodiments, the wild type immunoglobulin hinge region sequences are human.

Accordingly, in certain embodiments, a hinge is a wild type human immunoglobulin hinge region (*e.g.*, human immunoglobulin hinge regions as set forth in SEQ ID NOs:851-857). Exemplary human wild type immunoglobulin hinge regions are set forth in SEQ ID NOS:851 (IgA1 hinge), 852 (IgA2 hinge), 853 (IgD hinge), 854 (IgG1 hinge), 855 (IgG2 hinge), 856 (IgG3 hinge) and 857 (IgG4 hinge). In certain other embodiments, one or more amino acid residues may be added at the amino- or carboxy- terminus of a wild type immunoglobulin hinge region as part of a fusion protein construct design. For example, additional junction amino acid residues at the hinge amino-terminus can be "RT," "RSS," "TG," or "T", or at the hinge carboxy-terminus can be "SG", or a hinge deletion can be combined with an addition, such as ΔP with "SG" added at the carboxyl terminus.

According to crystallographic studies, an IgG hinge domain can be functionally and structurally subdivided into three regions: the upper hinge region, the core or middle hinge region, and the lower hinge region (Shin *et al.* (1992) *Immunol. Rev.* 130:87). Exemplary upper hinge regions include EPKSCDKTHT (SEQ ID NO:830) as found in IgG1, ERKCCVE (SEQ ID NO:831) as found in IgG2, ELKTPLGDTT HT (SEQ ID NO:832) or EPKSCDTPPP (SEQ ID NO:833) as found in IgG3, and ESKYGPP (SEQ ID NO:834) as found in IgG4. Exemplary middle hinge regions include CPPCP (SEQ ID NO:835) as found in IgG1 and IgG2, CPRCP (SEQ ID NO:836) as found in IgG3, and CPSCP (SEQ ID NO:837) as found in IgG4. While IgG1, IgG2, and IgG4 antibodies each appear to have a single upper and middle hinge, IgG3 has four in tandem – one of ELKTPLGDTT HTCPRCP (SEQ ID NO:838) and three of EPKSCDTPPP CPRCP (SEQ ID NO:839).

IgA and IgD antibodies appear to lack an IgG-like core region, and IgD appears to have two upper hinge regions in tandem (*see* SEQ ID NOS:840 and 841). Exemplary wild type upper hinge regions found in IgA1 and IgA2 antibodies are set forth in SEQ ID NOS:842 and 843.

IgE and IgM antibodies, in contrast, instead of a typical hinge region have a CH2 region with hinge-like properties. Exemplary wild-type CH2 upper hinge-like sequences of IgE and IgM are set forth in SEQ ID NO:844 (VCSRDFTPPT VKILQSSSDG GGHFPPTIQL LCLVSGYTPG TINITWLEDG QVMDVDLSTA STTQEGELAS TQSELTLSQK HWLSDRTYTC QVTYQGHTFE DSTKKCA) and SEQ ID NO:845 (VIAELPPKVS VFVPPRDGFF GNPRKSKLIC QATGFSPRQI QVSWLREGKQ VGSGVTTDQV QAEAKESGPT TYKVTSTLTI KESDWLGQSM FTCRVDHRGL TFQQNASSMC VP), respectively.

In certain embodiments, a hinge is an altered immunoglobulin hinge in which one or more cysteine residues in a wild type immunoglobulin hinge region is substituted with one or more other amino acid residues (*e.g.*, serine or alanine). For example, a hinge may be an altered immunoglobulin hinge based on or derived from a wild type human IgG1 hinge as set forth in SEQ ID NO:854, which from amino terminus to carboxyl terminus comprises the upper hinge region (EPKSCDKTHT, SEQ ID NO:830) and the core hinge region (CPPCP, SEQ ID NO:835). Exemplary altered immunoglobulin hinges include an immunoglobulin human IgG1 hinge region having one, two or three cysteine residues found in a wild type human IgG1 hinge substituted by one, two or three different amino acid residues (*e.g.*, serine or alanine). An altered immunoglobulin hinge may additionally have a proline substituted with another amino acid (*e.g.*, serine or alanine). For example, the above-described altered human IgG1 hinge may additionally have a proline located carboxyl terminal to the three cysteines of wild type human IgG1 hinge region substituted by another amino acid residue (*e.g.*, serine, alanine). Preferably the prolines of the core hinge region are not substituted. Exemplary altered immunoglobulin hinges are set forth in SEQ ID NOS: 865-903. A preferred altered IgG1 hinge is an altered human IgG1 hinge in which the first cysteine is substituted by serine. The sequence of this preferred altered IgG1 hinge is set forth in SEQ ID NO:878, and is referred to as the " human IgG1 SCC-P hinge" or "SCC-P hinge." In certain embodiments, one or more amino acid residues (*e.g.*, "RT," "RSS," or "T") may be added at the amino-or carboxy-terminus of a mutated immunoglobulin hinge region as part of a fusion protein construct design.

In certain embodiments, a hinge polypeptide comprises or is a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identical to a wild type

immunoglobulin hinge region, such as a wild type human IgG1 hinge, a wild type human IgG2 hinge, or a wild type human IgG4 hinge.

Alternative hinge and linker sequences that can be used as connecting regions may be crafted from portions of cell surface receptors that connect IgV-like or IgC-like domains. Regions between IgV-like domains where the cell surface receptor contains multiple IgV-like domains in tandem and between IgC-like domains where the cell surface receptor contains multiple tandem IgC-like regions could also be used as connecting regions or linker peptides. In certain embodiments, hinge and linker sequences are from 5 to 60 amino acids long, and may be primarily flexible, but may also provide more rigid characteristics, may contain primarily α helical structure with minimal β sheet structure. Preferably, sequences are stable in plasma and serum and are resistant to proteolytic cleavage. In some embodiments, sequences may contain a naturally occurring or added motif such as CPPCP (SEQ ID NO:835) that confers the capacity to form a disulfide bond or multiple disulfide bonds to stabilize the C-terminus of the molecule. In other embodiments, sequences may contain one or more glycosylation sites. Examples of hinge and linker sequences include interdomain regions between IgV-like and IgC-like or between IgC-like or IgV-like domains of CD2, CD4, CD22, CD33, CD48, CD58, CD66, CD80, CD86, CD96, CD150, CD166, and CD244. Alternative hinges may also be crafted from disulfide-containing regions of Type II receptors from non-immunoglobulin superfamily members, such as CD69, CD72, and CD161.

In some embodiments, a hinge linker has a single cysteine residue for formation of an interchain disulfide bond. In other embodiments, a linker has two cysteine residues for formation of interchain disulfide bonds. In further embodiments, a hinge linker is derived from an immunoglobulin interdomain region (*e.g.*, an antibody hinge region) or a Type II C-type lectin stalk region (derived from a Type II membrane protein; *see, e.g.*, exemplary lectin stalk region sequences set forth in of PCT Application Publication No. WO 2007/146968, such as SEQ ID NOS:111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 253, 255, 257, 259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 287, 289, 297, 305, 307, 309-311, 313-331, 346, 373-377, 380, or 381 from that publication), which sequences are hereby incorporated by reference.

In further embodiments, a hinge present in a single chain polypeptide may be a hinge that is not based on or derived from an

immunoglobulin hinge (*i.e.*, not a wild type immunoglobulin hinge or an altered immunoglobulin hinge). In certain embodiments, these types of non-immunoglobulin based hinges are used on or near the carboxyl end (*e.g.*, located carboxyl terminal to Fc region portions) of the antagonist binding fusion proteins disclosed herein. Examples for such hinges include peptides of about five to about 150 amino acids of the interdomain or stalk region of type II C-lectins or CD molecules, preferably having about eight to 25 amino acids, more preferably having seven to 18 amino acids, and derivatives thereof.

The "interdomain or stalk region" of a type II C-lectin or CD molecule refers to the portion of the extracellular domain of the type II C-lectin or CD molecule that is located between the C-type lectin-like domain (CTLD; *e.g.*, similar to CTLD of natural killer cell receptors) and the transmembrane domain. For example, in the human CD94 molecule (GenBank Accession No. AAC50291.1, PRI November 30, 1995), the extracellular domain corresponds to amino acid residues 34-179, whereas the CTLD corresponds to amino acid residues 61-176. Accordingly, the interdomain or stalk region of the human CD94 molecule includes amino acid residues 34-60, which is found between the membrane and the CTLD (*see* Boyington *et al.*, Immunity 10:75, 1999; for descriptions of other stalk regions, *see also* Beavil *et al.*, Proc. Nat'l. Acad. Sci. USA 89:753, 1992; and Figdor *et al.*, Nature Rev. Immunol. 2:77, 2002). These type II C-lectin or CD molecules may also have from six to 10 junction amino acids between the stalk region and the transmembrane region or the CTLD. In another example, the 233 amino acid human NKG2A protein (GenBank Accession No. P26715.1, PRI June 15, 2010) has a transmembrane domain ranging from amino acids 71-93 and an extracellular domain ranging from amino acids 94-233. The CTLD is comprised of amino acids 119-231, and the stalk region comprises amino acids 99-116, which is flanked by junctions of five and two amino acids. Other type II C-lectin or CD molecules, as well as their extracellular ligand-bind domains, interdomain or stalk regions, and CTLDs are known in the art (*see*, *e.g.*, GenBank Accession Nos. NP_001993.2; AAH07037.1, PRI July 15, 2006; NP_001773.1, PRI June 20, 1010; AAL65234.1, PRI January 17, 2002, and CAA04925.1, PRI November 14, 2006, for the sequences of human CD23, CD69, CD72, NKG2A and NKG2D and their descriptions, respectively).

