Bispecific antibodies against human TWEAK and human IL17 and uses thereof

The present invention relates to Bispecific antibodies against human TWEAK and human IL17 (bispecific TWEAK-IL17 antibodies), methods for their production, pharmaceutical compositions containing said antibodies, and uses thereof.

Background of the Invention

Human TWEAK (UniProtKB O43508, TNF-related weak inducer of apoptosis; SEQ ID NO: 68) is a cell surface associated type II transmembrane protein. TWEAK is described in Chicheportiche, Y., et al., J. Biol. Chem. 272 (1997) 32401-32410; Marsters, S.A., et al., Curr. Biol. 8 (1998) 525-528; Lynch, C.N., et al., J. Biol. Chem. 274 (1999) 8455-8459. The active form of TWEAK is a soluble homotrimer. Human and murine TWEAK show 93 % sequence identity in receptor binding domain. The TWEAK receptor Fn14 (fibroblast growth factor inducible 14 kDa protein) is a 129 amino acid (aa) type I transmembane protein consisting of one single cystein rich domain in ligand binding domain (SEQ ID NO: 98). Signaling of TWEAK occurs via NF-KB pathway activation. TWEAK mRNA is expressed in a variety of tissues and found in most major organs like heart, brain, skeletal muscle, and pancreas, tissues related to the immune system like spleen, lymph nodes, and thymus. Fn14 mRNA has been detected in heart, brain, lung, placenta, vascular EC and smooth muscle cells. TWEAK-null and Fnl4-null knockout mice are viable, healthy and fertile and have more natural killer cells and display an enhanced innate inflammatory response. TWEAK is involved in apoptosis, proliferation, angiogenesis, ischemic penumbra, cerebral edema, multiple sclerosis.

Anti-TWEAK antibodies are mentioned in WO 1998/005783, WO 2000/042073, WO 2003/086311, WO 2006/130429, WO 2006/130374, WO 2006/122187, WO 2006/089095, WO 2006/088890, WO 2006/052926.

Human IL-17A (CTLA-8, Swiss Prot Q16552, further named as IL-17 or IL17; SEQ ID NO: 70)) is a pro-inflammatory cytokine produced by a subset of memory T cells (called Th17) that has been implicated in the pathogenesis of MS. IL-17A plays a role in the induction of other inflammatory cytokines, chemokines and adhesion molecules. Treatment of animals with IL-17A neutralizing antibodies decreases disease incidence and severity in autoimmune encephalomyelitis

(Komiyama, Y. et al., J. Immunol. 177 (2006) 566-573). IL-17A is over-expressed in the cerebrospinal fluid of MS patients (Hellings, P.W. et al., Am. J. Resp. Cell Mol. Biol. 28 (2003) 42-50; Matusevicius, D. et al., Multiple Sclerosis 5 (1999) 101-104; WO 2005/051422). In addition, IL-17A neutralizing antibodies reduce severity and incidence of mouse RA model of collagen induced arthritis, and high levels of IL-17A can be detected in the synovial fluid of inflamed joints from RA patients (Ziolkowska, M,. et al., J. Immunol. 164 (2000) 2832-2838; Kotake, S., et al., J. Clin. Invest. 103 (1999) 1345-1352; Hellings, P.W., et al., Am. J. Resp. Cell Mol. Biol. 28 (2003) 42-50).

WO 96/17939, US 5,716,623; WO 95/18826; WO 97/15320; WO 99/35276 and WO 00/69436 WO 95/18826 US 6,274,711, US 6,274,711, WO 97/15320, US 6,063,372, WO 2006/013107 and WO 2008/02115 relate to IL-17A and antibodies against IL-17A. WO 2010/102251 relates IL17 binding proteins.

Bispecific antibodies

A wide variety of recombinant antibody formats have been developed in the recent past, e.g. tetravalent bispecific antibodies by fusion of, e.g., an IgG antibody format and single chain domains (see e.g. Coloma, M.J., et al., Nature Biotech 15 (1997) 159-163; WO 2001/077342; and Morrison, S.L., Nature Biotech 25 (2007) 1233-1234).

Also several other new formats wherein the antibody core structure (IgA, IgD, IgE, IgG or IgM) is no longer retained such as dia-, tria- or tetrabodies, minibodies, several single chain formats (scFv, Bis-scFv), which are capable of binding two or more antigens, have been developed (Holliger, P., et al., Nature Biotech 23 (2005) 1126-1136; Fischer, N., Léger, O., Pathobiology 74 (2007) 3-14; Shen, J., et al., Journal of Immunological Methods 318 (2007) 65-74; Wu, C., et al., Nature Biotech. 25 (2007) 1290-1297).

All such formats use linkers either to fuse the antibody core (IgA, IgD, IgE, IgG or IgM) to a further binding protein (e.g. scFv) or to fuse e.g. two Fab fragments or scFvs (Fischer, N., Léger, O., Pathobiology 74 (2007) 3-14). It has to be kept in mind that one may want to retain effector functions, such as e.g. complement-dependent cytotoxicity (CDC) or antibody dependent cellular cytotoxicity (ADCC), which are mediated through the Fc receptor binding, by maintaining a high degree of similarity to naturally occurring antibodies.

In WO 2007/024715 are reported dual variable domain immunoglobulins as engineered multivalent and multispecific binding proteins. A process for the preparation of biologically active antibody dimers is reported in US 6,897,044. Multivalent FV antibody construct having at least four variable domains which are linked with each over via peptide linkers are reported in US 7,129,330. Dimeric and multimeric antigen binding structures are reported in US 2005/0079170. Tri- or tetra-valent monospecific antigen-binding protein comprising three or four Fab fragments bound to each other covalently by a connecting structure, which protein is not a natural immunoglobulin are reported in US 6,511,663. In WO 2006/020258 tetravalent bispecific antibodies are reported that can be efficiently expressed in prokaryotic and eukaryotic cells, and are useful in therapeutic and diagnostic methods. A method of separating or preferentially synthesizing dimers which are linked via at least one interchain disulfide linkage from dimers which are not linked via at least one interchain disulfide linkage from a mixture comprising the two types of polypeptide dimers is reported in US 2005/0163782. Bispecific tetravalent receptors are reported in US 5,959,083. Engineered antibodies with three or more functional antigen binding sites are reported in WO 2001/077342.

Multispecific and multivalent antigen-binding polypeptides are reported in WO 1997/001580. WO 1992/004053 reports homoconjugates, typically prepared from monoclonal antibodies of the IgG class which bind to the same antigenic determinant are covalently linked by synthetic cross-linking. Oligomeric monoclonal antibodies with high avidity for antigen are reported in WO 1991/06305 whereby the oligomers, typically of the IgG class, are secreted having two or more immunoglobulin monomers associated together to form tetravalent or hexavalent IgG molecules. Sheep-derived antibodies and engineered antibody constructs are reported in US 6,350,860, which can be used to treat diseases wherein interferon gamma activity is pathogenic. In US 2005/0100543 are reported targetable constructs that are multivalent carriers of bi-specific antibodies, i.e., each molecule of a targetable construct can serve as a carrier of two or more bi-specific antibodies. Genetically engineered bispecific tetravalent antibodies are reported in WO 1995/009917. In WO 2007/109254 stabilized binding molecules that consist of or comprise a stabilized scFv are reported.

WO 2008/106131 relates to bispecific antibodies against IL23 and IL17 or TNF. WO 2007/027761 relates to bispecific antibodies against IL23 and IL17. WO 2010/003108 relates TNFalpha antagonist multitarget binding proteins.

Summary of the Invention

One aspect of the invention is a bispecific antibody comprising a first antigen-binding site that specifically binds to human TWEAK and a second antigen-binding site that specifically binds to human IL17.

In one embodiment of the invention the bispecific antibody inhibits

a) TWEAK induced proliferation of human fibroblast-like synoviocytes-rheumatoid arthritis (HFLS-RA) with an IC50 value of 0.2 nM or lower; and

b) IL17 induced IL6 cytokine stimulation of human fibroblast-like synoviocytes-rheumatoid arthritis (HFLS-RA) with an IC50 value of 3.0 nM or lower; and

c) IL17 induced IL8 cytokine stimulation of human fibroblast-like synoviocytes-rheumatoid arthritis (HFLS-RA) with an IC50 value of 2.0 nM or lower.

In one embodiment of the invention the bispecific antibody comprising a first antigen-binding site that specifically binds to human TWEAK and a second antigen-binding site that specifically binds to human IL17 is a bivalent, bispecific antibody.

In one embodiment of the invention the bispecific antibody comprising a first antigen-binding site that specifically binds to human TWEAK and a second antigen-binding site that specifically binds to human IL17 is characterized in that

i) said first antigen-binding site comprises

a) CDR1H of SEQ ID NO:17, CDR2H of SEQ ID NO:18, CDR3H of SEQ ID NO:19, and CDR1L of SEQ ID NO:20, CDR2L of SEQ ID NO:21, CDR3L of SEQ ID NO:22; or

b) CDR1H of SEQ ID NO:1, CDR2H of SEQ ID NO:2, CDR3H of SEQ ID NO:3, and CDR1L of SEQ ID NO:4, CDR2L of SEQ ID NO:5, CDR3L of SEQ ID NO:6; or

c) CDR1H of SEQ ID NO:9, CDR2H of SEQ ID NO:10, CDR3H of SEQ ID NO:11, and CDR1L of SEQ ID NO:12, CDR2L of SEQ ID NO:13, CDR3L of SEQ ID NO:14; and

ii) said second antigen-binding site comprises

CDR1H of SEQ ID NO:47, CDR2H of SEQ ID NO:48, CDR3H of SEQ ID NO:49, and CDR1L of SEQ ID NO:50, CDR2L of SEQ ID NO:51, CDR3L of SEQ ID NO:52.

In one embodiment of the invention is a chimeric or humanized variant of the such bispecific antibody.

In one embodiment of the invention the bispecific antibody comprising a first antigen-binding site that specifically binds to human TWEAK and a second antigen-binding site that specifically binds to human IL17 is characterized in that

i) said first antigen-binding site comprises

a variable heavy chain domain (VH) of SEQ ID NO:25, of SEQ ID NO:26, of SEQ ID NO:27, of SEQ ID NO:28, of SEQ ID NO:29, of SEQ ID NO:30, of SEQ ID NO:31, of SEQ ID NO:32, of SEQ ID NO:33, of SEQ ID NO:34, or of SEQ ID NO:35, and a variable light chain domain of SEQ ID NO:26, of SEQ ID NO:37, of SEQ ID NO:38, of SEQ ID NO:39, of SEQ ID NO:40, of SEQ ID NO:41, of SEQ ID NO:42, of SEQ ID NO:43, of SEQ ID NO:44, of SEQ ID NO:45, or of SEQ ID NO:46; and

ii) said second antigen-binding site comprises

a variable heavy chain domain (VH) of SEQ ID NO:55, or of SEQ ID NO:56, and a variable light chain domain of SEQ ID NO:57, or of SEQ ID NO:58.

In one embodiment of the invention the bispecific antibody comprising a first antigen-binding site that specifically binds to human TWEAK and a second antigen-binding site that specifically binds to human IL17 is characterized in that

i) said first antigen-binding site comprises

a variable heavy chain domain (VH) of SEQ ID NO:28, and a variable light chain domain of SEQ ID NO:37; and

ii) said second antigen-binding site comprises

a) a variable heavy chain domain (VH) of SEQ ID NO:56, and a variable light chain domain of SEQ ID NO:58; or

b) a variable heavy chain domain (VH) of SEQ ID NO:55, and a variable light chain domain of SEQ ID NO:57.

In one embodiment of the invention the bispecific antibody comprising a first antigen-binding site that specifically binds to human TWEAK and a second antigen-binding site that specifically binds to human IL17 is characterized in that

i) said first antigen-binding site comprises

a variable heavy chain domain (VH) of SEQ ID NO:28, and a variable light chain domain of SEQ ID NO:37; and

ii) said second antigen-binding site comprises

a variable heavy chain domain (VH) of SEQ ID NO:56, and a variable light chain domain of SEQ ID NO:58.

In one embodiment of the invention the bispecific antibody comprising a first antigen-binding site that specifically binds to human TWEAK and a second antigen-binding site that specifically binds to human IL17 is characterized in that

i) said first antigen-binding site comprises

a variable heavy chain domain (VH) of SEQ ID NO:28, and a variable light chain domain of SEQ ID NO:37; and

ii) said second antigen-binding site comprises

a variable heavy chain domain (VH) of SEQ ID NO:55, and a variable light chain domain of SEQ ID NO:57.

In one embodiment the bispecific antibody which binds to TWEAK and IL17 and being characterized by the above mentioned amino acid sequences and amino acid

sequence fragments is of human IgG1 isotype, in one embodiment with mutations L234A and L235A, in one embodiment with mutations L234A, L235A and P329G.

In one embodiment the bispecific antibody which binds to TWEAK and IL17 and being characterized by the above mentioned amino acid sequences and amino acid sequence fragments is of human IgG4 isotype, in one embodiment with mutations S228P and L235E, in one embodiment with mutations S228P, L235E and P329G.

A further embodiment of the invention is a pharmaceutical composition comprising a bispecific antibody according to the invention.

A further embodiment of the invention is the use of a bispecific antibody according to the invention for the manufacture of a pharmaceutical composition.

A further embodiment of the invention is a nucleic acid encoding a bispecific antibody according to the invention.

A further embodiment of the invention is a nucleic acid encoding a heavy chain variable domain and/or a light chain variable domain of a bispecific antibody according to the invention.

The invention further provides expression vectors containing nucleic acid according to the invention capable of expressing said nucleic acid in a prokaryotic or eukaryotic host cell, and host cells containing such vectors for the recombinant production of such an antibody.

The invention further comprises a prokaryotic or eukaryotic host cell comprising a vector according to the invention.

The invention further comprises a method for the production of a recombinant chimeric, human or humanized antibody according to the invention, characterized by expressing a nucleic acid according to the invention in a prokaryotic or eukaryotic host cell and recovering said antibody from said cell or the cell culture supernatant. The invention further comprises the antibody obtainable by such a recombinant method.

Antibodies according to the invention show benefits for patients in need of a TWEAK and IL17 targeting therapy. The antibodies according to the invention have new and inventive properties causing a benefit for a patient suffering from a cancer disease, especially suffering from colon, lung, or pancreatic cancer or from

inflammatory diseases, especially from autoimmune diseases, rheumatoid arthritis, psoratic arthritis, muscle diseases, e.g. muscular dystrophy, multiple sclerosis, chronic kidney diseases, bone diseases, e.g. bone degeneration in multiple myeloma, systemic lupus erythematosus, lupus nephritis, and vascular injury.

A further embodiment of the invention is a bispecific antibody according to the invention for use in the treatment of cancer, inflammatory diseases, autoimmune diseases, rheumatoid arthritis, psoratic arthritis, muscle diseases, e.g. muscular dystrophy, multiple sclerosis, chronic kidney diseases, bone diseases, e.g. bone degeneration in multiple myeloma, systemic lupus erythematosus, lupus nephritis, and vascular injury, especially for the treatment of systemic lupus erythematosus, lupus nephritis.

A further embodiment of the invention is a bispecific antibody according to the invention for manufacture of a medicament for the treatment of cancer, inflammatory diseases, autoimmune diseases, rheumatoid arthritis, psoratic arthritis, muscle diseases, e.g. muscular dystrophy, multiple sclerosis, chronic kidney diseases, bone diseases, e.g. bone degeneration in multiple myeloma, systemic lupus erythematosus, lupus nephritis, and vascular injury, especially for the treatment of systemic lupus erythematosus, lupus nephritis.

The invention further provides a method for treating a patient suffering from cancer, especially from colon, lung, or pancreatic cancer or from inflammatory diseases, especially from autoimmune diseases, rheumatoid arthritis, psoratic arthritis, muscle diseases, e.g. muscular dystrophy, multiple sclerosis, chronic kidney diseases, bone diseases, e.g. bone degeneration in multiple myeloma, systemic lupus erythematosus, lupus nephritis, and vascular injury, comprising administering to a patient diagnosed as having such a disease (and therefore being in need of such a therapy) an effective amount of the bispecific antibody which binds to TWEAK and IL17 according to the invention. The antibody is administered preferably in a pharmaceutical composition.

The invention further comprises a pharmaceutical composition comprising an antibody according to the invention, optionally together with a buffer and/or an adjuvant useful for the formulation of antibodies for pharmaceutical purposes.

The invention further provides a pharmaceutical composition comprising an antibody according to the invention in a pharmaceutically acceptable carrier. In one

embodiment, the pharmaceutical composition may be included in an article of manufacture or kit.

The bispecific antibodies according to the invention show benefits for human patients in need of a TWEAK and IL17 targeting therapy and have valuable properties.

The antibodies according to the invention have valuable properties causing a benefit for a patient suffering from such a disease, especially suffering from inflammatory diseases such as rheumatoid arthritis or lupus nephritis. Such valuable properties include the simultaneous inhibition of both TWEAK and IL17 leading to complementary treatment benefits. There is a persistent high unmet medical need in RA as many patients fail to achieve a target of remission. Bispecific antibodies targeting both TWEAK and IL17 offer an opportunity for improved efficacy by inhibiting IL-17 and TWEAK thus providing complementary anti-inflammatory and anti-angiogenic activity and optimal impact on bone formation/resorption. TWEAK expression is significantly increased in synovial tissue of RA and PsA patients – mainly in $CD55^+$ synoviocytes and $CD168^+$ macrophages. (Van Kuijk, et al., Ann Rheum Dis 69 (2010) 301-304). IL-17A is produced in inflamed synovial tissue with lower IL-17 in TNF responders and higher IL-17 levels in TNF-non-responders (Moran, et al., Arthritis & Rheumatism 11 (2009) R113). In addition, available data supports a role for IL-17 and TWEAK in kidney inflammation with effects on mesangial cells, tubular epithelial cells, endothelial cells, elevated chemokines, cytokines, neutrophil infiltration and positive correlations of IL-17 and TWEAK with disease activity. Urinary TWEAK levels correlate with renal SLEDAI scores, TWEAK is elevated during flares and specific to renal disease (Schwartz, et al., J Autoimmunity 27 (2006) 242-25). In addition, TWEAK blockade reduces kidney inflammation in in a model of acute kidney injury (AKI) (Sanz, et al., J Amer Soc Nephrol 19 (2008) 695-703). Elevated IL-17 is detected in tissues from lupus patients and IL-17 is detected in affected kidneys from patients with SLE (Crispin, et al., J Immunol. 181 (2008) 8761–8766).

Low viscosity and high stability in terms of aggregation (>55°C) makes such antibodies suitable for high concentration formulation for a possible subcutaneous application (He, F., et al., J Pharm Sci.100 (2010) 1330-1340).

In one embodiment the bispecific antibodies according to the invention are additionally characterized by one or more of the following properties (as determined in Examples 4, 10, 11, 16, 17 and 19): the bispecific antibody

a) shows no cross reactivity with IL17B, IL17C, IL17D, IL17F (which means that the binding to IL17B, IL17C, IL17D and IL17F is 0% compared to the binding to IL17A , which is set as 100%);

b) inhibits IL17 induced IL8 cytokine stimulation of CCD-25SK cells with an IC50 value of 2.0 nM or lower (e.g. with an IC50 value between 2.0 nM and 0.0 nM);

c) inhibits IL17 induced IL6 cytokine stimulation of CCD-25SK cells with an IC50 value of 5.0 nM or lower (e.g. with an IC50 value between 5.0 nM and 0.0 nM); preferably with an IC50 value of 2.0 nM or lower;

d) human TWEAK/human Fn14 interaction with an IC50 value of 4.0 [ng/ml] or lower (e.g. with an IC50 value between 4.0 [ng/ml] and 0.0 [ng/ml]); preferably with an IC50 value of 3.0 [ng/ml] or lower;

e) binds to human TWEAK with an KD value of binding affinity of 0.1 nM or lower, and binds to human IL-17 with an KD value of binding affinity of 0.3 nM or lower; and /or

f) is capable to simultaneously bind to human <TWEAK> and human <IL17>, wherein the signal intensity (in RU) (in a surface plasmon resonance assay) of the binding of the bispecific TWEAK/IL17 antibody to a 1:1 mixture from human <TWEAK> and human <IL17> is at least the same or higher compared to the sum of a) the signal intensity (in RU) of the binding of the bispecific TWEAK/IL17 antibody to human <TWEAK> alone and b) the signal intensity (in RU) of the binding of the bispecific TWEAK/IL17 antibody to human <IL17> alone.

Description of the Figures

Figure 1a and b Two exemplary bispecific, bivalent antibody formats for the bispecific <TWEAK/IL17> antibodies according to the invention are shown.

Figure 2 One exemplary bispecific, tetravalent antibody format for the bispecific <TWEAK/IL17> antibodies according to the invention is shown.

Detailed Description of the Invention

As used herein, "antibody" refers to a binding protein that comprises antigen-binding sites. The terms “binding site” or “antigen-binding site” as used herein denotes the region(s) of an antibody molecule to which a ligand (i. e. the antigen) actually binds. The term “antigen-binding site” include antibody heavy chain variable domains (VH) and/or an antibody light chain variable domains (VL), or pairs of VH/VL, and can be derived from whole antibodies or antibody fragments such as single chain Fv, a VH domain and/or a VL domain, Fab, or (Fab)2. In one embodiment of the current invention each of the antigen-binding sites comprises an antibody heavy chain variable domain (VH) and/or an antibody light chain variable domain (VL), and preferably is formed by a pair consisting of an antibody light chain variable domain (VL) and an antibody heavy chain variable domain (VH). The antibody according to the invention is preferably a humanized antibody, chimeric antibody, or further genetically engineered antibody as long as the characteristic properties according to the invention are retained.

The antigen-binding site, and especially heavy chain variable domains (VH) and/or antibody light chain variable domains (VL), that specifically bind to human TWEAK can be derived a) from known anti-TWEAK antibodies as described in e.g. WO 1998/005783, WO 2000/042073, WO 2003/086311, WO 2006/130429, WO 2006/130374, WO 2006/122187, WO 2006/089095, WO 2006/088890, WO 2006/052926, WO 2010/115555 or PCT Application No. PCT/EP2011/067070; or b) from new anti-TWEAK antibodies obtained e.g. by de novo immunization methods using inter alia either the human TWEAK protein or nucleic acid or fragments thereof or by phage display methods.

The antigen-binding site, and especially heavy chain variable domains (VH) and/or antibody light chain variable domains (VL), that specifically bind to human IL17 can be derived a) from known anti- IL17 antibodies as described in e.g. WO 96/17939, US 5,716,623; WO 95/18826; WO 97/15320; WO 99/35276, WO 00/69436, WO 95/18826, US 6,274,711, US 6,063,372, WO 2006/013107, WO 2008/02115, WO 2010/102251 or WO 2010/034443; or b) from new anti-IL17 antibodies obtained e.g. by de novo immunization methods using inter alia either the human IL17 protein or nucleic acid or fragments thereof or by phage display methods.

