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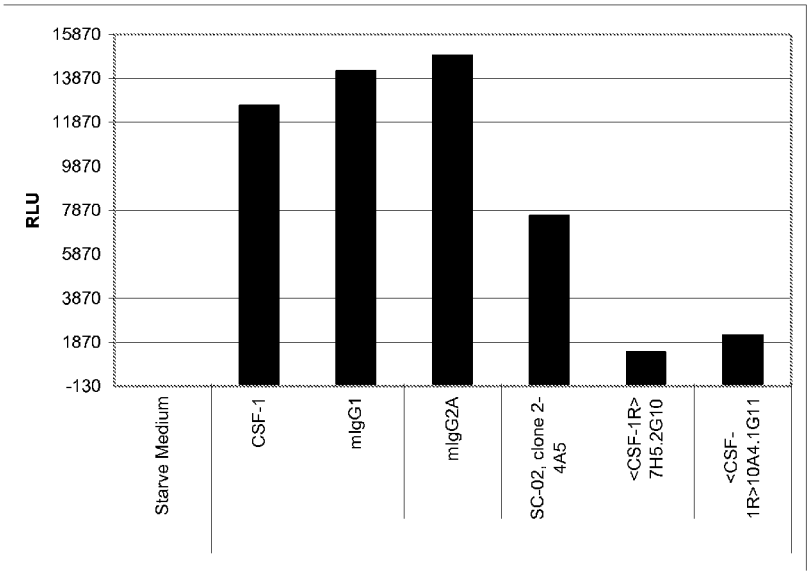
(54) Title: ANTIBODIES AGAINST HUMAN CSF-IR AND USES THEREOF

Fig. l



(57) Abstract: The present invention relates to antibodies against human CSF-IR (CSF-IR antibody), methods for their production, pharmaceutical compositions containing said antibodies, and uses thereof.

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Antibodies against human CSF-IR and uses thereof

Field of the invention

The present invention relates to antibodies against human CSF-IR (CSF-IR antibody), methods for their production, pharmaceutical compositions containing said antibodies, and uses thereof.

Background of the Invention

The CSF-1 receptor (CSF-IR; synonyms: M-CSF receptor; Macrophage colony-stimulating factor 1 receptor, EC 2.7.10.1, Fms proto-oncogene, c-fms, Swiss Prot P07333, CD1 15) is known since 1986 (Coussens, L., et al, Nature 320 (1986) 277-280). CSF-IR is a growth factor and encoded by the c-fms proto-oncogene (reviewed e.g. in Roth, P., and Stanley, E.R., Curr. Top. Microbiol. Immunol. 181 (1992) 141-67).

CSF-IR is the receptor for M-CSF (macrophage colony stimulating factor, also called CSF-1) and mediates the biological effects of this cytokine (Sherr, C.J., et al.,Cell 4 1 (1985) 665-676). The cloning of the colony stimulating factor-1 receptor (also called c-fms) was described for the first time in Roussel, M.F., et al, Nature 325 (1987) 549-552. In that publication, it was shown that CSF-IR had transforming potential dependent on changes in the C-terminal tail of the protein including the loss of the inhibitory tyrosine 969 phosphorylation which binds Cbl and thereby regulates receptor down regulation (Lee, P.S., et al, Embo J. 18 (1999) 3616-3628).

CSF-IR is a single chain, transmembrane receptor tyrosine kinase (RTK) and a member of the family of immunoglobulin (Ig) motif containing RTKs characterized by repeated Ig domains in the extracellular portion of the receptor. The intracellular protein tyrosine kinase domain is interrupted by a unique insert domain that is also present in the other related RTK class III family members that include the platelet derived growth factor receptors (PDGFR), stem cell growth factor receptor (c-Kit) and fins-like cytokine receptor (FLT3). In spite of the structural homology among this family of growth factor receptors, they have distinct tissue-specific functions. CSF-IR is mainly expressed on cells of the monocytic lineage and in the female reproductive tract and placenta. In addition expression of CSF-IR has been reported in Langerhans cells in skin, a subset of smooth muscle cells (Inaba, T., et al, J. Biol. Chem. 267 (1992) 5693-5699),

B cells (Baker, A.H., et al, Oncogene 8 (1993) 371-378) and microglia (Sawada, M., et al, Brain Res. 509 (1990) 119-124).

The main biological effects of CSF-1R signaling are the differentiation, proliferation, migration, and survival of hematopoietic precursor cells to the macrophage lineage (including osteoclast). Activation of CSF-1R is mediated by its ligand, M-CSF. Binding of M-CSF to CSF-1R induces the formation of homodimers and activation of the kinase by tyrosine phosphorylation (Stanley, E.R., et al, Mol. Reprod. Dev. 46 (1997) 4-10). Further signaling is mediated by the p85 subunit of PI3K and Grb2 connecting to the PI3K/AKT and Ras/MAPK pathways, respectively. These two important signaling pathways can regulate proliferation, survival and apoptosis. Other signaling molecules that bind the phosphorylated intracellular domain of CSF-1R include STAT1, STAT3, PLCy, and Cbl (Bourette, R.P., Rohrschneider, L.R., Growth Factors 17 (2000) 155-166).

CSF-1R signaling has a physiological role in immune responses, in bone remodeling and in the reproductive system. The knockout animals for either M-CSF-1 (Pollard, J.W., Mol. Reprod. Dev. 46 (1997) 54-61) or CSF-1R (Dai, X.M., et al, Blood 99 (2002) 111-120) have been shown to have osteopetrotic, hematopoietic, tissue macrophage, and reproductive phenotypes consistent with a role for CSF-1R in the respective cell types.

Sherr, C.J., et al, Blood 73 (1989) 1786-1793 relates to some antibodies against CSF-1R that inhibit the CSF-1 activity (see Sherr, C.J., et al, Blood 73 (1989) 1786-1793). Ashmun, R.A., et al, Blood 73 (1989) 827-837 relates to CSF-1R antibodies. Lenda, D., et al, Journal of immunology 170 (2003) 3254-3262 relates to reduced macrophage recruitment, proliferation, and activation in CSF-1-deficient mice results in decreased tubular apoptosis during renal inflammation. Kitaura, H., et al., Journal of dental research 87 (2008) 396-400 refers to an anti-CSF-1 antibody which inihibits orthodontic tooth movement. WO 2001/030381 mentions CSF-1 activity inhibitors including antisense nucleotides and antibodies while disclosing only CSF-1 antisense nucleotides. WO 2004/045532 relates to metastases and bone loss prevention and treatment of metastatic cancer by a M-CSF antagonist disclosing as antagonist anti-CSF-1-antibodies only. WO 2005/046657 relates to the treatment of inflammatory bowel disease by anti-CSF-1-antibodies. US 2002/0141994 relates to inhibitors of colony stimulating factors. WO 2006/096489 relates to the treatment of rheumatoid arthritis by anti-CSF-1-antibodies.

WO 2009/026303 and WO 2009/1 12245 relate to anti-CSF-lR antibodies.

Summary of the Invention

The invention comprises an antibody binding to human CSF-1R, characterized in binding to same epitope as the deposited antibody DSM ACC2922.

In one embodiment the antibody is characterized in comprising as heavy chain variable domain CDR3 region a CDR3 region of SEQ ID NO: 1, SEQ ID NO: 9 , or SEQ ID NO: 17.

In one embodiment the antibody is characterized in that

a) the heavy chain variable domain comprises a CDR3 region of SEQ ID NO: 1, a CDR2 region of SEQ ID NO: 2, and a CDR1 region of SEQ ID NO:3, and the light chain variable domain comprises a CDR3 region of SEQ ID NO: 4, a CDR2 region of SEQ ID NO:5, and a CDR1 region of SEQ ID NO:6, or

b) the heavy chain variable domain comprises a CDR3 region of SEQ ID NO: 9, a CDR2 region of SEQ ID NO: 10, and a CDR1 region of SEQ ID NO: 11, and the light chain variable domain comprises a CDR3 region of SEQ ID NO: 12, a CDR2 region of SEQ ID NO: 13, and a CDR1 region of SEQ ID NO: 14; or

c) the heavy chain variable domain comprises a CDR3 region of SEQ ID NO: 17, a CDR2 region of SEQ ID NO: 18, and a CDR1 region of SEQ ID NO: 19, and i the light chain variable domain comprises a CDR3 region of SEQ ID NO:20, a CDR2 region of SEQ ID NO: 21, and a CDR1 region of SEQ ID NO: 22; or

d) a CDR grafted, humanized or T cell epitope depleted antibody variant of the antibodies of a), b) or c).

In one embodiment the antibody is characterized in comprising

a) the amino acid sequence of the heavy chain variable domain is SEQ ID NO: 7, and the amino acid sequence of the light chain variable domain is SEQ ID NO:8, or

b) the amino acid sequence of the heavy chain variable domain is SEQ ID NO: 15, and the amino acid sequence of the light chain variable domain is SEQ ID NO: 16, or

c) the amino acid sequence of the heavy chain variable domain is SEQ ID NO: 23, and the amino acid sequence of the light chain variable domain is SEQ ID NO:24 or

d) a CDR grafted, humanized or T cell epitope depleted antibody variant of the antibodies of a), b) or c).

In one embodiment the antibody binding to human CSF-IR and being characterized by the above mentioned amino acid sequences and amino acid sequence fragments is of human IgGl subclass or is of human IgG4 subclass.

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

The invention further comprises a pharmaceutical composition characterized in comprising the antibody binding to human CSF-IR being characterized by the the above mentioned epitope binding properties or alternatively by the above mentioned amino acid sequences and amino acid sequence fragments.

The invention further comprises the use an of an antibody characterized characterized in comprising the antibody binding to human CSF-IR being characterized by the the above mentioned epitope binding properties or alternatively by the above mentioned amino acid sequences and amino acid sequence fragments for the manufacture of a pharmaceutical composition.

The invention further comprises the use of an antibody characterized in comprising the antibody binding to human CSF-IR being characterized by the the above mentioned epitope binding properties or alternatively by the above mentioned amino acid sequences and amino acid sequence fragments for the treatment of a CSF-IR mediated diseases.

The invention further comprises the use of an antibody characterized in comprising the antibody binding to human CSF-IR being characterized by the the above mentioned epitope binding properties or alternatively by the above mentioned amino acid sequences and amino acid sequence fragments for the treatment of cancer.

The invention further comprises the use of an antibody characterized in comprising the antibody binding to human CSF-IR being characterized by the the above mentioned epitope binding properties or alternatively by the above mentioned amino acid sequences and amino acid sequence fragments for the treatment of bone loss.

The invention further comprises the of an antibody characterized in comprising the antibody binding to human CSF-IR being characterized by the the above mentioned epitope binding properties or alternatively by the above mentioned amino acid sequences and amino acid sequence fragments for the prevention or treatment of metastasis.

The invention further comprises the of an antibody characterized in comprising the antibody binding to human CSF-IR being characterized by the the above mentioned epitope binding properties or alternatively by the above mentioned amino acid sequences and amino acid sequence fragments for treatment of inflammatory diseases.

One aspect of the invention is an antibody binding to human CSF-IR, characterized in comprising as heavy chain variable domain CDR3 region a CDR3 region of SEQ ID NO: 1, SEQ ID NO: 9 , or SEQ ID NO: 17.

Another aspect of the invention is an antibody binding to human CSF-IR, characterized in that

a) the heavy chain variable domain comprises a CDR3 region of SEQ ID NO: 1, a CDR2 region of SEQ ID NO: 2, and a CDR1 region of SEQ ID NO:3, and the light chain variable domain comprises a CDR3 region of SEQ ID NO: 4, a CDR2 region of SEQ ID NO:5, and a CDR1 region of SEQ ID NO:6, or

b) the heavy chain variable domain comprises a CDR3 region of SEQ ID NO: 9, a CDR2 region of SEQ ID NO: 10, and a CDR1 region of SEQ ID NO: 11, and the light chain variable domain comprises a CDR3 region of SEQ ID NO: 12, a CDR2 region of SEQ ID NO: 13, and a CDR1 region of SEQ ID NO: 14; or

c) the heavy chain variable domain comprises a CDR3 region of SEQ ID NO: 17, a CDR2 region of SEQ ID NO: 18, and a CDR1 region of SEQ ID NO:

19, and i the light chain variable domain comprises a CDR3 region of SEQ ID NO:20, a CDR2 region of SEQ ID NO: 21, and a CDR1 region of SEQ ID NO: 22; or

d) a CDR grafted, humanized or T cell epitope depleted antibody variant of the antibodies of a), b) or c).

In one embodiment the antibody is characterized in comprising

a) the amino acid sequence of the heavy chain variable domain is SEQ ID NO: 7, and the amino acid sequence of the light chain variable domain is SEQ ID NO:8, or

b) the amino acid sequence of the heavy chain variable domain is SEQ ID NO: 15, and the amino acid sequence of the light chain variable domain is SEQ ID NO: 16, or

c) the amino acid sequence of the heavy chain variable domain is SEQ ID NO: 23, and the amino acid sequence of the light chain variable domain is SEQ ID NO:24, or

d) a CDR grafted, humanized or T cell epitope depleted antibody variant of the antibodies of a), b) or c).

In one aspect of the invention the antibodies according to the invention bind to human CSF-IR with an affinity of at least 10^{-8} mol/1 to 10^{-12} mol/1.