A "derivative" of an interdomain or stalk region, or fragment thereof, of a type II C-lectin or CD molecule refers to an about an eight to about 150 amino acid sequence in which one, two, or three amino acids of the stalk

region of a wild type type II C-lectin or CD molecule have a deletion, insertion, substitution, or any combination thereof, preferably the one or more changes are substitutions or the one or more mutations include only one deletion. In further embodiments, a derivative of an interdomain or stalk region is more resistant to proteolytic cleavage as compared to the wild-type interdomain or stalk region sequence, such as those derived from about eight to about 20 amino acids of NKG2A, NKG2D, CD23, CD64, CD72, or CD94.

In certain embodiments, interdomain or stalk region hinges have seven to 18 amino acids and can form an α-helical coiled coil structure. In certain embodiments, interdomain or stalk region hinges contain 0, 1, 2, 3, or 4 cysteines. Exemplary interdomain or stalk region hinges are peptide fragments of the interdomain or stalk regions, such as ten to 150 amino acid fragments from the stalk regions of CD69, CD72, CD94, NKG2A and NKG2D, as set forth in SEQ ID NOS:858-864. Additional exemplary stalk region or interdomain hinges useful in the fusion proteins of this disclosure include those as set forth in SEQ ID NOS:990-999, 907-931, and 933.

In certain embodiments, hinge sequences have 5 to 150 amino acids, 5 to 10 amino acids, 10 to 20 amino acids, 20 to 30 amino acids, 30 to 40 amino acids, 40 to 50 amino acids, 50 to 60 amino acids, 5 to 60 amino acids, 5 to 40 amino acids, preferably have 8 to 20, more preferably have 10 to 15 amino acids, and may be primarily flexible, but may also provide more rigid characteristics or may contain primarily α-helical structure with minimal β-sheet structure. The lengths or the sequences of the hinges may affect the binding affinities of the binding domains to which the hinges are directly or indirectly (via another region or domain) connected as well as one or more activities of the Fc region portions to which the hinges are directly or indirectly connected.

Preferably, hinge sequences are stable in plasma and serum and are resistant to proteolytic cleavage. In certain embodiments, the first lysine in the IgG1 upper hinge region is mutated to minimize proteolytic cleavage, preferably the lysine is substituted with methionine, threonine, alanine or glycine, or is deleted (*see, e.g.*, SEQ ID NOS:934-989, which may include junction amino acids at the amino terminus, preferably RT). Additional exemplary hinges are set forth in SEQ ID NOS:1000-1106.

In certain embodiments where a single chain polypeptide comprises a binding domain at or near its carboxyl terminus, a hinge may be present to link the binding domain with another portion of the single chain polypeptide (*e.g.*, an Fc region portion or other domain). Preferably, such a

hinge is a non-immunoglobulin hinge (*i.e.*, a hinge not based on or derived from a wild type immunoglobulin hinge) and may be a stalk region of a type II C-lectin or CD molecule, an interdomain region that connect IgV-like or IgC-like domains of a cell surface receptor, or a derivative or functional variant thereof. Exemplary carboxyl terminal hinges, sometimes referred to as "back-end" hinges, includes those set forth in SEQ ID NOS:990-999, 907-931, and 933.

In one aspect, fusion proteins of this disclosure comprise a TNF-α antagonist as described elsewhere herein (TNF-α binding domain), IL6, IL6R, or IL6xR, in the form of a SMIP protein. Methods for making SMIP proteins are described herein and are known in the art (*see* U.S. Patent Publication Nos. 2003/0133939, 2003/0118592, and 2005/0136049). In certain embodiments, a fusion protein has a polypeptide binding domain specific for TNF-α or for an IL6xR complex that binds to the IL6xR with a higher affinity than either IL6 or IL6Rα alone, and competes with gp130 for binding to the sIL6xR complex or enhances gp130 binding with sIL6xR, wherein the fusion protein comprises from amino-terminus to carboxy-terminus, (a) the polypeptide binding domain, fused to (b) a first linker comprising a hinge region polypeptide, fused to (b) an immunoglobulin heavy chain CH2 constant region or sub-region polypeptide, fused to (c) an immunoglobulin heavy chain CH3 constant region or sub-region polypeptide. Alternatively, a SMIP protein structure can be illustrated as follows: N-BD-L1-CH2CH3-C, wherein N is the amino-terminus of the fusion protein, BD is the anti-IL6xR complex binding domain or scFv, L1 is a linker, CH2 and CH3 are immunoglobulin constant heavy regions 2 and 3, and C is the carboxy-terminus of the fusion protein. In some embodiments, the linker is a $(Gly_4Ser)_n$ wherein n is an integer of 1 to 6, such as Linker 46 (SEQ ID NO:542), or the linker is an IgG1, IgA or IgE hinge region, a mutant IgG1 hinge region having zero, one, or two cysteine residues, such as Linker 47 (SEQ ID NO:543), or Linker 80 (SEQ ID NO:576). In some embodiments, the fusion protein will be fused, via linker or not, to a domain other than an immunoglobulin constant region or sub-region so that the fusion protein remains primarily or substantially a single chain polypeptide in a composition.

In further embodiments, a SMIP fusion protein of this disclosure has a binding domain that comprises a light chain variable region containing CDR1, CDR2, and CDR3 sequences that are each at least 80% to 100% identical to at least one light chain variable region CDR1, CDR2, and CDR3 as set forth in any one of SEQ ID NOS:373-434 and 799-804, respectively, wherein each CDR has from zero to three amino acid changes, and comprises

a heavy chain variable region containing CDR1, CDR2, and CDR3 sequences that are each at least 80% to 100% identical to at least one heavy chain variable region CDR1, CDR2, and CDR3 as set forth in any one of SEQ ID NOS:435-496 and 805-810, respectively, wherein each CDR has from zero to three amino acid changes. In still further embodiments, a SMIP fusion protein of this disclosure has an amino acid sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an amino acid sequence set forth in any one of SEQ ID NOS:671-694, with or without a leader peptide sequence.

In still other embodiments, SMIP polypeptides can have a binding region or domain that is an IL6 antagonist, wherein IL6 cis- and trans-signaling is measurably inhibited. In certain embodiments, an IL6 antagonist according to this disclosure does not inhibit signaling of gp130 family cytokines other than IL6.

In further embodiments, fusion proteins of this disclosure comprise an IL6 antagonist binding domain in the form of a PIMS protein wherein the binding domain is disposed at the carboxy-terminus of the fusion protein. Constructs and methods for making PIMS proteins are described in PCT Publication No. WO 2009/023386. In general, a PIMS molecule is a single-chain polypeptide comprising, in amino-terminal to carboxy-terminal orientation, an intervening domain (*e.g.*, an immunoglobulin constant sub-region derived from that includes a CH2 and CH3 domain from the same (preferred) or different animal species, immunoglobulin isotype and/or immunoglobulin sub-class), a linker peptide (*e.g.*, an immunoglobulin hinge region or stalk region of type II C-lectin), and a specific binding domain. In some embodiments, a PIMS molecule further contains an amino-terminally disposed immunoglobulin hinge region, and the amino-terminal hinge region may be the same as, or different than, the linker found between the dimerization domain and the binding domain. In some embodiments, an amino-terminally disposed linker contains a naturally occurring or added motif (such as CPPC) to promote the formation of at least one disulfide bond to stabilize the amino-terminus of a multimerized molecule. Thus, exemplary schematic organizations of some PIMS molecules include N-dimerization domain-linker-binding domain-C or N-hinge linker-dimerization domain-linker-binding domain-C. In some embodiments, the fusion protein will have an intervening domain wherein the fusion protein remains primarily or substantially as a single chain

polypeptide in a composition or is found primarily or substantially as a dimer in a composition.

In certain embodiments, a fusion protein has a IL6 antagonist polypeptide binding domain that binds an IL6xR complex with a higher affinity than either IL6 or IL6Rα alone, and competes with gp130 for binding to the sIL6xR complex or enhances gp130 binding to sIL6xR complex, wherein, from carboxy-terminus to amino-terminus, (a) the polypeptide binding domain is fused to a first linker, (b) the first linker is fused to an immunoglobulin heavy chain CH3 constant region or sub-region polypeptide, and (c) the CH3 constant region or sub-region polypeptide is fused to an immunoglobulin heavy chain CH2 constant region or sub-region polypeptide, and (d) the CH2 constant region or sub-region polypeptide is fused to a second linker. In further embodiments, a PIMS fusion protein of this disclosure has a binding domain that comprises a light chain variable region containing CDR1, CDR2, and CDR3 sequences that are each at least 80% to 100% identical to at least one light chain variable region CDR1, CDR2, and CDR3 as set forth in any one of SEQ ID NOS:373-434 and 799-804, respectively, wherein each CDR has from zero to three amino acid changes, and comprises a heavy chain variable region containing CDR1, CDR2, and CDR3 sequences that are each at least 80% to 100% identical to at least one heavy chain variable region CDR1, CDR2, and CDR3 as set forth in any one of SEQ ID NOS:435-496 and 805-810, respectively, wherein each CDR has from zero to three amino acid changes.

Polynucleotides, Expression Vectors, and Host Cells

This disclosure provides polynucleotides (isolated or purified or pure polynucleotides) encoding the fusion proteins of this disclosure, vectors (including cloning vectors and expression vectors) comprising such polynucleotides, and cells (*e.g.*, host cells) transformed or transfected with a polynucleotide or vector according to this disclosure.

In certain embodiments, a polynucleotide (DNA or RNA) encoding a binding domain of this disclosure, or a fusion protein containing one or more such binding domains is contemplated. Expression cassettes encoding SMIP, PIMS or other fusion constructs are provided in the examples appended hereto.