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Human IL-17A (CTLA-8, Swiss Prot Q16552, further named as IL-17. IL17; SEQ ID NO: 70)) is a pro-inflammatory cytokine produced by a subset of memory T cells (called Th17) that has been implicated in the pathogenesis of MS. IL-17A plays a role in the induction of other inflammatory cytokines, chemokines and adhesion molecules. Treatment of animals with IL-17A neutralizing antibodies decreases disease incidence and severity in autoimmune encephalomyelitis (Komiyama, Y. et al., J. Immunol. 177 (2006) 566-573). IL-17A is over-expressed in the cerebrospinal fluid of MS patients (Hellings, P.W. et al., Am. J. Resp. Cell Mol. Biol. 28 (2003) 42-50; Matusevicius, D. et al., Multiple Sclerosis 5 (1999) 101-104; WO 2005/051422). In addition, IL-17A neutralizing antibodies reduce severity and incidence of mouse RA model of collagen induced arthritis, and high levels of IL-17A can be detected in the synovial fluid of inflamed joints from RA patients (Ziolkowska, M. et al., J. Immunol. 164 (2000) 2832-2838; Kotake, S., et al., J. Clin. Invest. 103 (1999) 1345-1352; Hellings, P.W. et al., Am. J. Resp. Cell Mol. Biol. 28 (2003) 42-50).

Antibody specificity refers to selective recognition of the antibody for a particular epitope of an antigen. Natural antibodies, for example, are monospecific.

"Bispecific antibodies" according to the invention are antibodies which have two different antigen-binding specificities. Where an antibody has more than one specificity, the recognized epitopes may be associated with a single antigen or with more than one antigen. Antibodies of the present invention are specific for two different antigens, i.e. TWEAK as first antigen and IL17 as second antigen.

The term "monospecific" antibody as used herein denotes an antibody that has one or more binding sites each of which bind to the same epitope of the same antigen.

The term "valent" as used within the current application denotes the presence of a specified number of binding sites in an antibody molecule. As such, the terms "bivalent", "tetravalent", and "hexavalent" denote the presence of two binding site, four binding sites, and six binding sites, respectively, in an antibody molecule. The bispecific antibodies according to the invention are at least "bivalent" and may be "trivalent" or "multivalent" (e.g. "tetravalent" or "hexavalent"). In one embodiment the bispecific antibody according to the invention is bivalent, trivalent or tetravalent. In one embodiment said bispecific antibody is bivalent. In one embodiment said bispecific antibody is trivalent. In one embodiment said bispecific antibody is tetravalent.

Antibodies of the present invention have two or more binding sites and are bispecific. That is, the antibodies may be bispecific even in cases where there are more than two binding sites (i.e. that the antibody is trivalent or multivalent). Bispecific antibodies of the invention include, for example, multivalent single chain antibodies, diabodies and triabodies, as well as antibodies having the constant domain structure of full length antibodies to which further antigen-binding sites (e.g., single chain Fv, a VH domain and/or a VL domain, Fab, or (Fab)2,) are linked via one or more peptide-linkers. The antibodies can be full length from a single species, or be chimerized or humanized. For an antibody with more than two antigen binding sites, some binding sites may be identical, so long as the protein has binding sites for two different antigens. That is, whereas a first binding site is specific for a TWEAK, a second binding site is specific for IL17, and vice versa.

The terms "monoclonal antibody" or "monoclonal antibody composition" as used herein refer to a preparation of antibody molecules of a single amino acid composition.

The term "humanized antibody" refers to antibodies in which the framework and/or "complementary determining regions" (CDR) have been modified to comprise the

CDR of an immunoglobulin of different species as compared to that of the parent immunoglobulin. In a preferred embodiment, a non-human (e.g. mouse, rabbit or hamster) CDR is grafted into the framework region of a human antibody to prepare the "humanized antibody". See, e.g., Riechmann, L., et al., Nature 332 (1988) 323-327; and Neuberger, M.S., et al., Nature 314 (1985) 268-270.

The term "chimeric antibody" refers to a monoclonal antibody comprising a variable region, i.e., binding region, from mouse and at least a portion of a constant region derived from a different source or species, usually prepared by recombinant DNA techniques. Chimeric antibodies comprising for example a mouse variable region and a human constant region. Such mouse/human chimeric antibodies are the product of expressed immunoglobulin genes comprising DNA segments encoding rat immunoglobulin variable regions and DNA segments encoding human immunoglobulin constant regions. Other forms of "chimeric antibodies" encompassed by the present invention are those in which the class or subclass has been modified or changed from that of the original antibody. Such "chimeric" antibodies are also referred to as "class-switched antibodies." Methods for producing chimeric antibodies involve conventional recombinant DNA and gene transfection techniques now well known in the art. See, e.g., Morrison, S.L., et al., Proc. Natl. Acad Sci. USA 81 (1984) 6851-6855; US Patent Nos. 5,202,238 and 5,204,244.

As used herein, the terms, "binds to", "binding" or "specifically binding" refers to the binding of the bispecific antibody to an epitope of the antigen (either human TWEAK or human IL17) with sufficient affinity such that the antibody is useful as a therapeutic agent in targeting human TWEAK and/or human IL17 according to the invention. The binding of the bispecific antibody to an epitope of the antigen (either human TWEAK or human IL17) can be measured in an in vitro assay, preferably in an plasmon resonance assay (e.g. BIAcore, GE-Healthcare Uppsala, Sweden) with purified wild-type human antigen (preferably with IL17A homodimer for the human IL17 antigen) (see e.g. Example 19). The affinity of the binding is defined by the terms ka (rate constant for the association of the antibody from the antibody/antigen complex), kd (dissociation constant), and KD (kd/ka). A bispecific antibody comprising a first antigen-binding site that specifically binds to human TWEAK and a second antigen-binding site that specifically binds to human IL17 refers to bispecific antibody with a first antigen-binding site which specifically binds to human TWEAK with a binding affinity (KD) of 1.0×10^{-8} M or less, e.g. from 1.0×10^{-8} M to 1.0×10^{-13} M (in one embodiment from 1.0×10^{-9}

M to 1.0 x 10^{-13} M), and with a second antigen-binding site which specifically binds to human IL17 with a binding affinity (KD) of 1.0 x 10^{-8} M or less, e.g. from 1.0 x 10^{-8} M to 1.0 x 10^{-13} M (in one embodiment from 1.0 x 10^{-9} M to 1.0 x 10^{-13} M).

The term "epitope" denotes a protein determinant capable of specifically binding to an antibody. Epitopes usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually epitopes have specific three dimensional structural characteristics, as well as specific charge characteristics. Conformational and nonconformational epitopes are distinguished in that the binding to the former but not the latter is lost in the presence of denaturing solvents.

One embodiment of the invention is a bispecific antibody comprising a first antigen-binding site that specifically binds to human TWEAK and a second antigen-binding site that specifically binds to human IL17, characterized in that

a) the first antigen-binding site binds to the same epitope on human TWEAK as an antibody which comprises a CDR1H of SEQ ID NO:17, CDR2H of SEQ ID NO:18, CDR3H of SEQ ID NO:19, and CDR1L of SEQ ID NO:20, CDR2L of SEQ ID NO:21, CDR3L of SEQ ID NO:22; and

b) the second antigen-binding site binds to the same epitope on human IL17 as an antibody which comprises a CDR1H of SEQ ID NO:47, CDR2H of SEQ ID NO:48, CDR3H of SEQ ID NO:49, and CDR1L of SEQ ID NO:50, CDR2L of SEQ ID NO:51, CDR3L of SEQ ID NO:52.

One embodiment of the invention is a bispecific antibody comprising a first antigen-binding site that specifically binds to human TWEAK and a second antigen-binding site that specifically binds to human IL17, characterized in that

a) the first antigen-binding site binds to the same epitope on human TWEAK as an antibody which comprises a variable heavy chain domain (VH) of SEQ ID NO:28, and a variable light chain domain of SEQ ID NO:37; and

b) the second antigen-binding site binds to the same epitope on human IL17 as an antibody which comprises a variable heavy chain domain (VH) of SEQ ID NO:56, and a variable light chain domain of SEQ ID NO:58.

One embodiment of the invention is a bispecific antibody comprising a first antigen-binding site that specifically binds to human TWEAK and a second antigen-binding site that specifically binds to human IL17, characterized in that

a) the first antigen-binding site competes for binding to the same epitope on human TWEAK as an antibody which comprises a CDR1H of SEQ ID NO:17, CDR2H of SEQ ID NO:18, CDR3H of SEQ ID NO:19, and CDR1L of SEQ ID NO:20, CDR2L of SEQ ID NO:21, CDR3L of SEQ ID NO:22; and

b) the second antigen-binding site competes for binding to the same epitope on human IL17 as an antibody which comprises a CDR1H of SEQ ID NO:47, CDR2H of SEQ ID NO:48, CDR3H of SEQ ID NO:49, and CDR1L of SEQ ID NO:50, CDR2L of SEQ ID NO:51, CDR3L of SEQ ID NO:52.

One embodiment of the invention is a bispecific antibody comprising a first antigen-binding site that specifically binds to human TWEAK and a second antigen-binding site that specifically binds to human IL17, characterized in that

a) the first antigen-binding site competes for binding to the same epitope on human TWEAK as an antibody which comprises a variable heavy chain domain (VH) of SEQ ID NO:28, and a variable light chain domain of SEQ ID NO:37; and

b) the second antigen-binding site competes for binding to the same epitope on human IL17 as an antibody which comprises a variable heavy chain domain (VH) of SEQ ID NO:56, and a variable light chain domain of SEQ ID NO:58.

Antibodies which compete for binding to the same epitope (and thus are likely to bind to the same epitope) cane identified by Surface Plasmon Resonance competition assay as described e.g. in Example 7.

The "variable domain" (variable domain of a light chain (V_L), variable domain of a heavy chain (V_H)) as used herein denotes each of the pair of light and heavy chain domains which are involved directly in binding the antibody to the antigen. The variable light and heavy chain domains have the same general structure and each domain comprises four framework (FR) regions whose sequences are widely conserved, connected by three "hypervariable regions" (or complementary determining regions, CDRs). The framework regions adopt a β-sheet conformation and the CDRs may form loops connecting the β-sheet structure. The CDRs in each

chain are held in their three-dimensional structure by the framework regions and form together with the CDRs from the other chain the antigen binding site. The antibody's heavy and light chain CDR3 regions play a particularly important role in the binding specificity/affinity of the antibodies according to the invention and therefore provide a further object of the invention.

The term "antigen-binding portion of an antibody" when used herein refers to the amino acid residues of an antibody which are responsible for antigen-binding. The antigen-binding portion of an antibody comprises amino acid residues from the "complementary determining regions" or "CDRs". "Framework" or "FR" regions are those variable domain regions other than the hypervariable region residues as herein defined. Therefore, the light and heavy chain variable domains of an antibody comprise from N- to C-terminus the domains FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4. Especially, CDR3 of the heavy chain is the region which contributes most to antigen binding and defines the antibody's properties. CDR and FR regions are determined according to the standard definition of Kabat et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991) and/or those residues from a "hypervariable loop".

The term"CDR1H" denotes the CDR1 region of the heavy chain variable region calculated according to Kabat (Kabat et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991)). CDR2L, CDR3H, etc. mean the respective regions from the heavy(H) or light(L) chain. For example, an antigen binding site characterized by comprising CDR1H of SEQ ID NO:3 means that the antigen binding site comprises this amino acid sequence as a heavy chain variable chain CDR1 region in its variable heavy chain. For example, an antigen binding site characterized by comprising CDR1H of SEQ ID NO:1, CDR2H of SEQ ID NO:2, CDR3H of SEQ ID NO:3 means that the antigen binding sites comprises in its heavy chain as sequence of CDR1 SEQ ID NO:1, as sequence of CDR2 SEQ ID NO:2, and as sequence of CDR3 SEQ ID NO:3.

The terms "nucleic acid" or "nucleic acid molecule" as used herein are intended to include DNA molecules and RNA molecules. A nucleic acid molecule may be single-stranded or double-stranded, but preferably is double-stranded DNA.

The term "amino acid" as used within this application denotes the group of naturally occurring carboxy α-amino acids comprising alanine (three letter code: ala, one letter code: A), arginine (arg, R), asparagine (asn, N), aspartic acid (asp, D), cysteine (cys, C), glutamine (gln, Q), glutamic acid (glu, E), glycine (gly, G), histidine (his, H), isoleucine (ile, I), leucine (leu, L), lysine (lys, K), methionine (met, M), phenylalanine (phe, F), proline (pro, P), serine (ser, S), threonine (thr, T), tryptophan (trp, W), tyrosine (tyr, Y), and valine (val, V).

A nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are colinear, and, in the case of a secretory leader, contiguous and in reading frame. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

As used herein, the expressions "cell", "cell line", and "cell culture" are used interchangeably and all such designations include progeny. Thus, the words "transformants" and "transformed cells" include the primary subject cell and cultures derived therefrom without regard for the number of transfers. It is also understood that all progeny may not be precisely identical in DNA content, due to deliberate or inadvertent mutations. Variant progeny that have the same function or biological activity as screened for in the originally transformed cell are included.

The "Fc part" of an antibody is not involved directly in binding of an antibody to an antigen, but exhibits various effector functions. An "Fc part of an antibody" is a term well known to the skilled artisan and defined on the basis of papain cleavage of antibodies. Depending on the amino acid sequence of the constant region of their heavy chains, antibodies or immunoglobulins are divided into the classes: IgA, IgD, IgE, IgG and IgM, and several of these may be further divided into subclasses (isotypes; the expressions "isotype" or "subclass" are used interchangeable herein), e.g. IgG1, IgG2, IgG3, and IgG4, IgA1, and IgA2. According to the heavy chain constant regions the different classes of immunoglobulins are called α, δ, ε, γ, and

μ, respectively. The Fc part of an antibody is directly involved in ADCC (antibody-dependent cell-mediated cytotoxicity) and CDC (complement-dependent cytotoxicity) based on complement activation, C1q binding and Fc receptor binding. Complement activation (CDC) is initiated by binding of complement factor C1q to the Fc part of most IgG antibody subclasses. While the influence of an antibody on the complement system is dependent on certain conditions, binding to C1q is caused by defined binding sites in the Fc part. Such binding sites are known in the state of the art and described, e.g., by Boackle, R.J., et al., Nature 282 (1979) 742-743; Lukas, T.J., et al., J. Immunol. 127 (1981) 2555-2560; Brunhouse, R. and Cebra, J.J., Mol. Immunol. 16 (1979) 907-917; Burton, D.R., et al., Nature 288 (1980) 338-344; Thommesen, J.E., et al., Mol. Immunol. 37 (2000) 995-1004; Idusogie, E.E., et al., J. Immunol. 164 (2000) 4178-4184; Hezareh, M. et al., J. Virology 75 (2001) 12161-12168; Morgan, A., et al., Immunology 86 (1995) 319-324; EP 0 307 434. Such binding sites are, e.g., L234, L235, D270, N297, E318, K320, K322, P331, and P329 (numbering according to EU index of Kabat used for the numbering of the constant domains, Kabat, et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991)).

Antibodies of subclass IgG1, IgG2 and IgG3 usually show complement activation and C1q and C3 binding, whereas IgG4 does not activate the complement system and does not bind C1q and C3.

In one embodiment the antibody according to the invention is characterized in that the constant chains are of human origin. Such constant chains are well known in the state of the art and e.g. described by Kabat (see e.g. Johnson, G. and Wu, T.T., Nucleic Acids Res. 28 (2000) 214-218 and Kabat et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991)). For example, useful human heavy chain constant regions comprises an amino acid sequence SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63 or SEQ ID NO: 64 (human IgG1 subclass allotypes (Caucasian and Afroamerican or mutants L234A/L235A, and L234A/L235A/P329G), SEQ ID NO: 65, SEQ ID NO: 66, or SEQ ID NO: 67 (human IgG4 subclass or mutants L234A/L235A, and L234A/L235A/P329G). For example, a useful human light chain constant region comprises an amino acid sequence of a kappa-light chain constant region of SEQ ID NO:59 or an amino acid sequence of a lambda-light chain constant region of SEQ ID NO:60. In one embodiment the antibody according to the invention comprises a Fc part derived from human origin and

preferably all other parts of the human constant regions. As used herein the term "Fc part derived from human origin" denotes a Fc part which is either a Fc part of a human antibody of the subclass IgG1, IgG2, IgG3 or IgG4, in one embodiment a Fc part from human IgG1 subclass, a mutated Fc part from human IgG1 subclass (preferably with a mutations L234A and L235A, or L234A, L235A and P329G), a Fc part from human IgG4 subclass or a mutated Fc part from human IgG4 subclass (preferably with a mutations S228P and L235E, or S228P, L235E and P329G). In one embodiment the bispecific antibody comprise the human heavy chain constant regions of SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63 or SEQ ID NO: 64 (human IgG1 subclass allotypes (Caucasian and Afroamerican or mutants L234A/L235A, and L234A/L235A/P329G), SEQ ID NO: 65, SEQ ID NO: 66, or SEQ ID NO: 67 (human IgG4 subclass or mutants L234A/L235A, and L234A/L235A/P329G) (numbering according to the EU index of Kabat et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991). These human heavy chain constant regions can comprise additionally modifications and or mutations (see e.g. the knobs and hole mutations as described below or other modification which enhance the heterodimerization. In one embodiment the bispecific antibody comprises two heavy chain constant regions wherein in one of the two CH3 domains the mutations Y349C, T366W and in the other of the two CH3 domains the mutations S354C, T366S, L368A, Y407V are additionally comprised in the amino acid sequences of SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63 or SEQ ID NO: 64 (human IgG1 subclass allotypes (Caucasian and Afroamerican or mutants L234A/L235A, and L234A/L235A/P329G), SEQ ID NO: 65, SEQ ID NO: 66, or SEQ ID NO: 67 (human IgG4 subclass or mutants L234A/L235A, and L234A/L235A/P329G) (numbering according to the EU index of Kabat et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991).

In one embodiment the antibody according to the invention is of human IgG1 subclass or of human IgG4 subclass. In one embodiment the antibody according to the invention is of human IgG1 subclass. In one embodiment the antibody according to the invention is of human IgG4 subclass.

In one embodiment the bispecific antibody specifically binding to human TWEAK and human IL17 according to the invention is a bispecific, bivalent antibody with two different specifities as described e.g. in WO 2009/080251, WO 2009/080252, WO 2009/080253 or Schaefer, W., et al., PNAS 108 (2011) 11187-92

("CrossMabs" or "domain exchanged antibodies"- see Example 14, and exemplary figure 1a; <Tweak-IL-17> #2, <Tweak-IL-17> #24 which have the format described in WO 2009/080253), in WO 2011/117330 ("bispecific one-armed scFab antibodies" see Example 14; <Tweak-IL-17> #4, <Tweak-IL-17> #20, <Tweak-IL-17> #21, <Tweak-IL-17> #23; see also and exemplary figure 1a), in Ridgway, J.B., Protein Eng. 9 (1996) 617-621; WO 96/027011; WO 98/050431, Merchant, A.M., et al., Nature Biotech 16 (1998) 677-681; Atwell, S., et al., J. Mol. Biol. 270 (1997) 26-35, EP 1 870 459 A1, Muda, M., et al , Protein Engineering, Design & Selection 24 (2011) 447–454, WO 2010/129304, WO 2011/028952, WO 2012/009544 and the like (which are all incorporated by references).

Typically such bispecific, bivalent antibody often comprise a Fc part and comprise two different heavy chain or heavy chain-like peptides which form heterodimers. To enforce the formation of such heterodimers (and reduce the formation of homodimeric by-products) the CH3 (and/or CH2) domains are modified in way that the formation of the heterodimer is preferred. There are different modifications known in the art to enhance such formation of the heterodimer , as described e.g. in WO 96/027011, Ridgway, J.B., et al., Protein Eng 9 (1996) 617–621; Merchant, A.M., et al., Nat Biotechnol 16 (1998) 677-681, WO 96/027011, WO 98/050431, US 2010/0015133, WO 2007/147901, WO 2009/089004, WO 2010/129304 and Muda, M., et al., Protein Engineering, Design & Selection 24 (2011) 447–454.

In one embodiment of the invention the bispecific, bivalent antibody comprises a Fc part derived from human origin and preferably all other parts of the human constant regions wherein the CH3 (and/or CH2) domains of the bispecific, bivalent antibody are altered by one or more modifications to enhance the formation of the heterodimers.

Thus one embodiment of the invention is a bispecific, bivalent antibody comprising a first antigen-binding site that specifically binds to human TWEAK and a second antigen-binding site that specifically binds to human IL17, wherein the bispecific, antibody is characterized in comprising the amino acid sequences of SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, and SEQ ID NO: 79.

One embodiment of the invention is a bispecific, bivalent antibody comprising a first antigen-binding site that specifically binds to human TWEAK and a second antigen-binding site that specifically binds to human IL17, wherein the bispecific,

antibody is characterized in comprising the amino acid sequences of SEQ ID NO: 80, SEQ ID NO: 81, and SEQ ID NO: 82.

One embodiment of the invention is a bispecific, bivalent antibody comprising a first antigen-binding site that specifically binds to human TWEAK and a second antigen-binding site that specifically binds to human IL17, wherein the bispecific, antibody is characterized in comprising the amino acid sequences of SEQ ID NO: 85, SEQ ID NO: 86, and SEQ ID NO: 87.

One embodiment of the invention is a bispecific, bivalent antibody comprising a first antigen-binding site that specifically binds to human TWEAK and a second antigen-binding site that specifically binds to human IL17, wherein the bispecific, antibody is characterized in comprising the amino acid sequences of SEQ ID NO: 88, SEQ ID NO: 89, and SEQ ID NO: 90.

One embodiment of the invention is a bispecific, bivalent antibody comprising a first antigen-binding site that specifically binds to human TWEAK and a second antigen-binding site that specifically binds to human IL17, wherein the bispecific, antibody is characterized in comprising the amino acid sequences of SEQ ID NO: 91, SEQ ID NO: 92, and SEQ ID NO: 93.

One embodiment of the invention is a bispecific, bivalent antibody comprising a first antigen-binding site that specifically binds to human TWEAK and a second antigen-binding site that specifically binds to human IL17, wherein the bispecific, antibody is characterized in comprising the amino acid sequences of SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, and SEQ ID NO: 97.

In one embodiment of the invention the CH3 domains of the bispecific, bivalent antibody are altered by the “knob-into-holes” technology which is described in detail with several examples in e.g. WO 96/027011, WO98/050431, Ridgway, J.B., et al., Protein Eng 9 (1996) 617–621; and Merchant, A.M., et al., Nat Biotechnol 16 (1998) 677-681. In this method the interaction surfaces of the two CH3 domains are altered to increase the heterodimerisation of both heavy chains containing these two CH3 domains. Each of the two CH3 domains (of the two heavy chains) can be the “knob”, while the other is the “hole”. The introduction of a disulfide bridge stabilizes the heterodimers (Merchant, A.M, et al., Nature Biotech 16 (1998) 677-681; Atwell, S., et al. J. Mol. Biol. 270 (1997) 26–35) and increases the yield.