In one aspect of the invention the antibodies according to the invention is a humanized antibody.

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. Preferably the nucleic acid encodes a heavy chain of an antibody binding to human CSF-IR, characterized in comprising as heavy chain CDR3 region a CDR3 region of SEQ ID NO: 1, SEQ ID NO: 9 , or SEQ ID NO: 17.

A further embodiment of the invention is a nucleic acid encoding an antibody according to the invention characterized in that

a) the heavy chain variable domain comprises a CDR3 region of SEQ ID NO: 1, a CDR2 region of SEQ ID NO: 2, and a CDR1 region of SEQ ID NO:3,

and the light chain variable domain comprises a CDR3 region of SEQ ID NO: 4, a CDR2 region of SEQ ID NO:5, and a CDR1 region of SEQ ID NO:6, or

b) the heavy chain variable domain comprises a CDR3 region of SEQ ID NO: 9, a CDR2 region of SEQ ID NO: 10, and a CDR1 region of SEQ ID NO: 11, and the light chain variable domain comprises a CDR3 region of SEQ ID NO: 12, a CDR2 region of SEQ ID NO: 13, and a CDR1 region of SEQ ID NO: 14; or

c) the heavy chain variable domain comprises a CDR3 region of SEQ ID NO: 17, a CDR2 region of SEQ ID NO: 18, and a CDR1 region of SEQ ID NO: 19, and i the light chain variable domain comprises a CDR3 region of SEQ ID NO:20, a CDR2 region of SEQ ID NO: 21, and a CDR1 region of SEQ ID NO: 22; or

d) a CDR grafted, humanized or T cell epitope depleted antibody variant of the antibodies of a), b) or c).

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 CSF-1R targeting therapy. The antibodies according to the invention have new and inventive properties causing a benefit for a patient suffering from a tumor disease, especially suffering from cancer.

The invention further provides a method for treating a patient suffering from cancer, 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 human CSF-IR 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 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 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 pharmaceutical compositions 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 Growth inhibition of BeWo tumor cells in 3D culture under treatment with different anti-CSF-lR monoclonal antibodies at a concentration of 10µg/ml.
X axis: viability mean relative light units (RLU) corresponding to the ATP-content of the cells (CellTiterGlo assay).
Y axis: tested probes: Minimal Medium (0.5 % FBS), mouse IgGl (mlgGl, ^g/ml), mouse IgG2a (mIgG2a ^g/ml), CSF-1 only, <CSF-1R>7H5.2G10, <CSF-1R>10A4.1G1 1, and SC-02, clone 2-4A5.
Highest inhibition of CSF-1 induced growth was observed with the anti-CSF-lR antibodies according to the invention.

Detailed Description of Embodiments of the Invention

I. Definitions

The term "antibody" encompasses the various forms of antibodies including but not being limited to whole antibodies, antibody fragments, humanized antibodies, chimeric antibodies, T cell epitope depleted antibodies, and further genetically engineered antibodies 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 Houston, 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 binding to CSF-IR, namely being able to assemble together with **aVL** domain, or of a V_L domain binding to CSF-IR, namely being able to assemble together with a V_H domain to a functional antigen binding site and thereby providing the property.

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 "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 a mouse variable region and a human constant region are especially preferred. Such rat/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 5,202,238 and US 5,204,244.

The term "CDR-grafted variant" as used within the current application denotes a variable domain of an antibody comprising complementary determining regions (CDRs or hypervariable regions) from one source or species and framework regions (FRs) from a different source or species, usually prepared by recombinant DNA techniques. CDR-grafted variants of variable domains comprising murine CDRs and a human FRs are preferred.

The term "T-cell epitope depleted variant" as used within the current application denotes a variable domain of an antibody which was modified to remove or reduce immunogenicity by removing human T-cell epitopes (peptide sequences within the variable domains with the capacity to bind to MHC Class II molecules). By this method interactions between amino acid side chains of the variable domain 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 term "humanized variant" as used within the current application denotes a variable domain of an antibody, which is reconstituted from the complementarity determining regions (CDRs) of non-human origin, e.g. from a non-human species, and from the framework regions (FRs) of human origin, and which has been further modified in order to also reconstitute or improve the binding affinity and specifity of the original non-human variable domain. Such humanized variants are usually prepared by recombinant DNA techniques. The reconstitution of the affinity and specifity of the parent non-human variable domain is the critical step, for which different methods are currently used. In one method it is determined whether it is beneficial to introduce mutations, so called backmutations, in the non-human CDRs as well as in the human FRs. The suited positions for such backmutations can be identified e.g. by sequence or homology analysis, by choosing the human framework (fixed frameworks approach; homology matching or best-fit), by using consensus sequences, by selecting FRs from several different human mAbs, or by replacing non-human residues on the three dimensional surface with the most common residues found in human mAbs ("resurfacing" or "veneering").

The antibodies according to the invention include, in addition, such antibodies having "conservative sequence modifications", nucleotide and amino acid sequence

modifications which do not affect or alter the above-mentioned characteristics of the antibody according to the invention. Modifications can be introduced by standard techniques known in the art, such as site-directed mutagenesis and PCR-mediated mutagenesis. Conservative amino acid substitutions include ones in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g. glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in a human anti-CSF-lR antibody can be preferably replaced with another amino acid residue from the same side chain family.

Amino acid substitutions can be performed by mutagenesis based upon molecular modeling as described by Riechmann, L., et al, Nature 332 (1988) 323-327 and Queen, C , et al, Proc. Natl. Acad. Sci. USA 86 (1989) 10029-10033.

The term "CSF-1R" as used herein refers to human CSF-1R (SEQ ID No: 31) CSF-1R (synonyms: CSF-1 receptor M-CSF receptor; Macrophage colony-stimulating factor 1 receptor, EC 2.7.10.1, Fms proto-oncogene, c-fms, Swiss Prot P07333, CD1 15,) is known since 1986 (Coussens, L., et al, Nature 320 (1986) 277-280). CSF-1R is a growth factor and encoded by the c-fms proto-oncogene (reviewed e.g. in Roth, P. and Stanley, E.R., Curr. Top. Microbiol. Immunol. 181 (1992) 141-67).

CSF-1 R is the receptor for M-CSF (macrophage colony stimulating factor, also called CSF-1) and mediates the biological effects of this cytokine (Sherr, C.J., et al., Cell 4 1 (1985) 665-676). The cloning of the colony stimulating factor-1 receptor (also called c-fms) was described for the first time in Roussel, M.F., et al, Nature 325 (1987) 549-552. In that publication, it was shown that CSF-1R had transforming potential dependent on changes in the C-terminal tail of the protein including the loss of the inhibitory tyrosine 969 phosphorylation which binds Cbl and thereby regulates receptor down regulation (Lee, P.S., et al, Embo J. 18 (1999) 3616-3628).

CSF-1R is a single chain, transmembrane receptor tyrosine kinase (RTK) and a member of the family of immunoglobulin (Ig) motif containing RTKs characterized by repeated Ig domains in the extracellular portion of the receptor. The intracellular protein tyrosine kinase domain is interrupted by a unique insert domain that is also present in the other related RTK class III family members that include the platelet derived growth factor receptors (PDGFR), stem cell growth factor receptor (c-Kit) and fins-like cytokine receptor (FLT3). In spite of the structural homology among this family of growth factor receptors, they have distinct tissue-specific functions. CSF-IR is mainly expressed on cells of the monocytic lineage and in the female reproductive tract and placenta. In addition expression of CSF-IR has been reported in Langerhans cells in skin, a subset of smooth muscle cells (Inaba, T., et al, J. Biol. Chem. 267 (1992) 5693-5699), B cells (Baker, A.H., et al, Oncogene 8 (1993) 371-378) and microglia (Sawada, M., et al, Brain Res. 509 (1990) 119-124).

As used herein, the terms "binding to human CSF-IR" or "that binds to human CSF-IR" or anti-CSF-lR" are used interchangeable and refer to an antibody specifically binding to the human CSF-IR antigen. The binding affinity is of KD-value of 1.0 x 10^{-8} mol/1 or lower at 35 °C, preferably of a KD-value of 1.0 x10^{-9} mol/1 or lower at 35 °C. The binding affinity is determined with a standard binding assay at 35 °C, such as surface plasmon resonance technique (Biacore®) (see Example 4).

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. Preferably an antibody according to the invention binds specifically to native but not to denatured CSF-IR.

The term "binding to the same epitope as the deposited antibody DSM ACC2922" as used herein refers to an anti-CSF-lR antibody of the invention that binds to the same epitope on CSF-IR to which the antibody <CSF-1R>7H5.2G10 (deposit no. DSM ACC2922) binds. The epitope binding property of an anti-CSF-lR antibody of the present invention may be determined using techniques known in the art. The CSF-IR antibody is measured by Surface Plasmon Resonance (SPR) at 25 °C in an

in vitro competitive binding inhibition assay to determine the ability of the test antibody to inhibit binding of antibody <CSF-1R>7H5.2G10 (deposit no. DSM ACC2922) to CSF-1R. This can be investigated by a BIAcore assay (Pharmacia Biosensor AB, Uppsala, Sweden) as e.g. in Example 5. In Example 5 the percentage (%) of expected binding response of the CSF-1R antibody of the invention competing with the bound the antibody <CSF-1R>7H5.2G10 (deposit no. DSM ACC2922) is calculated by "100 * relativeResponse(general_stability_early) / rMax", where rMax is calculated by "relativeResponse(general_stability_late) * antibody molecular weight / antigen molecular weight" as described in BIAcore assay epitope mapping instructions. A minimal binding response is also calculated from the pairs of identical antibody 1 and 2 (see Example 5). Thereof the obtained maximal value + 50 % is set as threshold for significant competion and thus significant binding to the same epitope (see Example 5 for antibody <CSF-1R>7H5.2G10 calculated threshold is 7+3.5= 10.5). Thus an antibody binding to human CSF-1R, characterized in "binding to the same epitope as <CSF-1R>7H5.2G10 (deposit no. DSM ACC2922)" has a percentage (%) of expected binding response of lower than 10.5 (%expected binding response < 10.5).

In one aspect the antibodies according to the invention compete with deposited antibody DSM ACC2922 for binding to human CSF-1R. Such binding competion may be determined using techniques known in the art. The CSF-1R antibody is measured at 25 °C by Surface Plasmon Resonance (SPR) in an in vitro competitive binding inhibition assay to determine the ability of the test antibody to inhibit binding of antibody <CSF-1R>7H5.2G10 (deposit no. DSM ACC2922) to human CSF-1R. This can be investigated by a BIAcore assay (Pharmacia Biosensor AB, Uppsala, Sweden) as e.g. in Example 5.

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 refer 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, E., A., 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 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 a-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 (gin, 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).

An "immunoconjugate" is an antibody conjugated to one or more heterologous molecule(s), including but not limited to a cytotoxic agent.

An "individual" or "subject" is a mammal. Mammals include, but are not limited to, domesticated animals (e.g., cows, sheep, cats, dogs, and horses), primates (e.g., humans and non-human primates such as monkeys), rabbits, and rodents (e.g., mice and rats). In certain embodiments, the individual or subject is a human.

An "isolated" antibody is one which has been separated from a component of its natural environment. In some embodiments, an antibody is purified to greater than

95 % or 99 % purity as determined by, for example, electrophoretic (e.g., SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatographic (e.g., ion exchange or reverse phase HPLC). For review of methods for assessment of antibody purity, see, e.g., Flatman et al, J. Chromatogr. B 848:79-87 (2007).

An "isolated" nucleic acid refers to a nucleic acid molecule that has been separated from a component of its natural environment. An isolated nucleic acid includes a nucleic acid molecule contained in cells that ordinarily contain the nucleic acid molecule, but the nucleic acid molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location.

"Isolated nucleic acid encoding an anti-CSF-lR antibody" refers to one or more nucleic acid molecules encoding antibody heavy and light chains (or fragments thereof), including such nucleic acid molecule(s) in a single vector or separate vectors, and such nucleic acid molecule(s) present at one or more locations in a host cell.

"Native antibodies" refer to naturally occurring immunoglobulin molecules with varying structures. For example, native IgG antibodies are heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light chains and two identical heavy chains that are disulfide-bonded. From N- to C-terminus, each heavy chain has a variable region (VH), also called a variable heavy domain or a heavy chain variable domain, followed by three constant domains (CHI, CH2, and CH3). Similarly, from N- to C-terminus, each light chain has a variable region (VL), also called a variable light domain or a light chain variable domain, followed by a constant light (CL) domain. The light chain of an antibody may be assigned to one of two types, called kappa (κ) and lambda (λ), based on the amino acid sequence of its constant domain.

The term "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, combination therapy, contraindications and/or warnings concerning the use of such therapeutic products.

"Percent (%) amino acid sequence identity" with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering

any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU5 10087. The ALIGN-2 program is publicly available from Genentech, Inc., South San Francisco, California, or may be compiled from the source code. The ALIGN-2 program should be compiled for use on a UNIX operating system, including digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

100 times the fraction X/Y

where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program.

TT. Comnositions and Methods

In one aspect, the invention is based, in part, on to same epitope as the deposited antibody DSM ACC2922. Antibodies of the invention are useful, e.g., for the diagnosis or treatment of cancer, of inflammatory diseases or of bone loss; or for the prevention or treatment of metastasis.

