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[Continued on next page]

(54) Title: METHODS OF TREATING IMMUNE DISORDERS WITH SINGLE DOMAIN ANTIBODIES AGAINST TNF-ALPHA

(57) Abstract: The invention relates to the TNFX binding molecule ozoralizumab (ATN-103), methods of using this molecule to treat immune disorders, including rheumatoid arthritis, and specific dosing regimens for the treatment of rheumatoid arthritis.

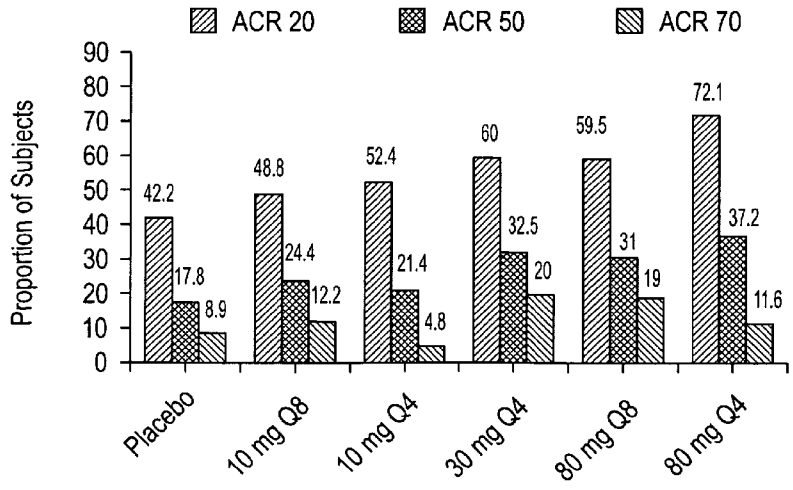

Figure 1

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METHODS OF TREATING IMMUNE DISORDERS WITH SINGLE DOMAIN ANTIBODIES AGAINST TNF -ALPHA

Field of Invention

The present invention relates to polypeptides comprising single domain antigen binding (SDAB) molecules against Tumor Necrosis Factor alpha (TNFa) and methods of using SDAB molecules to treat immune disorders such as rheumatoid arthritis.

Background of the Invention

SDABs against TNFa are described in, for example, PCT publications WO 04/041862 and WO 06/122786. These anti-TNFa SDABs may be used for the prevention and/or treatment of diseases and disorders associated with and/or mediated by TNFa, such as inflammation, rheumatoid arthritis, Crohn's disease, ulcerative colitis, inflammatory bowel syndrome, multiple sclerosis, Addison's disease, autoimmune hepatitis, autoimmune parotitis, diabetes type 1, epididymitis, glomerulonephritis, Graves' disease, Guillain-Barre syndrome, Hashimoto's disease, hemolytic anemia, systemic lupus erythematosus, male infertility, multiple sclerosis, myasthenia gravis, pemphigus, psoriasis, rheumatic fever, sarcoidosis, scleroderma, Sjogren's syndrome, spondyloarthropathies, thyroiditis, and vasculitis.

The implementation of anti-TNF therapeutics in the treatment regimen has improved the disease burden and quality of life for patients with inflammatory conditions, but many of these therapies require regular, e.g. weekly, injections of the drugs by a healthcare professional. Frequent office visits and injections pose a significant burden on the healthcare system, patients and their caregivers. Reducing these visits and providing the patients and caregivers with a self-administered option could result in significant personal and economical profits. Not every anti-TNF therapeutic will be a candidate for reduced treatment frequency or self-injections, and therefore there is a need for novel anti-TNF therapeutics that are efficacious when administered over longer intervals (e.g. monthly to bi-monthly) and/or as self-injection.

Patients who receive anti–tumor necrosis factor (TNF) drugs are at risk for serious infections (SI) due to bacterial, mycobacterial, fungal, viral, parasitic, and other opportunistic pathogens. These infections may be related either to the underlying disease or to medications taken as treatment. Serious infections (SI) result in hospitalization or death or require medication such as antibiotics. Accordingly, there is a need to minimize serious infections, while retaining drug efficacy.

Summary of the Invention

The present invention addresses one or more of the above mentioned needs. Treating human subjects suffering from rheumatoid arthritis with certain dosage regimens of a polypeptide comprising an anti-TNFa SDAB resulted in statistically significant improvements in the subjects' disease state. Accordingly, in some embodiments, the invention comprises a method of treating an immune disorder in a human in need thereof, comprising administering to the human multiple 30-200 mg doses of a polypeptide comprising two anti-TNFa SDABs and an anti-human serum albumin (HSA) SDAB, wherein the doses are separated in time by at least about four weeks. Modelling indicated that the polypeptide comprising an anti-TNFa SDAB was efficacious over prolonged intervals of time when increasing the dose, without clinically significant increased adverse effects. Comparative modelling with contemporaneous anti-TNF inhibitors further revealed that the polypeptide comprising an anti-TNFa SDAB combined a favourable efficacy without impact on risk-profile of SI effects. Accordingly, in some embodiments, the invention comprises a method of treating an immune disorder in a human in need thereof, comprising administering to the human multiple 30-400 mg doses of a polypeptide comprising two anti-TNFa SDABs and an anti-human serum albumin (HSA) SDAB, wherein the doses are separated in time by at least about four weeks, such as about 8 weeks or 2 months. In some embodiments, the present invention comprises a polypeptide comprising two anti-tumor necrosis factor alpha (TNFa) SDABs and an anti-human serum albumin (HSA) SDAB for use in treating an immune disorder in a human in need thereof, by administering to the human multiple 30-400 mg doses of said polypeptide, wherein the doses are at least about every four weeks apart.

In some embodiments, the anti-TNFa SDAB comprises 3 complementary determining regions (CDR1, CDR2, and CDR3) wherein

(a) CDR1 comprises

(i) the amino acid sequence DYWMY (SEQ ID NO:22);

(ii) an amino acid sequence that has at least 80% sequence identity with DYWMY (SEQ ID NO:22); or

(iii) an amino acid sequence that has only 1 amino acid difference from DYWMY (SEQ ID NO:22);

(b) CDR2 comprises

(i) the amino acid sequence EINTNGLITKYPDSVKG (SEQ ID NO:23);

(ii) an amino acid sequence that has at least 80%, 90%, or 95% sequence identity with EINTNGLITKYPDSVKG (SEQ ID NO:23); or

(iii) an amino acid sequence that has 2 or 1 amino acid differences from EINTNGLITKYPDSVKG (SEQ ID NO:23); and

(c) CDR3 comprises

(i) the amino acid sequence SPSGFN (SEQ ID NO:24);

(ii) an amino acid sequence that has at least 80% sequence identity with SPSGFN (SEQ ID NO:24); or

(iii) an amino acid sequence that has 1 amino acid difference from SPSGFN (SEQ ID NO:24).

In some embodiments, the anti-HSA SDAB comprises 3 CDRs (CDR1, CDR2, and CDR3) wherein

(a) CDR1 comprises

(i) the amino acid sequence SFGMS (SEQ ID NO:25);

(ii) an amino acid sequence that has at least 80% sequence identity with SFGMS (SEQ ID NO:25); or

(iii) an amino acid sequence that has only 1 amino acid difference from SFGMS (SEQ ID NO:25);

(b) CDR2 comprises

(i) the amino acid sequence SISGSGSDTLYADSVKG (SEQ ID NO:26);

(ii) an amino acid sequence that has at least 80%, 90%, or 95% sequence identity with SISGSGSDTLYADSVKG (SEQ ID NO:26); or

(iii) an amino acid sequence that has 2 or 1 amino acid differences from SISGSGSDTLYADSVKG (SEQ ID NO:26); and

(c) CDR3 comprises

(i) the amino acid sequence GGSLSR (SEQ ID NO:27);

(ii) an amino acid sequence that has at least 80% sequence identity with

10 GGSLSR (SEQ ID NO:27); or

(iii) an amino acid sequence that has 1 amino acid difference from GGSLSR (SEQ ID NO:27).

In particular embodiments, at least one of the anti-TNFa SDABs comprises an amino acid sequence with at least 80%, 90%, 95%, or 99% sequence identity to SEQ ID NO:2 (TNF30). In particular embodiments, the anti-HSA SDAB comprises an amino acid sequence having at least 80%, 90%, 95%, or 95% sequence identity to SEQ ID NO:5 (ALB8). In particular embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO:1 (ozoralizumab). In particular embodiments, at least one of the SDABs is humanized.

In some embodiments, each of the two anti-TNFa SDABs and anti-HSA SDAB are linked via linkers. The linkers may be amino acid sequences, which may be selected from the group consisting of SEQ ID NO:6 and SEQ ID NO:7.

The doses of the invention may be separated in time by at least about 2 weeks, 3 weeks, 4 weeks, 1 month, 5 weeks, 6 weeks, 7 weeks, 8 weeks, or 2 months.

The doses of the invention may comprise 10, 30, 80, 100, 120, 160, 180, 200, 225, 250, 275, 300, 320, 350, 375 or 400 mg of the SDAB molecule and may be administered intravenously, subcutaneously, orally, peritoneally, nasally, or sublingually.

The SDAB molecules of the invention may be formulated into a pharmaceutically acceptable formulation. In some embodiments, the formulation comprises

(a) a SDAB molecule at a concentration of about 1 mg/ml to 250 mg/ml, such as about 10 mg/ml to 250 mg/ml;

(b) a lyoprotectant chosen from sucrose, sorbitol, or trehalose at a concentration of about 5% to about 10%;

(c) a surfactant chosen from polysorbate-80 or poloxamer-188 at a concentration of about 0.01% to 0.6%; and

(d) a buffer chosen from Histidine buffer at a concentration of about 10 to 20 mM or a Tris buffer at a concentration of about 20 mM such that the pH of the formulation is about 5.0 to 7.5.

In some embodiments, the formulation comprises 1 mg/ml, 10 mg/ml, 30 mg/ml, 80 mg/ml, 100 mg/ml, 160 mg/ml 180 mg/ml, 200 mg/ml, or even 250 mg/ml of a polypeptide, 20 mM Histidine, 10% (w/v) sucrose, and 0.02% polysorbate-80 at pH 6.0.

In some embodiments, the formulation comprises 1 mg/ml, 10 mg/ml, 30 mg/ml, 80 mg/ml, 100 mg/ml, 160 mg/ml 180 mg/ml, 200 mg/ml, or even 250 mg/ml of a polypeptide, 20 mM Histidine, 7.5% (w/v) sucrose, and 0.01% polysorbate-80 at pH 6.0.

The SDAB molecules of the invention may be administered to treat an immune disorder and/or the polypeptides of the invention may be used for treating an immune disorder, wherein the immune disorder is selected from the group consisting of: inflammation, arthritis, including rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, juvenile idiopathic arthritis and osteoarthritis, COPD, asthma, inflammatory bowel diseases including Crohn's disease and ulcerative colitis, multiple sclerosis, Addison's disease, Autoimmune hepatitis, Autoimmune parotitis, Diabetes Type I, Epididymitis,

Glomerulonephritis, Graves' disease, Guillain -Barre syndrome, Hashimoto's disease, Hemolytic anemia, Systemic lupus erythematosus, Male infertility, Multiple sclerosis, Myasthenia Gravis, Pemphigus, Psoriasis, Hidradenitis suppurativa, Rheumatic fever, Sarcoidosis, Scleroderma, Sjogren's syndrome, Spondyloarthropathies, Thyroiditis, and Vasculitis.

The humans treated with the SDAB molecules of the invention may be concurrently treated with methotrexate.

In a particular embodiment, the invention contemplates a method of treating rheumatoid arthritis in a human in need thereof, comprising administering to the human 80 mg of a polypeptide comprising the amino acid sequence of SEQ ID NO:1 about once every four weeks, wherein the human is concurrently treated with methotrexate.

In a further particular embodiment, the invention contemplates a method of treating rheumatoid arthritis in a human in need thereof, comprising administering to the human 160, 200, 320 or 400 mg of a polypeptide comprising the amino acid sequence of SEQ ID NO:1 about once every four weeks, and optionally wherein the human is concurrently treated with methotrexate.

In another particular embodiment, the invention contemplates a method of treating rheumatoid arthritis in a human in need thereof, comprising administering to the human 320 or 400 mg of a polypeptide comprising the amino acid sequence of SEQ ID NO:1 about once every eight weeks or once every month, and optionally wherein the human is concurrently treated with methotrexate.

In a further embodiment, the invention relates to a polypeptide comprising the amino acid sequence of SEQ ID NO:1 for use in treating rheumatoid arthritis in a human in need thereof, by administering to the human 80 mg of a polypeptide about every four weeks, wherein the human is concurrently treated with methotrexate.

In a further embodiment, the invention relates to a polypeptide comprising the amino acid sequence of SEQ ID NO:1 for use in treating rheumatoid arthritis in a human in need thereof, by administering to the human 160, 200, 320 or 400 mg of a polypeptide comprising the amino acid sequence of SEQ ID NO:1 about once every four weeks, and optionally wherein the human is concurrently treated with methotrexate.

In another particular embodiment, the invention contemplates a polypeptide comprising the amino acid sequence of SEQ ID NO:1 for use in treating rheumatoid arthritis in a human in need thereof, by administering to the human 320 or 400 mg of a polypeptide comprising the amino acid sequence of SEQ

ID NO:1 about once every eight weeks or once every month, and optionally wherein the human is concurrently treated with methotrexate.

In a further embodiment, the invention relates to a polypeptide for use in treating an immune disorder in a human in need thereof, wherein said polypeptide competes with the polypeptide comprising the amino acid sequence of SEQ ID NO:1 (ozoralizumab).

DESCRIPTION OF THE INVENTION

Description of the Drawings

Figure 1 shows the percentage of patients in each of 6 treatment groups dosed with ozoralizumab achieving the ACR-20, 50, and 70 criteria for treatment of rheumatoid arthritis at weeks 8 and 16. In the every 4 week dosing regimen, subjects were dosed at day 1, week 4, 8, and 12 with either ozoralizumab or placebo. In the every 8 week regimen, subjects were dosed at day 1 and week 8 with ozoralizumab and placebo was administered at week 4 and 12.

Figure 2 shows the ACR 20 response rate over time by treatment group (LOCF).

Figure 3 shows the DAS28 response rate over time by treatment group (LOCF).

Figure 4 shows Median (95% PI) placebo-corrected dose-ACR20 response of ATN-103. Dashed lines show the median simulated responses of indicated comparator treatment.

Figure 5 shows median (95% PI) simulated DAS response of ATN-103. Dashed lines show median response of indicated comparator treatment.

Figure 6 shows utility by drug in terms of %ACR20 and %SI. Each bubble covers the 95% PI of simulated responses for each drug at its indicated dosing regimen.

Definitions

In order that the present invention may be more readily understood, certain terms are first defined. Additional definitions are set forth throughout the detailed description.

As used herein, the articles "a" and "an" refer to one or to more than one (e.g., to at least one) of the grammatical object of the article.

The term "or" is used herein to mean, and is used interchangeably with, the term "and/or," unless context clearly indicates otherwise.

The terms "proteins" and "polypeptides" are used interchangeably herein, and encompass the SDAB molecules of the invention.

"About" and "approximately" shall generally mean an acceptable degree of error for the quantity measured given the nature or precision of the measurements. Exemplary degrees of error are within 20 percent (%), typically, within 10%, and more typically, within 5% of a given value or range of values.

When a modifying term such as "about," "approximately," "at least," or "at most" precedes a list of items, the term is intended to modify each of the items in the list. For example, the phrase "at least about 10% or 20%" should be interpreted as "at least about 10% or at least about 20%."

In the context of nucleotide sequence, the term "substantially identical" is used herein to refer to a first nucleic acid sequence that contains a sufficient or minimum number of nucleotides that are identical to aligned nucleotides in a second nucleic acid sequence such that the first and second nucleotide sequences encode a polypeptide having common functional activity, or encode a common structural polypeptide domain or a common functional polypeptide activity, e.g., nucleotide sequences having at least about 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to a reference sequence.

