Antibodies against human TWEAK and uses thereof

The present invention relates to antibodies against human TWEAK (TWEAK 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: 60) 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 aa type I transmembane protein consisting of one single cystein rich domain in ligand binding domain. 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.

Summary of the Invention

The invention comprises an antibody binding to human TWEAK, characterized in comprising as heavy chain variable domain CDR3 region (CDR3H) selected from the group consisting of SEQ ID NO: 8, 16 or 24.

The invention comprises a chimeric, humanized or T-cell epitope depleted variant of an antibody comprising a variable light chain of SEQ ID NO:1 and a variable heavy chain of SEQ ID NO:5, of an antibody comprising a variable light chain of

SEQ ID NO:9 and a variable heavy chain of SEQ ID NO:13 or of an antibody comprising a variable light chain of SEQ ID NO:17 and a variable heavy chain of SEQ ID NO:21.

In one embodiment the antibody is characterized by comprising CDR1H of SEQ ID NO:6, CDR2H of SEQ ID NO:7, and CDR3H of SEQ ID NO:8.

In one embodiment is characterized by comprising CDR1H of SEQ ID NO:14, CDR2H of SEQ ID NO:15, and CDR3H of SEQ ID NO:16.

In one embodiment the antibody is characterized by comprising CDR1H of SEQ ID NO:22, CDR2H of SEQ ID NO:23, and CDR3H of SEQ ID NO:24.

In one embodiment the antibody is characterized by comprising CDR1H of SEQ ID NO:6, CDR2H of SEQ ID NO:7, CDR3H of SEQ ID NO:8 and CDR1L of SEQ ID NO:2, CDR2L of SEQ ID NO:3, CDR3L of SEQ ID NO:4.

In one embodiment the antibody is characterized by comprising CDR1H of SEQ ID NO:14, CDR2H of SEQ ID NO:15, CDR3H of SEQ ID NO:16 and CDR1L of SEQ ID NO:10, CDR2L of SEQ ID NO:11, CDR3L of SEQ ID NO:12.

In one embodiment the antibody is characterized by comprising CDR1H of SEQ ID NO:22, CDR2H of SEQ ID NO:23, CDR3H of SEQ ID NO:24 and CDR1L of SEQ ID NO:18, CDR2L of SEQ ID NO:19, CDR3L of SEQ ID NO:20.

In one embodiment the antibody is characterized by comprising as light chain variable domain sequence a sequence selected of the group consisting of SEQ ID NO:1, 9, 17, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 56, or 58.

In one embodiment the antibody is characterized by comprising as heavy chain variable domain sequence a sequence selected from the group consisting of SEQ ID NO:5, 13, 21, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 57, or 59.

In one embodiment the antibody is characterized by comprising as light chain variable domain sequence a sequence selected from the group consisting of SEQ ID NO:17, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36 and as heavy chain variable domain sequence a sequence selected from the group consisting of SEQ ID NO:21, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, or 48.

Preferred is a combination of light and heavy variable chains selected from the group consisting of: 1/5, 9/13, 17/21, 25/37, 26/38, 27/39, 28/40, 29/41, 30/42, 31/43, 32/44; 33/45, 34/46, 35/47; 36/48, 56/57, 58/59.(e.g. 17/21 means an antibody comprising variable light chain of SEQ ID NO: 17 and variable heavy chain SEQ ID NO: 21).

One embodiment of the invention is an antibody binding to human TWEAK, characterized by comprising by comprising CDR1H of SEQ ID NO:6, CDR2H of SEQ ID NO:7, CDR3H of SEQ ID NO:8 and CDR1L of SEQ ID NO:2, CDR2L of SEQ ID NO:3, CDR3L of SEQ ID NO:4.

One embodiment of the invention is an antibody binding to human TWEAK, characterized by comprising CDR1H of SEQ ID NO:14, CDR2H of SEQ ID NO:15, CDR3H of SEQ ID NO:16 and CDR1L of SEQ ID NO:10, CDR2L of SEQ ID NO:11, CDR3L of SEQ ID NO:12.

One embodiment of the invention is an antibody binding to human TWEAK, characterized by comprising CDR1H of SEQ ID NO:22, CDR2H of SEQ ID NO:23, CDR3H of SEQ ID NO:24 and CDR1L of SEQ ID NO:18, CDR2L of SEQ ID NO:19, CDR3L of SEQ ID NO:20.