Exemplary Anti-CSF-lR Antibodies

In one aspect, the invention provides antibodies that bind to human CSF-IR. In certain embodiments, the anti-CSF-lR antibody is characterized in binding to same epitope as the deposited antibody DSM ACC2922.

Another aspect of the invention is an antibody binding to human CSF-IR, characterized in that

a) the heavy chain variable domain comprises a CDR3 region of SEQ ID NO: 1, a CDR2 region of SEQ ID NO: 2, and a CDR1 region of SEQ ID NO:3, and the light chain variable domain comprises a CDR3 region of SEQ ID NO: 4, a CDR2 region of SEQ ID NO:5, and a CDR1 region of SEQ ID NO:6, or

b) the heavy chain variable domain comprises a CDR3 region of SEQ ID NO: 9, a CDR2 region of SEQ ID NO: 10, and a CDR1 region of SEQ ID NO: 11, and the light chain variable domain comprises a CDR3 region of SEQ ID NO: 12, a CDR2 region of SEQ ID NO: 13, and a CDR1 region of SEQ ID NO: 14; or

c) the heavy chain variable domain comprises a CDR3 region of SEQ ID NO: 17, a CDR2 region of SEQ ID NO: 18, and a CDR1 region of SEQ ID NO: 19, and i the light chain variable domain comprises a CDR3 region of SEQ ID NO:20, a CDR2 region of SEQ ID NO: 21, and a CDR1 region of SEQ ID NO: 22; or

d) a CDR grafted, humanized or T cell epitope depleted antibody variant of the antibodies of a), b) or c).

Another aspect of the invention is an antibody binding to human CSF-IR, characterized in that

a) the heavy chain variable domain comprises a CDR3 region of SEQ ID NO: 1, a CDR2 region of SEQ ID NO: 2, and a CDR1 region of SEQ ID NO:3, and the light chain variable domain comprises a CDR3 region of SEQ ID NO: 4, a CDR2 region of SEQ ID NO:5, and a CDR1 region of SEQ ID NO:6, or

b) the heavy chain variable domain comprises a CDR3 region of SEQ ID NO: 9, a CDR2 region of SEQ ID NO: 10, and a CDR1 region of SEQ ID NO: 11, and the light chain variable domain comprises a CDR3 region of SEQ ID NO: 12, a CDR2 region of SEQ ID NO: 13, and a CDR1 region of SEQ ID NO: 14; or

c) the heavy chain variable domain comprises a CDR3 region of SEQ ID NO: 17, a CDR2 region of SEQ ID NO: 18, and a CDR1 region of SEQ ID NO: 19, and i the light chain variable domain comprises a CDR3 region of SEQ ID NO:20, a CDR2 region of SEQ ID NO: 21, and a CDR1 region of SEQ ID NO: 22; or

d) a CDR grafted, humanized or T cell epitope depleted antibody variant of the antibodies of a), b) or c); and

having one or more of the following properties (determined in assays as described in Example 2, 3, 4, 6, 7 and 8):

- the anti-CSF-lR antibody inhibits CSF-1 binding to CSF-IR with an IC50 of 75 ng/ml or lower, in one embodiment with an IC50 of 50 ng/ml or lower;

- the anti-CSF-lR antibody inhibits CSF-l-induced CSF-IR phosphorylation (in NIH3T3-CSF-1R recombinant cells) with an IC50 of 100 ng/ml or lower, in one embodiment with an IC50 of 50 ng/ml or lower;

- the anti-CSF-lR antibody inhibits the growth of recombinant NIH3T3 cells expressing human CSF-IR (SEQ ID No: 15) by 80 % or more (as compared to the absence of antibody), preferably by 90 % or more;

- the anti-CSF-lR antibody inhibits the growth of BeWo tumor cells (ATCC CCL-98) by 70 % or more (at a antibody concentration of 10µg/ml ;and as compared to the absence of antibody), preferably by 80 % or more;

- the anti-CSF-lR antibody inhibits macrophage differentiation. (In one embodiment the anti-CSF-lR antibody inhibits the survival of monocytes with an IC50 of 1.5 nM or lower, preferably with an IC50 of 1.0 nM or lower); or

- the anti-CSF-lR antibody is binding to human CSF-1R with a binding affinity of KD = 2.0 x 10^{-9} mol/l or lower at 35 °C.

In another aspect, an anti-CSF-lR antibody according to the invention comprises in the heavy chain variable domain (VH) sequence a) a CDRIH having an amino acid sequence identical to, or comprising 1, 2, or 3 amino acid residue substitutions relative to SEQ ID NO:3, SEQ ID NO:l l or SEQ ID NO:19, b) a CDR2H having an amino acid sequence identical to, or comprising 1, 2, or 3 amino acid residue substitutions relative to SEQ ID NO:2, SEQ ID NO:10 or SEQ ID NO:18, and c) a CDR3H having an amino acid sequence identical to, or comprising 1, 2, or 3 amino acid residue substitutions relative to SEQ ID NO:l, SEQ ID NO:9 or SEQ ID NO:17.

In certain embodiments, a heavy chain variable domain (VH) sequence comprising a) a CDRIH having an amino acid sequence identical to, or comprising 1, 2, or 3 amino acid residue substitutions relative to SEQ ID NO:3, SEQ ID NO: 11 or SEQ ID NO: 19, b) a CDR2H having an amino acid sequence identical to, or comprising 1, 2, or 3 amino acid residue substitutions relative to SEQ ID NO:2, SEQ ID NO:10 or SEQ ID NO:18, and c) a CDR3H having an amino acid sequence identical to, or comprising 1, 2, or 3 amino acid residue substitutions relative to SEQ ID NO:l, SEQ ID NO:9 or SEQ ID NO:17, contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti CSF-1R antibody comprising that sequence retains the ability to bind to CSF-lR.

In another aspect, an anti-CSF-lR antibody according to the invention comprises in the light chain variable domain (VL) sequence a) a CDRIL having an amino acid sequence identical to, or comprising 1, 2, or 3 amino acid residue substitutions relative to SEQ ID NO:6, SEQ ID NO: 14 or SEQ ID NO:22, b) a CDR2L having an amino acid sequence identical to, or comprising 1, 2, or 3 amino acid residue substitutions relative to SEQ ID NO:5, SEQ ID NO:13 or SEQ ID NO:21, and c) a CDR3L having an amino acid sequence identical to, or comprising 1, 2, or 3 amino acid residue substitutions relative to SEQ ID NO:4, SEQ ID NO: 12 or SEQ ID NO:20.

In certain embodiments, a light chain variable domain (VL) sequence comprising a) a CDR1L having an amino acid sequence identical to, or comprising 1, 2, or 3 amino acid residue substitutions relative to SEQ ID NO:6, SEQ ID NO: 14 or SEQ ID NO:22, b) a CDR2L having an amino acid sequence identical to, or comprising 1, 2, or 3 amino acid residue substitutions relative to SEQ ID NO:5, SEQ ID NO: 13 or SEQ ID NO:21, and c) a CDR3L having an amino acid sequence identical to, or comprising 1, 2, or 3 amino acid residue substitutions relative to SEQ ID NO:4, SEQ ID NO: 12 or SEQ ID NO:20, contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti CSF-IR antibody comprising that sequence retains the ability to bind to CSF-lR.

In another aspect, an anti-CSF-lR antibody according to the invention

- comprises in the heavy chain variable domain (VH) sequence a) a CDR1H having an amino acid sequence identical to, or comprising 1, 2, or 3 amino acid residue substitutions relative to SEQ ID NO:3, b) a CDR2H having an amino acid sequence identical to, or comprising 1, 2, or 3 amino acid residue substitutions relative to SEQ ID NO:2, and c) a CDR3H having an amino acid sequence identical to, or comprising 1, 2, or 3 amino acid residue substitutions relative to SEQ ID NO:l, and comprises in the light chain variable domain (VL) sequence d) a having an amino acid sequence identical to, or comprising 1, 2, or 3 amino acid residue substitutions relative to SEQ ID NO:6, e) a CDR2L having an amino acid sequence identical to, or comprising 1, 2, or 3 amino acid residue substitutions relative to SEQ ID NO:5, and f) a CDR3L having an amino acid sequence identical to, or comprising 1, 2, or 3 amino acid residue substitutions relative to SEQ ID NO:4; or

- comprises in the heavy chain variable domain (VH) sequence a) a CDR1H having an amino acid sequence identical to, or comprising 1, 2, or 3 amino acid residue substitutions relative to SEQ ID NO:l 1, b) a CDR2H having an amino acid sequence identical to, or comprising 1, 2, or 3 amino acid residue substitutions relative to SEQ ID NO: 10, and c) a CDR3H having an amino acid sequence identical to, or comprising 1, 2, or 3 amino acid residue substitutions relative to SEQ ID NO:9, and comprises in the light chain variable domain (VL) sequence d) a CDR1L having an amino acid sequence identical to, or comprising 1, 2, or 3 amino acid residue

substitutions relative to SEQ ID NO: 14, e) a CDR2L having an amino acid sequence identical to, or comprising 1, 2, or 3 amino acid residue substitutions relative to SEQ ID NO: 13, and f) a CDR3L having an amino acid sequence identical to, or comprising 1, 2, or 3 amino acid residue substitutions relative to SEQ ID NO: 12; or

- comprises in the heavy chain variable domain (VH) sequence a) a CDR1H having an amino acid sequence identical to, or comprising 1, 2, or 3 amino acid residue substitutions relative to SEQ ID NO: 19, b) a CDR2H having an amino acid sequence identical to, or comprising 1, 2, or 3 amino acid residue substitutions relative to SEQ ID NO: 18, and c) a CDR3H having an amino acid sequence identical to, or comprising 1, 2, or 3 amino acid residue substitutions relative to SEQ ID NO: 17, and comprises in the light chain variable domain (VL) sequence d) a CDRIL having an amino acid sequence identical to, or comprising 1, 2, or 3 amino acid residue substitutions relative to SEQ ID NO:22, e) a CDR2L having an amino acid sequence identical to, or comprising 1, 2, or 3 amino acid residue substitutions relative to SEQ ID NO:21, and f) a CDR3L having an amino acid sequence identical to, or comprising 1, 2, or 3 amino acid residue substitutions relative to SEQ ID NO:20.

In another aspect, an anti-CSF-lR antibody according to the invention

- comprises in the heavy chain variable domain (VH) sequence a) a CDR1H having an amino acid sequence identical to, or comprising 1, 2, or 3 amino acid residue substitutions relative to SEQ ID NO:3, b) a CDR2H having an amino acid sequence identical to, or comprising 1, 2, or 3 amino acid residue substitutions relative to SEQ ID NO:2, and c) a CDR3H having an amino acid sequence identical to, or comprising 1, 2, or 3 amino acid residue substitutions relative to SEQ ID NO:l, and comprises in the light chain variable domain (VL) sequence d) a CDRIL having an amino acid sequence identical to, or comprising 1, 2, or 3 amino acid residue substitutions relative to SEQ ID NO:6, e) a CDR2L having an amino acid sequence identical to, or comprising 1, 2, or 3 amino acid residue substitutions relative to SEQ ID NO:5, and f) a CDR3L having an amino acid sequence identical to, or comprising 1, 2, or 3 amino acid residue substitutions relative to SEQ ID NO:4; or

- comprises in the heavy chain variable domain (VH) sequence a) a CDR1H having an amino acid sequence identical to, or comprising 1, 2, or 3 amino acid residue substitutions relative to SEQ ID NO:l 1, b) a CDR2H having an amino acid sequence identical to, or comprising 1, 2, or 3 amino acid residue substitutions relative to SEQ ID NO: 10, and c) a CDR3H having an amino acid sequence identical to, or comprising 1, 2, or 3 amino acid residue substitutions relative to SEQ ID NO:9, and comprises in the light chain variable domain (VL) sequence d) a CDR1L having an amino acid sequence identical to, or comprising 1, 2, or 3 amino acid residue substitutions relative to SEQ ID NO: 14, e) a CDR2L having an amino acid sequence identical to, or comprising 1, 2, or 3 amino acid residue substitutions relative to SEQ ID NO: 13, and f) a CDR3L having an amino acid sequence identical to, or comprising 1, 2, or 3 amino acid residue substitutions relative to SEQ ID NO: 12; or

- comprises in the heavy chain variable domain (VH) sequence a) a CDR1H having an amino acid sequence identical to, or comprising 1, 2, or 3 amino acid residue substitutions relative to SEQ ID NO: 19, b) a CDR2H having an amino acid sequence identical to, or comprising 1, 2, or 3 amino acid residue substitutions relative to SEQ ID NO: 18, and c) a CDR3H having an amino acid sequence identical to, or comprising 1, 2, or 3 amino acid residue substitutions relative to SEQ ID NO: 17, and comprises in the light chain variable domain (VL) sequence d) a CDR1L having an amino acid sequence identical to, or comprising 1, 2, or 3 amino acid residue substitutions relative to SEQ ID NO:22, e) a CDR2L having an amino acid sequence identical to, or comprising 1, 2, or 3 amino acid residue substitutions relative to SEQ ID NO:21, and f) a CDR3L having an amino acid sequence identical to, or comprising 1, 2, or 3 amino acid residue substitutions relative to SEQ ID NO:20; and

the anti-CSF-lR antibody has one or more of the following properties (determined in assays as described in Example 2, 3, 4, 6, 7 and 8):

- the anti-CSF-lR antibody inhibits CSF-1 binding to CSF-IR with an IC50 of 75 ng/ml or lower, in one embodiment with an IC50 of 50 ng/ml or lower;

- the anti-CSF-lR antibody inhibits CSF-l-induced CSF-1R phosphorylation (in NIH3T3-CSF-1R recombinant cells) with an IC50 of 100 ng/ml or lower, in one embodiment with an IC50 of 50 ng/ml or lower;

- the anti-CSF-lR antibody inhibits the growth of recombinant NIH3T3 cells expressing human CSF-1R (SEQ ID No: 15) by 80 % or more (as compared to the absence of antibody), preferably by 90 % or more;

- the anti-CSF-lR antibody inhibits the growth of BeWo tumor cells (ATCC CCL-98) by 70 % or more (at a antibody concentration of 10µg/ml ;and as compared to the absence of antibody), preferably by 80 % or more;

- the anti-CSF-lR antibody inhibits macrophage differentiation. (In one embodiment the anti-CSF-lR antibody inhibits the survival of monocytes with an IC50 of 1.5 nM or lower, preferably with an IC50 of 1.0 nM or lower); or

- the anti-CSF-lR antibody is binding to human CSF-1R with a binding affinity of KD = 2.0 x 10^{-9} mol/1 or lower at 35 °C.