Also included in the invention are fragments, derivatives, analogs, or variants of polypeptides of the invention, and any combination thereof. The terms "fragment", "variant", "derivative" and "analog" when referring to proteins of the present invention include any polypeptides which retain at least some of the functional properties of the corresponding native SDAB molecule. Fragments of polypeptides of the present invention include proteolytic and deletion fragments, in addition to specific antigen binding fragments discussed elsewhere herein. Variants of the polypeptides of the present invention include fragments as described above, and also polypeptides with altered amino acid sequences due to amino acid substitutions, deletions, or insertions. Variants may occur naturally or be non-naturally occurring. Non-naturally occurring variants may be produced using art-known mutagenesis techniques. Variant polypeptides may comprise conservative or non-conservative amino acid substitutions, deletions, or additions. Derivatives of the fragments of the present invention are polypeptides that have been altered so as to exhibit additional features not found on the native polypeptide. Examples include fusion proteins. Variant polypeptides may also be referred to herein as "polypeptide analogs." As used herein, a "derivative" of a polypeptide refers to a subject polypeptide having one or more residues chemically derivatized by reaction of a functional side group. Also included as "derivatives" are those polypeptides that contain one or more naturally occurring amino acid derivatives of the twenty standard amino acids.

For example, 4-hydroxyproline may be substituted for proline; 5-hydroxylysine may be substituted for lysine; 3-methylhistidine may be substituted for histidine; homoserine may be substituted for serine; and ornithine may be substituted for lysine.

The term "functional variant" refers to polypeptides that have a substantially identical amino acid sequence to the naturally-occurring sequence, or are encoded by a substantially identical nucleotide sequence, and are capable of having one or more activities of the naturally occurring sequence.

Calculations of homology or sequence identity between sequences (the terms are used interchangeably herein) are performed as follows.

To determine the percent identity of two amino acid sequences, or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). In a typical embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of the length of the reference sequence.

The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology").

The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In one embodiment, the percent identity between two amino acid sequences is determined using the algorithm described in Needleman & Wunsch, *J. Mol. Biol.* 48:444-453(1970), which has been incorporated into the GAP program in the GCG software package (available on the internet at gcg.com), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available on the internet at gcg.com), using a NWSgapdna.CMP matrix and a gap

weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. One typical set of parameters (and the one that should be used unless otherwise specified) are a Blossum 62 scoring matrix with a gap penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5.

The percent identity between two amino acid or nucleotide sequences can be determined using the algorithm described in E. Meyers and W. Miller *CABIO*, 4:11-17(1989) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

The nucleic acid and protein sequences described herein can be used as a "query sequence" to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, et al. *J. Mol. Biol.* 215:403-10(1990). BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to a nucleic acid molecules featured in the invention. BLAST protein searches can be performed with the XBLAST program, score=50, wordlength=3 to obtain amino acid sequences homologous to a protein (S EQ ID NO:1) molecule featured in the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al., *Nucleic Acids Res.* 25:3389-25 3402(1997). When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used.

A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine).

The term "SDAB" refers to a single domain antigen binding molecule as described in WO04/041862 and WO 06/122786. The term "SDAB molecule" refers to a polypeptide or other molecule comprising one or more SDABs. The terms "SDAB" and "SDAB molecule" may be used interchangeably to refer to a single single domain antigen binding unit (e.g., TNF30 or ALB8) or multi-valent single domain antigen binding domains (e.g., TNF55, TNF56, or ozoralizumab).

A "CDR" of a variable domain are amino acid residues within the hypervariable region that are identified in accordance with the definitions of the Kabat, Chothia, the cumulation of both Kabat and Chothia, AbM, contact, and/or conformational definitions or any method of CDR determination well known in the art. Antibody CDRs may be identified as the hypervariable regions originally defined by Kabat et al. See, e.g., Kabat et al., 1992, Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, NIH, Washington D.C. The positions of the CDRs may also be identified as the structural loop structures originally described by Chothia and others. See, e.g., Chothia et al., 1989, Nature 342:877-883. Other approaches to CDR identification include the "AbM definition," which is a compromise between Kabat and Chothia and is derived using Oxford Molecular's AbM antibody modeling software (now Accelrys®), or the "contact definition" of CDRs based on observed antigen contacts, set forth in MacCallum et al., 1996, J. Mol. Biol., 262:732-745. In another approach, referred to herein as the "conformational definition" of CDRs, the positions of the CDRs may be identified as the residues that make enthalpic contributions to antigen binding. See, e.g., Makabe et al., 2008, Journal of Biological Chemistry, 283:1156-1166. Still other CDR boundary definitions may not strictly follow one of the above approaches, but will nonetheless overlap with at least a portion of the Kabat CDRs, although they may be shortened or lengthened in light of prediction or experimental findings that particular residues or groups of residues or even entire CDRs do not significantly impact antigen binding. As used herein, a CDR may refer to CDRs defined by any approach known in the art, including combinations of approaches. The methods used herein may utilize CDRs defined according to any of these approaches. For any given embodiment containing more than one CDR, the CDRs may be defined in accordance with any of Kabat, Chothia, extended, AbM, contact, and/or conformational definitions.

Detailed Description of the Invention

Single Domain Antigen Binding (SDAB) Molecules

Single domain antigen binding (SDAB) molecules include molecules whose complementary determining regions are part of a single domain polypeptide. Examples include, but are not limited to, heavy chain variable domains, binding molecules naturally devoid of light chains, single domains derived from conventional 4-chain antibodies, engineered domains and single domain scaffolds other than those derived from antibodies. SDAB molecules may be any of the art, or any future single domain molecules. SDAB molecules may be derived from any species including, but not limited to mouse, human, camel, llama, fish, shark, goat, rabbit, and bovine.

According to one aspect of the invention, a SDAB molecule is a naturally occurring single domain antigen binding molecule known as heavy chain devoid of light chains. Such single domain molecules are disclosed, for example, in WO 94/04678 and Hamers-Casterman et al. *Nature* 363:446-448 (1993). For clarity reasons, this variable domain derived from a heavy chain molecule naturally devoid of light chain may be described as a VHH to distinguish it from the conventional VH of four chain immunoglobulins. Such a VHH molecule can be derived from Camelidae species, for example in camel, llama, dromedary, alpaca, and guanaco. Other species besides Camelidae may produce heavy chain molecules naturally devoid of light chain; such VHHs are within the scope of the invention.

The SDAB molecules can be recombinant, CDR-grafted, humanized, camelized, deimmunized and/or in vitro generated (e.g., selected by phage display), as described in more detail below.

The term "antigen-binding" is intended to include the part of a polypeptide, e.g., a single domain molecule described herein, that comprises determinants that form an interface that binds to a target antigen, or an epitope thereof. With respect to proteins (or protein mimetics), the antigen-binding site typically includes one or more loops (of at least four amino acids or amino acid mimics) that form an interface that binds to the target antigen. Typically, the antigen binding site of the polypeptide, e.g., the single domain antibody molecule, includes at least one or two CDRs, or more typically at least three, four, five, or six CDRs.

The term "immunoglobulin variable domain" is frequently understood in the art as being identical or substantially identical to a VL or a VH domain of human or animal origin. It shall be recognized that immunoglobulin variable domains may have evolved in certain species, e.g., sharks and llama, to differ in amino acid sequence from human or mammalian VL or VH.

However, these domains are still primarily involved in antigen binding. The term "immunoglobulin variable domain" typically includes at least one or two CDRs, or more typically at least three CDRs.

A "constant immunoglobulin domain" or "constant region" is intended to include an immunoglobulin domain that is identical to or substantially similar to a CL, CH1, CH2, CH3, or CH4 domain of human or animal origin. See e.g. Hasemann & Capra, Immunoglobulins: Structure and Function, in William E. Paul, ed., Fundamental Immunology, Second Edition, 209, 210-218 (1989). The term "Fc region" refers to the Fc portion of the constant immunoglobulin domain that includes immunoglobulin domains CH2 and CH3 or immunoglobulin domains substantially similar to these.

In certain embodiments, the SDAB molecule is a monovalent, or a multispecific molecule (e.g., a bivalent, trivalent, or tetravalent molecule). In other embodiments, the SDAB molecule is a monospecific, bispecific, trispecific, or tetraspecific molecule. Whether a molecule is "monospecific" or "multispecific," e.g., "bispecific," refers to the number of different epitopes with which a binding polypeptide reacts. Multispecific molecules may be specific for different epitopes of a target polypeptide described herein or may be specific for a target polypeptide as well as for a heterologous epitope, such as a heterologous polypeptide or solid support material.

As used herein the term "valency" refers to the number of potential binding domains, e.g., antigen binding domains, present in an SDAB molecule. Each binding domain specifically binds one epitope. When an SDAB molecule comprises more than one binding domain, each binding domain may specifically bind the same epitope, for an antibody with two binding domains, termed "bivalent monospecific," or to different epitopes, for an SDAB molecule with two binding domains, termed "bivalent bispecific." An SDAB molecule may also be bispecific and bivalent for each specificity (termed "bispecific tetravalent molecules"). Bispecific bivalent molecules, and methods of making them, are described, for instance in U.S. Patent Nos. 5,731,168; 5,807,706; 5,821,333; and U.S. Publication Nos. 2003/020734 and 2002/0155537, the disclosures of all of which are incorporated by reference herein. Bispecific tetravalent molecules and methods of making them are described, for instance, in WO 02/096948 and WO00/44788, the disclosures of both of which are incorporated by reference herein. Further examples of multispecific and multivalent molecules are provided in WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; U.S. Patent Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; and 5,601,819; Tutt et al, *J. Immunol.* 147:60-69 (1991); and Kostelny et al., *J.Immunol.* 148: 1547-1553 (1992).

In certain embodiments, the SDAB molecule is a single chain fusion polypeptide comprising one or more single domain molecules devoid of a complementary variable domain or an immunoglobulin constant, e.g., Fc, region, that binds to one or more target antigens. An exemplary target antigen recognized by the antigen-binding polypeptides includes tumor necrosis factor alpha (TNFa). In certain embodiments, the antigen-binding single domain molecule binds to a serum protein, e.g., a human serum proteins chosen from one or more of serum albumin (e.g., human serum albumin (HSA)) or transferin.

TNFa

Tumor necrosis factor alpha (TNFa) is known in the art to the associated with inflammatory disorders such as rheumatoid arthritis, Crohn's disease, ulcerative colitis, and multiple sclerosis. Both TNFa and its

receptors (CD120a andCD120b) have been studied in great detail. TNFa in its bioactive form is a trimer. Several strategies to antagonize the action of TNFa using anti-TNFa antibodies have been developed and are currently commercially available, such as Remicade® and Humira®. Numerous examples of TNFa-binding single domain antigen binding molecules are disclosed in WO 04/041862, WO 04/041865, and WO06/122786, the contents of all of which are incorporated by reference herein in their entirety.

Additional examples of single domain antigen binding molecules are disclosed in US2006/286066, US2008/0260757, WO 06/003388, US2005/0271663, and US2006/0106203, the contents of all of which are incorporated by reference herein in their entirety. In particular embodiments, the SDAB molecules of the invention include SDABs comprising an amino acid sequence of Table 1, or an amino acid sequence with about 80%, 85%, 90%, 95%, or 99% sequence identity with a sequence of Table 1. In alternative embodiments, the SDAB molecules of the invention may comprise an amino acid sequence with 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 amino acid differences from a sequence of Table 1.

In other embodiments, mono-, bi-, tri- and other multi-specific single domain antibodies against TNFa and a serum protein, e.g., HSA, are contemplated. Numerous examples of multispecific TNFa and HSA binding polypeptides are disclosed in WO 06/122786, the contents of which are incorporated by reference herein. Multi-specific polypeptides of the invention may comprise an amino acid sequence of Table 1, or an amino acid sequence with about 80%, 85%, 90%, 95%, or 99% sequence identity with a sequence of Table 1. In alternative embodiments, the multi-specific SDAB molecules of the invention may comprise an amino acid sequence with 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 amino acid differences from a sequence of Table 1.

In specific embodiments, the TNFa-binding SDAB molecule comprises one or more of the SDABs disclosed in WO 06/122786. For example, the TNFa-binding SDAB molecule can be a monovalent, bivalent, or trivalent TNFa-binding polypeptide disclosed in WO 06/122786.

Exemplary TNFa-binding SDABs disclosed in WO 06/122786 include, but are not limited to, TNF1, TNF2, TNF3, and humanized forms thereof (e.g., TNF29, TNF30, TNF31, TNF32, TNF33). Additional examples of monovalent TNFa-binding SDABs are disclosed in Table 8 of WO 2006/122786. Exemplary bivalent TNFa-binding SDAB molecules include, but are not limited to, TNF55 and TNF56, which comprise two TNF30 SDABs linked via a peptide linker to form a single fusion polypeptide (disclosed in WO 06/122786). Additional examples of bivalent TNFa-binding SDAB molecules are disclosed in Table 19 of WO 06/122786 as TNF4, TNF5, TNF6, TNF7, TNF8).

In particular embodiments, the anti-TNFa SDAB comprises 3 complementary determining regions (CDR1, CDR2, and CDR3) wherein: CDR1 comprises the amino acid sequence DYWMY (SEQ ID NO:22); an amino acid sequence that has at least 80% sequence identity with DYWMY (SEQ ID NO:22); or an amino acid sequence that has only 1 amino acid difference from DYWMY (SEQ ID 25 NO:22); CDR2 comprises the amino acid sequence EINTNGLITKYPDSVKG (SEQ ID NO:23); an amino acid sequence that has at least 80%, 90%, or 95% sequence identity with EINTNGLITKYPDSVKG (SEQ ID NO:23); or an amino acid sequence that has 2 or 1 amino acid differences from EINTNGLITKYPDSVKG (SEQ ID NO:23); and CDR3 comprises the amino acid sequence SPSGFN (SEQ ID NO:24); an amino acid sequence that has at least 80% sequence identity with SPSGFN (SEQ ID NO:24); or an amino acid sequence that has 1 amino acid difference from SPSGFN (SEQ ID NO:24).

In some embodiments, the HSA-binding SDAB comprises an amino acid sequence of Table 1, or an amino acid sequence with about 80%, 85%, 90%, 95%, or 99% sequence identity with a sequence of Table 1. In alternative embodiments, the anti-HSA SDAB of the invention may comprise an amino acid sequence with 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 amino acid differences from a sequence of Table 1.

In other embodiments, the HSA-binding SDAB molecule comprises one or more of the SDABs disclosed in WO 06/122786. For example, the HSA-binding SDAB molecule can be a monovalent, bivalent, trivalent HSA-binding SDAB molecule disclosed in WO 06/122786. In other embodiments, the HSA-binding SDAB molecule can be a monospecific or a multispecific molecule having at least one of the binding specificities bind to HSA. Exemplary HSA-binding SDABs include, but are not limited to, ALB1 and humanized forms thereof (e.g., ALB6, ALB7, ALB8, ALB9, ALB10), disclosed in WO 06/122786.

In particular embodiments, the anti-HSA SDAB comprises 3 CDRs (CDR1, CDR2, and CDR3) wherein CDR1 comprises the amino acid sequence SFGMS (SEQ ID NO:25); an amino acid sequence that has at least 80% sequence identity with SFGMS (SEQ ID NO:25); or an amino acid sequence that has only 1 amino acid difference from SFGMS (SEQ ID NO:25); CDR2 comprises he amino acid sequence SISGSGSDTLYADSVKG (SEQ ID NO:26); an amino acid sequence that has at least 80%, 90%, or 95% sequence identity with SISGSGSDTLYADSVKG (SEQ ID NO:26); or an amino acid sequence that has 2 or 1 amino acid differences from SISGSGSDTLYADSVKG (SEQ ID NO:26); and CDR3 comprises the amino acid sequence GGSLSR (SEQ ID NO:27); an amino acid sequence that has at least 80% sequence identity with GGSLSR (SEQ ID NO:27); or an amino acid sequence that has 1 amino acid difference from GGSLSR (SEQ ID NO:27).

