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(54) Title: ANTI-TNF-/ANTI-IL-23 BISPECIFIC ANTIBODIES

(57) Abstract: Bispecific antibodies are provided that bind Tumor Necrosis Factor alpha (TNF-alpha) and the p19 subunit of Interleukin-23 (IL-23p19) and are characterized as having high affinity and strong simultaneous neutralizing properties to both TNF-alpha and IL-23. The bispecific antibodies of the invention are useful for treating various autoimmune diseases including Inflammatory Bowel Disease, such as Crohns Disease and Ulcerative Colitis, Axial Spondyloarthritis, Rheumatoid Arthritis and Psoriatic Arthritis.



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ANTI-TNF- / ANTI-IL-23 BISPECIFIC ANTIBODIES

The present invention is in the field of medicine, particularly in the novel field of bispecific antibodies directed against Tumor Necrosis Factor alpha (TNF α) and
5 Interleukin-23 (IL-23). The bispecific antibodies of the present invention are expected to be useful in treating autoimmune diseases including Inflammatory Bowel Disease (IBD), such as Crohn's Disease (CD) and Ulcerative Colitis (UC), Axial Spondyloarthritis (AxSpA), Rheumatoid Arthritis (RA) and Psoriatic Arthritis (PsA).

Autoimmune diseases arise from the body's production of an immune response
10 against its own tissue. Autoimmune diseases are often chronic and can be debilitating and even life-threatening. IBD, which generically represents a group of disorders such as CD and UC, is a common chronic relapsing autoimmune disease characterized pathologically by intestinal inflammation and epithelial injury. Other forms of chronic autoimmune diseases, such as RA, AxSpA and PsA, may affect the axial and / or
15 peripheral skeleton.

Interleukin 23 (IL-23) is a heterodimeric cytokine believed to be important in the activation of a range of inflammatory cells required for the induction of chronic inflammation. IL-23, which is an upstream regulator of IL-6, IL-17, GM-CSF and IL-22 secretion, is composed of a p19 subunit (IL23p19) covalently paired to a p40 subunit (the
20 p40 subunit is shared with cytokine IL-12). Additionally, IL-23 has been implicated as playing an important role in both memory / pathogenic T-cell inflammatory response as well as playing a role in the regulation of innate lymphoid cell inflammatory activity. There is evidence that IL-23 regulation of the cytokines IL-6, IL-17, GM-CSF and IL-22 is associated with inflammatory diseases including IBD, RA and other autoimmune
25 diseases.

Tumor Necrosis Factor alpha (TNF α) is a pleiotropic homotrimeric cytokine which is primarily secreted by monocytes and macrophages, but also known to be produced by CD4⁺ and CD8⁺ peripheral blood T lymphocytes. TNF α is expressed in both a soluble and transmembrane form (the membrane-bound precursor form can be
30 proteolytically cleaved into a soluble homotrimer by metalloproteinase TNF alpha converting enzyme (TACE)). TNF α is believed to play a role in the regulation of immune cells and be important in systemic inflammation, specifically in acute phase

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inflammatory reactions. Excess amounts of TNF α have been associated with various forms of autoimmune diseases, including RA, CD and psoriasis.

Current FDA approved treatments for autoimmune diseases such as IBD include corticosteroids, often used to treat acute inflammation, and bioproducts, many of which
5 (such as REMICADE[®], ENBREL[®] and HUMIRA[®]) attempt to target and neutralize TNF α in the body. Another bioproduct approved for treatment of PsA includes STELARA[®] which attempts to target the shared p40 subunit of cytokines IL-12 and IL-23. Current treatments have demonstrated efficacy for reducing symptoms and slowing progression of some autoimmune diseases in a subset of patients. However, a large
10 percentage of patients are nonresponsive to currently available treatments (for example, induction of remission occurs in only 30-50% of CD patients treated with TNF α neutralization, and loss of response to TNF α neutralization occurs in between 23 and 46% of patients following 12 months of treatment). Thus, there remains a need for alternative therapies for treatment of autoimmune diseases, including IBD (such as CD and UC), RA,
15 AxSpA and PsA. Preferably, such alternative therapies will be capable of demonstrating efficacy in a large percentage of patients non-responsive to currently available treatments.

One approach to such alternative therapies may include the co-administration of two different bioproducts (e.g., antibodies). Co-administration requires either injections of two separate products or a single injection of a co-formulation of two different
20 antibodies. While two injections permit flexibility of dose amounts and timing, it is inconvenient to patients both for compliance and pain. Further, while a co-formulation might provide some flexibility of dose amounts, it is often quite challenging or impossible to find formulation conditions having acceptable viscosity in solution (at relatively high concentration) and that permit chemical and physical stability of both antibodies due to
25 different molecular characteristics of the two antibodies. Additionally, co-administration and co-formulation involve the additive costs of two different drug therapies which can increase patient and / or payor costs. Thus, there remains a need for alternative therapies for treatment of autoimmune diseases and preferably such alternative therapies will comprise a bispecific antibody.

30 U.S. Patent Publication Number 2012/0251541 A1 discloses antibody-like binding proteins having a cross-over dual variable ("CODV") configuration with an antigen binding site to a TNF α target and an antigen binding site to an IL23/IL12 target (e.g., the

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shared p40 subunit). U.S. Patent Number 7,872,102 discloses an antibody to the p19 subunit of IL-23 useful in the treatment of autoimmune diseases such as multiple sclerosis. U.S. Patent Number 6,090,382 discloses human antibodies to human TNF α . WO2011/070339 discloses a method of co-administrating, and a co-formulation of, anti-
5 TNF α and anti-IL-23p40 antibodies for CD, RA, PsA, AxSpA and psoriasis. Despite these disclosures, high affinity neutralizing bispecific antibodies that specifically bind and neutralize both TNF α and the p19 subunit of IL-23 were not described in any of these applications.

Furthermore, significant problems associated with chemical and physical stability
10 were encountered when building a bispecific antibody of the present invention. Many changes were required in the starting bispecific antibody to sufficiently overcome a myriad of issues, such as expressing a physically stable molecule, stabilizing the VH/VL interface of the single chain fragment variable region (scFv), increasing thermal and salt-dependent stability, decreasing aggregation, increasing solubility at high concentrations,
15 and rebalancing the electrostatic distribution in the binding surfaces of the bispecific antibody, all while maintaining binding affinity for both targeted antigens; TNF α and the p19 subunit of IL-23, respectively.

Therefore, a need still exists for a single bispecific antibody that neutralizes both human TNF α and human IL-23, where the bispecific antibody specifically targets the p19
20 subunit of human IL-23 and does not specifically neutralize IL-12 (which shares a common p40 subunit with IL-23). It is desirable to provide a bispecific antibody that is thermally stable, physically stable, exhibits low aggregation, and neutralizes human TNF α and human IL23p19. It is also desirable to provide a pharmaceutical composition including a single bispecific antibody that neutralizes both human TNF α and human
25 IL23p19, thereby avoiding the challenges of finding formulation conditions that must satisfy the different molecular characteristics of two different, separate antibodies. The present invention therefore seeks to address one or more of the above mentioned problems.

The present invention provides a bispecific antibody having an immunoglobulin G
30 antibody (IgG) that binds tumor necrosis factor alpha (TNF α) conjugated to two single chain variable fragments (scFv) that bind the p19 subunit of IL-23 (IL23p19). According to a bispecific antibody of the present invention, the IgG has two heavy chains (HC) and

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two light chains (LC), each HC having a heavy chain variable region (HCVR1) with heavy chain complementarity determining regions (HCDR) 1-3 and each LC having a light chain variable region (LCVR1) with light chain complementarity determining regions (LCDR) 1-3. According to a bispecific antibody of the present invention, the amino acid sequence of HCDR1 is SEQ ID NO:9, the amino acid sequence of HCDR2 is SEQ ID NO:10, the amino acid sequence of HCDR3 is SEQ ID NO:11, the amino acid sequence of LCDR1 is SEQ ID NO:15, the amino acid sequence of LCDR2 is SEQ ID NO:16, and the amino acid sequence of LCDR3 is SEQ ID NO:17. The scFv of bispecific antibodies of the present invention have a heavy chain variable region (HCVR2), with HCDRs 4-6, and a light chain variable region (LCVR2) with LCDRs 4-6. According to a bispecific antibody of the present invention the amino acid sequence of HCDR4 is SEQ ID NO:12, the amino acid sequence of HCDR5 is SEQ ID NO:13, the amino acid sequence of HCDR6 is SEQ ID NO:14, the amino acid sequence of LCDR4 is SEQ ID NO:18, the amino acid sequence of LCDR5 is SEQ ID NO:19, and the amino acid sequence of LCDR6 is SEQ ID NO:20. Further, bispecific antibodies of the present invention have each scFv independently conjugated to the IgG antibody via a polypeptide linker (Linker 1 (L1)) covalently attached to the C-terminus of each IgG HC and the N-terminus of HCVR2 of each scFv. Additionally, bispecific antibodies of the present invention have the HCVR2 of each scFv covalently attached to the LCVR2 of the same scFv via a second polypeptide linker (Linker 2 (L2)) covalently attached to the C-terminus of the HCVR2 and the N-terminus of the LCVR2 of the same scFv. According to a bispecific antibody of the present invention, the amino acid sequence of L1 is SEQ ID NO:21 and the amino acid sequence of L2 is SEQ ID NO:22.

The present invention also provides a bispecific antibody having an IgG that binds TNF α conjugated to two scFvs that bind IL23p19, where the IgG has two heavy chains and two light chains, each heavy chain having a heavy chain variable region (HCVR1) and each light chain having a light chain variable region (LCVR1). According to a bispecific antibody of the present invention, the amino acid sequence of HCVR1 is SEQ ID NO: 5 and the amino acid sequence of LCVR1 is SEQ ID NO:7. Additionally, each scFv has a heavy chain variable region (HCVR2) and a light chain variable region (LCVR2). According to a bispecific antibody of the present invention, the amino acid sequence of HCVR2 is SEQ ID NO: 6 and the amino acid sequence of LCVR2 is SEQ ID

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NO:8. Additionally, each scFv is independently conjugated to the IgG antibody via a polypeptide linker (L1) covalently attached to the C-terminus of each IgG HC and the N-terminus of HCVR2 of each scFv. Further, the HCVR2 of each scFv is covalently attached to the LCVR2 of the same scFv via a second polypeptide linker (L2) covalently attached to the C-terminus of the HCVR2 and the N-terminus of the LCVR2 of the same scFv.

The present invention further provides a bispecific antibody having an IgG that binds TNF α conjugated to two scFvs that bind IL23p19, where the IgG has two HCs and two LCs. According to a bispecific antibody of the present invention, the amino acid sequence of HC is SEQ ID NO:23 and the amino acid sequence of LC is SEQ ID NO:2. Additionally, each scFv has a heavy chain variable region (HCVR2) and a light chain variable region (LCVR2). According to a bispecific antibody of the present invention, the amino acid sequence of HCVR2 is SEQ ID NO: 6 and the amino acid sequence of LCVR2 is SEQ ID NO:8. Additionally, each scFv is independently conjugated to the IgG antibody via a polypeptide linker (L1) covalently attached to the C-terminus of each IgG HC and the N-terminus of HCVR2 of each scFv. Further, the HCVR2 of each scFv is covalently attached to the LCVR2 of the same scFv via a second polypeptide linker (L2) covalently attached to the C-terminus of the HCVR2 and the N-terminus of the LCVR2 of the same scFv. According to a bispecific antibody of the present invention, the amino acid sequence of L1 is SEQ ID NO:21 and the amino acid sequence of L2 is SEQ ID NO:22.