Recombinant Methods and Compositions

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) 151-161; Werner, R.G., 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 alkaline/SDS treatment, CsCl banding, column chromatography, agarose gel electrophoresis, and others well known in the art. See Ausubel, F., et al, ed. Current Protocols in Molecular Biology, Greene Publishing and Wiley Interscience, New York (1987).

Expression in NSO 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., Christensen, K., in Cytotechnology 30 (1999) 71-83, and by Schlaeger, E.-J., in J. Immunol. Methods 194 (1996) 191-199.

The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to utilize promoters, enhancers and polyadenylation signals.

Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. 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 contiguous, 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, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

Monoclonal antibodies are suitably separated from the culture medium by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, 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-CSF-lR 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-CSF-lR 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.

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 exhibit various effector functions. A "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 in the classes: IgA, IgD, IgE, IgG and IgM, and several of these may be further divided into subclasses (isotypes), e.g. IgGl, IgG2, IgG3, and IgG4, IgAl, and IgA2. According to the heavy chain constant regions the different classes of immunoglobulins are called a ,

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, Clq binding and Fc receptor binding. Complement activation (CDC) is initiated by binding of complement factor Clq 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 Clq 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 Boakle, R.J., et al, Nature 282 (1979) 742-743, Lukas, T.J., et al, J. Immunol. 127 (1981) 2555-2560, Brunhouse, R., Cenbra, 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 0307434. Such binding sites are e.g. L234, L235, D270, N297, E318, K320, K322, P331 and P329 (numbering according to EU index of Kabat, E.A., see below). Antibodies of subclass IgGl, IgG2 and IgG3 usually show complement activation and Clq and C3 binding, whereas IgG4 do not activate the complement system and do not bind Clq 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 IgGl, IgG2, IgG3 or IgG4, preferably a Fc part from human IgGl subclass, a mutated Fc part from human IgGl 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). Mostly preferred are the human heavy chain constant regions of SEQ ID NO:27 (human IgGl subclass), SEQ ID NO: 28 (human IgGl subclass with mutations L234A and L235A) , SEQ ID NO:29 human IgG4 subclass), or SEQ ID NO:30 (human IgG4 subclass with mutation S228P).

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, E.A., (see e.g. Johnson, G., 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: 25. 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: 26. It is further

preferred that the antibody is of mouse origin and comprises the antibody variable sequence frame of a mouse antibody according to Kabat.

Immunoconjugates

The invention also provides immunoconjugates comprising an anti-CSF-lR antibody herein conjugated to one or more cytotoxic agents, such as chemotherapeutic agents or drugs, growth inhibitory agents, toxins (e.g., protein toxins, enzymatically active toxins of bacterial, fungal, plant, or animal origin, or fragments thereof), or radioactive isotopes.

In one embodiment, an immunoconjugate is an antibody-drug conjugate (ADC) in which an antibody is conjugated to one or more drugs, including but not limited to a maytansinoid (see U.S. Patent Nos. 5,208,020, 5,416,064 and European Patent EP 0 425 235 Bl); an auristatin such as monomethylauristatin drug moieties DE and DF (MMAE and MMAF) (see U.S. Patent Nos. 5,635,483 and 5,780,588, and 7,498,298); a dolastatin; a calicheamicin or derivative thereof (see U.S. Patent Nos. 5,712,374, 5,714,586, 5,739,1 16, 5,767,285, 5,770,701, 5,770,710, 5,773,001, and 5,877,296; Hinman et al, Cancer Res. 53:3336-3342 (1993); and Lode et al, Cancer Res. 58:2925-2928 (1998)); an anthracycline such as daunomycin or doxorubicin (see Kratz et al, Current Med. Chem. 13:477-523 (2006); Jeffrey et al, Bioorganic & Med. Chem. Letters 16:358-362 (2006); Torgov et al, Bioconj. Chem. 16:717-721 (2005); Nagy et al, Proc. Natl. Acad. Sci. USA 97:829-834 (2000); Dubowchik et al, Bioorg. & Med. Chem. Letters 12:1529-1532 (2002); King et al, J. Med. Chem. 45:4336-4343 (2002); and U.S. Patent No. 6,630,579); methotrexate; vindesine; a taxane such as docetaxel, paclitaxel, larotaxel, tesetaxel, and ortataxel; a trichothecene; and CC1065.

In another embodiment, an immunoconjugate comprises an antibody as described herein conjugated to an enzymatically active toxin or fragment thereof, including but not limited to diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from Pseudomonas aeruginosa), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, Phytolaca americana proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes.

In another embodiment, an immunoconjugate comprises an antibody as described herein conjugated to a radioactive atom to form a radioconjugate. A variety of

radioactive isotopes are available for the production of radioconjugates. Examples include At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, Pb^{212} and radioactive isotopes of Lu. When the radioconjugate is used for detection, it may comprise a radioactive atom for scintigraphic studies, for example tc99m or 1123, or a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic resonance imaging, mri), such as iodine-123 again, iodine-131, indium-I 11, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron.

Conjugates of an antibody and cytotoxic agent may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HC1), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as l,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al., Science 238:1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO 94/1 1026. The linker may be a "cleavable linker" facilitating release of a cytotoxic drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, photolabile linker, dimethyl linker or disulfide-containing linker (Chari et al, Cancer Res. 52:127-131 (1992); U.S. Patent No. 5,208,020) may be used.

The immunuoconjugates or ADCs herein expressly contemplate, but are not limited to such conjugates prepared with cross-linker reagents including, but not limited to, BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SIAB, SMCC, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, and sulfo-SMPB, and SVSB (succinimidyl-(4-vinylsulfone)benzoate) which are commercially available (e.g., from Pierce Biotechnology, Inc., Rockford, IL., U.S.A).

Therapeutic Methods and Compositions

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.

One preferred embodiment of the invention are the CSF-1R antibodies of the present invention for use in the treatment of "CSF-1R mediated diseases" or the CSF-1R antibodies of the present invention for use for the manufacture of a medicament in the treatment of "CSF-1R mediated diseases", which can be described as follows:

There are 3 distinct mechanisms by which CSF-1R signaling is likely involved in tumor growth and metastasis. The first is that expression of CSF-ligand and receptor has been found in tumor cells originating in the female reproductive system (breast, ovarian, endometrium, cervical) (Scholl, S.M., et al, J. Natl. Cancer Inst. 86 (1994) 120-126; Kacinski, B.M., Mol. Reprod. Dev. 46 (1997) 71-74; Ngan, H.Y., et al, Eur. J. Cancer 35 (1999) 1546-1550; Kirma, N., et al, Cancer Res 67 (2007) 1918-1926) and the expression has been associated with breast cancer xenograft growth as well as poor prognosis in breast cancer patients. Two point mutations were seen in CSF-1R in about 10-20 % of acute myelocytic leukemia, chronic myelocytic leukemia and myelodysplasia patients tested in one study, and one of mutations was found to disrupt receptor turnover (Ridge, S.A., et al, Proc. Natl. Acad. Sci USA 87 (1990) 1377-1380). However the incidence of the mutations could not be confirmed in later studies (Abu-Duhier, F.M., et al., Br. J. Haematol. 120 (2003) 464-470). Mutations were also found in some cases of hepatocellular cancer (Yang, D.H., et al., Hepatobiliary Pancreat. Dis. Int. 3 (2004) 86-89) and idiopathic myelofibrosis (Abu-Duhier, F., M., et al., Br. J. Haematol. 120 (2003) 464-470).

Pigmented villonodular synovitis (PVNS) and Tenosynovial Giant cell tumors (TGCT) can occur as a result of a translocation that fuses the M-CSF gene to a collagen gene COL6A3 and results in overexpression of M-CSF (West, R.B., et al, Proc. Natl. Acad. Sci. USA 103 (2006) 690-695). A landscape effect is proposed to be responsible for the resulting tumor mass that consists of monocytic cells attracted by cells that express M-CSF. TGCTs are smaller tumors that can be

relatively easily removed from fingers where they mostly occur. PVNS is more aggressive as it can recur in large joints and is not as easily controlled surgically.

The second mechanism is based on blocking signaling through M-CSF/CSF-1R at metastatic sites in bone which induces osteoclastogenesis, bone resorption and osteolytic bone lesions. Breast, multiple myeloma and lung cancers are examples of cancers that have been found to metastasize to the bone and cause osteolytic bone disease resulting in skeletal complications. M-CSF released by tumor cells and stroma induces the differentiation of hematopoietic myeloid monocyte progenitors to mature osteoclasts in collaboration with the receptor activator of nuclear factor kappa-B ligand-RANKL. During this process, M-CSF acts as a permissive factor by giving the survival signal to osteoclasts (Tanaka, S., et al, J. Clin. Invest. 91 (1993) 257-263). Inhibition of CSF-1R activity during osteoclast differentiation and maturation with a anti-CSF-lR antibody is likely to prevent unbalanced activity of osteoclasts that cause osteolytic disease and the associated skeletal related events in metastatic disease. Whereas breast, lung cancer and multiple myeloma typically result in osteolytic lesions, metastasis to the bone in prostate cancer initially has an osteoblastic appearance in which increased bone forming activity results in 'woven bone' which is different from typical lamellar structure of normal bone. During disease progression bone lesions display a significant osteolytic component as well as high serum levels of bone resorption and suggests that anti-resorptive therapy may be useful. Bisphosphonates have been shown to inhibit the formation of osteolytic lesions and reduced the number of skeletal-related events only in men with hormone-refractory metastatic prostate cancer but at this point their effect on osteoblastic lesions is controversial and bisphosphonates have not been beneficial in preventing bone metastasis or hormone responsive prostate cancer to date. The effect of anti-resorptive agents in mixed osteolytic/osteoblastic prostate cancer is still being studied in the clinic (Choueiri, M.B., et al, Cancer Metastasis Rev. 25 (2006) 601-609; Vessella, R.L. and Corey, E., Clin. Cancer Res. 12 (20 Pt 2) (2006) 6285s-6290s).

The third mechanism is based on the recent observation that tumor associated macrophages (TAM) found in solid tumors of the breast, prostate, ovarian and cervical cancers correlated with poor prognosis (Bingle, L., et al, J. Pathol. 196 (2002) 254-265; Pollard, J.W., Nat. Rev. Cancer 4 (2004) 71-78). Macrophages are recruited to the tumor by M-CSF and other chemokines. The macrophages can then contribute to tumor progression through the secretion of angiogenic factors, proteases and other growth factors and cytokines and may be blocked by inhibition

of CSF-1R signaling. Recently it was shown by Zins, K., et al, Cancer Res. 67 (2007) 1038-1045) that expression of siRNA of Tumor necrosis factor alpha (TNF alpha), M-CSF or the combination of both would reduce tumor growth in a mouse xenograft model between 34 % and 50 % after intratumoral injection of the respective siRNA. SiRNA targeting the TNF alpha secreted by the human SW620 cells reduced mouse M-CSF levels and led to reduction of macrophages in the tumor. In addition treatment of MCF7 tumor xenografts with an antigen binding fragment directed against M-CSF did result in 40 % tumor growth inhibition, reversed the resistance to chemotherapeutics and improved survival of the mice when given in combination with chemotherapeutics (Paulus, P., et al., Cancer Res. 66 (2006) 4349-4356).