One embodiment of the invention is an antibody binding to human TWEAK, characterized by comprising a) a variable light chain of SEQ ID NO: 27 and a variable heavy chain of SEQ ID NO: 41, b) a variable light chain of SEQ ID NO: 27 and a variable heavy chain of SEQ ID NO: 44, c) a variable light chain of SEQ ID NO: 27 and a variable heavy chain of SEQ ID NO: 45, d), a variable light chain of SEQ ID NO: 26 and a variable heavy chain of SEQ ID NO: 47, e), a variable light chain of SEQ ID NO: 29 and a variable heavy chain of SEQ ID NO: 44, f), a variable light chain of SEQ ID NO: 31 and a variable heavy chain of SEQ ID NO: 45, or g) a variable light chain of SEQ ID NO: 29 and a variable heavy chain of SEQ ID NO: 41.

One embodiment of the invention is an antibody binding to human TWEAK, characterized by comprising a) a variable light chain of SEQ ID NO: 27 and a variable heavy chain of SEQ ID NO: 41, b) a variable light chain of SEQ ID NO: 27 and a variable heavy chain of SEQ ID NO: 44, c) a variable light chain of SEQ ID NO: 27 and a variable heavy chain of SEQ ID NO: 45, d) a variable light chain of SEQ ID NO: 26 and a variable heavy chain of SEQ ID NO: 47, e) a variable light chain of SEQ ID NO: 29 and a variable heavy chain of SEQ ID NO: 44, f) a

variable light chain of SEQ ID NO: 31 and a variable heavy chain of SEQ ID NO: 45, g) a variable light chain of SEQ ID NO: 29 and a variable heavy chain of SEQ ID NO: 41, or h) a variable light chain of SEQ ID NO: 29 and a variable heavy chain of SEQ ID NO: 39.

These antibodies are designated as follows in the application.

Antibody	SEQ ID NOs
27	27, 41
28	27, 44
29	27, 45
30	26, 47
31	29, 44
32	31, 45
33	29, 41
34	29, 39

One embodiment of the invention is an antibody binding to human TWEAK, characterized by comprising a variable light chain of SEQ ID NO: 27 and a variable heavy chain of SEQ ID NO: 41, b) a variable light chain of SEQ ID NO: 27 and a variable heavy chain of SEQ ID NO: 45, c) a variable light chain of SEQ ID NO: 29 and a variable heavy chain of SEQ ID NO: 44, d) a variable light chain of SEQ ID NO: 29 and a variable heavy chain of SEQ ID NO: 41, or e) a variable light chain of SEQ ID NO: 29 and a variable heavy chain of SEQ ID NO: 39.

In one embodiment the antibody binding to TWEAK 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 + L235A. In one embodiment the antibody binding to TWEAK and being characterized by the above mentioned amino acid sequences and amino acid sequence fragments is of human IgG4 isotype, preferably with a mutations S228P and L235E.

An antibody according to the invention specifically binds to human TWEAK and preferably shows a half-life of a complex between soluble murine TWEAK (amino

acids 81-225 of SEQ ID NO: 61) and antibody of 15 minutes or more at 25°C, measured by Biacore and binds to murine TWEAK and inhibits the interaction between murine TWEAK and Fn14 with an IC_{50} value of 4.9 ng/ml or lower.

Preferably an antibody according to the invention shows a half-life of a complex between soluble human TWEAK (amino acids 99-249 of SEQ ID NO: 60) 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.

Preferably an antibody according to the invention inhibits the interaction between human TWEAK and Fn14 with an IC_{50} value of 3.5 ng/ml or lower, preferably 2.5 ng/ml or lower. As used herein, IC_{50} means the amount of antibody that blocks 50% of the interaction between human TWEAK and Fn14.

The antibody is in one embodiment of human IgG1 isotype, in one embodiment with mutations L234A + L235A. In one embodiment the antibody according to the invention is of human IgG4 isotype, preferably with a mutations S228P and L235E. Preferably the antibody is a humanized or human antibody.

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

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

A further embodiment of the invention is an antibody according to the invention for manufacture of a medicament for the treatment of cancer, 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 an antibody according to the invention for manufacture of a medicament 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.

