Antibody against the CSF-1R

Description

[0001] The CSF-1 (Colony-Stimulating Factor-1) is a cytokine expressed particularly by various types of cells. It is a differentiation, growth and survival factor for cells of the mononuclear phagocyte lineage which express the receptor for CSF-1 (CSF-1R) (SHERR. Colony-stimulating factor-1 receptor. *blood.* 1990, vol.75, no.1, p.1-12.). CSF-1R is a tyrosine kinase receptor encoded by the *c-fms* protoonogene containing an intracellular kinase domain and a ligand-binding extracellular region organized in five immunoglobulin-like subdomains. Response to CSF-1 results in increased survival, growth, differentiation, and reversible changes in function. The *c-fms* gene is itself a macrophage differentiation marker. The extent of *c-fms* expression is stronger than that of other macrophage-specific genes including lysozyme and a macrophage-specific protein tyrosine phosphatase. (HUME, et al. Regulation of CSF-1 receptor expression. *Molecular reproduction and development.* 1997, vol.46, no.1, p.46-52.).

[0002] In addition to cells of the mononuclear phagocyte lineage, the CSF-1R is also expressed by many types of human tumors. In breast cancer, CSF-1R expression is associated with larger tumor sizes and decreased survival (KLUGER, et al. Macrophage colony-stimulating factor-1 receptor expression is associated with poor outcome in breast cancer by large cohort tissue microarray analysis. *Clinical cancer research* . 2004, vol.10, no.1, p.173-7. ; SCHOLL, et al. Anti-colony-stimulating factor-1 antibody staining in primary breast adenocarcinomas correlates with marked inflammatory cell infiltrates and prognosis. *Journal of the National Cancer Institute.* 1994, vol.86, no.2, p.120-6.). In epithelial ovarian cancer, the majority of primary tumors and metastases strongly express the CSF-1R, and metastases frequently co-express CSF-1 and CSF-1R. The CSF-1R is also expressed by tumor-infiltrating macrophages (CHAMBERS, et al. Overexpression of epithelial macrophage colony-stimulating factor (CSF-1) and CSF-1 receptor: a poor prognostic factor in epithelial ovarian cancer,

contrasted with a protective effect of stromal CSF-1. *Clinical Cancer Research.* 1997, vol.3, no.6, p.999-1007.). In ovarian and endometrial cancers, Northerm blot analysis shows that the vast majority of tumors co-express CSF-1 and CSF-1R , while CSF-1R expression is only weakly detected in normal endometrial tissue samples (BAÏOCCHI, et al. Expression of the macrophage colony-stimulating factor and its receptor in gynecologic malignancies. *Cancer.* 1991, vol.67, no.4, p.990-6.). In cervical carcinomas, CSF-1R expression is up-regulated both in tumor stroma and in tumor epithelium, compared with normal endometrium (KIRMA, et al. Elevated expression of the oncogene c-fms and its ligand, the macrophage colony-stimulating factor-1, in cervical cancer and the role of transforming growth factor-beta1 in inducing c-fms expression. *Cancer res..* 2007, vol.67, no.5, p.1918-26.). In renal carcinoma, infiltration of tumor-associated macrophages expressing high levels of CSF-1R is associated with tumor progression (HEMMERLEIN, et al. Expression of acute and late-stage inflammatory antigens, c-fms, CSF-1, and human monocytic serine esterase 1, in tumor-associated macrophages of renal cell carcinomas. *Cancer immunology, immunotherapy .* 2000, vol.49, no.9, p.485-92.). CSF-1R is expressed by close to 100% prostatic intraepithelial neoplasia or cancer samples (IDE, et al. Expression of colony-stimulating factor 1 receptor during prostate development and prostate cancer progression. *Proc. Natl. Acad. Sci. U.S.A..* 2002, vol.99, no.22, p.14404-9.). CSF-1R expression has also been detected in acute myeloblastic leukemias and B-cell chronic lymphocytic leukemias (RAMBALDI, et al. Expression of the macrophage colony-stimulating factor and c-fms genes in human acute myeloblastic leukemia cells. *Journal of Clinical Investigation.* 1988, vol.81, no.4, p.1030-5.).

[0003] Work done by immunohistochemistry and *in situ* hybridization has demonstrated specificity of the expression of CSF-1 in invasive breast cancer cells while such production is not observed in intra-canal or non-invasive tumor cells (SCHOLL, et al. Anti-colony-stimulating factor-1 antibody staining in primary breast adenocarcinomas correlates with marked inflammatory cell infiltrates and prognosis. *Journal of the National*

Cancer Institute. 1994, vol.86, no.2, p.120-6. ; TANG, et al. M-CSF (monocyte colony stimulating factor) and M-CSF receptor expression by breast tumour cells: M-CSF mediated recruitment of tumour infiltrating monocytes?. *Journal of cellular biochemistry.* 1992, vol.50, no.4, p.350-6.). Production of CSF-1 by invasive tumor cells correlates with its increase in concentration in the plasma of patients, where it can exceed 1000 pg/ml compared with less than 300 pg/ml in normal subjects. High serum concentration correlates with advanced stages of the disease and unfavorable short term prognostic (SCHOLL, et al. Circulating levels of colony-stimulating factor 1 as a prognostic indicator in 82 patients with epithelial ovarian cancer. *British journal of cancer.* 1994, vol.69, no.2, p.342-6. ; SCHOLL. Circulating levels of the macrophage colony stimulating factor CSF-1 in primary and metastatic breast cancer patients. A pilot study. *Breast cancer research and treatment.* 1996, vol.39, no.3, p.275-83.). Moreover, it has been demonstrated that CSF-1 stimulates mobility and invasiveness of tumor cells (DORSCH, et al. Macrophage colony-stimulating factor gene transfer into tumor cells induces macrophage infiltration but not tumor suppression. *European journal of immunology.* 1993, vol.23, no.1, p.186-90. ; WANG, et al. Induction of monocyte migration by recombinant macrophage colony-stimulating factor. *Journal of immunology* . 1988, vol.141, no.2, p.575-9. ; FILDERMAN, et al. Macrophage colony-stimulating factor (CSF-1) enhances invasiveness in CSF-1 receptor-positive carcinoma cell lines. *Cancer res.*. 1992, vol.52, no.13, p.3661-6.).

[0004] CSF-1 also has a chemotactic effect on precursors of the myeloid line, which facilitates infiltration of monocytes in the tumor. However, the presence of these monocytes is not sufficient to observe destruction of the tumor by the immune system. (DORSCH, et al. Macrophage colony-stimulating factor gene transfer into tumor cells induces macrophage infiltration but not tumor suppression. *European journal of immunology.* 1993, vol.23, no.1, p.186-90.). It appears that at the high serum contents commonly found in patients suffering from tumors of the breast, ovaries or pancreas, CSF-1 orients the differentiation of these monocytes into

macrophages and not into dendritic cells capable of presenting tumoral antigens and thus initiating an efficient cytotoxic immune response directed against tumor cells (SCHOLL. Circulating levels of the macrophage colony stimulating factor CSF-1 in primary and metastatic breast cancer patients. A pilot study. *Breast cancer research and treatment.* 1996, vol.39, no.3, p.275-83. ; BARON, et al. Modulation of MHC class II transport and lysosome distribution by macrophage-colony stimulating factor in human dendritic cells derived from monocytes. *Journal of cell science.* 2001, vol.114, no.pt5, p.999-1010.).

[0005] CSF-1 is also essential for proliferation and differentiation of osteoclasts. (CECCHINI, et al. Role of CSF-1 in bone and bone marrow development. *Molecular reproduction and development.* 1997, vol.46, no.1, p.75-83.). Osteoclasts are multinucleated cells that express the CSF-1R, deriving from hematopoietic precursors that are primarily responsible for the degradation of mineralized bone during bone development, homeostasis and repair. In various skeletal disorders such as osteoporosis, hypercalcemia of malignancy, rheumatoid arthritis, tumor metastases and Paget's disease, bone resorption by osteoclasts exceeds bone formation by osteoblasts leading to decreased bone mass, skeletal fragility and bone fracture.(BRUZZANITI, et al. Molecular regulation of osteoclast activity. *Reviews in endocrine .* 2006, vol.7, no.1-2, p.123-39.). For example, patients with advanced breast cancer frequently develop metastasis to bone. Bone metastasis results in intractable pain and a high risk of fractures due to tumor-driven bone loss (osteolysis), which is caused by increased osteoclast activity(CICEK, et al. Breast cancer bone metastasis and current small therapeutics. *Cancer metastasis reviews.* 2006, vol.25, no.4, p.635-44.). It has been shown that osteolysis is linked to a high level of circulating CSF-1(KITAURA, et al. The journal of clinical investigation. *M-CSF mediates TNF-induced inflammatory osteolysis.* 2005, vol.115, no.12, p.3418-27.) .

[0006] The CSF-1 pathway is also involved in mediating intestinal inflammation in disease such as Inflammatory bowel disease (MARSHALL, et al. Blockade of colony stimulating factor-1 (CSF-I) leads to inhibition of DSS-

induced colitis. *Inflammatory bowel diseases.* 2007, vol.13, no.2, p.219-24.), in mediating macrophage proliferation during acute allograft rejection (JOSE, et al. Blockade of macrophage colony-stimulating factor reduces macrophage proliferation and accumulation in renal allograft rejection. *American journal of transplantation* . 2003, vol.3, no.3, p.294-300. and in HIV-1 replication in infected macrophage (KUTZA, et al. Macrophage colony-stimulating factor antagonists inhibit replication of HIV-1 in human macrophages. *Journal of immunology* . 2000, no.164, p.4955-4960.

[0007] For these reasons, the inhibition of the CSF-1 activity by various compounds has been proposed for the treatment of cancer and bone degradation.

Background Art

[0008] WO 01/30381 relates to the use of inhibitors of the CSF-1 activity in the production of medicaments for the treatment of tumor diseases. The two proposed approaches for the inhibition of the CSF-1 activity are the suppression of the CSF-1 activity itself, and the suppression of the activity of the CSF-1R. Neutralizing antibodies against CSF-1 or its receptor are preferred as the inhibitors of CSF-1 activity.

[0009] WO 03/059395 describes combination products comprising a substance capable of inhibiting CSF-1 activity and a substance having a cytotoxic activity for the treatment of cancer.

[0010] WO 2005/068503 discloses a method for preventing and treating osteolysis, cancer metastasis and bone loss associated with cancer metastasis by administering an antibody against CSF-1 to a subject.

[0011] EP 1488792 A relates to the use of mono- and/or bicyclic aryl or heteroaryl quinazoline compounds which exhibit selective inhibition of differentiation, proliferation or mediator release by effectively inhibiting CSF-1R tyrosine kinase activity. This application also relates to the use of such compounds for the manufacture of a medicament for inhibiting abnormal cell proliferation.