TAMs are only one example of an emerging link between chronic inflammation and cancer. There is additional evidence for a link between inflammation and cancer as many chronic diseases are associated with an increased risk of cancer, cancers arise at sites of chronic inflammation, chemical mediators of inflammation are found in many cancers; deletion of the cellular or chemical mediators of inflammation inhibits development of experimental cancers and long-term use of anti-inflammatory agents reduce the risk of some cancers. A link to cancer exists for a number of inflammatory conditions among- those H.pylori induced gastritis for gastric cancer, Schistosomiasis for bladder cancer, HHVX for Kaposi's sarcoma, endometriosis for ovarian cancer and prostatitis for prostate cancer (Balkwill, F., et al, Cancer Cell 7 (2005) 211-217). Macrophages are key cells in chronic inflammation and respond differentially to their microenvironment. There are two types of macrophages that are considered extremes in a continuum of functional states: M1 macrophages are involved in Type 1 reactions. These reactions involve the activation by microbial products and consequent killing of pathogenic microorganisms that result in reactive oxygen intermediates. On the other end of the extreme are M2 macrophages involved in Type 2 reactions that promote cell proliferation, tune inflammation and adaptive immunity and promote tissue remodeling, angiogenesis and repair (Mantovani, A., et al, Trends Immunol. 25 (2004) 677-686). Chronic inflammation resulting in established neoplasia is usually associated with M2 macrophages. A pivotal cytokine that mediates inflammatory reactions is TNF alpha that true to its name can stimulate anti-tumor immunity and hemorrhagic necrosis at high doses but has also recently been found to be expressed by tumor cells and acting as a tumor promoter (Zins, K., et al, Cancer Res. 67 (2007) 1038-1045; Balkwill, F., Cancer Metastasis Rev. 25 (2006)

409-416). The specific role of macrophages with respect to the tumor still needs to be better understood including the potential spatial and temporal dependence on their function and the relevance to specific tumor types.

Thus one embodiment of the invention are the CSF-1 R antibodies of the present invention for use in the treatment of cancer. The term "cancer" as used herein may be, for example, lung cancer, non small cell lung (NSCL) cancer, bronchioloalviolar cell lung cancer, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, gastric cancer, colon cancer, breast cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, prostate cancer, cancer of the bladder, cancer of the kidney or ureter, renal cell carcinoma, carcinoma of the renal pelvis, mesothelioma, hepatocellular cancer, biliary cancer, neoplasms of the central nervous system (CNS), spinal axis tumors, brain stem glioma, glioblastoma multiforme, astrocytomas, schwanomas, ependymonas, medulloblastomas, meningiomas, squamous cell carcinomas, pituitary adenoma, lymphoma, lymphocytic leukemia, including refractory versions of any of the above cancers, or a combination of one or more of the above cancers. Preferably such cancer is a breast cancer, ovarian cancer , cervical cancer, lung cancer or prostate cancer. Preferably such cancers are further characterized by CSF-1 or CSF-IR expression or overexpression. One further embodiment the invention are the CSF-IR antibodies of the present invention for use in the simultaenoues treatment of primary tumors and new metastases.

Thus another embodiment of the invention are the CSF-IR antibodies of the present invention for use in the treatment of periodontitis, histiocytosis X, osteoporosis, Paget's disease of bone (PDB), bone loss due to cancer therapy, periprosthetic osteolysis, glucocorticoid-induced osteoporosis, rheumatoid arthritis, psiratic arthritis, osteoarthritis, inflammatory arthridities, and inflammation.

Rabello, D., et al, Biochem. Biophys. Res. Commun. 347 (2006) 791-796 has demonstrated that SNPs in the CSF1 gene exhibited a positive association with

aggressive periodontitis: an inflammatory disease of the periodontal tissues that causes tooth loss due to resorption of the alveolar bone.

Histiocytosis X (also called Langerhans cell histiocytosis, LCH) is a proliferative disease of Langerhans dendritic cells that appear to differentiate into osteoclasts in bone and extraosseous LCH lesions. Langerhans cells are derived from circulating monocytes. Increased levels of M-CSF that have been measured in sera and lesions where found to correlate with disease severity (da Costa, C.E., et al, J. Exp. Med. 201 (2005) 687-693). The disease occurs primarily in a pediatric patient population and has to be treated with chemotherapy when the disease becomes systemic or is recurrent.

The pathophysiology of osteoporosis is mediated by loss of bone forming osteoblasts and increased osteoclast dependent bone resorption. Supporting data has been described by Cenci et al showing that an anti-M-CSF antibody injection preserves bone density and inhibits bone resorption in ovariectomized mice (Cenci, S., et al, J. Clin. Invest. 105 (2000) 1279-1287). Recently a potential link between postmenopausal bone loss due to estrogen deficiency was identified and found that the presence of TNF alpha producing T-cell affected bone metabolism (Roggia, C , et al., Minerva Med. 95 (2004) 125-132). A possible mechanism could be the induction of M-CSF by TNF alpha in vivo. An important role for M-CSF in TNF-alpha-induced osteoclastogenesis was confirmed by the effect of an antibody directed against M-CSF that blocked the TNF alpha induced osteolysis in mice and thereby making inhibitors of CSF-IR signaling potential targets for inflammatory arthritis (Kitaura, H., et al, J. Clin. Invest. 115 (2005) 3418-3427).

Paget's disease of bone (PDB) is the second most common bone metabolism disorder after osteoporosis in which focal abnormalities of increased bone turnover lead to complications such as bone pain, deformity, pathological fractures and deafness. Mutations in four genes have been identified that regulate normal osteoclast function and predispose individuals to PDB and related disorders: insertion mutations in TNFRSFl lA, which encodes receptor activator of nuclear factor (NF) kappaB (RANK)-a critical regulator of osteoclast function, inactivating mutations of TNFRSF1 1B which encodes osteoprotegerin (a decoy receptor for RANK ligand), mutations of the sequestosome 1 gene (SQSTM1), which encodes an important scaffold protein in the NFkappaB pathway and mutations in the valosin-containing protein (VCP) gene. This gene encodes VCP, which has a role in targeting the inhibitor of NFkappaB for degradation by the proteasome

(Daroszewska, A., Ralston, S.H., Nat. Clin. Pract. Rheumatol. 2 (2006) 270-277). Targeted CSF-1R inhibitors provide an opportunity to block the deregulation of the RANKL signaling indirectly and add an additional treatment option to the currently used bisphosphonates.

Cancer therapy induced bone loss especially in breast and prostate cancer patients is an additional indication where a targeted CSF-1R inhibitor could prevent bone loss (Lester, J.E., et al, Br. J. Cancer 94 (2006) 30-35). With the improved prognosis for early breast cancer the long-term consequences of the adjuvant therapies become more important as some of the therapies including chemotherapy, irradiation, aromatase inhibitors and ovary ablation affect bone metabolism by decreasing the bone mineral density, resulting in increased risk for osteoporosis and associated fractures (Lester, J.E., et al, Br. J. Cancer 94 (2006) 30-35). The equivalent to adjuvant aromatase inhibitor therapy in breast cancer is androgen ablation therapy in prostate cancer which leads to loss of bone mineral density and significantly increases the risk of osteoporosis-related fractures (Stoch, S.A., et al, J. Clin. Endocrinol. Metab. 86 (2001) 2787-2791).

Targeted inhibition of CSF-1R signaling is likely to be beneficial in other indications as well when targeted cell types include osteoclasts and macrophages e.g. treatment of specific complications in response to joint replacement as a consequence of rheumatoid arthritis. Implant failure due to periprosthetic bone loss and consequent loosing of prostheses is a major complication of joint replacement and requires repeated surgery with high socioeconomic burdens for the individual patient and the health-care system. To date, there is no approved drug therapy to prevent or inhibit periprosthetic osteolysis (Drees, P., et al., Nat. Clin. Pract. Rheumatol. 3 (2007) 165-171).

Glucocorticoid-induced osteoporosis (GIOP) is another indication in which a CSF-1R inhibitor could prevent bone loss after longterm glucocorticocosteroid use that is given as a result of various conditions among those chronic obstructive pulmonary disease, asthma and rheumatoid arthritis (Guzman-Clark, J.R., et al., Arthritis Rheum. 57 (2007) 140-146; Feldstein, A.C., et al, Osteoporos. Int. 16 (2005) 2168-2174).

Rheumatoid arthritis, psioratic arthritis and inflammatory arthridities are in itself potential indications for CSF-1R signaling inhibitors in that they consist of a macrophage component and to a varying degree bone destruction (Ritchlin, C.T., et

al., J. Clin. Invest. 111 (2003) 821-831). Osteoarthritis and rheumatoid arthritis are inflammatory autoimmune disease caused by the accumulation of macrophages in the connective tissue and infiltration of macrophages into the synovial fluid, which is at least partially mediated by M-CSF. Campbell, I.K., et al., J. Leukoc. Biol. 68 (2000) 144-150, demonstrated that M-CSF is produced by human-joint tissue cells (chondrocytes, synovial fibroblasts) in vitro and is found in synovial fluid of patients with rheumatoid arthritis, suggesting that it contributes to the synovial tissue proliferation and macrophage infiltration which is associated with the pathogenesis of the disease. Inhibition of CSF-IR signaling is likely to control the number of macrophages in the joint and alleviate the pain from the associated bone destruction. In order to minimize adverse affects and to further understand the impact of the CSF-IR signaling in these indications, one method is to specifically inhibit CSF-IR without targeting a myriad other kinases, such as Raf kinase.

Recent literature reports correlate increased circulating M-CSF with poor prognosis and atherosclerotic progression in chronic coronary artery disease (Saitoh, T., et al, J. Am. Coll. Cardiol. 35 (2000) 655-665; Ikonomidis, I., et al, Eur. Heart. J. 26 (2005) 1618-1624); M-CSF influences the atherosclerotic process by aiding the formation of foam cells (macrophages with ingested oxidized LDL) that express CSF-IR and represent the initial plaque (Murayama, T., et al., Circulation 99 (1999) 1740-1746).

Expression and signaling of M-CSF and CSF-IR is found in activated microglia. Microglia, which are resident macrophages of the central nervous system, can be activated by various insults, including infection and traumatic injury. M-CSF is considered a key regulator of inflammatory responses in the brain and M-CSF levels increase in HIV-1, encephalitis, Alzheimer's disease (AD) and brain tumors. Microgliosis as a consequence of autocrine signaling by M-CSF/CSF-1R results in induction of inflammatory cytokines and nitric oxides being released as demonstrated by e.g. using an experimental neuronal damage model (Hao, A.J., et al, Neuroscience 112 (2002) 889-900; Murphy, G.M., Jr., et al, J. Biol. Chem. 273 (1998) 20967-20971). Microglia that have increased expression of CSF-IR are found to surround plaques in AD and in the amyloid precursor protein V717F transgenic mouse model of AD (Murphy, G.M., Jr., et al, Am. J. Pathol. 157 (2000) 895-904). On the other hand op/op mice with fewer microglia in the brain resulted in fibrilar deposition of A-beta and neuronal loss compared to normal control suggesting that microglia do have a neuroprotective function in the

development of AD lacking in the op/op mice (Kaku, M., et al., Brain Res. Brain Res. Protoc. 12 (2003) 104-108).

Expression and signaling of M-CSF and CSF-1R is associated with inflammatory bowel disease (IBD) (WO 2005/046657). The term "inflammatory bowel disease" refers to serious, chronic disorders of the intestinal tract characterised by chronic inflammation at various sites in the gastrointestinal tract, and specifically includes ulcerative colitis (UC) and Crohn's disease.

The invention the antibody characterized in comprising the antibody binding to human CSF-1R being characterized by the above mentioned epitope binding properties or alternatively by the above mentioned amino acid sequences and amino acid sequence fragments for the treatment of cancer.

The invention the antibody characterized in comprising the antibody binding to human CSF-1R being characterized by the above mentioned epitope binding properties or alternatively by the above mentioned amino acid sequences and amino acid sequence fragments for the treatment of bone loss.

The invention comprises the antibody characterized in comprising the antibody binding to human CSF-1R being characterized by the above mentioned epitope binding properties or alternatively by the above mentioned amino acid sequences and amino acid sequence fragments for the prevention or treatment of metastasis.

The invention comprises the antibody characterized in comprising the antibody binding to human CSF-1R being characterized by the above mentioned epitope binding properties or alternatively by the above mentioned amino acid sequences and amino acid sequence fragments for treatment of inflammatory diseases.

The invention comprises the use of an antibody characterized in comprising the antibody binding to human CSF-1R being characterized by the above mentioned epitope binding properties or alternatively by the above mentioned amino acid sequences and amino acid sequence fragments for the treatment of cancer or alternatively for the manufacture of a medicament for the treatment of cancer.

The invention comprises the use of an antibody characterized in comprising the antibody binding to human CSF-1R being characterized by the above mentioned epitope binding properties or alternatively by the above mentioned amino acid

sequences and amino acid sequence fragments for the treatment of bone loss or alternatively for the manufacture of a medicament for the treatment of bone loss.

The invention comprises the use of an antibody characterized in comprising the antibody binding to human CSF-1R being characterized by the above mentioned epitope binding properties or alternatively by the above mentioned amino acid sequences and amino acid sequence fragments for the prevention or treatment of metastasis or alternatively for the manufacture of a medicament for the prevention or treatment of metastasis.

The invention comprises the use of an antibody characterized in comprising the antibody binding to human CSF-1R being characterized by the above mentioned epitope binding properties or alternatively by the above mentioned amino acid sequences and amino acid sequence fragments for treatment of inflammatory diseases or alternatively for the manufacture of a medicament for the treatment of inflammatory diseases. In one embodiment the antibodies according to the invention inhibit CSF-1 binding to CSF-1R with an IC50 of 75 ng/ml or lower, in one embodiment with an IC50 of 50 ng/ml or lower. The IC50 of inhibition of CSF-1 binding to CSF-1R can be determined as shown in Example 2.