In other embodiments, two or more of the single domain molecules of the SDAB molecules are fused, with or without a linking group, as a genetic or a polypeptide fusion. The linking group can be any linking group apparent to those of skill in the art. For instance, the linking group can be a biocompatible polymer with a length of 1 to 100 atoms. In one embodiment, the linking group includes or consists of polyglycine, polyserine, polylysine, polyglutamate, polyisoleucine, or polyarginine residues, or a combination thereof. For example, the polyglycine or polyserine linkers can include at least five, seven eight, nine, ten, twelve, fifteen, twenty, thirty, thirty-five and forty glycine and serine residues. Exemplary linkers that can be used include Gly-Ser repeats, for example, (Gly)4-Ser (SEQ ID NO:19) repeats of at one, two, three, four, five, six, seven or more repeats. In some embodiments, the linker has the following sequences: (Gly)4-Ser-(Gly)3-Ser (SEQ ID NO: 20) or ((Gly)4-Ser)n (SEQ ID NO: 21), where n is 4, 5, or 6. In some embodiments, the linker comprises SEQ ID NO:6 (GS9) or SEQ ID NO:7 (GS30).

In one exemplary embodiment, the SDAB molecules of the invention may be composed of a single chain polypeptide fusion of two SDAB domains (e.g., two camelid variable regions) that bind to a target antigen, e.g., TNFa, and one single domain antibody molecule (e.g., a camelid variable region) that binds to a serum protein, e.g., HSA.

A polypeptide referred to herein as "ozoralizumab" is a humanized, trivalent, bi-specific TNFa-inhibiting fusion protein. This fusion protein is derived from camelids and has a high degree of sequence and structural homology to human immunoglobulin VH domains. Its single polypeptide chain is composed of two binding domains to TNFa and one to HSA, with two nine amino acid G-S linkers connecting the domains. A detailed description of ozoralizumab is provided in WO 06/122786 (where it is referred to at TNF60), the contents of which are incorporated by reference herein.

Table 1

Name	SEQ ID NO	Sequence
Ozoralizumab (TNF60)	1	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLE WVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTA VYYCARSPSGFNRGQGTLVTVSS ggggsgggs EVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLE WVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTA VYYCTIGGSLSRSSQGTLVTVSS Ggggsgggs EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLE WVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTA VYYCARSPSGFNRGQGTLVTVSS

TNF1	2	QVQLVESGGGLVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLE WVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLKPEDTA LYYCARSPSGFNRGQGTQVTVSS
TNF2	3	QVQLVESGGGLVQAGGSLRLSCAASGRTFSEPSGYTYTIGWFRQAP GKEREFVARIYWSSGLTYYADSVKGRFTISRDIAKNTVDLLMNSLK PEDTAVYYCAARDGIPTSRSVGSYNYWGQGTQVTVSS
TNF3	4	EVQLVESGGGLVQAGGSLSLSCSASGRSLSNYYMGWFRQAPGKERE LLGNISWRGYN1YYKDSVKGRFTISRDDAKNTIYLQMNRLKPEDTA VYYCAASILPLSDDPGWNTYWGQGTQVTVSS
TNF30	5	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLE WVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTA VYYCARSPSGFNRGQGTLVTVSS
GS9	6	GGGGSGGGS
GS30	7	GGGGSGGGGSGGGGSGGGGSGGGGSGGGGS
ALB1	8	AVQLVESGGGLVQPGNSLRLSCAASGFTFRSFGMSWVRQAPGKEPE WVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLKPEDTA VYYCTIGGSLSRSSQGTQVTVSS
ALB2	9	AVQLVESGGGLVQGGGSLRLACAASERIFDLNLMGWYRQGPGNERE LVATCITVG.DSTNYADSVKGRFTISMDYTKQTVYLHMNSLRPEDT GLYYCKIRRTWHSELWGQGTQVTVSS
ALB8	10	EVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEW VSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVY YCTIGGSLSRSSQGTLVTVSS
TNF1-GS9- TNF1 (TNF4)	11	QVQLVESGGGLVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLE WVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLKPEDTA LYYCARSPSGFNRGQGTQVTVSSGGGGSGGGSQVQLVESGGGLVQP GGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEINTNGLITKY PDSVKGRFTISRDNAKNTLYLQMNSLKPEDTALYYCARSPSGFNRG QGTQVTVSS
TNF2-GS9- TNF2 (TNF5)	12	QVQLVESGGGLVQAGGSLRLSCAASGRTFSEPSGYTYTIGWFRQAP GKEREFVARIYWSSGLTYYADSVKGRFTISRDIAKNTVDLLMNSLK PEDTAVYYCAARDGIPTSRSVGSYNYWGQGTQVTVSSGGGGSGGGS QVQLVESGGGLVQAGGSLRLSCAASGRTFSEPSGYTYTIGWFRQAP GKEREFVARIYWSSGLTYYADSVKGRFTISRDIAKNTVDLLMNSLK PEDTAVYYCAARDGIPTSRSVGSYNYWGQGTQVTVSS
TNF3-GS9- TNF3 (TNF6)	13	EVQLVESGGGLVQAGGSLSLSCSASGRSLSNYYMGWFRQAPGKERE LLGNISWRGYN1YYKDSVKGRFTISRDDAKNTIYLQMNRLKPEDTA VYYCAASILPLSDDPGWNTYWGQGTQVTVSSGGGGSGGGSEVQLVE SGGGLVQAGGSLSLSCSASGRSLSNYYMGWFRQAPGKERELLGNIS WRGYNIYYKDSVKGRFTISRDDAKNTIYLQMNRLKPEDTAVYYCAA SILPLSDDPGWNTYWGQGTQVTVSS

TNF1-GS30-TNF1 (TNF7)	14	QVQLVESGGGLVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLE WVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLKPEDTA LYYCARSPSGFNRGQGTQVTVSSGGGGSGGGGSGGGGSGGGGSGGG GSGGGGSQVQLVESGGGLVQPGGSLRLSCAASGFTFSDYWMYWVRQ APGKGLEWVSE1NTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNS LKPEDTALYYCARSPSGFNRGQGTQVTVSS
TNF2-GS30-TNF2 (TNF8)	15	QVQLVESGGGLVQAGGSLRLSCAASGRTFSEPSGYTYTIGWFRQAP GKEREFVARIYWSSGLTYYADSVKGRFTISRD1AKNTVDLLMNSLK PEDTAVYYCAARDGIPTSRSVGSYNYWGQGTQVTVSSGGGGSGGGG SGGGGSGGGGSGGGGSGGGGSQVQLVESGGGLVQAGGSLRLSCAAS GRTFSEPSGYTYTIGWFRQAPGKEREFVARIYWSSGLTYYADSVKG RFTISRDIAKNTVDLLMNSLKPEDTAVYYCAARDGIPTSRSVGSYN YWGQGTQVTVSS
TNF3-GS30-TNF3 (TNF9)	16	EVQLVESGGGLVQAGGSLSLSCSASGRSLSNYYMGWFRQAPGKERE LLGNISWRGYNIYYKDSVKGRFTISRDDAKNTIYLQMNRLKPEDTA VYYCAASILPLSDDPGWNTYWGQGTQVTVSSGGGGSGGGGSGGGGS GGGGSGGGGSGGGGSEVQLVESGGGLVQAGGSLSLSCSASGRSLSN YYMGWFRQAPGKERELLGNISWRGYN1YYKDSVKGRFTISRDDAKN TIYLQMNRLKPEDTAVYYCAASILPLSDDPGWNTYWGQGTQVTVSS
TNF30-GS30-TNF30-C (TNF55)	17	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLE WVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTA VYYCARSPSGFNRGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGG GSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFTFSDYWMYWVRQ APGKGLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNS LRPEDTAVYYCARSPSGFNRGQGTLVTVSC
TNF30-GS30-TNF30-gggC (TNF56)	18	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLE WVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTA VYYCARSPSGFNRGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGG GSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFTFSDYWMYWVRQ APGKGLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNS LRPEDTAVYYCARSPSGFNRGQGTLVTVSSgggC
(Gly)4-Ser	19	(G)4-S
(Gly)4-Ser-(Gly)3-Ser	20	(G)4-S-(G)3-S
((Gly)4-Ser)n	21	((G)4-S)n, wherein n is 4, 5, or 6
TNF CDR1	22	DYWMY
TNF CDR2	23	EINTNGLITKYPDSVKG
TNF CDR3	24	SPSGFN
HSA CDR1	25	SFGMS
HSA CDR2	26	SISGSGSDTLYADSVKG
HSA CDR3	27	GGSLSR

Preparation of SDAB Molecules

The SDAB molecules of the invention may comprise of one or more single domain molecules (e.g., SDABs) that are recombinant, CDR-grafted, humanized, camelized, de-immunized, and/or in vitro generated (e.g., selected by phage display). Techniques for generating antibodies and SDAB molecules, and modifying them recombinantly are known in the art and are described in detail below.

Numerous methods known to those skilled in the art are available for obtaining antibodies. For example, monoclonal antibodies may be produced by generation of hybridomas in accordance with known methods. Hybridomas formed in this manner are then screened using standard methods, such as enzyme-linked immunosorbent assay (ELISA) and surface plasmon resonance (BIACORE™) analysis, to identify one or more hybridomas that produce an SDAB that specifically binds with a specified antigen. Any form of the specified antigen may be used as the immunogen, e.g., recombinant antigen, naturally occurring forms, any variants or fragments thereof, as well as antigenic peptide thereof.

One exemplary method of making antibodies and SDAB molecules includes screening protein expression libraries, e.g., phage or ribosome display libraries. Phage display is described, for example, in U.S. Patent No. 5,223,409; Smith Science 228: 1315-1317(1985); WO 92/18619; WO 91/17271; WO 92/20791; WO 92/15679; WO 93/01288; WO 92/01047; WO92/09690; and WO 90/02809.

In addition to the use of display libraries, the specified antigen can be used to immunize a non-human animal, e.g., a rodent, e.g., a mouse, hamster, or rat. In one embodiment, the non-human animal includes at least a part of a human immunoglobulin gene. For example, it is possible to engineer mouse strains deficient in mouse antibody production with large fragments of the human Ig loci. Using the hybridoma technology, antigen-specific monoclonal antibodies derived from the genes with the desired specificity may be produced and selected. See, e.g., XENOMOUSE™, Green et al Nature Genetics 7:13-21 (1994), US20030070185, WO 96/34096, and PCT Application No. PCT/US96/05928.

In another embodiment, a SDAB molecule is obtained from the non-human animal, and then modified, e.g., humanized, deimmunized, and/or chimerized. These SDAB molecules may be produced using recombinant DNA techniques known in the art. A variety of approaches for making chimeric antibodies and SDAB molecules have been described. See e.g., Morrison et al, *Proc. Natl. Acad. Sci* U.S.A. 81:6851(1985); Takeda et al, *Nature* 314:452(1985); U.S. Patent Nos. 4,816,567 and 4,816,397; European Patent Publications EP171496 and 0173494; and United Kingdom Patent GB 2177096B. Humanized SDAB molecules may also be produced, for example, using transgenic mice that express human immunoglobulin genes, but are incapable of expressing the endogenous mouse immunoglobulin genes.

Winter describes an exemplary CDR-grafting method that may be used to prepare the humanized SDAB molecule described herein (U.S. Patent No. 5,225,539). All of the CDRs of a particular SDAB molecule may be replaced with at least a portion of a non-human CDR, or only some of the CDRs may be replaced with non-human CDRs. It is only necessary to replace the number of CDRs required for binding of the SDAB molecule to a predetermined antigen.

Humanized SDAB molecules can be generated by replacing sequences of the variable domain that are not directly involved in antigen binding with equivalent sequences from human variable domains. Exemplary methods for generating humanized antibodies or fragments thereof are provided by Morrison *Science* 229:1202-1207(1985); Oi et al. *BioTechniques* 4:214(1986); and U.S. Patent Nos. 5,585,089; 5,693,761; 5,693,762; 5,859,205; and 6,407,213.

Those methods include isolating, manipulating, and expressing the nucleic acid sequences that encode all or part of immunoglobulin variable domains from at least one of a heavy or light chain. Such nucleic acids may be obtained from a hybridoma producing an SDAB molecule against a predetermined target, as described above, as well as from other sources. The recombinant DNA encoding the humanized SDAB molecule, can then be cloned into an appropriate expression vector.

In certain embodiments, a humanized SDAB molecule is optimized by the introduction of conservative substitutions, consensus sequence substitutions, germline substitutions and/or back mutations. Such altered immunoglobulin molecules can be made by any of several techniques known in the art, (e.g., Teng et al, *Proc. Natl. Acad. Sci U.S.A.* 80:7308-7312(1983); Kozbor et al, *Immunology Today* 4:7279(1983); Olsson et al, *Meth. Enzymol,* 92:3-16(1982)), and may be made according to the teachings of WO92/06193 or EP 0239400. Techniques for humanizing SDAB molecules are also provided in WO 06/122786.

An SDAB molecule may also be modified by specific deletion of human T cell epitopes or "deimmunization" by the methods disclosed in WO 98/52976 and WO 00/34317. Briefly, the heavy chain variable domains of a SDAB molecule can be analyzed for peptides that bind to MHC Class II; these peptides represent potential T-cell epitopes (as defined in WO 98/52976 and WO 00/34317). For detection of potential T-cell epitopes, a computer modeling approach termed "peptide threading" can be applied, and in addition a database of human MHC class II binding peptides can be searched for motifs present in the VH and VL sequences, as described in WO 98/52976 and WO 00/34317. These motifs bind to any of the 18 major MHC class II DR allotypes, and thus constitute potential T cell epitopes. Potential T-cell epitopes detected can be eliminated by substituting small numbers of amino acid residues in the

variable domains, or preferably, by single amino acid substitutions. Typically, conservative substitutions are made.

Often, but not exclusively, an amino acid common to a position in human germline antibody sequences may be used. Human germline sequences, e.g., are disclosed in Tomlinson et al., *J. Mol. Biol.* 227:776-798(1992); Cook et al., *Immunol. Today* 16(5):237-242(1995); Chothia et al., *J. Mol. Biol.* 227:799-817(1992); and Tomlinson et al., *EMBO J.* 14:4628-4638(1995). The V BASE directory provides a comprehensive directory of human immunoglobulin variable region sequences (compiled by Tomlinson, LA. et al. MRC Centre for Protein Engineering, Cambridge, UK). These sequences can be used as a source of human sequence, e.g., for framework regions and CDRs. Consensus human framework regions can also be used, e.g., as described in U.S. 6,300,064. The SDAB molecules can be produced by living host cells that have been genetically engineered to produce the protein. Methods of genetically engineering cells to produce proteins are well known in the art. See e.g. Ausubel et al., eds. (1990), Current Protocols in Molecular Biology (Wiley, New York). Such methods include introducing nucleic acids that encode and allow expression of the protein into living host cells. These host cells can be bacterial cells, fungal cells, or, preferably, animal cells grown in culture. Bacterial host cells include, but are not limited to, Escherichia coli cells. Examples of suitable E. coli strains include: HB101, DH5a, GM2929, JM109, KW251, NM538, NM539, and any E. coli strain that fails to cleave foreign DNA. Fungal host cells that can be used include, but are not limited to, Saccharomyces cerevisiae, Pichia pastoris and Aspergillus cells. A few examples of animal cell lines that can be used are CHO, VERO, BHK, HeLa, Cos, MDCK, 293, 3T3, and WI38. New animal cell lines can be established using methods well know by those skilled in the art (e.g., by transformation, viral infection, and/or selection). Optionally, the protein can be secreted by the host cells into the medium.

Modified SDAB molecules

The formulations of the invention may contain at least one SDAB molecule having an amino acid sequence that differs from the amino acid sequence of a naturally occurring domain, e.g., VH domain, by least one amino acid position in one of the framework regions. It shall be understood that the amino acid sequences of the some of the SDAB molecules of the invention, such as the humanized SDAB molecules, can differ by at least one amino acid position in at least one of the framework regions from the amino acid sequences of naturally occurring domain, e.g., a naturally occurring VHH domains.

The invention also includes formulations of derivatives of the SDAB molecules. Such derivatives can generally be obtained by modification, and in particular by chemical and/or 5 biological (e.g., enzymatic) modification, of the SDAB molecules and/or of one or more of the amino acid residues that form the SDAB molecules disclosed herein. Examples of such modifications, as well as examples of amino acid residues within the SDAB molecule sequence that can be modified in such a manner (i.e. either on the protein backbone but preferably on a side chain), methods and techniques that can be used to introduce such modifications and the potential uses and advantages of such modifications will be clear to the skilled person.