[0012] US 2005059113 relates to antibodies and antigen-binding portions thereof that specifically bind to aCSF-1. The invention also relates to human anti-CSF-1 antibodies and antigen-binding portions thereof. This application

invention also provides gene therapy methods using nucleic acid molecules encoding the heavy and/or light immunoglobulin molecules that comprise the human anti-CSF-1 antibodies.

Disclosure of Invention

[0013] The present invention relates to an antibody that specifically binds to the CSF-1R.

[0014] As used throughout the entire application, the terms "a" and "an" are used in the sense that they mean "at least one", "at least a first", "one or more" or "a plurality" of the referenced components or steps, unless the context clearly dictates otherwise. For example, the term "a cell" includes a plurality of cells, including mixtures thereof.

[0015] The term "and/or" wherever used herein includes the meaning of "and", "or" and "all or any other combination of the elements connected by said term".

[0016] The term "about" or "approximately" as used herein means within 20%, preferably within 10%, and more preferably within 5% of a given value or range.

[0017] As used herein, the terms "comprising" and "comprise" are intended to mean that the kits of parts, products, compositions and methods include the referenced components or steps, but not excluding others. "Consisting essentially of" when used to define products, compositions and methods, shall mean excluding other components or steps of any essential significance. Thus, a composition consisting essentially of the recited components would not exclude trace contaminants and pharmaceutically acceptable carriers. "Consisting of" shall mean excluding more than trace elements of other components or steps.

[0018] As used herein, the term "specifically binds to" refers to a binding reaction which is determinative of the presence of a target protein in the presence of a heterogeneous population of proteins and other biologics. Thus, under designated assay conditions, the antibody according to the invention bind preferentially to at least part of the CSF-1R and do not bind in a significant amount to other components present in a test sample. Specific binding between the antibody according to the invention and the CSF-1R target

means that the binding affinity is of at least 10^3 M^{-1}, and preferably 10^5 M^{-1}, 10^6 M^{-1}, 10^7 M^{-1}, 10^8 M^{-1}, 10^9 M^{-1} or 10^{10} M^{-1}.

[0019] As used herein, the term "CSF-1R" refers to the human CSF1 receptor. The human CSF-1 receptor has been sequenced and its amino acid sequence is depicted in SEQ ID NO: 29.

[0020] As used herein, "antibody" or "Ab" is used in the broadest sense. Therefore, an "antibody" or "Ab" can be naturally occurring or man-made such as monoclonal antibodies (mAbs) produced by conventional hybridoma technology, recombinant technology and/or a functional fragment thereof. Antibodies of the present invention are meant to include both intact immunoglobulin molecules for example a polyclonal antibody, a monoclonal antibody (mAb), a monospecific antibody, a bispecific antibody, a polyspecific antibody, a human antibody, an animal antibody (e.g. camelid antibody), chimeric antibodies, as well as portions, fragments, regions, peptides and derivatives thereof (provided by any known technique, such as, but not limited to, enzymatic cleavage, peptide synthesis, or recombinant techniques), such as, for example, immunoglobulin devoid of light chains (see for example US 6,005,079), Fab, Fab', F $(ab')_2$, Fv, scFv, antibody fragment, diabody , Fd, CDR regions, or any portion or peptide sequence of the antibody that is capable of binding antigen or epitope.. An antibody is said to be "capable of binding" a molecule if it is capable of specifically reacting with the molecule to thereby bind the molecule to the antibody. Antibody fragments or portions may lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding than an intact antibody. Examples of antibody may be produced from intact antibodies using methods well known in the art, for example by proteolytic cleavage with enzymes such as papain (to produce Fab fragments) or pepsin (to produce.$F(ab')_2$ fragments). See e.g., Wahl et al., 24 J. Nucl. Med. 316-25 (1983). Portions of antibodies may be made by any of the above methods, or may be made by expressing a portion of the recombinant molecule. For example, the CDR region(s) of a recombinant antibody may be isolated and subcloned into the appropriate expression

vector.

[0021] As used herein, the term "variable region" refers to the variable region, or domain, of the light chain (VL) or heavy chain (VH) which contain the determinants for binding recognition specificity. The variable domains are involved in antigen recognition and form the antigen binding site. As used herein, the term "framework region" refers to portions of light and heavy chain variable regions that are at least 85 % homologous (i.e., other than the CDR's) among different antibodies in the same specie. As used herein, the term "homologous» refers to a comparison of the amino acids of two polypeptides which, when aligned by using the Smith-Waterman algorithm (SMITH, et al. Identification of common molecular subsequences. *Journal of Molecular Biology.* 1981, no.147, p.195-7.), have approximately the designated percentage of the same amino acids. For example, "85% homologous" refers to a comparison of the amino acids of two polypeptides which when optimally aligned have 85% amino acid identity. The variable region of both the heavy and light chain is divided into segments comprising four framework sub-regions (FR1, FR2, FR3, and FR4), interrupted by three stretches of hypervariable sequences, or the complementary determining regions (CDR's), as defined in Kabat's database (Kabat et al., op. cit.), with the CDR1 positioned between FR1 and FR2, CDR2 between FR2 and FR3, and CDR3 between FR3 and FR4. Without specifying the particular sub-regions as FR1, FR2, FR3 or FR4, a framework region as referred by others, represents the combined FR's within the variable region of a single, naturally occurring immunoglobulin chain. As used herein, a FR represents one of the four sub-regions, and FR's represents two or more of the four sub-regions constituting a framework region. The sequences of the framework regions of different light or heavy chains are relatively conserved within a species. The framework region of an antibody is the combined framework regions of the constituent light and heavy chains and serves to position and align the CDR's. The CDR's are primarily responsible for forming the binding site of an antibody conferring binding specificity and affinity to an epitope of an antigen. Within the variable regions of the H or L chains that provide

for the antigen binding regions are smaller sequences dubbed "hypervariable" because of their extreme variability between antibodies of differing specificity. Such hypervariable regions are also referred to as "complementarity determining regions" or "" regions. These CDR regions account for the basic specificity of the antibody for a particular antigenic determinant structure. The CDRs represent non-contiguous stretches of amino acids within the variable regions but, regardless of species, the positional locations of these critical amino acid sequences within the variable heavy and light chain regions have been found to have similar locations within the amino acid sequences of the variable chains. The variable heavy and light chains of all antibodies each have 3 CDR regions, each non-contiguous with the others (termed L1, L2, L3, H1, H2, H3) for the respective light (L) and heavy (H) chains. The accepted CDR regions have been described by Kabat et al, 252 J. Biol. Chem. 6609-16 (1977), and CDR loops may be identified by applying these rules during an examination of a linear amino acid sequence. The rules for defining the CDR -H3 loop can vary, however (see Chapter 4, Antibody Engineering: Methods & Protocols, (Lo, ed. Humana Press, Totowa, NJ, 2004)), and the actual boundaries of some CDR -H3 loops may not be identified without experimental techniques such as circular dichroism, nuclear magnetic resonance, or X-ray crystallography.In all mammalian species, antibody peptides contain constant (i.e., highly conserved) and variable regions, and, within the latter, there are the CDRs and the so-called "framework regions" made up of amino acid sequences within the variable region of the heavy or light chain but outside the CDRs. The CDR regions can also be defined using Chothia nomenclature (CHOTHIA and LESK. Canonical structures for the hypervariable regions of immunoglobulins (1987) J Mol Biol. 1987 Aug 20;196(4):901-17). Therefore, in certain embodiments, the CDRs are Kabat defined CDRs, and in other embodiments, the CDRs are Chothia defined CDRs. Regarding the antigenic determinate recognized by the CDR regions of the antibody, this is also referred to as the "epitope." In other words, epitope refers to that portion of any molecule capable of being recognized by, and bound by, an antibody (the

corresponding antibody binding region may be referred to as a paratope). In general, epitopes consist of chemically active surface groupings of molecules, for example, amino acids or sugar side chains, and have specific three-dimensional structural characteristics as well as specific charge characteristics.

[0022] The term "monoclonal antibody" or "mAb" as used herein refers to an antibody that is derived from a single clone. Monoclonal antibodies can be prepared using hybridoma techniques such as those disclosed in HARLOW. Antibodies: A Laboratory manual. 2nd edition. Cold Spring Harbor: Laboratory press, 1988. and HAMMERLING, et al. Monoclonal Antibodies and T Cell Hybridomas. New York: Elsevier, 1981. p.563-681. .

[0023] As used herein, the term "human antibody" refers to an antibody having variable and constant regions derived from or closely matching human germline immunoglobulin sequences. The human antibody of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis in vitro or by somatic mutation in vivo). Thus, as used herein, the term "human antibody" refers to an antibody in which substantially every part of the protein is substantially similar to a human germline antibody. "Substantially similar" refers to an antibody having a nucleic acid sequence which is at least 80, preferably 85, more preferably 90 and even more preferably 95% homologous to the nucleic acid sequence a human germline antibody.

[0024] As used herein, the term "Fab" refers to regions of antibody molecules which include the variable region of the heavy chain and light chain and which exhibit binding activity. "Fab" includes aggregates of one heavy and one light chain (commonly known as Fab), whether any of the above are covalently or non-covalently aggregated so long as the aggregation is capable of selectively reacting with a particular antigen or antigen family. The Fab fragment is a heterodimer comprising a VL and a second polypeptide comprising the VH and CH1 domains. In a preferred embodiment the antibody is an Fab' fragment. By Fab' fragments differ from Fab fragments in that the Fab' fragment contains a few residues at

the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody "hinge region".

[0025] "$F(ab')_2$" refers to an antibody fragment obtained by the pepsin treatment of an antibody or to the equivalent protein obtained by other techniques such as recombinant technologies. $F(ab')_2$ fragment has two antigen-combining sites and is still capable of cross-linking an antigen.

[0026] "Fv" is the minimum antibody fragment that contains a complete antigen recognition and binding site. This region consists of a dimer of one heavy- and one light- chain variable domain in tight, non-covalent association. It is in this configuration that the three CDRs of each variable domain interact to define an antigen binding site on the surface of the VH VL dimer. Collectively, the six CDRs confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

[0027] "Single-chain Fv" or "scFv" comprise the VH and VL domains of antibody, wherein these domains are present in a single polypeptide chain. Preferably, the scFv further comprises a polypeptide linker between the VH and VL domains which enables the scFv to form the desired structure for antigen binding (LENNARD. Standard protocols for the construction of scFv libraries. *Methods in molecular biology*. 2002, no.178, p.59-71.).

[0028] As used herein, the term "antibody fragment" refers to one or more fragments of an antibody that retain the ability to specifically bind to the CSF-1R.

[0029] The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (VH) connected to a light-chain variable domain (VL) in the same polypeptide chain (VH VL). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies may bind to one ore more than one epitope. Diabodies are more fully described in POLJAK. Production

and structure of diabodies. *Structure.* 1994, vol.2, no.12, p.1121-3. , HUDSON, et al. High avidity scFv multimers; diabodies and triabodies. *Journal of immunological methods.* 1999, vol.231, no.1-2, p.177-89. and KIPRIYANOV. Generation of bispecific and tandem diabodies. *Methods in molecular biology* . 2002, no.178, p.317-31. .