In a preferred aspect of the invention all bispecific antibodies according to the invention are characterized in that

the CH3 domain of one heavy chain and the CH3 domain of the other heavy chain each meet at an interface which comprises an original interface between the antibody CH3 domains;

wherein said interface is altered to promote the formation of the bispecific antibody, wherein the alteration is characterized in that:

a) the CH3 domain of one heavy chain is altered,

so that within the original interface the CH3 domain of one heavy chain that meets the original interface of the CH3 domain of the other heavy chain within the bispecific antibody,

an amino acid residue is replaced with an amino acid residue having a larger side chain volume, thereby generating a protuberance within the interface of the CH3 domain of one heavy chain which is positionable in a cavity within the interface of the CH3 domain of the other heavy chain

and

b) the CH3 domain of the other heavy chain is altered,

so that within the original interface of the second CH3 domain that meets the original interface of the first CH3 domain within the bispecific antibody

an amino acid residue is replaced with an amino acid residue having a smaller side chain volume, thereby generating a cavity within the interface of the second CH3 domain within which a protuberance within the interface of the first CH3 domain is positionable.

Thus the antibody according to invention is preferably characterized in that

the CH3 domain of the heavy chain of the full length antibody of a) and the CH3 domain of the heavy chain of the full length antibody of b) each meet at an interface which comprises an alteration in the original interface between the antibody CH3 domains;

wherein i) in the CH3 domain of one heavy chain

an amino acid residue is replaced with an amino acid residue having a larger side chain volume, thereby generating a protuberance within the interface of the CH3 domain of one heavy chain which is positionable in a cavity within the interface of the CH3 domain of the other heavy chain

and wherein ii) in the CH3 domain of the other heavy chain

an amino acid residue is replaced with an amino acid residue having a smaller side chain volume, thereby generating a cavity within the interface of the second CH3 domain within which a protuberance within the interface of the first CH3 domain is positionable.

Preferably said amino acid residue having a larger side chain volume is selected from the group consisting of arginine (R), phenylalanine (F), tyrosine (Y), tryptophan (W).

Preferably said amino acid residue having a smaller side chain volume is selected from the group consisting of alanine (A), serine (S), threonine (T), valine (V).

In one aspect of the invention both CH3 domains are further altered by the introduction of cysteine (C) as amino acid in the corresponding positions of each CH3 domain such that a disulfide bridge between both CH3 domains can be formed.

In one embodiment, the bispecific, bivalent antibody comprises a T366W mutation in the CH3 domain of the "knobs chain" and T366S, L368A, Y407V mutations in the CH3 domain of the "hole chain". An additional interchain disulfide bridge between the CH3 domains can also be used (Merchant, A.M, et al., Nature Biotech 16 (1998) 677-681) e.g. by introducing a Y349C mutation into the CH3 domain of the "knobs chain" and a E356C mutation or a S354C mutation into the CH3 domain of the "hole chain" (numbering according to the EU index of Kabat et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991)).

In another embodiment, the bispecific, bivalent antibody according to the invention comprises Y349C, T366W mutations in one of the two CH3 domains and E356C, T366S, L368A, Y407V mutations in the other of the two CH3 domains. In a another preferred embodiment the bispecific antibody comprises Y349C, T366W

mutations in one of the two CH3 domains and S354C, T366S, L368A, Y407V mutations in the other of the two CH3 domains (the additional Y349C mutation in one CH3 domain and the additional E356C or S354C mutation in the other CH3 domain forming a interchain disulfide bridge) (numbering always according to EU index of Kabat; (Kabat, E.A., et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991))). But also other knobs-in-holes technologies as described by EP 1 870 459 A1, can be used alternatively or additionally. Thus another example for the bispecific antibody are R409D; K370E mutations in the CH3 domain of the "knobs chain" and D399K; E357K mutations in the CH3 domain of the "hole chain" (numbering always according to EU index of Kabat; (Kabat, E.A., et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991)).

In another embodiment the bispecific, bivalent antibody comprises a T366W mutation in the CH3 domain of the "knobs chain" and T366S, L368A, Y407V mutations in the CH3 domain of the "hole chain" and additionally R409D; K370E mutations in the CH3 domain of the "knobs chain" and D399K; E357K mutations in the CH3 domain of the "hole chain".

In another embodiment the bispecific, bivalent antibody comprises Y349C, T366W mutations in one of the two CH3 domains and S354C, T366S, L368A, Y407V mutations in the other of the two CH3 domains or said bispecific antibody comprises Y349C, T366W mutations in one of the two CH3 domains and S354C, T366S, L368A, Y407V mutations in the other of the two CH3 domains and additionally R409D; K370E mutations in the CH3 domain of the "knobs chain" and D399K; E357K mutations in the CH3 domain of the "hole chain". Such knob and hole mutations in the CH3 domain are typically used in human heavy chain constant regions of SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63 or SEQ ID NO: 64 (human IgG1 subclass allotypes (Caucasian and Afroamerican or mutants L234A/L235A, and L234A/L235A/P329G), SEQ ID NO: 65, SEQ ID NO: 66, or SEQ ID NO: 67 (human IgG4 subclass or mutants L234A/L235A, and L234A/L235A/P329G) (numbering according to the EU index of Kabat et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991)).

Thus in one embodiment, the bispecific antibody according to the invention comprises human heavy chain constant regions of SEQ ID NO: 61, SEQ ID NO: 62,

SEQ ID NO: 63 or SEQ ID NO: 64 (human IgG1 subclass allotypes (Caucasian and Afroamerican or mutants L234A/L235A, and L234A/L235A/P329G), SEQ ID NO: 65, SEQ ID NO: 66, or SEQ ID NO: 67 (human IgG4 subclass or mutants L234A/L235A, and L234A/L235A/P329G) further including such "knob" and "hole" mutations in the CH3 domain (e.g. Y349C, T366W mutations in one of the two CH3 domains and S354C, T366S, L368A, Y407V mutations in the other of the two CH3 domains) (numbering according to the EU index of Kabat et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991)).

Such bivalent bispecific antibody specifically binding to human TWEAK and human IL17 according to the invention, have especially valuable properties such as low viscosity and high stability (so that they can be produced without high aggregation and in good yields). Such bivalent bispecific antibodies with their low viscosity and high stability are especially useful in highly concentrated formulations/compositions which can be used e.g. in a subcutaneous administration.

Thus one embodiment of the invention is a bispecific, bivalent antibody comprising a first antigen-binding site that specifically binds to human TWEAK and a second antigen-binding site that specifically binds to human IL17 characterized in that

a) the viscosity at 100 mg/ml is 4.0 mPa·s or lower (and/ or the viscosity at 70 mg/ml is 3.0 mPa·s or lower and /or the viscosity at 150 mg/ml is 8.5 mPa·s or lower) (as determined in Example 18)

b) aggregation temperature is 55 °C or higher (as determined in Example 18)

The aggregation temperature refers to the DLS aggregation onset temperature (see Example 18).

In one embodiment said bispecific antibody is trivalent using e.g. formats based on a full length antibody specifically binding to one of the two antigens TWEAK or IL17, to which only at one C-terminus of one heavy chain a scFab fragment is fused which specifically binds to the other of the two antigens TWEAK or IL17, including knobs–into holes technology, as described e.g. in WO 2010/112193 or e.g. formats based on a full length antibody specifically binding to one of the two antigens TWEAK or IL17, to which at one C-terminus of one heavy chain a VH or VH-CH1 fragment and at the other C-terminus of the second heavy chain a VL or VL-CL fragment is fused which specifically binds to the other of the two antigens

TWEAK or IL17, including knobs–into holes technology, as described e.g. in WO 2010/115589 or WO 2011/028952.

In one embodiment the bispecific antibody specifically binding to human TWEAK and human IL17 according to the invention is a tetravalent antibody ("four binding arms") with two different specifities as described e.g. in WO 2007/024715, or WO 2007/109254, WO 2010/112193, WO 2010/145792 or WO 2010/145793 (see also Example 14; <Tweak-IL-17> #5).

One embodiment of the invention is a bispecific, tetravalent antibody comprising a first antigen-binding site that specifically binds to human TWEAK and a second antigen-binding site that specifically binds to human IL17, wherein the bispecific, antibody is characterized in comprising the amino acid sequences of SEQ ID NO: 83, and SEQ ID NO: 84.

The bispecific, bivalent antibody formats of WO 2011/117330 ("bispecific one-armed scFab antibodies") and the bispecific, tetravalent antibody formats of WO 2010/112193 comprise single chain Fab fragments (scFab) in which the Fab heavy and light chain fragments are linked via a peptide linker (see Fig 1b and 2, as well as WO 2011/117330 and WO 2010/112193). The peptide linker is typically a peptide with amino acid sequences, which is preferably of synthetic origin and has a length of at least 30 amino acids, preferably a length of 30 to 50 amino acids (in one embodiment with a length of 32 to 40 amino acids). In one embodiment said linker is (GxS)n with G = glycine, S = serine, (x =3, n= 8, 9 or 10 and m= 0, 1, 2 or 3) or (x = 4 and n= 6, 7 or 8 and m= 0, 1, 2 or 3). In one embodiment said peptide linker is (G4S)6G2.

The bispecific, tetravalent antibody formats of WO 2007/024715, or WO 2007/109254, WO 2010/112193, WO 2010/145792 or WO 2010/145793 comprise peptide connectors to link the antigen binding site to a full length antibody. Typically such peptide connector is a peptide with amino acid sequences, which is preferably of synthetic origin and has a length of at least 5 amino acids, preferably with a length of 5 to 100, (in one embodiment with a length of 10 to 50 amino acids; in one embodiment with a length of 10 to 50 amino acids). In one embodiment said peptide connector is (GxS)n or (GxS)nGm with G = glycine, S = serine, and (x = 3, n= 3, 4, 5 or 6, and m= 0, 1, 2 or 3) or (x = 4,n= 2, 3, 4 or 5 and m= 0, 1, 2 or 3). In one embodiment said peptide connector is (G3S)3 or (G4S)2.

A further embodiment of the invention is a method for the production of a bispecific antibody according to the invention, characterized in that the sequence of a nucleic acid encoding the heavy chain of an antibody according to the invention and the nucleic acid encoding the light chain of said antibody are inserted into one or two expression vector(s), said vector(s) is/are inserted in a eukaryotic host cell, the encoded antibody is expressed and recovered from the host cell or the supernatant.

The antibodies according to the invention are preferably produced by recombinant means. Such methods are widely known in the state of the art and comprise protein expression in prokaryotic and eukaryotic cells with subsequent isolation of the antibody polypeptide and usually purification to a pharmaceutically acceptable purity. For the protein expression nucleic acids encoding light and heavy chains or fragments thereof are inserted into expression vectors by standard methods. Expression is performed in appropriate prokaryotic or eukaryotic host cells, such as CHO cells, NS0 cells, SP2/0 cells, HEK293 cells, COS cells, yeast, or E. coli cells, and the antibody is recovered from the cells (from the supernatant or after cells lysis).

Recombinant production of antibodies is well-known in the state of the art and described, for example, in the review articles of Makrides, S.C., Protein Expr. Purif. 17 (1999) 183-202; Geisse, S., et al., Protein Expr. Purif. 8 (1996) 271-282; Kaufman, R.J., Mol. Biotechnol. 16 (2000) 151-160; Werner, R.G., Arzneimittelforschung (Drug Res.) 48 (1998) 870-880.

The antibodies may be present in whole cells, in a cell lysate, or in a partially purified, or substantially pure form. Purification is performed in order to eliminate other cellular components or other contaminants, e.g., other cellular nucleic acids or proteins, by standard techniques, including column chromatography and others well known in the art. See Ausubel, F. et al. (eds.), Current Protocols in Molecular Biology, Greene Publishing and Wiley Interscience, New York (1987).

Expression in NS0 cells is described by, e.g., Barnes, L.M., et al., Cytotechnology 32 (2000) 109-123; Barnes, L.M., et al., Biotech. Bioeng. 73 (2001) 261-270. Transient expression is described by, e.g., Durocher, Y., et al., Nucl. Acids. Res. 30 (2002) E9. Cloning of variable domains is described by Orlandi, R., et al., Proc. Natl. Acad. Sci. USA 86 (1989) 3833-3837; Carter, P., et al., Proc. Natl. Acad. Sci. USA 89 (1992) 4285-4289; Norderhaug, L., et al., J. Immunol. Methods 204

(1997) 77-87. A preferred transient expression system (HEK 293) is described by Schlaeger, E.-J. and Christensen, K., in Cytotechnology 30 (1999) 71-83, and by Schlaeger, E.-J., in J. Immunol. Methods 194 (1996) 191-199.

Monoclonal antibodies are suitably separated from the culture medium by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, dialysis, or affinity chromatography. DNA and RNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures. The hybridoma cells can serve as a source of such DNA and RNA. Once isolated, the DNA may be inserted into expression vectors, which are then transfected into host cells, such as HEK 293 cells, CHO cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of recombinant monoclonal antibodies in the host cells.

Nucleic acid molecules encoding amino acid sequence variants of anti-TWEAK /anti-IL17 bispecific antibody are prepared by a variety of methods known in the art. These methods include, but are not limited to, isolation from a natural source (in the case of naturally occurring amino acid sequence variants) or preparation by oligonucleotide-mediated (or site-directed) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared variant or a non-variant version of anti-TWEAK /anti-IL17 bispecific antibody.

The heavy and light chain variable domains according to the invention are combined with sequences of promoter, translation initiation, constant region, 3' untranslated region, polyadenylation, and transcription termination to form expression vector constructs. The heavy and light chain expression constructs can be combined into a single vector, co-transfected, serially transfected, or separately transfected into host cells which are then fused to form a single host cell expressing both chains.

The bispecific TWEAK/IL17 antibody, especially the bispecific bivalent antibodies have valuable properties such good developability and producibility, (e.g. no hotspots are contained which require specific production conditions), good titers and yields and are producible in high amounts and with relatively low impurities (> 60 % Monomer after Protein A (SE-HPLC) with an estimated purity after 2nd column (ESI-MS) > 80 %) (see Example 14 and 15).

Pharmaceutical Compositions

Pharmaceutical compositions of a bispecific TWEAK/IL17 antibody as described herein are prepared by mixing such antibody having the desired degree of purity with one or more optional pharmaceutically acceptable carriers (Remington's Pharmaceutical Sciences, 16th edition, Osol, A. (ed.) (1980)), in the form of lyophilized formulations or aqueous solutions. Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyl dimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as poly(vinylpyrrolidone); amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG). Exemplary pharmaceutically acceptable carriers herein further include interstitial drug dispersion agents such as soluble neutral-active hyaluronidase glycoproteins (sHASEGP), for example, human soluble PH-20 hyaluronidase glycoproteins, such as rhuPH20 (HYLENEX®, Baxter International, Inc.). Certain exemplary sHASEGPs and methods of use, including rhuPH20, are described in US Patent Publication Nos. 2005/0260186 and 2006/0104968. In one aspect, a sHASEGP is combined with one or more additional glycosaminoglycanases such as chondroitinases.

Exemplary lyophilized antibody formulations are described in US Patent No. 6,267,958. Aqueous antibody formulations include those described in US Patent No. 6,171,586 and WO 2006/044908, the latter formulations including a histidine-acetate buffer.

The formulation herein may also contain more than one active ingredients as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other.

The bispecific antibodies specifically binding to human TWEAK and human IL17 according to the invention (especially the bispecific, bivalent), have especially valuable properties such as low viscosity and high stability (so that they can be produced without high aggregation and in good yields) (see Example 18). Such bivalent bispecific antibodies with their low viscosity and high stability are especially useful in highly concentrated formulations/compositions which can be used e.g. in a subcutaneous administration.

The bispecific antibodies according to the invention may be administered as the sole active ingredient or in conjunction with, e.g. as an adjuvant to or in combination to, other drugs e.g. immunosuppressive or immunomodulating agents or other anti-inflammatory agents, e.g. for the treatment or prevention of diseases mentioned above. For example, the bispecific antibodies as described herein may be used in combination with DMARD, e.g. Gold salts, sulphasalazine, antimalarias, methotrexate, D-penicillamine, azathioprine, mycophenolic acid, cyclosporine A, tacrolimus, sirolimus, minocycline, leflunomide, glococorticoids; a calcineurin inhibitor, e.g. cyclosporin A or FK 506; a modulator of lymphocyte recirculation, e.g. FTY720 and FTY720 analogs; a mTOR inhibitor, e.g. rapamycin, 40-O-(2-hydroxyethyl)-rapamycin, CCI779, ABT578, AP23573 or TAFA-93; an ascomycin having immuno-suppressive properties, e.g. ABT-281, ASM981, etc.; corticosteroids; cyclophosphamide; azathioprene; methotrexate; leflunomide; mizoribine; mycophenolic acid; myco-pheno-late mofetil; 15-deoxyspergualine or an immunosuppressive homologue, analogue or derivative thereof; immunosuppressive monoclonal antibodies, e.g., monoclonal antibodies to leukocyte receptors, e.g., MHC, CD2, CD3, CD4, CD7, CD8, CD25, CD28, CD40. CD45, CD58, CD80, CD86 or their ligands; other immunomodulatory compounds, e.g. a recombinant binding molecule having at least a portion of the extracellular domain of CTLA4 or a mutant thereof, e.g. an at least extracellular portion of CTLA4 or a mutant thereof joined to a non-CTLA4 protein sequence, e.g. CTLA4Ig (for ex. designated ATCC 68629) or a mutant thereof, e.g. LEA29Y; adhesion molecule inhibitors, e.g. LFA-1 antagonists, ICAM-1 or -3 antagonists, VCAM-4 antagonists or VLA-4 antagonists; or a chemotherapeutic agent, e.g. paclitaxel, gemcitabine, cisplatinum, doxorubicin or 5-fluorouracil; anti TNF agents, e.g. monoclonal antibodies to TNF, e.g. infliximab, adalimumab, CDP870, or receptor constructs to TNF-RI or TNF-RII, e.g. Etanercept, PEG-TNF-RI; blockers of proinflammatory cytokines, IL-1 blockers, e.g. Anakinra or IL-1 trap, AAL160, ACZ 885, IL-6 blockers; chemokines blockers, e.g. inhibitors or

activators of proteases, e.g. metalloproteases, anti-IL-15 antibodies, anti-IL-6 antibodies, anti-IL-23 antibodies, anti-CD20 antibodies, NSAIDs, such as aspirin or an anti-infectious agent (the list not limited to the agent mentioned).

A bispecific antibody according to the present invention may be provided in combination or addition with one or more of the following agents:

- an antagonist of cytokine function, (e.g. an agent which act on cytokine signaling pathways such as a modulator of the SOCS system), such as an alpha-, beta-, and/or gamma-interferon; modulators of insulin-like growth factor type I (IGF-I), its receptors and associated binding proteins; interleukins (IL) e.g. one or more of IL-1 to 33, and/or an interleukin antagonist or inhibitor such as anakinra; inhibitors of receptors of interleukin family members or inhibitors of specific subunits of such receptors; a tumor necrosis factor alpha (TNF-.alpha.) inhibitor such as an anti-TNF monoclonal antibody (for example infliximab; adalimumab, and/or CDP-870), and/or a TNF receptor antagonist e.g. an immunoglobulin molecule (such as etanercept) and/or a low-molecular-weight agent such as pentoxyfylline;

- a modulator of B cells, e.g. a monoclonal antibody targeting B- lymphocytes (such as CD20 (rituximab) or MRA-aIL16R) or T-lymphocytes (e.g. CTLA4-Ig, HuMax 11-15 or Abatacept);

- a modulator that inhibits osteoclast activity, for example an antibody to RANKL;

- a modulator of chemokine or chemokine receptor function such as an antagonist of CCRl, CCR2, CCR2A, CCR2B, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CCRlO and CCRIl (for the C-C family); CXCRl, CXCR2,

CXCR3, CXCR4 and CXCR5 and CXCR6 (for the C-X-C family) and CX3CRl for the C-X3-C family;

- an inhibitor of matrix metalloproteases (MMPs), i.e., one or more of the stromelysins, the collagenases, and the gelatinases, as well as aggrecanase; especially collagenase-1 (MMP1), collagenase-2 (MMP8), collagenase-3 (MMP13) , stromelysin-1 (MMP3) , stromelysin-2 (MMP10), and/or stromelysin-3 (MMP11) and/or MMP9 and/or MMP12, e.g. an agent such as doxycycline; a leukotriene biosynthesis inhibitor, 5-lipoxygenase (5-LO) inhibitor or 5-lipoxygenase activating protein (FLAP) antagonist such as;

zileuton; ABT-761; fenleuton; tepoxalin; Abbott-79175; Abbott- 85761; N- (5-substituted) -thiophene-2-alkylsulfonamides; 2, 6-di-tert-butylphenolhydrazones; methoxytetrahydropyrans such as Zeneca ZD- 2138; the compound SB-210661; a pyridinyl-substituted 2- cyanonaphthalene compound such as L-739,010; a 2-cyanoquinoline compound such as L-746,530; indole and/or a quinoline compound such as MK-591, MK-886, and/or BAY x 1005; a receptor antagonist for leukotrienes (LT) B4, LTC4, LTD4, and LTE4, selected from the group consisting of the phenothiazin-3-ls such as L-651,392; amidino compounds such as CGS-25019c; benzoxalamines such as ontazolast; benzenecarboximidamides such as BIIL 284/260; and compounds such as zafirlukast, ablukast, montelukast, pranlukast, verlukast (MK-679) , RG-12525, Ro-245913, iralukast (CGP 45715A) , and BAY x 7195; a phosphodiesterase (PDE) inhibitor such as a methylxanthanine, e.g. theophylline and/or aminophylline; and/or a selective PDE isoenzyme inhibitor e.g. a PDE4 inhibitor and/or inhibitor of the isoform PDE4D, and/or an inhibitor of PDE5;

- a histamine type 1 receptor antagonist such as cetirizine, loratadine, desloratadine, fexofenadine, acrivastine, terfenadine, astemizole, azelastine, levocabastine, chlorpheniramine, promethazine, cyclizine, and/or mizolastine (generally applied orally, topically or parenterally);

- a proton pump inhibitor (such as omeprazole) or gastroprotective histamine type 2 receptor antagonist; - an antagonist of the histamine type 4 receptor; an alpha-l/alpha-2 adrenoceptor agonist vasoconstrictor sympathomimetic agent, such as propylhexedrine, phenylephrine, phenylpropanolamine, ephedrine, pseudoephedrine, naphazoline hydrochloride, oxymetazoline hydrochloride, tetrahydrozoline hydrochloride, xylometazoline hydrochloride, tramazoline hydrochloride, and ethylnorepinephrine hydrochloride; an anticholinergic agent, e.g. a muscarinic receptor (Ml, M2, and M3) antagonist such as atropine, hyoscine, glycopyrrrolate, ipratropium bromide, tiotropium bromide, oxitropium bromide, pirenzepine, and telenzepine;

- a beta-adrenoceptor agonist (including beta receptor subtypes 1- 4) such as isoprenaline, salbutamol, formoterol, salmeterol, terbutaline, orciprenaline, bitolterol mesylate, and/or pirbuterol, e.g. a chiral enantiomer thereof;

- a chromone, e.g. sodium cromoglycate and/or nedocromil sodium;

- a glucocorticoid, such as flunisolide, triamcinolone acetonide, beclomethasone dipropionate, budesonide, fluticasone propionate, ciclesonide, and/or mometasone furoate; an agent that modulate nuclear hormone receptors such as a PPAR;

- an immunoglobulin (Ig) or Ig preparation or an antagonist or antibody modulating Ig function such as anti-IgE (e.g. omalizumab);