For example, such a modification may involve the introduction (e.g. by covalent linking or in any other suitable manner) of one or more functional groups, residues or moieties into or onto the SDAB molecule, and in particular of one or more functional groups, residues or moieties that confer one or more desired properties or functionalities to the SDAB molecules. Example of such functional groups will be clear to the skilled person.

For example, such modification may comprise the introduction (e.g. by covalent binding or in any other suitable manner) of one or more functional groups that that increase the half-life, the solubility and/or the absorption of the SDAB molecule, that reduce the immunogenicity and/or the toxicity of the SDAB molecule, that eliminate or attenuate any undesirable side effects of the SDAB molecule, and/or that confer other advantageous properties to and/or reduce the undesired properties of the SDAB molecule; or any combination of two or more of the foregoing. Examples of such functional groups and of techniques for introducing them will be clear to the skilled person, and can generally comprise all functional groups and techniques mentioned in the general background art cited hereinabove as well as the functional groups and techniques known per se for the modification of pharmaceutical proteins, and in particular for the modification of antibodies or antibody fragments (including ScFvs and single domain antibodies), for which reference is for example made to Remington's Pharmaceutical Sciences, 16th ed., Mack Publishing Co., Easton, PA (1980). Such functional groups may for example be linked directly (for example covalently) to a SDAB molecule of the invention, or optionally via a suitable linker or spacer, as will again be clear to the skilled person. One widely used techniques for increasing the half-life and/or the reducing immunogenicity of pharmaceutical proteins comprises attachment of a suitable pharmacologically acceptable polymer, such as poly(ethyleneglycol) (PEG) or derivatives thereof (such as methoxypoly(ethyleneglycol) or mPEG). Generally, any suitable form of pegylation can be used, such as the pegylation used in the art for antibodies and antibody fragments (including but not limited to (single) domain antibodies and ScFvs); reference is made to for example Chapman, *Nat. Biotechnol.*, 54:531-545

(2002); Veronese and Harris, *Adv. Drug Deliv. Rev.* 54:453-456 (2003); Harris and Chess, *Nat. Rev. Drug. Discov.*, 2, (2003); and WO 04/060965. Various reagents for pegylation of proteins are also commercially available, for example from Nektar Therapeutics, USA.

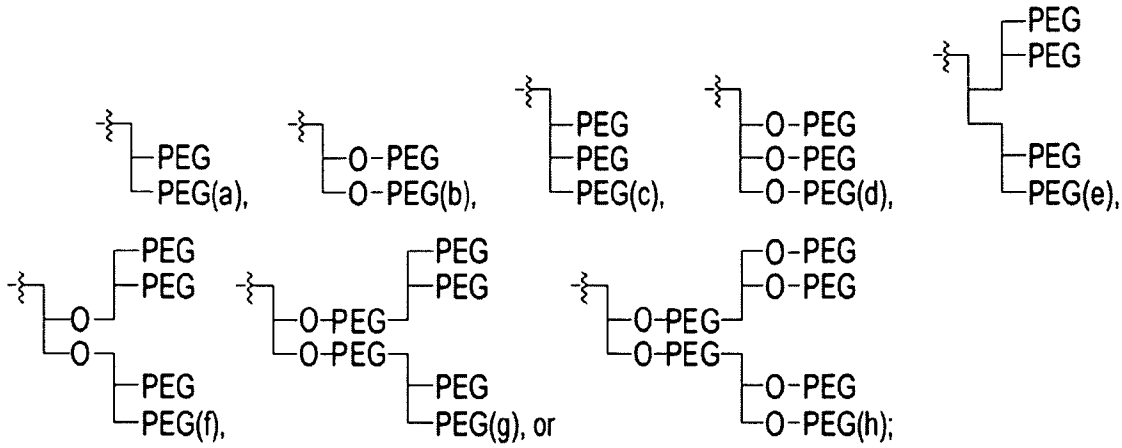
Preferably, site-directed pegylation is used, in particular via a cysteine-residue (see for example Yang et al., *Protein Engineering* 16(10):761-770 (2003)). For example, for this purpose, PEG may be attached to a cysteine residue that naturally occurs in an SDAB molecule, an SDAB molecule may be modified so as to suitably introduce one or more cysteine residues for attachment of PEG, or an amino acid sequence comprising one or more cysteine residues for attachment of PEG may be fused to the N-and/or C-terminus of a SDAB of the invention, all using techniques of protein engineering known per se to the skilled person.

Preferably, for the SDAB molecule, a PEG is used with a molecular weight of more than 5000, such as more than 10,000 and less than 200,000, such as less than 100,000; for example in the range of 20,000-80,000.

With regard to pegylation, it should be noted that generally, the invention also encompasses any SDAB molecule that has been pegylated at one or more amino acid positions, preferably in such a way that said pegylation either (1) increases the half- life in vivo; (2) reduces immunogenicity; (3) provides one or more further beneficial properties known per se for pegylation; (4) does not essentially affect the affinity of the SDAB molecule (e.g. does not reduce said affinity by more than 90%, preferably not by more than 50%, and by no more than 10%, as determined by a suitable assay, such as those described in the Examples below); and/or (4) does not affect any of the other desired properties of the SDAB molecule. Suitable PEG-groups and methods for attaching them, either specifically or non-specifically, will be clear to the skilled person.

Pegylated SDABs are disclosed, for example in US Provisional Application No. 61/265,307, filed July 16, 2011, which is incorporated by reference herein.

In some embodiments, the Pegylated SDAB comprises a modified SDAB molecule linked to a PEG polymer, wherein the PEG polymer molecule is a branched PEG polymer molecule selected from the group consisting of formulas (a)-(h):



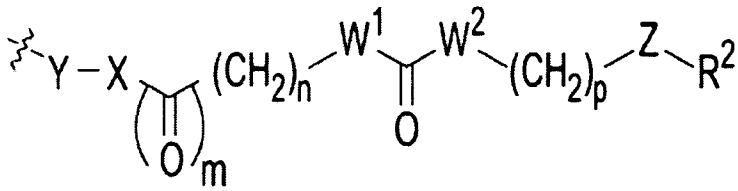
In some embodiments, the Pegylated SDAB comprises:

(i) one or more single antigen binding domains that bind to one or more targets;

(ii) a non-peptidic linker; and

(iii) one or more polymer molecules,

wherein the non-peptidic linker is a moiety of formula (I):



wherein

W1 and W2 are each independently selected from a bond or NR1;

Y is a bond, C1-4 alkylene substituted with 0-2 occurrences of Ra or a pyrrolidine-2,5-dione;

X is O, a bond or is absent;

Z is O, NR3, S or a bond;

R1 and R3 are each independently hydrogen or C1-6 alkyl;

R2 is absent or is one or more polymer moieties;

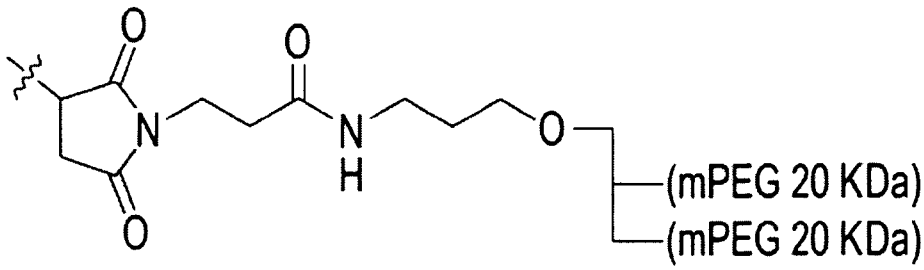
Ra is selected from hydroxyl, C1-4 alkyl or C1-4 alkoxy;

m is 0 or 1;

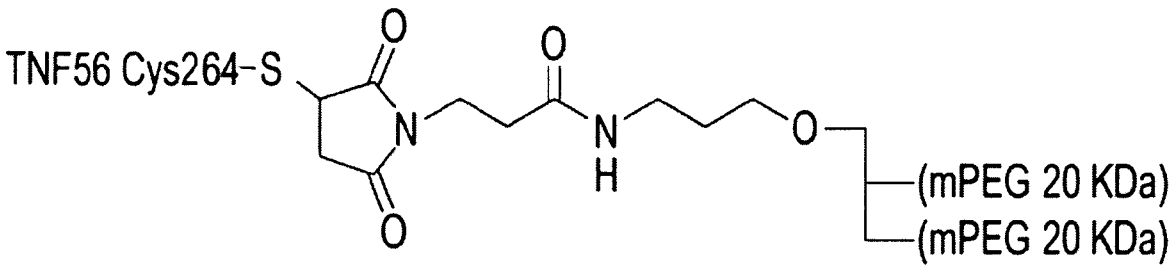
n is 0, 1, 2 or 3;

p is 0, 1, 2, 3 or 4.

In additional embodiments, the Pegylated SDAB is linked to the PEG via a linker represented by the following formula:



In particular embodiments, the modified SDAB molecule comprises the following structure:



Another, usually less preferred modification comprises N-linked or 0-linked glycosylation, usually as part of co-translational and/or post-translational modification, depending on the host cell used for expressing the SDAB molecule.

Methods of making SDABs

SDAB molecules can be produced by living host cells that have been genetically engineered to produce the protein. Methods of genetically engineering cells to produce proteins are well known in the art. See e.g. Ausubel et al., eds. (1990), Current Protocols in Molecular Biology (Wiley, New York). Such methods include introducing nucleic acids that encode and allow expression of the protein into living host cells. These host cells can be bacterial cells, fungal cells, or animal cells grown in culture. Bacterial host cells include, but are not limited to, Escherichia coli cells. Examples of suitable E. coli strains include: HB101, DH5a, GM2929, JM109, KW251, NM538, NM539, and any E. coli strain that fails to cleave foreign DNA. Fungal host cells that can be used include, but are not limited to, Saccharomyces cerevisiae, Pichia pastoris and Aspergillus cells. A few examples of animal cell lines that can be used are CHO, VERO, BHK, HeLa, Cos, MDCK, 293, 3T3, and WI38. New animal cell lines can be established using methods well know by those skilled in the art (e.g., by transformation, viral infection, and/or selection). Optionally, the protein can be secreted by the host cells into the medium.

In some embodiments, the SDAB molecules can be produced in bacterial cells, e.g., E. coli cells. For example, if the SDAB is encoded by sequences in a phage display vector that includes a suppressible stop codon between the display entity and a bacteriophage protein (or fragment thereof), the vector nucleic acid can be transferred into a bacterial cell that cannot suppress a stop codon. In this case, the SDAB is not fused to the gene III protein and is secreted into the periplasm and/or media.

The SDAB molecules can also be produced in eukaryotic cells. In one embodiment, the SDAB molecules are expressed in a yeast cell such as Pichia (see, e.g., Powers et al. *J Immunol Methods* 251:123-35 (2001)), Hansenula, or Saccharomyces.

In one embodiment, SDAB molecules are produced in mammalian cells. Typical mammalian host cells for expressing the clone antibodies or antigen-binding fragments thereof include Chinese Hamster Ovary (CHO cells) (including dhfr – CHO cells, described in Urlaub and Chasin, Proc. Natl. Acad. Sci. USA 77:4216-4220(1980), used with a DHFR selectable marker, e.g., as described in Kaufman and Sharp, Mol. Biol. 159:601-621 (1982)), lymphocytic cell lines, e.g., NS0 myeloma cells and SP2 cells, COS cells, and a cell from a transgenic animal, e.g., a transgenic mammal. For example, the cell is a mammary epithelial cell.

In addition to the nucleic acid sequences encoding the SDAB molecule, the recombinant expression vectors may carry additional sequences, such as sequences that regulate replication of the vector in host cells (e.g., origins of replication) and selectable marker genes. The selectable marker gene facilitates selection of host cells into which the vector has been introduced (see e.g., U.S. Patent Nos. 4,399,216; 4,634,665; and 5,179,017). For example, typically the selectable marker gene confers resistance to drugs, such as G418, hygromycin, or methotrexate, on a host cell into which the vector has been introduced.

In an exemplary system for recombinant expression of the SDAB molecule, a recombinant expression vector encoding the single domain antibody chain is introduced into dhfr- CHO cells by calcium phosphate-mediated transfection. Within the recombinant expression vector, the antibody genes are operatively linked to enhancer/promoter regulatory elements (e.g., derived from SV40, CMV, adenovirus and the like, such as a CMV enhancer/AdMLP promoter regulatory element or an SV40 enhancer/AdMLP promoter regulatory element) to drive high levels of transcription of the genes. The recombinant expression vector also carries a DHFR gene, which allows for selection of CHO cells that have been transfected with the vector using methotrexate selection/amplification. The selected transformant host cells can be cultured to allow for expression of the antibody chains and intact single domain is recovered from the culture medium. Standard molecular biology techniques can be used to prepare the

recombinant expression vector, transfect the host cells, select for transformants, culture the host cells and recover the antibody molecule from the culture medium. For example, some SDAB molecules can be isolated by affinity chromatography.

In one embodiment, the SDAB molecule is purified as described in WO 10/056550. In an exemplary embodiment, the SDAB is purified from one or more contaminants by: contacting a mixture of SDAB and contaminant(s) with a Protein A-based support and/or an ion exchange support, under conditions that allow the SDAB to bind to or adsorb to the support; removing one or more contaminants by washing the bound support under conditions where the SDAB remains bound to the support, and selectively eluting the SDAB from the support by eluting the adsorbed SDAB molecule with an elution buffer.

SDAB molecules can also be produced by a transgenic animal. For example, U.S. Patent No. 5,849,992 describes a method of expressing an antibody in the mammary gland of a transgenic mammal. A transgene is constructed that includes a milk-specific promoter and nucleic acids encoding the antibody molecule and a signal sequence for secretion. The milk produced by females of such transgenic mammals includes, secreted therein, the single domain of interest. The antibody molecule can be purified from the milk, or for some applications, used directly.

Formulations

The SDABs of the invention may be formulated into any pharmaceutically acceptable formulation. The formulation may be liquid or dry. The formulation may be generated via mixing, drying, lyophilization, vacuum drying, or any known method for formulating pharmaceutical compositions.

A pharmaceutical formulation can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable to high protein concentration. Sterile injectable solutions can be prepared by incorporating an agent described herein in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating an agent described herein into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated below. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants.

Prolonged absorption of injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

A formulation of an SDAB molecule includes an SDAB molecule, a compound that can serve as a cryoprotectant, and a buffer. The pH of the formulation is generally pH 5.5 – 7.0, preferably about pH 6. In some embodiments, a formulation is stored as a liquid. In other embodiments, a formulation is prepared as a liquid and then is dried, e.g., by lyophilization or spray-drying, prior to storage. A dried formulation can be used as a dry compound, e.g., as an aerosol or powder, or reconstituted to its original or another concentration, e.g., using water, a buffer, or other appropriate liquid.

The SDAB molecule purification process is designed to permit transfer of an SDAB molecule into a formulation suitable for long-term storage as a frozen liquid and subsequently for freeze-drying (e.g., using a histidine/sucrose formulation). The formulation is lyophilized with the protein at a specific concentration. The lyophilized formulation can then be reconstituted as needed with a suitable diluent (e.g., water) to resolubilize the original formulation components to a desired concentration, generally the same or higher concentration compared to the concentration prior to lyophilization.

The lyophilized formulation may be reconstituted to produce a formulation that has a concentration that differs from the original concentration (i.e., before lyophilization), depending upon the amount of water or diluent added to the lyophilate relative to the volume of liquid that was originally freeze- dried. Suitable formulations can be identified by assaying one or more parameters of antibody integrity.

The SDABs of the invention may be formulated as described in WO 10/077422. The SDABs of the invention may be formulated by the following exemplary process: lyophilizing a 10 mixture of a SDAB, a lyoprotectant, a surfactant, and a buffer; and reconstituting the lyophilized mixture in diluents, thereby preparing the formulation. In particular embodiments, the SDABs of the invention are formulated by the following process: expressing the SDAB in a cell culture; purifying the SDAB via a chromatography purification step and/or a ultrafiltration/diafiltration step; adjusting the concentration of the SDAB to about 10 to 250 mg/ml in a formulation containing sucrose at a concentration of about 5-10%, polysorbate-80 at a concentration of about 0.01-0.02%, and a Histidine buffer at a concentration of about 10-20 mM or a Tris buffer at a concentration of about 20 mM, such that the pH of the formulation is about 5 to 7.5.