[0030] Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies. However, these fragments can now be produced directly by recombinant host cells. Fab, Fv and scFv antibody fragments can all be expressed in and secreted from *E. coli*, thus allowing the production of large amounts of these fragments. Techniques for the production of antibody fragments will be apparent to the skilled in the art. In other embodiments, the antibody of choice is a single chain Fv fragment (scFv).

[0031] As used herein "Domain Antibodies" (dAbs) consist in the smallest functional binding units of antibodies, corresponding to the variable regions of either the heavy (VH) or light (VL) chains of the antibodies. Domain Antibodies have a molecular weight of approximately 13 kDa, or less than one-tenth the size of a full antibody.

[0032] As used herein, "Fd" refers to an antibody fragment that consists of the VH and CH1 domains.

[0033] The term "antibody" or "Ab" also refers to other antibody fragment well known to the one skilled in the art, for example those described in HOLLIGER, et al. Engineered antibody fragments and the rise of single domains. *Nature biotechnology.* 2005, vol.23, no.9, p.1126-36. and HOOGENBOOM, et al. Natural and designer binding sites made by phage display technology. *Immunology today.* 2000, vol.21, no.8, p.371-8.

[0034] According to one embodiment, the antibody of the Invention binds specifically to CSF-1R and comprises :

(i) at least one CDR wherein said CDR is comprising at least five consecutive amino acids of the sequence starting in position 45 and finishing in position 54 of SEQ ID NO:2, of the sequence starting in position 66 and finishing in position 87 of

SEQ ID NO:2 or of the sequence starting in position 117 and finishing in position 126 of SEQ ID NO:2;

or ,

(ii) at least one CDR wherein said CDR is comprising at least five consecutive amino acids of the sequence starting in position 44 and finishing in position 56 of SEQ ID NO:4, of the sequence starting in position 66 and finishing in position 76 of SEQ ID NO:4 or of the sequence starting in position 109 and finishing in position 117 of SEQ ID NO:4.

[0035] According to another embodiment, the antibody of the Invention binds specifically to CSF-1R and comprises at least one CDR wherein said CDR is, independently from one another, selected in the group of CDRs comprising at least five consecutive amino acids :

- of the sequence starting in position 45 and finishing in position 54 of SEQ ID NO:2,
- of the sequence starting in position 66 and finishing in position 87 of SEQ ID NO:2,
- of the sequence starting in position 117 and finishing in position 126 of SEQ ID NO:2,
- of the sequence starting in position 44 and finishing in position 56 of SEQ ID NO:4,
- of the sequence starting in position 66 and finishing in position 76 of SEQ ID NO:4
- or of the sequence starting in position 109 and finishing in position 117 of SEQ ID NO:4.

[0036] According to a preferred embodiment, the antibody of the Invention binds specifically to CSF-1R and comprises 2, 3, 4 or 5 and even more preferably 6 CDRs wherein said CDRs are, independently from one another, selected in the group of CDRs comprising at least five consecutive amino acids :

- of the sequence starting in position 45 and finishing in position 54 of SEQ ID NO:2,
- of the sequence starting in position 66 and finishing in position

87 of SEQ ID NO:2,

- of the sequence starting in position 117 and finishing in position 126 of SEQ ID NO:2,
- of the sequence starting in position 44 and finishing in position 56 of SEQ ID NO:4,
- of the sequence starting in position 66 and finishing in position 76 of SEQ ID NO:4
- or of the sequence starting in position 109 and finishing in position 117 of SEQ ID NO:4.

[0037] According to a another preferred embodiment, the antibody of the Invention binds specifically to CSF-1R and comprises :

(iii) 2 and even more preferably 3 CDRs wherein said CDRs are, independently from one another, selected in the group of CDRs comprising at least five consecutive amino acids :

- of the sequence starting in position 45 and finishing in position 54 of SEQ ID NO:2,
- of the sequence starting in position 66 and finishing in position 87 of SEQ ID NO:2,
- of the sequence starting in position 117 and finishing in position 126 of SEQ ID NO:2,

or

(iv) 2 and even more preferably 3 CDRs wherein said CDRs are, independently from one another, selected in the group of CDRs comprising at least five consecutive amino acids :

- of the sequence starting in position 44 and finishing in position 56 of SEQ ID NO:4,
- of the sequence starting in position 66 and finishing in position 76 of SEQ ID NO:4
- or of the sequence starting in position 109 and finishing in position 117 of SEQ ID NO:4.

[0038] According to another embodiment, the antibody of the Invention binds

specifically to CSF-1R and comprises :

(i) at least one CDR selected, independently from one another, in the group of the CDR as set forth in :

- the sequence starting in position 45 and finishing in position 54 of SEQ ID NO:2,
- the sequence starting in position 66 and finishing in position 87 of SEQ ID NO:2 and
- the sequence starting in position 117 and finishing in position 126 of SEQ ID NO:2;

or

(ii) at least one CDR selected, independently from one another, in the group of the CDR as set forth in :

- the sequence starting in position 44 and finishing in position 56 of SEQ ID NO:4,
- the sequence starting in position 66 and finishing in position 76 of SEQ ID NO:4 and
- the sequence starting in position 109 and finishing in position 117 of SEQ ID NO:4.

[0039] According to another embodiment, the antibody of the Invention binds specifically to CSF-1R and comprises at least one CDR selected, independently from one another, in the group of the CDR as set forth in :

- the sequence starting in position 45 and finishing in position 54 of SEQ ID NO:2,
- the sequence starting in position 66 and finishing in position 87 of SEQ ID NO:2,
- the sequence starting in position 117 and finishing in position 126 of SEQ ID NO:2,
- the sequence starting in position 44 and finishing in position 56 of SEQ ID NO:4,
- the sequence starting in position 66 and finishing in position 76 of SEQ ID NO:4 and
- the sequence starting in position 109 and finishing in position 117 of SEQ ID NO:4.

[0040] According to another embodiment, the antibody of the Invention binds specifically to CSF-1R and comprises 2, 3, 4 or 5 and even more preferably 6 CDRs selected, independently from one another, in the group of the CDR as set forth in :

- the sequence starting in position 45 and finishing in position 54 of SEQ ID NO:2,
- the sequence starting in position 66 and finishing in position 87 of SEQ ID NO:2,
- the sequence starting in position 117 and finishing in position 126 of SEQ ID NO:2,
- the sequence starting in position 44 and finishing in position 56 of SEQ ID NO:4,
- the sequence starting in position 66 and finishing in position 76 of SEQ ID NO:4 and
- the sequence starting in position 109 and finishing in position 117 of SEQ ID NO:4.

[0041] According to a another preferred embodiment, the antibody of the Invention binds specifically to CSF-1R and comprises :

(iv) 2 and even more preferably 3 CDRs wherein said CDRs are, independently from one another, selected in the group of the CDRs as set forth in :

- the sequence starting in position 45 and finishing in position 54 of SEQ ID NO:2,
- the sequence starting in position 66 and finishing in position 87 of SEQ ID NO:2,
- the sequence starting in position 117 and finishing in position 126 of SEQ ID NO:2,

or

(iv) 2 and even more preferably 3 CDRs wherein said CDRs are, independently from one another, selected in the group of the CDRs as set forth in :

- the sequence starting in position 44 and finishing in position 56 of SEQ ID NO:4,

- the sequence starting in position 66 and finishing in position 76 of SEQ ID NO:4,
- the sequence starting in position 109 and finishing in position 117 of SEQ ID NO:4.

[0042] According to one embodiment, the antibody of the Invention binds specifically to CSF-1R and comprises (i) at least one CDR comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 11, 12, or 13; or (ii) at least one CDR comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 14, 15 or 16.

[0043] According to another embodiment, the antibody of the Invention binds specifically to CSF-1R and comprises at least one CDR comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 11, 12, 13, 14, 15 or 16.

[0044] According to a preferred embodiment, the antibody of the Invention binds specifically to CSF-1R and comprises 2, 3, 4, 5 and even more preferably 6 CDRs comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 11, 12, 13, 14, 15 or 16.

[0045] According to another embodiment, the antibody of the Invention binds specifically to CSF-1R and comprises (i) at least one CDR as set forth in any one of SEQ ID NOs: 11, 12 or 13; or (ii) at least one CDR set forth in any one of SEQ ID NOs: 14, 15 or 16.

[0046] According to another embodiment, the antibody of the Invention binds specifically to CSF-1R and comprises at least one CDR as set forth in any one of SEQ ID NOs: 11, 12, 13, 14, 15 or 16.

[0047] According to one preferred embodiment, the antibody of the Invention binds specifically to CSF-1R and comprises 2, 3, 4, 5 and even more preferably 6 CDRs as set forth in any one of SEQ ID NOs: 11, 12, 13, 14, 15 or 16.

[0048] According to another embodiment, the antibody of the Invention binds specifically to CSF-1R and comprises (i) at least one CDR comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 17, 18 or 19; or (ii) at least one CDR comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 20, 21 or 22.

[0049] According to another embodiment, the antibody of the Invention binds specifically to CSF-1R and comprises at least one CDR comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 17, 18, 19, 20, 21 or 22.

[0050] According to one preferred embodiment, the antibody of the Invention binds specifically to CSF-1R and comprises 2, 3, 4, 5 and even more preferably 6 CDRs comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 17, 18, 19, 20, 21 or 22.

[0051] According to another embodiment, the antibody of the Invention binds specifically to CSF-1R and comprises (i) at least one CDR as set forth in any one of SEQ ID NOs: 17, 18 or 19; or (ii) at least one CDR as set forth in any one of SEQ ID NOs: 20, 21 or 22.

[0052] According to another embodiment, the antibody of the Invention binds specifically to CSF-1R and comprises at least one CDR set forth in any one of SEQ ID NOs: 17, 18, 19, 20, 21 or 22.

[0053] According to one preferred embodiment, the antibody of the Invention binds specifically to CSF-1R and comprises 2, 3, 4, 5 and even more preferably 6 CDRs as set forth in any one of SEQ ID NOs: 17, 18, 19, 20, 21 or 22.

[0054] According to another embodiment, the antibody of the Invention binds specifically to CSF-1R and comprises (i) at least one CDR comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 23, 24 or 25; or (ii) at least one CDR comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 26, 27 or 28.

[0055] According to another embodiment, the antibody of the Invention binds specifically to CSF-1R and comprises at least one CDR comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 23, 24, 25, 26, 27 or 28.

[0056] According to one preferred embodiment, the antibody of the Invention binds specifically to CSF-1R and comprises 2, 3, 4, 5 and even more preferably 6 CDRs comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 23, 24, 25, 26, 27 or 28.

[0057] According to another embodiment, the antibody of the Invention binds

specifically to CSF-1R and comprises (i) at least one CDR as set forth in any one of SEQ ID NOs: 23, 24 or 25; or (ii) at least one CDR as set forth in any one of SEQ ID NOs: 26, 27 or 28.