The present disclosure also relates to vectors that include a polynucleotide of this disclosure and, in particular, to recombinant expression constructs. In one embodiment, this disclosure contemplates a vector comprising a polynucleotide encoding a binding domain of this disclosure or a

polypeptide comprising such a binding domain, *e.g.*, a SMIP, PIMS, SCORPION, Xceptor or other mono, bi- or multi-functional fusion protein, along with other polynucelotide sequences that cause or facilitate transcription, translation, and processing of such binding domain-encoding sequences.

Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described, for example, in Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, NY, (1989). Exemplary cloning/expression vectors include cloning vectors, shuttle vectors, and expression constructs, that may be based on plasmids, phagemids, phasmids, cosmids, viruses, artificial chromosomes, or any nucleic acid vehicle known in the art suitable for amplification, transfer, and/or expression of a polynucleotide contained therein

As used herein, "vector" means a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. Exemplary vectors include plasmids, yeast artificial chromosomes, and viral genomes. Certain vectors can autonomously replicate in a host cell, while other vectors can be integrated into the genome of a host cell and thereby are replicated with the host genome. In addition, certain vectors are referred to herein as "recombinant expression vectors" (or simply, "expression vectors"), which contain nucleic acid sequences that are operatively linked to an expression control sequence and, therefore, are capable of directing the expression of those sequences.

In certain embodiments, expression constructs are derived from plasmid vectors. Illustrative constructs include modified pNASS vector (Clontech, Palo Alto, CA), which has nucleic acid sequences encoding an ampicillin resistance gene, a polyadenylation signal and a T7 promoter site; pDEF38 and pNEF38 (CMC ICOS Biologics, Inc.), which have a CHEF1 promoter; and pD18 (Lonza), which has a CMV promoter. Other suitable mammalian expression vectors are well known (*see, e.g.*, Ausubel *et al.*, 1995; Sambrook *et al.*, supra; *see also*, *e.g.*, catalogs from Invitrogen, San Diego, CA; Novagen, Madison, WI; Pharmacia, Piscataway, NJ). Useful constructs may be prepared that include a dihydrofolate reductase (DHFR)-encoding sequence under suitable regulatory control, for promoting enhanced production levels of the fusion proteins, which levels result from gene amplification following application of an appropriate selection agent (*e.g.*, methotrexate).

Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell,

and a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence, as described above. A vector in operable linkage with a polynucleotide according to this disclosure yields a cloning or expression construct. Exemplary cloning/expression constructs contain at least one expression control element, *e.g.*, a promoter, operably linked to a polynucleotide of this disclosure. Additional expression control elements, such as enhancers, factor-specific binding sites, terminators, and ribosome binding sites are also contemplated in the vectors and cloning/expression constructs according to this disclosure. The heterologous structural sequence of the polynucleotide according to this disclosure is assembled in appropriate phase with translation initiation and termination sequences. Thus, for example, the fusion protein-encoding nucleic acids as provided herein may be included in any one of a variety of expression vector constructs as a recombinant expression construct for expressing such a protein in a host cell.

The appropriate DNA sequence(s) may be inserted into a vector, for example, by a variety of procedures. In general, a DNA sequence is inserted into an appropriate restriction endonuclease cleavage site(s) by procedures known in the art. Standard techniques for cloning, DNA isolation, amplification and purification, for enzymatic reactions involving DNA ligase, DNA polymerase, restriction endonucleases and the like, and various separation techniques are contemplated. A number of standard techniques are described, for example, in Ausubel *et al.* (1993 Current Protocols in Molecular Biology, Greene Publ. Assoc. Inc. & John Wiley & Sons, Inc., Boston, MA); Sambrook *et al.* (1989 Molecular Cloning, Second Ed., Cold Spring Harbor Laboratory, Plainview, NY); Maniatis *et al.* (1982 Molecular Cloning, Cold Spring Harbor Laboratory, Plainview, NY); Glover (Ed.) (1985 DNA Cloning Vol. I and II, IRL Press, Oxford, UK); Hames and Higgins (Eds.), (1985 Nucleic Acid Hybridization, IRL Press, Oxford, UK); and elsewhere.

The DNA sequence in the expression vector is operatively linked to at least one appropriate expression control sequence (*e.g.*, a constitutive promoter or a regulated promoter) to direct mRNA synthesis. Representative examples of such expression control sequences include promoters of eukaryotic cells or their viruses, as described above. Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and

promoter is well within the level of ordinary skill in the art, and preparation of certain particularly preferred recombinant expression constructs comprising at least one promoter or regulated promoter operably linked to a nucleic acid encoding a protein or polypeptide according to this disclosure is described herein.

Variants of the polynucleotides of this disclosure are also contemplated. Variant polynucleotides are at least 90%, and preferably 95%, 99%, or 99.9% identical to one of the polynucleotides of defined sequence as described herein, or that hybridizes to one of those polynucleotides of defined sequence under stringent hybridization conditions of 0.015M sodium chloride, 0.0015M sodium citrate at about 65-68ºC or 0.015M sodium chloride, 0.0015M sodium citrate, and 50% formamide at about 42ºC. The polynucleotide variants retain the capacity to encode a binding domain or fusion protein thereof having the functionality described herein.

The term "stringent" is used to refer to conditions that are commonly understood in the art as stringent. Hybridization stringency is principally determined by temperature, ionic strength, and the concentration of denaturing agents such as formamide. Examples of stringent conditions for hybridization and washing are 0.015M sodium chloride, 0.0015M sodium citrate at about 65-68°C or 0.015M sodium chloride, 0.0015M sodium citrate, and 50% formamide at about 42°C (*see* Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. 1989).

More stringent conditions (such as higher temperature, lower ionic strength, higher formamide, or other denaturing agent) may also be used; however, the rate of hybridization will be affected. In instances wherein hybridization of deoxyoligonucleotides is concerned, additional exemplary stringent hybridization conditions include washing in 6x SSC, 0.05% sodium pyrophosphate at 37°C (for 14-base oligonucleotides), 48°C (for 17-base oligonucleotides), 55°C (for 20-base oligonucleotides), and 60°C (for 23-base oligonucleotides).

A further aspect of this disclosure provides a host cell transformed or transfected with, or otherwise containing, any of the polynucleotides or vector/expression constructs of this disclosure. The polynucleotides or cloning/expression constructs of this disclosure are introduced into suitable cells using any method known in the art, including transformation, transfection and transduction. Host cells include the cells of a subject undergoing ex vivo

cell therapy including, for example, ex vivo gene therapy. Eukaryotic host cells contemplated as an aspect of this disclosure when harboring a polynucleotide, vector, or protein according to this disclosure include, in addition to a subject's own cells (*e.g.*, a human patient's own cells), VERO cells, HeLa cells, Chinese hamster ovary (CHO) cell lines (including modified CHO cells capable of modifying the glycosylation pattern of expressed multivalent binding molecules, *see* US Patent Application Publication No. 2003/0115614), COS cells (such as COS-7), W138, BHK, HepG2, 3T3, RIN, MDCK, A549, PC12, K562, HEK293 cells, HepG2 cells, N cells, 3T3 cells, *Spodoptera frugiperda* cells (*e.g.*, Sf9 cells), *Saccharomyces cerevisiae* cells, and any other eukaryotic cell known in the art to be useful in expressing, and optionally isolating, a protein or peptide according to this disclosure. Also contemplated are prokaryotic cells, including *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhimurium*, a Streptomycete, or any prokaryotic cell known in the art to be suitable for expressing, and optionally isolating, a protein or peptide according to this disclosure. In isolating protein or peptide from prokaryotic cells, in particular, it is contemplated that techniques known in the art for extracting protein from inclusion bodies may be used. The selection of an appropriate host is within the scope of those skilled in the art from the teachings herein. Host cells that glycosylate the fusion proteins of this disclosure are contemplated.

The term "recombinant host cell" (or simply "host cell") refers to a cell containing a recombinant expression vector. It should be understood that 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.

A recombinant host cells can be cultured in a conventional nutrient medium modified as appropriate for activating promoters, selecting transformants, or amplifying particular genes. The culture conditions for particular host cells selected for expression, such as temperature, pH and the like, will be readily apparent to the ordinarily skilled artisan. Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman (1981) Cell 23:175, and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors will

comprise an origin of replication, a suitable promoter and, optionally, enhancer, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5′-flanking nontranscribed sequences, for example, as described herein regarding the preparation of multivalent binding protein expression constructs. DNA sequences derived from the SV40 splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements. Introduction of the construct into the host cell can be effected by a variety of methods with which those skilled in the art will be familiar, including calcium phosphate transfection, DEAE-Dextran-mediated transfection, or electroporation (Davis *et al*. (1986) Basic Methods in Molecular Biology).

In one embodiment, a host cell is transduced by a recombinant viral construct directing the expression of a protein or polypeptide according to this disclosure. The transduced host cell produces viral particles containing expressed protein or polypeptide derived from portions of a host cell membrane incorporated by the viral particles during viral budding.

Compositions and Methods of Use

To treat human or non-human mammals suffering a disease state associated with TNF-α and IL6 dysregulation, a composition comprising a TNF-α antagonist binding protein and an IL6 antagonist binding protein of this disclosure is administered to the subject in an amount that is effective to ameliorate symptoms of the disease state following a course of one or more administrations. Being polypeptides, the binding proteins of this disclosure can be suspended or dissolved in a pharmaceutically acceptable diluent, optionally including a stabilizer of other pharmaceutically acceptable excipients, which can be used for intravenous administration by injection or infusion, as more fully discussed below.