Methods of Treatment

SDAB molecules can be administered to a subject (e.g., a human subject) alone or combination with a second agent, e.g., a second therapeutically or pharmacologically active agent, to treat or prevent (e.g., reduce or ameliorate one or more symptoms associated with) a TNFa associated disorder, e.g., inflammatory or autoimmune disorders.

The SDAB molecules of the invention, such as polypeptides comprising two anti-tumor necrosis factor alpha (TNFa) SDABs and an anti-human serum albumin (HSA) SDAB, can be used for treating or preventing an immune disorder in a human in need thereof, alone or in combination with a second agent as described herein.

The term "treating" refers to administering a therapy in an amount, manner, and/or mode effective to improve a condition, symptom, or parameter associated with a disorder or to prevent progression of a disorder, to either a statistically significant degree or to a degree detectable to one skilled in the art. In the case of therapeutic use, the treatment may improve, cure, maintain, or decrease duration of, the disorder or condition in the subject. In therapeutic uses, the subject may have a partial or full manifestation of the symptoms. In a typical case, treatment improves the disorder or condition of the subject to an extent detectable by a physician, or prevents worsening of the disorder or condition. An effective amount, manner, or mode can vary depending on the subject and may be tailored to the subject.

As used herein, the terms "subject" and "patient" are used interchangeably. As used herein, the terms "subject" and "subjects" refer to an animal, e.g., a mammal including a nonprimate (e.g., a cow, pig, horse, donkey, goat, camel, cat, dog, guinea pig, rat, mouse, sheep) and a primate (e.g., a monkey, such as a cynomolgus monkey, gorilla chimpanzee and a human).

Non-limiting examples of immune disorders that can be treated include, but are not limited to, autoimmune disorders, e.g., arthritis (including rheumatoid arthritis, juvenile rheumatoid arthritis, osteoarthritis, psoriatic arthritis, lupus-associated arthritis or ankylosing spondylitis), scleroderma, systemic lupus erythematosis, Sjogren's syndrome, vasculitis, multiple sclerosis, autoimmune thyroiditis, dermatitis (including atopic dermatitis and eczematous dermatitis), myasthenia gravis, inflammatory bowel disease (IBD), Crohn's disease, colitis, diabetes mellitus (type I); inflammatory conditions of, e.g., the skin (e.g., psoriasis); acute inflammatory conditions (e.g., endotoxemia, sepsis and septicemia, toxic shock syndrome and infectious disease); transplant rejection and allergy. In one embodiment, the TNFa associated disorder is, an arthritic disorder, e.g., a disorder chosen from one or more of rheumatoid

arthritis, juvenile rheumatoid arthritis (RA) (e.g., moderate to severe rheumatoid arthritis), osteoarthritis, psoriatic arthritis, or ankylosing spondylitis, polyarticular juvenile idiopathic arthritis (JIA); or psoriasis, ulcerative colitis, Crohn's disease, inflammatory bowel disease, and/or multiple sclerosis.

In certain embodiments, the SDAB molecules (or formulations) are administered or used for administration in combination with a second therapeutic agent. For example, for TNF-SDABs, the second agent may be an anti-TNF antibody or TNF-binding fragment thereof, wherein the second TNF antibody has a different epitope specificity than the TNF-binding SDAB molecule of the formulation. Other non-limiting examples of agents that can be co-formulated with TNF-binding SDAB include, for example, a cytokine inhibitor, a growth factor inhibitor, an immunosuppressant, an anti-inflammatory agent, a metabolic inhibitor, an enzyme inhibitor, a cytotoxic agent, and a cytostatic agent. In one embodiment, the additional agent is a standard treatment for arthritis, including, but not limited to, nonsteroidal anti-inflammatory agents (NSAIDs); corticosteroids, including prednisolone, prednisone, cortisone, and triamcinolone; and disease modifying anti-rheumatic drugs (DMARDs), such as methotrexate, hydroxychloroquine (Plaquenil) and sulfasalazine, leflunomide (Arava®), tumor necrosis factor inhibitors, including etanercept (Enbrel®), infliximab (Remicade®) (with or without methotrexate), and adalimumab (Humira®), anti-CD20 antibody (e.g., Rituxan®), soluble interleukin-1 receptor, such as anakinra (Kineret), gold, minocycline (Minocin®), penicillamine, and cytotoxic agents, including azathioprine, cyclophosphamide, and cyclosporine. Such combination therapies may advantageously utilize lower dosages of the administered therapeutic agents, thus avoiding possible toxicities or complications associated with the various monotherapies.

When the additional agent is methotrexate, the dose of methotrexate may range from about 7.5 to about 25 mg weekly.

The SDAB molecule can be administered or used for administration in the form of a liquid solution (e.g., injectable and infusible solutions). Such compositions can be administered by a parenteral mode (e.g., subcutaneous, intraperitoneal, or intramuscular injection), or by inhalation. The phrases "parenteral administration" and "administered parenterally" as used herein mean modes of administration other than enteral and topical administration, usually by injection, and include, subcutaneous or intramuscular administration, as well as intravenous, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcuticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion. In one embodiment, the formulations described herein are administered subcutaneously.

The present inventors hypothesized that the bispecific interaction sites of ozoralizumab with TNFα is particularly useful for treating immune disorders. Accordingly, the present invention relates to a method of treating an immune disorder in a human in need thereof, comprising administering to the human a polypeptide competing with the polypeptide comprising the amino acid sequence of SEQ ID NO:1 (ozoralizumab).

Dosage regimens

Ozoralizumab showed efficacy in treating rheumatoid arthritis when administered subcutaneously with at least four weeks between doses. In contrast, the recommended dosing for Humira® is administration every two weeks and Remicade® must be administered intravenously. Accordingly the dosage regimens of the instant invention provide advantages over the state of the art.

The SDAB molecules of the invention have shown efficacy in treating disease at doses of 30 mg and 80 mg administered every 4 weeks. Modeling based on these efficacy results indicated that at higher doses (e.g., 120-200 mg or 200-400 mg), the SDAB molecules of the invention are efficacious in treating disease when administered every 6 or 8 weeks or 2 months. This advantageous profile results in a decreased injection burden.

In addition, modeling also indicated that the serious infections (SI) of the ozoralizumab treatment were lower than with etanercept and infliximab. In particular, compared to the prior art anti-TNFa inhibitors, the SDAB molecules of the present invention demonstrate a very favourable benefit (efficacy) to risk (SI effects) ratio.

Accordingly, the SDAB molecules of the invention may be administered every 4, 6, or 8 weeks at doses ranging from 30-200 mg. Particular efficacious doses are 30-400 mg. In particular embodiments, the dose comprises about 5, 10, 15, 20, 25, 30, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 320, 350, 375 or 400 mg of a SDAB molecule.

The single doses of about 30-200 mg or even 30-400 mg may be administered approximately every day, every other day, twice a week, every 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 weeks, or every 1 or 2 months.

In particular embodiments, a dose of about 30, 80, 120, 160, 200, 240, 280, 320, 360 or 400 mg of a polypeptide comprising the amino acid sequence of SEQ ID NO:1 (ozoralizumab) is administered to human subjects approximately every 4, 6, or 8 weeks or 2 months. In some embodiments, the subjects are suffering from rheumatoid arthritis.

In additional embodiments, the dose of the SDAB molecule is about 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 205, 210, 215, 220, 225, 230, 235, 240, 245, 250, 255, 260, 265, 270, 275, 280, 285, 290, 295, 300, 305, 310, 315, 320, 325, 330, 335, 340, 345, 350, 355, 360, 365, 370, 375, 380, 385, 390, 395 or 400 mg.

In some embodiments, the dose is about 3.6, 10.8, 36, 72, 144, or 288 mg.

In particular embodiments, the SDAB molecule is PEGylated and the dose is about 3.6, 10.8, 36, 72, 144, or 288 mg.

For administration to juvenile patients, the dose should be adjusted to the weight of the patient. In particular embodiments the dose is about 0.1, 0.38, 1, 2, 3, 3.5, 4, 4.5 or 5 mg/kg.

The subjects may be concurrently treated with methotrexate. In some embodiments, the subjects may have been receiving methotrexate for at least about 6 or 12 weeks prior to the initial dosing with ozoralizumab. The methotrexate may be administered by any suitable route, and the dose may range from about 7.5 to 25 mg weekly.

Methods of determining efficacy

The efficacy of any particular SDAB molecule or dosing regimen may be determined by methods available to those of skill in the art. Briefly, during a clinical trial, the patients may be observed by medical personnel and the state of disease is assessed by any combination of criteria. The improvement of a patient's disease state is determined based on these criteria at numerous time points and the combination of these determinations on a patient population is plotted to assess the efficacy of treatment.

In exemplary embodiments, disease progression for rheumatoid arthritis may be measured by any or all of the criteria set forth in Table 2.

Table 2

ACR criteria for classification of RA	Arnett et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum.1988:31:315-241. Hochberg et al., American College of Rheumatology 1991 revised criteria for the classification of global functional status in rheumatoid arthritis. Arthritis Rheum. 1992;35:498-502.
ACR Preliminary Definition of Improvement in RA (ACR20, 50, 70)	Felson, et al., American College of Rheumatology preliminary definition of improvement in rheumatoid arthritis. Arthritis Rheum 1995; 38:727-735
Joint Assessment—28 Joints	Starz et al., J Musculoskeletal Med 28(3):1-7
NY Heart Association (NYHA) classification	The Criteria Committee of the New York Heart Association. Nomenclature and Criteria for Diagnosis of Diseases of the Heart and Great Vessels. 9th ed. Boston, Mass: Little, Brown & Co; 1994:253-256.
Duration of morning stiffness	Khan et al., J Rheum 36(11):2435-42 (2009)
Health Assessment Questionnaire Disability Index (HAQ-DI)	Bruce & Fries, Health Qual Life Outcomes 1:20 (2003)
SF-36	http://www.sf-36.org/demos/SF-36.html QualityMetric Incorporated
Physician and Patient Global Assessment	Reid & Miller, Clinical Trials in Rheumatoid Arthritis and Osteoarthritis, Springer, 2008
General Health Visual Analog Scale	Massy-Westropp et al, J Hand Ther 18(1):30-33 (2005)
Disease Activity Score 28 (DAS 28)	Van der Heijde et al., J. Rheumatol 1993; 20: 579-81 Van Gestel et al., Arthritis Rheum 1998; 41:1845-1850 Prevoo et al., Arthritis Rheum 1995; 38:44-8. Aletaha et al., Arthritis Res 2005; 7:R796-R806. The DAS-CRP. UMC Sint Radbound Nijmegen DAS website. Available at http://www.das-score.nl . (last date accessed 30 Apr 200
EULAR Response criteria	van Gestel et al., Arthritis Rheum. 1998; 41(10):1845-1850.
Pain visual analog scale	Callahan et al., Arthritis Rheum 30(6):630-36 (1987)

EXAMPLES

Example 1

A seamless phase 1/2, randomized, stratified, double-blind, placebo-controlled study in rheumatoid arthritis (RA) patients was performed with ozoralizumab. There were a total of 254 subjects randomized to the study; of which one subject was not treated and the remaining 253 subjects were included in the modified intent-to-treat (mITT) population as well as the safety population. Each subject was randomly assigned to only one treatment group, stratified based on TNF inhibitor (TNFi) naïve or prior TNFi use. There were a total of six treatment groups, described in Table 3, with 40-45 patients randomized in each group.

Table 3

Group	Treatment
1	10 mg every 4 weeks (total of 4 doses)
2	10 mg every 8 weeks (placebo given at the intervening 4 week visit) (total of 2 doses)
3	30 mg every 4 weeks (total of 4 doses)
4	80 mg every 4 weeks (total of 4 doses)
5	80 mg every 8 weeks (placebo given at the intervening 4 week visit) (total of 2 doses)
6	Placebo every 4 weeks (total of 4 doses)

Baseline characteristics in these six groups were generally similar; the only small but statistically significant difference (p=0.048) observed among groups was in DAS28 with the highest mean (6.59) DAS28 in the 80 mg Q4 group. The subject ages ranged from 18 to 79 (mean of 52.1) and the majority of the subjects (80.2%) were female (as expected for a clinical trial in RA patients). The overall mean of disease duration was 8.3 years, and 28.9% of subjects had received prior TNFi treatment.

The primary population for efficacy analysis was the mITT population, defined as all randomized subjects who received at least one dose of ozoralizumab. All patients were concurrently treated with methotrexate at doses of 7.5 to 25 mg weekly.

Each subject received a subcutaneous (SC) injection of a single dose level of ozoralizumab or placebo at the indicated time points.

The primary comparisons of interest are the comparisons of each ATN-103 treatment group versus placebo.

Table 4 shows the American College of Rheumatology 20 (ACR20) response rates at week 16.

Table 4

Treatment	N	n(%)	P value		Placebo-Adjusted Difference and its Confidence Interval (30% percentile, 80% percentile)
			OZORALIZUMAB v placebo	overall	(OZORALIZUMAB - Placebo)
Placebo	45	19 (42.2)		0.120	
10 mg Q4	42	22 (52.4)	0.130		10.2 (9.3, 24.7)
10 mg Q8	41	20 (48.8)	0.390		6.6 (3.6, 19.6)
30 mg Q4	40	24 (60.0)	0.122		17.8 (9.4, 25.0)
80 mg Q4	43	31 (72.1)	0.006		29.9 (23.5, 38.7)
80 mg Q8	42	25 (59.5)	0.101		17.3 (12.2, 27.9)

The P-value was obtained based on stratified (TNFi naive or prior TNFi use) Cochran-Mantel-Haenszel Test. The minimum risk (MR) weighting method proposed by Mehrotra and Railkar is used for calculation of the stratified (TNFi naive or prior TNFi use) confidence interval for the placebo-adjusted difference. Last observation carried forward (LOCF) approach was used to impute any missing data of ACR components before the ACR20 response is derived.

Figure 1 shows the percentage of subjects achieving the ACR20, ACR50, and ACR70 criteria at week 8 and week 16. Some of the dosing regimens had an advantage over placebo in the following endpoints: ACR20 (Figures 1 & 2), ACR50 (Figure 1), DAS28 (Figure 3), ACR-N, TJC, SJC, Pain VAS, HAQ-DI, CRP, Physician Global Assessment, Patient Global Assessment, General Health VAS, and EULAR response (data not shown).

Briefly, as shown in Figure 1, at Week 8, for 10 mg Q8, 36.6% of subjects achieved ACR20, 9.8% of subjects achieved ACR50, and 0% of subjects achieved ACR70; for 10 mg Q4, 45.2% of subjects achieved ACR20, 11.9% of subjects achieved ACR50, and 7.1% of subjects achieved ACR70; for 30 mg Q4, 50% of subjects achieved ACR20, 32.5% of subjects achieved ACR50, and 15% of subjects achieved ACR70; for 80 mg Q8, 54.8% of subjects achieved ACR20, 23.8% of subjects achieved ACR50, and 4.8% of subjects achieved ACR70; and for 80 mg Q4, 65.1% of subjects achieved ACR20, 25.6% of subjects achieved ACR50, and 2.3% of subjects achieved ACR70. At Week 16, for 10 mg Q8, 48.8% of subjects achieved ACR20, 24.4% of subjects achieved ACR50, and 8.9% of subjects achieved ACR70; for 10 mg Q4, 52.4% of subjects achieved ACR20, 21.4% of subjects achieved ACR50, and 4.8% of subjects achieved ACR70; for 30 mg Q4, 60% of subjects achieved ACR20, 32.5% of subjects achieved ACR50, and 20% of subjects achieved ACR70; for 80 mg Q8, 59.5% of subjects achieved ACR20, 31% of subjects achieved ACR50, and

19% of subjects achieved ACR70; and for 80 mg Q4, 72.1% of subjects achieved ACR20, 37.2% of subjects achieved ACR50, and 11.6% of subjects achieved ACR70.