A further embodiment of the invention an antibody according to the invention for manufacture of a medicament for the treatment of colon, lung, or pancreatic cancer.

A further embodiment of the invention is the use of an antibody according to the invention for manufacture of a medicament for the treatment of cancer, 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 the use of an antibody according to the invention for manufacture of a medicament 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.

A further embodiment of the invention is the use of an antibody according to the invention for manufacture of a medicament for the treatment of colon, lung, or pancreatic cancer.

A further embodiment of the invention is a nucleic acid encoding an 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 an 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 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 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 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 further provides a method for treating 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, comprising administering to a patient diagnosed as having such a disease (and therefore being in need of such a therapy) an effective amount of an antibody binding to TWEAK according to the invention. The antibody is administered preferably in a pharmaceutical composition.

A further embodiment of the invention is a method 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, characterized by administering to the patient an antibody according to the invention.

The invention further comprises the use of an antibody according to the invention 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, and for the manufacture of a pharmaceutical composition according to the invention. In addition, the invention comprises a method for the manufacture of a pharmaceutical composition according to the invention.

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.

Brief description of the figures

Figure 1: Inhibition of collagen-induced arthritis, a murine model of rheumatoid arthritis, by antibodies according to the invention

Detailed Description of the Invention

The term "antibody" encompasses the various forms of antibody structures including, but not being limited to, whole antibodies, bispecific antibodies and antibody fragments. 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.

"Antibody fragments" comprise a portion of a full length antibody, preferably the variable domain thereof, or at least the antigen binding site thereof. Examples of antibody fragments include diabodies, single-chain antibody molecules, and multispecific antibodies formed from antibody fragments. scFv antibodies are, e.g., described in Huston, J.S., Methods in Enzymol. 203 (1991) 46-88. In addition, antibody fragments comprise single chain polypeptides having the characteristics of a V_H domain, namely being able to assemble together with a V_L domain, or of a V_L domain binding to TWEAK, namely being able to assemble together with a V_H domain to a functional antigen binding site.

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 rabbit 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.

The term “T cell epitope depleted antibody" refers to antibodies that were modified to remove or reduce immunogenicity by removing human T cell epitopes (peptide sequences within proteins with the capacity to bind to MHC Class II molecules). By this method, interactions between amino acid side chains of the peptide and specific binding pockets with the MHC class II binding groove are identified. The identified immunogenic regions are mutated to eliminate immunogenicity. Such methods are described in general in, e.g., WO 98/52976.

The terms “anti-TWEAK antibody” and “an antibody that specifically binds to TWEAK” according to the invention refer to an antibody that is capable of binding

TWEAK with sufficient affinity such that the antibody is useful as a therapeutic agent in targeting TWEAK according to the invention. An antibody that binds specifically to TWEAK has a dissociation constant (Kd) of 10^{-9} M or less, e.g. from 10^{-9} M to 10^{-13} M.

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. CDR2L, CDR3H, etc. mean the respective regions from the heavy(H) or light(L) chain. For example, an antibody characterized by comprising CDR1H of SEQ ID NO:6 means that the antibody comprises this amino acid sequence as a heavy chain variable chain CDR1 region in its variable

heavy chain. For example, an antibody characterized by comprising CDR1H of SEQ ID NO:6, CDR2H of SEQ ID NO:7, CDR3H of SEQ ID NO:8 means that the antibody comprises in its heavy chain as sequence of CDR1 SEQ ID NO:6, as sequence of CDR2 SEQ ID NO:7, and as sequence of CDR3 SEQ ID NO:8.

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, see below). 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 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, preferably a Fc part from human IgG1 subclass, a mutated Fc part from human IgG1 subclass (preferably with a mutation on L234A + L235A), a Fc part from human IgG4 subclass or a mutated Fc part from human IgG4 subclass (preferably with a mutation on S228P and L235E). Mostly preferred are the human heavy chain constant regions of SEQ ID NO: 51 or SEQ ID NO: 52 (human IgG1

subclass), SEQ ID NO: 53 (human IgG1 subclass with mutations L234A and L235A) , SEQ ID NO: 54 (human IgG4 subclass), or SEQ ID NO: 55 (human IgG4 subclass with mutations S228P and L235E).