A pharmaceutically effective dose is that dose required to prevent, inhibit the occurrence of, or treat (alleviate a symptom to some extent, preferably all symptoms of) a disease state. The pharmaceutically effective dose depends on the type of disease, the composition used, the route of administration, the type of subject being treated, the physical characteristics of the specific subject under consideration for treatment, concurrent medication, and other factors that those skilled in the medical arts will recognize. For example, an amount between 0.1 mg/kg and 100 mg/kg body weight (which can be administered as a single dose, or in multiple doses given hourly, daily,

weekly, monthly, or any combination thereof that is an appropriate interval) of active ingredient may be administered depending on the potency of a binding domain polypeptide or multi-specific protein fusion of this disclosure.

In certain aspects, compositions of antagonist binding proteins are provided by this disclosure. Compositions of this disclosure generally comprise one or more type of binding domain protein in combination with a pharmaceutically acceptable carrier, excipient, or diluent. Such carriers will be nontoxic to recipients at the dosages and concentrations employed. Pharmaceutically acceptable carriers for therapeutic use are well known in the pharmaceutical art, and are described, for example, in Remington's Pharmaceutical Sciences, Mack Publishing Co. (A.R. Gennaro (Ed.) 1985). For example, sterile saline and phosphate buffered saline at physiological pH may be used. Preservatives, stabilizers, dyes and the like may be provided in the pharmaceutical composition. For example, sodium benzoate, sorbic acid, or esters of *p*-hydroxybenzoic acid may be added as preservatives. *Id.* at 1449. In addition, antioxidants and suspending agents may be used. *Id.* The compounds of the present disclosure may be used in either the free base or salt forms, with both forms being considered as being within the scope of the present disclosure.

Pharmaceutical compositions may also contain diluents such as buffers, antioxidants such as ascorbic acid, low molecular weight (less than about 10 residues) polypeptides, proteins, amino acids, carbohydrates (*e.g.*, glucose, sucrose, or dextrins), chelating agents (*e.g.*, EDTA), glutathione or other stabilizers or excipients. Neutral buffered saline or saline mixed with nonspecific serum albumin are exemplary appropriate diluents. Preferably, product is formulated as a lyophilizate using appropriate excipient solutions as diluents.

In certain embodiments, cis-signaling of IL6 is minimally or not inhibited, *i.e.*, any inhibition of cis-signaling is not substantial, meaning that inhibition is non-existent, asymptomatic, or not detectable. The extent of inhibition of IL6 trans-signaling can vary, but in general trans-signaling is altered to an extent that has a positive effect on symptoms of a disease state mediated by or associated with such signaling. In certain embodiments, inhibition of trans-signaling of IL6 by binding domain polypeptides or fusion proteins thereof of this disclosure can retard, stop, or reverse disease progression.

Compositions of this disclosure can be used to treat disease states in human and non-human mammals that are mediated by TNF-α and IL6 signaling.

Increased production of IL-6, and thus IL-6 signaling, has been implicated in various disease processes, including Alzheimer's disease, autoimmunity (*e.g.*, rheumatoid arthritis, SLE), inflammation, myocardial infarction, Paget's disease, osteoporosis, solid tumors (*e.g.*, colon cancer, RCC prostatic and bladder cancers), certain neurological cancers, B-cell malignancies (*e.g.*, Castleman's disease, some lymphoma subtypes, chronic lymphocytic leukemia, and, in particular, malignant melanoma). In some instances, IL-6 is implicated in proliferation pathways because it acts with other factors, such as heparin-binding epithelial growth factor and hepatocyte growth factor (*see*, *e.g.*, Grant *et al*. (2002) Oncogene 21:460; Badache and Hynes (2001) Cancer Res. 61:383; Wang *et al*. (2002) Oncogene 21:2584). Similarly, the TNF superfamily is known to be involved in a variety of disorders, such as cancer (tumorigenesis, including proliferation, migration, metastasis), autoimmunity (SLE, diabetes), chronic heart failure, bone resorption, and atherosclerosis, to name a few (*see*, *e.g.*, Aggarwal (2003) Nature Rev. 3:745; Lin *et al*. (2008) Clin. Immunol. 126:13).

Compositions comprising the antagonist binding domains of this disclosure are useful in treating autoimmune and other disorders including, rheumatoid arthritis, psoriasis, systemic lupus erythematosus (SLE), and inflammatory bowel disease (IBD), including Crohn's disease and ulcerative colitis.

Compositions comprising the TNF-α antagonist binding protein and the IL6 antagonist binding protein of this disclosure may be administered simultaneously (in the same formulation or concurrently in separate formulations, with concurrently referring to agents administered within 30 minutes of each other) or sequentially (from 30 minutes up to one week following administration of the first antagonist binding protein).

Also contemplated is the administration of binding protein compositions of this disclosure in combination with a second agent. A second agent may be one accepted in the art as a standard treatment for a particular disease state, such as inflammation and autoimmunity. Exemplary second agents contemplated include cytokines, growth factors, steroids, NSAIDs, DMARDs, chemotherapeutics, radiotherapeutics, or other active and ancillary agents, or any combination thereof.

"Pharmaceutically acceptable salt" refers to a salt of a binding domain polypeptide or fusion protein of this disclosure that is pharmaceutically acceptable and that possesses the desired pharmacological activity of the parent compound. Such salts include the following: (1) acid addition salts, formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like; or formed with organic acids such as acetic acid, propionic acid, hexanoic acid, cyclopentanepropionic acid, glycolic acid, pyruvic acid, lactic acid, malonic acid, succinic acid, malic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, 3-(4-hydroxybenzoyl)benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, 1,2-ethane-disulfonic acid, 2-hydroxyethanesulfonic acid, benzenesulfonic acid, 4-chlorobenzenesulfonic acid, 2-naphthalenesulfonic acid, 4-toluenesulfonic acid, camphorsulfonic acid, 4-methylbicyclo[2.2.2]-oct-2-ene-1-carboxylic acid, glucoheptonic acid, 3-phenylpropionic acid, trimethylacetic acid, tertiary butylacetic acid, lauryl sulfuric acid, gluconic acid, glutamic acid, hydroxynaphthoic acid, salicylic acid, stearic acid, muconic acid, and the like; or (2) salts formed when an acidic proton present in the parent compound either is replaced by a metal ion, *e.g.*, an alkali metal ion, an alkaline earth ion, or an aluminum ion; or coordinates with an organic base such as ethanolamine, diethanolamine, triethanolamine, N-methylglucamine, or the like.

In particular illustrative embodiments, a binding protein of this disclosure is administered intravenously by, for example, bolus injection or infusion. Routes of administration in addition to intravenous include oral, topical, parenteral (*e.g.*, sublingually or buccally), sublingual, rectal, vaginal, intranasal, and perispinal. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intrasternal, intracavernous, intrathecal, intrameatal, intraurethral injection or infusion techniques. The pharmaceutical composition is formulated so as to allow the active ingredients contained therein to be bioavailable upon administration of the composition to a patient. Compositions that will be administered to a patient take the form of one or more dosage units, where for example, a tablet may be a single dosage unit, and a container of one or more compounds of this disclosure in aerosol form may hold a plurality of dosage units.

For oral administration, an excipient and/or binder may be present, such as sucrose, kaolin, glycerin, starch dextrans, cyclodextrins, sodium alginate, ethyl cellulose, and carboxy methylcellulose. Sweetening

agents, preservatives, dye/colorant, flavor enhancer, or any combination thereof may optionally be present. A coating shell may also optionally be used.

In a composition intended to be administered by injection, one or more of a surfactant, preservative, wetting agent, dispersing agent, suspending agent, buffer, stabilizer, isotonic agent, or any combination thereof may optionally be included.

For nucleic acid-based formulations, or for formulations comprising expression products according to this disclosure, about 0.01 μg/kg to about 100 mg/kg body weight will be administered, for example, by the intradermal, subcutaneous, intramuscular, or intravenous route, or by any route known in the art to be suitable under a given set of circumstances. A preferred dosage, for example, is about 1 μg/kg to about 20 mg/kg, with about 5 μg/kg to about 10 mg/kg particularly preferred. It will be evident to those skilled in the art that the number and frequency of administration will be dependent upon the response of the host.

The pharmaceutical compositions of this disclosure may be in any form that allows for administration to a patient, such as, for example, in the form of a solid, liquid, or gas (aerosol). The composition may be in the form of a liquid, *e.g.*, an elixir, syrup, solution, emulsion or suspension, for administration by any route described herein.

A liquid pharmaceutical composition as used herein, whether in the form of a solution, suspension or other like form, may include one or more of the following components: sterile diluents such as water for injection, saline solution (*e.g.*, physiological saline), Ringer's solution, isotonic sodium chloride, fixed oils such as synthetic mono- or digylcerides that may serve as the solvent or suspending medium, polyethylene glycols, glycerin, propylene glycol or other solvents; antibacterial agents such as benzyl alcohol or methyl paraben; antioxidants such as ascorbic acid or sodium bisulfite; buffers such as acetates, citrates or phosphates; chelating agents such as ethylenediaminetetraacetic acid; and agents for the adjustment of tonicity such as sodium, chloride, or dextrose. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic. Physiological saline is a preferred additive. An injectable pharmaceutical composition is preferably sterile.

It may also be desirable to include other components in the preparation, such as delivery vehicles including aluminum salts, water-in-oil emulsions, biodegradable oil vehicles, oil-in-water emulsions, biodegradable

microcapsules, and liposomes. Examples of adjuvants for use in such vehicles include N-acetylmuramyl-L-alanine-D-isoglutamine (MDP), lipopolysaccharides (LPS), glucan, IL-12, GM-CSF, γ-interferon, and IL-15.

While any suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions of this disclosure, the type of carrier will vary depending on the mode of administration and whether a sustained release is desired. For parenteral administration, the carrier may comprise water, saline, alcohol, a fat, a wax, a buffer, or any combination thereof. For oral administration, any of the above carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, magnesium carbonate, or any combination thereof, may be employed.