[0058] According to another embodiment, the antibody of the Invention binds specifically to CSF-1R and comprises at least one CDR as set forth in any one of SEQ ID NOs: 23, 24, 25, 26, 27 or 28.

[0059] According to one preferred embodiment, the antibody of the Invention binds specifically to CSF-1R and comprises 2, 3, 4, 5 and even more preferably 6 CDRs as set forth in any one of SEQ ID NOs: 23, 24, 25, 26, 27 or 28.

[0060] According to another embodiment, the antibody of the Invention binds specifically to CSF-1R and comprises a variable region, wherein said variable region comprises the CDRs as set forth in :

- the sequence starting in position 45 and finishing in position 54 of SEQ ID NO:2,
- the sequence starting in position 66 and finishing in position 87 of SEQ ID NO:2 and
- the sequence starting in position 117 and finishing in position 126 of SEQ ID NO:2.

[0061] According to another embodiment, the antibody of the Invention binds specifically to CSF-1R and comprises a variable region, wherein said variable region comprises the CDRs as set forth in :

- the sequence starting in position 44 and finishing in position 56 of SEQ ID NO:4,
- the sequence starting in position 66 and finishing in position 76 of SEQ ID NO:4 and
- the sequence starting in position 109 and finishing in position 117 of SEQ ID NO:4.

[0062] According to another embodiment, the antibody of the Invention binds specifically to CSF-1R and comprises a variable region, wherein said variable region comprises the three CDRs as set forth in SEQ ID NOs: 11, 12, and 13.

[0063] According to another embodiment, the antibody of the Invention binds

specifically to CSF-1R and comprises a variable region, wherein said variable region comprises the three CDRs as set forth in SEQ ID NOs: 14, 15, and 16.

[0064] According to another embodiment, the antibody of the Invention binds specifically to CSF-1R and comprises a variable region, wherein said variable region comprises the three CDRs set forth in SEQ ID NOs: 17, 18, and 19.

[0065] According to another embodiment, the antibody of the Invention binds specifically to CSF-1R and comprises a variable region, wherein said variable region comprises the three CDRs as set forth in SEQ ID NOs: 20, 21, and 22.

[0066] According to another embodiment, the antibody of the Invention binds specifically to CSF-1R and comprises a variable region, wherein said variable region comprises the three CDRs as set forth in SEQ ID NOs: 23, 24, and 25.

[0067] According to another embodiment, the antibody of the Invention binds specifically to CSF-1R and comprises a variable region, wherein said variable region comprises the three CDRs as set forth in SEQ ID NOs: 26, 27, and 28.

[0068] In preferred embodiments, the said variable region further comprises one, more preferably two, even more preferably three and definitely preferably four framework region, and more preferably human FR. As used herein, a "human FR" is a framework region that is at least 75 % homologous to the framework region of a naturally occurring human antibody.

[0069] According to one preferred embodiment, the antibody of the Invention binds specifically to CSF-1R and comprises a variable region, wherein the variable region comprises an amino acid sequence as set forth in SEQ ID NO:6.

[0070] In a more preferred embodiment, the antibody of the Invention binds specifically to CSF-1R and comprises a variable region, wherein the variable region is as set forth in SEQ ID NO:6.

[0071] In another preferred embodiment, the antibody of the Invention binds specifically to CSF-1R and comprises a variable region, wherein the

variable region comprises an amino acid sequence as set forth in SEQ ID NO:9.

[0072] In another more preferred embodiment, the antibody of the Invention binds specifically to CSF-1R and comprises a variable region, wherein the variable region is as set forth in SEQ ID NO:9.

[0073] In another embodiment, the antibody of the Invention binds specifically to CSF-1R and comprises two variable regions, wherein the variable regions are, independently from one another, selected in the group of

(i) variable regions comprising the CDRs as set forth in :
- the sequence starting in position 45 and finishing in position 54 of SEQ ID NO:2,
- the sequence starting in position 66 and finishing in position 87 of SEQ ID NO:2 and
- the sequence starting in position 117 and finishing in position 126 of SEQ ID NO:2;

(ii) variable regions comprising the CDR as set forth in :
- the sequence starting in position 44 and finishing in position 56 of SEQ ID NO:4,
- the sequence starting in position 66 and finishing in position 76 of SEQ ID NO:4 and
- the sequence starting in position 109 and finishing in position 117 of SEQ ID NO:4;

(ii) variable regions comprising the three CDRs set forth in SEQ ID NOs: 11, 12, and 13;

(iii) variable regions comprising the three CDRs set forth in SEQ ID NOs: 14, 15, and 16;

(iv) variable regions comprising the three CDRs set forth in SEQ ID NOs: 17, 18, and 19;

(v) variable regions comprising the three CDRs set forth in SEQ ID NOs: 20, 21, and 22;

(vi) variable regions comprising the three CDRs set forth in SEQ ID NOs: 23, 24, and 25

and

(vii) variable regions comprising the three CDRs set forth in SEQ ID NOs: 26, 27 and 28.

[0074] According to a preferred embodiment, the antibody of the Invention binds specifically to CSF-1R and comprises :

(i) a first variable region, wherein said variable region comprises :

- the CDR as set forth in the sequence starting in position 45 and finishing in position 54 of SEQ ID NO:2,
- the CDR as set forth in the sequence starting in position 66 and finishing in position 87 of SEQ ID NO:2 and
- the CDR as set forth in the sequence starting in position 117 and finishing in position 126 of SEQ ID NO:2;

and

(ii) a second variable region, wherein said variable region comprises :

- the CDR as set forth in the sequence starting in position 44 and finishing in position 56 of SEQ ID NO:4,
- the CDR as set forth in the sequence starting in position 66 and finishing in position 76 of SEQ ID NO:4 and
- the CDR as set forth in the sequence starting in position 109 and finishing in position 117 of SEQ ID NO:4.

[0075] According to a preferred embodiment, the antibody of the Invention binds specifically to CSF-1R and comprises :

(i) a variable region comprising the three CDRs as set forth in SEQ ID NOs: 11, 12, and 13, and

(ii) a variable region comprising the three CDRs as set forth in SEQ ID NOs: 14, 15, and 16.

[0076] According to a preferred embodiment, the antibody of the Invention binds specifically to CSF-1R and comprises :

(i) a variable region comprising the three CDRs as set forth in SEQ ID NOs: 17, 18, and 19, and

(ii) a variable region comprising the three CDRs as set forth in SEQ ID NOs: 20, 21, and 22.

[0077] According to a preferred embodiment, the antibody of the Invention binds

specifically to CSF-1R and comprises :

(i) a variable region comprising the three CDRs as set forth in SEQ ID NOs: 23, 24, and 25, and

(ii) a variable region comprising the three CDRs as set forth in SEQ ID NOs: 26, 27, and 28.

[0078] According to a preferred embodiment, the antibody of the Invention binds specifically to CSF-1R and comprises :

(i) a variable region as set forth in SEQ ID NO:6 and

(ii) a variable region as set forth in SEQ ID NO:9.

[0079] According to a preferred embodiment, the antibody of the Invention binds specifically to CSF-1R and comprises :

(i) a heavy-chain variable region comprising :

- the CDR as set forth in the sequence starting in position 45 and finishing in position 54 of SEQ ID NO:2,
- the CDR as set forth in the sequence starting in position 66 and finishing in position 87 of SEQ ID NO:2 and
- the CDR as set forth in the sequence starting in position 117 and finishing in position 126 of SEQ ID NO:2;

and

(ii) a light-chain variable region comprising :

- the CDR as set forth in the sequence starting in position 44 and finishing in position 56 of SEQ ID NO:4,
- the CDR as set forth in the sequence starting in position 66 and finishing in position 76 of SEQ ID NO:4 and
- the CDR as set forth in the sequence starting in position 109 and finishing in position 117 of SEQ ID NO:4.

[0080] According to another embodiment, the antibody of the Invention binds specifically to CSF-1R and comprises (i) a heavy-chain variable region comprising the three CDRs as set forth in SEQ ID NOs: 11, 12, and 13, and (ii) a light-chain variable region comprising the three CDRs as set forth in SEQ ID NOs: 14, 15, and 16.

[0081] According to another embodiment, the antibody of the Invention binds specifically to CSF-1R and comprises (i) a heavy-chain variable region

comprising the three CDRs as set forth in SEQ ID NOs: 17, 18, and 19, and (ii) a light-chain variable region comprising the three CDRs as set forth in SEQ ID NOs: 20, 21, and 22.

[0082] According to another embodiment, the antibody of the Invention binds specifically to CSF-1R and comprises (i) a heavy-chain variable region comprising the three CDRs as set forth in SEQ ID NOs: 23, 24, and 25, and (ii) a light-chain variable region comprising the three CDRs as set forth in SEQ ID NOs: 26, 27, and 28.

[0083] According to a preferred embodiment, the antibody of the Invention binds specifically to CSF-1R and comprises (i) a heavy-chain variable region as set forth in SEQ ID NO:6 and (ii) a light-chain variable region as set forth in SEQ ID NO:9.

[0084] According to another preferred embodiment, the antibody of the Invention binds specifically to CSF-1R and is a scFv, wherein said scFv comprises :

(i) a variable regions comprising :

- the CDR as set forth in the sequence starting in position 45 and finishing in position 54 of SEQ ID NO:2,
- the CDR set forth in the sequence starting in position 66 and finishing in position 87 of SEQ ID NO:2 and
- the CDR set forth in the sequence starting in position 117 and finishing in position 126 of SEQ ID NO:2;

and

(ii) a variable region comprising :

- the CDR set forth in the sequence starting in position 44 and finishing in position 56 of SEQ ID NO:4,
- the CDR set forth in the sequence starting in position 66 and finishing in position 76 of SEQ ID NO:4 and
- the CDR set forth in the sequence starting in position 109 and finishing in position 117 of SEQ ID NO:4.

[0085] According to another preferred embodiment, the antibody of the Invention binds specifically to CSF-1R and is a scFv, wherein said scFv comprises :

(i) a variable region comprising the three CDRs as set forth in SEQ ID NOs: 11, 12, and 13, and

(ii) a variable region comprising the three CDRs as set forth in SEQ ID NOs: 14, 15, and 16.

[0086] According to another preferred embodiment, the antibody of the Invention binds specifically to CSF-1R and is a scFv, wherein said scFv comprises :

(i) a variable region comprising the three CDRs as set forth in SEQ ID NOs: 17, 18, and 19, and

(ii) a variable region comprising the three CDRs as set forth in SEQ ID NOs: 20, 21, and 22.

[0087] According to another preferred embodiment, the antibody of the Invention binds specifically to CSF-1R and is a scFv, wherein said scFv comprises

(i) a variable region comprising the three CDRs as set forth in SEQ ID NOs: 23, 24, and 25, and

(ii) a variable region comprising the three CDRs as set forth in SEQ ID NOs: 26, 27, and 28.