The relationship of the various regions and linkers of an exemplified bispecific antibody of the present invention is as follows (numbering of amino acids applies linear numbering; assignment of amino acids to variable domains is based on the International Immunogenetics Information System[®] available at www.imgt.org; assignment of amino acids to CDR domains is based on the well-known Kabat and North numbering conventions as reflected in Table 1):

Table 1: Amino acid regions of an exemplified bispecific antibody of the present invention.

SEQ ID NO:1	SEQ ID NO:2
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	Region	Positions		Region	Positions
HCVR1 TNF α	FRH1-1	1-22	LCVR1 TNF α	FRL1-1	1-23
	HCDR1	23-35		LCDR1	24-34
	FRH1-2	36-49		FRL1-2	35-48
	HCDR2	50-66		LCDR2	49-56
	FRH1-3	67-96		FRL1-3	57-88
	HCDR3	97-110		LCDR3	89-97
	FRH1-4	111-121		FRL1-4	98-107
Constant	CH	122-449	Constant	CL	108-214
Linker	L1	450-464			
HCVR2 IL-23	FRH2-1	465-486			
	HCDR4	487-499			
	FRH2-2	500-513			
	HCDR5	514-530			
	FRH2-3	531-560			
	HCDR6	561-568			
	FRH2-4	569-579			
Linker	L2	580-599			
LCVR2 IL-23	FRL2-1	600-622			
	LCDR4	623-633			
	FRL2-2	634-647			
	LCDR5	648-655			
	FRL2-3	656-687			
	LCDR6	688-696			
	FRL2-4	697-706			
CDR	Starting Amino Acid Pos. Definition		Ending Amino Acid Pos. Definition		
HCDR1	North		Kabat		
HCDR2	Kabat		Kabat		
HCDR3	North		Kabat		
HCDR4	North		Kabat		
HCDR5	Kabat		Kabat		
HCDR6	North		Kabat		
LCDR1	Kabat		Kabat		
LCDR2	North		Kabat		
LCDR3	Kabat		Kabat		
LCDR4	Kabat		Kabat		
LCDR5	North		Kabat		
LCDR6	Kabat		Kabat		

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The present invention further provides a bispecific antibody wherein each of the HCs form an inter-chain disulfide bond with each of the LCs; wherein each of the HCs forms an inter-chain disulfide bond with the other HC; and wherein each of the scFvs
5 forms an intra-chain disulfide bond between HCVR2 and LCVR2. According to the exemplified bispecific antibody of the present invention presented in Table 1, an inter-chain disulfide bond of each of the HCs and each of the LCs forms between cysteine residue 148 (of SEQ ID NO:1) of the HC, and cysteine residue 214 (of SEQ ID NO:2) of the LC; at least two inter-chain disulfide bonds form between the two HCs, the first inter-
10 chain disulfide bond forming between cysteine residue 230 (of SEQ ID NO: 1) of the HC and cysteine residue 230 (of SEQ ID NO: 1) of the other HC, the second inter-chain disulfide bond forming between cysteine residue 233 (of SEQ ID NO: 1) of the HC and cysteine residue 233 (of SEQ ID NO: 1) of the other HC; and an intra-chain disulfide bond of the scFv is formed between cysteine residue 508 (of SEQ ID NO: 1) of the
15 HCVR2 and cysteine residue 699 (of SEQ ID NO:1) of the LCVR2.

According to some embodiments of the present invention, a bispecific antibody comprising glycosylation of the HC is provided. According to the exemplified bispecific antibody of the present invention presented in Table 1, glycosylation of the HC occurs at the asparagine residue 301 of SEQ ID NO: 1.

20 The present invention also provides a DNA molecule comprising a polynucleotide sequence encoding a polypeptide chain comprising a HC, scFv, L1 and L2 of the bispecific antibody of present invention. According to an embodiment of the present invention, the amino acid sequence of the encoded polypeptide chain is SEQ ID NO:1.

The present invention also provides a DNA molecule comprising a polynucleotide
25 sequence encoding a second polypeptide chain comprising a LC of the bispecific antibody of the present invention. According to an embodiment of the present invention, the amino acid sequence of the second encoded polypeptide chain is SEQ ID NO:2.

The present invention also provides a DNA molecule comprising a polynucleotide sequence encoding a polypeptide chain comprising a HC, a scFv, L1 and L2 of the
30 bispecific antibody of present invention, and comprising a second polynucleotide sequence encoding a second polypeptide chain comprising a LC of the bispecific antibody of the present invention. According to an embodiment of the present invention, the amino

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acid sequence of the encoded polypeptide chain comprising a HC, scFv, L1 and L2 is SEQ ID NO:1 and an expression vector containing the DNA sequence of SEQ ID NO: 3 encodes the polypeptide chain. Also, according to an embodiment of the present invention, the amino acid sequence of the second encoded polypeptide chain is SEQ ID
5 NO:2 and an expression vector containing the DNA sequence of SEQ ID NO: 4 encodes the second polypeptide chain.

The present invention also provides a mammalian cell comprising a DNA molecule of the present invention, wherein the cell is capable of expressing a bispecific antibody of the present invention, said bispecific antibody comprising an IgG that binds
10 TNF α conjugated to two scFvs that bind IL23p19.

The present invention also provides a mammalian cell transformed with a DNA molecule(s) of the present invention, wherein the cell is capable of expressing a bispecific antibody of the present invention, said bispecific antibody comprising an IgG that binds TNF α conjugated to two scFvs that bind IL23p19.

15 The present invention also provides a process for producing a bispecific antibody of the present invention, the process comprising cultivating a mammalian cell of the present invention under conditions such that the bispecific antibody is expressed, and recovering the expressed bispecific antibody. The present invention also provides a bispecific antibody according to the present invention produced by said process.

20 The present invention also provides a method of treating autoimmune diseases comprising administering to a patient in need thereof an effective amount of a bispecific antibody of the present invention.

The present invention also provides a method of treating IBD, such as CD and UC, comprising administering to a patient in need thereof a therapeutically effective
25 amount of a bispecific antibody of the present invention.

The present invention also provides a method of treating RA, AxSpA and PsA comprising administering to a patient in need thereof a therapeutically effective amount of a bispecific antibody of the present invention.

The present invention also provides a bispecific antibody of the present invention
30 for use in therapy.

The present invention also provides a bispecific antibody of the present invention for use in the treatment of autoimmune diseases including IBD, such as CD and UC.

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The present invention also provides a bispecific antibody of the present invention for use in the treatment of autoimmune diseases including RA, AxSpA and PsA.

The present invention also provides a pharmaceutical composition comprising a bispecific antibody of the present invention and one or more pharmaceutically acceptable
5 carriers, diluents or excipients.

Another embodiment of the present invention comprises use of a bispecific antibody of the present invention in the manufacture of a medicament for the treatment of ulcerative colitis and Crohn's disease.

An additional embodiment of the present invention comprises use of a bispecific
10 antibody of the present invention in the manufacture of a medicament for the treatment of rheumatoid arthritis, psoriatic arthritis, and axial spondyloarthritis.

Definitions

When used herein the term "bispecific antibody" refers to a molecule comprising
15 an immunoglobulin G antibody (IgG) conjugated to two single chain variable fragments (scFv). As referred to herein, a bispecific antibody of the present invention comprises one scFv covalently linked to the carboxy-terminus of a HC of the IgG by a polypeptide linker (L1) and the other scFv covalently linked to the carboxy-terminus of the other HC of the IgG by a polypeptide linker (L1). The IgG and scFvs of a bispecific antibody of
20 the present invention specifically bind different antigens (TNF α and the p19 subunit of IL-23, respectively).

When used herein, the term "immunoglobulin G antibody" (IgG), refers to an immunoglobulin molecule comprised of four polypeptide chains; two heavy chains (HC) and two light chains (LC) interconnected by disulfide bonds. The amino-terminal portion
25 of each of the four polypeptide chains includes a variable region of about 100-110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each of the four polypeptide chains defines a constant region primarily responsible for effector function. The carboxy-terminal portion of each heavy chain is covalently attached to one of the single chain variable fragments (scFv) via a polypeptide
30 linker (L1).

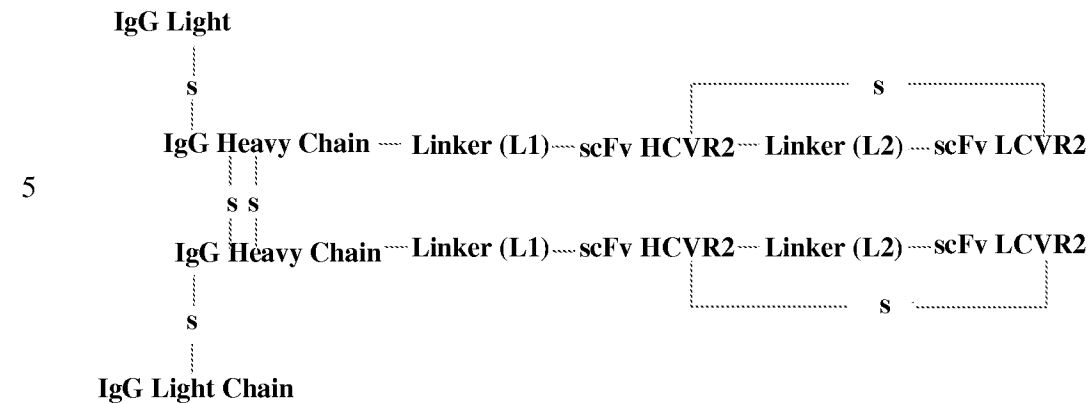
The light chains (LC) of the IgG of the bispecific antibody of the present invention are classified as kappa or lambda and characterized by a particular constant

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region as known in the art. The heavy chains (HC) of the IgG according to the present invention is classified as gamma, which defines the isotype (e.g., as an IgG). The isotype may be further divided into subclasses (e.g., IgG₁, IgG₂, IgG₃, and IgG₄). Each HC type is characterized by a particular constant region known in the art. Each HC is comprised of an N-terminal heavy chain variable region ("HCVR") and a heavy chain constant region (CH). The CH for IgG, according to the present invention, is comprised of three domains (CH1, CH2, and CH3). Each light chain of the IgG is comprised of a light chain variable region (LCVR) and a light chain constant region (CL). The HCVR and LCVR regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each HCVR and LCVR of the IgGs according to the present invention are composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1-1, CDR1, FR1-2, CDR2, FR1-3, CDR3, FR1-4. Herein the 3 CDRs of the HC are referred to as "HCDR1, HCDR2 and HCDR3" and the 3 CDRs of the LC are referred to as "LCDR1, LCDR2 and LCDR3." The CDRs contain most of the residues which form specific interactions with the antigen. The functional ability of an antibody to bind a particular antigen is largely influenced by the six CDRs.