This disclosure contemplates a dosage unit comprising a pharmaceutical composition of this disclosure. Such dosage units include, for example, a single-dose or a multi-dose vial or syringe, including a two-compartment vial or syringe, one comprising the pharmaceutical composition of this disclosure in lyophilized form and the other a diluent for reconstitution. A multi-dose dosage unit can also be, *e.g.*, a bag or tube for connection to an intravenous infusion device.

This disclosure also contemplates a kit comprising a pharmaceutical composition in a unit dose or multi-dose container, *e.g.*, a vial, and a set of instructions for administering the composition to patients suffering a disorder as described herein.

All U.S. patents, U.S. patent application publications, U.S. patent applications, foreign patents, foreign patent applications, non-patent publications, tables, sequences, webpages, or the like referred to in this specification, are incorporated herein by reference, in their entirety. The following examples are intended to illustrate, but not limit, this disclosure.

EXAMPLES

Sequences:

Amino acid sequences of exemplary SMIP molecules having an anti-IL6xR binding domain are provided in SEQ ID NOS:671-694, with the corresponding nucleotide expression cassettes of the fusion proteins being provided in SEQ ID NOS:761-784 (note the mature proteins will lack the signal peptide sequence found in SEQ ID NOS:671-694). Specifically, SMIP fusion proteins TRU(S6)-1004, 1007, 1008, 1013, 1018, 1019, 1029, and 1038 are provided in SEQ ID NOs:672, 673, 674, 676, 678, 679, 684 and 685, respectively.

Amino acid sequences of exemplary TNF-α antagonists comprise amino acids 23-257 of SEQ ID NO:846 or amino acids 31-211 of SEQ ID NO:847 (with or without the native leader peptide sequence included in these sequence).

EXAMPLE 1

PRETREATMENT OF MICE WITH TNF-α ANTAGONIST AND ANTI-MIL-6 BLOCKS EARLY BIOMARKER RESPONSE TO EXOGENOUS MTNF-α MORE POTENTLY THAN PRETREATMENT OF MICE WITH EITHER ALONE

In this Example, A2-XT6 (a multi-target binding molecule with the TNFR extracellular domain (ECD) at the N-terminal end and the anti-hyperIL6 scFv at the carboxy-terminal end; SEQ ID NO:608) plus anti-mIL-6 antibody - mediated blockade of biomarker response in normal BALB/c mice was investigated. Note that A2-XT6 binds to human IL6 but does not bind to murine IL6. Accordingly, in this example, this molecule functions as a TNF-α antagonist only.

The ability of TNF-α and IL-6 antagonist proteins disclosed herein to reduce TNF-α-induced production of serum amyloid A (SAA) protein in mice was examined as described below. Serum amyloid A (SAA) protein is one of the major acute-phase proteins in humans and mice. Prolonged elevation of plasma SAA levels is found in chronic inflammation and leads to amyloidosis which affects the liver, kidney and spleen (Rienhoff *et al.* (1990) Mol. Biol. Med. 7:287). Both IL-6 and TNF have been shown to induce SAA when administered alone (Benigni *et al.*, (1996) Blood 87:1851; Ramadori *et al.*, (1988) Eur. J. Immunol. 18:1259).

Experiments were designed as outlined below in Table 1 to determine whether pretreatment of mice with A2-XT6 and anti-mIL-6 antibody given in combination can block the biomarker response to exogenous mTNF-α more potently than pretreatment of mice with A2-XT6 only.

Table 1: Study Design

Group	Retro-orbital Injection At T= -1hr	IV Dose (ug)		IP Injection At T= 0 hr	IP Dose (ug)		Timepoint (hr after mTNF-a injection)	# mice
G-1	PBS	NA		PBS	NA		2hr & 24 hr	4+4
G-2	PBS	NA		mTNF-a	0.5		2hr & 24 hr	4+4
G-3	A2-XT6	300		mTNF-a	0.5		2hr & 24 hr	4+4
G-4	A2-XT6 + anti-mIL-6 Ab	300+200		mTNF-a	0.5		2hr & 24 hr	4+4
G-5	ENBREL®	200		mTNF-a	0.5		2hr & 24 hr	4+4
G-6	ENBREL® + anti-mIL-6 Ab	200+200		mTNF-a	0.5		2hr & 24 hr	4+4
G-7	Anti-mIL-6 Ab	200		mTNF-a	0.5		2hr & 24 hr	4+4
								56

Mice were female BALB/C; 12.5 weeks of age

Readouts were mouse serum amyloid A (SAA) ELISA (see Figures 1A and 1B). Other readouts may also be carried out, such as: Mouse Cytokine/Chemokine Luminex Assay (2hr timepoint only); (TNF-α, Eotaxin, G-CSF, IL-1a, IL-6, IP-10, KC. MCP-1, MIP-1B, MIG, and RANTES); Mouse Soluble Cytokine Receptor Luminex Assay; (sIL-1R1, sIL-6R, sgp130, sTNFR1, and sTNFR2).

The following reagents were used for these experiments: ENBREL®: Amgen/Wyeth, Lot# P109374, 25mg/0.5mL; A2-XT6: Trubion, Zeu080a, Lot#867MBD090505, and endotoxin level: <0.051 EU/mg; Anti-mIL-6 antibody (clone:MP520F3): R&D, cat#MAB406, and endotoxin level: 0.33 EU/mg; Mouse TNF-α: R&D, Cat#410-MT/CF, Lot# CS080807A, and endotoxin level: 3.12 EU/mg; PBS: Gibco, Cat#14190-136.

As shown in Figure 1, panel A, pretreatment of mice with A2-XT6 and anti-mIL-6 antibody given in combination results in lower SAA than in mice

pretreated with either antagonist alone, indicating that such pretreatment can block the biomarker response to exogenous mTNF-α more potently than pretreatment of mice with A2-XT6 or anti-mIL-6 antibody alone at 2 hours post-TNF-alpha injection. The block observed was more effective than ENBREL® alone and as effective as pretreatment with ENBREL® in combination with anti-mIL-6 antibody at 2 hours post-injection. Lower SAA levels were still observed at 24 hours post-TNF-alpha injection in mice pretreated with A2-XT6 and anti-mIL-6 antibody as compared to A2-XT6 alone. However, serum SAA levels at 24 hours post-injection for mice pretreated with anti-mIL-6 antibody alone were equivalent to those observed in mice pretreated with the combination of antagonists.

These results indicate that pretreatment with the combination of a TNF-α antagonist and an IL-6 antagonist provides an effective early block of the signaling response to exogenous TNF-α that is more potent than pretreatment with either antagonist alone. Accordingly, these results provide support for the use of the combination therapy in a variety of autoimmune settings associated with TNF-α and IL6, including rheumatoid arthritis, SLE, psoriasis, inflammatory bowel disease and other such disorders.

EXAMPLE 2

ANIMAL MODELS FOR RHEUMATOID ARTHRITIS

The therapeutic efficacy of any of the compositions comprising antagonist binding molecules disclosed herein is examined in at least one of two murine models of rheumatoid arthritis (RA), namely the collagen induced arthritis (CIA) and glucose-6-phosphate isomerase (G6PI) models. Each of these models has been shown to be useful for predicting efficacy of certain classes of therapeutic drugs in RA (*see* Holmdahl (2000) Arthritis Res. 2:169; Holmdahl (2006) Immunol. Lett. 103:86; Holmdahl (2007) Methods Mol. Med. 136:185; McDevitt, H. (2000) Arthritis Res. 2:85; Kamradt and Schubert (2005) Arthritis Res. Ther. 7:20).

(i) <u>CIA Model</u>

The CIA model is a well-characterized mouse model of arthritis in terms of its pathogenesis and immunological basis. In addition, it is a widely used model of RA and is an acceptable model to persons of skill in the art for investigating potential new therapeutics for RA (Jirholt *et al.* (2001) Arthritis Res. 3:87; Van den Berg, W.B. (2002) Curr. Rheumatol. Rep. 4:232; Rosloniec

(2003) Collagen-Induced Arthritis. In Current Protocols in Immunology, eds. Coligan *et al.*, John Wiley & Sons, Inc, Hoboken, NJ).

In the CIA model, arthritis is induced by immunization of male DBA/1 mice with collagen II (CII) in Complete Freund's Adjuvant (CFA). Specifically, mice are injected intradermally/ subcutaneously with CII in CFA on Day -21 and boosted with CII in Incomplete Freund's Adjuvant (IFA) on Day 0. Mice develop clinical signs of arthritis within days of the boost with CII/IFA. A subset of mice (0% to 10%) immunized with CII/CFA develop signs of arthritis on or around Day 0 without a boost and are excluded from the experiments. In some CIA experiments, the boost is omitted and mice are instead treated with Xceptor or control starting 21 days after immunization with CII/CFA (*i.e.* the day of first treatment is Day 0).

Mice are treated with the compositions described herein, vehicle (PBS), or negative or positive control in a preventative and/or therapeutic regimen. Preventative treatment starts on Day 0 and continues through the peak of disease in control (untreated) mice. Therapeutic treatment starts when the majority of mice show mild signs of arthritis. ENBREL®, which has been shown to have good efficacy in both the CIA and G6PI-induced models of arthritis, is used as a positive control. Data collected in every experiment includes clinical scores and cumulative incidence of arthritis. Clinical signs of arthritis in the CIA model are scored using a scale from 0 to 4 as shown in Table 2 below.

Table 2

Score	Observations
0	No apparent swelling or redness
1	Swelling/redness in one to three digits
2	Redness and/or swelling in more than three digits, mild swelling extending into the paw, swollen or red ankle, or mild swelling/redness of forepaw
3	Swollen paw with mild to moderate redness
4	Extreme redness and swelling in entire paw

(ii) G6PI Model

In the G6PI model, arthritis is induced by immunization of DBA/1 mice with G6PI in adjuvant (Kamradt and Schubert (2005) Arthritis Res. Ther. 7:20; Schubert *et al.*, (2004) J. Immunol. 172:4503; Bockermann, R. *et al.* (2005) Arthritis Res. Ther. 7:R1316; Iwanami *et al.*, (2008) Arthritis Rheum.