As shown in Figure 3, at week 4, the observed mean change from baseline (placebo) for the DAS28 criteria was 1.39% for 10 mg Q4, 1.05% for 10 mg Q8, 1.62% for 30 mg Q4, 1.63% for 80 mg Q4, and 1.67% for 80 mg Q8. At week 8, the observed mean change from baseline for the DAS28 criteria was 1.59% for 10 mg Q4, 1.01% for 10 mg Q8, 1.78% for 30 mg Q4, 2.00% for 80 mg Q4, and 1.85% for 80 mg Q8. At week 12, the observed mean change from baseline for the DAS28 criteria was 1.61% for 10 mg Q4, 1.72% for 10 mg Q8, 2.31% for 30 mg Q4, 2.30% for 80 mg Q4, and 2.20% for 80 mg Q8. At week 16, the observed mean change from baseline for the DAS28 criteria was 1.60% for 10 mg Q4, 1.70% for 10 mg Q8, 2.09 % for 30 mg Q4, 2.46% for 80 mg Q4, and 1.93% for 80 mg Q8. At week 20, the observed mean change from baseline for the DAS28 criteria was 1.02% for 10 mg Q4, 1.03% for 10 mg Q8, 1.62% for 30 mg Q4, 2.00% for 80 mg Q4, and 1.36% for 80 mg Q8.

Overall, the safety profile of ozoralizumab appears to be comparable to that of other TNF inhibitor (TNFi) agents. Reported SAEs were also consistent with the safety profile of other anti-TNF agents (data not shown).

Example 2: Comparative Effectiveness of the Nanobody® ATN-103 versus five marketed anti-TNFs in Rheumatoid Arthritis

2.1 Objectives:

Ozoralizumab (ATN-103), a novel tumor necrosis factor inhibitor (TNFi), is a humanized, trivalent, bispecific Nanobody containing two human TNF-binding domains linked to a human serum albumin-binding domain. A model-based meta analysis (MBMA) was done to assess the comparative effectiveness of various doses/dosing regimens of ATN-103 versus five marketed Tumor Necrosis Factor-alpha blocking (anti-TNFa) drugs with respect to efficacy (ACR20/50/70, DAS, HAQ) and safety/tolerability (rate of serious infections, %SI) and to evaluate the effect of explanatory covariates.

2.2 Methods:

The model-based comparative efficacy and safety analysis was conducted across 5 comparators (infliximab, adalimumab, etanercept, certolizumab pegol and golimumab) using published data, summarized at a study-arm level, and in-house Phase IIa data.

In particular, the ACR20/50/70 dataset included data from 7,474 subjects, participating in 20 trials with a total of 63 arms. DAS was observed in 21 arms in 8 trials. The serious infection rate dataset included data from 6,209 subjects, participating in 14 trials with a total of 46 arms. The datasets contained measures of drug exposure (dose strengths, dosing intervals), baseline disease severity covariates (tender and swollen joint count, observed DAS, patients' and physicians' global assessment, CRP concentration, disease duration), characteristics of the trial populations (percent anti-TNFα experienced subjects, and geography (Asian versus non-Asian subjects).

The three ACR endpoints and the DAS endpoints were described using joint ACR20/50/70 and DAS models, respectively.

2.3 Results:

2.3.1 Simulated dose-AC20/50/70 response

The expected dose-response relationship of ATN-103 and five comparator drugs following 24 weeks of treatment adjunctive to continued DMARD background medication was simulated using the final ACR20/50/70 model in a typical patient population (TNF experienced with a mean baseline swollen joint count of 19 and 21% ACR20 responders in the placebo group). Figure 4 shows the predicted dose-response relationship of ATN-103 for ACR20 response at week 24 compared to other anti-TNFα drugs in the population.

The simulations suggest that 80 mg Q4W ATN-103 treatment is comparable to 95% of the response to adalimumab and golimumab, while 200 mg ATN-103 Q4W is predicted to produce a response similar to 95% of the response to etanercept.

2.3.2 Simulated dose-DAS response

The expected dose-response relationship of ATN-103 and five comparator drugs for DAS %CfB following 24 weeks of treatment adjunctive to ongoing DMARD background medication was simulated. The DAS placebo response was assumed to be -16% change from baseline for DAS, reflecting the mean of the unstructured mean placebo model parameter estimates (data not shown).

Figure 5 shows the expected dose-response relationship of ATN-103 for DAS responses compared to other anti-TNFα drugs. At 200 mg Q4W, the simulations indicate that ATN-103 is superior to golimumab and certolizumab in terms of DAS %CfB.

2.3.3 Simulated serious infection rates

A simulated placebo-corrected %SI suggests that the expected infection rate of ATN-103 is comparable to that of adalimumab and golimumab, while certolizumab, etanercept, and infliximab may have higher serious infection rates (data not shown).

Figure 6 provides an integrated overview of the expected efficacy (ACR) and safety (%SI) profile of ATN-103 versus other anti-TNFα drugs. In particular, Figure 6 shows the 95% prediction intervals of simulated efficacy (ACR20) of anti-TNFα drugs at relevant dosing regimen and corresponding simulated 95% prediction intervals of the serious infection rates. An optimal efficacy/safety utility is shown towards the upper left corner (high efficacy, low serious infection rate), decreasing towards the lower right corner. The bubble plots show a considerable overlap in utility between the anti-TNFα drugs.

This representation of simulated efficacy and safety suggests that 200 mg ATN-103 Q4W may be well positioned versus other anti-TNFα drugs.

2.3.4 Dosage regimen of 400 mg Q8W

The model suggests that the response is not dependent upon the treatment regimen, and similar effects can be obtained for 200 mg Q4W regimen or a 400 mg Q8W.

2.4 Conclusions:

The comparative effectiveness of ATN-103 versus five anti-TNFa drugs for efficacy (ACR/DAS) and safety/tolerability (serious infection rate) was evaluated in a model-based meta-analysis. Compared to other anti-TNFα drugs, ATN-103 shows a favourable combination of efficacy and SI effects.

Model-based simulations indicated that 200 mg ATN-103 Q4W or 400 mg ATN-103 Q8W are similar in efficacy to etanercept, adalimumab and infliximab.

CLAIMS

1. A polypeptide comprising two anti-tumor necrosis factor alpha (TNFa) SDABs and an anti-human serum albumin (HSA) SDAB for use in treating an immune disorder in a human in need thereof, by administering to the human multiple 30-400 mg doses of said polypeptide, wherein the doses are at least about every four weeks apart.

2. The polypeptide of claim 1, wherein each anti-TNFa SDAB comprises 3 complementary determining regions (CDR1, CDR2, and CDR3) wherein

(a) CDR1 comprises

(i) the amino acid sequence DYWMY (SEQ ID NO:22);

(ii) an amino acid sequence that has at least 80% sequence identity with DYWMY (SEQ ID NO:22); or

(iii) an amino acid sequence that has only 1 amino acid difference from DYWMY (SEQ ID NO:22);

(b) CDR2 comprises

(i) the amino acid sequence EINTNGLITKYPDSVKG (SEQ ID NO:23);

(ii) an amino acid sequence that has at least 80%, 90%, or 95% sequence identity with EINTNGLITKYPDSVKG (SEQ ID NO:23); or

(iii) an amino acid sequence that has 2 or 1 amino acid differences from EINTNGLITKYPDSVKG (SEQ ID NO:23); and

(c) CDR3 comprises

(i) the amino acid sequence SPSGFN (SEQ ID NO:24);

(ii) an amino acid sequence that has at least 80% sequence identity with SPSGFN (SEQ ID NO:24); or

(iii) an amino acid sequence that has 1 amino acid difference from SPSGFN (SEQ 5 ID NO:24).

3. The polypeptide of claim 1 or 2, wherein CDR1 comprises the amino acid sequence DYWMY (SEQ ID NO:22), CDR2 comprises the amino acid sequence EINTNGLITKYPDSVKG (SEQ ID NO:23), and CDR3 comprises the amino acid sequence SPSGFN (SEQ ID NO:24).

4. The polypeptide of any one of claims 1 to 3, wherein at least one of the anti-TNFa SDABs comprises an amino acid sequence with at least 80%, 90%, 95%, or 99% sequence identity to SEQ ID NO:2 (TNF30).

5. The polypeptide of any one of claims 1 to 4, wherein each anti-TNFa SDAB comprises an amino acid sequence having at least 80%, 90%, 95%, or 95% sequence identity to SEQ ID NO:2 (TNF30).

6. The polypeptide of any one of claims 1 to 5, wherein the anti-HSA SDAB comprises 3 CDRs (CDR1, CDR2, and CDR3) wherein

(a) CDR1 comprises

(i) the amino acid sequence SFGMS (SEQ ID NO:25);

(ii) an amino acid sequence that has at least 80% sequence identity with SFGMS (SEQ ID NO:25); or

(iii) an amino acid sequence that has only 1 amino acid difference from SFGMS (SEQ ID NO:25);

(b) CDR2 comprises

(i) the amino acid sequence SISGSGSDTLYADSVKG (SEQ ID NO:26);

(ii) an amino acid sequence that has at least 80%, 90%, or 95% sequence identity with SISGSGSDTLYADSVKG (SEQ ID NO:26); or

(iii) an amino acid sequence that has 2 or 1 amino acid differences from SISGSGSDTLYADSVKG (SEQ ID NO:26); and

(c) CDR3 comprises

(i) the amino acid sequence GGSLSR (SEQ ID NO:27);

(ii) an amino acid sequence that has at least 80% sequence identity with GGSLSR (SEQ ID NO:27); or

(iii) an amino acid sequence that has 1 amino acid difference from GGSLSR (SEQ ID NO:27).

7. The polypeptide of claim 6, wherein CDR1 comprises the amino acid sequence SFGMS (SEQ ID NO:25), CDR2 comprises the amino acid sequence SISGSGSDTLYADSVKG (SEQ ID NO:26), and CDR3 comprises the amino acid sequence GGSLSR (SEQ ID NO:27).

8. The polypeptide of claim 6 or 7, wherein the anti-HSA SDAB comprises an amino acid sequence having at least 80%, 90%, 95%, or 95% sequence identity to SEQ ID NO:5 (ALB8).

9. The polypeptide of any one of claims 1 to 8, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:1 (ozoralizumab).

10. The polypeptide of any one of claims 1 to 9, wherein at least one of the SDABs is humanized.

11. The polypeptide of any one of claims 1 to 10, wherein each of the two anti-TNF SDABs and anti-HSA SDAB are linked via linkers, wherein each linker is selected from the group consisting of the amino acid sequences of SEQ ID NO:6 and SEQ ID NO:7.

12. The polypeptide of any one of claims 1 to 11, wherein the doses are separated in time by at least about one month.

13. The polypeptide of any one of claims 1 to 36, wherein the doses are separated in time by at least about 6 weeks.

14. The polypeptide of any one of claims 1 to 13, wherein the doses are separated in time by at least about 8 weeks.

15. The polypeptide of any one of claims 1 to 14, wherein the doses are selected from the group consisting of about 10, 30, 80, 100, 120, 140, 160, 180, 200, 250, 275, 300, 320, 350, 375 and 400 mg of the SDAB molecule.

16. The polypeptide of any one of claims 1 to 15, wherein the polypeptide is administered subcutaneously or intravenously.

17. The polypeptide of any one of claims 1 to 16, wherein the human is treated concurrently with methotrexate.

18. The polypeptide of claim 1 to 17, wherein the polypeptide is formulated into a pharmaceutically acceptable formulation.

19. The polypeptide of claim 18, wherein the formulation comprises

(a) a polypeptide of claim 1 at a concentration of about 10 mg/ml to 250 mg/ml;

(b) a lyoprotectant chosen from sucrose, sorbitol, or trehalose at a concentration of about 5% to about 10%;

(c) a surfactant chosen from polysorbate-80 or poloxamer-188 at a concentration of about 0.01% to 0.6%; and

(d) a buffer chosen from Histidine buffer at a concentration of about 10 to 20 mM or a Tris buffer at a concentration of about 20 mM such that the pH of the formulation is about 5.0 to 7.5.

20. The polypeptide of claim 19, wherein the formulation comprises 100 mg/ml of the polypeptide of claim 1, 20 mM Histidine, 7.5% (w/v) sucrose, and 0.01% polysorbate-80 at pH 6.0.

21. The polypeptide of any one of claims 1 to 20, wherein the immune disorder is selected from the group consisting of: inflammation, arthritis, including rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, juvenile idiopathic arthritis and osteoarthritis, COPD, asthma, inflammatory bowel diseases including Crohn's disease and ulcerative colitis, multiple sclerosis, Addison's disease, Autoimmune hepatitis, Autoimmune parotitis, Diabetes Type I, Epididymitis, Glomerulonephritis, Graves' disease, Guillain -Barre syndrome, Hashimoto's disease, Hemolytic anemia, Systemic lupus erythematosus, Male infertility, Multiple sclerosis, Myasthenia Gravis, Pemphigus, Psoriasis, Hidradenitis suppurativa, Rheumatic fever, Sarcoidosis, Scleroderma, Sjogren's syndrome, Spondyloarthropathies, Thyroiditis, and Vasculitis.

22. The polypeptide of claim 21, wherein the immune disorder is rheumatoid arthritis.

23. A polypeptide comprising the amino acid sequence of SEQ ID NO:1 for use in treating rheumatoid arthritis in a human in need thereof, by administering to the human 80 mg of a polypeptide about every four weeks, wherein the human is concurrently treated with methotrexate.

24. A polypeptide comprising the amino acid sequence of SEQ ID NO:1 for use in treating rheumatoid arthritis in a human in need thereof, by administering to the human 80 mg of a polypeptide about every eight weeks, wherein the human is concurrently treated with methotrexate.

25. A polypeptide for use in treating an immune disorder in a human in need thereof, wherein said polypeptide competes with the polypeptide comprising the amino acid sequence of SEQ ID NO:1 (ozoralizumab) of claim 9.

26 A method of treating an immune disorder in a human in need thereof, comprising administering to the human multiple 30-400 mg doses of a polypeptide comprising two anti-tumor necrosis factor alpha (TNFa) SDABs and an anti-human serum albumin (HSA) SDAB, wherein the doses are at least about every four weeks apart.

27. The method of claim 26, wherein each anti-TNFa SDAB comprises 3 complementary determining regions (CDR1, CDR2, and CDR3) wherein

(a) CDR1 comprises

(i) the amino acid sequence DYWMY (SEQ ID NO:22);

(ii) an amino acid sequence that has at least 80% sequence identity with DYWMY (SEQ ID NO:22); or

(iii) an amino acid sequence that has only 1 amino acid difference from DYWMY (SEQ ID NO:22);

(b) CDR2 comprises

(i) the amino acid sequence EINTNGLITKYPDSVKG (SEQ ID NO:23);

(ii) an amino acid sequence that has at least 80%, 90%, or 95% sequence identity with EINTNGLITKYPDSVKG (SEQ ID NO:23); or

(iii) an amino acid sequence that has 2 or 1 amino acid differences from EINTNGLITKYPDSVKG (SEQ ID NO:23); and

(c) CDR3 comprises

(i) the amino acid sequence SPSGFN (SEQ ID NO:24);

(ii) an amino acid sequence that has at least 80% sequence identity with SPSGFN (SEQ ID NO:24); or

(iii) an amino acid sequence that has 1 amino acid difference from SPSGFN (SEQ ID NO:24).

28. The method of claim 26 or 27, wherein CDR1 comprises the amino acid sequence DYWMY (SEQ ID NO:22), CDR2 comprises the amino acid sequence EINTNGLITKYPDSVKG (SEQ ID NO:23), and CDR3 comprises the amino acid sequence SPSGFN (SEQ ID NO:24).

29. The method of any one of claims 26 to 28, wherein at least one of the anti-TNFa SDABs comprises an amino acid sequence with at least 80%, 90%, 95%, or 99% sequence identity to SEQ ID NO:2 (TNF30).

30. The method of any one of claims 26 to 29, wherein each anti-TNFa SDAB comprises an amino acid sequence having at least 80%, 90%, 95%, or 95% sequence identity to SEQ ID NO:2 (TNF30).