As referred to herein, the term "single chain variable fragment" (scFv), refers to a polypeptide chain comprising a heavy chain variable region (HCVR2) and a light chain variable region (LCVR2) connected via a polypeptide linker (L2). Additionally, as referred to herein (and as represented in the following schematic), the HCVR2 of each scFv is: a.) covalently linked, at its N-terminus, to the C-terminus of one HC of the IgG via a polypeptide linker (L1); and b.) covalently linked, at its C-terminus, to the N-terminus of the LCVR2 of the same scFv via a second polypeptide linker (L2). Further, each scFv of the present invention includes a disulfide bond formed between a cysteine residue of HCVR2 and a cysteine residue of LCVR2 of the same polypeptide chain (as represented in the following schematic):

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HCVR2 and LCVR2 of the scFv can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each HCVR2 and LCVR2 of the IgGs according to the present invention are each composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR2-1, CDR4, FR2-2, CDR5, FR2-3, CDR6, FR2-4. Herein the 3 CDRs of HCVR2 of the scFv are referred to as “HCDR4, HCDR5 and HCDR6” and the 3 CDRs of LCVR2 of the scFv are referred to as “LCDR4, LCDR5 and LCDR6.” The CDRs contain most of the residues which form specific interactions with the antigen. The functional ability of a scFv to bind a particular antigen is largely influenced by the six CDRs.

The variable regions of each light / heavy chain pair of an IgG and scFv, respectively, according to the present invention form an antigen-binding site of the bispecific antibody. According to the present invention, the IgG has two antigen binding sites which are the same (but are different than the antigen binding sites of the scFv) and each scFv has an antigen binding site (which is the same as the antigen binding site of the other scFv). As used herein, the “antigen-binding portion” or “antigen-binding site” or “antigen-binding region” or “antigen-binding fragment” refers interchangeably to that portion of an IgG or scFv molecule, within the variable region, which contains the amino acid residues that interact with an antigen and confer to the bispecific antibody specificity and affinity for an antigen. This antibody portion includes the framework amino acid residues necessary to maintain the proper conformation of the antigen-binding residues.

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Preferably, the framework regions of the bispecific antibodies of the invention are of human origin or substantially of human origin.

A “parent antibody” or “parental antibody,” as used interchangeably herein, is an antibody encoded by an amino acid sequence which is used in the preparation of one of the IgG and scFv of the bispecific antibody, for example through amino acid substitutions and structural alteration. The parent antibody may be a murine, chimeric, humanized or human antibody.

The terms “Kabat numbering” or “Kabat labeling” are used interchangeably herein. These terms, which are recognized in the art, refer to a system of numbering amino acid residues which are more variable (i.e., hypervariable) than other amino acid residues in the heavy and light chains variable regions of an antibody (Kabat, et al., *Ann. NY Acad. Sci.* 190:382-93 (1971); Kabat et al., *Sequences of Proteins of Immunological Interest*, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242 (1991)).

The terms “North numbering” or “North labeling” are used interchangeably herein. These terms, which are recognized in the art, refer to a system of numbering amino acid residues which are more variable (i.e., hypervariable) than other amino acid residues in the heavy and light chains variable regions of an antibody and is based, at least in part, on affinity propagation clustering with a large number of crystal structures, as described in (North et al., *A New Clustering of Antibody CDR Loop Conformations*, *Journal of Molecular Biology*, 406:228-256 (2011)).

The terms “patient,” “subject,” and “individual,” used interchangeably herein, refer to an animal, preferably the term refers to humans. In certain embodiments, the subject, preferably a human, is further characterized with a disease or disorder or condition (e.g., an autoimmune disorder) that would benefit from a decreased level or decreased bioactivity of both IL-23 and TNF α . In another embodiment the subject, preferably a human, is further characterized as being at risk of developing a disorder, disease or condition that would benefit from a decreased level or decreased bioactivity of both IL-23 and TNF α .

30

Bispecific Antibody Engineering

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Significant problems associated with chemical and physical stability were encountered when constructing a bispecific antibody of the present invention. Problems encountered included poor to no expression, poor purification recovery, low thermostability, high salt-dependent aggregation, diabody formation (and challenges in
5 reducing diabodies through purification), high solution viscosity, low binding affinity and cross-reactivity.

For example, initial attempts in constructing a bispecific antibody according to the present invention included constructs in which a parental IL-23 antibody (the IL-23 antibody described in U.S. Patent No. 7,872,102) comprised the IgG antibody portion and
10 a parental TNF α antibody (Adalimumab, see for example U.S. Patent No. 6,090,382) comprised the scFv portion of the bispecific antibody. Other initially attempted constructs included the parental IL-23 antibody comprising the scFv portion while the TNF α antibody comprised the IgG portion of the bispecific antibody. Additionally, initial
15 constructs included the scFv portion being conjugated to the IgG portion in various configurations, including at the amino-terminus or the carboxyl terminus for both the heavy and light chains, respectively. Also, initial constructs included the scFv portion varying in arrangement of the HCVR2 and LCVR2 (e.g., IgG portion (C or N terminus) – linker 1 – LCVR2 or HCVR2 – linker 2 – the other of LCVR2 or HCVR2). Further, parental IL-23 antibody constructs included combinations of heavy chain germline
20 frameworks VH 5-51 and 1-69, and light chain germline frameworks VK 02, VK 12 and VK B3. Parental TNF α antibody constructs (when comprising the IgG portion) included an IgG4 subclass structure having three amino acid mutations (from native IgG4) within the constant region (CH). Initial constructs were cloned into a human IgG4-Fc mammalian expression vector. However, initially produced bispecific constructs as
25 (described above) exhibited one or more chemical and / or physical problem(s) described above.

Electrostatic surface of the bispecific antibody was calculated and charged patches were identified and disrupted. Extensive protein stability studies were performed and the constructed bispecific antibodies were screened for thermostability properties as well as
30 TNF α and IL-23 binding (relative to the respective parental antibody) properties.

Chemical and physical modifications were therefore made to improve chemical and physical stability of the bispecific antibody of the present invention. Modifications to

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the parental IL-23 antibody, in scFv format, were made in HCDR4, HCDR5, LCDR4, LCDR5 and LCDR6 to improve chemical and physical stability. Constructed HCVR and LCVR were combined into the IL-23 scFv format according to the following formula: TNF α IgG (C-term.) – L1 – HCVR2 – L2 – LCVR2. A disulfide bond, for stabilizing the

5 IL-23 scFv, was engineered between the HCVR2 (G508C) and the LCVR2 (G699C) of the IL-23 scFv (numbering of amino acids applies linear numbering based on exemplified bispecific antibody presented in Table 1). Additionally, the parental TNF α antibody, in an IgG portion of the bispecific antibody, was engineered to an IgG1 subclass for improved biochemical behavior, including solubility and reduced aggregation. The

10 engineered IL-23 scFv construct and TNF α IgG construct, comprising these chemical and physical modifications, were inserted into an IgG1 expression vector.

A bispecific antibody containing a TNF α IgG, as an IgG1 subclass, and an IL-23 scFv with six CDR mutations (relative to the parental IL-23 antibody: HCVR at K28P and T54V; and LCVR at L30G, L53K, E54L and M90Q, assignment of amino acid

15 numbering is based on the well-known Kabat numbering conventions)(as represented in the exemplified bispecific antibody reflected in Table 1: HCVR at K492P and T522V; and LCVR at L629G, L653K, E654L and M689Q, numbering of amino acids is based on linear numbering) was identified as improving the expression, affinity (for IL-23 relative to the parental molecule) and thermostability issues demonstrated in initial constructs.

20 Additionally, these mutations resulted in a significantly reduced clearance rate in cynolomolgus monkeys. None of the above modifications were identified in initial characterizations of the parental single antibodies.

Bispecific Antibody Binding

25 The bispecific antibodies of the present invention bind both human TNF α and human IL23p19 and neutralize at least one human TNF α bioactivity and at least one human IL-23 bioactivity in vitro or in vivo. The bispecific antibodies of the present invention are potent inhibitors of IL-23 in the presence and absence of TNF α in vitro. The bispecific antibodies of the present invention are potent inhibitors of both soluble and

30 membrane-bound TNF α in the presence or absence of IL-23 in vitro.

The bispecific antibodies of the invention are further characterized as having a binding affinity (K_D) for human TNF α in the range of 96 ± 3.5 pM and human IL23p19 in

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the range of 121 ± 8.5 pM at 37°C. The bispecific antibodies effectively neutralize soluble as well as membrane-bound TNF α and this neutralization is not affected by the presence of saturating amounts of human IL-23. The bispecific antibodies effectively neutralize human IL-23 and this neutralization is not affected by the presence of

5 saturating amounts of human TNF α .

Bispecific Antibody Expression

Expression vectors capable of directing expression of genes to which they are operably linked are well known in the art. Expression vectors can encode a signal peptide

10 that facilitates secretion of the polypeptide(s) from a host cell. The signal peptide can be an immunoglobulin signal peptide or a heterologous signal peptide. The first polypeptide chain (comprising a HC, scFv, L1 and L2) and the second polypeptide chain (comprising a LC) may be expressed independently from different promoters to which they are operably linked in one vector or, alternatively, the first and second polypeptide chains

15 may be expressed independently from different promoters to which they are operably linked in two vectors – one expressing the first polypeptide chain and one expressing the second polypeptide chain.

A host cell includes cells stably or transiently transfected, transformed, transduced or infected with one or more expression vectors expressing a first polypeptide chain, a

20 second polypeptide chain or both a first and a second polypeptide chain of the invention. Creation and isolation of host cell lines producing a bispecific antibody of the invention can be accomplished using standard techniques known in the art. Mammalian cells are preferred host cells for expression of bispecific antibodies. Particular mammalian cells are HEK 293, NS0, DG-44, and CHO. Preferably, the bispecific antibodies are secreted

25 into the medium in which the host cells are cultured, from which the bispecific antibodies can be recovered or purified.

It is well known in the art that mammalian expression of antibodies results in glycosylation. Typically, glycosylation occurs in the Fc region of the antibody at a highly conserved N-glycosylation site. N-glycans typically attach to asparagine. By way of

30 example, each HC of exemplified bispecific antibody presented in Table 1 is glycosylated at asparagine residue 301 of SEQ ID NO:1.