- other systemic or topically-applied anti-inflammatory agent, e.g. thalidomide or a derivative thereof, a retinoid, dithranol, and/or calcipotriol;

- combinations of aminosalicylates and sulfapyridine such as sulfasalazine, mesalazine, balsalazide, and olsalazine; and immunomodulatory agents such as the thiopurines, and corticosteroids such as budesonide;

- an antibacterial agent e.g. a penicillin derivative, a tetracycline, a macrolide, a beta-lactam, a fluoroquinolone, metronidazole, and/or an inhaled aminoglycoside; and/or an antiviral agent e.g. acyclovir, famciclovir, valaciclovir, ganciclovir, cidofovir; amantadine, rimantadine; ribavirin; zanamavir and/or oseltamavir; a protease inhibitor such as indinavir, nelfinavir, ritonavir, and/or saquinavir; a nucleoside reverse transcriptase inhibitor such as didanosine, lamivudine, stavudine, zalcitabine, zidovudine; a non-nucleoside reverse transcriptase inhibitor such as nevirapine, efavirenz; a cardiovascular agent such as a calcium channel blocker, beta- adrenoceptor blocker, angiotensin-converting enzyme (ACE) inhibitor, angiotensin-2 receptor antagonist; lipid lowering agent such as a statin, and/or fibrate; a modulator of blood cell morphology such as pentoxyfylline; a thrombolytic, and/or an anticoagulant e.g. a platelet aggregation inhibitor;

- a CNS agent such as an antidepressant (such as sertraline) , anti-Parkinsonian drug (such as deprenyl, L-dopa, ropinirole, pramipexole, MAOB inhibitor such as selegine and rasagiline, comP inhibitor such as tasmar, A-2 inhibitor, dopamine reuptake inhibitor, NMDA antagonist, nicotine agonist, dopamine agonist and/or inhibitor of neuronal nitric oxide synthase), and an anti-Alzheimer's drug such as donepezil, rivastigmine, tacrine, COX-2 inhibitor, propentofylline or metrifonate; an agent for the treatment of acute and chronic pain, e.g. a centrally or peripherally-acting analgesic such as an opioid analogue or derivative, carbamazepine, phenytoin, sodium valproate, amitryptiline or other antidepressant agent, paracetamol, or nonsteroidal anti-inflammatory agent; a

parenterally or topically-applied (including inhaled) local anaesthetic agent such as lignocaine or an analogue thereof; an anti-osteoporosis agent e.g. a hormonal agent such as raloxifene, or a biphosphonate such as alendronate;

(i) a tryptase inhibitor; (ii) a platelet activating factor (PAF) antagonist; (iii) an interleukin converting enzyme (ICE) inhibitor; (iv) an IMPDH inhibitor; (v) an adhesion molecule inhibitors including VLA-4 antagonist; (vi) a cathepsin; (vii) a kinase inhibitor e.g. an inhibitor of tyrosine kinases (such as Btk, Itk, Jak3 MAP examples of inhibitors might include Gefitinib, Imatinib mesylate), a serine / threonine kinase (e.g. an inhibitor of MAP kinase such as p38, JNK, protein kinases A, B and C and IKK), or a kinase involved in cell cycle regulation (e.g. a cylin dependent kinase) ; (viii) a glucose-6 phosphate dehydrogenase inhibitor; (ix) a kinin-B. subl . - and/or B.sub2. -receptor antagonist; (x) an anti-gout agent, e.g., colchicine; (xi) a xanthine oxidase inhibitor, e.g., allopurinol; (xii) a uricosuric agent, e.g., probenecid, sulfinpyrazone, and/or benzbromarone; (xiii) a growth hormone secretagogue; (xiv) transforming growth factor (TGF.beta.) ; (xv) platelet-derived growth factor (PDGF) ; (xvi) fibroblast growth factor, e.g., basic fibroblast growth factor (bFGF) ; (xvii) granulocyte macrophage colony stimulating factor (GM-CSF) ; (xviii) capsaicin cream; (xix) a tachykinin NK. subl. and/or NK.sub3. receptor antagonist such NKP-608C, SB-233412 (talnetant) , and/or D-4418; (xx) an elastase inhibitor e.g. UT-77 and/or ZD-0892; (xxi) a TNF-alpha converting enzyme inhibitor (TACE) ; (xxii) induced nitric oxide synthase (iNOS) inhibitor or (xxiii) a chemoattractant receptor- homologous molecule expressed on TH2 cells, (such as a CRTH2 antagonist) (xxiv) an inhibitor of a P38 (xxv) agent modulating the function of Toll-like receptors (TLR) and (xxvi) an agent modulating the activity of purinergic receptors such as P2X7; (xxvii) an inhibitor of transcription factor activation such as NFkB, API, and/or STATS.

An inhibitor may be specific or may be a mixed inhibitor, e.g. an inhibitor targeting more than one of the molecules (e.g. receptors) or molecular classes mentioned above.

The bispecific antibody could also be used in association with a chemotherapeutic agent or another tyrosine kinase inhibitor in coadministration or in the form of an immuno-conjugate. Fragments of said antibody could also be used in bispecific antibodies obtained by recombinant mechanisms or biochemical coupling, and then associating the specificity of the above described antibody with the specificity of other antibodies able to recognize other molecules involved in the activity for

which IL-17 is associated. For treatment of an inflammatory disease, a bispecific antibody of the invention may be combined with one or more agents such as:- Non-steroidal anti-inflammatory agents (hereinafter NSAIDs) including non-selective cyclo-oxygenase (COX)-I / COX-2 inhibitors whether applied topically or systemically (such as piroxicam, diclofenac, propionic acids such as naproxen, flurbiprofen, fenoprofen, ketoprofen and ibuprofen, fenamates such as mefenamic acid, indomethacin, sulindac, azapropazone, pyrazolones such as phenylbutazone, salicylates such as aspirin); selective COX-2 inhibitors (such as meloxicam, celecoxib, rofecoxib, valdecoxib, lumarocoxib, parecoxib and etoricoxib); cyclo-oxygenase inhibiting nitric oxide donors (CINODs); glucocorticosteroids (whether administered by topical, oral, intramuscular, intravenous, or intra- articular routes); methotrexate, leflunomide; hydroxychloroquine, d- penicillamine, auranofin or other parenteral or oral gold preparations; analgesics; diacerein; intra-articular therapies such as hyaluronic acid derivatives; and nutritional supplements such as glucosamine.

A bispecific antibody of the invention can also be used in combination with an existing therapeutic agent for the treatment of cancer. Suitable agents to be used in combination include: (i) antiproliferative/antineoplastic drugs and combinations thereof, as used in medical oncology, such as alkylating agents (for example cis-platin, carboplatin, cyclophosphamide, nitrogen mustard, melphalan, chlorambucil, busulphan and nitrosoureas); antimetabolites (for example antifolates such as fluoropyrimidines like 5-fluorouracil and tegafur, raltitrexed, methotrexate, cytosine arabinoside, hydroxyurea, gemcitabine and paclitaxel; antitumour antibiotics (for example anthracyclines like adriamycin, bleomycin, doxorubicin, daunomycin, epirubicin, idarubicin, mitomycin-C, dactinomycin and mithramycin); antimitotic agents (for example vinca alkaloids like vincristine, vinblastine, vindesine and vinorelbine and taxoids like taxol and taxotere); and topoisomerase inhibitors (for example epipodophyllotoxins like etoposide and teniposide, amsacrine, topotecan and camptothecins);

(ii) cytostatic agents such as antioestrogens (for example tamoxifen, toremifene, raloxifene, droloxifene and iodoxyfene), oestrogen receptor down regulators (for example fulvestrant), antiandrogens

(for example bicalutamide, flutamide, nilutamide and cyproterone acetate), LHRH antagonists or LHRH agonists (for example goserelin, leuprorelin and buserelin), progestogens (for example megestrol acetate), aromatase inhibitors (for example as

anastrozole, letrozole, vorazole and exemestane) and inhibitors of 5.alpha.-reductase such as finasteride;

(iii) Agents which inhibit cancer cell invasion (for example metalloproteinase inhibitors like marimastat and inhibitors of urokinase plasminogen activator receptor function); (iv) inhibitors of growth factor function, for example such inhibitors include growth factor antibodies, growth factor receptor antibodies (for example the anti-erbb2 antibody trastuzumab and the anti-erbb1 antibody cetuximab [C225]), farnesyl transferase inhibitors, tyrosine kinase inhibitors and serine/threonine kinase inhibitors, for example inhibitors of the epidermal growth factor family (for example EGFR family tyrosine kinase inhibitors such as N- (3-chloro-4-fluorophenyl) -7-methoxy-6- (3- morpholinopropoxy) quinazolin-4-amine (gefitinib, AZD1839) , N- (3- ethynylphenyl) -6, 7-bis (2-methoxyethoxy) quinazolin-4-amine (erlotinib, OSI-774) and 6-acrylamido-N- (3-chloro-4-fluorophenyl) -7- (3- morpholinopropoxy) quinazolin-4-amine (CI 1033)), for example inhibitors of the platelet-derived growth factor family and for example inhibitors of the hepatocyte growth factor family; (v) antiangiogenic agents such as those which inhibit the effects of vascular endothelial growth factor, (for example the anti-vascular endothelial cell growth factor antibody bevacizumab, compounds such as those disclosed in International Patent Applications WO 97/22596, WO 97/30035, WO 97/32856 and WO 98/13354, each of which is incorporated herein in its entirety) and compounds that work by other mechanisms (for example linomide, inhibitors of integrin .alpha.v.beta.3 function and angiostatin); (vi) vascular damaging agents such as combretastatin A4 and compounds disclosed in International Patent Applications WO 99/02166, WO 00/40529, WO 00/41669, WO 01/92224, WO 02/04434 and WO 02/08213, each of which is incorporated herein in its entirety; (vii) antisense therapies, for example those which are directed to the targets listed above, such as ISIS 2503, an anti-ras antisense; (viii) gene therapy approaches, including for example approaches to replace aberrant genes such as aberrant p53 or aberrant BRCA1 or BRCA2, GDEPT (gene directed enzyme pro-drug therapy) approaches such as those using cytosine deaminase, thymidine kinase or a bacterial nitroreductase enzyme and approaches to increase patient tolerance to chemotherapy or radiotherapy such as multi-drug resistance gene therapy; and (ix) immunotherapeutic approaches, including for example ex vivo and in vivo approaches to increase the immunogenicity of patient tumor cells, such as transfection with cytokines such as interleukin 2, interleukin 4 or granulocyte macrophage colony stimulating factor, approaches to decrease T cell

anergy, approaches using transfected immune cells such as cytokine transfected dendritic cells, approaches using cytokine transfected tumor cell lines and approaches using anti-idiotypic antibodies.

Such active ingredients are suitably present in combination in amounts that are effective for the purpose intended.

Active ingredients may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methyl methacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences, 16th edition, Osol, A. (ed.) (1980).

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semi-permeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g. films, or microcapsules.

The compositions to be used for in vivo administration are generally sterile. Sterility may be readily accomplished, e.g., by filtration through sterile filtration membranes.

The bispecific antibody according to the invention, especially the bispecific bivalent antibody, have valuable biological properties (determined in assays as described in Examples 3, 4, 10, 11, 16, 17 and 19):

A) the bispecific TWEAK/IL17 antibody inhibits

a) TWEAK induced proliferation of human fibroblast-like synoviocytes-rheumatoid arthritis (HFLS-RA) with an IC50 value of 0.2 nM or lower (e.g. with an IC50 value between 0.2 nM and 0.0 nM); preferably with an IC50 value of 0.1 nM or lower (as determined in Example 17 as IC50/per valency); and

b) IL17 induced IL6 cytokine stimulation of human fibroblast-like synoviocytes-rheumatoid arthritis (HFLS-RA) with an IC50 value of 3.0 nM or lower (e.g. with an IC50 value between 3.0 nM and 0.0 nM);

preferably with an IC50 value of 2.0 nM or lower (as determined in Example 16); and

c) IL17 induced IL8 cytokine stimulation of human fibroblast-like synoviocytes-rheumatoid arthritis (HFLS-RA)with an IC50 value of 2.0 nM or lower (e.g. with an IC50 value between 2.0 nM and 0.0 nM); preferably with an IC50 value of 1.5 nM or lower (as determined in Example 16);

B) the bispecific TWEAK/IL17 antibody is capable to simultaneously bind to human <TWEAK> and human <IL17>, wherein the signal intensity (in RU) (in a surface plasmon resonance assay (Example 19) of the binding of the bispecific TWEAK/IL17 antibody to a 1:1 mixture from human <TWEAK> and human <IL17> is at least the same or higher compared to the sum of a) the signal intensity (in RU) of the binding of the bispecific TWEAK/IL17 antibody to human <TWEAK> alone and b) the signal intensity (in RU) of the binding of the bispecific TWEAK/IL17 antibody to human <IL17> alone (as determined in Example 19);

C) the bispecific TWEAK/IL17 antibody shows no cross reactivity with IL17B, IL17C, IL17D, IL17F (which means that the binding to IL17B, IL17C, IL17D and IL17F is 0% compared to the binding to IL17A , which is set as 100%) (as determined in Example 10);

D) the bispecific TWEAK/IL17 antibody inhibits IL17 induced IL6 cytokine stimulation of CCD-25SK cells with an IC50 value of 2.0 nM or lower (e.g. with an IC50 value between 2.0 nM and 0.0 nM); (as determined in Example 11);

E) the bispecific TWEAK/IL17 antibody inhibits IL17 induced IL8 cytokine stimulation of CCD-25SK cells with an IC50 value of 5.0 nM or lower (e.g. with an IC50 value between 5.0 nM and 0.0 nM); preferably with an IC50 value of 2.0 nM or lower; (as determined in Example 11);

F) the bispecific TWEAK/IL17 antibody inhibits human TWEAK/human Fn14 interaction with an IC50 value of 4.0 [ng/ml] or lower (e.g. with an IC50 value between 4.0 [ng/ml] and 0.0 [ng/ml]); preferably with an IC50 value of 3.0 [ng/ml] or lower; (as determined in Example 4);

G) the bispecific TWEAK/IL17 antibody binds to human TWEAK with an KD value of binding affinity of 0.1 nM or lower, and binds to human IL-17 with an KD value of binding affinity of 0.3 nM or lower; (as determined in Example 19); and /or

H) the bispecific TWEAK/IL17 antibody shows a half-life of a complex between soluble human TWEAK (amino acids 99-249 of SEQ ID NO: 68) and antibody of 100 minutes or more at 25°C, measured by Biacore (as determined in Example 19).

The term "human fibroblast-like synoviocytes-rheumatoid arthritis (HFLS-RA)" refers to human adult fibroblast-like synoviocytes obtained from RA patients (human fibroblast-like synoviocytes-rheumatoid arthritis (HFLS-RA)), e.g. to HFLS-RA (Cat. #408RA-05a) obtainable from Cell Applications Inc. (San Diego, CA, USA). Human Fibroblast-Like Synoviocytes-Rheumatoid Arthritis (HFLS-RA) are isolated from synovial tissues obtained from patients with Rheumatoid Arthritis (RA). They are cryopreserved at second passage and can be cultured and propagated at least 5 population doublings. HFLS are long known for their role in joint destruction by producing cytokines and metalloproteinases that contribute to cartilage degradation (Firestein, G.S., et al., J. Immunol. 149 (1992) 1054; Firestein, G.S., et al., Arthritis and Rheumatism 37(5) (1994) 644) Proinflammatory cytokines induce the proliferation, collagenase and aggrecanase production and GM-CSF secretion on HFLS (Alvaro, J.M., et al., J. Clin. Immunol. 13(3) (1993) 212; Yamanishi, Y., et al., J. Immunol. 168(3) (2002) 1405). Ongoing arthritis research also has shown that HFLS express apoptosis and P53 mutations (Firestein, G.S., et al., J. Clin. Invest. 96 (1995) 1631; Firestein, G.S., et al., Am. J. Pathol. 148(6) (1996) 2143). We provide two types of HFLS that are useful cellular models for studying the differences between HFLS-RA and HFLS-OA, such as the expression and regulation of proteases (Firestein, G.S., et al., Am. J. Pathol. 148(6) (1996) 2143) and integrin subunits (Rinaldi, N., et al., Ann. Rheum. Dis. 56(12) (1997) 729).

The "IL17 induced IL6 or IL8 cytokine stimulation" refers to the human IL6 or human IL8 cytokine stimulation by human IL17. The IC50 values for the TWEAK induced proliferation of human fibroblast-like synoviocytes-rheumatoid arthritis (HFLS-RA) and for the IL17 induced IL6 cytokine stimulation of human fibroblast-like synoviocytes-rheumatoid arthritis (HFLS-RA) are calculated as IC50 values per valency (per binding arm against the respective antigen (IL17 in

Example 16 and TWEAK in Example 17). This mean that for a bispecific, bivalent antibody which has one binding arm for each antigen (= which is monovalent for each antigen), the IC50 values are identical to the determined IC50 values (of Examples 16 and 17). To compare such monovalent binding with that of a bispecific, tetravalent antibody which has two binding arms for each antigen (= which is bivalent for each antigen), the IC50 value is calculated with the assumption that the double molar concentration of binding arms was used to achieve the IC50, so the IC50 value is doubled (to get the corresponding 50% inhibitory concentration per binding arm).

The invention comprises a method for the treatment of a patient in need of therapy, characterized by administering to the patient a therapeutically effective amount of an antibody according to the invention.

The invention comprises the use of an antibody according to the invention for the preparation of a medicament for the treatment of cancer, especially colon, lung, or pancreatic cancer or for the treatment of autoimmune diseases, rheumatoid arthritis, psoratic arthritis, muscle diseases, e.g. muscular dystrophy, multiple sclerosis, chronic kidney diseases, bone diseases, e.g. bone degeneration in multiple myeloma, systemic lupus erythematosus, lupus nephritis, and vascular injury.

The invention comprises the use of an antibody according to the invention for the preparation of a medicament for the treatment of systemic lupus erythematosus, or lupus nephritis.

The invention comprises the use of an antibody according to the invention for the treatment of cancer or inflammatory diseases, preferably for the treatment of colon, lung, or pancreatic cancer or for the treatment of autoimmune diseases, rheumatoid arthritis, psoratic arthritis, muscle diseases, e.g. muscular dystrophy, multiple sclerosis, chronic kidney diseases, bone diseases, e.g. bone degeneration in multiple myeloma, systemic lupus erythematosus, lupus nephritis, and vascular injury.

The invention comprises the use of an antibody according to the invention for the treatment of cancer or inflammatory diseases, preferably for the treatment of systemic lupus erythematosus, or lupus nephritis. The invention comprises the use of the bispecific antibodies specifically binding to human TWEAK and human IL17 according to the invention for the treatment (or the bispecific antibodies for use in the treatment) of a patient suffering from cancer, especially from colon, lung,

or pancreatic cancer or from autoimmune diseases, rheumatoid arthritis, psoratic arthritis, psoriasis, muscle diseases, e.g. muscular dystrophy, multiple sclerosis, chronic kidney diseases, bone diseases, e.g. bone degeneration in multiple myeloma, systemic lupus erythematosus, lupus nephritis, and vascular injury.

The invention comprises the use of the bispecific antibodies specifically binding to human TWEAK and human IL17 according to the invention for the treatment (or the bispecific antibodies for use in the treatment) of a patient suffering from systemic lupus erythematosus or lupus nephritis.

The invention comprises the use of the bispecific antibodies specifically binding to human TWEAK and human IL17 according to the invention for the treatment (or the bispecific antibodies for use in the treatment) of a variety of inflammatory, immune and proliferative disorders, including rheumatoid arthritis (RA), osteoarthritis, rheumatoid arthritis osteoporosis, inflammatory fibrosis (e.g. scleroderma, lung fibrosis, and cirrhosis), gingivitis, periodontitis or other inflammatory periodontal diseases, inflammatory bowel disorders (e.g. Crohn's disease, ulcerative colitis and inflammatory bowel disease), asthma (including allergic asthma), allergies, chronic obstructive pulmonary disease (COPD), multiple sclerosis, psoriasis and cancer, ankylosing spondylitis, systemic scleroris, psioriatic arthritis, inflammatory arthritis, osteoarthritis, inflammatory joint disease, autoimmune disease including autoimmune vasculitis, multiple sclerosis, lupus, diabetes (e.g., insulin diabetes), inflammatory bowel disease, transplant rejection, graft vs. host disease, and inflammatory conditions resulting from strain, sprain, cartilage damage, trauma, orthopedic surgery, infection or other disease processes. Other diseases influenced by the dysfunction of the immune system are encompassed within the scope of the invention, including but not limited to, allergies.

Bispecific antibodies of the invention are particularly useful for the treatment, prevention, or amelioration of autoimmune disease and of inflammatory conditions, in particular inflammatory conditions with an aetiology including an autoimmune component such as arthritis (for example rheumatoid arthritis, arthritis chronica progrediente and arthritis deformans) and rheumatic diseases, including inflammatory conditions and rheumatic diseases involving bone loss, inflammatory pain, spondyloarhropathies including ankolsing spondylitis, Reiter syndrome, reactive arthritis, psoriatic arthritis, and enterophathis arthritis, hypersensitivity (including both airways hypersensitivity and dermal hypersensitivity) and allergies.

Specific auto-immune diseases for which the bispecific antibodies as described herein may be employed include autoimmune haematological disorders (including e.g. hemolytic anaemia, aplastic anaemia, pure red cell anaemia and idiopathic thrombocytopenia), systemic lupus erythematosus, inflammatory muscle disorders, polychondritis, sclerodoma, Wegener granulomatosis, dermatomyositis, chronic active hepatitis, myasthenia gravis, psoriasis, Steven-Johnson syndrome, idiopathic sprue, autoimmune inflammatory bowel disease (including e.g. ulcerative colitis, Crohn's disease and Irritable Bowel Syndrome), endocrine ophthalmopathy, Graves' disease, sarcoidosis, multiple sclerosis, primary biliary cirrhosis, juvenile diabetes (diabetes mellitus type I), uveitis (anterior and posterior), keratoconjunctivitis sicca and vernal keratoconjunctivitis, interstitial lung fibrosis, psoriatic arthritis and glomerulonephritis (with and without nephrotic syndrome, e.g. including idiopathic nephrotic syndrome or minimal change nephropathy), tumors, multiple sclerosis, inflammatory disease of skin and cornea, myositis, loosening of bone implants, metabolic disorders, such as atherosclerosis, diabetes, and dislipidemia. The bispecific antibodies according to the invention are also useful for the treatment, prevention, or amelioration of asthma, bronchitis, pneumoconiosis, pulmonary emphysema, and other obstructive or inflammatory diseases of the airways.

The invention comprises also a method for the treatment of a patient suffering from such disease.

The invention further provides a method for the manufacture of a pharmaceutical composition comprising an effective amount of an antibody according to the invention together with a pharmaceutically acceptable carrier and the use of the antibody according to the invention for such a method.

The invention also provides the use of an antibody according to the invention in an effective amount for the manufacture of a pharmaceutical agent, preferably together with a pharmaceutically acceptable carrier, for the treatment of a patient suffering from cancer, especially from colon, lung, or pancreatic cancer or from autoimmune diseases, rheumatoid arthritis, psoratic arthritis, muscle diseases, e.g. muscular dystrophy, multiple sclerosis, chronic kidney diseases, bone diseases, e.g. bone degeneration in multiple myeloma, systemic lupus erythematosus, lupus nephritis, and vascular injury.