31. The method of any one of claims 26 to 30, wherein the anti-HSA SDAB comprises 3 CDRs (CDR1, CDR2, and CDR3) wherein

(a) CDR1 comprises

(i) the amino acid sequence SFGMS (SEQ ID NO:25);

(ii) an amino acid sequence that has at least 80% sequence identity with SFGMS (SEQ ID NO:25); or

(iii) an amino acid sequence that has only 1 amino acid difference from SFGMS (SEQ ID NO:25);

(b) CDR2 comprises

(i) the amino acid sequence SISGSGSDTLYADSVKG (SEQ ID NO:26);

(ii) an amino acid sequence that has at least 80%, 90%, or 95% sequence identity with SISGSGSDTLYADSVKG (SEQ ID NO:26); or

(iii) an amino acid sequence that has 2 or 1 amino acid differences from SISGSGSDTLYADSVKG (SEQ ID NO:26); and

(c) CDR3 comprises

(i) the amino acid sequence GGSLSR (SEQ ID NO:27);

(ii) an amino acid sequence that has at least 80% sequence identity with GGSLSR (SEQ ID NO:27); or

(iii) an amino acid sequence that has 1 amino acid difference from GGSLSR (SEQ ID NO:27).

32. The method of claim 31, wherein CDR1 comprises the amino acid sequence SFGMS (SEQ ID NO:25), CDR2 comprises the amino acid sequence SISGSGSDTLYADSVKG (SEQ ID NO:26), and CDR3 comprises the amino acid sequence GGSLSR (SEQ ID NO:27).

33. The method of claim 31 or 32, wherein the anti-HSA SDAB comprises an amino acid sequence having at least 80%, 90%, 95%, or 95% sequence identity to SEQ ID NO:5 (ALB8).

34. The method of any one of claims 26 to 33, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:1 (ozoralizumab).

35. The method of any one of claims 26 to 34, wherein at least one of the SDABs is humanized.

36. The method of any one of claims 26 to 35, wherein each of the two anti-TNF SDABs and anti-HSA SDAB are linked via linkers, wherein each linker is selected from the group consisting of the amino acid sequences of SEQ ID NO:6 and SEQ ID NO:7.

37. The method of any one of claims 26 to 36, wherein the doses are separated in time by at least about one month.

38. The method of any one of claims 26 to 37, wherein the doses are separated in time by at least about 6 weeks.

39. The method of any one of claims 26 to 38, wherein the doses are separated in time by at least about 8 weeks.

40. The method of any one of claims 26 to 39, wherein the doses are selected from the group consisting of about 10, 30, 80, 100, 120, 140, 160, 180, 200, 250, 275, 300, 320, 350, 375 and 400 mg of the SDAB molecule.

41. The method of any one of claims 26 to 40, wherein the polypeptide is administered subcutaneously or intravenously.

42. The method of any one of claims 26 to 41, wherein the human is treated concurrently with methotrexate.

43. The method of claim 26 to 42, wherein the polypeptide is formulated into a pharmaceutically acceptable formulation.

44. The method of claim 43, wherein the formulation comprises

(a) a polypeptide of claim 26 at a concentration of about 10 mg/ml to 250 mg/ml;

(b) a lyoprotectant chosen from sucrose, sorbitol, or trehalose at a concentration of about 5% to about 10%;

(c) a surfactant chosen from polysorbate-80 or poloxamer-188 at a concentration of about 0.01% to 0.6%; and

(d) a buffer chosen from Histidine buffer at a concentration of about 10 to 20 mM or a Tris buffer at a concentration of about 20 mM such that the pH of the formulation is about 5.0 to 7.5.

45. The method of claim 44, wherein the formulation comprises 100 mg/ml of the polypeptide of claim 1, 20 mM Histidine, 7.5% (w/v) sucrose, and 0.01% polysorbate-80 at pH 6.0.

46. The method of any one of claims 26 to 45, wherein the immune disorder is selected from the group consisting of: inflammation, arthritis, including rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, juvenile idiopathic arthritis and osteoarthritis, COPD, asthma, inflammatory bowel diseases including Crohn's disease and ulcerative colitis, multiple sclerosis, Addison's disease, Autoimmune hepatitis, Autoimmune parotitis, Diabetes Type I, Epididymitis, Glomerulonephritis, Graves' disease, Guillain -Barre syndrome, Hashimoto's disease, Hemolytic anemia, Systemic lupus erythematosus, Male infertility, Multiple sclerosis, Myasthenia Gravis, Pemphigus, Psoriasis, Hidradenitis suppurativa, Rheumatic fever, Sarcoidosis, Scleroderma, Sjogren's syndrome, Spondyloarthropathies, Thyroiditis, and Vasculitis.

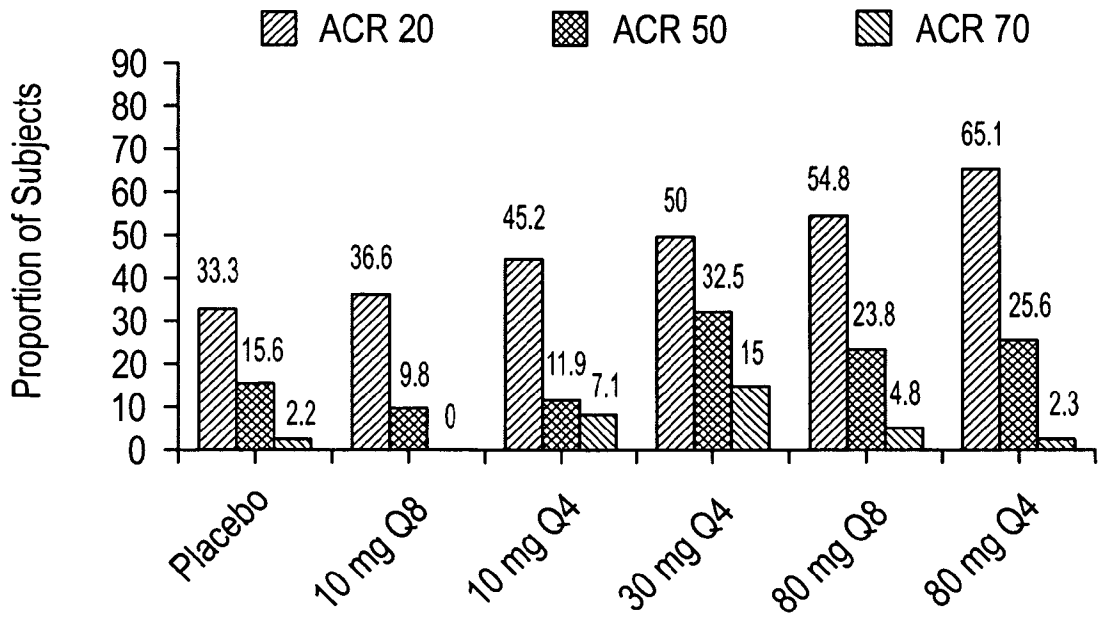
47. The method of claim 46, wherein the immune disorder is rheumatoid arthritis.

48. A method of treating rheumatoid arthritis in a human in need thereof, comprising administering to the human 80 mg of a polypeptide comprising the amino acid sequence of SEQ ID NO:1 about every four weeks, wherein the human is concurrently treated with methotrexate.

49. A method of treating rheumatoid arthritis in a human in need thereof, comprising administering to the human 80 mg of a polypeptide comprising the amino acid sequence of SEQ ID NO:1 about every eight weeks, wherein the human is concurrently treated with methotrexate.

50. A method of treating an immune disorder in a human in need thereof, comprising administering to the human a polypeptide competing with the polypeptide comprising the amino acid sequence of SEQ ID NO:1 (ozoralizumab) of claim 34.

Week 8



Week 16

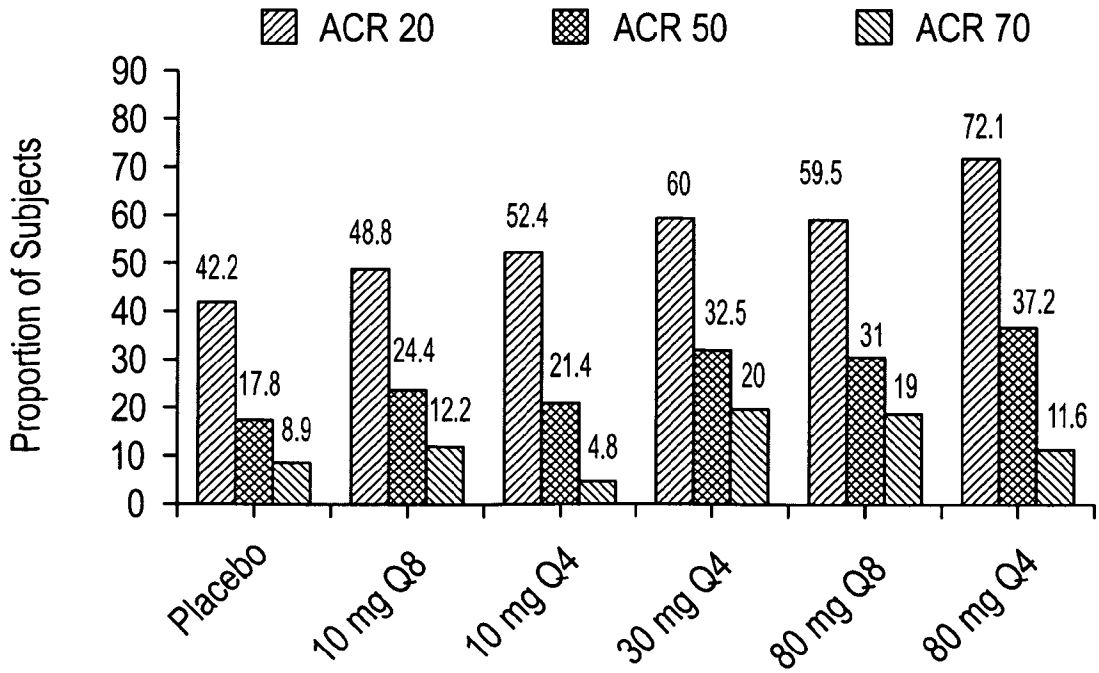
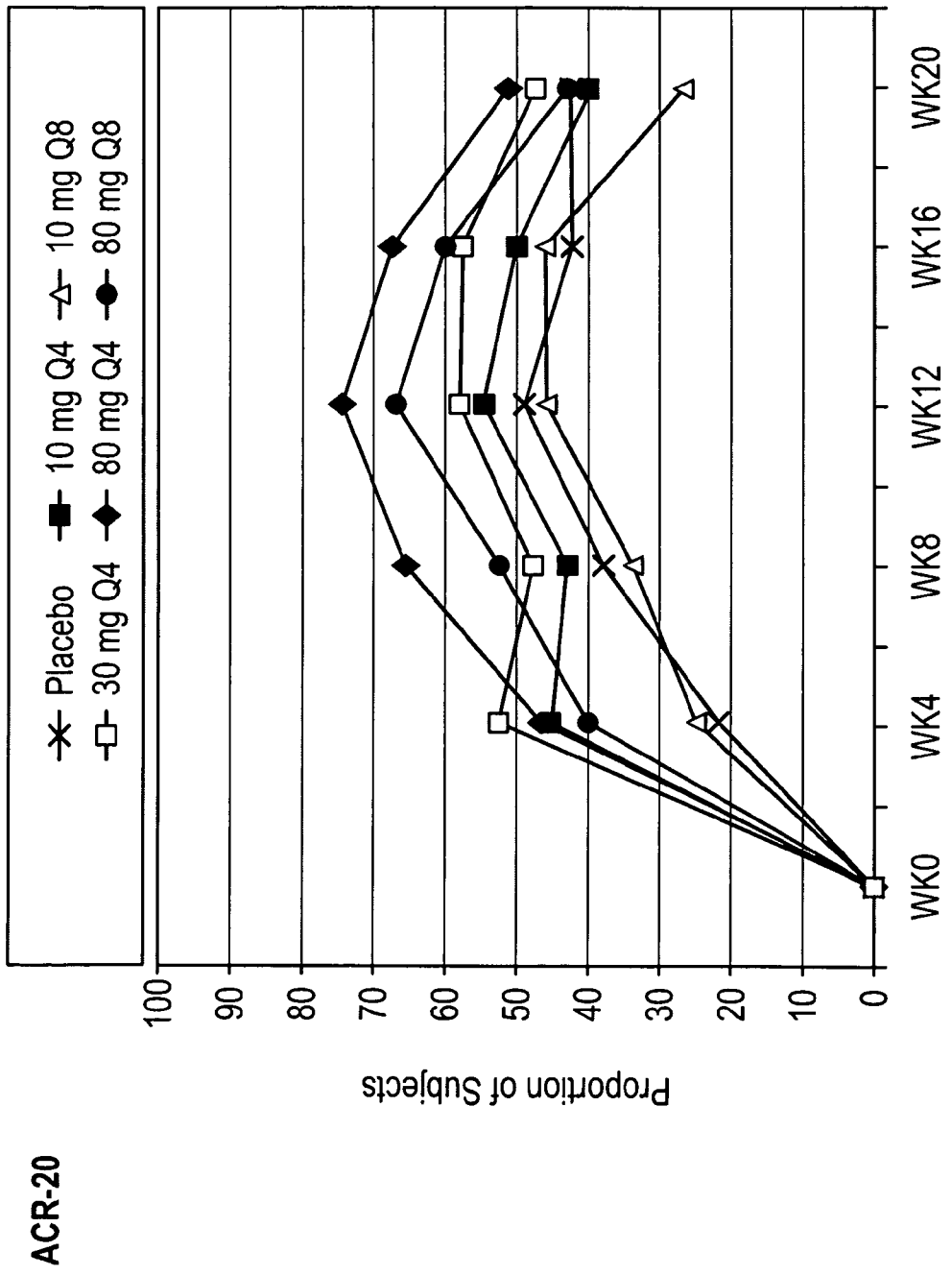


Figure 1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90
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70
60
50
40
30
20
10
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WK0
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WK8
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WK16
WK20
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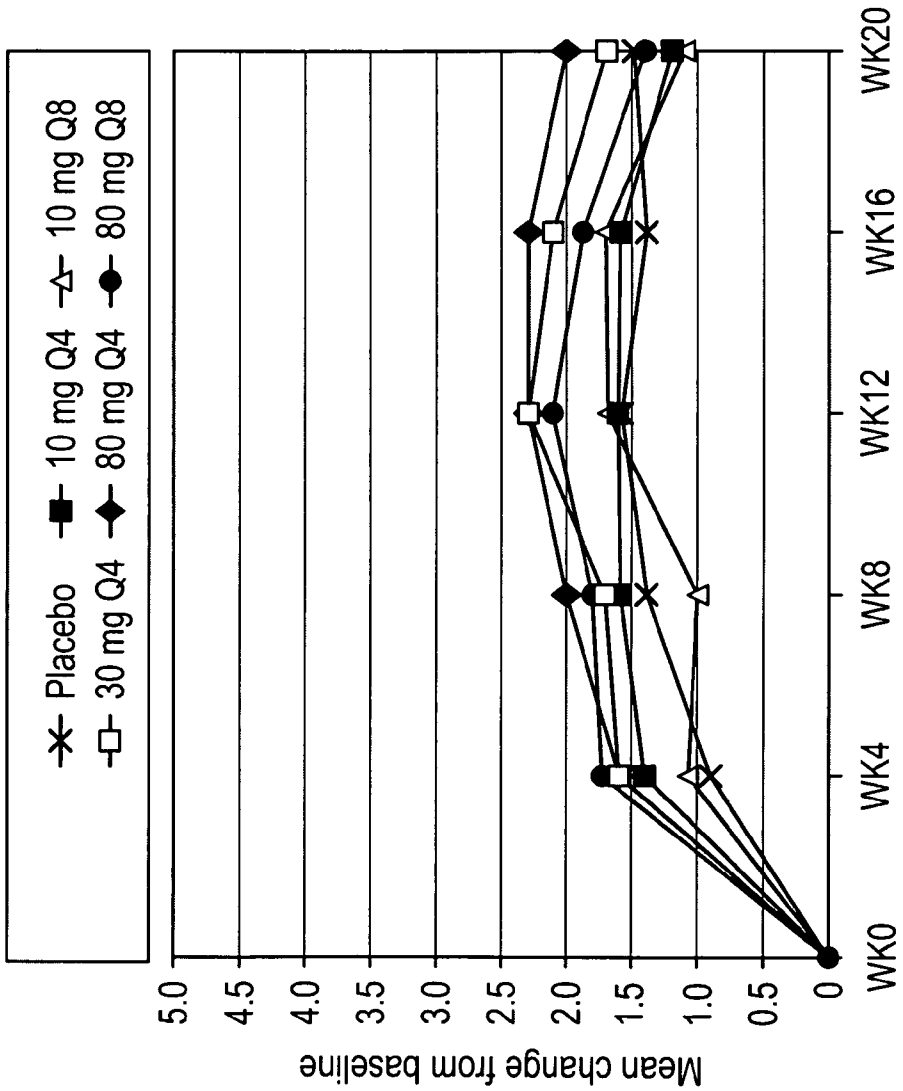
80 mg Q4
80 mg Q8