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A particular DNA polynucleotide sequence encoding a first polypeptide chain (comprising a HC, scFv, L1 and L2 of the present invention and having an amino acid sequence of SEQ ID NO:1) is:

```
atggagacagacacactcctgctatgggtactgctgctctgggttccaggctccactggc
5 gaggtgcagctggtggagtctgggggaggccttggtacagcctgggaggtccctgagactc
tctgtgcagcctctggattcacctttgatgactatgccatgcactgggtccgccaggct
ccagggaaggggctggagtgggtgtcagctattacttggaatagtggtcacatagactac
gcagactccgtggaggggccggttcaccatctccagagacaatgccagaactccctgtat
10 ctgcaaatgaacagcctgagagccgaggacacggccgtatattactgtgcgaaagtgagc
tacctgagtactgcctccagcctggactactggggccaaggaaccctgggtcacctgtcc
tcagcctccaccaagggcccatcggtcttcccgtagcaccctcctccaagagcacctct
gggggcacagcggccctgggctgcctgggtcaaggactacttccccgaaccgggtgacgggtg
tcgtggaactcaggcgccctgaccagcggcgtgcacaccttcccggctgtcctacagtcc
tcaggactctactccctcagcagcgtgggtgaccgtgccctccagcagcttgggcacccag
15 acctacatctgcaacgtgaatcacaagcccagcaacaccaaggtggacaagaaagttgag
cccaaactcttgtagacaaaactcacacatgccaccgtgccagcacctgaactcctgggg
ggaccgtcagtccttcctcttccccccaaaacccaaggacaccctcatgatctcccggacc
cctgaggtcacatgcgtgggtgggtggacgtgagccacgaagaccctgaggtcaagttcaac
tggtacgtggacggcgtggaggtgcataatgccaaagacaaagccgcgaggagcagtac
20 aacagcacgtaccgtgtgggtcagcgtcctcaccgtcctgcaccaggactggctgaatggc
aaggagtacaagtgaaggtctccaacaaagccctcccagcccccatcgagaaaaccatc
tcaaagccaaagggcagccccgagaaccacaggtgtacaccctgcccccatcccgggac
gagctgaccaagaaccaggtcagcctgacctgcctgggtcaaaggcttctatcccagcgac
atcgccgtggagtgggagagcaatgggcagccggagaaactacaagaccacgcccccc
25 gtgctggactccgacggctccttcttctctatagcaagctcacctgggacaagagcagg
tggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactac
acgcagaagagcctctccctgtctccgggtggcggaggctccgggggagggggtagcgga
ggaggggggatcccaggtgcagctggtgcagtctggggctgaggtgaagaagcctgggtcc
tcggtgaaggtctcctgcaaggcttctggatatccattcactcgctatgttatgcactgg
30 gtgcgacaggccctggacaatgccttgagtggatgggatataattaatccttacaatgat
gggtgtgaactacaatgagaagttcaaaggcagagtcacgattaccgcggacgaatccacg
agcacagcctacatggagctgagcagcctgagatctgaggacacggccgtgtattactgt
gcgagaaactgggacacaggcctctgggggcaagggaccacgggtcacctctcctcaggc
ggcggagggtctggcggaggtggtagtgggtggcgggtggatcagggggaggcggtatctgac
35 atccagatgaccagctctccatcctcctgtctgcacatctgtaggagacagagtcaccatc
acttgcaaggcaagtgaccacattggcaaatttttaacttggtatcagcagaaaccaggg
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aaagcccctaagctcctgatctatggtgcaaccagcaagctgactggggtcccatcaagg
 ttcagtggcagtggtatctgggacagatttcactctcaccatcagcagctctgcaacctgaa
 gatcttgcgaacttactactgtcaacagatattggagtactccgttcacgttcggatgcggg
 accaaggtggaaataaaa (SEQ ID NO:3).

- 5 A particular DNA polynucleotide sequence encoding a second polypeptide chain (comprising a LC of the present invention having an amino acid sequence of SEQ ID NO:2) is:

atggagacagacacactcctgctatgggtactgctgctctgggttcaggatccactggc
 gacatccagatgacccagctctccatcctccctgtctgcatctgtaggagacagagtcacc
 10 atcacttgccgggagagtcagggcattcgcaattatttagcctggtatcagcagaaacca
 gggaaagctcctaagctcctgatctatgctgcatccactttgcaatcaggggtcccatct
 cggttcagtggcagtggtatctgggacagatttcactctcaccatcagcagcctgcagcct
 gaagatggtgcaacttattactgtcaacgctataaaccgtgcccccttacacgttcggccaa
 gggaccaaggtggaaatcaaacggactgtggctgcaccatctgtcttcatcttcccgcca
 15 tctgatgagcagttgaaatctggaactgcctctgttggtgtgctgctgaataacttctat
 ccagagaggccaaagtacagtggagggtggataacgcctccaatcgggtaactccag
 gagagtgtcacagagcaggacagcaaggacagcacctacagcctcagcagcaccctgacg
 ctgagcaaagcagactacgagaaacacaaagtctacgcctgcgaagtcacccatcagggc
 ctgagctcgcccgtcacaaagagcttcaacaggggagagtgc (SEQ ID NO:4).

- 20 Medium, into which a bispecific antibody has been secreted, may be purified by conventional techniques. For example, the medium may be applied to and eluted from a Protein A or G column using conventional methods. Soluble aggregate and multimers may be effectively removed by common techniques, including size exclusion, hydrophobic interaction, ion exchange, or hydroxyapatite chromatography. The product
 25 may be immediately frozen, for example at -70°C, or may be lyophilized.

- In some instances, process for producing a bispecific antibody of the present invention may result in the formation of diabodies. Diabodies are bivalent formations of scFv in which HCVR2 and LCVR2 regions are expressed on a single polypeptide chain, but instead of the variable domains pairing with complementary domains of the same
 30 polypeptide chain, the variable domains pair with complementary domains of the other polypeptide chain or a different molecule. For example, if the bispecific antibody comprises two first polypeptides (for convenience, 1A and 1B, where each of 1A and 1B comprise a HC, a scFv, L1 and L2), and two second polypeptides (for convenience, 2A and 2B, where each of 2A and 2B comprise a LC), HCVR2 of 1A pairs with

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complementary domains of LCVR2 of 1B instead of pairing with LCVR2 of 1A. As described herein, it may be beneficial to purify out diabodies from the bispecific antibodies described above. Diabody content can be greater than 10% after cellular expression and can be reduced to less than 3% after purification.

5

Therapeutic Uses

As used herein, “treatment” and/or “treating” are intended to refer to all processes wherein there may be a slowing, interrupting, arresting, controlling, or stopping of the progression of the disorders described herein, but does not necessarily indicate a total
10 elimination of all disorder symptoms. Treatment includes administration of a bispecific antibody of the present invention for treatment of a disease or condition in a mammal, particularly in a human, that would benefit from a decreased level of TNF α and / or IL-23 or decreased bioactivity of TNF α and / or IL-23, and includes: (a) inhibiting further progression of the disease, *i.e.*, arresting its development; and (b) relieving the disease,
15 *i.e.*, causing regression of the disease or disorder or alleviating symptoms or complications thereof.

The bispecific antibody of the present invention is expected to treat autoimmune diseases, including IBD (such as CD and UC), AxSpA, RA and PsA.

Pharmaceutical Composition

A bispecific antibody of the invention can be incorporated into a pharmaceutical composition suitable for administration to a patient. A bispecific antibody of the invention may be administered to a patient alone or with a pharmaceutically acceptable carrier and / or diluent in single or multiple doses. Such pharmaceutical compositions are
25 designed to be appropriate for the selected mode of administration, and pharmaceutically acceptable diluents, carrier, and / or excipients such as dispersing agents, buffers, surfactants, preservatives, solubilizing agents, isotonicity agents, stabilizing agents and the like are used as appropriate. Said compositions can be designed in accordance with conventional techniques disclosed in, *e.g.*, Remington, The Science and Practice of
30 Pharmacy, 22nd Edition, Loyd V, Ed., Pharmaceutical Press, 2012, which provides a compendium of formulation techniques as are generally known to practitioners. Suitable carriers for pharmaceutical compositions include any material which, when combined

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with a bispecific antibody of the invention, retains the molecule's activity and is non-reactive with the patient's immune system. A pharmaceutical composition of the present invention comprises a bispecific antibody and one or more pharmaceutically acceptable carriers, diluents or excipients.

5 A pharmaceutical composition comprising a bispecific antibody of the present invention can be administered to a patient at risk for or exhibiting diseases or disorders as described herein using standard administration techniques.

 A pharmaceutical composition of the invention contains an "effective" or "therapeutically effective" amount, as used interchangeably herein, of a bispecific
10 antibody of the invention. An effective amount refers to an amount necessary (at dosages and for periods of time and for the means of administration) to achieve the desired therapeutic result. An effective amount of the bispecific antibody may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the antibody or antibody portion to elicit a desired response in the individual. An
15 effective amount is also one in which any toxic or detrimental effect of the bispecific antibody, are outweighed by the therapeutically beneficial effects.

Examples

 Except as noted otherwise, the exemplified bispecific antibody referred to
20 throughout the Examples refers to the exemplified bispecific antibody of the present invention set forth in Table 1 above.

Bispecific Antibody Expression and Purification

 The exemplified bispecific antibody is expressed and purified essentially as
25 follows. A glutamine synthetase (GS) expression vector containing the DNA of SEQ ID NO:3 (encoding a polypeptide chain, comprising the IgG HC, the scFv HCVR2 and LCVR2 and linkers L1 and L2, and having the amino acid sequence of SEQ ID NO:1) and SEQ ID NO:4 (encoding a second polypeptide chain, comprising the IgG LC, and having the amino acid sequence of SEQ ID NO:2) is used to transfect a Chinese hamster
30 cell line, CHO (GS knockout, clone 1D3), by electroporation. The expression vector encodes an SV Early (Simian Virus 40E) promoter and the gene for GS. Expression of GS allows for the biochemical synthesis of glutamine, an amino acid required by the

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CHO cells. Post-transfection, cells undergo bulk selection with 50 μ M L-methionine sulfoximine (MSX). The inhibition of GS by MSX is utilized to increase the stringency of selection. Cells with integration of the expression vector cDNA into transcriptionally active regions of the host cell genome can be selected against CHO wild type cells, which

5 express an endogenous level of GS. Transfected pools are plated at low density to allow for close-to-clonal outgrowth of stable expressing cells. The masterwells are screened for bispecific antibody expression and then scaled up in serum-free, suspension cultures to be used for production. Clarified medium, into which the exemplified bispecific antibody has been secreted, is applied to a Protein A affinity column that has been equilibrated

10 with a compatible buffer such as phosphate buffered saline (pH 7.4). The column is washed to remove nonspecific binding components. The bound bispecific antibody is eluted, for example, by pH gradient (such as 0.1 M sodium phosphate buffer pH 6.8 to 0.1 M sodium citrate buffer pH 2.5) and neutralized with Tris, pH 8 buffer. Bispecific antibody fractions are detected, such as by SDS-PAGE or analytical size-exclusion, and

15 then are pooled. Soluble aggregate and multimers may be effectively removed by common techniques including size exclusion, hydrophobic interaction, ion exchange, or hydroxyapatite chromatography. The bispecific antibody is concentrated and / or sterile filtered using common techniques. The purity of the exemplified bispecific antibody after these chromatography steps is greater than 98.6% (monomer). The bispecific antibody

20 may be immediately frozen at -70°C or stored at 4°C for several months.

Bispecific Antibody Solubility and Stability Analysis

The exemplified bispecific antibody is formulated in 10mM citrate pH 6.0. The bispecific antibody is concentrated to 1 mg/mL, 50 mg/mL and 100 mg/mL (using