The invention also provides the use of an antibody according to the invention in an effective amount for the manufacture of a pharmaceutical agent, preferably together with a pharmaceutically acceptable carrier, for the treatment of a patient suffering from of autoimmune disease and of inflammatory conditions, in particular inflammatory conditions with an aetiology including an autoimmune component such as arthritis (for example rheumatoid arthritis, arthritis chronica progrediente and arthritis deformans) and rheumatic diseases, including inflammatory conditions and rheumatic diseases involving bone loss, inflammatory pain, spondyloarhropathies including ankolsing spondylitis, Reiter syndrome, reactive arthritis, psoriatic arthritis, and enterophathis arthritis, hypersensitivity (including both airways hypersensitivity and dermal hypersensitivity) and allergies. Specific auto-immune diseases for which the bispecific antibodies as described herein may be employed include autoimmune haematological disorders (including e.g. hemolytic anaemia, aplastic anaemia, pure red cell anaemia and idiopathic thrombocytopenia), systemic lupus erythematosus, inflammatory muscle disorders, polychondritis, sclerodoma, Wegener granulomatosis, dermatomyositis, chronic active hepatitis, myasthenia gravis, psoriasis, Steven-Johnson syndrome, idiopathic sprue, autoimmune inflammatory bowel disease (including e.g. ulcerative colitis, Crohn's disease and Irritable Bowel Syndrome), endocrine ophthalmopathy, Graves' disease, sarcoidosis, multiple sclerosis, primary biliary cirrhosis, juvenile diabetes (diabetes mellitus type I), uveitis (anterior and posterior), keratoconjunctivitis sicca and vernal keratoconjunctivitis, interstitial lung fibrosis, psoriatic arthritis and glomerulonephritis (with and without nephrotic syndrome, e.g. including idiopathic nephrotic syndrome or minimal change nephropathy), tumors, multiple sclerosis, inflammatory disease of skin and cornea, myositis, loosening of bone implants, metabolic disorders, such as atherosclerosis, diabetes, and dislipidemia. The bispecific antibodies according to the invention are also useful for the treatment, prevention, or amelioration of asthma, bronchitis, pneumoconiosis, pulmonary emphysema, and other obstructive or inflammatory diseases of the airways.

In another aspect, the present invention provides a composition, e.g. a pharmaceutical composition, containing a bispecific TWEAK/IL17 antibody as described herein, formulated together with a pharmaceutically acceptable carrier, e.g., for use in any of the above therapeutic methods.

As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and

absorption/resorption delaying agents, and the like that are physiologically compatible. Preferably, the carrier is suitable for injection or infusion.

A composition of the present invention can be administered by a variety of methods known in the art. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results.

Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is known in the art. In addition to water, the carrier can be, for example, an isotonic buffered saline solution.

In another embodiment, a pharmaceutical formulation comprises any of the bispecific TWEAK/IL17 antibodies provided herein and at least one additional therapeutic agent, e.g., as described below.

Antibodies of the invention can be used either alone or in combination with other agents in a therapy. For instance, an antibody of the invention may be co-administered with at least one additional therapeutic agent. In certain embodiments, an additional therapeutic agent is a. immunosuppressive or immunomodulating agents or other anti-inflammatory agents. For example, the bispecific antibodies as described herein may be used in combination with DMARD, e.g. Gold salts, sulphasalazine, antimalarias, methotrexate, D-penicillamine, azathioprine, mycophenolic acid, cyclosporine A, tacrolimus, sirolimus, minocycline, leflunomide, glococorticoids; a calcineurin inhibitor, e.g. cyclosporin A or FK 506; a modulator of lymphocyte recirculation, e.g. FTY720 and FTY720 analogs; a mTOR inhibitor, e.g. rapamycin, 40-O-(2-hydroxyethyl)-rapamycin, CCI779, ABT578, AP23573 or TAFA-93; an ascomycin having immuno-suppressive properties, e.g. ABT-281, ASM981, etc.; corticosteroids; cyclophosphamide; azathioprene; methotrexate; leflunomide; mizoribine; mycophenolic acid; myco-pheno-late mofetil; 15-deoxyspergualine or an immunosuppressive homologue, analogue or derivative thereof; immunosuppressive monoclonal antibodies, e.g., monoclonal antibodies to leukocyte receptors, e.g., MHC, CD2, CD3, CD4, CD7, CD8, CD25, CD28, CD40. CD45, CD58, CD80, CD86 or their ligands; other immunomodulatory compounds, e.g. a recombinant binding molecule having at least a portion of the extracellular domain of CTLA4 or a mutant thereof, e.g. an at least extracellular portion of CTLA4 or a mutant thereof joined to a non-CTLA4

protein sequence, e.g. CTLA4Ig (for ex. designated ATCC 68629) or a mutant thereof, e.g. LEA29Y; adhesion molecule inhibitors, e.g. LFA-1 antagonists, ICAM-1 or -3 antagonists, VCAM-4 antagonists or VLA-4 antagonists; or a chemotherapeutic agent, e.g. paclitaxel, gemcitabine, cisplatinum, doxorubicin or 5-fluorouracil; anti TNF agents, e.g. monoclonal antibodies to TNF, e.g. infliximab, adalimumab, CDP870, or receptor constructs to TNF-RI or TNF-RII, e.g. Etanercept, PEG-TNF-RI; blockers of proinflammatory cytokines, IL-1 blockers, e.g. Anakinra or IL-1 trap, AAL160, ACZ 885, IL-6 blockers; chemokines blockers, e.g. inhibitors or activators of proteases, e.g. metalloproteases, anti-IL-15 antibodies, anti-IL-6 antibodies, anti-IL-23 antibodies, anti-CD20 antibodies, NSAIDs, such as aspirin or an anti-infectious agent (the list not limited to the agent mentioned).

Such combination therapies noted above encompass combined administration (where two or more therapeutic agents are included in the same or separate formulations), and separate administration, in which case, administration of the antibody of the invention can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent and/or adjuvant.

An antibody of the invention (and any additional therapeutic agent) can be administered by any suitable means, including parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. Dosing can be by any suitable route, e.g. by injections, such as intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic. Various dosing schedules including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.

Antibodies of the invention would be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The antibody need not be, but is optionally formulated with one or more agents currently used to prevent or treat the disorder in question. The effective amount of such other agents depends on the amount of antibody present in the formulation, the type of disorder

or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as described herein, or about from 1 to 99 % of the dosages described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate.

For the prevention or treatment of disease, the appropriate dosage of an antibody of the invention (when used alone or in combination with one or more other additional therapeutic agents) will depend on the type of disease to be treated, the type of antibody, the severity and course of the disease, whether the antibody is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody, and the discretion of the attending physician. The antibody is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 1 μg/kg to 15 mg/kg (e.g. 0.5mg/kg - 10 mg/kg) of antibody can be an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. One typical daily dosage might range from about 1 μg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment would generally be sustained until a desired suppression of disease symptoms occurs. One exemplary dosage of the antibody would be in the range from about 0.05 mg/kg to about 10 mg/kg. Thus, one or more doses of about 0.5 mg/kg, 2.0 mg/kg, 4.0 mg/kg or 10 mg/kg (or any combination thereof) may be administered to the patient. Such doses may be administered intermittently, e.g. every week or every three weeks (e.g. such that the patient receives from about two to about twenty, or e.g. about six doses of the antibody). An initial higher loading dose, followed by one or more lower doses may be administered. An exemplary dosing regimen comprises administering.. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

Regardless of the route of administration selected, the compounds of the present invention, which may be used in a suitable hydrated form, and/or the pharmaceutical compositions of the present invention, are formulated into pharmaceutically acceptable dosage forms by conventional methods known to those of skill in the art.

Actual dosage levels of the active ingredients in the pharmaceutical compositions of the present invention may be varied so as to obtain an amount of the active

ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient (effective amount). The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present invention employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

Articles of Manufacture

In another aspect of the invention, an article of manufacture containing materials useful for the treatment, prevention and/or diagnosis of the disorders described above is provided. The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, IV solution bags, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is by itself or combined with another composition effective for treating, preventing and/or diagnosing the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an antibody of the invention. The label or package insert indicates that the composition is used for treating the condition of choice. Moreover, the article of manufacture may comprise (a) a first container with a composition contained therein, wherein the composition comprises an antibody of the invention; and (b) a second container with a composition contained therein, wherein the composition comprises a further cytotoxic or otherwise therapeutic agent. The article of manufacture in this embodiment of the invention may further comprise a package insert indicating that the compositions can be used to treat a particular condition. Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

Description of the Sequences

SEQ ID NO:	1	CDR1H <TWEAK> 301
SEQ ID NO:	2	CDR2H <TWEAK> 301
SEQ ID NO:	3	CDR3H <TWEAK> 301
SEQ ID NO:	4	CDR1L <TWEAK> 301
SEQ ID NO:	5	CDR2L <TWEAK> 301
SEQ ID NO:	6	CDR3L <TWEAK> 301
SEQ ID NO:	7	Rabbit variable heavy chain domain (VH) <TWEAK> 301
SEQ ID NO:	8	Rabbit variable light chain domain (VL) <TWEAK> 301
SEQ ID NO:	9	CDR1H <TWEAK> 304
SEQ ID NO:	10	CDR2H <TWEAK> 304
SEQ ID NO:	11	CDR3H <TWEAK> 304
SEQ ID NO:	12	CDR1L <TWEAK> 304
SEQ ID NO:	13	CDR2L <TWEAK> 304
SEQ ID NO:	14	CDR3L <TWEAK> 304
SEQ ID NO:	15	Rabbit variable heavy chain domain (VH) <TWEAK> 304
SEQ ID NO:	16	Rabbit variable light chain domain (VL) <TWEAK> 304
SEQ ID NO:	17	CDR1H <TWEAK> 305
SEQ ID NO:	18	CDR2H <TWEAK> 305
SEQ ID NO:	19	CDR3H <TWEAK> 305

SEQ ID NO:	20	CDR1L <TWEAK> 305
SEQ ID NO:	21	CDR2L <TWEAK> 305
SEQ ID NO:	22	CDR3L <TWEAK> 305
SEQ ID NO:	23	Rabbit variable heavy chain domain (VH) <TWEAK> 305
SEQ ID NO:	24	Rabbit variable light chain domain (VL) <TWEAK> 305
SEQ ID NO:	25	Humanized variant of VH, <TWEAK> 305-HC1
SEQ ID NO:	26	Humanized variant of VH, <TWEAK> 305-HC2
SEQ ID NO:	27	Humanized variant of VH, <TWEAK> 305-HC3
SEQ ID NO:	28	Humanized variant of VH, <TWEAK> 305-HC4
SEQ ID NO:	29	Humanized variant of VH, <TWEAK> 305-HC5
SEQ ID NO:	30	Humanized variant of VH, <TWEAK> 305-HC6
SEQ ID NO:	31	Humanized variant of VH, <TWEAK> 305-HC7
SEQ ID NO:	32	Humanized variant of VH, <TWEAK> 305-HC8
SEQ ID NO:	33	Humanized variant of VH, <TWEAK> 305-HC9
SEQ ID NO:	34	Humanized variant of VH, <TWEAK> 305-HC10
SEQ ID NO:	35	Humanized variant of VH, <TWEAK> 305-HC11
SEQ ID NO:	36	Humanized variant of VL, <TWEAK> 305-LC1
SEQ ID NO:	37	Humanized variant of VL, <TWEAK> 305-LC2
SEQ ID NO:	38	Humanized variant of VL, <TWEAK> 305-LC3
SEQ ID NO:	39	Humanized variant of VL, <TWEAK> 305-LC4

SEQ ID NO:	40	Humanized variant of VL, <TWEAK> 305-LC5
SEQ ID NO:	41	Humanized variant of VL, <TWEAK> 305-LC6
SEQ ID NO:	42	Humanized variant of VL, <TWEAK> 305-LC7
SEQ ID NO:	43	Humanized variant of VL, <TWEAK> 305-LC8
SEQ ID NO:	44	Humanized variant of VL, <TWEAK> 305-LC9
SEQ ID NO:	45	Humanized variant of VL, <TWEAK> 305-LC10
SEQ ID NO:	46	Humanized variant of VL, <TWEAK> 305-LC11
SEQ ID NO:	47	CDR1H <IL17> 9C6-2B6
SEQ ID NO:	48	CDR2H <IL17> 9C6-2B6
SEQ ID NO:	49	CDR3H <IL17> 9C6-2B6
SEQ ID NO:	50	CDR1L <IL17> 9C6-2B6
SEQ ID NO:	51	CDR2L <IL17> 9C6-2B6
SEQ ID NO:	52	CDR3L <IL17> 9C6-2B6
SEQ ID NO:	53	Mouse variable heavy chain domain (VH), <IL17> 9C6-2B6
SEQ ID NO:	54	Mouse variable light chain domain (VL) <IL17> 9C6-2B6
SEQ ID NO:	55	Humanized variant of VH, <IL17> 9C6-2B6-HC134
SEQ ID NO:	56	Humanized variant of VH, <IL17> 9C6-2B6-HC136
SEQ ID NO:	57	Humanized variant of VL, <IL17> 9C6-2B6-LC134
SEQ ID NO:	58	Humanized variant of VL, <IL17> 9C6-2B6-LC136
SEQ ID NO	59	Human kappa light chain constant region

SEQ ID NO	60	Human lambda light chain constant region
SEQ ID NO	61	Human IgG1 (Caucasian Allotype) constant region
SEQ ID NO	62	Human IgG1 (Afroamerican Allotype) constant region
SEQ ID NO	63	Human IgG1 L234A/L235A Mutant (Caucasian Allotype)
SEQ ID NO	64	Human IgG1 L234A/L235A/P329G Mutant (Caucasian Allotype)
SEQ ID NO	65	Human IgG4 constant region
SEQ ID NO	66	Human IgG4 S228P/L235E Mutant
SEQ ID NO	67	Human IgG4 S228P/L235E/P329G Mutant
SEQ ID NO	68	Human TWEAK
SEQ ID NO	69	Murine TWEAK
SEQ ID NO	70	Human IL17 (IL17A)
SEQ ID NO	71	Human IL17B
SEQ ID NO	72	Human IL17C
SEQ ID NO	73	Human IL17D
SEQ ID NO	74	Human IL17E
SEQ ID NO	75	Human IL17F
SEQ ID NO	76	Bispecific <Tweak-IL-17> #2 antibody- heavy chain construct 1
SEQ ID NO	77	Bispecific <Tweak-IL-17> #2 antibody- heavy chain construct 2
SEQ ID NO	78	Bispecific <Tweak-IL-17> #2 antibody- light chain construct 1
SEQ ID NO	79	Bispecific <Tweak-IL-17> #2 antibody- light chain construct 2

SEQ ID NO	80	Bispecific <Tweak-IL-17> #4 antibody- heavy chain construct 1
SEQ ID NO	81	Bispecific <Tweak-IL-17> #4 antibody- heavy chain construct 2
SEQ ID NO	82	Bispecific <Tweak-IL-17> #4 antibody- light chain construct 1
SEQ ID NO	83	Bispecific <Tweak-IL-17> #5 antibody-heavy chain construct
SEQ ID NO	84	Bispecific <Tweak-IL-17> #5 antibody- light chain construct
SEQ ID NO	85	Bispecific <Tweak-IL-17> #20 antibody- heavy chain construct 1
SEQ ID NO	86	Bispecific <Tweak-IL-17> #20 antibody- heavy chain construct 2
SEQ ID NO	87	Bispecific <Tweak-IL-17> #20 antibody- light chain construct 1
SEQ ID NO	88	Bispecific <Tweak-IL-17> #21 antibody- heavy chain construct 1
SEQ ID NO	89	Bispecific <Tweak-IL-17> #21 antibody- heavy chain construct 2
SEQ ID NO	90	Bispecific <Tweak-IL-17> #21 antibody- light chain construct 1
SEQ ID NO	91	Bispecific <Tweak-IL-17> #23 antibody- heavy chain construct 1
SEQ ID NO	92	Bispecific <Tweak-IL-17> #23 antibody- heavy chain construct 2
SEQ ID NO	93	Bispecific <Tweak-IL-17> #23 antibody- light chain construct 1
SEQ ID NO	94	Bispecific <Tweak-IL-17> #24 antibody- heavy chain construct 1
SEQ ID NO	95	Bispecific <Tweak-IL-17> #24 antibody- heavy chain construct 2
SEQ ID NO	96	Bispecific <Tweak-IL-17> #24 antibody- light chain construct 1
SEQ ID NO	97	Bispecific <Tweak-IL-17> #24 antibody- light chain construct 2
SEQ ID NO	98	Human Fn14 (TWEAK receptor)
SEQ ID NO	99	Human IL6

SEQ ID NO	100	Human IL8

In the following, embodiments of the invention are listed:

1. A bispecific antibody comprising a first antigen-binding site that specifically binds to human TWEAK and a second antigen-binding site that specifically binds to human IL17.

2. The bispecific, bivalent antibody according to embodiment 1, wherein the bispecific antibody inhibits
a) TWEAK induced proliferation of human fibroblast-like synoviocytes-rheumatoid arthritis (HFLS-RA) with an IC50 value of 0.2 nM or lower; and
b) IL17 induced IL6 cytokine stimulation of human fibroblast-like synoviocytes-rheumatoid arthritis (HFLS-RA) with an IC50 value of 3.0 nM or lower; and
c) IL17 induced IL8 cytokine stimulation of human fibroblast-like synoviocytes-rheumatoid arthritis (HFLS-RA) with an IC50 value of 2.0 nM or lower.

3. The bispecific, bivalent antibody according to embodiment 1, characterized in that the bispecific antibody is bivalent.

4. The bispecific antibody according to any of embodiments 1 to 3, characterized in that

i) said first antigen-binding site comprises
a) CDR1H of SEQ ID NO:17, CDR2H of SEQ ID NO:18, CDR3H of SEQ ID NO:19, and CDR1L of SEQ ID NO:20, CDR2L of SEQ ID NO:21, CDR3L of SEQ ID NO:22; or
b) CDR1H of SEQ ID NO:1, CDR2H of SEQ ID NO:2, CDR3H of SEQ ID NO:3, and CDR1L of SEQ ID NO:4, CDR2L of SEQ ID NO:5, CDR3L of SEQ ID NO:6; or
c) CDR1H of SEQ ID NO:9, CDR2H of SEQ ID NO:10, CDR3H of SEQ ID NO:11, and CDR1L of SEQ ID NO:12, CDR2L of SEQ ID NO:13, CDR3L of SEQ ID NO:14; and

ii) said second antigen-binding site comprises
CDR1H of SEQ ID NO:47, CDR2H of SEQ ID NO:48, CDR3H of SEQ ID NO:49, and CDR1L of SEQ ID NO:50, CDR2L of SEQ ID NO:51, CDR3L of SEQ ID NO:52.

5. A chimeric or humanized variant of the bispecific, antibody according to embodiment 4.

6. The bispecific antibody according to any of embodiments 1 to 3, characterized in that

i) said first antigen-binding site comprises
a variable heavy chain domain (VH) of SEQ ID NO:25, of SEQ ID NO:26, of SEQ ID NO:27, of SEQ ID NO:28, of SEQ ID NO:29, of SEQ ID NO:30, of SEQ ID NO:31, of SEQ ID NO:32, of SEQ ID NO:33, of SEQ ID NO:34, or of SEQ ID NO:35, and a variable light chain domain of SEQ ID NO:26, of SEQ ID NO:37, of SEQ ID NO:38, of SEQ ID NO:39, of SEQ ID NO:40, of SEQ ID NO:41, of SEQ ID NO:42, of SEQ ID NO:43, of SEQ ID NO:44, of SEQ ID NO:45, or of SEQ ID NO:46; and

ii) said second antigen-binding site comprises
a variable heavy chain domain (VH) of SEQ ID NO:55, or of SEQ ID NO:56, and a variable light chain domain of SEQ ID NO:57, or of SEQ ID NO:58.

7. The bispecific antibody according to any of embodiments 1 to 3, characterized in that

i) said first antigen-binding site comprises
a variable heavy chain domain (VH) of SEQ ID NO:28, and a variable light chain domain of SEQ ID NO:37; and

ii) said second antigen-binding site comprises
a) a variable heavy chain domain (VH) of SEQ ID NO:56, and a variable light chain domain of SEQ ID NO:58; or
b) a variable heavy chain domain (VH) of SEQ ID NO:55, and a variable light chain domain of SEQ ID NO:57.

8. The bispecific antibody according to any of embodiments 1 to 3, characterized in that

i) said first antigen-binding site comprises
a variable heavy chain domain (VH) of SEQ ID NO:28, and a variable light chain domain of SEQ ID NO:37; and

ii) said second antigen-binding site comprises
a variable heavy chain domain (VH) of SEQ ID NO:56, and a variable light chain domain of SEQ ID NO:58.

9. The bispecific antibody according to any of embodiments 1 to 3, characterized in that

i) said first antigen-binding site comprises
a variable heavy chain domain (VH) of SEQ ID NO:28, and a variable light chain domain of SEQ ID NO:37; and

ii) said second antigen-binding site comprises
a variable heavy chain domain (VH) of SEQ ID NO:55, and a variable light chain domain of SEQ ID NO:57.

10. The bispecific antibody according to any of the preceding embodiments, characterized in that it is of IgG1 or IgG4 subclass.

11. The bispecific antibody according to any of the preceding embodiments, characterized in being of IgG1 subclass with the mutations L234A and L235A (numbering according to the EU index of Kabat).

12. The bispecific antibody according to any of the preceding embodiments, characterized in being of IgG1 subclass with the mutations L234A, L235A and P329G (numbering according to the EU index of Kabat).

13. The bispecific antibody according to any of the preceding embodiments, characterized in being of IgG4 subclass with the mutations S228P and L235E (numbering according to the EU index of Kabat).

14. The bispecific antibody according to any of the preceding embodiments, characterized in being of IgG4 subclass with the mutations S228P, L235E and P329G (numbering according to the EU index of Kabat).

15. Pharmaceutical composition characterized by comprising an antibody according to embodiments 1 to 14.

16. Use of an antibody according to embodiments 1 to 14 for the manufacture of a pharmaceutical composition.

17. An antibody according to embodiments 1 to 14 for use in the treatment of cancer, or inflammatory diseases, autoimmune diseases, rheumatoid arthritis, psoratic arthritis, muscle diseases, e.g. muscular dystrophy, multiple sclerosis, chronic kidney diseases, bone diseases, e.g. bone degeneration in multiple myeloma, systemic lupus erythematosus, lupus nephritis, and vascular injury.

18. Use of an antibody according to embodiments 1 to 14 for manufacture of a medicament for the treatment of cancer, or inflammatory diseases, autoimmune diseases, rheumatoid arthritis, psoratic arthritis, muscle diseases, e.g. muscular dystrophy, multiple sclerosis, chronic kidney diseases, bone diseases, e.g. bone degeneration in multiple myeloma, systemic lupus erythematosus, lupus nephritis, and vascular injury.

19. Nucleic acid encoding an antibody according to embodiments 1 to 14.

20. Expression vectors characterized by comprising a nucleic acid according to embodiment 19 for the expression of the bispecific antibody according to embodiments 1 to 14 in a prokaryotic or eukaryotic host cell.