Figure 2


Placebo
10 mg Q4
10 mg Q8
30 mg Q4
80 mg Q4
80 mg Q8
Mean change from baseline
5.0
4.5
4.0
3.5
3.0
2.5
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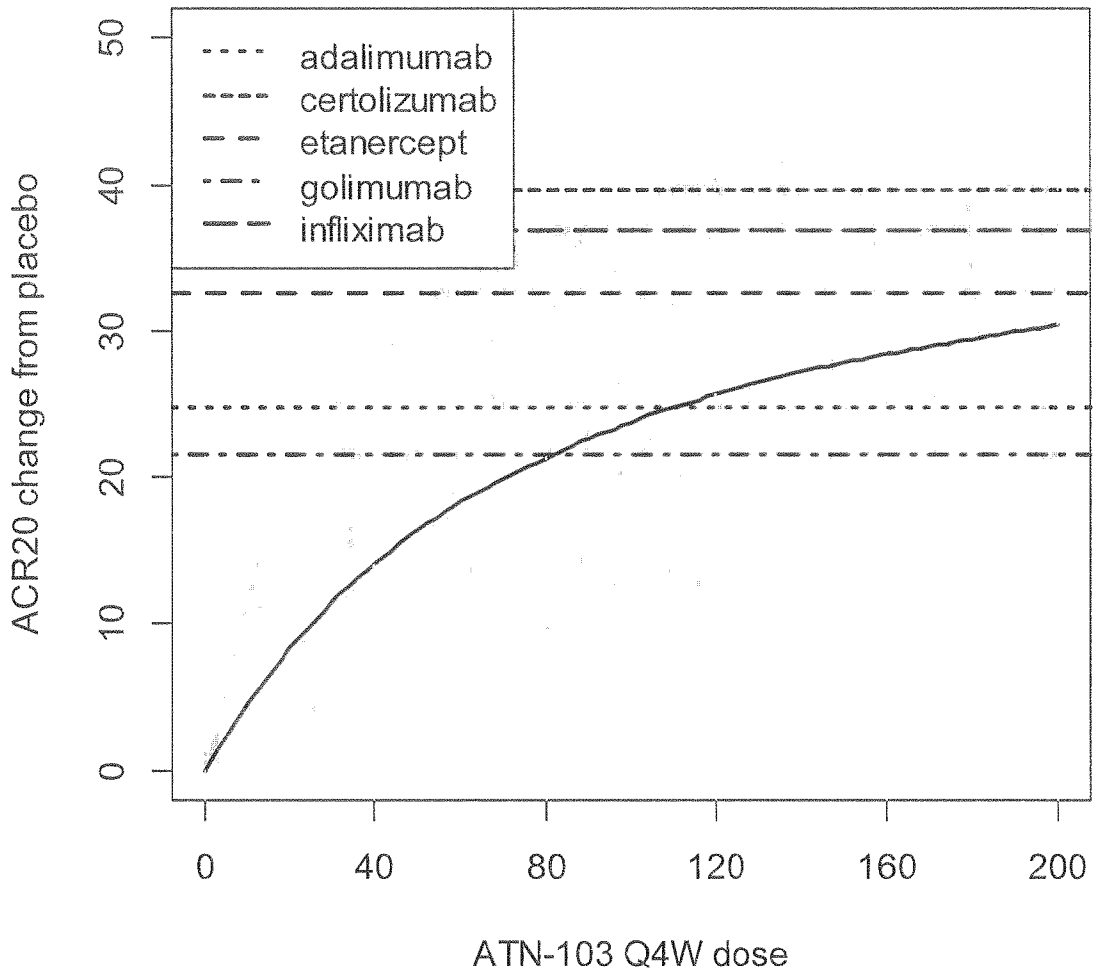
1.0
0.5
0
WK0
WK4
WK8
WK12
WK16
WK20
DAS28

Figure 3


adalimumab
certolizumab
etanercept
golimumab
infliximab
ACR20 change from placebo
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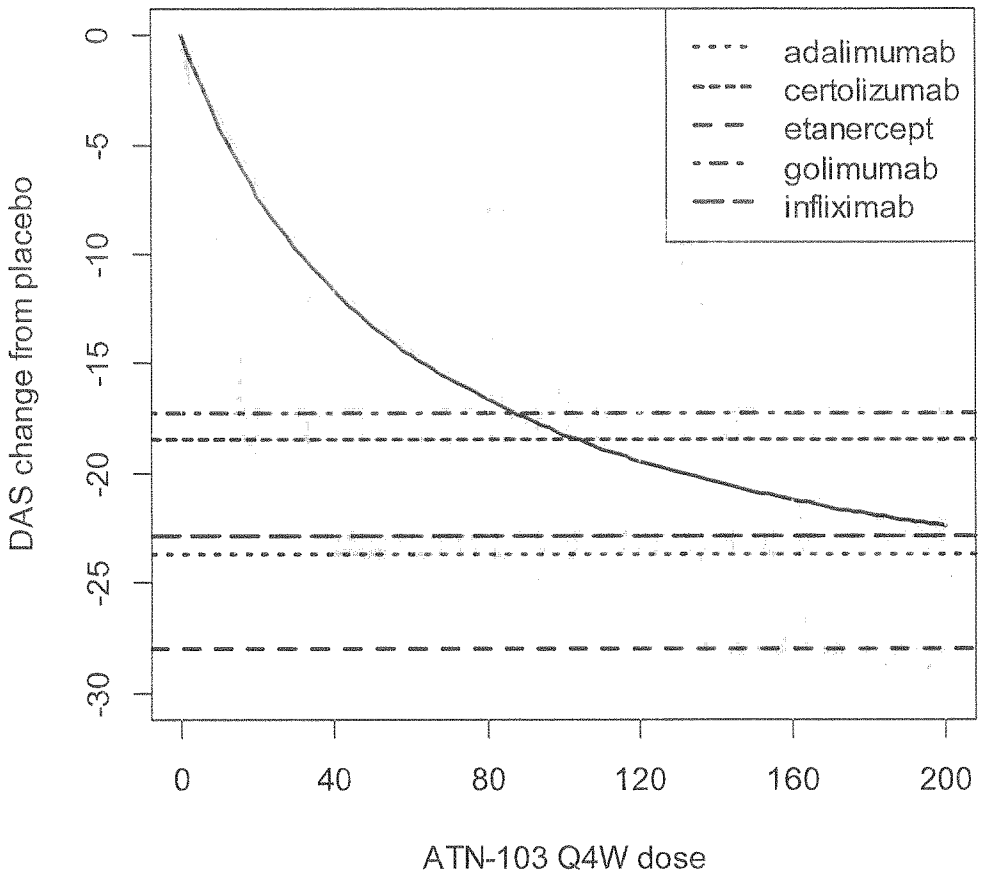
10
20
30
40
50
0
40
80
120
160
200
ATN-103 Q4W dose

FIGURE 4


adalimumab
certolizumab
etanercept
golimumab
infliximab
DAS change from placebo
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-10
-15
-20
-25
-30
0
40
80
120
160
200
ATN-103 Q4W dose

FIGURE 5

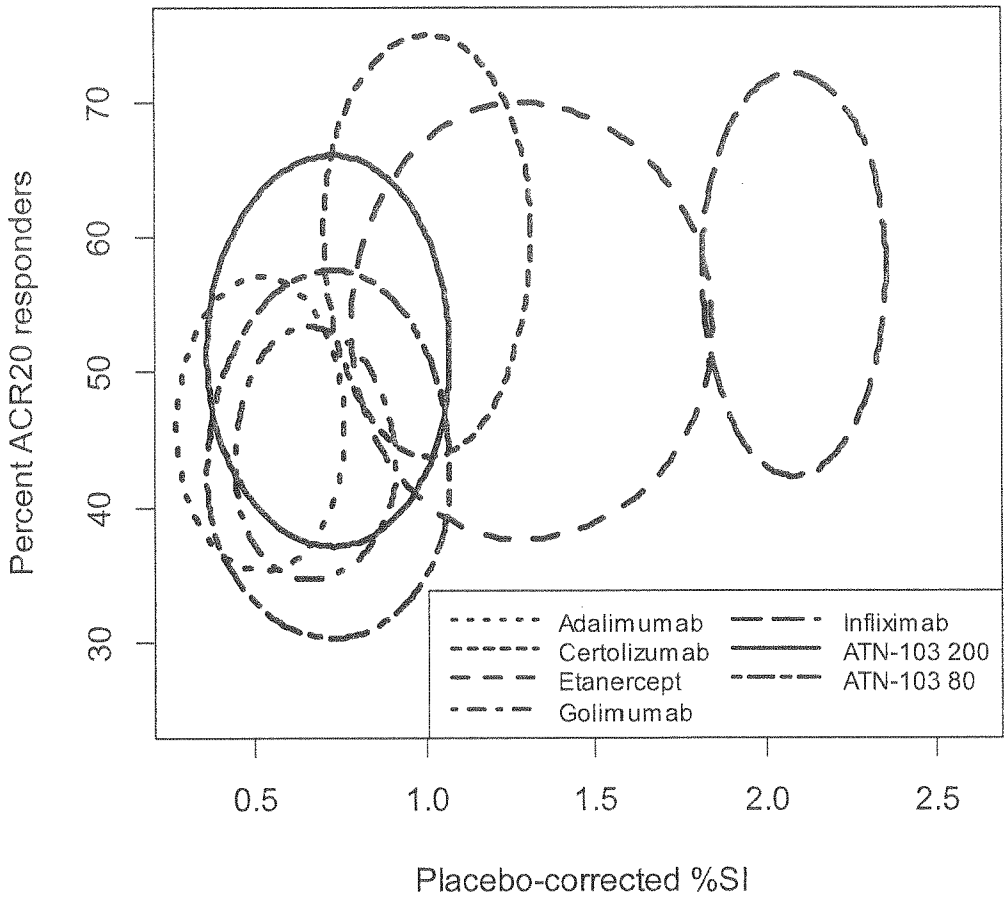
Percent ACR20 responders
30
40
50
60
70
0.5
1.0
1.5
2.0
2.5
Placebo-corrected %SI
Adalimumab
Certolizumab
Etanercept
Golimumab
Infliximab
ATN-103 200
ATN-103 80

FIGURE 6

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/055830

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/18 C07K16/24 C07K16/46 A61K39/395 A61P37/06
A61P19/02
ADD.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Wyeth: "NCT00959036: Study Evaluating Multiple Doses Of ATN-103 In Subjects With Active Rheumatoid Arthritis", ClinicalTrials.gov , 17 February 2011 (2011-02-17), XP002678652, Retrieved from the Internet: URL:http://clinicaltrials.gov/ct2/show/NCT00959036 [retrieved on 2012-06-26] the whole document ----- -/--	1-50

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 June 2012	19/07/2012

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Bumb, Peter

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Wyeth: "NCT00916110: Study Of Single Dose Of ATN-103 Administered To Healthy Japanese Male Subjects", ClinicalTrials.gov , 17 February 2011 (2011-02-17), XP002678653, Retrieved from the Internet: URL:http://clinicaltrials.gov/ct2/show/NCT00916110 [retrieved on 2012-06-26] the whole document	1-50
Y	WO 2006/122786 A2 (ABLYNX NV [BE]; BEIRNAERT ELS [BE]) 23 November 2006 (2006-11-23) examples 60-62	1-50
Y	WO 2010/077422 A2 (WYETH LLC [US]; FERNANDEZ JASON E [US]; DIXON DANIEL A [US]; PAULSON A) 8 July 2010 (2010-07-08) the whole document	19,20, 44,45
X	WEYCKER D ET AL: "Retrospective study of the costs of care during the first year of therapy with etanercept or infliximab among patients aged >=65 years with rheumatoid arthritis", CLINICAL THERAPEUTICS, vol. 27, no. 5, 1 May 2005 (2005-05-01), pages 646-656, XP027641215, EXCERPTA MEDICA, PRINCETON, NJ, US ISSN: 0149-2918 [retrieved on 2005-05-01] the whole document	25,50
Y,P	Ablynx: "PFIZER'sS ANTI-TNF-ALPHA NANOBODY (ATN-103) SUCCESSFULLY COMPLETESA PHASE II STUDY AND DEMONSTRATES CLINICAL PROOF-OF-CONCEPT (POC) IN PATIENTS WITH RA", hugin.info: Regulated information , 17 May 2011 (2011-05-17), XP002678654, Retrieved from the Internet: URL:http://hugin.info/137912/R/1516627/452958.pdf [retrieved on 2012-06-26] the whole document	1-50

-/--

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y,P	Fleischmann et al.: "A Multiple Ascending Dose/Proof of Concept Study of ATN-103 (ozoralizumab) in Rheumatoid Arthritis Subjects on a Background of Methotrexate.", Blackwellpublishing.com: ACR/ARHP Meeting Arthritis & Rheumatism, vol. 63, no. Suppl. 10 October 2011 (2011-10), XP002678655, Retrieved from the Internet: URL:http://www.blackwellpublishing.com/acrmeeting/abstract.asp?MeetingID=781&id=97379 [retrieved on 2012-06-26] the whole document -----	1-50
Y,P	Udata et al.: "Pharmacokinetic-Pharmacodynamic Modeling of Ozoralizumab (ATN-103), a Novel Humanized Nanobody Tumor Necrosis Factor Inhibitor for Rheumatoid Arthritis", Blackwellpublishing.com: ACR/ARHP Meeting Arthritis & Rheumatism, vol. 63, no. Suppl. 10 October 2011 (2011-10), XP002678656, Retrieved from the Internet: URL:http://www.blackwellpublishing.com/acrmeeting/abstract.asp?MeetingID=781&id=95999 [retrieved on 2012-06-26] the whole document -----	1-50

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2012/055830

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2006122786 A2	23-11-2006	AT 537188 T	15-12-2011
		AT 549357 T	15-03-2012
		AU 2006249144 A1	23-11-2006
		BR PI0609224 A2	18-10-2011
		CA 2608770 A1	23-11-2006
		CN 101248087 A	20-08-2008
		CR 9519 A	17-10-2008
		DK 1888640 T3	18-06-2012
		DK 1888641 T3	23-04-2012
		EC SP077910 A	26-03-2008
		EP 1888640 A2	20-02-2008
		EP 1888641 A1	20-02-2008
		EP 2172484 A2	07-04-2010
		EP 2365000 A2	14-09-2011
		EP 2479191 A2	25-07-2012
		ES 2379283 T3	24-04-2012
		ES 2384164 T3	02-07-2012
		JP 2008539772 A	20-11-2008
		KR 20080011395 A	04-02-2008
		NI 200700291 A	12-05-2008
		NZ 562695 A	27-05-2011
		PT 1888641 E	19-03-2012
		SI 1888641 T1	31-05-2012
		US 2009028880 A1	29-01-2009
		WO 2006122786 A2	23-11-2006
		WO 2006122787 A1	23-11-2006
		ZA 200709867 A	29-10-2008
		ZA 200901011 A	28-04-2010
WO 2010077422 A2	08-07-2010	AR 073997 A1	15-12-2010
		AU 2009333791 A1	08-07-2010
		CA 2738243 A1	08-07-2010
		CN 102271707 A	07-12-2011
		EP 2362767 A2	07-09-2011
		JP 2012507553 A	29-03-2012
		KR 20110061646 A	09-06-2011
		US 2010137213 A1	03-06-2010
		WO 2010077422 A2	08-07-2010