[0088] According to a more preferred embodiment, the antibody of the Invention binds specifically to CSF-1R and is a scFv, wherein said scFv comprises :

(i) a variable region as set forth in SEQ ID NO:6 and

(ii) a variable region as set forth in SEQ ID NO:9.

[0089] According to another preferred embodiment, the antibody of the Invention binds specifically to CSF-1R and is a scFv, wherein said scFv comprises :

(i) a heavy-chain variable region comprising :

- the CDR set forth in the sequence starting in position 45 and finishing in position 54 of SEQ ID NO:2,
- the CDR set forth in the sequence starting in position 66 and finishing in position 87 of SEQ ID NO:2 and
- the CDR set forth in the sequence starting in position 117 and finishing in position 126 of SEQ ID NO:2

and

(ii) a light-chain variable region comprising :

- the CDR set forth in the sequence starting in position 44 and finishing in position 56 of SEQ ID NO:4,
- the CDR set forth in the sequence starting in position 66 and finishing in position 76 of SEQ ID NO:4 and

- the CDR set forth in the sequence starting in position 109 and finishing in position 117 of SEQ ID NO:4.

[0090] According to another preferred embodiment, the antibody of the Invention binds specifically to CSF-1R and is a scFv, wherein said scFv comprises :

(i) a heavy-chain variable region comprising the three CDRs as set forth in SEQ ID NOs: 11, 12, and 13, and

(ii) a light-chain variable region comprising the three CDRs as set forth in SEQ ID NOs: 14, 15, and 16.

[0091] According to another preferred embodiment, the antibody of the Invention binds specifically to CSF-1R and is a scFv, wherein said scFv comprises :

(i) a heavy-chain variable region comprising the three CDRs as set forth in SEQ ID NOs: 17, 18, and 19, and

(ii) a light-chain variable region comprising the three CDRs as set forth in SEQ ID NOs: 20, 21, and 22.

[0092] According to another preferred embodiment, the antibody of the Invention binds specifically to CSF-1R and is a scFv, wherein said scFv comprises :

(i) a heavy-chain variable region comprising the three CDRs as set forth in SEQ ID NOs: 23, 24, and 25, and

(ii) a light-chain variable region comprising the three CDRs as set forth in SEQ ID NOs: 26, 27, and 28.

[0093] According to a more preferred embodiment, the antibody of the Invention binds specifically to CSF-1R and is a scFv, wherein said scFv comprises :

(i) the heavy-chain variable region as set forth in SEQ ID NO:6 and

(ii) the light-chain variable region as set forth in SEQ ID NO:9.

In an even more preferred embodiment, the scFv is provided wherein at least one amino acid is substituted (according to Table 1 and Table 2) within the amino acid sequence as set forth in SEQ ID NO 6 and 9. In a definitely preferred embodiment the human antibody is provided wherein all the amino acid depicted in Table 1 and Table 2 are substituted (according to Table 1 and Table 2) within the amino acid sequence as set forth in SEQ ID NO 6 and 9.

[0094] According to another preferred embodiment, the antibody of the Invention

binds specifically to CSF-1R and comprises the heavy chain as set forth in SEQ ID NO:2.

[0095] According to another preferred embodiment, the antibody of the Invention binds specifically to CSF-1R and comprises the light chain as set forth in SEQ ID NO:4.

[0096] According to a more preferred embodiment, the antibody of the Invention binds specifically to CSF-1R and comprises the heavy chain as set forth in SEQ ID NO:2 and the light chain as set forth in SEQ ID NO:4.

[0097] According to an even more preferred embodiment, the antibody of the Invention binds specifically to CSF-1R and comprises two heavy chains as set forth in SEQ ID NO:2 and two light chain as set forth in SEQ ID NO:4. This particular antibody will be named CXIIG6 throughout the present application.

[0098] According to another preferred embodiment, the present invention relates to a human antibody, that specifically binds to CSF-1R, comprising :

(i) a heavy-chain variable region comprising :

- the CDR as set forth in the sequence starting in position 45 and finishing in position 54 of SEQ ID NO:2,
- the CDR as set forth in the sequence starting in position 66 and finishing in position 87 of SEQ ID NO:2 and
- the CDR as set forth in the sequence starting in position 117 and finishing in position 126 of SEQ ID NO:2;

and

(ii) a light-chain variable region comprising :

- the CDR as set forth in the sequence starting in position 44 and finishing in position 56 of SEQ ID NO:4,
- the CDR as set forth in the sequence starting in position 66 and finishing in position 76 of SEQ ID NO:4 and
- the CDR as set forth in the sequence starting in position 109 and finishing in position 117 of SEQ ID NO:4.

[0099] According to another embodiment, the present invention relates to an human antibody, that specifically binds to CSF-1R, comprising :

(i) a heavy-chain variable region comprising the three CDRs as

set forth in SEQ ID NOs: 11, 12, and 13, and

(ii) a light-chain variable region comprising the three CDRs as set forth in SEQ ID NOs: 14, 15, and 16.

[00100] According to another embodiment, the present invention relates to an human antibody, that specifically binds to CSF-1R, comprising :

(i) a heavy-chain variable region comprising the three CDRs as set forth in SEQ ID NOs: 17, 18, and 19, and

(ii) a light-chain variable region comprising the three CDRs as set forth in SEQ ID NOs: 20, 21, and 22.

[0101] According to another embodiment, the present invention relates to an human antibody, that specifically binds to CSF-1R, comprising :

(i) a heavy-chain variable region comprising the three CDRs as set forth in SEQ ID NOs: 23, 24, and 25, and

(ii) a light-chain variable region comprising the three CDRs as set forth in SEQ ID NOs: 26, 27, and 28.

[0102] According to a preferred embodiment, the present invention relates to an human antibody, that specifically binds to CSF-1R, comprising :

(i) the heavy-chain variable region set forth in SEQ ID NO:6 and

(ii) the light-chain variable region set forth in SEQ ID NO:9.

In a more preferred embodiment, the human antibody is provided wherein at least one amino acid is substituted (according to Table 1 and Table 2) within the amino acid sequence as set forth in SEQ ID NO: 6 and 9. In a even more preferred embodiment the human antibody is provided wherein all the amino acid depicted in Table 1 and Table 2 are substituted (according to Table 1 and Table 2) within the amino acid sequence as set forth in SEQ ID NO: 6 and 9.

Table 1

SEQ ID NO:6	position	Preferred substitution
K	3	Q
E	5	V
M	18	L
K	19	R
W	33	Y
S	40	A
E	42	G

M	43	K
A	49	G
E	50	R
I	51	T
A	59	T
F	61	E
E	64	A
S	79	N
V	81	L
R	89	K
P	90	T, A
G	94	A
I	95	V
T	99	A
N	107	Y, V
T	113	L
L	114	V

Table 2

SEQ ID NO:9	Position	Preferred substitution
A	9	S
V	13	A
E	17	D
T	18	R
E	27	Q
N	28	G
Q	40	P, D
S	43	A
Q	45	K
V	48	L
H	49	Y
N	53	R
A	55	E
D	56	S
Q	70	D
Y	71	F
S	72	T
K	74	T
N	76	S
S	80	P
G	84	A
S	85	T
H	90	Q
G	100	Q

[0103] The antibody, more specifically the human antibody, according to the

invention may be of different isotypes, such as IgG, IgA, IgM or IgE. In a preferred embodiment the antibody, more specifically the human antibody, according to the invention is an IgG.

[0104] In a related embodiment, the human antibody comprises a modified or unmodified constant region of a human IgGI, IgG2, IgG3 or IgG4. In a preferred embodiment, the constant region is human IgG1 or IgG4, which may optionally be modified to enhance or decrease certain properties.

[0105] In the case of IgG1, modifications to the constant region, particularly the hinge or CH2 region, may increase or decrease effector function, including ADCC and/or CDC activity. In other embodiments, an IgG2 constant region is modified to decrease antibody-antigen aggregate formation. In the case of IgG4, modifications to the constant region, particularly the hinge region, may reduce the formation of half-antibodies.

[0106] The desired binding affinity may be retained even though one or more of the amino acids in the antibody are mutated. These variants have at least one amino acid in the antibody replaced by a different residue. According to another embodiment, the present invention provides an antibody that specifically binds to CSF1 as above described in which at least one of the amino acid comprised in the CDR(s) is conservatively substituted. Conservative substitutions are shown in Table 3.

Table 3

Original Amino Acid	Preferred conservative substitution	More preferred conservative substitution
A	V, L, I	V
R	K, Q, N	K
N	Q, H, D, K, R	Q
D	E, N	E
C	S, A	S
Q	N, E	N
E	D, Q	D
G	A	A
H	N, Q, K, R	R
I	L, V, M, A, F	L
L	I, V, M, A, F	I
K	R, Q, N	R
M	L, F, I	L
F	W, L, V, I, A, Y	Y

Original Amino Acid	Preferred conservative substitution	More preferred conservative substitution
P	A	A
S	T	T
T	V, S	S
W	Y, F	Y
Y	W, F, T, S	F
V	L, M, F, A	L

[0107] The present invention also relates to a process of modifying the antibody of the invention by affinity maturation.

[0108] As used herein, "affinity maturation" refers to the substitution of one or more amino acid comprised in one or more CDRs, said substitution resulting in an improvement in the affinity of the antibody to CSF-1R, compared to a parent antibody which does not possess those substitution(s). Affinity maturation processes are known in the art. See for example methods disclosed in MARKS, et al. By-passing immunization: building high affinity human antibodies by chain shuffling. *Biotechnology*. 1992, vol.10, no.7, p.779-83. ; BARBAS, et al. In vitro evolution of a neutralizing human antibody to human immunodeficiency virus type 1 to enhance affinity and broaden strain cross-reactivity. *Proceedings of the National Academy of Sciences of the United States of America.* 1994, vol.91, no.9, p.3809-13. ; SCHIER. Identification of functional and structural amino-acid residues by parsimonious mutagenesis. *Gene.* 1996, vol.169, no.2, p.147-55. ; YELTON. Affinity maturation of the BR96 anti-carcinoma antibody by codon-based mutagenesis. *J. immunol..* 1995, vol.155, no.4, p.1994-2004. ; JACKSON, et al. In vitro antibody maturation. Improvement of a high affinity, neutralizing antibody against IL-1 beta. *J. immunol..* 1995, vol.154, no.7, p.3310-9. and HAWKINS, et al. Selection of phage antibodies by binding affinity. Mimicking affinity maturation. *Journal of molecular biology.* 1992, vol.226, no.3, p.889-96.

[0109] The present invention also relates to an antibody, that specifically binds to CSF-1R, obtained by affinity maturation as previously described.

[0110] In another embodiment, the present invention provides variants of the antibody previously described, having an amino acid sequence which is at

least 80%, preferably at least 85%, more preferably at least 90%, and even more preferably at least 98% homologous to the amino acid sequence of the previously described antibody.