21. Prokaryotic or eukaryotic host cell comprising a vector according to embodiment 20.

22. Method for the production of a recombinant antibody according to embodiments 1 to 14, characterized by expressing a nucleic acid according to embodiment 19 in a prokaryotic or eukaryotic host cell and recovering said antibody from said cell or the cell culture supernatant.

23. Method for the treatment of a patient suffering from cancer or from inflammatory diseases, autoimmune diseases, rheumatoid arthritis, psoratic arthritis, muscle diseases, e.g. muscular dystrophy, multiple sclerosis, chronic kidney diseases, bone diseases, e.g. bone degeneration in multiple myeloma, systemic lupus erythematosus, lupus nephritis, and vascular injury,

characterized by administering to the patient an antibody according to embodiments 1 to 14.

The following examples, figures and sequence listing are provided to aid the understanding of the present invention, the true scope of which is set forth in the appended claims. It is understood that modifications can be made in the procedures set forth without departing from the spirit of the invention.

Examples

Materials & general methods

General information regarding the nucleotide sequences of human immunoglobulins light and heavy chains is given in: Kabat, E.A., et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991). Amino acids of antibody constant chains are numbered and referred to according to EU index according to Kabat (Kabat, E.A., et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD, (1991)).

Recombinant DNA techniques

Standard methods were used to manipulate DNA as described in Sambrook, J., et al., Molecular Cloning: A Laboratory Manual; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989). The molecular biological reagents were used according to the manufacturer's instructions.

Gene synthesis

Desired gene segments can be prepared from oligonucleotides made by chemical synthesis. The gene segments, which are flanked by singular restriction endonuclease cleavage sites, were assembled by annealing and ligation of oligonucleotides including PCR amplification and subsequently cloned via the indicated restriction sites e.g. KpnI/ SacI or AscI/PacI into a pPCRScript (Stratagene) based pGA4 cloning vector. The DNA sequences of the subcloned gene fragments were confirmed by DNA sequencing.

Gene synthesis fragments were ordered according to given specifications at Geneart (Regensburg, Germany). All gene segments encoding light and heavy chains of Tweak/IL-17 bispecific antibodies were synthesized with a 5'-end DNA sequence coding for a leader peptide which targets proteins for secretion in eukaryotic cells, and unique restriction sites at the 5' and 3' ends of the synthesized

gene. DNA sequences carrying disulfide stabilized "knobs-into-hole" modified heavy chains were designed with S354C and T366W mutations in the "knobs" heavy chain and Y349C, T366S, L368A and Y407V mutations in the "hole" heavy chain.

DNA sequence determination

DNA sequences were determined by double strand sequencing performed at MediGenomix GmbH (Martinsried, Germany) or Sequiserve GmbH (Vaterstetten, Germany).

DNA and protein sequence analysis and sequence data management

The GCG's (Genetics Computer Group, Madison, Wisconsin) software package version 10.2 and Infomax's Vector NT1 Advance suite version 11.5 was used for sequence creation, mapping, analysis, annotation and illustration.

Expression vectors

For the expression of the described antibodies variants of expression plasmids for transient expression (e.g. in HEK293 EBNA or HEK293-F cells) or for stable expression (e.g. in CHO cells) based either on a cDNA organization with a CMV-Intron A promoter or on a genomic organization with a CMV promoter were applied.

In case of IgG4_SPLE the intron between the CH1 domain and the hinge domain was removed, keeping the remainder of the antibody gene in a genomic organization. The intron-deleted version of IgG4_SPLE no longer shows hingeless antibodies as a result of a splice artefact commonly seen in IgG4_SPLE encoded in total genomic organization.

Beside the antibody expression cassette the vectors contained:

- an origin of replication which allows replication of this plasmid in E. coli, and
- a ß-lactamase gene which confers ampicillin resistance in E. coli.

The transcription unit of the antibody gene is composed of the following elements:

- unique restriction site(s) at the 5' end
- the immediate early enhancer and promoter from the human cytomegalovirus,
- followed by the Intron A sequence in the case of the cDNA organization,

- a 5'-untranslated region of a human antibody gene,
- an immunoglobulin heavy chain signal sequence,
- the human antibody chain (heavy chain, modified heavy chain or light chain) either as cDNA or as genomic organization with an the immunoglobulin exon-intron organization
- a 3' untranslated region with a polyadenylation signal sequence, and
- unique restriction site(s) at the 3'end. For transient and stable transfections larger quantities of the plasmids were prepared by plasmid preparation from transformed E. coli cultures (Nucleobond AX, Macherey-Nagel).

Cell culture techniques

Standard cell culture techniques were used as described in Current Protocols in Cell Biology (2000), Bonifacino, J.S., Dasso, M., Harford, J.B., Lippincott-Schwartz, J. and Yamada, K.M. (eds.), John Wiley & Sons, Inc..

Transient transfections in HEK293-F system

Recombinant immunoglobulin variants were expressed by transient transfection of human embryonic kidney 293-F cells using the FreeStyle™ 293 Expression System according to the manufacturer's instruction (Invitrogen, USA). Briefly, suspension FreeStyle™ 293-F cells were cultivated in FreeStyle™ 293 Expression medium at 37°C/8 % CO_2 and the cells were seeded in fresh medium at a density of 1-2x10^6 viable cells/ml on the day of transfection. DNA-293fectin™ complexes were prepared in Opti-MEM® I medium (Invitrogen, USA) using 325 µl of 293fectin™ (Invitrogen, Germany) and 250 µg of heavy and light chain plasmid DNA in a 1:1 molar ratio for a 250 ml final transfection volume for monospecific parent antibodies. "Knobs-into-hole" DNA-293fectin complexes with two heavy chains and one light chain were prepared in Opti-MEM® I medium (Invitrogen, USA) using 325 µl of 293fectin™ (Invitrogen, Germany) and 250 µg of "Knobs-into-hole" heavy chain 1 and 2 and light chain plasmid DNA generally in a 1:1:1 molar ratio for a 250 ml final transfection volume (For format described in WO2011/117330 ("bispecific one-armed scFab antibodies")). For expression yield and product quality optimization the ratio can be varied. DNA-293fectin complexes were prepared in Opti-MEM® I medium (Invitrogen, USA) using 325 µl of 293fectin™ (Invitrogen, Germany) and 250 µg of "Knobs-into-hole" heavy chain 1 and 2 and light chain 1 and 2 plasmid DNA in a 1:1:1:1 molar ratio for a 250 ml final transfection volume (For the format described in WO 2009/080253 ("CrossMabs" or "CH1-CL domain exchanged antibodies")). For expression yield and product quality optimization the ratio can be varied. Antibody containing cell

culture supernatants were harvested 7 days after transfection by centrifugation at 14000 g for 30 minutes and filtered through a sterile filter (0.22 µm). Supernatants were stored at -20° C until purification.

Protein determination

The protein concentration of purified antibodies and derivatives was determined by determining the optical density (OD) at 280 nm, using the molar extinction coefficient calculated on the basis of the amino acid sequence according to Pace, C.N., et. al., Protein Science 4 (1995) 2411-1423.

Antibody concentration determination in supernatants

The concentration of antibodies and derivatives in cell culture supernatants was estimated by immunoprecipitation with Protein A Agarose-beads (Roche). 60 µL Protein A Agarose beads are washed three times in TBS-NP40 (50 mM Tris, pH 7.5, 150 mM NaCl, 1 % Nonidet-P40). Subsequently, 1 -15 mL cell culture supernatant are applied to the Protein A Agarose beads pre-equilibrated in TBS-NP40. After incubation for at 1 h at room temperature the beads are washed on an Ultrafree-MC-filter column (Amicon] once with 0.5 mL TBS-NP40, twice with 0.5 mL 2x phosphate buffered saline (2xPBS, Roche) and briefly four times with 0.5 mL 100 mM Na-citrate pH 5,0. Bound antibody is eluted by addition of 35 µl NuPAGE® LDS Sample Buffer (Invitrogen). Half of the sample is combined with NuPAGE® Sample Reducing Agent or left unreduced, respectively, and heated for 10 min at 70°C. Consequently, 20 µl are applied to an 4-12 % NuPAGE® Bis-Tris SDS-PAGE (Invitrogen) (with MOPS buffer for non-reduced SDS-PAGE and MES buffer with NuPAGE® Antioxidant running buffer additive (Invitrogen) for reduced SDS-PAGE) and stained with Coomassie Blue.

The concentration of antibodies and derivatives in cell culture supernatants was measured by Protein A-HPLC chromatography. Briefly, cell culture supernatants containing antibodies and derivatives that bind to Protein A were applied to a HiTrap Protein A column (GE Healthcare) in 50 mM K2HPO4, 300 mM NaCl, pH 7.3 and eluted from the matrix with 550 mM acetic acid, pH 2.5 on a Dionex HPLC-System. The eluted protein was quantified by UV absorbance and integration of peak areas. A purified standard IgG1 antibody served as a standard.

Alternatively, the concentration of antibodies and derivatives in cell culture supernatants was measured by Sandwich-IgG-ELISA. Briefly, StreptaWell High Bind Strepatavidin A-96 well microtiter plates (Roche) were coated with

100 µL/well biotinylated anti-human IgG capture molecule F(ab')2<h-Fcgamma> BI (Dianova) at 0.1 µg/mL for 1 h at room temperature or alternatively over night at 4°C and subsequently washed three times with 200 µL/well PBS, 0.05 % Tween (PBST, Sigma). 100 µL/well of a dilution series in PBS (Sigma) of the respective antibody containing cell culture supernatants was added to the wells and incubated for 1-2 h on a microtiterplate shaker at room temperature. The wells were washed three times with 200 µL/well PBST and bound antibody was detected with 100 µl F(ab')2<hFcgamma>POD (Dianova) at 0.1 µg/mL as detection antibody for 1-2 h on a microtiterplate shaker at room temperature. Unbound detection antibody was washed away three times with 200 µL/well PBST and the bound detection antibody was detected by addition of 100 µL ABTS/well. Determination of absorbance was performed on a Tecan Fluor Spectrometer at a measurement wavelength of 405 nm (reference wavelength 492 nm).

Purification of bispecific antibodies

Bispecific antibodies were purified from cell culture supernatants by affinity chromatography using Protein A-Sepharose™ (GE Healthcare, Sweden) and Superdex200 size exclusion chromatography. Briefly, sterile filtered cell culture supernatants were applied on a HiTrap ProteinA HP (5 ml) column equilibrated with PBS buffer (10 mM Na_2HPO_4, 1 mM KH_2PO_4, 137 mM NaCl and 2.7 mM KCl, pH 7.4). Unbound proteins were washed out with equilibration buffer. Antibody and antibody variants were eluted with 0.1 M citrate buffer, pH 2.8, and the protein containing fractions were neutralized with 0.1 ml 1 M Tris, pH 8.5. Then, the eluted protein fractions were pooled, concentrated with an Amicon Ultra centrifugal filter device (MWCO: 30 K, Millipore) to a volume of 3 ml and loaded on a Superdex200 HiLoad 120 ml 16/60 gel filtration column (GE Healthcare, Sweden) equilibrated with 20mM Histidin, 140 mM NaCl, pH 6.0. Fractions containing purified bispecific antibodies with less than 5 % high molecular weight aggregates were pooled and stored as 1.0 mg/ml aliquots at -80°C.

SDS-PAGE

The NuPAGE® Pre-Cast gel system (Invitrogen) was used according to the manufacturer's instruction. In particular, 4-20 % NuPAGE® Novex® TRIS-Glycine Pre-Cast gels and a Novex® TRIS-Glycine SDS running buffer were used. Reducing of samples was achieved by adding NuPAGE® sample reducing agent prior to running the gel.

Analytical size exclusion chromatography

Size exclusion chromatography for the determination of the aggregation and oligomeric state of antibodies was performed by HPLC chromatography. Briefly, Protein A purified antibodies were applied to a Tosoh TSKgel G3000SW column in 300 mM NaCl, 50 mM KH2PO4/K2HPO4, pH 7.5 on an Agilent HPLC 1100 system or to a Superdex 200 column (GE Healthcare) in 2 x PBS on a Dionex HPLC-System. The eluted protein was quantified by UV absorbance and integration of peak areas. BioRad Gel Filtration Standard 151–1901 served as a standard.

Mass spectrometry

The total deglycosylated mass of the bispecific antibodies was determined and confirmed via electrospray ionization mass spectrometry (ESI-MS). Moreover potential sideproducts such as LC and HC mispairing were detected and relatively quantified. Briefly, 100 µg purified antibodies at a protein concentration of up to 3 mg/ml were deglycosylated with 14 or 28 U N-Glycosidase F (Roche) in 100 mM NaH2PO4/Na2HPO4, pH 7 at 37 or 45°C for 16 or 2 h and subsequently desalted via HPLC on a Sephadex G25 column (GE Healthcare). The mass of the respective heavy and light chains was determined by ESI-MS after deglycosylation and reduction. In brief, 50 µg antibody in 115 µl were incubated at 37°C for 30 min with 60 µl 0.5 M TCEP in 4 M Guanidine-hydrochloride and 50 µl 8 M Guanidine-hydrochloride and subsequently desalted. The total mass and the mass of the reduced heavy and light chains were determined via ESI-MS on a maXis UHR-TOF (Bruker) MS system equipped with a TriVersa NanoMate (Advion) source.

Example 1

Generation of TWEAK antigen binding sites via immunization (Generation of parent TWEAK antibodies from which the TWEAK antigen binding sites for the TWEAK/IL17 bispecific antibodies can be derived)

Immunization of rabbits with human/murine TWEAK

New Zealand White rabbits (Oryctolagus cuniculus) were immunized with 400 µg of recombinant human TWEAK at day 0 with complete Freund's adjuvant, with 200 µg of human TWEAK at days 21, 43 and 65 with incomplete Freund's adjuvant and with 200 µg of murine TWEAK at day 85 with incomplete Freund's adjuvant. All immunizations were done subcutaneously at several sites. Sera were prepared at days 77 and 98 for titer determination. The final boost was done by

intravenous injection of 200 µg of human and 200 µg of murine soluble TWEAK and antibodies were selected based on their ability to bind human and mouse TWEAK (Example 2), neutralize human and mouse TWEAK-Fn14 interaction (Examples 4 and 5), and inhibit IL8 secretion (Example 6). In addition, the half-life of the antibody-TWEAK complex was investigated (Example 3). Anti-tumor efficacy of the antibody was tested in B16BL6 (murine melanoma; metastatic lung subline of B16), SJSA (osteosarcoma, ATCC CRL-2098) and HCT-116 (colon, ATCC CCL-247) xenograft models.

Example 2

Binding to human and mouse TWEAK (ELISA)

Binding of parent anti-TWEAK antibodies to human and mouse TWEAK was determined by ELISA. Human or mouse recombinant TWEAK were immobilized on a 384-well Nunc Maxisorp plate at 1 µg/ml, 25 µl/well, in 0.5 M carbonate coating buffer, pH 9.5, by incubation overnight at 2-8°C. Blocking of the plate with PBS/1 % BSA for 1 h at room temperature was followed by two wash steps (0.1 % Tween® 20 in PBS) and incubation with anti-TWEAK antibodies at different concentrations in blocking buffer or hybridoma supernatants of said antibodies for 1 h at room temperature. After further four washes, antibodies were detected with anti-rabbit-HRP antibody diluted 1:5000 in blocking buffer, for 1 h at room temperature. Signal was developed by addition of ABTS® (Roche Diagnostics GmbH) for 10-30 minutes after another four wash steps. Absorbance was read out at 405 nm.

Example 3

Half-Life determination of the antibody-TWEAK complexes using Biacore

A Biacore 2000 instrument was used with a Biacore streptavidin coated sensor mounted into the system. The system buffer HBS-ET (10 mM HEPES pH 7.4, 150 mM NaCl, 1mM EDTA, 0.05 % Tween® 20) was used at a flow rate of 100 µl/min. The sample buffer was the system buffer. Biotinylated human soluble TWEAK (amino acids 99-249 of SEQ ID NO: 68) and biotinylated murine soluble TWEAK (amino acids 81-225 of SEQ ID NO: 69) was immobilized on different flow cells on the SA sensor at 150 RU each. The flow cell FC1 was used as a blank reference cell. Each antibody was injected into the system as an analyte at 100 nM at 100 µl/min for 2 min association time. The dissociation of the immunecomplexes were monitored for 5 min. The sensor surface was washed with

HBS-ET for 10 seconds and regenerated using 2x2 minutes injections with 10 mM glycine pH 2.25. This procedure was done at 25°C. The kinetically rate limiting step of the complex dissociation phase in the interval [240s-300s] was taken to calculate the dissociation rate kd [1/s] (Biacore Evaluation Software 4.0). According to the equation t1/2 diss = ln(2) / (60 x kd), the half-life of the immunocomplexes in minutes was calculated. Results are shown in tables 1a and 1b, as well as in table 2b.

Table 1a:

Antibody		Human TWEAK t/2 diss [min] 25 °C	Murine TWEAK t/2 diss [min] 25 °C
<TWEAK> 301chi	Chimer[1)]	110	n.d.
<TWEAK> 304chi	Chimer[1)]	37	n.d.
<TWEAK> 305chi	Chimer[1)]	147	39
Chimeric P2D10	Chimer[1)]	76	41

[1)] : human constant regions of the human kappa light chain constant region of SEQ ID NO:59 and the human IgG1 constant region of SEQ ID NO: 61

In a further experiment the half-Life of the antibody-TWEAK complexes (t/2 diss [min] at 25°C) of the chimeric <TWEAK> 305chi and the chimeric version of P2D10 of WO 2006/130374 was determined (both chimeric antibodies have as human constant regions the human kappa light chain constant region of SEQ ID NO:59 and the human IgG1 constant region of SEQ ID NO: 61).

Table 1b:

Antibody		Human TWEAK t/2 diss [min] 25 °C	Murine TWEAK t/2 diss [min] 25 °C
<TWEAK> 305chi	Chimer[1)]	148	41
Chimeric P2D10	Chimer[1)]	76	41

[1)]: human constant regions of the human kappa light chain constant region of SEQ ID NO:59 and the human IgG1 constant region of SEQ ID NO: 61

The antibodies according to the invention show a valuable properties like a half-life of a complex between soluble human TWEAK (amino acids 99-249 of SEQ ID NO: 68) and antibody of 100 minutes or more, preferably of 110 minutes or more at 25°C, measured by Biacore. Anti-TWEAK antibodies showing such half-life are especially preferred for use in the treatment of autoimmune diseases, rheumatoid arthritis, psoratic arthritis, muscle diseases, e.g. muscular dystrophy, multiple sclerosis, chronic kidney diseases, bone diseases, e.g. bone degeneration in multiple myeloma, systemic lupus erythematosus, lupus nephritis, and vascular injury.

Example 4

Neutralization of TWEAK-Fn14 interaction (human)

Blocking of human TWEAK/human Fn14 interaction was shown by receptor interaction ELISA. 96-well Maxisorp® plates (Nunc) were coated with 100 µl 1 µg/ml human Fn14:Fc (extracellular domain of human Fn14 (amino acids 1-75 of SEQ ID NO: 98) fused to Fc portion of human IgG1) in PBS per well for 1.5 h at room temperature and blocked with a solution of 5 % FBS in PBS for 30 minutes at room temperature under shaking. In the meantime, human Flag-tagged soluble TWEAK (amino acids 106-249) at 2.5 ng/ml in blocking solution was incubated with different concentrations of anti-TWEAK antibodies or hybridoma supernatant for 2 h at room temperature under shaking. After washing the Fn14-coated plate once with wash buffer (0.1 % Tween® 20 in PBS), 100 µl of the TWEAK-antibody solution were transferred to each well and the plate was incubated for 1 h at room temperature, followed by four washes with wash buffer. Wells were filled with 100 µl of anti-FLAG-HRP detection antibody, diluted 1:5000 in blocking buffer, and incubated for 1 h at room temperature. After four more wash steps, the

signal was developed by addition of 100 µl 3,3,5,5-Tetramethylbenzidine (TMB) solution for approximately ten minutes. The reaction was stopped by adding 100 µl of 1 N HCl, and absorbance measured at 450 nm (reference wavelength 620 nm). Results are shown in table 2a and b.

Example 5

Neutralization of TWEAK-Fn14 interaction (mouse)

The mouse TWEAK/mouse Fn14 interaction ELISA followed a similar principle as described for the human proteins but used a different detection system, as mouse soluble TWEAK was not tagged. Briefly, Maxisorp plates were coated with mouse Fn14:Fc (extracellular domain of mouse Fn14 (amino acids 1-75 of SEQ ID NO: 98) fused to Fc portion of human IgG1) as described above for human Fn14:Fc, followed by blocking and washing. Mouse soluble TWEAK at 4 ng/ml was pre-incubated with anti-TWEAK-antibodies or hybridoma supernatant in blocking buffer and 100 µl of the mixture were added per well of the Fn14-coated plate. After 1 h of incubation at room temperature and four washes, biotinylated anti-mouse TWEAK antibody at 125 ng/ml in blocking buffer was added for 1 h at room temperature, followed by another four wash steps. The TWEAK antibody was detected by incubation with streptavidin-HRP, diluted 1:5000 in blocking buffer, for 30 minutes at room temperature. Signal was developed and absorbance measured as described above. Results are shown in table 2a and 2b below.

Example 6

IL-8 Secretion ELISA

Blocking of TWEAK activity by anti-TWEAK antibodies in a cellular system was shown in an IL-8 secretion assay using A375 melanoma cells. 10,000 A375 cells (ATCC #CRL1619) were seeded per well of 96-well cell culture plate in 100 µl of growth medium (DMEM with 4.5 g/L glucose, with pyruvate and GlutaMAX™ /10 % FBS) and incubated at 37°C/5 % CO_2 for 48 h. Human recombinant soluble TWEAK was pre-incubated at 300 ng/ml with different concentrations of anti-TWEAK antibodies in growth medium for 30 minutes at room temperature. Then, 50 µl of the mixture were added to each well of the cell plate, followed by another 48 h-incubation to allow for IL-8 secretion. 20 µl of the cell supernatant were removed after centrifuging the plate for five minutes at 200 x g and mixed with 980 µl of RD5P Calibrator Diluent from the "CXCL8

Quantikine ELISA" kit (R&D Systems). IL-8 was detected by the ELISA according to the manufacturer's instructions. Results are shown in table 2a and 2b.