25 Amicon U.C. filters, Millipore, catalog # UFC903024). Samples concentrated to 1 mg/mL are incubated at one of 4 and 25°C over a period of 4 weeks and samples concentrated to 50 and 100 mg/mL are incubated with 25 or 150 mM NaCl at one of 4 and 25°C over a period of 4 weeks.

Following incubation, samples are analyzed for percent high molecular weight

30 (%HMW) with size exclusion chromatography (SEC) using a TSKgel Super SW3000 (Tosoh Bioscience product # 18675) column. 50 mM sodium phosphate + 350 mM NaCl, pH 7.0 is used as the mobile phase running at 0.4 mL/min for 15 minutes. A volume of

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5 μ L (5 μ g) of the concentrated antibody is injected into the column and the detection is measured at 214nm. For high concentration samples, a volume of 1 μ L (50 μ g or 100 μ g) is injected into the column and the detection is measured at 280nm. Chromatograms are analyzed using ChemStation and % high molecular weight (HMW) is calculated using the ratio of AUC of the peaks eluted before the monomer peak to total AUC. Results are summarized in Table 2.

Table 2: Summary of change in percentage of high molecular weight species from starting control over 4 weeks measured by SE-HPLC.

Sample Concentration	Incubation Temperature	Change in % of HMW by SE-HPLC
1 mg/mL	4°C	0
1 mg/mL	25°C	0.5
50 mg/mL	4°C	0.2
50 mg/mL	25°C	0.7
100 mg/mL	4°C	0.1
100 mg/mL	25°C	0.7

10

Sample degradation of samples concentrated to 1 mg/mL is also analyzed with capillary isoelectric focusing (cIEF) analysis using a Beckman-PA800 plus system according to manufacturer instructions. Results are summarized in Table 3.

15 Table 3: Summary of change in sample degradation over 4 weeks measured by cIEF.

Sample Concentration	Incubation Temperature	% Sample Degradation
1 mg/mL	4°C	0.7
1 mg/mL	25°C	1.7

20 Samples concentrated to 1 mg/mL and stored at 40°C are also analyzed for identification of peptide bond cleavage with liquid chromatography-mass spectrometry (LC-MS) analysis using a Thermo Scientific Ion Trap LC-MS system according to manufacturer instructions. No region of the bispecific antibody demonstrated peptide bond cleavage of greater than 2%. Only the hinge region (1.8%) and linker L1 (1.1%) of

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the first encoded polypeptide chain (SEQ ID NO:1 of the exemplified bispecific antibody) demonstrated peptide bond cleavage of greater than 1%.

Solubility of the exemplified bispecific antibody is analyzed at 4, -5 and -10°C after one week incubation period. Solubility is assessed with bispecific antibody
5 concentrated to between 100 and 150 mg/mL (using Amicon U.C. filters, Millipore, catalog # UFC903024) and formulated in 10mM citrate at pH 6.0 including 150mM NaCl. The exemplified bispecific antibody exhibited good solubility and lacked phase separation following the incubation period.

Viscosity of the exemplified bispecific antibody is analyzed at room temperature.
10 Viscosity is assessed with bispecific antibody concentrated to 100 mg/mL (using Amicon U.C. filters, Millipore, catalog # UFC903024) and formulated in 10mM citrate at pH 6.0 including 150mM NaCl. The exemplified bispecific antibody exhibited good viscosity, 4.2cp at 100 mg/mL, whereas other initial attempted constructs demonstrated unacceptably high viscosity (>9cP).

15 Preliminary studies with a bispecific antibody comprising parental TNF α antibody and parental IL-23 were not possible due to poor bispecific antibody expression, bispecific antibody degradation / clipping, unacceptably high solution viscosity at relatively high concentrations and low affinity (relative to the parental antibodies). However, preliminary studies with the exemplified bispecific antibody of the present
20 invention demonstrate unexpected beneficial properties, including expression of the bispecific antibody and unexpected beneficial chemical and physical stability and solubility properties. As demonstrated by the preliminary data, the bispecific antibody of the present invention exhibits low HMW aggregation, good solubility, good viscosity in solution, increased physical stability including low degradation levels and low peptide
25 bond cleavage, and maintains affinity for both TNF α and the p19 subunit of IL-23.

Bispecific Antibody Binding Affinity to IL-23 and TNF α

Binding affinity and binding stoichiometry of the exemplified bispecific antibody to human IL-23 and human TNF α is determined using a surface plasmon resonance assay
30 on a Biacore T100 instrument primed with HBS-EP+ (10 mM Hepes pH7.4 + 150 mM NaCl + 3 mM EDTA + 0.05% (w/v) surfactant P20) running buffer and analysis temperature set at 37°C. A CM5 chip (Biacore P/N BR-100530) containing immobilized

-23-

protein A (generated using standard NHS-EDC amine coupling) on all four flow cells (Fc) is used to employ a capture methodology. Antibody samples are prepared at 10 $\mu\text{g/mL}$ by dilution into running buffer. Human IL-23 are prepared at final concentrations of 20.0, 10.0, 5.0, 2.5, 1.25, 0.62 and 0 (blank) nM by dilution into running buffer.

- 5 Human TNF α are prepared at final concentrations of 50.0, 25.0, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39, 0.19 and 0 (blank) nM by dilution into running buffer.

Each analysis cycle consists of (1) capturing antibody samples on separate flow cells (Fc2 and Fc3); (2) injection of each human IL-23 concentration over all Fc at 80 $\mu\text{L/min}$ for 200 seconds followed by return to buffer flow for 1800 seconds to monitor dissociation phase; (3) injection of each human TNF α concentration over all Fc at 50 $\mu\text{L/min}$ for 250 seconds followed by return to buffer flow for 1200 seconds to monitor dissociation phase; (4) regeneration of chip surfaces with injection of 10 mM glycine, pH 1.5, for 60 seconds at 80 $\mu\text{L/min}$ over all cells; and (5) equilibration of chip surfaces with a 10 μL (60-sec) injection of HBS-EP+. Data are processed using standard double-
 10 referencing and fit to a 1:1 binding model using Biacore T100 Evaluation software, version 2.0.3, to determine the association rate (k_{on} , $\text{M}^{-1}\text{s}^{-1}$ units), dissociation rate (k_{off} , s^{-1} units), and R_{max} (RU units). The equilibrium dissociation constant (K_D) is calculated from the relationship $K_D = k_{off}/k_{on}$, and is in molar units.

- 20 Table 4: Binding affinity to human IL-23 and human TNF α by the exemplified bispecific antibody at 37°C.

Antigen	k_{on} Avg $\pm$ SD $\text{M}^{-1}\text{s}^{-1}$	k_{off} Avg $\pm$ SD s^{-1} (10^{-4})	K_D Avg $\pm$ SD pM	n
Human IL-23	$8.3 (\pm 0.8) \times 10^5$	1.0 ± 0.0	121 ± 8.5	3
Human TNF α	$4.1 (\pm 0.2) \times 10^6$	4.0 ± 0.2	96 ± 3.5	3

These results demonstrate that the exemplified bispecific antibody of the present invention binds human IL-23 and human TNF α with high affinity at 37°C.

25

Simultaneous Binding of IL-23 and TNF α

A BIAcore T100 instrument is used to determine whether human IL-23 and human TNF α can bind to the bispecific antibody simultaneously. Except as noted, all

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reagents and materials are purchased from BIAcore AB (Uppsala, Sweden). All measurements are performed at 37°C. HBS-EP+ buffer (150 mM sodium chloride, 3 mM EDTA, 0.05 % (w/v) surfactant P-20, and 10 mM HEPES, pH7.4) is used as the running buffer and sample buffer. Protein A is immobilized on flow cells 1 and 2 of a CM5 sensor chip using an amine coupling kit. The exemplified bispecific antibody (diluted to 1 µg/ml) is first captured on flow cell 2 (with a 4 second injection at 80 µl/min, yielding 95.3 response units (Δ RU) of bispecific antibody capture), followed by injection of human TNFα at 100 nM for 5 min to saturate TNFα binding site (a binding signal of 32.4 Δ RU is observed). Flow cell 1 is a protein A only control. After binding of TNFα (flow cell 2), human IL-23 at 100 nM is then injected for 5 min and additional binding signal (of 57.3 Δ RU) is observed. Chip surface is then regenerated using 10mM Glycine pH 1.5. The same process is repeated except with a reverse order of human IL-23 first followed by human TNFα. These results demonstrate that the exemplified bispecific antibody of the present invention can bind human IL-23 and human TNFα simultaneously as shown by the increase in response units (initial 32.4 RU from TNFα and then additional 57.3 RU from IL-23) from the two ligands binding to the bispecific antibody.

Inhibition of IL-23-Mediated Stat 3 Phosphorylation *in Vitro* from Kit225 Cells

Kit225 cells are a human T cell lymphocytic leukemia cell line that naturally expresses the IL-23 receptor. Incubation of Kit225 cells with human IL-23 results in the rapid increase of phosphorylation of Stat3 mediated by IL-23R/JAK kinase, which can be measured using commercially available ELISA.

Kit225 cells are routinely cultured in assay medium (RPMI 1640 containing 10% FBS, 10 ng/ml human IL-2, and 1x penicillin plus puromycin). On the day of assay, the cells are harvested, washed with large volume of serum free RPMI 1640 medium, then resuspended in serum free RPMI 1640 at 5×10^6 per ml. 500,000 Kit225 cells per well (in 100µL) are added to the wells of a U-bottom cultured 96 well plate. The 96 well plate is placed in an incubator and the cells are starved for 3 hours at 37°C.

A dose range of the exemplified bispecific antibody from 100nM to 0.5pM, with 1:3 dilutions, is evaluated (MW of bispecific antibody is 200 kDa). Each test concentration of exemplified bispecific antibody is pre-incubated with (2 ng/ml)

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recombinant human IL-23 for 30 minutes at 37°C. Assay medium is used for “medium alone” and “medium with 2 ng/ml IL-23” controls. An IL-23 neutralizing antibody (parental IL-23 antibody described in U.S. Patent No. 7,872,102), targeting the p19 subunit of IL-23, is used as a positive control in the assay and tested at the same molar
5 range as the bispecific antibody. Control antibodies are also pre-incubated with (2 ng/ml) recombinant human IL-23 for 30 minutes at 37°C. Following pre-incubation, antibody/IL-23 mixtures are transferred to Kit225 cells and incubated for 15 minutes at 37 °C.

At the end of the assay, the plates are centrifuged (3000 rpm for 2 minutes at 4 °C) and the supernatant is then discarded. Lysis buffer (100µL, containing
10 phosphate/proteinase inhibitor) is added to the cells and then incubated on ice for 5 minutes. Following incubation, the cells are centrifuged (3000 rpm for 5 minutes at 4°C). After centrifugation, 100µL of supernatant is transferred to 96 well plates (pre-coated with anti-Stat 3 antibody) for overnight incubation (4°C). On the day of measuring Stat 3 phosphorylation by ELISA, the 96 well plates are warmed at room temperature and
15 ELISA to determine Stat 3 phosphorylation levels is carried out according to manufacturer instructions (Cell Signaling Inc., Cat#7146). Following ELISA reactions, the 96 well plates are read at 450 nm on a microplate reader (Molecular Devices SpectraMax 190). Results are expressed as the concentration where 50% of the IL-23-induced Stat 3 phosphorylation is inhibited (IC₅₀) by either bispecific antibody or the