Preferably 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.

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). For example, a useful human heavy chain constant region comprises an amino acid sequence of SEQ ID NO:51 or 28. 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:49. It is further preferred that the antibody is of rabbit origin and comprises the antibody variable sequence frame of a rabbit antibody according to Kabat (see e.g. Sequences of Proteins of Immunological Interest, Kabat, E.A. et al., 5th edition, DIANE Publishing (1992)).

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 therapy.

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 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.

A further embodiment of the invention is a method for the production of an antibody against TWEAK, 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 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 humanized anti-TWEAK 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.

In another aspect, the present invention provides a composition, e.g. a pharmaceutical composition, containing one or a combination of monoclonal antibodies, or the antigen-binding portion thereof, of the present invention, formulated together with a pharmaceutically acceptable carrier.

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.

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.

The invention comprises the use of the antibodies according to the invention 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 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 further 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 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.

Description of the Sequences

Table 1:
Antibody 301

Rabbit variable domain of light chain (VL)	SEQ ID NO:1
CDR1L	SEQ ID NO:2
CDR2L	SEQ ID NO:3
CDR3L	SEQ ID NO:4
Rabbit variable domain of heavy chain (VH)	SEQ ID NO:5
CDR1H	SEQ ID NO:6
CDR2H	SEQ ID NO:7
CDR3H	SEQ ID NO:8
Chimeric light chain 301	SEQ ID NO:56
Chimeric heavy chain 301	SEQ ID NO:57

Table 2:
Antibody 304

Rabbit variable domain of light chain (VL)	SEQ ID NO:9
CDR1L	SEQ ID NO:10
CDR2L	SEQ ID NO:11
CDR3L	SEQ ID NO:12
Rabbit variable domain of heavy chain (VH)	SEQ ID NO:13
CDR1H	SEQ ID NO:14
CDR2H	SEQ ID NO:15
CDR3H	SEQ ID NO:16
Chimeric light chain 304	SEQ ID NO:58
Chimeric heavy chain 304	SEQ ID NO:59

Table 3:

Antibody 305

Rabbit variable domain of light chain (VL)	SEQ ID NO:17
CDR1L	SEQ ID NO:18
CDR2L	SEQ ID NO:19
CDR3L	SEQ ID NO:20
Rabbit variable domain of heavy chain (VH)	SEQ ID NO:21
CDR1H	SEQ ID NO:22
CDR2H	SEQ ID NO:23
CDR3H	SEQ ID NO:24
Chimeric light chain 305	SEQ ID NO:25
Humanized variant of VL, 305-LC1	SEQ ID NO:26
Humanized variant of VL, 305-LC2	SEQ ID NO:27
Humanized variant of VL, 305-LC3	SEQ ID NO:28
Humanized variant of VL, 305-LC4	SEQ ID NO:29
Humanized variant of VL, 305-LC5	SEQ ID NO:30
Humanized variant of VL, 305-LC6	SEQ ID NO:31
Humanized variant of VL, 305-LC7	SEQ ID NO:32
Humanized variant of VL, 305-LC8	SEQ ID NO:33
Humanized variant of VL, 305-LC9	SEQ ID NO:34
Humanized variant of VL, 305-LC10	SEQ ID NO:35
Humanized variant of VL, 305-LC11	SEQ ID NO:36
Chimeric heavy chain 305	SEQ ID NO:37
Humanized variant of VH, 305-HC1	SEQ ID NO:38
Humanized variant of VH, 305-HC2	SEQ ID NO:39
Humanized variant of VH, 305-HC3	SEQ ID NO:40
Humanized variant of VH, 305-HC4	SEQ ID NO:41
Humanized variant of VH, 305-HC5	SEQ ID NO:42
Humanized variant of VH, 305-HC6	SEQ ID NO:43
Humanized variant of VH, 305-HC7	SEQ ID NO:44
Humanized variant of VH, 305-HC8	SEQ ID NO:45
Humanized variant of VH, 305-HC9	SEQ ID NO:46
Humanized variant of VH, 305-HC10	SEQ ID NO:47
Humanized variant of VH, 305-HC11	SEQ ID NO:48

Table 4a:

Human constant regions

Human kappa light chain	SEQ ID NO:49
Human lambda light chain	SEQ ID NO:50
Human IgG1 (Caucasian Allotype)	SEQ ID NO:51
Human IgG1 (Afroamerican Allotype)	SEQ ID NO:52
Human IgG1 LALA-Mutant (Caucasian Allotype)	SEQ ID NO:53
Human IgG4	SEQ ID NO:54
Human IgG4 SPLE-Mutant	SEQ ID NO:55

Table 4b:

Human and Murine TWEAK

Human TWEAK	SEQ ID NO:60
Murine TWEAK	SEQ ID NO:61

The following examples 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.