Table 2a:

Antibody		Inhibition of TWEAK-Fn14 Interaction		Inhibition of IL-8 Secretion IC50 [ng/ml]
		Human TWEAK IC50 [ng/ml]	Murine TWEAK IC50 [ng/ml]	
<TWEAK> 301	Rabbit	3.4	4.7	128
TW-304	Rabbit	2.8	3.6	109
<TWEAK> 305	Rabbit	2.5	3.3	99
<TWEAK> 301	Chimer	2.8	6.4	121
<TWEAK> 304chi	Chimer	2.6	4.4	122
<TWEAK> 305chi	Chimer	2.6	4.9	104

Table 2b:

Humanized <TWEAK> 305- antibody	SEQ ID NOs VH, and VL	Interaction Inhibition		Inhibition of IL8-Secretion [ng/ml]	Biacore t/2 diss. [min]			
		human IC50 [ng/ml]	murine IC50 [ng/ml]		25 °C human	25 °C murine	37 °C human	37 °C murine
27	28, 37	2.3	8.2	197	110	19	147	19
28	31, 37	1.5	6.5	165	87	19	115	17
29	32, 37	1.8	5.7	78	146	22	195	21
30	34, 36	2.3	7.6	207	50	17	45	15
31	31, 39	1.7	7.1	94	115	19	151	19
32	32,41	1.5	5.4	134	67	17	50	20
33	28, 39	1.7	6.7	26	127	19	99	15
34	26, 39	1.9	5.9	24	158	22	185	20

Example 7

Determination of the epitope region of <TWEAK> 301, <TWEAK> 304, <TWEAK> 305 (abbreviated as TW-301, TW-304, TW-305)

A Biacore 2000 instrument was used together with the Biacore Evaluation Software 4.0. The sample and system buffer was HBS-ET pH 7.4. Due to strong unspecific binding of the TWEAK analyte to the sensor surface epitope mapping of individual antibodies could not be done as usual by a Biacore cross-competition experiment as described by Johne, B., et al., J. Immun. Meth. 160 (1993) 191-198.

Because of the individual biochemical properties of the TWEAK protein another method had to be developed using TWEAK as ligand. Biotinylated TWEAK was immobilized on the streptavidin-coated chip surface and epitope coverage of consecutively injected antibodies (antibody 1) was measured. The aim was to detect the relative binding levels of a secondary antibody (antibody 2) in the presence of an already bound primary antibody. From these relative binding levels a quotient was calculated (Ab2/Ab1, molar ratio given in %, table 3).

5 nM of biotinylated TWEAK was immobilized at 20 µl/min for 1 min on a streptavidin coated sensor flow cell. Primary and secondary mAbs were consecutively injected at 10 µl/min for 4 min into the system at 100 nM each until saturation of the respective TWEAK epitopes was achieved. As a reference an SA coated flow cell was used.

The system was washed with HBS-ET for 20 sec at 30 µl/min followed by two regeneration steps with 1 min at 30 µl/min 6 M GuadHCl and 100 mM HCl. These regeneration steps stripped off the bound mAbs from the sensor surface and immobilized biotinylated TWEAK was irreversibly denatured. The process was repeated by the immobilization of native biotinylated TWEAK protein (feed batch mode) on the same flow cell until the streptavidin sensor surface was completely saturated by biotinylated TWEAK.

Table 3:

MR %	Antibody 2		
Antibody 1	TW-301chi	TW-304chi	TW-305chi
TW-301chi	0	6	9
TW-304chi	0	0	3
TW-305chi	0	1	0

The crossblocking experiment shows accessibility values of the respective antibodies smaller than 10 %, which is within the noise of this assay. It is clearly shown, that TW-301chi, TW-304chi and TW-305chi bind to the same epitope region.

Example 8

In vivo-inhibition of collagen-induced arthritis (murine model of rheumatoid arthritis) -Antibody <TWEAK> 305- (chimeric; TW305)) inhibits collagen-induced arthritis, a murine model of rheumatoid arthritis.

Male DBA1/J mice (Jackson Laboratory, Bal Harbor, Maine), 6 to 8 weeks old, were immunized with type II bovine collagen in complete Freund's adjuvant and again in incomplete Freund's adjuvant 3 weeks later (boost, day 0). Mice were administered with chimeric antibody <TWEAK> 305- (=TW305,) (10 mg/kg, n=12), Enbrel (10 mg/kg, n=12) or vehicle (phosphate buffered saline, n=12) every other day starting the day before the boost. Mice were examined for arthritis on day 0, 2, 5, 7, 9, 12 and 14 after the boost. Severity of arthritis was scored based on the

following criteria: 1= swelling and/or redness of one digit; 2= swelling in two or more joints; 3= gross swelling of the paw with more than two joints involved; 4= severe arthritis of the entire paw and digits. Compared with vehicle, TW-305 significantly reduced clinical scores ($p<0.05$, day 14), by a similar magnitude to that of the TNF blocker Enbrel.

Example 9

Generation of IL17 antigen binding sites via immunization (Generation of parent IL17 antibodies from which the IL17 antigen binding sites for the TWEAK/IL17 bispecific antibodies can be derived)

Immunization was performed within 20 weeks using 5 female Balb/c mice using 250 (1x) and 100 µg (3x) recombinant human IL17 from Peprotech (http://www.peprotech.com; Cat.No.: 200-17 in 1% PBS with 1% Albumin) per mouse. Hybridoma generation. The mouse lymphocytes were isolated and fused with a mouse myeloma cell line using PEG based standard protocols to generate hybridomas. The resulting hybridomas were then screened for the production of antigen-specific antibodies. From resulting hybridomas mouse clone <IL17> 9C6-2B6 was selected using the binding to IL-17 subtypes measured by ELISA and a cytokine release assay (via the inhibition of IL-17A induced hIL-6 and hIL-8 release. Humanization of mouse clone <IL17> 9C6-2B6 resulted in the humanized variants <IL17> 9C6-2B6-134 (with the humanized variant of VH, <IL17> 9C6-2B6-HC134 and humanized variant of VL, <IL17> 9C6-2B6-LC134 of SEQ ID No. 55 and 57) and <IL17> 9C6-2B6 (with the humanized variant of VH, <IL17> 9C6-2B6-HC136 and humanized variant of VL, <IL17> 9C6-2B6-LC136 of SEQ ID No. 56 and 58).

Example 10

Binding to IL-17 and Crossreactivity with IL17 subtypes measured by ELISA

NUNC® Maxisorp plates (96-well) are coated with recombinant human IL-17 (Peprotech # 200-17, www.peprotech.com) at a concentration of 0,5µg/ml in PBS (100ml/well). Plates are incubated at 37°C on an orbital shaker with agitation for 2 hours. Thereafter coating solution is removed and 100µl/well PBSTC (phosphate buffered saline, 0,0 5% Tween®20, 2 % chicken serum) is added. Plates are incubated at room temperature for 1 hour. Blocking solution is removed and samples (blank: PBSTC, samples (10µg/ml in PBS): anti-human IL-17 antibodies <IL17> 9C6-2B6, <IL17> 9C6-2B6-134, <IL17> 9C6-2B6-136, Mab 16-7178-85

of eBioscience (www.ebioscience.com); MAB 317 of R&D Systems (www.rndsystems.com), NVP-AIN-497 (WO 2006/013107); are added to the plate (100µl/well). Plates are incubated at room temperature with agitation. Samples are removed, plates are washed three times with 200µl/well PBST (phosphate buffered saline, 0,05 % Tween® 20) and second antibody (Goat anti-mouse IgG, Fc gamma, HRP conjugate; Chemicon AP127P, www.millipore.com) for the detection of mouse antibodies or Goat Anti-human IgG, Fc gamma, HRP conjugate (Chemicon AP113P) for the detection of humanized antibodies is added. The second antibody is diluted 1:10000 in PBSTC and plates are incubated for 1 hour at room temperature with agitation. Second antibody is removed, plates are washed three times with 200µl/well PBST (phosphate buffered saline, 0,05 % Tween®20) and 100µl/well ABTS® (Roche Diagnostics GmbH) is added. Optical density is measured at 405/492nm in relation to IL-17A binding (set as 100 %). Binding to other human IL-17 subtypes (IL-17B, IL-17C, IL-17D, IL-17E and IL-17F) were performed with the same assay format. Results are shown in table 4.The results show that the antibody with the most similar binding behavior against the different IL17 subtypes is MAB 317 of R&D Systems (www.rndsystems.com).

Table 4:

IL17-Antibody	Binding (IL-17A binding set as 100)					
	IL17A	IL17B	IL17C	IL17D	IL17E	IL17F
<IL17> 9C6-2B6	100	0	0	0	0	0
<IL17> 9C6-2B6-134	100	0	0	0	0	0
<IL17> 9C6-2B6-136	100	0	0	0	0	0
<IL17> Mab 317	100	0	0	0	0	0
<IL17> 16-7178-85	100	7	97	6	5	5
<IL17> NVP-AIN-497	100	2	2	0	3	62

Example 11

Cytokine release assay, inhibition of IL-17A induced hIL-6 and hIL-8 release in CCD-25SK cells

The assay is performed as detection of hIL-8 production of CCD-25SK cells (skin fibroblasts, ATCC No: CRL-1474) after stimulation with IL-17A and TNF-alpha with preincubation of anti-IL-17 antibodies. CCD-25SK cells have the IL-17 receptor. Soluble IL-17A binds to the these IL-17 receptor. Antibodies against

IL-17A bind to IL-17A. The mechanism is only working in the presence of TNFalpha. Through the binding of IL-17A to the IL-17 receptor, the cells produce hIL-6 and hIL-8 which can be detected by ELISA as a read out. The measured hIL-6 and hIL-8 give the information in which concentrations anti-IL-17 antibodies inhibit the stimulation of CCD-25SK cells by IL-17.

CCD-25SK cells were seeded with a cell density of $2,5x10^4$ cells/well in a 48-well plate (volume 0,45ml/well) and incubated for 24h at 37°C and 5%CO_2. After overnight incubation the cells were treated with anti-IL-17 antibodies for 30 minutes with end concentrations of 9000; 3000;1000; 333,3; 111,1; 37,03; 12,34; and 4,11 ng/ml. Each antibody dilution series was made with medium, 50µl/well (10x concentrated). After 30 min the cells were stimulated with a mixture of 10ng/ml IL-17A and 50pg/ml TNF-alpha. 50µl/well (10x concentrated) and incubated for 24h at 37°C and 5%CO_2. After overnight incubation the supernatants were transferred to 96-well plates and frozen at -20°C as intermediates for hIL-8 ELISA.

hIL-6 and hIL-8 ELISA was performed as follows.100µl diluted capture antibody was added to each well and incubated overnight at 4°C. Dilutions were made with coating buffer. Plates were aspirated, washed with 200µl/well for 3 times, blocked with 200µl/well assay diluent, and incubated for 1h at RT. The plates were aspirated and washed with 200µl/well for 3 times. 100µl standard and samples were added and incubated for 2h at RT. Standard dilution series: 400pg/ml; 200pg/ml; 100pg/ml; 50pg/ml; 25pg/ml; 12,5pg/ml; 6,3pg/ml and assay diluent as negative control. Sample dilution was 1:200. Plates were aspirated and washed with 250µl/well for 4 times. 100µl conjugate was added to each well. The conjugate was prepared with detection antibody and enzyme reagent 1:250 diluted in assay diluent. Plates were aspirated and washed with 250µl/well for 6 times. 100µl substrate was added to each well and incubated for 12 minutes. After incubation the reaction was stopped with 50µl/well 1M H_2SO_4. Read out was performed at 450nm within 30 min with λ correction at 570nm. Results are shown in table 5 (IC_{50} values measured in relation to a maximal inhibition of 80%).

Table 5:

Antibody	IL-6 Inhibition IC50 (nM)	IL-8 Inhibition IC50 (nM)
<IL17> 9C6-2B6	1.6	4.8
<IL17> 9C6-2B6-134	n.d.	4.5
<IL17> 9C6-2B6-136	n.d.	1.8
Mab 317	2.8	n.d

Example 12

Crossreactivity with Cynomolgous IL-17A (Binding Assay)

Relative binding to human and cynomolgous IL17A was determined. The binding assay was performed according to example 2. The results for two separates experiments (one in which mouse IL17 antibodies were compared and one in which human and humanized IL17 antibodies were compared) are shown in tables 6a and 6b.

Table 6a:

IL17 Antibody **(mouse)**	Relative binding to human IL-17A in %	Relative binding to cynomolgous IL-17A in %
Mab 317 (R&D)	100	100
eBio64CAP17	122	124
9C6/2B6	127	134

Table 6b:

IL17 Antibody **(human/ized)**	Relative binding to human IL-17A	Relative binding to cynomolgous IL-17A
NVP-AIN-497	100	100
9C6/2B6-134	108	n.d
9C6/2B6-136	108	149

Example 13

Cynomolgous monkey (Maccaca Fasicularis) cytokine release assay, inhibition of cynomolgous IL-17A induced IL-6 and IL-8 production

Cynomolgous dermal fibroblasts (CDF) cells produce cynomolgous IL-6 and IL-8 in response to human or cynomolgous IL-17A stimulation. The assay is performed to measure the inhibition of this cynomolgous IL-17A stimulated IL-6 and IL-8 production by CDF cells following preincubation of the cells with anti-IL-17 antibodies raised against human IL-17 prior to stimulation.

CDF cells are seeded with a cell density of $2x10^5$ cells/ml in a volume of 0.5ml in a 48-well plate, and incubated overnight at 37°C and 5 %CO_2 to adhere. After overnight incubation , the media is replaced with 400µl fresh media and the cells are treated with anti-IL-17 antibodies for 30 minutes across a range of antibody concentrations (10000, 3000, 1000, 300, 100, 30, 10, 3, 0 ng/ml). Each antibody dilution series is made with medium using 50µl/well (10x concentrated). After 30 min the cells are stimulated with 100ng/ml IL-17A (50µl of 1000ng/ml 10x concentration) and incubated overnight (18h) at 37°C and 5 %CO_2. After the incubation period, supernatants are transferred into fresh tubes and either analyzed immediately or stored at -80°C until analysis by ELISA. hIL-6 and hIL-8 ELISA were shown to be cross-reactive with their respective cynomolgous cytokines and are used to quantitate cytokine levels. For the ELISA's 100µl diluted capture antibody is added to each well and incubated overnight at 4°C. Dilutions are made with coating buffer. Plates are aspirated, washed with 200µl/well for 3 times, blocked with 200µl/well assay diluent, and incubated for 1h at RT. The plates are aspirated and washed with 200µl/well for 3 times. 100µl standard and samples are

added and incubated for 2h at RT according to the manufacturer's instructions. Plates are aspirated and washed with 250µl/well for at least 3 times. 100µl conjugate is added to each well. The conjugate is prepared with detection antibody and enzyme reagent 1:250 diluted in assay diluent. Plates are aspirated and washed with 250µl/well for at least 3 times. 100µl substrate was added to each well and incubated until sufficient color had developed for reading. After incubation the reaction is stopped with 50µl/well 1M H_2SO_4 and read on the plate reader at a wavelength of 450nm within 30 min.

Example 14

Expression and purification of bispecific <TWEAK-IL-17> antibody molecules <Tweak-IL-17> #2, <Tweak-IL-17> #4, <Tweak-IL-17> #20, <Tweak-IL-17> #21, <Tweak-IL-17> #23, <Tweak-IL-17> #24, <Tweak-IL-17> #5

Light and heavy chains of the following bispecific antibodies <Tweak-IL-17> #2, <Tweak-IL-17> #4, <Tweak-IL-17> #20, <Tweak-IL-17> #21, <Tweak-IL-17> #23, <Tweak-IL-17> #5 (based on the antigen binding sites (VH/VL) as described in the Table 7 below) were constructed in genomic, partly genomic or cDNA-derived expression vectors as described. For the bispecific, bivalent antibodies <Tweak-IL-17> #2, <Tweak-IL-17> #24 the format described in WO 2009/080253 ("CrossMabs" or "CH1-CL domain exchanged antibodies") has been used. For the bispecific, bivalent <Tweak-IL-17> #4, <Tweak-IL-17> #20, <Tweak-IL-17> #21, <Tweak-IL-17> #23 the format described in WO2011/117330 ("bispecific one-armed scFab antibodies") has been used, and for <Tweak-IL-17> #5 the bispecific, tetravalent format, wherein the scFabs are fused at the N-terminus of the heavy chains described in WO 2010/112193 has been used. WO 2009/080253, WO 2011/117330 and WO 2010/112193 are incorporated herein by reference).

The plasmids were amplified in E. coli, purified, and subsequently HEK293 cells were transfected for transient expression of recombinant proteins. After 7 days of cultivation, the supernatants of HEK293 cells were harvested, filtered and the bispecific bivalent antibodies were purified.

Table 7: Construction of different <Tweak/IL-17> bispecific antibodies based on the corresponding TWEAK and IL17 VH and VL domains, and the respective IgG constant region (with mutations, if present; numbering according to the EU index of Kabat)

	VH <TWEAK>	**VL <TWEAK>**	**VH <IL17>**	**VL <IL17>**	**IgG subtype with mutation(s)**
<Tweak-IL-17> #2	SEQ ID NO: 28	SEQ ID NO: 37	SEQ ID NO: 56	SEQ ID NO: 58	IgG1 with Y349C, T366W mutations in one of the two CH3 domains and S354C, T366S, L368A, Y407V mutations in the other of the two CH3 domains
<Tweak-IL-17> #4	SEQ ID NO: 28	SEQ ID NO: 37	SEQ ID NO: 56	SEQ ID NO: 58	IgG1 with Y349C, T366W mutations in one of the two CH3 domains and S354C, T366S, L368A, Y407V mutations in the other of the two CH3 domains
<Tweak-IL-17> #5	SEQ ID NO: 28	SEQ ID NO: 37	SEQ ID NO: 56	SEQ ID NO: 58	IgG1
<Tweak-IL-17> #20	SEQ ID NO: 28	SEQ ID NO: 37	SEQ ID NO: 56	SEQ ID NO: 58	IgG4 with a) mutations S228P and L235E; and b) with Y349C, T366W mutations in one of the two CH3 domains and S354C, T366S, L368A, Y407V mutations in the other of the two CH3 domains

	VH <TWEAK>	**VL <TWEAK>**	**VH <IL17>**	**VL <IL17>**	**IgG subtype with mutation(s)**
<Tweak-IL-17> #21	SEQ ID NO: 28	SEQ ID NO: 37	SEQ ID NO: 55	SEQ ID NO: 57	IgG4 with a) mutations S228P and L235E; and b) with Y349C, T366W mutations in one of the two CH3 domains and S354C, T366S, L368A, Y407V mutations in the other of the two CH3 domains
<Tweak-IL-17> #23	SEQ ID NO: 28	SEQ ID NO: 37	SEQ ID NO: 55	SEQ ID NO: 57	IgG1 with a) mutations L234A, L235A and P329G; and b) with Y349C, T366W mutations in one of the two CH3 domains and S354C, T366S, L368A, Y407V mutations in the other of the two CH3 domains
<Tweak-IL-17> #24	SEQ ID NO: 28	SEQ ID NO: 37	SEQ ID NO: 55	SEQ ID NO: 57	IgG1 with a) mutations L234A, L235A and P329G; and b) with Y349C, T366W mutations in one of the two CH3 domains and S354C, T366S, L368A, Y407V mutations in the other of the two CH3 domains

Bispecific antibodies were purified from cell culture supernatants by affinity chromatography using MabSelect SuRe™ (GE Healthcare, Sweden). The subsequent chromatographic steps (size exclusion chromatography (Superdex200

HiLoad 120 ml 16/60 gel filtration column, GE Healthcare, Sweden) or ion exchange chromatography (MacroPrep CHT™ TypeII 10 ml, Bio-Rad plus size exclusion chromatography) was chosen in respect to the individual product related sideproducts of the bispecific antibodies after MabSelect SuRe™ chromatography.

Briefly, sterile filtered cell culture supernatants were captured on a MabSelect SuRe resin equilibrated with PBS buffer (10 mM Na_2HPO_4, 1 mM KH_2PO_4, 137 mM NaCl and 2.7 mM KCl, pH 7.4), washed with equilibration buffer and eluted with 25 mM sodium citrate at pH 3.0. The eluted protein fractions were pooled and neutralized with 2M Tris, pH 9.0. The antibody pools were prepared for hydrophobic interaction chromatography by rebuffering in 10 mM NaH_2PO_4, 20 mM MES, 50 mM NaCl, 0.1 mM $CaCl_2$, pH 7.5. After equilibration of the CHT column with equilibration buffer (10 mM NaH_2PO_4, 20 mM MES, 50 mM NaCl, 0.1 mM $CaCl_2$, pH 7.5), the antibodies were applied to the CHT column, washed with equilibration buffer and eluted in an linear gradient to 10 mM NaH_2PO_4, 20 mM MES, 500 mM NaCl, 0.1 mM $CaCl_2$, pH 7.5. The bispecific antibody containing fractions (from ion exchange chromatography or MabSelect SuRe affinity chromatography) were pooled and further purified by size exclusion chromatography using a Superdex 200 26/60 GL (GE Healthcare, Sweden) column equilibrated with 20 mM histidine, 140 mM NaCl, pH 6.0. The bispecific antibody containing fractions were pooled, concentrated to the required concentration using Vivaspin ultrafiltration devices (Sartorius Stedim Biotech S.A., France) and stored at -80°C.

Example 15

SDS-CE and analytical SEC of bispecific molecules

SDS-CE

Purity, antibody integrity and molecular weight of bispecific and control antibodies were analyzed by CE-SDS using microfluidic Labchip technology (Caliper Life Science, USA). 5 µl of protein solution was prepared for CE-SDS analysis using the HT Protein Express Reagent Kit according manufacturer's instructions and analyzed on LabChip GXII system using a HT Protein Express Chip. Data were analyzed using LabChip GX Software.

Analytical size exclusion chromatography

Size exclusion chromatography for the determination of the aggregation and oligomeric state of antibodies was performed by HPLC chromatography. Briefly,

purified antibodies at various levels of the purification process were applied to a Tosoh TSKgel G3000SW column in 300 mM NaCl, 50 mM KH2PO4/K2HPO4, pH 7.5 on an Agilent HPLC 1100 system or to a Superdex 200 column (GE Healthcare) in 2 x PBS on a Dionex HPLC-System. The eluted protein was quantified by UV absorbance and integration of peak areas. BioRad Gel Filtration Standard 151–1901 served as a standard.

Table 8: Yield, purity profile and aggregation tendencies (aggregate tendencies are reflected by % monomers after Protein A)

	Titer (mg/l)	**Light chain mispairing (CE-SDS)**	**% Monomer Protein A (SE-HPLC)**	**Estimated purity after 2nd column (ESI-MS)**
<TweakIL-17>#4	44	~18%	~70%	<80%
<Tweak-IL-17> #5	13	~10%	~60%	>90%
<Tweak-IL-17>#20	65	~9%	~80%	>95%
<Tweak-IL-17>#21	25	~4%	~70%	>95%
<Tweak-IL-17>#23	73	12%	~60%	<80%
<Tweak-IL-17> #2	8	~10%	>90%	>90%
<Tweak-IL-17 #24	8	n.a.	~80%	<80%

Example 16

Inhibition of IL-17 induced cytokine stimulation of human synovial fibroblasts

The anti-IL-17 component of different <Tweak-IL-17> bispecific antibodies was tested for inhibition of an IL-17-induced production of pro-inflammatory cytokines (e.g. human IL-6, human IL-8) by human adult fibroblast-like synoviocytes obtained from RA patients (HFLS-RA). After establishment of a dose-response response of different RA-FLS donors, the potency of several lead candidates was assessed.

HFLS-RA (Cat. #408RA-05a) were purchased from Cell Applications Inc. (San Diego, CA, USA; German distributor: tebu-bio, Offenbach, Germany). Cells were thawed, expanded in Synoviocyte Growth Medium (Cell Applications, Inc.; Cat. #415-500), detached with Accutase (PAA Laboratories GmbH, Pasching, Austria; Cat. #L11-007) before approx. $2x10^4$ HFLS-RA cells/well were seeded in 200µl/well medium in 96wF cell culture plates (Costar/Corning Life Sciences, Amsterdam, The Netherlands; Cat. #3596).