20 positive control and is calculated using a 4 parameter sigmoidal fit of the data (GraphPad Prism).

The results demonstrate that the exemplified bispecific antibody of the present invention inhibited human IL-23 induced Stat 3 phosphorylation in Kit225 cells in a concentration-dependent manner. The inhibition was comparable to that observed with
25 the positive control IL-23p19 antibody (with an IC₅₀ for bispecific antibody of 158 ± 26 pM versus 75 ± 7 pM for the positive control IL-23p19 antibody (average IC₅₀ ± SEM from three independent experiments)). Negative control antibody did not inhibit Stat 3 phosphorylation in Kit225 cells at any concentration tested. The exemplified bispecific antibody of the present invention effectively neutralized IL-23.

Inhibition of Soluble TNFα-Induced Cytotoxicity in L929 Cells *in Vitro*

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L929 cells are mouse fibrosarcoma cells that naturally express the TNF receptor. Incubation of L929 cells with human TNF α results in rapid cell death due to excessive formation of reactive oxygen intermediates. Cell death can be measured using MTT cytotoxicity assay, where mitochondrial succinate dehydrogenase in viable cells reduces
5 tetrazolium salt into formazan product, which can be detected with a microplate reader (Molecular Devices SpectriMax 190).

A dose range from 20 nM to 10 pM is evaluated (MW of the bispecific antibody is 200 kDa). Each test concentration of exemplified bispecific antibody (100 μ L) is added to wells containing 200 pg/mL recombinant human TNF α and 6.25 μ g/mL actinomycin-
10 D. Testing is carried out in duplicate wells per treatment. A TNF α antibody, Adalimumab with IgG1 isotype (parental TNF α antibody, see for example U.S. Patent No. 6,090,382), is used as a positive control in the assay. A human IgG1 isotype control antibody is used as a negative control. Control antibodies are tested at the same molar dose range as exemplified bispecific antibody. Plates containing antibody mixtures are
15 incubated for 60 minutes at room temperature.

L929 cells are routinely cultured in assay medium (1xDMEM Cellgro, 10% FBS, 1% Pen-Strep, 1% MEM essential amino acids, 1% L-glutamine, 1% sodium pyruvate). On the day of the assay, the cells are rinsed with 1x PBS (no Ca⁺⁺ or Mg⁺⁺) and detached from culture flasks with 0.25% trypsin + EDTA. The trypsin is inactivated with assay
20 medium. L929 cells are centrifuged at 215xg for 5 minutes at RT. The cell pellet is resuspended in assay medium. Cell density is measured with a hemocytometer, and 10,000 L929 cells (in 100 μ L) are added to 96-well plates and placed in a tissue culture incubator (37°C, 95% relative humidity, 5% CO₂) over night. The antibody/TNF α /actinomycin-D mixture is transferred to the 96 well plates with L929 adherent cells and
25 incubated (37°C, 95% relative humidity, 5% CO₂) 18 hours. The assay medium is removed and the MTT substrate mixture is added to the wells (120 μ L). The plates are placed at 37°C, 95% relative humidity, 5% CO₂ for 3 hours. The cell death is determined by reading the plates at 490 nm on a microplate reader (Molecular Devices SpectraMax 190). Results are expressed as the concentration where 50% of the TNF α induced
30 response is inhibited (IC₅₀) (average of three independent experiments $\pm$ SEM) by either

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the bispecific antibody or the positive control antibody, calculated using a 4 parameter sigmoidal fit of the data (GraphPad Prism).

The results demonstrate that the exemplified bispecific antibody of the present invention inhibited human TNF α -induced killing of L929 cells in a concentration-
5 dependent manner with an IC₅₀ of 233 ± 20 pM. This inhibition was comparable to that observed with the positive control antibody, Adalimumab (IC₅₀ = 250 ± 24 pM), whereas the negative control antibody did not inhibit human TNF α . The exemplified bispecific of the present invention effectively neutralized human TNF α .

10 **Inhibition of Membrane Bound Human TNF α -Induced Cytotoxicity in L929 Cells *in Vitro***

In order to study the ability of the bispecific antibody to inhibit membrane bound TNF α , known cleavage sites of TNF α are inactivated using a set of mutations previously demonstrated to allow expression of bioactive TNF α on cell surface (Mueller et. al. 1999)
15 in the absence of TNF α cleavage. The non-cleavable TNF α construct is stably transfected to Chinese hamster ovary (CHO) cells. These cells express membrane bound TNF α as shown by flow cytometry. Incubation of L929 cells with CHO cells expressing human non-cleavable bound TNF α results in rapid L929 cell death.

CHO cells expressing membrane bound human TNF α are routinely maintained in
20 selection medium (AM2001 media, an internal CHO growth media without MSX, 8mM glutamine, GS supplement, HT supplement with 500 μ g/mL G418). On the day of the assay, the cells are counted, rinsed with 1x PBS (no Ca⁺⁺ or Mg⁺⁺), centrifuged at 215 x g for 5 min and re-suspended at 50,000 cells/mL in L929 assay medium together with Actinomycin-D (6.25 μ g/mL final concentration). 500 cells (in 10 μ L) of cell suspension
25 are added to each concentration of antibody mixtures that were incubated for 60 minutes at 37°C, 95% relative humidity, 5% CO₂. The mixtures containing antibody, membrane bound human TNF α CHO cells, actinomycin-D mixtures are transferred to 96 well plates with L929 adherent cells and incubated 18 hours at 37°C, 95% relative humidity, 5% CO₂. The cell death is measured using an MTT cytotoxicity assay as described above for
30 soluble TNF α L929 assay. Results are expressed as the concentration where 50% of the TNF α induced response is inhibited (IC₅₀) (average of 3 independent experiments $\pm$ SEM) by either the bispecific or the positive control antibody.

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The results demonstrate that the exemplified bispecific antibody of the present invention inhibited killing of L929 cells by human non-cleavable membrane bound TNF α CHO cells in a concentration-dependent manner with an IC₅₀ of 755 $\pm$ 297 pM. This inhibition was comparable to that observed with the positive control antibody (Adalimumab with IgG1 isotype, IC₅₀ = 581 $\pm$ 201 pM), whereas the negative control antibody did not inhibit human TNF α . The exemplified bispecific antibody of the present invention effectively neutralized membrane bound human TNF α .

Inhibition of Human IL-23-Induced mIL-22 Production *in Vivo*

Administration of human IL-23 induces expression of mouse IL-22 (mIL-22) in normal Balb C mice *in vivo*. This human IL-23-induced expression of mIL-22, *in vivo*, is blocked by the bispecific antibody of the present invention (which does not cross react with either mouse IL-23 or mouse TNF α).

Normal Balb C mice (N=8) are injected intraperitoneally with either 50 nmole/kg of exemplified bispecific antibody (molecular weight 200kDa) or with a negative control antibody (human IgG1 isotype antibody, 50 nmole/kg, molecular weight 150kDa). Three days following injection, the mice are challenged by intraperitoneal injection of 50 nmol/kg of human IL-23. Five hours post IL-23 challenge the mice are sacrificed and plasma is collected. Collected plasma is analyzed by commercial ELISA (eBioscience, Cat.# 88-7422-86), according to manufacturer's instructions, for mouse IL-22 expression.

Table 5: Inhibition of human IL-23-induced mIL-22 production *in vivo* (relative to negative control antibody).

	Bispecific Antibody + IL-23	Naïve mouse	Negative Control Antibody (human IgG1 isotype antibody) + IL-23
mIL22 Level (pg/mL)	1.3 $\pm$ 2.6	0 $\pm$ 0	132 $\pm$ 51

The results demonstrate that the exemplified bispecific antibody of the present invention blocks the human IL-23-induced increase in mIL-22 expression. This inhibition is comparable to mouse IL-22 levels observed in naïve mice (p<0.0001, ANOVA followed by Turkey's Multiple Comparison test), whereas the negative control

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antibody did not inhibit the human IL-23-induced increase in expression of mIL-22. The bispecific antibody of the present invention effectively neutralized human IL-23.

Inhibition of Human TNF α -Induced Production of CXCL1 *in Vivo*

- 5 Administration of human TNF α induces a rapid and transient increase of mouse CXCL1 levels in plasma in regular Balb C mice, *in vivo*. This human TNF α -induced increase of mouse CXCL1 levels, *in vivo*, is blocked by the exemplified bispecific antibody of the present invention (which does not cross react with either mouse IL-23 or mouse TNF α).
- 10 Regular Balb C mice (N=8) are injected intraperitoneally with either: (a) 18 nmole/kg of bispecific antibody; (b) 18 nmole/kg of positive control anti-TNF α antibody (Adalimumab with IgG1 isotype); or (c) 18 nmole/kg of negative control antibody (human IgG1 isotype antibody). Three days following injection, the mice are challenged by intraperitoneal injection of 18 nmol/kg of human TNF α . Two hours post TNF α
- 15 challenge the mice are sacrificed and plasma is collected. Collected plasma is analyzed by commercial MSD assay (Masol Scale Discovery, Cat.# K152QTG-1), according to manufacturer's instructions, for mouse CXCL1 levels.

20 Table 6: Inhibition of human TNF α -induced CXCL1 production *in vivo* (relative to negative control antibody).

	Bispecific Antibody + TNFα	Positive Control Antibody (anti-TNFα antibody) + TNFα	Negative Control Antibody (human IgG1 isotype antibody) + TNFα	Naïve mouse
CXCL1 Level (pg/mL)	622 $\pm$ 147	633 $\pm$ 162	2420 $\pm$ 850	16 $\pm$ 4.4

- The results demonstrate that the exemplified bispecific antibody of the present invention significantly inhibited human TNF α -induced CXCL1 production relative to animals that received the negative control antibody ($p < 0.0001$, ANOVA followed by
- 25 Turkey's Multiple Comparison test). The reduction in CXCL1 production with the bispecific antibody was comparable to that observed with the positive control antibody.

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Thus, the exemplified bispecific antibody of the present invention effectively neutralized biological effects induced by human TNF α in mouse.

Comparison of Antibody Clearance with Parental IL-23 Antibody

5 In order to compare serum pharmacokinetics of the bispecific antibody with the serum pharmacokinetics of the parental IL-23 antibody (IL-23 antibody described in U.S. Patent No. 7,872,102), male Cynomolgus monkeys are administered either the 5 mg/kg of exemplified bispecific antibody (intravenous (N=2); or subcutaneous (N=2)) or 5 mg/kg parental IL-23 antibody (intravenous (N=3)). Exemplified bispecific antibody is prepared
10 in solution of 10 mM citrate (pH 6.0), 150 mM NaCl, 0.02% Tween 80. Parental IL-23 antibody is prepared in solution of phosphate-buffered saline (pH ~7.4).

 Prior to administration, approximately 1.5mL of blood is collected from each Cynomolgus monkey. Post administration, blood (approximately 1.5mL) is collected at 1 (intravenous only), 6, 12, 24, 48, 72, 96, 168, 240, 336, 432, 504, 600 and 672 hours post
15 administration. Blood samples are collected intravenously from a femoral vein into tubes serum separator tubes (e.g., containing no anticoagulant) and processed to serum.