58:754; Matsumoto *et al.*, (2008) Arthritis Res. Ther. 10:R66). G6PI is an enzyme present in virtually all cells in the body and it is not known why immunization induces a joint specific disease. A number of agents, such as CTLA4-Ig, TNF antagonists (*e.g.* ENBREL®) and anti-IL6 receptor monoclonal antibody, have been shown to inhibit development of arthritis in the G6PI model.

Male DBA/1 mice are immunized with G6PI in Complete Freund's Adjuvant (CFA) in order to induce arthritis. Specifically, mice are injected intradermally/ subcutaneously with G6PI in CFA on Day 0 and develop clinical signs of arthritis within days of the immunization. As with the CIA model discussed above, mice are treated with antagonists as described herein, vehicle (PBS), or negative or positive control in a preventative and/or therapeutic regimen. Preventative treatment starts on Day 0 and continues through the peak of disease in control mice. Therapeutic treatment starts when the majority of mice show mild signs of arthritis. ENBREL®, which has been shown to have good efficacy in both the CIA and G6PI-induced models of arthritis, is used as a positive control. Data collected in every experiment includes clinical scores and cumulative incidence of arthritis. Clinical signs of arthritis in the G6PI model are scored using a scale similar to that employed for the CIA model.

EXAMPLE 3

IN VIVO EFFICACY OF TNF-α + IL-6 ANTAGONISTS IN A MURINE MODEL FOR RHEUMATOID ARTHRITIS

The G6PI model as described in Example 2 was used to study the *in vivo* efficacy of the combination of a TNF-α antagonist (ENBREL®) and an IL-6 antagonist (anti-mIL6 antibody) in reducing severity of arthritis symptoms.

The randomized and blinded study was designed as follows: on Day -3, ID and temperature transponders were implanted. On Day -1 mice were weighed, temperatures taken, and mice were randomized. On Day 0 mice were weighed, temperatures taken; indicated emulsion was injected (G6PI and CFA) subcutaneously in the base of the tail. After immunization, mice were injected with the indicated amount of protein (RITUXAN®, ENBREL®, anti-mouse IL-6 (20F3), or combination of ENBREL® and anti-mouse IL-6). See Table 3 below.

Table 3: IP Dosing

Group	Treatment	IP Dosing (μg)	Days	Number of mice
A	Untreated	---	---	5
B	RITUXAN®	154 QOD	0, 3, 5, 7, 10, 12, 14	12
C	ENBREL®	100 QOD	0, 3, 5, 7, 10, 12, 14	12
D		10 QOD	0, 3, 5, 7, 10, 12, 14	12
E	Anti-mouse IL-6 (20F3)	154 QOD	0, 3, 5, 7, 10, 12, 14	12
F		15.4 QOD	0, 3, 5, 7, 10, 12, 14	12
G	ENBREL® + anti-mouse IL-6	10 QOD (Enbrel®) 15.4 QOD (anti-mouse IL-6)	0, 3, 5, 7, 10, 12, 14	12
H	Unprimed (naïve)	---	---	5

Weights and temperatures were recorded 6 days per week. Arthritis was scored 3 times per week (or more if necessary). Arthritis scores were determined by grading each paw and adding the four scores together. Scores ranged from 0 (no arthritis) to 4 (severe swelling and redness). A mouse must have had a clinical score of 2 or more on two consecutive scoring sessions in order to have arthritis. A mouse was considered to have moderate to severe arthritis if it had a total paw score of 10 or more at any point during the observation period.

As shown in Figure 3, the mean level of arthritis observed in mice treated with the combination of ENBREL® and anti-mIL6 antibody was reduced as compared to control mice and was slightly lower than that observed in mice treated with 100μg ENBREL® alone. Similarly, as shown in Figures 4 and 5, mean body weight in mice treated with the combination of ENBREL® and anti-mIL6 antibody was maintained. The combination was as effective for preventing weight loss as 100μg ENBREL®. At earlier time points in the study (*e.g.*, Day 6), the combination treatment was more effective than 100μg ENBREL® at preventing weight loss.

The study results are summarized in Table 4 below. In particular, treatment with a combination of ENBREL® at 10μg and anti-mIL6 antibody significantly reduced the incidence of arthritis as compared to treatment with 100μg ENBREL® alone.

Table 4: G6PI Model Study Results – Summary

Group	Incidence of Arthritis (%)	p Value vs Control Group	Incidence of Moderate to Severe Arthritis (%)	p Value vs Control Group	Number of Mice Euthanized (Reason)
Untreated	4/5 (92%)	N/A	1/5 (20%)	N/A	1 Severe arthritis
RITUXAN®	11/12 (92%)	N/A	6/12 (50%)	N/A	4 Severe arthritis
ENBREL® (100 µg)	3/12 (25%)	**0.0028****	1/12 (8%)	0.0686	0
ENBREL® (10 µg)	11/12 (92%)	1.0000	3/12 (25%)	0.4003	2 Severe arthritis
Anti-mouse IL-6 (154 µg)	8/12 (67%)	0.3168	3/12 (25%)	0.4003	1 Severe arthritis
Anti-mouse IL-6 (15.4 µg)	11/12 (92%)	1.0000	2/12 (17%)	0.1930	1 Severe arthritis
ENBREL® (10 µg) + anti-mouse IL-6 (15.4 µg)	4/12 (33%)	**0.0094****	0/12 (0%)	**0.0137****	0

p Value is from a contingency analysis (Fisher's exact test) for indicated group versus the RITUXAN® group.

Thus, these studies show that treatment with a combination of TNF-α antagonist and IL6 antagonist is much more effective at reducing severity and incidence of arthritis when compared to either therapeutic administered alone in a well-established pre-clinical animal model. Such combination treatment may be effective for the treatment of a variety of autoimmune diseases associated with TNF-α and IL6 in humans.

From the foregoing it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

CLAIMS

What is claimed is:

1. A method for treating rheumatoid arthritis comprising administering to a subject in need thereof a therapeutically effective amount of a combination of an IL-6 antagonist and a TNF-α antagonist.

2. A method for treating SLE comprising administering to a subject in need thereof a therapeutically effective amount of a combination of an IL-6 antagonist and a TNF-α antagonist.

3. A method for treating psoriasis comprising administering to a subject in need thereof a therapeutically effective amount of a combination of an IL-6 antagonist and a TNF-α antagonist.

4. A method for treating inflammatory bowel disease comprising administering to a subject in need thereof a therapeutically effective amount of a combination of an IL6 antagonist and a TNF-α antagonist.

5. A method according to any one of claims 1-4 wherein the IL-6 antagonist comprises a binding domain specific for an IL6/IL6R (IL6xR) complex, said binding domain specific for IL6xR comprising an amino acid sequence at least 80% identical to one or more light chain variable regions as listed in SEQ ID NOs:373-434 and 799-804 and an amino acid sequence that is at least 80% identical to one or more of the heavy chain variable regions as listed in SEQ ID NOs:435-496 and 805-810.

6. The method according to any one of claims 1-4 wherein the TNF-α antagonist comprises a binding domain specific for TNF-α, wherein the binding domain specific for TNF-α comprises amino acids 23-257 of SEQ ID NO:846 or amino acids 31-211 of SEQ ID NO:847.

7. The method according to any one of claims 1-4 wherein the IL-6 antagonist comprises a binding domain specific for an IL6/IL6R (IL6xR) complex, said binding domain specific for IL6xR comprising an amino acid sequence at least 80% identical to one or more light chain variable regions as

listed in SEQ ID NOs:373-434 and 799-804 and an amino acid sequence that is at least 80% identical to one or more of the heavy chain variable regions as listed in SEQ ID NOs:435-496 and 805-810; and

wherein the TNF-α antagonist comprises a binding domain specific for TNF-α and comprising amino acids 23-257 of SEQ ID NO:846 or amino acids 31-211 of SEQ ID NO:847.

8. The method of any one of claims 5-7 wherein the IL-6 antagonist and TNF-α antagonist both comprise an antibody, or an antigen binding domain thereof, a Fab, or a scFv.

9. The method of any one of claims 5-7 wherein the IL-6 antagonist is an antibody and the TNF-α antagonist is a small modular immunopharmaceutical (SMIP) protein.

10. The method of any one of claims 5-7 wherein the IL-6 antagonist is a SMIP protein and the TNF-α antagonist is an antibody.

11. The method of any one of claims 5-7 wherein the IL-6 antagonist and the TNF-α antagonist are both SMIP proteins.

12. The method of claim 7 wherein the binding domain of the IL-6 antagonist and the TNF-α antagonist are an antibody or antigen binding domain thereof, a Fab, or a scFv.

13. The method of any one of claims 5 and 7wherein the binding domain of the IL-6 antagonist comprises a light chain variable region containing CDR1, CDR2, and CDR3 sequences that are each at least 80% identical to at least one light chain variable region CDR1, CDR2, and CDR3 as set forth in SEQ ID NOS:373-434 and 799-804, respectively.

14. The method of any one of claims 5 and 7 wherein the binding domain of the IL-6 antagonist comprises a heavy chain variable region containing CDR1, CDR2, and CDR3 sequences that are each at least 80% identical to at least one heavy chain variable region CDR1, CDR2, and CDR3 as set forth in SEQ ID NOS:435-496 and 805-810, respectively.