[0111] In another embodiment, the antibody according to the invention specifically binds to more than one epitope. For example, the antibody according to the invention may bind to two different epitopes of CSF-1R. Alternatively, the antibody according to the invention can be able to bind to CSF-1R and to another molecule. As used herein, antibodies that specifically bind to more than one epitope can be cross-linked antibodies. For example, an antibody can be coupled to avidin, the other to biotin. Cross-linked antibodies may be made using any convenient cross-linking methods well known in the art. Techniques for generating bispecific antibodies from antibody fragments have also been described, see for example BRENNAN, et al. Preparation of bispecific antibodies by chemical recombination of monoclonal immunoglobulin G1 fragments. *Science.* 1985, vol.229, no.4708, p.81-3. and SHALABY, et al. Development of humanized bispecific antibodies reactive with cytotoxic lymphocytes and tumor cells overexpressing the HER2 protooncogene. *The Journal of experimental medicine.* 1992, vol.175, no.1, p.217-25. KOSTELNY, et al. Formation of a bispecific antibody by the use of leucine zippers. *J. immunol..* 1992, vol.148, no.5, p.1547-33. .

[0112] According to a preferred embodiment, the antibody that specifically binds to more than one epitope according to the invention is a diabody.

[0113] According to another preferred embodiment, the antibody that specifically binds to more than one epitope according to the invention is a linear antibody as described in ZAPATA, et al. Engineering linear F(ab')2 fragments for efficient production in Escherichia coli and enhanced antiproliferative activity. *Protein engineering.* 1995, vol.8, no.10, p.1057-62. .

[0114] The antibody according to the invention may be glycosylated or non-glycosylated.

[0115] As used herein, the term "glycosylation" refers to the presence of carbohydrate units that are covalently attached to the antibody.

[0116] In another embodiment, the antibody according to the invention is conjugated to a radiosensitizer agent, a receptor and/or a cytotoxic agent

[0117] As used herein, the term "radiosensitizer" refers to a molecule that makes cells more sensitive to radiation therapy. Radiosensitizer includes, but are not limited to, metronidazole, misonidazole, desmethylmisonidazole, pimonidazole, etanidazole, nimorazole, mitomycin C, RSU 1069, SR 4233, E09, RB 6145, nicotinamide, 5-bromodeoxyuridine (BUdR), 5-iododeoxyuridine(IUdR), bromodeoxycytidine, fluorodeoxyuridine (FUdR), hydroxyurea and cisplatin.

[0118] As used herein, the term "receptor" refers to a compound able to specifically bind to a ligand. According to a preferred embodiment of the invention, the receptor is biotin.

[0119] As used herein, the term cytoxic agent refers to a compound that is directly toxic to cells, preventing their reproduction or growth. According to a preferred embodiment, the cytotoxic agent used in the context of the present invention is chosen from the group comprising cancer therapeutic agent, toxin (e. g., an enzymatically active toxin of bacterial, fungal, plant or animal origin, or fragments thereof), or a radioactive isotope.

[0120] In another embodiment, the antibody according to the invention is conjugated to a labelling agent

[0121] As used herein, "a labelling agent" refers to a detectable compound. The labelling agent may be detectable by itself (e. g., radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical modification of a substrate compound which is detectable.

[0122] As used herein, the term "conjugated" means that the antibody according to the invention and the labelling agent are covalently or non-covalently linked.

[0123] "Covalent link" refers to coupling through reactive functional groups, optionally with the intermediary use of a cross linker or other activating agent (see for example HERMANSON. Bioconjugate techniques. Academic press, 1996.). The antibody according to the invention and/or the conjugated agent may be modified in order to allow their coupling via, for example, substitution on an activated carbonyl group (including those

activated in situ) or on an imidoester, via addition on an unsaturated carbonyl group, by reductive amination, nucleophilic substitution on a saturated carbon atom or on a heteroatom, by reaction on aromatic cycles, In particular, coupling may be done using homobifunctional or heterobifunctional cross-linking reagents. Homobifunctional cross linkers including glutaraldehyde, succinic acid and bis-imidoester like DMS (dimethyl suberimidate) can be used to couple amine groups which may be present on the various moieties. Numerous examples are given in HERMANSON. Bioconjugate techniques. Academic press, 1996. p.118-228. which are well known by those of the art. Heterobifunctional cross linkers include those having both amine reactive and sulfhydryl-reactive groups, carbonyl-reactive and sulfhydryl-reactive groups and sulfhydryl-reactive groups and photoreactive linkers. Suitable heterobifunctional cross-linkers are, for example, described in HERMANSON. Bioconjugate techniques. Academic press, 1996. p.229-285. . Examples are, for example, SPDP (N-succinimidyl 3-(2-pyridyldithio) propionate), SMBP (succinimidyl-4-(p-maleimidophenyl) butyrate), SMPT (succinimidyloxycarbonyl--methyl-(-2-pyridyldithio) toluene), MBS (m-maleimidobenzoyl-N-hydroxysuccinimide ester), SIAB (N-succinimidyl (4 iodoacetyl) aminobenzoate), GMBS (γ-maleimidobutyryloxy) succinimide ester), SIAX (succinimidyl-6- iodoacetyl amino hexonate, SIAC (succinimidyl-4-iodoacetyl amino methyl), NPIA (p-nitrophenyl iodoacetate). Other examples are useful to couple carbohydrate-containing molecules (e.g. env glycoproteins, antibodies) to sulfydryl-reactive groups. Examples include MPBH (4-(4-N maleimidophenyl) butyric acid hydrazide) and PDPH (4-(N- maleimidomethyl) cyclohexane-1-carboxyl-hydrazide (M2C2H and 3-2(2-pyridyldithio) proprionyl hydrazide).

[0124] According to another embodiment, the present invention relates to a nucleic acid sequence coding the antibody of the invention.

[0125] The term "nucleic acid sequence" refers to a linear sequence of nucleotides. The nucleotides are either a linear sequence of polyribonucleotides or polydeoxyribonucleotides, or a mixture of both. Examples of polynucleotides in the context of the present invention include

single and double stranded DNA, single and double stranded RNA, and hybrid molecules that have both mixtures of single and double stranded DNA and RNA. Further, the polynucleotides of the present invention may have one or more modified nucleotides.

[0126] According to a preferred embodiment of the invention, the nucleic acid sequence according to the invention is comprised in a vector.

[0127] The vector can be of plasmid or viral origin and can, where appropriate, be combined with one or more substances which improve the transfectional efficiency and/or stability of the vector. These substances are widely documented in the literature which is available to the skilled person (see, for example, FELGNER, et al. Cationic liposome mediated transfection. *Proceedings of the Western Pharmacology Society.* 1989, vol.32, p.115-21. ; HODGSON, et al. Virosomes: cationic liposomes enhance retroviral transduction. *Nature biotechnology.* 1996, vol.14, no.3, p.339-42. ; REMY, et al. Gene transfer with a series of lipophilic DNA-binding molecules. *Bioconjugate chemistry.* 1994, vol.5, no.6, p.647-54.). By way of non-limiting illustration, the substances can be polymers, lipids, in particular cationic lipids, liposomes, nuclear proteins or neutral lipids. These substances can be used alone or in combination. A combination which can be envisaged is that of a recombinant plasmid vector which is combined with cationic lipids (DOGS, DC-CHOL, spermine-chol, spermidine-chol, etc.), lysophospholipides (for example Hexadecylphosphocholine) and neutral lipids (DOPE).

[0128] According to a preferred embodiment, the cationic lipids which can be used in the present invention are the cationic lipids describes in EP 901463 B and more preferably pcTG90.

[0129] The choice of the plasmids which can be used within the context of the present invention is huge. They can be cloning vectors and/or expression vectors. In a general manner, they are known to the skilled person and, while a number of them are available commercially, it is also possible to construct them or to modify them using the techniques of genetic manipulation. Examples which may be mentioned are the plasmids which are derived from pBR322 (Gibco BRL), pUC (Gibco BRL), pBluescript

(Stratagene), pREP4, pCEP4 (Invitrogene) or p Poly (LATHE, et al. Plasmid and bacteriophage vectors for excision of intact inserts. *Gene.* 1987, vol.57, no.2-3, p.193-201.). Preferably, a plasmid which is used in the context of the present invention contains an origin of replication which ensures that replication is initiated in a producer cell and/or a host cell (for example, the ColE1 origin will be chosen for a plasmid which is intended to be produced in E. coli and the oriP/EBNA1 system will be chosen if it desired that the plasmid should be self-replicating in a mammalian host cell, LUPTON, et al. Mapping genetic elements of Epstein-Barr virus that facilitate extrachromosomal persistence of Epstein-Barr virus-derived plasmids in human cells. *Molecular and cellular biology.* 1985, vol.5, no.10, p.2533-42. ; YATES, et al. Stable replication of plasmids derived from Epstein-Barr virus in various mammalian cells. *Nature.* 1985, vol.313, no.6005, p.812-5.). The plasmid can additionally comprise a selection gene which enables the transfected cells to be selected or identified (complementation of an auxotrophic mutation, gene encoding resistance to an antibiotic, etc.). Naturally, the plasmid can contain additional elements which improve its maintenance and/or its stability in a given cell (cer sequence, which promotes maintenance of a plasmid in monomeric form (SUMMERS, et al. Multimerization of high copy number plasmids causes instability: ColE1 encodes a determinant essential for plasmid monomerization and stability. *Cell.* 1984, vol.36, no.4, p.1097-103. , sequences for integration into the cell genome).

[0130] With regard to a viral vector, it is possible to envisage a vector which is derived from a poxvirus (vaccinia virus, in particular MVA, canarypoxvirus, etc.), from an adenovirus, from a retrovirus, from a herpesvirus, from an alphavirus, from a foamy virus or from an adenovirus-associated virus. It is possible to use replication competent or replication deficient viral vectors. Preference will be given to using a vector which does not integrate. In this respect, adenoviral vectors and vectors deriving from poxvirus and more preferably vaccinia virus and MVA are very particularly suitable for implementing the present invention.

[0131] According to a preferred embodiment, the viral vector according to the

invention derives from a Modified Vaccinia Virus Ankara (MVA). MVA vectors and methods to produce such vectors are fully described in European patents EP 83286 A and EP 206920 A , as well as in SUTTER, et al. Nonreplicating vaccinia vector efficiently expresses recombinant genes. *Proc. Natl. Acad. Sci. U.S.A.*. 1992, vol.89, no.22, p.10847-51. . According to a more preferred embodiment, the nucleic acid sequence according to the invention may be inserted in deletion I, II, III, IV, V and VI of the MVA vector and even more preferably in deletion III (MEYER, et al. Mapping of deletions in the genome of the highly attenuated vaccinia virus MVA and their influence on virulence. *The Journal of general virology.* 1991, vol.72, no.Pt5, p.1031-8. ; SUTTER, et al. A recombinant vector derived from the host range-restricted and highly attenuated MVA strain of vaccinia virus stimulates protective immunity in mice to influenza virus. *Vaccine.* 1994, vol.12, no.11, p.1032-40.).