Example 1

Description of immunization

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 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: 60) and biotinylated murine soluble TWEAK (amino acids 81-225 of SEQ ID NO: 61) 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 5a and 5b, as well as in table 6b.

Table 5a:

Antibody		Human TWEAK t/2 diss [min] 25 °C	Murine TWEAK t/2 diss [min] 25 °C
TW-301chi	Chimer[1)]	110	n.d.
TW-304chi	Chimer[1)]	37	n.d.
TW-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:49 and the human IgG1 constant region of SEQ ID NO: 51

In a further experiment the half-Life of the antibody-TWEAK complexes (t/2 diss [min] at 25 °C) of the chimeric TW-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:49 and the human IgG1 constant region of SEQ ID NO: 51)

Table 5b:

Antibody		Human TWEAK t/2 diss [min] 25 °C	Murine TWEAK t/2 diss [min] 25 °C
TW-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:49 and the human IgG1 constant region of SEQ ID NO: 51

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: 60) 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) 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 6.

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) 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 6a and 6b.

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 6a and 6b.

Table 6a:

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

Table 6b:

Antibody	SEQ ID NOs	Interaction Inhibition Human IC50 [ng/ml]	Interaction Inhibition Murine IC50 [ng/ml]	IL8-Secretion [ng/ml]	Biacore t/2 diss. [min] 25 °C human	25 °C murine	37 °C human	37 °C murine
27	27, 41	2.3	8.2	197	110	19	147	19
28	27, 44	1.5	6.5	165	87	19	115	17
29	27, 45	1.8	5.7	78	146	22	195	21
30	26, 47	2.3	7.6	207	50	17	45	15
31	29, 44	1.7	7.1	94	115	19	151	19
32	31, 45	1.5	5.4	134	67	17	50	20
33	29, 41	1.7	6.7	26	127	19	99	15
34	29, 39	1.9	5.9	24	158	22	185	20

Example 7

Determination of the epitope region of 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 7).

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 7:

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 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 305 (=TW305, see Figure 1) (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. Results are shown in Figure 1.

Patent Claims

1. Antibody binding to human TWEAK, characterized by comprising as heavy chain variable domain CDR3H selected from the group consisting of SEQ ID NO: 8, 16 or 24.

2. Chimeric, humanized or T-cell epitope depleted variant
a) of an antibody comprising a variable light chain of SEQ ID NO:1 and a variable heavy chain of SEQ ID NO:5,
b) of an antibody comprising a variable light chain of SEQ ID NO:9 and a variable heavy chain of SEQ ID NO:13 or
c) of an antibody comprising a variable light chain of SEQ ID NO:17 and a variable heavy chain of SEQ ID NO:21.

3. Antibody according to claim 1, characterized

a) by comprising CDR1H of SEQ ID NO:6, CDR2H of SEQ ID NO:7, CDR3H of SEQ ID NO:8 and CDR1L of SEQ ID NO:2, CDR2L of SEQ ID NO:3, CDR3L of SEQ ID NO:4, or
b) by comprising CDR1H of SEQ ID NO:14, CDR2H of SEQ ID NO:15, CDR3H of SEQ ID NO:16 and CDR1L of SEQ ID NO:10, CDR2L of SEQ ID NO:11, CDR3L of SEQ ID NO:12, or
c) by comprising CDR1H of SEQ ID NO:22, CDR2H of SEQ ID NO:23, CDR3H of SEQ ID NO:24 and CDR1L of SEQ ID NO:18, CDR2L of SEQ ID NO:19, CDR3L of SEQ ID NO:20.