In one embodiment the antibodies according to the invention inhibit CSF-1-induced CSF-1R phosphorylation (in NIH3T3-CSF-1R recombinant cells) with an IC50 of 150 ng/ml or lower, in one embodiment with an IC50 of 100 ng/ml or lower, in one embodiment with an IC50 of 50 ng/ml or lower. in one embodiment with an IC50 of 25 ng/ml or lower The IC50 of CSF-l-induced CSF-1R phosphorylation can be determined as shown in Example 3.

In one embodiment the antibodies according to the invention inhibit the growth of recombinant NIH3T3 cells expressing human CSF-1 R (SEQ ID No: 15) by 80 % or more (as compared to the absence of antibody), preferably by 90 % or more. The % growth inhibition is determined as shown in Example 6 wherein the % survival is measured. From the % survival the % growth inhibition are calculated as follows: % growth inhibition = 100 -% survival. E.g. <CSF-1R>7G5.3B6 shows a growth inhibition of wt human CSF-1R expressing NIH3T3 cells of 100 -2 = 98 %.

In one embodiment the antibodies according to the invention stimulate the growth of recombinant NIH3T3 cells expressing human mutant CSF-1R L301S Y969F (SEQ ID No: 16) by 5 % or more (as compared to the absence of antibody), in one embodiment by 20 % or more. The % growth stimulation is determined as shown

in Example 6 wherein the % survival is measured. From the % survival the % growth stimulation are calculated as follows: % growth stimulation = - (100 -% survival). E.g. <CSF-1R>7G5.3B6 shows a growth stimulation of mutant human CSF-1R expressing NIH3T3 cells of -(100 -0) = -(100-1 12) % = +12 %.

In one embodiment the antibodies according to the invention inhibit the growth of BeWo tumor cells (ATCC CCL-98) by 70 % or more (at a antibody concentration of 10µg/ml ;and as compared to the absence of antibody), preferably by 80 % or more. The % growth inhibition is determined as shown in Example 7. E.g. <CSF-1R>7G5.3B6 shows a growth inhibition of BeWo tumor cells of 89 %.

In one embodiment the antibodies according to the invention inhibit macrophage differentiation. In one embodiment the antibodies according to the invention inhibit the survival of monocytes with an IC50 of 1.5 nM or lower, preferably with an IC50 of 1.0 nM or lower,. The inhibition of the survival of monocytes is determined as shown in Example 8.

A further embodiment of the invention is a method for the production of an antibody against CSF-1R characterized in that the sequence of a nucleic acid encoding the heavy chain of a human IgGl class antibody binding to human CSF-1R according to the invention said modified nucleic acid and the nucleic acid encoding the light chain of said antibody are inserted into an expression vector, said vector is inserted in a eukaryotic host cell, the encoded protein is expressed and recovered from the host cell or the supernant.

Pharmaceutical Formulations

The term "pharmaceutical formulation" refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

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.

A "pharmaceutically acceptable carrier" refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is nontoxic to a subject., A

pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative. 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 pancreas cancer.

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.

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.

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.

It is understood that any of the above articles of manufacture may include an immunoconjugate of the invention in place of or in addition to an anti-CSF-lR antibody.

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.

Antibody Deposition

Cell line	Deposition No.	Date of Deposit
<CSF-1R>7H5.2G10	DSM ACC2922	10.06.2008

Description of the Sequences

SEQ ID NO: 1 heavy chain CDR3, <CSF-1R>7H5.2G10
SEQ ID NO: 2 heavy chain CDR2, <CSF-1R>7H5.2G10
SEQ ID NO: 3 heavy chain CDR1, <CSF-1R>7H5.2G10
SEQ ID NO: 4 light chain CDR3, <CSF-1R>7H5.2G10
SEQ ID NO: 5 light chain CDR2, <CSF-1R>7H5.2G10
SEQ ID NO: 6 light chain CDR1, <CSF-1R>7H5.2G10
SEQ ID NO: 7 heavy chain variable domain, <CSF-1R>7H5.2G10
SEQ ID NO: 8 light chain variable domain, <CSF-1R>7H5.2G10
SEQ ID NO: 9 heavy chain CDR3, <CSF-1R>10A4.1G1 1
SEQ ID NO: 10 heavy chain CDR2, <CSF-1R>10A4.1G1 1
SEQ ID NO: 11 heavy chain CDR1, <CSF-1R>10A4.1G1 1
SEQ ID NO: 12 light chain CDR3, <CSF-1R>10A4.1G1 1
SEQ ID NO: 13 light chain CDR2, <CSF-1R>10A4.1G1 1
SEQ ID NO: 14 light chain CDR1, <CSF-1R>10A4.1G1 1
SEQ ID NO: 15 heavy chain variable domain, <CSF-1R>10A4.1G1 1
SEQ ID NO: 16 light chain variable domain, <CSF-1R>10A4.1G1 1

SEQ ID NO: 17	heavy chain CDR3, <CSF-1R>6G4.1C8
SEQ ID NO: 18	heavy chain CDR2, <CSF-1R>6G4.1C8
SEQ ID NO: 19	heavy chain CDR1, <CSF-1R>6G4.1C8
SEQ ID NO: 20	light chain CDR3, <CSF-1R>6G4.1C8
SEQ ID NO: 21	light chain CDR2, <CSF-1R>6G4.1C8
SEQ ID NO: 22	light chain CDR1, <CSF-1R>6G4.1C8
SEQ ID NO: 23	heavy chain variable domain, <CSF-1R>6G4.1C8
SEQ ID NO: 24	light chain variable domain, <CSF-1R>6G4.1C8
SEQ ID NO: 25	gamma 1 heavy chain constant region
SEQ ID NO: 26	κ light chain constant region
SEQ ID NO: 27	human heavy chain constant region derived from IgGl
SEQ ID NO: 28	human heavy chain constant region derived from IgG1 mutated on L234A and L235A
SEQ ID NO: 29	human heavy chain constant region derived from IgG4
SEQ ID NO: 30	human heavy chain constant region derived from IgG4 mutated onS228P
SEQ ID NO: 31	wildtype CSF-1R (wt CSF-1R)
SEQ ID NO: 32	mutant CSF-1R L301S Y969F

The following examples, sequence listing and figures 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.

III. Examples

Example_1

Generation of a hybridoma cell line producing anti-CSF-lR antibodies

Immunization procedure of NMRI mice

NMRI mice were immunized with an expression vector pDisplay™ (Invitrogen, USA) encoding the extracellular domain of huCSF-lR by utilizing electroporation. Every mouse was 4 times immunized with 100µg DNA. When serum titers of anti-huCSF-lR were found to be sufficient, mice were additionally boosted once with 50µg of a l:lmixture huCSF-lR ECD/huCSF-lR ECDhuFc chimera in 200 µï PBS intravenously (i.v.) 4 and 3 days before fusion.

Antigen specific ELISA

Anti-CSF-lR titers in sera of immunized mice were determined by antigen specific ELISA.

0.3 µg/ml huCSF-lR-huFc chimera (soluble extracellular domain) was captured on a streptavidin plate (MaxiSorb; MicroCoat, DE, Cat.No. 11974998/MC1099) with 0.1 mg/ml biotinylated anti Fey (Jackson ImmunoResearch., Cat.No. 109-066-098) and horse radish peroxidase (HRP)-conjugated $F(ab')_2$ anti mouse IgG (GE Healthcare, UK, Cat.No.NA93 10V) diluted 1/800 in PBS/0.05 % Tween20/0.5 % BSA was added. Sera from all taps were diluted 1/40 in PBS/0.05 % Tween20/0.5 % BSA and serially diluted up to 1/1638400. Diluted sera were added to the wells. Pre-tap serum was used as negative control. A dilution series of mouse anti-human CSF-IR Mab3291 (R&D Systems, UK) from 500 ng/ml to 0,25 ng/ml was used as positive control. All components were incubated together for 1,5 hours, Wells were washed 6 times with PBST (PBS/0.2 % Tween20) and assays were developed with freshly prepared ABTS® solution (1 mg/ml) (ABTS: 2,2'-azino bis (3-ethylbenzthiazoline-6-sulfonic acid) for 10 minutes at RT. Absorbance was measured at 405 nm.

Hybridoma generation

The mouse lymphocytes can be isolated and fused with a mouse myeloma cell line using PEG based standard protocols to generate hybridomas. The resulting hybridomas are then screened for the production of antigen-specific antibodies. For example, single cell suspensions of splenic derived lymphocytes from immunized mice are fused to Ag8 non-secreting mouse myeloma cells P3X63Ag8.653 (ATCC, CRL-1580) with 50 % PEG. Cells are plated at approximately 10^4 in flat bottom 96 well micro titer plate, followed by about two weeks incubation in selective medium. Individual wells are then screened by ELISA for human anti-CSF-lR monoclonal IgM and IgG antibodies. Once extensive hybridoma growth occurs, the antibody secreting hybridomas are replated, screened again, and if still positive for human IgG, anti-CSF-lR monoclonal antibodies, can be subcloned by FACS. The stable subclones are then cultured in vitro to produce antibody in tissue culture medium for characterization.

Culture of hybridomas

Generated muMAb hybridomas were cultured in RPMI 1640 (PAN - Catalogue No. (Cat. No.) P04- 17500) supplemented with 2 mM L-glutamine (GIBCO - Cat.

No.35050-038), 1 mM Na-Pyruvat (GIBCO - Cat. No.l 1360-039), lx NEAA (GIBCO - Cat. No.l 1140-035), 10 % FCS (PAA - Cat. No.A 15-649), lx Pen Strep (Roche - Cat. No.1074440), lx Nutridoma CS (Roche - Cat. No.1363743), 50 µM Mercaptoethanol (GIBCO - Cat. No.3 1350-010) and 50 U/ml IL 6 mouse (Roche - Cat. No.l 444 581) at 37°C and 5 % $C0_2$.

Example 2

Inhibition of CSF-1 binding to CSF-IR (ELISA)

The test was performed on 384 well microtiter plates (MicroCoat, DE, Cat.No. 464718) at RT. After each incubation step plates were washed 3 times with PBST.

At the beginning, plates were coated with 0.5 mg/ml goat $F(ab')_2$ biotinylated anti Fey (Jackson ImmunoResearch., Cat.No. 109-006- 170) for 1 hour (h).

Thereafter the wells were blocked with PBS supplemented with 0.2 % Tween®-20 and 2 % BSA (Roche Diagnostics GmbH, DE) for 0.5 h. 75 ng/ml of huCSF-lR-huFc chimera (soluble extracellular domain) was immobilized to plate for 1 h. Then dilutions of purified antibodies in PBS/0.05 % Tween20/0.5 % BSA were incubated for 1 h. After adding a mixture of 3 ng/ml CSF-1 (Biomol, DE, Cat.No.60530), 50ng/ml biotinylated anti CSF-1 clone BAF216 (R&D Systems,UK) and 1:5000 diluted streptavidin HRP (Roche Diagnostics GmbH, DE, Cat.No.1 1089153001) for 1 h the plates were washed 6 times with PBST. Anti CSF-IR SC-02, clone 2-4A5 (Santa Cruz Biotechnology, US), which inhibits the ligand- receptor interaction, was used as positive control. Plates were developed with freshly prepared BM blue® POD substrate solution (BM blue®: 3,3'-5,5'-Tetramethylbenzidine, Roche Diagnostics GmbH, DE, Cat.No. 11484281001) for 30 minutes at RT. Absorbance was measured at 370 nm. All anti-CSF-lR antibodies showed significant inhibition of the CSF-1 binding to CSF-IR (see Table 1). Anti CSF-IR SC-02, clone 2-4A5 (Santa Cruz Biotechnology, US), which inhibits the ligand- receptor interaction, was used as reference control.

Table 1: Calculated IC50 values for the inhibition of the CSF-l/CSF-lR interaction

Antibody	IC50 CSF-1 /CSF-1R Inhibition [ng/ml]
<CSF-1R>7H5.2G10	26.9
<CSF-1R>10A4.1G11	63.4
<CSF-1R>6G4.1C8	21.2
SC-02, clone 2-4A5	30.9

Example 3

Inhibition of CSF-l-induced CSF-1R phosphorylation in NIH3T3-CSF-1R recombinant cells

4.5xl0 ^{3} NIH 3T3 cells, retrovirally infected with an expression vector for full-length CSF-1R, were cultured in DMEM (PAA Cat. No.E15-01 1), 2mM L-glutamine (Sigma, Cat.No.G7513, 2mM Sodium pyruvate, lx nonessential aminoacids, 10 % FKS (PAA, Cat.No.Al 5-649) and 100µg/ml PenStrep (Sigma , Cat.No. P4333 [lOmg/ml]) until they reached confluency. Thereafter cells were washed with serum-free DMEM media (PAA Cat.No. E15-01 1) supplemented with sodium selenite [5ng/ml] (Sigma, Cat.No. S9133), transferrin [10µg/ml] (Sigma, Cat.No. T8158), BSA [400µg/ml] (Roche Diagnostics GmbH, Cat.No. 10735078), 4mM L-glutamine (Sigma, Cat.No.G7513), 2mM sodium pyruvate (Gibco, Cat.No. 11360), lx nonessential aminoacids (Gibco, Cat: 11140-035), 2-mercaptoethanol [0,05mM] (Merck, Cat.No. M7522), 100µg/ml and PenStrep (Sigma, Cat. No. P4333) and incubated in 30 µï of the same medium for 16 hours to allow for receptor up-regulation. 10 µï of diluted anti-CSR-lR antibodies were added to the cells for 1.5 h. Then cells were stimulated with 10 µï of 100 ng/ml huM-CSF-1 (Biomol Cat.No. 60530) for 5 min. After the incubation, supernatant was removed, cells were washed twice with 80 µï of ice-cold PBS and 50 µï of freshly prepared ice-cold lysis buffer (150mM NaCl/ 20mM Tris pH 7.5 / ImM EDTA/ ImM EGTA/ 1 % Triton X-100 *l* protease inhibitor tablet (Roche Diagnostics GmbH Cat.No. 1 836 170) per 10 ml buffer / 1Oµï/ml phosphatase inhibitor cocktail 1 (Sigma Cat.No. P-2850, lOOx Stock) / IOµI/ml protease inhibitor 1 (Sigma Cat.No.P-5726, lOOx Stock) /10µ1/ɱ1 1 M NaF) was added. After 30 minutes on ice the plates were shaken vigourously on a plateshaker for 3 minutes and then centrifuged 10 minutes at 2200 rpm (Heraeus Megafuge 10).