 Serum samples are analyzed by total human IgG ELISA utilizing AffiniPure F(ab')₂ Fragment Goat Anti-Human IgG (Jackson ImmunoResearch Laboratories, Inc.) as capture reagent coated on ELISA plates (Thermo Scientific™ Immulon® 4HBX). Serum
20 samples (100 μ L) are added to the individual wells of ELISA plate and incubated at 25°C for 60 mins. Following incubation, 100 μ L (10,000 fold dilution) mouse anti-human IgG Fc-HRP (Southern Biotech) is added to wells of ELISA plate for detection of exemplified bispecific antibody and 100 μ L (10,000 fold dilution) mouse anti-human IgG₄ Fc-HRP (Southern Biotech) is added to wells of ELISA plate for detection of parental IL-23
25 antibody. Unbound enzyme is removed via washing and 100 μ L TMB Microwell Peroxidase Substrate System (KPL) is added to individual wells of ELISA plate. Color development is stopped by addition of 100 μ L TMB Stop Solution (KPL) and optical density of the wells is measured at 450 nm with wavelength correction set to 630 nm.

 Standard curves for both exemplified bispecific antibody and parental IL-23
30 antibody, respectively, are generated by dilution of known amounts of exemplified bispecific antibody and parental IL-23 antibody, respectively, into 100% Cynomolgus monkey serum (BioreclamationIVT), followed by 5-fold dilution in blocker casein in PBS

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(Thermo Scientific™ Pierce™). Standard curve range of parental IL-23 antibody is 1.95-125 ng/mL (with an upper and lower limit of quantification of 50 ng/mL and 5 ng/mL, respectively); standard curve range of exemplified bispecific antibody is 15.63-1000 ng/mL (with an upper and lower limit of quantification of 450 ng/mL and 50 ng/mL, respectively).

Pharmacokinetic parameters (clearance values) are calculated using immunoreactivity versus time profile from time zero (administration of antibody) to 168 post administration (exemplified bispecific antibody) or 672 hours post administration (parental IL-23 antibody) and are determined via non-compartmental analysis using Phoenix (WinNonLin 6.3, Connect 1.3). Results are summarized in Table 8.

Table 8. Antibody clearance of exemplified bispecific antibody and parental IL-23 antibody in Cynomolgus monkey following single IV or subcutaneous administration.

Antibody Administered	IV Clearance (mL/hr/kg)	Subcutaneous Clearance (mL/hr/kg)
Exemplified bispecific Ab	0.23	0.34
Parental IL-23 Ab	1.17 ± 0.06	N.A.

The results demonstrate that the exemplified bispecific antibody has approximately 5-fold reduced antibody clearance compared to parental IL-23 antibody in Cynomolgus monkey.

Dual Neutralization of Murine IL-23 and Murine TNF α in an Anti-CD40 Antibody-Induced Murine Colitis Model

The bispecific antibody of the present invention does not bind murine IL-23 (mIL23) or murine TNF α (mTNF α). Therefore, in order to test therapeutic potential of dual targeting TNF α and IL-23 in a rodent model, a surrogate bispecific antibody (surrogate bispecific) is generated. The surrogate bispecific is constructed of an engineered chimeric anti-TNF α mIgG2a antibody (engineered chimeric surrogate antibody of Adalimumab targeting equivalent mTNF α epitope, in mouse, as IgG of the bispecific antibody of the present invention targets in human), having fused at the C-

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terminus of both heavy chains of the anti-TNF α IgG2a antibody an engineered anti-IL-23p19 scFv (engineered from a surrogate murine antibody targeting an equivalent IL23p19 epitope, in mouse, as the scFv of the bispecific antibody of the present invention targets in human). The binding affinity to mIL23 and mTNF α of the surrogate bispecific is measured, using surface plasmon resonance, to be 1.51 nM and 0.08 nM, respectively.

The therapeutic potential of dual targeting TNF α and IL-23 for colitis is tested in an anti-CD40 antibody-induced murine colitis model by comparing the therapeutic effect (colon weight v. length) of the surrogate bispecific to the therapeutic effect of an anti-TNF α single antibody alone and an anti-IL-23 single antibody alone. Rag1 knockout mice, upon injection of murine anti-CD40 antibody, develop severe, acute colitis which leads to wasting disease, gastrointestinal symptoms including diarrhea and anal inflammation, and weight loss up to 10-20% within 4 days post injection.

To determine the therapeutic potential of dual targeting TNF α and IL-23, three days prior to injection of anti-CD40 antibody, Rag1 knockout mice are administered one of: (a) surrogate bispecific (1.4mg/kg); (b) anti-TNF α antibody (1 mg/kg); (c) anti-IL-23 antibody (0.3mg/kg); or (d) control IgG2a antibody (1mg/kg) (these doses of antibody injection result in similar level of antibody exposure in vivo). Three days following injection, the mice are administered 200 μ g per mouse of murine anti-CD40 antibody. Four days after administration of anti-CD40 antibody, the mice are sacrificed and colon weight and length are measured (a ratio of colon weight to length is determined as a measure of colon inflammation greater than mice not administered anti-CD40 antibody).

Table 9: Change in ratio (colon weight : length).

	Surrogate bispecific Ab	Anti-IL-23 Ab	Anti-TNFα Ab	Murine IgG2a Ab
Change in colon weight : length (mg/mm)	2.68 $\pm$ 0.09	3.27 $\pm$ 0.09	3.18 $\pm$ 0.09	4 $\pm$ 0.098

The results demonstrate that dual blocking of TNF α and IL-23 by the surrogate bispecific antibody is superior for inhibiting colitis as compared to anti-IL-23 antibody and anti-TNF α antibody therapies alone (p<0.0001 compared to anti-IL-23 antibody alone; p<0.0004 compared to anti-TNF antibody alone, ABOVA followed by comparisons with control using Dunett's method). The inhibition of colitis with the

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surrogate bispecific was comparable to that observed with the control mice (not administered anti-CD40 antibody). Thus, dual blocking of TNF and IL-23 by the surrogate bispecific effectively inhibits colitis in mouse.

5 **Dual Neutralization of Murine IL-23 and Murine TNF α in a Murine GPI-Induced Rheumatoid Arthritis Model**

DBA/1 mice, upon administration of glucose-6-phosphate-isomerase (GPI), develop rheumatoid arthritis characterized by rapid swelling in the paws. GPI is a protein in the glycolytic pathway and autoantibodies against GPI have been demonstrated in both
10 human rheumatoid arthritis patients as well as murine models. Therapies targeting the TNF α or Th17 pathway alone have demonstrated efficacy in reducing joint swelling in the murine model (Matsumoto 2008, Iwanami 2008).

To demonstrate efficacy of dual neutralization of murine IL-23 and murine TNF α (by surrogate bispecific antibody, described above) when administered in an early
15 therapeutic mode, DBA/1 mice are injected with 400 μ g recombinant human GPI and complete Freund's adjuvant (CFA)(1:1 v/v, 2 subcutaneous injection sites at the base of the tail). Eight days following administration of GPI, the mice are administered (by twice weekly intra-peritoneal injection) one of: (a) control murine IgG2a antibody (90 μ g); (b) surrogate bispecific (4.2 μ g) + control murine IgG2a antibody (87 μ g); (c) surrogate
20 bispecific (12.6 μ g) + control murine IgG2a antibody (81 μ g); or (d) surrogate bispecific (42 μ g) + control murine IgG2a antibody (60 μ g). Three weeks (day 21) following administration of GPI, the mice are euthanized.

Starting on the day of administration of GPI (day 0) and days 2, 4, 7, 8, 9, 10, 11, 12, 14, 16, 18, and 21 thereafter, each paw is scored for severity of joint swelling based
25 on a 0-3 scoring system (0 = normal; 1 = erythema and slight swelling of major joint; 2 = moderate to severe swelling of the major joint; and 3 = severe swelling of entire paw). The clinical score represents the total score of all 4 paws (a maximum score being 12). The area under curve (AUC) is calculated by trapezoid method for clinical score over time from day 1 to day 21 (the end of study). Clinical score AUC data (shown in Table
30 10 as mean $\pm$ SEM) are fitted with a one-way ANOVA model for treatment groups. Test p values of interested comparisons are derived from model based T-test.

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Table 10: Clinical scores for GPI-induced arthritis mice treated with surrogate bispecific antibody (at various doses) or control antibody. ($p < 0.05$ from model based t-test)

	Control Ab only	Surrogate Bis. (4.2μg) + Control Ab (87μg)	Surrogate Bis. (12.6μg) + Control Ab (81μg)	Surrogate Bis. (42μg) + Control Ab (60μg)
Clinical Score AUC	105.3 $\pm$ 8.4	72.8 $\pm$ 14.5	32.9 $\pm$ 10.4	33.6 $\pm$ 8.9

The results demonstrate that dual blocking of TNF α and IL-23 by surrogate bispecific antibody reduces joint swelling of rheumatoid arthritis in a concentration-dependent manner. On day 9 (the day after treatment initiation with one of the control antibody or surrogate bispecific) mean clinical scores of mice treated with surrogate bispecific Ab are lower than control antibody-treated mice. Maximal clinical scores are attenuated by surrogate bispecific in a dose dependent manner, with clinical score AUCs for all three doses of surrogate bispecific-treated mice significantly lower than control antibody-treated mice (surrogate bispecific concentrations of 12.6 μ g and 42 μ g achieve the greatest percentage of attenuation).

Comparison of Dual Neutralization of Murine IL-23 and Murine TNF α in a Murine GPI-Induced Rheumatoid Arthritis Model with Neutralization of Murine IL-23 Alone and Murine TNF α Alone

In order to compare efficacy of dual neutralization of murine IL-23 and murine TNF α (by surrogate bispecific antibody) to single murine IL-23 and single murine TNF α treatments when administered in an early therapeutic mode, DBA/1 mice are injected with 400 μ g recombinant human GPI and CFA (1:1 v/v, 2 subcutaneous injection sites at the base of the tail). Eight days following administration of GPI, the mice are administered (by twice weekly intra-peritoneal injection) one of: (a) control murine IgG2a antibody (30 μ g); (b) murine anti-IL-23 antibody (30 μ g, an engineered surrogate murine antibody targeting an equivalent IL23p19 epitope, in mouse, as the scFv of the bispecific antibody of the present invention); (c) murine anti-TNF α antibody (30 μ g, an engineered chimeric surrogate antibody of Adalimumab targeting an equivalent mTNF α

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epitope, in mouse, as IgG of the bispecific antibody of the present invention targets in human); or (d) surrogate bispecific (42 μ g, described above). Twelve days (day 12) following administration of GPI, the mice are euthanized.

Starting on the day of administration of GPI (day 0) and days 2, 4, 7, 8, 9, 10, 11 and 12 thereafter, each paw is scored for severity of joint swelling based on a 0-3 scoring system (0 = normal; 1 = erythema and slight swelling of major joint; 2 = moderate to severe swelling of the major joint; and 3 = severe swelling of entire paw). The clinical score represents the total score of all 4 paws (a maximum score being 12). The area under curve (AUC) is calculated by trapezoid method for clinical score over time from day 8 (immunization with an antibody) to day 12 (end of study). Clinical score AUC data (shown in Table 11 as mean $\pm$ SEM) are fitted with a one-way ANOVA model for treatment groups. Test p values of interested comparisons are derived from model based T-test. Clinical scores for each individual mouse for days 8 through 12 are fitted with a repeated measurement model with the factors if treatment groups, measurement days and their interaction. Autoregression structure modeling is also applied (to account for correlation of the same animal repeatedly measured across days). Synergy for each is assessed by constructing contrasts on interaction for the Bliss test.