[0132] Retroviruses have the property of infecting, and in most cases integrating into, dividing cells and in this regard are particularly appropriate for use in relation to cancer. A recombinant retrovirus according to the invention generally contains the LTR sequences, an encapsidation region and the nucleotide sequence according to the invention, which is placed under the control of the retroviral LTR or of an internal promoter such as those described below. The recombinant retrovirus can be derived from a retrovirus of any origin (murine, primate, feline, human, etc.) and in particular from the MoMuLV (Moloney murine leukemia virus), MVS (Murine sarcoma virus) or Friend murine retrovirus (Fb29). It is propagated in an encapsidation cell line which is able to supply in trans the viral polypeptides gag, pol and/or env which are required for constituting a viral particle. Such cell lines are described in the literature (PA317, Psi CRIP GP + Am-12 etc.). The retroviral vector according to the invention can contain modifications, in particular in the LTRs (replacement of the promoter region with a eukaryotic promoter) or the encapsidation region (replacement with a heterologous encapsidation region, for example the VL3O type) (see US 5747323)

[0133] Preference will be also given to using an adenoviral vector which lacks all

or part of at least one region which is essential for replication and which is selected from the E1, E2, E4 and L1-L5 regions in order to avoid the vector being propagated within the host organism or the environment. A deletion of the E1 region is preferred. However, it can be combined with (an)other modification(s)-/deletion(s) affecting, in particular, all or part of the E2, E4 and/or L1-L5 regions, to the extent that the defective essential functions are complemented in trans by means of a complementing cell line and/or a helper virus. In this respect, it is possible to use second-generation vectors of the state of the art (see, for example, international applications WO 94/28152 and WO 97/04119). By way of illustration, deletion of the major part of the E1 region and of the E4 transcription unit is very particularly advantageous. For the purpose of increasing the cloning capacities, the adenoviral vector can additionally lack all or part of the non-essential E3 region. According to another alternative, it is possible to make use of a minimal adenoviral vector which retains the sequences which are essential for encapsidation, namely the 5' and 3' ITRs (Inverted Terminal Repeat), and the encapsidation region. The various adenoviral vectors, and the techniques for preparing them, are known (see, for example, GRAHAM, et al. Methods in molecular biology. Edited by MURREY. The human press inc, 1991. p.109-128.).

[0134] Furthermore, the origin of the adenoviral vector according to the invention can vary both from the point of view of the species and from the point of view of the serotype. The vector can be derived from the genome of an adenovirus of human or animal (canine, avian, bovine, murine, ovine, porcine, simian, etc.) origin or from a hybrid which comprises adenoviral genome fragments of at least two different origins. More particular mention may be made of the CAV-I or CAV-2 adenoviruses of canine origin, of the DAV adenovirus of avian origin or of the Bad type 3 adenovirus of bovine origin (ZAKHARCHUK, et al. Physical mapping and homology studies of egg drop syndrome (EDS-76) adenovirus DNA. *Archives of virology* . 1993, vol.128, no.1-2, p.171-6. ; SPIBEY, et al. Molecular cloning and restriction endonuclease mapping of two strains of canine adenovirus type 2. *The Journal of general virology* . 1989, vol.70, no.Pt 1, p.165-72. ;

JOUVENNE, et al. Cloning, physical mapping and cross-hybridization of the canine adenovirus types 1 and 2 genomes. *Gene.* 1987, vol.60, no.1, p.21-8. ; MITTAL, et al. Development of a bovine adenovirus type 3-based expression vector. *The Journal of general virology.* 1995, vol.76, no.Pt 1 , p.93-102.). However, preference will be given to an adenoviral vector of human origin which is preferably derived from a serotype C- adenovirus, in particular a type 2 or 5 serotype C adenovirus.

[0135] The term "replication-competent" as used herein refers to a viral vector capable of replicating in a host cell in the absence of any trans-complementation.

[0136] According to a preferred embodiment of the invention, the replication competent vector is a replication competent adenoviral vector. These replication competent adenoviral vectors are well known by the one skilled in the art. Among these, adenoviral vectors deleted in the E1b region coding the 55kD P53 inhibitor, as in the ONYX-015 virus (BISCHOFF, et al. An adenovirus mutant that replicates selectively in p53-deficient human tumor cells. *Science.* 1996, vol.274, no.5286, p.373-6. ; He HEISE, et al. An adenovirus E1A mutant that demonstrates potent and selective systemic anti-tumoral efficacy. *Nature Medicine.* 2000, vol.6, no.10, p.1134-9. ; WO 94/18992), are particularly preferred. Accordingly, this virus can be used to selectively infect and kill p53-deficient neoplastic cells. A person of ordinary skill in the art can also mutate and disrupt the p53 inhibitor gene in adenovirus 5 or other viruses according to established techniques. Adenoviral vectors deleted in the E1A Rb binding region can also be used in the present invention. For example, Delta24 virus which is a mutant adenovirus carrying a 24 base pair deletion in the E1A region (FUEYO, et al. A mutant oncolytic adenovirus targeting the Rb pathway produces anti-glioma effect in vivo. *Oncogene.* 2000, vol.19, no.1, p.2-12.). Delta24 has a deletion in the Rb binding region and does not bind to Rb. Therefore, replication of the mutant virus is inhibited by Rb in a normal cell. However, if Rb is inactivated and the cell becomes neoplastic, Delta24 is no longer inhibited. Instead, the mutant virus replicates efficiently and lyses the Rb-deficient cell.

[0137] An adenoviral vector according to the present invention can be generated in vitro in Escherichia coli (E. coli) by ligation or homologous recombination (see, for example, international application WO 96/17070) or else by recombination in a complementing cell line.

[0138] According to a preferred embodiment of the invention, the vector further comprises the elements necessary for the expression of the antibody according to the invention.

[0139] The elements necessary for the expression consist of all the elements which enable the nucleic acid sequence to be transcribed into RNA and the mRNA to be translated into polypeptide. These elements comprise, in particular, a promoter which may be regulable or constitutive. Naturally, the promoter is suited to the chosen vector and the host cell. Examples which may be mentioned are the eukaryotic promoters of the PGK (phosphoglycerate kinase), MT (metallothionein; MCIVOR. Human purine nucleoside phosphorylase and adenosine deaminase: gene transfer into cultured cells and murine hematopoietic stem cells by using recombinant amphotropic retroviruses. *Molecular and cellular biology.* 1987, vol.7, no.2, p.838-46.), α-1 antitrypsin, CFTR, surfactant, immunoglobulin, actin (TABIN, et al. Adaptation of a retrovirus as a eucaryotic vector transmitting the herpes simplex virus thymidine kinase gene. *Molecular and cellular biology.* 1982, vol.2, no.4, p.426-36.) and SRα (TAKEBE, et al. SR alpha promoter: an efficient and versatile mammalian cDNA expression system composed of the simian virus 40 early promoter and the R-U5 segment of human T-cell leukemia virus type 1 long terminal repeat. *Molecular and cellular biology.* 1988, vol.8, no.1, p.466-72.) genes, the early promoter of the SV40 virus (Simian virus), the LTR of RSV (Rous sarcoma virus), the HSV-I TK promoter, the early promoter of the CMV virus (Cytomegalovirus), the p7.5K pH5R, pK1L, p28 and p11 promoters of the vaccinia virus, chimeric promoters such as p11K7.5 and the E1A and MLP adenoviral promoters. The promoter can also be a promoter which stimulates expression in a tumor or cancer cell. Particular mention may be made of the promoters of the MUC-I gene, which is overexpressed in breast and prostate cancers (CHEN, et al. Breast cancer selective gene

expression and therapy mediated by recombinant adenoviruses containing the DF3/MUC1 promoter. *The Journal of clinical investigation.* 1995, vol.96, no.6, p.2775-82.), of the CEA (standing for carcinoma embryonic antigen) gene, which is overexpressed in colon cancers (SCHREWE, et al. Cloning of the complete gene for carcinoembryonic antigen: analysis of its promoter indicates a region conveying cell type-specific expression. *Molecular and cellular biology.* 1990, vol.10, no.6, p.2738-48.) of the tyrosinase gene, which is overexpressed in melanomas (VILE, et al. Use of tissue-specific expression of the herpes simplex virus thymidine kinase gene to inhibit growth of established murine melanomas following direct intratumoral injection of DNA. *Cancer res..* 1993, vol.53, no.17, p.3860-4.), of the ERBB-2 gene, which is overexpressed in breast and pancreatic cancers (HARRIS, et al. Gene therapy for cancer using tumour-specific prodrug activation. *Gene therapy.* 1994, vol.1, no.3, p.170-5.) and of the α-fetoprotein gene, which is overexpressed in liver cancers (KANAI, et al. In vivo gene therapy for alpha-fetoprotein-producing hepatocellular carcinoma by adenovirus-mediated transfer of cytosine deaminase gene. *Cancer res..* 1997, vol.57, no.3, p.461-5.). The cytomegalovirus (CMV) early promoter is very particularly preferred.

[0140] However, when a vector deriving from a Vaccinia Virus (as for example an MVA vector) is used, the promoter of the thymidine kinase 7.5K gene and the pH5R promoter are particularly preferred.

[0141] The necessary elements can furthermore include additional elements which improve the expression of the nucleic acid sequence according to the invention or its maintenance in the host cell. Intron sequences, secretion signal sequences, nuclear localization sequences, internal sites for the reinitiation of translation of IRES type, transcription termination poly A sequences, tripartite leaders and origins of replication may in particular be mentioned. These elements are known to the skilled person. Among secretion signal sequence, sequences encoding the polypeptides as set forth in SEQ ID NO 5 and/or 8 are particularly preferred.

[0142] The recombinant vector according to the invention can also comprise one or more additional genes of interest, with it being possible for these genes

to be placed under the control of the same regulatory elements (polycistronic cassette) or of independent elements. Genes which may in particular be mentioned are the genes encoding interleukins IL-2, IL-4, IL-7, IL-10, IL-12, IL-15, IL-18, chemokines as CCL19, CCL20, CCL21, CXCL-14, interferons, tumor necrosis factor (TNF), and factors acting on innate immunity and angiogenesis (for example PAI-1, standing for plasminogen activator inhibitor). In one particular embodiment, the recombinant vector according to the invention comprises the gene of interest encoding IL-2.

[0143] The present invention also relates to a cell comprising the nucleic acid sequence according to the invention. In a preferred embodiment, he cell according to the invention is eukaryotic cell and more preferably a mammalian cell. Mammalian cells available as hosts for expression are well known in the art and include many immortalized cell lines, such as but not limited to, Chinese Hamster Ovary (CHO) cells, Baby Hamster Kidney (BHK) cells and many others. Suitable additional eukaryotic cells include yeast and other fungi.

[0144] The present invention also relates to a process for producing an antibody according to the invention comprising culturing the cell according to the invention under conditions permitting expression of the antibody and purifying the antibody from the cell or medium surrounding the cell.