The presence of phosphorylated and total CSF-1 receptor in the cell lysate was analyzed with Elisa. For detection of the phosphorylated receptor the kit from R&D Systems (Cat. No. DYC3268-2) was used according to the instructions of the supplier. For detection of total CSF-1R 10 µï of the lysate was immobilized on plate by use of the capture antibody contained in the kit. Thereafter 1:750 diluted biotinylated anti CSF-1R antibody BAF329 (R&D Systems) and 1:1000 diluted streptavidin-HRP conjugate was added. After 60 minutes plates were developed with freshly prepared ABTS® solution and the absorbance was detected. Data were calculated as % of positive control without antibody and the ratio value phospho/total receptor expressed. The negative control was defined without addition of M-CSF-1. Anti CSF-IR SC-02, clone 2-4A5 (Santa Cruz Biotechnology, US, see also Sherr, C.J., et al, Cell 41 (1985) 665-676), which inhibits the ligand- receptor interaction, was used as reference control.

Table 2: Calculated IC_{50} values for the inhibition of CSF-1 receptor phosphorylation .

Antibody	IC50 CSF-1R Phosphorylation [ng/ml]
<CSF-1R>7H5.2G10	49.0
<CSF-1R>10A4.1G11	15.4
<CSF-1R>6G4.1C8	82.6
SC-02, clone 2-4A5	412.0

Example 4

Determination of the affinity of anti-CSF-lR antibodies to CSF-IR

Instrument: BIACORE® A100
Chip: CM5 (Biacore BR-1006-68)
Coupling: amine coupling
Buffer: PBS (Biacore BR-1006-72), pH 7.4, 35 °C

For affinity measurements 36 µg/ml anti mouse Fey antibodies (from goat, Jackson Immuno Reasearch JIR1 15-005-071) have been coupled to the chip surface for capturing the antibodies against CSF-IR. CSF-IR ECD (R&D-Systems 329-MR or in-house subcloned pCMV-presS-HisAvitag-hCSF-lR-ECD were added in various concentrations in solution. Association was measured by an CSF-lR-injection of 1.5 minutes at 35 °C; dissociation was measured by washing the chip surface with

buffer for 10 minutes at 35 °C. Anti CSF-IR SC-02, clone 2-4A5 (Santa Cruz Biotechnology, US; see also Sherr, C.J., et al, Cell 41 (1985) 665-676), which inhibits the ligand- receptor interaction, was used as reference control.

For calculation of kinetic parameters the Langmuir 1:1 model was used.

Table 3: Affinity data measured by SPR (BIACORE® A100) at 35 °C

Antibody	K_D (nM)	k_a (1/Ms)	k_d (1/s)	$t_{1/2}$ (min)
<CSF-1R>7H5.2G10	0.54	7.0E+05	3.8E-04	30.40
<CSF-1R>10A4.1G11	1.77	7.4E+05	1.3E-03	8.89
<CSF-1R>6G4.1C8	0.52	5.7E+05	2.9E-04	39.43
SC-02, clone 2-4A5	2.73	5.09E+05	1.39E-03	8.31

Example 5

Epitope Mapping of anti-CSF-lR monoclonal antibodies based on cross-competition by utilizing SPR

Instrument: BIACORE ® A100
Chip: CM5 (Biacore BR- 1006-68)
Coupling: amine coupling
Buffer: PBS PBS (Biacore BR-1006-72), pH 7.4, 25 °C

For epitope mapping assays via cross-competition 36 µg/ml anti mouse Fey antibodies or anti rat Fey antibodies (from goat, Jackson Immuno Research Cat.No. 115-005-071 and Cat. No.l 12-005-071) have been coupled to sensor chip surface for presentation of the antibody against CSF-IR. After capture from 5 µg/ml anti-CSF-lR monoclonal antibodies free binding capacities of capture antibodies have been blocked with 250 µg/ml mouse or rat immunoglobulins (Pierce Cat. No. 31202 and Pierce Cat. No.31233), followed by injection of 12.5 µg/ml CSF-IR (R**&D**-Systems Cat.No. 329-MR) for 2 min. Binding of second anti-CSF-lR antibody has been analyzed by injection for 2 min, dissociation was measured by washing with buffer for 5 minutes. The assay and the measurements were conducted at 25 °C. The specific binding of the second anti-CSF-lR antibody has been referenced against spot with the same chip setup up but only without injection of CSF-IR. The cross competition data have been calculated in percentage (%) of expected binding response of the second anti-CSF-lR antibody. The item "percentage (%) of expected binding response" for binding of the second

antibody was calculated by "100 * relativeResponse(general _stability _early) / rMax", where rMax is calculated by "relativeResponse(general_stability_late) * antibody molecular weight / antigen molecular weight" as described in Biacore epitope mapping instruction (for BIACORE® A100 instument).s.

The minimal binding response was also calculated from the pairs of identical antibody 1 and 2. Thereof the obtained maximal value + 50 % was set as threshold for significant binding competition (see table X e.g. for antibody <CSF-1R>7H5.2G10 calculated threshold is 7+3.5= 10.5). Thus an "anti-CSF-lR antibody binding to the same epitope as <CSF-1R>7H5.2G10" has a percentage (%) of expected binding response > 10.5.

The anti-CSF-lR SC-02, clone 2-4A5 (Santa Cruz Biotechnology, US, see also Sherr, C.J., et al, Cell 41 (1985) 665-676), which inhibit the ligand- receptor interaction, was used as reference control.

Table 4: The epitope mapping via cross-competition data of anti CSF-1R antibodies

	Antibody 2		
Antibody 1	<CSF-1R> 7H5.2G10	<CSF-1R> 10A4.1G11	SC-02, clone 2-4A5
<CSF-1R> 7H5.2G10	7	3	42
<CSF-1R> 10A4.1G11	5	-3	24
SC-02, clone 2-4A5	51	39	-2

The results indicate that the antibodies <CSF-1R>7H5.2G10, <CSF-1R>10A4.1G11, all bind to the same epitope, while e.g. SC-2-4A5 binds to another epitope and does not crossreact (crosscompete for binding) with the antibodies according to the invention.

Example_6

Growth inhibition of NIH3T3-CSF-1R recombinant cells in 3D culture under treatment with anti-CSF-lR monoclonal antibodies (CellTiterGlo-assay)

NIH 3T3 cells, retrovirally infected with either an expression vector for full-length wildtype CSF-1R (SEQ ID No: 31) or mutant CSF-1R L301S Y969F (SEQ ID No: 32), were cultured in DMEM high glucose media (PAA, Pasching, Austria) supplemented with 2mM L-glutamine, 2mM sodium pyruvate and non-essential

amino acids and 10 % fetal bovine serum (Sigma, Taufkirchen, Germany) on poly-HEMA (poly(2-hydroxyethylmethacrylate)) (Polysciences, Warrington, PA, USA)) coated dishes to prevent adherence to the plastic surface. Cells are seeded in medium replacing serum with 5ng/ml sodium selenite, lOmg/ml transferrin, 40(^g/ml BSA and 0.05 mM 2-mercaptoethanol. When treated with lOOng/ml huCSF-1 (Biomol, Hamburg, Germany) wtCSF-lR expressing cells form dense spheroids that grow three dimensionally, a property that is called anchorage independence. These spheroids resemble closely the three dimensional architecture and organization of solid tumors in situ. Mutant CSF-1R recombinant cells are able to form spheroids independent of the CSF-1 ligand. Spheroid cultures were incubated for 3 days in the presence of 10µg/ml antibody. The CellTiterGlo assay was used to detect cell viability by measuring the ATP-content of the cells.

Table 5:

Antibody	NIH3T Cells expressing wtCSF-1R % survival	NIH3T Cells expressing Mutant CSF-1R % survival
<CSF-1R>7H5.2G10	2	112
<CSF-1R>10A4.1G11	3	144
<CSF-1R>6G4.1C8	3	91
Antibody	**NIH3T Cells expressing wtCSF-1R % survival**	**NIH3T Cells expressing Mutant CSF-1R % survival**
SC-02, clone 2-4A5	62**	66***

** average of 15 different experiments,

*** average of 6 different experiments

Example_7

Growth inhibition of BeWo tumor cells in 3D culture under treatment with anti-CSF-lR monoclonal antibodies (CellTiterGlo-assay)

BeWo choriocarcinoma cells (ATCC CCL-98) were cultured in F12K media (Sigma, Steinheim, Germany) supplemented with 10 % FBS (Sigma) and 2mM L-glutamine. 5xl0 ^{4} cells/well were seeded in 96-well poly-HEMA (poly (2-hydroxy ethylmethacrylate)) coated plates containing F12K medium supplemented with 0.5 % FBS and 5 % BSA. Concomitantly, 200 ng/ml huCSF-1 and 10µg/ml of different anti-CSF-lR monoclonal antibodies were added and

incubated for 6 days. The CellTiterGlo assay was used to detect cell viability by measuring the ATP-content of the cells in relative light units (RLU). When BeWo spheroid cultures were treated with different anti-CSF-lR antibodies (10µg/ml) inhibition of CSF-1 induced growth was observed. To calculate antibody-mediated inhibition the mean RLU value of unstimulated BeWo cells was subtracted from all samples. Mean RLU value of CSF-1 stimulated cells was set arbitrarily to 100 %. Mean RLU values of cells stimulated with CSF-1 and treated with anti-CSF-lR antibodies were calculated in % of CSF-1 stimulated RLUs. The Table 6 shows the calculated data; Fig.l depicts mean RLU values. Each mean value was derived from triplicates.

Table 6:

Antibody	% inhibition 10µg/ml antibody concentration
CSF-1 only	0
<CSF-1R>7H5.2G10	89
<CSF-1R>10A4.1G11	83
SC-02, clone 2-4A5	40

Example 8

Inhibition of macrophage differentiation/monocyte survival under treatment with anti-CSF-lR monoclonal antibodies (CellTiterGlo-assay)

Monocytes isolated from peripheral blood using the RosetteSep™ Human Monocyte Enrichment Cocktail (StemCell Tech. - Cat. No. 15028). Enriched monocyte populations were seeded into 96 well microtiterplates (2.5×10^4 cells/well) in 100 µï RPMI 1640 (Gibco - Cat. No.31870) supplemented with 10 FCS (GIBCO - Cat. No.01 1-090014M), 4 mM L-glutamine (GIBCO - Cat. No.25030) and lx PenStrep (Roche Cat. No.l 074 440) at 37 °C and 5 % $C0_2$. When 150 ng/ml huCSF-1 was added to the medium, a clear differentiation into adherent macrophages could be observed. This differentiation could be inhibited by addition of anti-CSF-lR antibodies. Concomitantly, the monocyte survival is affected and could be analyzed by CellTiterGlo (CTG) analysis. From the concentration dependent inhibition of the survival of monocytes by antibody treatment an IC_{50} was calculated (see Table 7).

Table 7:

Antibody	ICso **[nM]**
<CSF-1R>7H5.2G10	1.0
<CSF-1R>10A4.1G1 1	0.4
SC-02, clone 2-4A5	2.4

PCT

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0-1	**Form PCT/RO/134 (SAFE) Indications Relating to Deposited Microorganism(s) or Other Biological Material (PCT Rule 13bis)**	
0-1-1	Prepared Using	PCT Online Filing Version 3.5.000.221 MT/FOP 20020701/0.20.5.9
0-2	**International Application No.**	
0-3	**Applicant's or agent's file reference**	26607 WO-WJ

1	**The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:**	
1-1	**Paragraph number**	**41**
1-3	**Identification of deposit**	
1-3-1	Name of depositary institution	**DSMZ DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH**
1-3-2	Address of depositary institution	**Inhoffenstr. 7B, D-38124 Braunschweig, Germany**
1-3-3	Date of deposit	**10 June 2008 (10.06.2008)**
1-3-4	Accession Number	**DSMZ ACC2922**
1-5	**Designated States for Which Indications are Made**	**All designations**

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Patent Claims

1. An antibody binding to human CSF-IR, characterized in binding to same epitope as the deposited antibody DSM ACC2922.

2. The antibody according to claim 1 characterized in comprising as heavy chain variable domain CDR3 region a CDR3 region of SEQ ID NO: 1, SEQ ID NO: 9 , or SEQ ID NO: 17.