4. Antibody binding to human TWEAK, characterized by comprising by comprising CDR1H of SEQ ID NO:6, CDR2H of SEQ ID NO:7, CDR3H of SEQ ID NO:8 and CDR1L of SEQ ID NO:2, CDR2L of SEQ ID NO:3, CDR3L of SEQ ID NO:4.

5. Antibody binding to human TWEAK, characterized by comprising CDR1H of SEQ ID NO:14, CDR2H of SEQ ID NO:15, CDR3H of SEQ ID NO:16 and CDR1L of SEQ ID NO:10, CDR2L of SEQ ID NO:11, CDR3L of SEQ ID NO:12.

6. Antibody binding to human TWEAK, characterized by comprising CDR1H of SEQ ID NO:22, CDR2H of SEQ ID NO:23, CDR3H of SEQ ID NO:24

and CDR1L of SEQ ID NO:18, CDR2L of SEQ ID NO:19, CDR3L of SEQ ID NO:20.

7. Antibody according to claim 6, characterized by comprising a) a variable light chain of SEQ ID NO: 27 and a variable heavy chain of SEQ ID NO: 41, b) a variable light chain of SEQ ID NO: 27 and a variable heavy chain of SEQ ID NO: 44, c) a variable light chain of SEQ ID NO: 27 and a variable heavy chain of SEQ ID NO: 45, d) a variable light chain of SEQ ID NO: 26 and a variable heavy chain of SEQ ID NO: 47, e) a variable light chain of SEQ ID NO: 29 and a variable heavy chain of SEQ ID NO: 44, f) a variable light chain of SEQ ID NO: 31 and a variable heavy chain of SEQ ID NO: 45, g) a variable light chain of SEQ ID NO: 29 and a variable heavy chain of SEQ ID NO: 41, or h) a variable light chain of SEQ ID NO: 29 and a variable heavy chain of SEQ ID NO: 39.

8. Antibody according to claim 6, characterized by comprising a variable light chain of SEQ ID NO: 27 and a variable heavy chain of SEQ ID NO: 41, b) a variable light chain of SEQ ID NO: 27 and a variable heavy chain of SEQ ID NO: 45, c) a variable light chain of SEQ ID NO: 29 and a variable heavy chain of SEQ ID NO: 44, d) a variable light chain of SEQ ID NO: 29 and a variable heavy chain of SEQ ID NO: 41, or e) a variable light chain of SEQ ID NO: 29 and a variable heavy chain of SEQ ID NO: 39.

9. Antibody according to claim 6, characterized by a combination of a variable light chain sequence/variable heavy chain sequence selected from the group consisting of SEQ ID NO:1/SEQ ID NO:5, 9/13, 17/21, 25/37, 26/38, 27/39, 28/40, 29/41, 30/42, 31/43, 32/44; 33/45, 34/46, 35/47, 36/48, 56/57, 58/59.

10. Antibody according to claim 6, characterized by comprising a variable heavy chain or variable light chain selected from the group of variable heavy or light chains of SEQ ID NO:17, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 56, 58, 21, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 57, or 59.

11. Pharmaceutical composition characterized by comprising an antibody according to claims 1 to 10.

12. Use of an antibody according to claims 1 to 10 for the manufacture of a pharmaceutical composition.

13. An antibody according to claims 1 to 10 for the treatment of cancer, or 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.

14. An antibody according to claims 1 to 10 for the treatment of colon, lung, or pancreatic cancer.

15. Use of an antibody according to claims 1 to 10 for manufacture of a medicament for the treatment of cancer, or 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.

16. Use of an antibody according to claims 1 to 10 for manufacture of a medicament for the treatment of colon, lung, or pancreatic cancer.

17. Nucleic acid encoding an antibody according to claims 1 to 10.

18. Expression vectors characterized by comprising a nucleic acid according to claim 17 for the expression of an antibody binding to TWEAK in a prokaryotic or eukaryotic host cell.

19. Prokaryotic or eukaryotic host cell comprising a vector according to claim 18.

20. Method for the production of a recombinant antibody according to claims 1 to 10, characterized by expressing a nucleic acid according to claim 17 in a prokaryotic or eukaryotic host cell and recovering said antibody from said cell or the cell culture supernatant.

21. Method for the treatment of a patient suffering from 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, characterized by administering to the patient an antibody according to claims 1 to 10.