Table 11: Clinical scores for GPI-induced arthritis mice treated with surrogate bispecific antibody, murine anti-IL-23 Ab, murine anti-TNF α Ab, or control antibody. (p<0.05 from model based t-test)

	Control mIgG2a Ab	anti-IL-23 Ab	anti-TNFα Ab	Surrogate bispecific
Clinical Score AUC	19.7 $\pm$ 3.6	18.4 $\pm$ 2.1	19.6 $\pm$ 4.1	6.1 $\pm$ 3.2

The results demonstrate that dual blocking of TNF α and IL-23 by surrogate bispecific antibody is superior for reducing joint swelling of rheumatoid arthritis as compared to anti-IL-23 antibody and anti-TNF α antibody therapies alone. On day 9 (the day after treatment initiation with one of the surrogate bispecific, anti-IL-23 antibody, anti-TNF α antibody or control antibody) mean clinical scores of mice treated with surrogate bispecific are lower than other treatment groups. Thus, dual blocking of TNF α

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and IL-23, by the surrogate bispecific, effectively reduces joint swelling of rheumatoid arthritis in mouse.

Sequences

5 Exemplified First Encoded Polypeptide; IgG HC, L1, scFv HCVR2, L2, and scFv

LCVR2 (SEQ ID NO:1)

EVQLVESGGG LVQPGRSLRL SCAASGFTFD DYAMHWVRQA PGKGLEWVSA ITWNSGHIDY
 ADSVEGRFTI SRDNAKNSLY LQMNSLRAED TAVYYCAKVS YLSTASSLDY WGQGTILVTVS
 SASTKGPSVF PLAPSSKSTS GGTAALGCLV KDYFPEPVTV SWNSGALTSG VHTFPAVLQS
 10 SGLYSLSSVV TVPSSSLGTQ TYICNVNHKP SNTKVDKKVE PKSCDKHTTC PPCPAPELLG
 GPSVFLFPPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY
 NSTYRVSVSL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP QVYTLPPSRD
 ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTPPP VLDSGGSFFL YSKLTVDKSR
 WQQGNVFCSS VMHEALHNHY TQKSLSLSPG GGGSGGGGSG GGGSQVQLVQ SGAEVKKPGS
 15 SVKVSCKASG YPFTRYVMHW VRQAPGQCLE WMGYINPYND GVNYNEKFKG RVTITADEST
 STAYMELSSL RSED TAVYYC ARNWD TGLWG QGTTVTVSSG GGGSGGGGSG GGGSGGGGSD
 IQMTQSPSSL SASVGDRVTI TCKASDHIGK FLTWYQQKPG KAPKLLIYGA TSKLTGVPSR
 FSGSGSGTDF LTISSSLQPE DFATYYCQQY WSTPFTFGCG TKVEIK

20 Exemplified Second Encoded Polypeptide; IgG LC (SEQ ID NO:2)

DIQMTQSPSS LSASVGDRVT ITCRASQGIR NYLAWYQQKP GKAPKLLIYA ASTLQSGVPS
 RFSGSGSGTD FTLTISSLQP EDVATYYCQR YNRAPYTFGQ GTKVEIKRTV AAPSVFIFPP
 SDEQLKSGTA SVVCLLNIFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYLSSTLT
 LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC

25

DNA Seq. Encoding Exemplified First Polypeptide (SEQ ID NO:3)

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t caggactctactccctcagcagcgtggtgaccgtgccctccagcagcttgggcacccag
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accaaggtggaataaaa

DNA Seq. Encoding Exemplified Second Polypeptide (SEQ ID NO:4)

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35 gaagatggtgcaacttattactgtcaacgctataaccgtgccccttacacgttcggccaa
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tctgatgagcagttgaaatctggaactgcctctgttggtgcctgctgaataacttctat
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ctgagcaaagcagactacgagaaacacaaagtctacgcctgcgaagtcacccatcagggc
5 ctgagctcgcccgtcacaaagagcttcaacaggggagagtgc

Exemplified IgG Heavy Chain Variable Region 1 (SEQ ID NO:5)

EVQLVESGGG LVQPGRSLRL SCAASGFTFD DYAMHWVRQA PGKGLEWVSA
ITWNSGHIDY ADSVEGRFTI SRDNAKNSLY LQMNSLRAED TAVYYCAKVS
10 YLSTASSLDY WQGTLTVTS S

Exemplified scFv Heavy Chain Variable Region 2 (SEQ ID NO:6)

QVQLVQ SGAEVKKPGS SVKVSCKASG YPFTRYVMHW VRQAPGQCLE
WMGYINPYND GVNYNEKFKG RVTITADEST STAYMELSSL RSED TAVYYC
15 ARNWD TGLWG QGTTVTVSS

Exemplified IgG Light Chain Variable Region 1 (SEQ ID NO:7)

DIQMTQSPSS LSASVGDRVT ITCRASQGIR NYLAWYQQKPK GKAPKLLIYA ASTLQSGVPS
RFSGSGSGTD FTLTISSLQP EDVATYYCQR YNRAPYTFGQ GTKVEIK
20

Exemplified scFv Light Chain Variable Region 2 (SEQ ID NO:8)

DIQMTQSPSSL SASVGDRVTI TCKASDHIGK FLTWYQQKPG KAPKLLIYGA
TSKLTGVPSR FSGSGSGTDF TLTISLQPE DFATYYCQY WSTPFTFGCG
TKVEIK
25

Exemplified HCDR1 (SEQ ID NO:9)

AASGFTFDDYAMH

Exemplified HCDR2 (SEQ ID NO:10)

30 AITWNSGHIDYADSVEG

Exemplified HCDR3 (SEQ ID NO:11)

AKVSYLSTASSLDY

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Exemplified HCDR4 (SEQ ID NO:12)

KASGYPFTRYVMH

5 **Exemplified HCDR5 (SEQ ID NO:13)**

YINPYNDGVNYNEKFKG

Exemplified HCDR6 (SEQ ID NO:14)

ARNWDTGL

10

Exemplified LCDR1 (SEQ ID NO:15)

RASQGIRNYLA

Exemplified LCDR2 (SEQ ID NO:16)

15 YAASTLQS

Exemplified LCDR3 (SEQ ID NO:17)

QRYNRAPYT

20 **Exemplified LCDR4 (SEQ ID NO:18)**

KASDHIGKFLT

Exemplified LCDR5 (SEQ ID NO:19)

YGATSKLT

25

Exemplified LCDR6 (SEQ ID NO:20)

QQYWSTPFT

Exemplified Polypeptide Linker 1 (SEQ ID NO:21)

30 GGGGSGGGGSGGGGS

Exemplified Polypeptide Linker 2 (SEQ ID NO:22)

GGGGSGGGGSGGGGSGGGGS

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Exemplified IgG Heavy Chain (SEQ ID NO:23)

EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITWNSGHIDY
ADSVETRFTISRDNANKNSLYLQMNSLRAEDTAVYYCAKVSYLSTASSLDYWGQGTLLTVS
5 SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS
SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLG
GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRD
ELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSR
10 WQQGNVFSCSVMHEALHNHYTQKSLSLSP

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WE CLAIM:

1. A bispecific antibody comprising an immunoglobulin G antibody (IgG) that binds tumor necrosis factor alpha (TNF α) conjugated to two single chain variable fragments (scFv) that bind the p19 subunit of IL-23 (IL23p19) wherein,
 - 5 a.) said IgG comprises two heavy chains (HC) and two light chains (LC), each HC comprises a heavy chain variable region (HCVR1) comprising heavy chain CDRs (HCDR) 1-3 and each LC comprises a light chain variable region (LCVR1) comprising light chain CDRs (LCDR) 1-3, wherein the amino acid sequence of HCDR1 is SEQ ID NO:9, the amino acid sequence of HCDR2 is SEQ ID NO:10,
10 the amino acid sequence of HCDR3 is SEQ ID NO:11, the amino acid sequence of LCDR1 is SEQ ID NO:15, the amino acid sequence of LCDR2 is SEQ ID NO:16, and the amino acid sequence of LCDR3 is SEQ ID NO:17; and
 - b.) each scFv comprises a heavy chain variable region (HCVR2) and a light chain variable region (LCVR2), the HCVR2 comprising HCDRs 4-6 and the LCVR2 comprising LCDRs 4-6, wherein the amino acid sequence of HCDR4 is SEQ ID
15 NO:12, the amino acid sequence of HCDR5 is SEQ ID NO:13, the amino acid sequence of HCDR6 is SEQ ID NO:14, the amino acid sequence of LCDR4 is SEQ ID NO:18, the amino acid sequence of LCDR5 is SEQ ID NO:19, and the amino acid sequence of LCDR6 is SEQ ID NO:20,
20 wherein each scFv is independently conjugated to said IgG antibody via a polypeptide linker (L1) covalently attached to the C-terminus of each IgG HC and the N-terminus of HCVR2 of each scFv, and the HCVR2 of each scFv is covalently attached to the LCVR2 of the same scFv via a second polypeptide linker (L2) covalently attached to the C-terminus of the HCVR2 and the N-terminus of the LCVR2 of the same scFv.
25
2. The bispecific antibody of Claim 1, wherein the amino acid sequence of HCVR1 of each HC is SEQ ID NO:5, the amino acid sequence of LCVR1 of each LC is SEQ ID NO:7, the amino acid sequence of HCVR2 of each scFv is SEQ ID NO:6, and the amino acid sequence of LCVR2 of each scFv is SEQ ID NO:8.
30
3. The bispecific antibody of any of Claims 1-2, wherein the amino acid sequence of each HC is SEQ ID NO:23, the amino acid sequence of each LC is SEQ ID NO:2, the

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amino acid sequence of HCVR2 of each scFv is SEQ ID NO:6, the amino acid sequence of LCVR2 of each scFv is SEQ ID NO:8, the amino acid sequence of L1 is SEQ ID NO:21, and the amino acid sequence of L2 is SEQ ID NO:22.

- 5 4. A DNA molecule comprising a polynucleotide sequence encoding a polypeptide chain comprising one HC, one scFv, one polypeptide linker and one second polypeptide linker of the bispecific antibody of any of Claims 1-3.
- 10 5. The DNA molecule of Claim 4, wherein the amino acid sequence of the encoded polypeptide chain is SEQ ID NO:1.
- 15 6. A DNA molecule comprising a polynucleotide sequence encoding a polypeptide chain comprising one LC of the bispecific antibody of any of Claims 1-3.
- 20 7. The DNA molecule of Claim 6, wherein the amino acid sequence of the encoded polypeptide chain is SEQ ID NO:2.
- 25 8. A DNA molecule comprising:
 - (i) a polynucleotide sequence encoding a polypeptide chain comprising one HC, one scFv, one polypeptide linker and one second polypeptide linker; and
 - (ii) a polynucleotide sequence encoding a polypeptide chain comprising one LC, of the bispecific antibody of any of Claims 1-3.
- 30 9. The DNA molecule of Claim 8, wherein the amino acid sequence of the encoded polypeptide chain comprising one HC, one scFv, one polypeptide linker and one second polypeptide linker is SEQ ID NO:1, and the amino acid sequence of the encoded polypeptide chain comprising one LC is SEQ ID NO:2.
- 35 10. A mammalian cell comprising the DNA molecule of any of Claims 4-9.
- 40 11. A mammalian cell of Claim 10, wherein the cell is capable of expressing the bispecific antibody of any of Claims 1-3.

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12. A process for producing the bispecific antibody of any of Claims 1-3, comprising:
a.) cultivating the mammalian cell of Claim 10 under conditions such that the
bispecific antibody is expressed; and
5 b.) recovering the expressed bispecific antibody.
13. A bispecific antibody produced by the process of Claim 12.
14. A method of treating ulcerative colitis or rheumatoid arthritis comprising
10 administering to a patient in need thereof a therapeutically effective amount of a
bispecific antibody of any of Claims 1-3.
15. A pharmaceutical composition comprising a bispecific antibody of any of Claims 1-3
and one or more pharmaceutically acceptable carriers, diluents, or excipients.
- 15 16. Use of the bispecific antibody of any of Claims 1-3 in the manufacture of a
medicament for the treatment of ulcerative colitis.
17. Use of the bispecific antibody of any of Claims 1-3 in the manufacture of a
20 medicament for the treatment of rheumatoid arthritis.
18. The bispecific antibody of any of Claims 1-3 for use in therapy.
19. The bispecific antibody of any of Claims 1-3 for use in the treatment of ulcerative
25 colitis.
20. The bispecific antibody of any of Claims 1-3 for use in the treatment of rheumatoid
arthritis.

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2015/058719

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07K16/24 C07K16/28 A61K39/395 A61K39/00
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 G01N A61K

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)

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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INTERNATIONAL SEARCH REPORT

International application No
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