3. The antibody according to claim 2, characterized in that

   a) the heavy chain variable domain comprises a CDR3 region of SEQ ID NO: 1, a CDR2 region of SEQ ID NO: 2, and a CDR1 region of SEQ ID NO:3, and the light chain variable domain comprises a CDR3 region of SEQ ID NO: 4, a CDR2 region of SEQ ID NO:5, and a CDR1 region of SEQ ID NO:6, or

   b) the heavy chain variable domain comprises a CDR3 region of SEQ ID NO: 9, a CDR2 region of SEQ ID NO: 10, and a CDR1 region of SEQ ID NO: 11, and the light chain variable domain comprises a CDR3 region of SEQ ID NO: 12, a CDR2 region of SEQ ID NO: 13, and a CDR1 region of SEQ ID NO: 14; or

   c) the heavy chain variable domain comprises a CDR3 region of SEQ ID NO: 17, a CDR2 region of SEQ ID NO: 18, and a CDR1 region of SEQ ID NO: 19, and i the light chain variable domain comprises a CDR3 region of SEQ ID NO:20, a CDR2 region of SEQ ID NO: 21, and a CDR1 region of SEQ ID NO: 22; or

   d) a CDR grafted, humanized or T cell epitope depleted antibody variant of the antibodies of a), b) or c).

4. The antibody according to claim 3, characterized in comprising

   a) the amino acid sequence of the heavy chain variable domain is SEQ ID NO: 7, and the amino acid sequence of the light chain variable domain is SEQ ID NO:8, or

   b) the amino acid sequence of the heavy chain variable domain is SEQ ID NO: 15, and the amino acid sequence of the light chain variable domain is SEQ ID NO: 16, or

c) the amino acid sequence of the heavy chain variable domain is SEQ ID NO: 23, and the amino acid sequence of the light chain variable domain is SEQ ID NO:24; or

d) a CDR grafted, humanized or T cell epitope depleted antibody variant of the antibodies of a), b) or c).

5. The antibody according to claims 1 to 4, characterized in that said antibody is of human IgG4 subclass or is of human IgGl subclass,

6. Pharmaceutical composition characterized in comprising an antibody or fragment according to claims 1 to 5.

7. The antibody according to claims 1 to 5 for the treatment of cancer.

8. The antibody according to claims 1 to 5 for the treatment of bone loss.

9. The antibody according to claims 1 to 5 for the prevention or treatment of metastasis.

10. The antibody according to claims 1 to 5 for the treatment of inflammatory diseases.

11. Nucleic acid encoding a heavy chain of an antibody binding to CSF-1R, characterized in that said antibody comprises a variable domain according to claim 2, 3 or 34.

12. Expression vectors characterized in comprising a nucleic acid according to claim 11 for the expression of an antibody binding to CSF-1R in a prokaryotic or eukaryotic host cell.

13. Prokaryotic or eukaryotic host cell comprising a vector according to claim 12.

14. Method for the production of a recombinant antibody according to claims 1 to 5, characterized by expressing a nucleic acid according to claim 11 in a prokaryotic or eukaryotic host cell and recovering said antibody from said cell or the cell culture supernatant.

15. An antibody binding to human CSF-1R, characterized in that

a) the heavy chain variable domain comprises a CDR3 region of SEQ ID NO: 1, a CDR2 region of SEQ ID NO: 2, and a CDR1 region of SEQ ID NO:3, and the light chain variable domain comprises a CDR3 region of SEQ ID NO: 4, a CDR2 region of SEQ ID NO:5, and a CDR1 region of SEQ ID NO:6, or

b) the heavy chain variable domain comprises a CDR3 region of SEQ ID NO: 9, a CDR2 region of SEQ ID NO: 10, and a CDR1 region of SEQ ID NO: 11, and the light chain variable domain comprises a CDR3 region of SEQ ID NO: 12, a CDR2 region of SEQ ID NO: 13, and a CDR1 region of SEQ ID NO: 14; or

c) the heavy chain variable domain comprises a CDR3 region of SEQ ID NO: 17, a CDR2 region of SEQ ID NO: 18, and a CDR1 region of SEQ ID NO: 19, and i the light chain variable domain comprises a CDR3 region of SEQ ID NO:20, a CDR2 region of SEQ ID NO: 21, and a CDR1 region of SEQ ID NO: 22; or

d) a CDR grafted, humanized or T cell epitope depleted antibody variant of the antibodies of a), b) or c).

16. The antibody according to claim 15, characterized in comprising

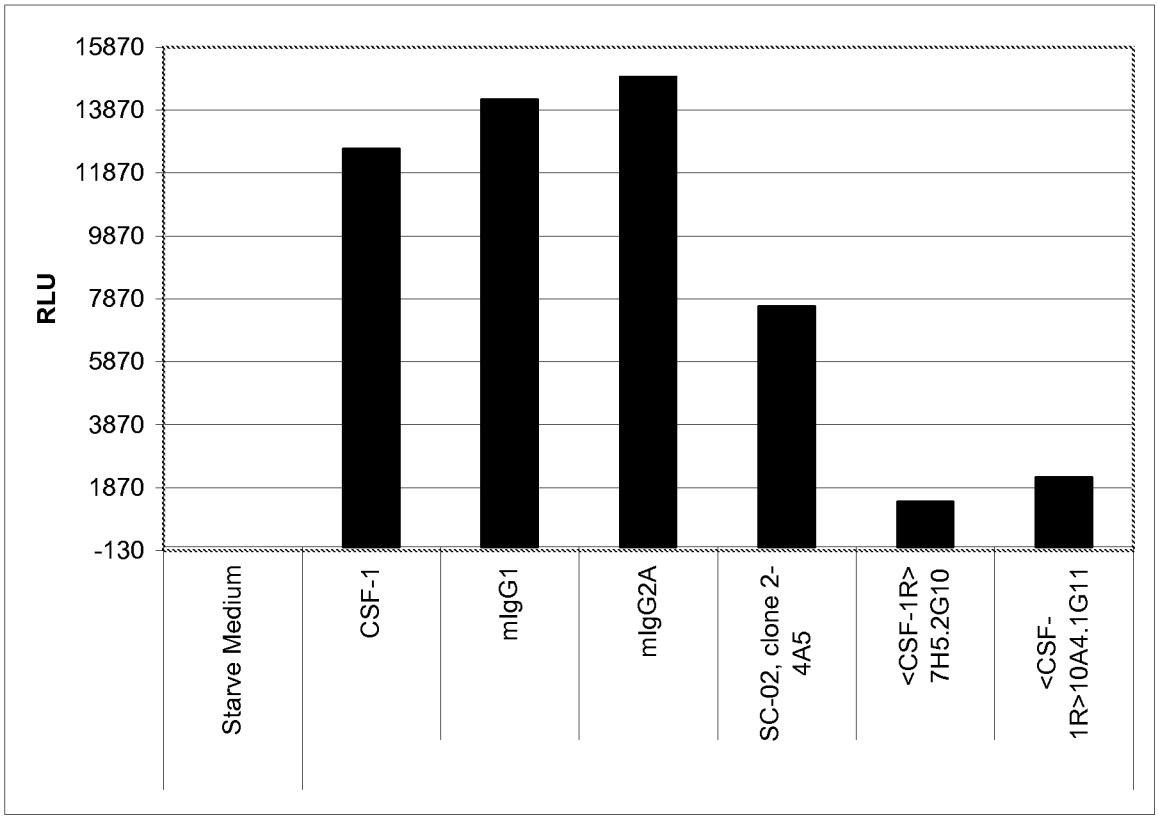
a) the amino acid sequence of the heavy chain variable domain is SEQ ID NO: 7, and the amino acid sequence of the light chain variable domain is SEQ ID NO:8, or

b) the amino acid sequence of the heavy chain variable domain is SEQ ID NO: 15, and the amino acid sequence of the light chain variable domain is SEQ ID NO: 16, or

c) the amino acid sequence of the heavy chain variable domain is SEQ ID NO: 23, and the amino acid sequence of the light chain variable domain is SEQ ID NO:24; or

d) a CDR grafted, humanized or T cell epitope depleted antibody variant of the antibodies of a), b) or c).

Fig. 1


15870
13870
11870
9870
7870
5870
3870
1870
-130
RLU
Starve Medium
CSF-1
mIgG1
mIgG2A
SC-02, clone 2-4A5
<CSF-1R> 7H5.2G10
<CSF-1R>10A4.1G11

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2011/053214

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/28 A61P35/00 A61P37/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

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

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal , BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2009/026303 A1 (AMGEN INC [US] ; BRASEL KENNETH ALLAN [US] ; FOSTER STEPHEN [US] ; CERRET) 26 February 2009 (2009-02-26) the whole document -----	1-16
X	WO 2009/112245 A1 (TRANSGENE SA [FR] ; HAEGEL HELENE [FR] ; THI0UDELLET CHRISTINE [FR] ; GEI) 17 September 2009 (2009-09-17) the whole document -----	1-16

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

[X] See patent family annex.

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

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 April 2011	28/04/2011

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

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ZHENG G ET AL: "Membrane-bound macrophage colony-stimulating factor and its receptor play adhesion molecule-like roles in leukemic cells", LEUKEMIA RESEARCH, NEW YORK, NY, US LNKD-DOI:10.1016/50145-2126(99)00192-7, vol. 24, no. 5, 1 May 2000 (2000-05-01), pages 375-383, XP002505573, ISSN: 0145-2126 the whole document	1-16
X	SHERR C J ET AL: "INHIBITION OF COLONY-STIMULATING FACTOR-1 ACTIVITY BY MONOCLONAL ANTIBODIES TO THE HUMAN CSF-1 RECEPTOR", BLOOD, AMERICAN SOCIETY OF HEMATOLOGY, US, vol. 73, no. 7, 15 May 1989 (1989-05-15), pages 1786-1793, XP000568719, ISSN: 0006-4971 the whole document	1-16
X	ASHMUN R A ET AL: "Monoclonal antibodies to the human CSF-1 receptor (c-fms proto-oncogene product) detect epitopes on normal mononuclear phagocytes and on human myeloid leukemic blast cells", BLOOD, AMERICAN SOCIETY OF HEMATOLOGY, US, vol. 73, no. 3, 15 February 1989 (1989-02-15), pages 827-837, XP002505053, ISSN: 0006-4971 the whole document	1-16
X	KITAURA H ET AL: "An anti-c-Fms antibody inhibits orthodontic tooth movement", JOURNAL OF DENTAL RESEARCH, INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH, US LNKD-DOI:10.1177/154405910808700405, vol. 87, no. 4, 1 April 2008 (2008-04-01), pages 396-400, XP008098947, ISSN: 0022-0345 the whole document	1-16
X	MURAYAMA T ET AL: "Intraperitoneal administration of anti-c-fms monoclonal antibody prevents initial events of atherogenesis but does not reduce the size of advanced lesions in apolipoprotein E-deficient mice", CIRCULATION, LIPPINCOTT WILLIAMS & WILKINS, US, vol. 99, no. 13, 6 April 1999 (1999-04-06), pages 1740-1746, XP002551089, ISSN: 0009-7322 the whole document	1-16

-/--

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PATEL S ET AL: "Colony-stimulating factor-1 receptor inhibitors for the treatment of cancer and inflammatory disease" , CURRENT TOPICS IN MEDICINAL CHEMISTRY, BENTHAM SCIENCE PUBLISHERS LTD, NETHERLANDS, vol . 9, no. 7, 1 January 2009 (2009-01-01) , pages 599-610, XP002570335 , ISSN: 1568-0266 the whole document	1-16
A	HAMILTON JOHN A: "Colony-stimulating factors in inflammation and autoimmunity" , NATURE REVIEWS. IMMUNOLOGY, NATURE PUBLISHING GROUP, GB LNKD-D01:10.1038/NRI2356, vol . 8, no. 7, 1 July 2008 (2008-07-01) , pages 533-544, XP002539696, ISSN: 1474-1733 [retrieved on 2008-06-13] the whole document	1-16
A	AHARINEJAD SEYEDH0SSEIN ET AL: "Colony-stimulating factor-1 blockade by antisense oligonucleotides and small interfering RNAs suppresses growth of human mammary tumor xenografts in mice" , CANCER RESEARCH, AMERICAN ASSOCIATION FOR CANCER REREARCH, US LNKD-D0I:10.1158/0008-5472 .CAN-04-0961 , vol . 64, no. 15, 1 August 2004 (2004-08-01) , pages 5378-5384, XP002505575 , ISSN: 0008-5472 the whole document	1-16

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2011/053214

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO 2009026303 A1	26-02-2009	AR	068347 A1	11-11-2009
		AU	2008288974 A1	26-02-2009
		CA	2696761 A1	26-02-2009
		CN	101802008 A	11-08-2010
		CR	11282 A	28-06-2010
		EA	201000357 A1	30-12-2010
		EP	2188313 A1	26-05-2010
		JP	2010536378 T	02-12-2010
		KR	20100056492 A	27-05-2010
		PE	10042009 A1	19-08-2009
		US	2009155164 A1	18-06-2009
WO 2009112245 A1	17-09-2009	AU	2009224955 A1	17-09-2009
		AU	2010257416 A1	20-01-2011
		CA	2718499 A1	17-09-2009
		CN	101970496 A	09-02-2011
		EP	2262836 A1	22-12-2010
		KR	20100122923 A	23-11-2010
		US	2011081353 A1	07-04-2011