Cells were pre-cultured for two days at 37°C, 5 %CO_2 before cytokines (and optionally antibodies) were added. Prior to the cytokine addition, medium was removed and 150µl/w of the corresponding cytokine (optional: antibody) dilution was added: 0-10µg/ml rec. human IL-17A (PeproTech, Hamburg, Germany; Cat. #200-17); 0-25 µg/ml rec. human TWEAK (R&D Systems, Wiesbaden, Germany; Cat. #1090-TW/CF), or 0-10µg/ml rec. human TNFα (R&D Systems; Cat. #210-TA/CF) was titrated in ten 1:10 dilution steps to obtain an ED50 value for the indicated cytokines. TWEAK was used as negative and TNF was used as positive control. The cells were incubated for 6h, 24h and 72h at 37°C, 5 % CO2, whereas 72 hrs was used for the following experiments as this incubation time gave the most robust cytokine response (on a protein level). Cells were pre-incubated with several antibodies at different concentrations (c_{fin}=0-150/500nM) for 30 min at 37°C, 5 % CO_2 before stimulation with 100ng/ml-10µg/ml TWEAK for additional 72 hrs was applied.

Table 9: Effective dose (ED50) values in ng/ml of cytokine induction by IL-17, Tweak and TNFalpha of synovial fibroblasts.

Cytokine	Human IL-6	Human IL-8	G-CSF
+IL-17A	1.0	5.0	2.1
+TWEAK	>100	>100	>100
+TNFalpha	0.2	0.7	0.4

Table 10: Inhibitory concentration (IC50) in nM of <IL-17> parent antibodies and <Tweak-IL-17> bispecific antibodies after cytokine induction by IL-17 of synovial fibroblasts calculated as inhibitory concentration IC50/per valency (= per IL17 binding arm) .

Antibody	IL-6 IC50 [nM]	IL-8 IC50 [nM]
<IL-17>9C6-2B6-134	3.56	2.20
<IL-17>9C6-2B6-136	1.62	1.74
<Tweak-IL-17>#4	1.72	1.10
<Tweak-IL-17>#20	1.38	0.90
<Tweak-IL-17>#5	1.32	1.12
<Tweak-IL-17>#21	2.71	1.71

Interestingly the bispecific antibodies according to the invention show an improved IC50 per binding valency compared to parent IL17 antibodies.

Cytokine determination via CBA

After this, approximately 120µl/w supernatant was transferred in 96w RB plates and stored at -20°C until cytokine analysis was performed. For this the Cytometric Bead Array (CBA) platform was used and in particular the production of IL-6 and IL-8 (or its inhibition) was analyzed. The assay was performed according to manufacturer´s instructions of the Human Soluble Protein Master Buffer Kit (BD Biosciences, Heidelberg, Germany, Cat. #558265) using the human IL-6 (BD Biosciences, Cat. #558276) and IL-8 (BD; Cat. #558277) flex sets. Plates were measured with a FACS Array and analyzed using FCAP software (both from BD).

Example 17

Inhibition of Tweak induced proliferation of human synovial fibroblasts

Human Adult Fibroblast-Like Synoviocytes obtained from RA patients (HFLS-RA; Cat. #408RA-05a) were purchased from Cell Applications Inc. (San Diego, CA, USA; German distributor: tebu-bio, Offenbach, Germany). Cells were thawed, expanded and – after detachment with Accutase (PAA Laboratories GmbH, Pasching, Austria; Cat. #L11-007) $2x10^4$ HFLS-RA cells/well were seeded in 100µl/well medium in 96wF white chimney plate (Greiner Bio-one, Frickenhausen, Germany; #655098) for subsequent CellTiter Glo Proliferation/Viability assay. In preliminary experiments the proliferation was assessed using the Click-iT EdU kit according the manufacturer's instructions (see below).

Recombinant human TWEAK (R&D Systems, Wiesbaden, Germany; Cat. #1090-TW/CF) was titrated from 0-6,000 ng/ml in 1:3 dilution steps with 100µl/w in triplicates to obtain the EC50 value of a dose-response-curve. The total volume per well was 200µl. Plates were then incubated for 72 hrs at 37°C, 5 % CO_2 until proliferation was measured.

In final experiments, cells were pre-incubated with indicated antibodies at different concentrations (c_{fin}=0-150nM) for 30 min at 37°C, 5 % CO_2. After this, cells were stimulated by adding 10-20 ng/ml rec. human TWEAK to each well (50µl/w) and culturing for additional 72 hrs. Stimulation with recombinant IL-17 or TNFalpha did not lead to a measurable induction of HFLS proliferation.

Proliferation assay

The CellTiter Glo kit (Promega GmbH, Mannheim, Germany; Cat. #G7571) was used to assess the proliferation as measured by general cell viability/activity. Briefly, substrate and buffers were thawed and substrate was dissolved in 10 ml buffer. For equilibration the plate was incubated for 30 min at room temperature, centrifuged, and 100µl cell supernatant was added to 100µl of the CellTiter Glo reagent (per well). Plates were shaked 2 min and, after signal equilibration for 10 min, luminescence was measured using a Tecan Infinite 2000 reader (Tecan, Crailsheim, Germany) with the following settings: 96 w F Greiner chimney white/luminescence/0-1000 ms.

In preliminary experiments the proliferation was assessed using the Click-iT EdU A647 kit according the manufacturer's instructions (Invitrogen, Cat. # A10208).

Table 11: HFLS and HFSL-RA were stimulated and proliferation was analyzed after 72 hrs.

	HFLS	HFLS-RA
Effective dose (ED50)	55 ng/ml	44 ng/ml
Proliferation compared to unstimulated	~3-fold	~3-fold

Table 12: Inhibition of Tweak induced HFLS proliferation by <Tweak-IL-17> bispecific antibodies calculated as inhibitory concentration IC50/per valency (= per Tweak binding arm) .

Antibody	IC50 (nM)
Humanized <Tweak>TW-305 (HC4,LC2)	0.152
<Tweak-IL-17>#2	0.065
<Tweak-IL-17>#4	0.157
<Tweak-IL-17>#20	0.080
<Tweak-IL-17>#5	0.152

Example 18

Small-scale Dynamic Light Scattering (DLS) -based viscosity measurement of <TWEAK-IL-17> antibody molecules.

Viscosity measurement was essentially performed as described in [He, F., et al., Analytical Biochemistry 399(1) (2009), 141-3]. Briefly, samples are concentrated to various protein concentrations in 200 mM arginine succinate, pH 5.5, before polystyrene latex beads (300 nm diameter) and Polysorbate 20 (0.02 % v/v) are added. Samples are transferred into an optical 384-well plate by centrifugation through a 0.4 µm filter plate and covered with paraffine oil. The apparent diameter of the latex beads is determined by dynamic light scattering at 25 °C. The viscosity of the solution can be calculated as $\eta = \eta_0(r_h/r_{h,0})$ (η: viscosity; η_0: viscosity of water; r_h: apparent hydrodynamic radius of the latex beads; $r_{h,0}$: hydrodynamic radius of the latex beads in water.

To allow comparison of various samples at the same concentration, viscosity-concentration data were fitted with the Mooney equation (Equation 1) [(Mooney, Colloid Sci, 1951; Monkos, Biochem. Biophys. Acta 1997)] and data interpolated accordingly.

$$\eta = \eta_0 \exp\left(\frac{S\Phi}{1 - K\Phi}\right)$$

(S: hydrodynamic interaction parameter of the protein; K: self-crowding factor; Φ: volume fraction of the dissolved protein)

For comparison the data of IL17 based bispecific antibody D2E7-B6-17.8 DVD-Ig (which binds TNF alpha as second specificity) described in WO 2010/102251 was also determined.

Table 13: Viscosity of bispecific antibodies was measured at various concentrations.

	Viscosity at 70 mg/ml	Viscosity at 100 mg/ml	Viscosity at 150 mg/ml
<Tweak-IL-17>#4	2.2 mPa·s	3.5 mPa·s	8.2 mPa·s
<Tweak-IL-17>#20	2.1 mPa·s	n.d.	n.d.
<Tweak-IL-17>#21	2.3 mPa·s	n.d.	n.d.
<Tweak-IL-17>#23	2.7 mPa·s	3.3 mPa·s	7.4 mPa·s
D2E7-B6-17.8 DVD-Ig	3.4 mPa·s	6.5 mPa·s	n.d.

Stability of bispecific antibodies

Samples are concentrated to a final concentration of 150 mg/mL in 200 mM arginine succinate, pH 5.5, sterile filtered and quiescently stored at 40°C for 4 days. Before and after storage, the content of high molecular weight (HMW) species is determined by size-exclusion chromatography. The difference in HMW content between the stored sample and a sample measured immediately after preparation is

reported as "HMW increase". For comparison the data of IL17 based bispecific antibody D2E7-B6-17.8 DVD-Ig (which binds TNF alpha as second specificity) described in WO 2010/102251 was also determined.

Table 14: Stability of bispecific antibodies was assessed by DLS and SE.-HPLC.

	<Tweak-IL-17>#20	<Tweak-IL-17>#21	<Tweak-IL-17>#23	D2E7-B6-17.8 DVD-Ig
DLS aggregation onset temperature (= aggregation temperature)	≈57°C	≈61°C	≈64°C	*≈54°C*
4 d storage at 40°C at 150 mg/ml (HMW increase)	< 5 area-%	n.d.	n.d.	n.d.

Example 19

Binding of the bispecific <TWEAK/IL17> antibodies according to the invention

The <IL17> and <TWEAK> binding affinity of bispecific antibodies and parent antibodies was measured by Surface Plasmon Resonance (SPR) using a BIAcore® T100 instrument (GE Healthcare) at 25°C. The BIAcore® system is well established for the study of molecule interactions. SPR-technology is based on the measurement of the refractive index close to the surface of a gold coated biosensor chip. Changes in the refractive index indicate mass changes on the surface caused by the interaction of immobilized ligand with analyte injected in solution. The mass increases if molecules bind immobilized ligands on the surface, and vice versa, the mass decreases in case of dissociation of the analyte from the immobilized ligand (reflecting complex dissociation). SPR allows a continuous real-time monitoring of ligand/analyte binding and thus the determination of the association rate constant (ka), the dissociation rate constant (kd), and of the equilibrium constant (KD).

IL17 binding affinity

Around 12000 resonance units (RU) of the capturing system (10 µg/ml goat anti human F(ab')2; Order Code: 28958325; GE Healthcare Bio-Sciences AB, Schweden) were coupled on a CM5 chip at pH 5.0 by using an amine coupling kit supplied by the GE Healthcare. The sample and system buffer was PBS-T (10 mM phosphate buffered saline including 0.05 % Tween20) pH 7.4. The bispecific

antibody was captured by injecting a 50nM solution for 1 min at a flow of 10 µl/min. Association was measured by injection human IL17 in various concentrations in solution for 3 min at a flow of 30 µl/min starting with 50nM in 1:1 dilutions. The dissociation phase was monitored for up to 5 min and triggered by switching from the sample solution to running buffer. The surface was regenerated by two times 60 sec. washing with a glycin pH 2.1 solution at a flow rate of 30 µl/min. Bulk refractive index differences were corrected by subtracting the response obtained from a goat anti human F(ab')2 surface. Blank injections are also subtracted (= double referencing). For calculation of apparent K_D and other kinetic parameters the Langmuir 1:1 model was used. Results are shown in Table 17 below.

Table 15: Binding affinity to human IL17

Antibody	IL-17 A/A* (app KD)	IL-17 A/F (KD)	IL-17 F/F (KD)
<IL17> 9C6-2B6-136	0.18 nM	0.26 nM	no binding
<Tweak-IL-17>#2	0.15 nM	0.25 nM	no binding
<Tweak-IL-17>#4	0.15 nM	0.27 nM	no binding
<Tweak-IL-17>#20	<0.2 nM	n.d.	no binding

TWEAK binding affinity

Due to strong unspecific binding of the TWEAK analyte to the sensor surface, a reverse setup - using TWEAK as ligand - was chosen. Around 100 resonance units (RU) of TWEAK was immobilized on the C1 chip surface at pH 5.0 using an amine coupling kit supplied by the GE Healthcare. The sample and system buffer was PBS-T (10 mM phosphate buffered saline including 0.05 % Tween20) pH 7.4. Association was measured by injection the bispecific antibody in various concentrations in solution for 3 min at a flow of 30 µl/min starting with 50nM in 1:1 dilutions. The dissociation phase was monitored for up to 10 min and triggered

by switching from the sample solution to running buffer. The surface was regenerated by three times 30 sec. washing with a 3M MgCl2 solution at a flow rate of 30 µl/min. Bulk refractive index differences were corrected by subtracting the response obtained from a blank-coupled surface. Blank injections are also subtracted (=double referencing). For calculation of apparent K_D and other kinetic parameters the Langmuir 1:1 model was used. Results are shown in the table 18 below.

Table 16: Binding affinity to human TWEAK

	Ligand	ka (1/Ms)	kd (1/s)	App. KD (M)	T1/2 (min)
<TWEAK> 305 (antibody 27 = humanized variant with HC4 LC2)	Tweak-Fc	2.27E+06	5.71E-05	2.52E-11	202.4
<TWEAK> 305 (antibody 27) -Fab		4.72E+06	7.09E-05	1.50E-11	163.0
<Tweak-IL-17>#4		4.26E+05	5.42E-05	1.27E-10	213.0
<Tweak-IL-17>#20		8.10E+05	5.31E-05	6.56E-11	217.6

Simultaneous binding of <TWEAK/IL17> antibodies to both targets, human TWEAK and human IL17

Around 12000 resonance units (RU) of the capturing system (10 µg/ml goat anti human F(ab')2; Order Code: 28958325; GE Healthcare Bio-Sciences AB, Sweden) were coupled to a CM5 chip (GE Healthcare BR-1005-30) at pH 5.0 by using an amine coupling kit supplied by the GE Healthcare. The sample and system buffer was PBS-T (10 mM phosphate buffered saline including 0.05 % Tween20) pH 7.4. The temperature of the flow cell was set to 25°C and of the sample block to 12°C. Before capturing, the flow cell was primed with running buffer twice. The bispecific antibody was captured by injecting a 50 nM solution for 60 sec at a flow of 10 µl/min. Independent binding of each ligand to the bispecific antibody was analyzed by determining the active binding capacity for each ligand, either added sequentially or simultaneously (flow of 30 µl/min):

1) Injection of human Tweak-Fc with a concentration of 50 nM for 120 sec (identifies the single binding of the antigen). 2) Injection of human IL17A/A with a concentration of 50 nM for 120 sec (identifies single binding of the antigen).

3) Injection of human Tweak-Fc with a concentration of 50 nM for 120 sec followed by an additional injection of human IL17A/A with a concentration of 50 nM (identifies binding of IL17A/A in the presence of Tweak). 4) Injection of human IL17A/A with a concentration of 50 nM for 120 sec followed by an additional injection of human Tweak-Fc with a concentration of 50 nM (identifies binding of Tweak in the presence of IL17A/A). 5) Co-Injection of human IL17A/A with a concentration of 50 nM and of human Tweak-Fc with a concentration of 50 nM for 120 sec (identifies the binding of Tweak and of IL17A/A at the same time).

The surface was regenerated by 2 times 60 sec washing with a Glycine pH 2.1 solution at a flow rate of 30 µl/min. Bulk refractive index differences were corrected by subtracting the response obtained from a goat anti human IgG surface. The bispecific antibody is able to bind both antigens mutual independently if the resulting final signal of the approaches 3, 4 & 5 equals the sum of the individual final signals of the approaches 1 and 2.

Table 17: Simultaneous of <TWEAK/IL17> antibodies to both targets <TWEAK> and <IL17>

	TWEAK-Fc addition (RU)	IL-17 addition (RU)	Addition of Tweak-Fc/ IL-17 mixture (RU)
<Tweak-IL-17>#4	88	42	135
<Tweak-IL-17>#20	139	42	186

Patent Claims

1. A bispecific antibody comprising a first antigen-binding site that specifically binds to human TWEAK and a second antigen-binding site that specifically binds to human IL17.

2. The bispecific, bivalent antibody according to claim 1, wherein the bispecific antibody inhibits
a) TWEAK induced proliferation of human fibroblast-like synoviocytes-rheumatoid arthritis (HFLS-RA) with an IC50 value of 0.2 nM or lower; and
b) IL17 induced IL6 cytokine stimulation of human fibroblast-like synoviocytes-rheumatoid arthritis (HFLS-RA) with an IC50 value of 3.0 nM or lower; and
c) IL17 induced IL8 cytokine stimulation of human fibroblast-like synoviocytes-rheumatoid arthritis (HFLS-RA) with an IC50 value of 2.0 nM or lower.

3. The bispecific, bivalent antibody according to claim 1, characterized in that the bispecific antibody is bivalent.

4. The bispecific antibody according to any of claims 1 to 3, characterized in that

i) said first antigen-binding site comprises
a) CDR1H of SEQ ID NO:17, CDR2H of SEQ ID NO:18, CDR3H of SEQ ID NO:19, and CDR1L of SEQ ID NO:20, CDR2L of SEQ ID NO:21, CDR3L of SEQ ID NO:22; or
b) CDR1H of SEQ ID NO:1, CDR2H of SEQ ID NO:2, CDR3H of SEQ ID NO:3, and CDR1L of SEQ ID NO:4, CDR2L of SEQ ID NO:5, CDR3L of SEQ ID NO:6; or
c) CDR1H of SEQ ID NO:9, CDR2H of SEQ ID NO:10, CDR3H of SEQ ID NO:11, and CDR1L of SEQ ID NO:12, CDR2L of SEQ ID NO:13, CDR3L of SEQ ID NO:14; and

ii) said second antigen-binding site comprises
CDR1H of SEQ ID NO:47, CDR2H of SEQ ID NO:48, CDR3H of SEQ ID NO:49, and CDR1L of SEQ ID NO:50, CDR2L of SEQ ID NO:51, CDR3L of SEQ ID NO:52.

5. A chimeric or humanized variant of the bispecific, antibody according to claim 4.

6. The bispecific antibody according to any of claims 1 to 3, characterized in that

i) said first antigen-binding site comprises
a variable heavy chain domain (VH) of SEQ ID NO:25, of SEQ ID NO:26, of SEQ ID NO:27, of SEQ ID NO:28, of SEQ ID NO:29, of SEQ ID NO:30, of SEQ ID NO:31, of SEQ ID NO:32, of SEQ ID NO:33, of SEQ ID NO:34, or of SEQ ID NO:35, and a variable light chain domain of SEQ ID NO:26, of SEQ ID NO:37, of SEQ ID NO:38, of SEQ ID NO:39, of SEQ ID NO:40, of SEQ ID NO:41, of SEQ ID NO:42, of SEQ ID NO:43, of SEQ ID NO:44, of SEQ ID NO:45, or of SEQ ID NO:46; and

ii) said second antigen-binding site comprises
a variable heavy chain domain (VH) of SEQ ID NO:55, or of SEQ ID NO:56, and a variable light chain domain of SEQ ID NO:57, or of SEQ ID NO:58.

7. The bispecific antibody according to any of claims 1 to 3, characterized in that

i) said first antigen-binding site comprises
a variable heavy chain domain (VH) of SEQ ID NO:28, and a variable light chain domain of SEQ ID NO:37; and

ii) said second antigen-binding site comprises
a) a variable heavy chain domain (VH) of SEQ ID NO:56, and a variable light chain domain of SEQ ID NO:58; or
b) a variable heavy chain domain (VH) of SEQ ID NO:55, and a variable light chain domain of SEQ ID NO:57.

8. The bispecific antibody according to any of claims 1 to 3, characterized in that

i) said first antigen-binding site comprises
a variable heavy chain domain (VH) of SEQ ID NO:28, and a variable light chain domain of SEQ ID NO:37; and

ii) said second antigen-binding site comprises
a variable heavy chain domain (VH) of SEQ ID NO:56, and a variable light chain domain of SEQ ID NO:58.

9. The bispecific antibody according to any of claims 1 to 3, characterized in that

i) said first antigen-binding site comprises
a variable heavy chain domain (VH) of SEQ ID NO:28, and a variable light chain domain of SEQ ID NO:37; and

ii) said second antigen-binding site comprises
a variable heavy chain domain (VH) of SEQ ID NO:55, and a variable light chain domain of SEQ ID NO:57.

10. The bispecific antibody according to any of the preceding claims, characterized in that it is of IgG1 or IgG4 subclass.

11. The bispecific antibody according to any of the preceding claims, characterized in being of IgG1 subclass with the mutations L234A and L235A (numbering according to the EU index of Kabat).

12. The bispecific antibody according to any of the preceding claims, characterized in being of IgG1 subclass with the mutations L234A, L235A and P329G (numbering according to the EU index of Kabat).

13. The bispecific antibody according to any of the preceding claims, characterized in being of IgG4 subclass with the mutations S228P and L235E (numbering according to the EU index of Kabat).

14. The bispecific antibody according to any of the preceding claims, characterized in being of IgG4 subclass with the mutations S228P, L235E and P329G (numbering according to the EU index of Kabat).

15. Pharmaceutical composition characterized by comprising an antibody according to claims 1 to 14.

16. Use of an antibody according to claims 1 to 14 for the manufacture of a pharmaceutical composition.

17. An antibody according to claims 1 to 14 for use in the treatment of cancer, or inflammatory diseases, autoimmune diseases, rheumatoid arthritis, psoratic arthritis, muscle diseases, e.g. muscular dystrophy, multiple sclerosis, chronic kidney diseases, bone diseases, e.g. bone degeneration in multiple myeloma, systemic lupus erythematosus, lupus nephritis, and vascular injury.

18. Use of an antibody according to claims 1 to 14 for manufacture of a medicament for the treatment of cancer, or inflammatory diseases, autoimmune diseases, rheumatoid arthritis, psoratic arthritis, muscle diseases, e.g. muscular dystrophy, multiple sclerosis, chronic kidney diseases, bone diseases, e.g. bone degeneration in multiple myeloma, systemic lupus erythematosus, lupus nephritis, and vascular injury.

19. Nucleic acid encoding an antibody according to claims 1 to 14.

20. Expression vectors characterized by comprising a nucleic acid according to claim 19 for the expression of the bispecific antibody according to claims 1 to 14 in a prokaryotic or eukaryotic host cell.

21. Prokaryotic or eukaryotic host cell comprising a vector according to claim 20.

22. Method for the production of a recombinant antibody according to claims 1 to 14, characterized by expressing a nucleic acid according to claim 19 in a prokaryotic or eukaryotic host cell and recovering said antibody from said cell or the cell culture supernatant.

23. Method for the treatment of a patient suffering from cancer or from inflammatory diseases, autoimmune diseases, rheumatoid arthritis, psoratic arthritis, muscle diseases, e.g. muscular dystrophy, multiple sclerosis, chronic kidney diseases, bone diseases, e.g. bone degeneration in multiple myeloma, systemic lupus erythematosus, lupus nephritis, and vascular injury, characterized by administering to the patient an antibody according to claims 1 to 14.