15. The method of any one of claims 5 and 7 wherein the binding domain of the IL-6 antagonist comprises a light chain variable region containing CDR1, CDR2, and CDR3 sequences that are each at least 80% identical to at least one light chain variable region CDR1, CDR2, and CDR3 as set forth in SEQ ID NOS:373-434 and 799-804, respectively, and comprises a heavy chain variable region containing CDR1, CDR2, and CDR3 sequences that are each at least 80% identical to at least one heavy chain variable region CDR1, CDR2, and CDR3 as set forth in SEQ ID NOS:435-496 and 805-810, respectively.

16. The method of any one of claims 5 and 7 wherein the IL6xR complex has an amino acid sequence as set forth in SEQ ID NO:606.

17. The method of claim 7 wherein the binding domains of the IL6 antagonist and the TNF-α antagonist are fused to (a) an immunoglobulin Fc domain or one or more CH domains of an immunoglobulin Fc domain, or (b) a serum protein binding protein.

18. The method of claim 17 wherein the one or more CH domains of an immunoglobulin Fc domain comprises a CH2 constant region and CH3 constant region, preferably IgG1 CH2 and CH3 domains.

19. The method of claim 18 wherein,

I) the IL6 antagonist comprises, from amino-terminus to carboxy-terminus, (a) the binding domain specific for IL6xR fused to a linker, (b) the linker fused to an immunoglobulin heavy chain CH2 constant region polypeptide, and (c) the CH2 constant region polypeptide fused to an immunoglobulin heavy chain CH3 constant region polypeptide; and

II) the TNF-α antagonist comprises, from amino-terminus to carboxy-terminus, (a) the binding domain specific for TNF-α fused to a linker, (b) the linker fused to an immunoglobulin heavy chain CH2 constant region polypeptide, and (c) the CH2 constant region polypeptide fused to an immunoglobulin heavy chain CH3 constant region polypeptide.

20. The method of claim 18 wherein,

I) the IL6 antagonist comprises, from carboxyl-terminus to amino-terminus, (a) the polypeptide binding domain specific for IL6xR fused to a first

linker, (b) the first linker fused to an immunoglobulin heavy chain CH3 constant region polypeptide, (c) the CH3 constant region polypeptide fused to an immunoglobulin heavy chain CH2 constant region polypeptide, and (d) the CH2 constant region polypeptide fused to a second linker; and

II) the TNF-α antagonist comprises, from carboxy-terminus to amino-terminus, (a) the binding domain specific for TNF-α fused to a first linker, (b) the first linker fused to an immunoglobulin heavy chain CH3 constant region polypeptide, (c) the CH3 constant region polypeptide fused to an immunoglobulin heavy chain CH2 constant region polypeptide, and (d) the CH2 constant region polypeptide fused to a second linker.

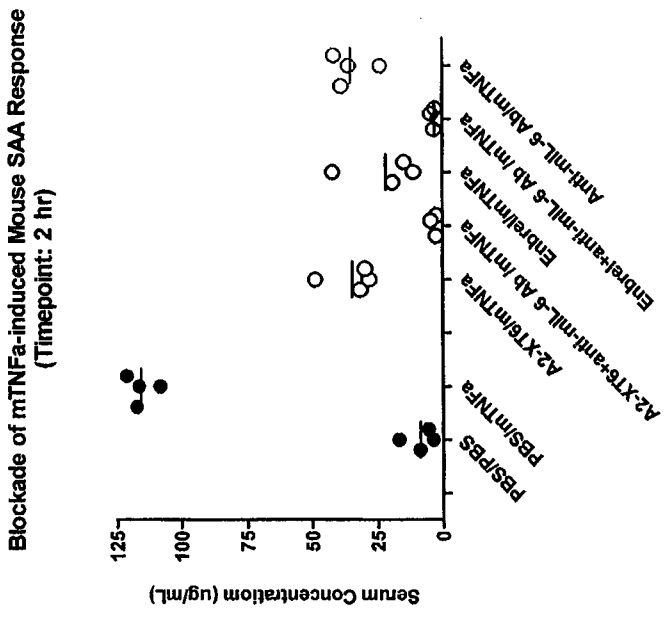
21. The method of claim 19 or 20 wherein the linker is an immunoglobulin hinge region polypeptide.

22. The method of claim 19 or 20 wherein the linker is selected from the group consisting of SEQ ID NO:497-604, 823-828, 830-845, and 851-1106.

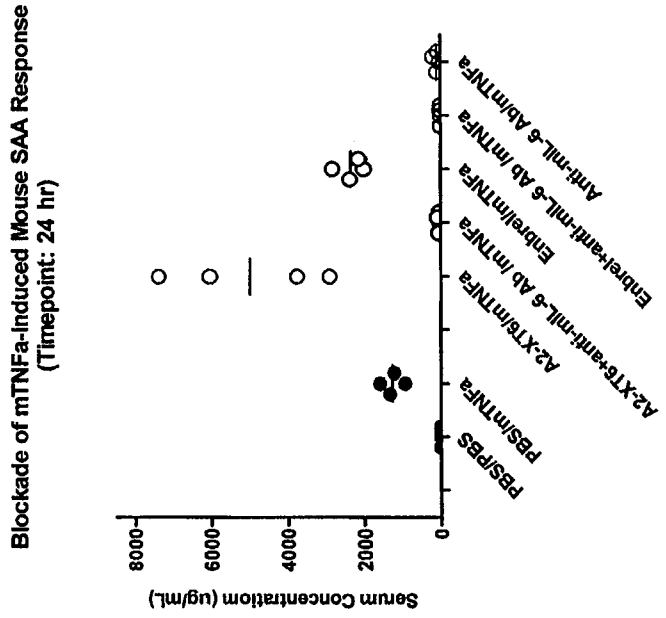
23. The method of claim 7, wherein: i) the IL-6 antagonist is administered simultaneously with the TNF-α antagonist in the same formulation; ii) the IL-6 antagonist is administered concurrently with the TNF-α antagonist in separate formulations within 30 minutes of each other; iii) the IL-6 antagonist is administered one week up to 30 minutes prior to administration of the TNF-α antagonist; or iv) the IL-6 antagonist is administered 30 minutes up to one week subsequent to administration of TNF-α antagonist.

24. The method of claim 20, wherein the first linker is a type II C-lectin stalk region or an immunoglobulin hinge region polypeptide and the second linker is an immunoglobulin hinge region polypeptide.

Figure 1


Blockade of mTNFa-induced Mouse SAA Response
(Timepoint: 2 hr)
Serum Concentration (ug/mL)
0
25
50
75
100
125
PBS/PBS
PBS/mTNFa
A2-XTb/mTNFa
Embrel/mTNFa
A2-XTb+anti-mIL-6 Ab /mTNFa
Embrel+anti-mIL-6 Ab/mTNFa
Anti-mIL-6 Ab/mTNFa

A


Blockade of mTNFa-induced Mouse SAA Response
(Timepoint: 24 hr)
Serum Concentration (ug/mL)
0
2000
4000
6000
8000
PBS/PBS
PBS/mTNFa
A2-XTb/mTNFa
Embrel/mTNFa
A2-XTb+anti-mIL-6 Ab /mTNFa
Embrel+anti-mIL-6 Ab/mTNFa
Anti-mIL-6 Ab/mTNFa

B

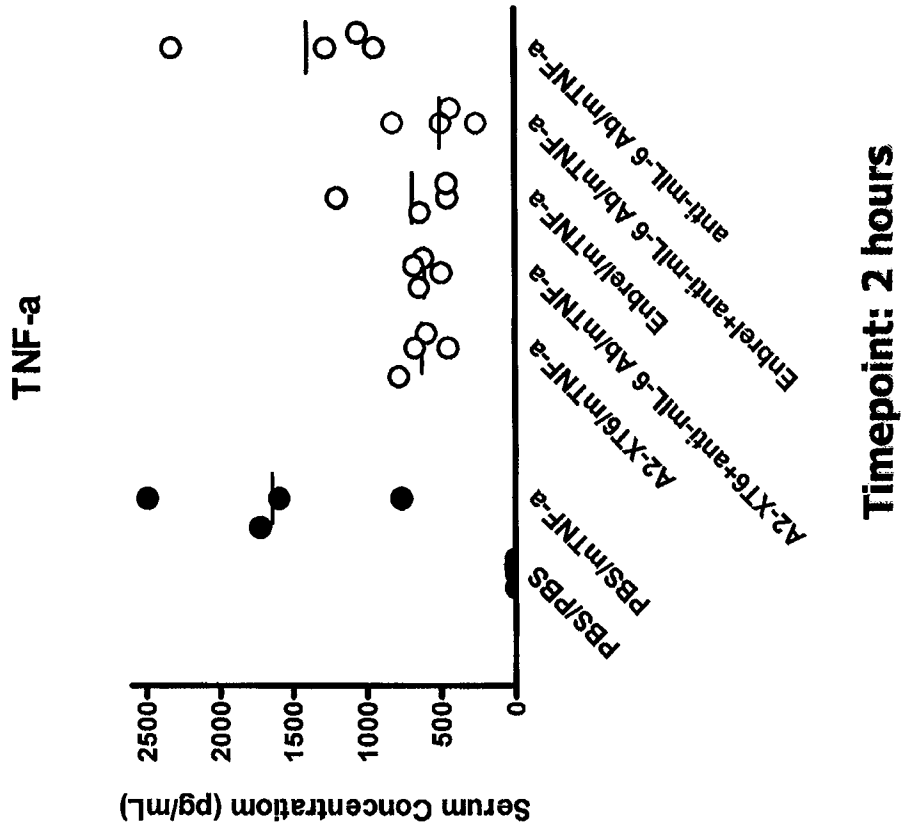

TNF-a
Serum Concentration (pg/mL)
2500
2000
1500
1000
500
0
PBS/PBS
PBS/mTNF-a
A2-XT6/mTNF-a
A2-XT6+anti-mIL-6 Ab/mTNF-a
Enbrel/mTNF-a
Enbrel+anti-mIL-6 Ab/mTNF-a
anti-mIL-6 Ab/mTNF-a
Timepoint: 2 hours

Figure 2

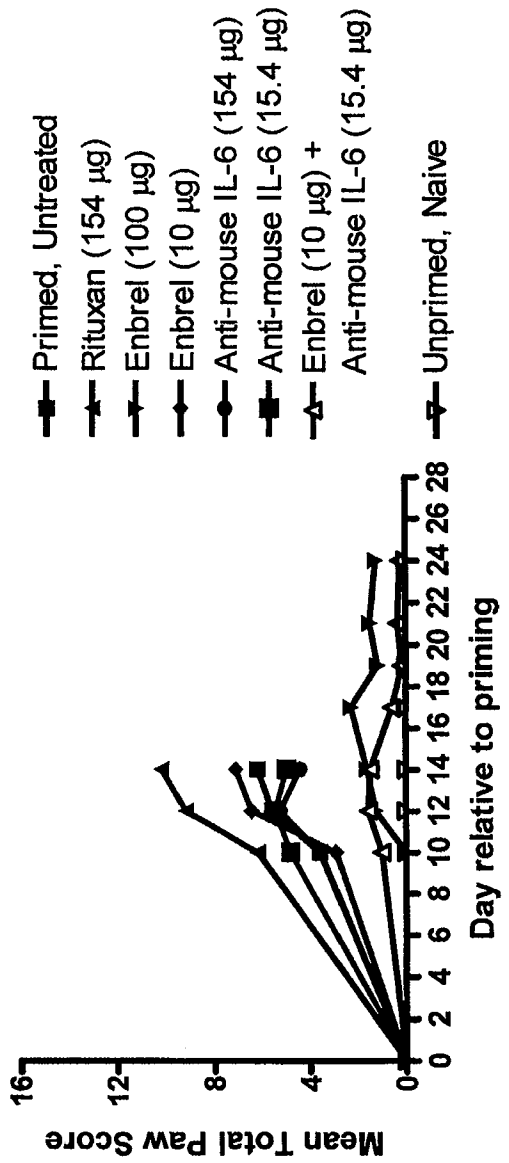
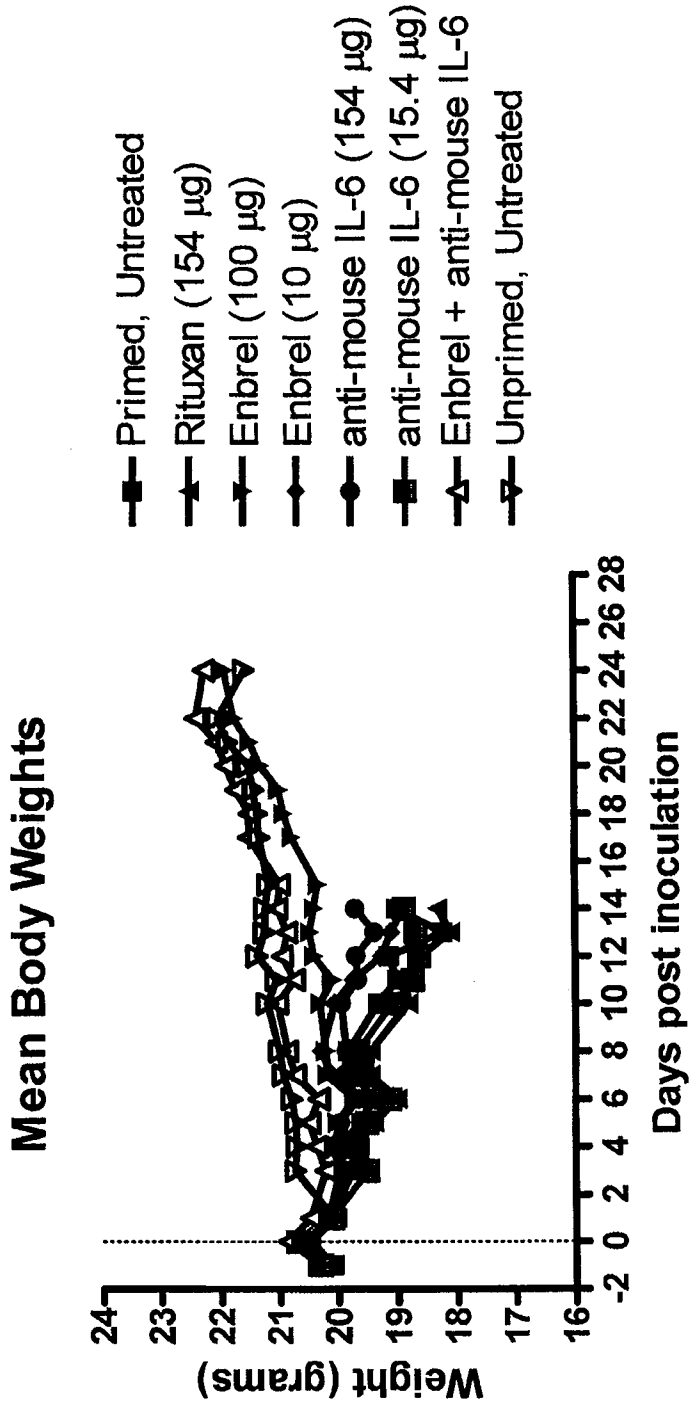

Mean Scores
Primed, Untreated
Rituxan (154 µg)
Enbrel (100 µg)
Enbrel (10 µg)
Anti-mouse IL-6 (154 µg)
Anti-mouse IL-6 (15.4 µg)
Enbrel (10 µg) + Anti-mouse IL-6 (15.4 µg)
Unprimed, Naive
Mean Total Paw Score
0
4
8
12
16
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28
Day relative to priming

Figure 3

Figure 4


Mean Body Weights
Primed, Untreated
Rituxan (154 µg)
Enbrel (100 µg)
Enbrel (10 µg)
anti-mouse IL-6 (154 µg)
anti-mouse IL-6 (15.4 µg)
Enbrel + anti-mouse IL-6
Unprimed, Untreated
Weight (grams)
24
23
22
21
20
19
18
17
16
-2 0 2 4 6 8 10 12 14 16 18 20 22 24 26 28
Days post inoculation

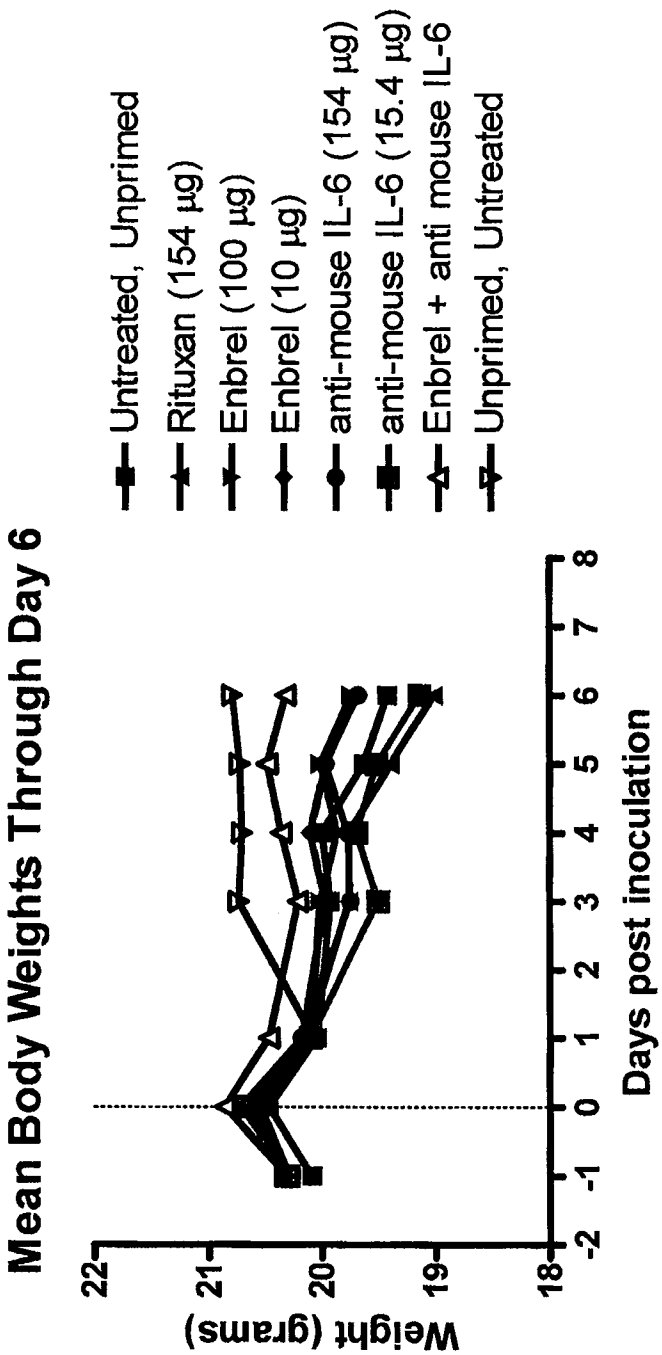

Mean Body Weights Through Day 6
Untreated, Unprimed
Rituxan (154 µg)
Enbrel (100 µg)
Enbrel (10 µg)
anti-mouse IL-6 (154 µg)
anti-mouse IL-6 (15.4 µg)
Enbrel + anti mouse IL-6
Unprimed, Untreated
Weight (grams)
22
21
20
19
18
-2
-1
0
1
2
3
4
5
6
7
8
Days post inoculation

Figure 5