[0145] In another embodiment, the present invention relates to a pharmaceutical composition comprising any one of the antibody, the nucleic acid sequence or the vector according to the invention and a pharmaceutically acceptable carrier. In a preferred embodiment, the pharmaceutical composition further comprises a compound of interest.

[0146] The pharmaceutically acceptable carrier is preferably isotonic, hypotonic or weakly hypertonic and has a relatively low ionic strength, such as for example a sucrose solution. Moreover, such a carrier may contain any solvent, or aqueous or partially aqueous liquid such as nonpyrogenic sterile water. The pH of the pharmaceutical composition is, in addition, adjusted and buffered so as to meet the requirements of use in vivo. The pharmaceutical composition may also include a pharmaceutically

acceptable diluent, adjuvant or excipient, as well as solubilizing, stabilizing and preserving agents. For injectable administration, a formulation in aqueous, nonaqueous or isotonic solution is preferred. It may be provided in a single dose or in a multidose in liquid or dry (powder, lyophilisate and the like) form which can be reconstituted at the time of use with an appropriate diluent.

[0147] The present invention also relates, to a kit of part comprising (i) a pharmaceutical composition, an antibody, a nucleic acid sequence or a vector according to the invention and, (ii) a compound of interest.

[0148] As used herein the term, "compound of interest" relates to a therapeutic compound and preferably to a cancer therapeutic agent or a compound useful in the treatment of bone mass decrease.

[0149] According to a preferred embodiment, the cancer therapeutic agent is chosen from the group comprising Abraxane (Paclitaxel Albumin-stabilized Nanoparticle Formulation), Adriamycin (Doxorubicin Hydrochloride), Adrucil (Fluorouracil), Aldara (Imiquimod), Alemtuzumab, Alimta (Pemetrexed Disodium), Aminolevulinic Acid, Anastrozole, Aprepitant, Arimidex (Anastrozole), Aromasin (Exemestane), Arranon (Nelarabine), Arsenic Trioxide, Avastin (Bevacizumab), Azacitidine, Bevacizumab, Bexarotene, Bortezomib, Campath (Alemtuzumab), Camptosar (Irinotecan Hydrochloride), Capecitabine, Carboplatin, Cetuximab, Cisplatin, Clafen (Cyclophosphamide), Clofarabine, Clofarex (Clofarabine), Clolar (Clofarabine), Cyclophosphamide, Cytarabine, Cytosar-U (Cytarabine), Cytoxan (Cyclophosphamide), Dacogen (Decitabine), Dasatinib, Decitabine, DepoCyt (Liposomal Cytarabine), DepoFoam (Liposomal Cytarabine), Dexrazoxane Hydrochloride, Docetaxel, Doxil (Doxorubicin Hydrochloride Liposome), Doxorubicin Hydrochloride, Doxorubicin Hydrochloride Liposome, Dox-SL (Doxorubicin Hydrochloride Liposome), Efudex (Fluorouracil), Ellence (Epirubicin Hydrochloride), Eloxatin (Oxaliplatin), Emend (Aprepitant), Epirubicin Hydrochloride, Erbitux (Cetuximab), Erlotinib Hydrochloride, Evacet (Doxorubicin Hydrochloride Liposome), Evista (Raloxifene Hydrochloride), Exemestane, Faslodex (Fulvestrant), Femara (Letrozole), Fluoroplex (Fluorouracil), Fluorouracil,

Fulvestrant, Gefitinib, Gemcitabine Hydrochloride, Gemtuzumab Ozogamicin, Gemzar (Gemcitabine Hydrochloride), Gleevec (Imatinib Mesylate), Herceptin (Trastuzumab), Hycamtin (Topotecan Hydrochloride), Imatinib Mesylate, Imiquimod, Iressa (Gefitinib), Irinotecan Hydrochloride, Ixabepilone, Ixempra (Ixabepilone), Keoxifene (Raloxifene Hydrochloride), Kepivance (Palifermin), Lapatinib Ditosylate, Lenalidomide, Letrozole, Levulan (Aminolevulinic Acid), LipoDox (Doxorubicin Hydrochloride Liposome), Liposomal Cytarabine, Methazolastone (Temozolomide), Methotrexate, Mylosar (Azacitidine), Mylotarg (Gemtuzumab Ozogamicin), Nanoparticle Paclitaxel (Paclitaxel Albumin-stabilized Nanoparticle Formulation), Nelarabine, Neosar (Cyclophosphamide), Nexavar (Sorafenib Tosylate), Nilotinib, Nolvadex (Tamoxifen Citrate), Oncaspar (Pegaspargase), Oxaliplatin, Paclitaxel, Paclitaxel Albumin-stabilized Nanoparticle Formulation, Palifermin, Panitumumab, Paraplat (Carboplatin), Paraplatin (Carboplatin), Pegaspargase, Pemetrexed Disodium, Platinol-AQ (Cisplatin), Platinol (Cisplatin), Raloxifene Hydrochloride, Revlimid (Lenalidomide), Rituxan (Rituximab), Rituximab, Sclerosol Intrapleural Aerosol (Talc), Sorafenib Tosylate, Sprycel (Dasatinib), Sterile Talc Powder (Talc), Steritalc (Talc), Sunitinib Malate, Sutent (Sunitinib Malate), Synovir (Thalidomide), Talc, Tamoxifen Citrate, Tarabine PFS (Cytarabine), Tarceva (Erlotinib Hydrochloride), Targretin (Bexarotene), Tasigna (Nilotinib), Taxol (Paclitaxel), Taxotere (Docetaxel), Temodar (Temozolomide), Temozolomide, Temsirolimus, Thalomid (Thalidomide), Thalidomide, Totect (Dexrazoxane Hydrochloride), Topotecan Hydrochloride, Torisel (Temsirolimus), Trastuzumab, Trisenox (Arsenic Trioxide), Tykerb (Lapatinib Ditosylate), Vectibix (Panitumumab), Velcade (Bortezomib), Vidaza (Azacitidine), Vorinostat, Xeloda (Capecitabine), Zinecard (Dexrazoxane Hydrochloride), Zoledronic Acid, Zolinza (Vorinostat) and Zometa (Zoledronic Acid).

[0150] According to a preferred embodiment of the invention the compound useful in the treatment of bone mass decrease is a biphosphonate, a selective oestrogen receptor modulators (SERMs), a parathyroid hormone (PTH) (e.g. teriparatide (Forteo)), strontium ranelate, Denosumab or

calcitonin, or a combination thereof. According to a more preferred embodiment, the biphosphonate is chosen from the group comprising Alendronate (Fosamax, Fosamax Plus D), Etidronate (Didronel), Ibandronate (Boniva), Pamidronate (Aredia), Risedronate (Actonel, Actonel W/Calcium), Tiludronate (Skelid), and Zoledronic acid (Reclast, Zometa). According to a more preferred embodiment, the SERMs is chosen from the group comprising raloxifene (Evista), bazedoxifene/premarin (Aprelal) and tamoxifen.

[0151] According to another embodiment, the present invention relates to the use of the antibody, the nucleic acid sequence, the vector, the pharmaceutical composition or the kit of parts according to the invention for the treatment of diseases associated to an increased osteoclast activity. Such disease comprised but are not limited to endocrinopathies (hypercortisolism, hypogonadism, primary or secondary hyperparathyroidism, hyperthyroidism), hypercalcemia, deficiency states (rickets/osteomalacia, scurvy, malnutrition), chronic diseases (malabsorption syndromes, chronic renal failure (renal osteodystrophy), chronic liver disease (hepatic osteodystrophy), drugs (glucocorticoids (glucocorticoid-induced osteoporosis), androgen deprivation therapy, aromatase inhibitor therapy, heparin, alcohol), and hereditary diseases (osteogenesis imperfecta, homocystinuria), osteoporosis, osteopetrosis, inflammation of bone associated with arthritis and rheumatoid arthritis, periodontal disease, fibrous dysplasia, and/or Paget's disease.

[0152] According to another embodiment, the present invention relates to the use of the antibody, the nucleic acid sequence, the vector, the pharmaceutical composition or the kit of parts according to the invention for the treatment of diseases associated to inflammation and/or autoimmunity. Such diseases comprise but are not limited to seronegative spondyloarthropathy (psoriatic arthritis, ankylosing spondylitis, reiters syndrome, spondyloarthropathy associated with inflammatory bowel disease), prosthetic joint loosening, connective tissue diseases (juvenile rheumatoid arthritis, rheumatoid arthritis, systemic lupus erythematosus (SLE) and lupus nephritis, scleroderma, Sjogren's syndrome, mixed connective tissue

disease, polymyositis, dermatomyositis), inflammatory bowel disease (e.g.Crohn's disease; ulcerative colitis), whipples disease, arthritis associated with granulomatous ileocolitis, inflammatory skin conditions (autoimmune bullous pemphigoid, autoimmune pemphigus vulgaris, eczema, dermatitis), inflammatory lung disease (alveolitis, pulmonary fibrosis, sarcoidoisis, asthma, bronchitis, bronchiolitis obliterans), inflammatory renal disease (glomerulonethritis, renal allograft rejection, renal tubular inflammation), atherosclerosis, systemic vasculitis (temporal arteritis/giant cell arteritis, takayasu arteritis, polyarteritis nodosa, Kawasaki disease, Wegener's granulomatosis, churg strauss syndrome, microscopic polyangiitis, necrotising glomerulonephritis, henoch schonlein purpura, essential cryoglobulinaemic vasculitis and other small vessel vasculitis, Behcets disease), macrophage activation diseases (macrophage activation syndrome (MAS), adult onset stills disease, haemophagocytic syndrome), polymyalgia rheumatica, primary biliary sclerosis, sclerosing cholangitis, autoimmune hepatitis, Type 1 Diabetes Mellitus, Hashimoto's thyroiditis, Graves' disease, multiple sclerosis (MS), Guillain-Barre syndrome, Addison's disease, and/or Raynaud's phenomenon, Goodpasture's syndrome.

[0153] According to another embodiment, the present invention relates to the use of the antibody, the nucleic acid sequence, the vector, the pharmaceutical composition or the kit of parts according to the invention for the treatment of cancer.

[0154] As used herein, the term “cancer” refers but is not limited to adenocarcinoma, acinic cell adenocarcinoma, adrenal cortical carcinomas, alveoli cell carcinoma, anaplastic carcinoma, basaloid carcinoma, basal cell carcinoma, bronchiolar carcinoma, bronchogenic carcinoma, renaladinol carcinoma, embryonal carcinoma, anometroid carcinoma, fibrolamolar liver cell carcinoma, follicular carcinomas, giant cell carcinomas, hepatocellular carcinoma, intraepidermal carcinoma, intraepithelial carcinoma, leptomanigio carcinoma, medullary carcinoma, melanotic carcinoma, menigual carcinoma, mesometonephric carcinoma, oat cell carcinoma, squamal cell carcinoma, sweat gland carcinoma,

transitional cell carcinoma, tubular cell carcinoma, amelioblastic sarcoma, angiolithic sarcoma, botryoid sarcoma, endometrial stroma sarcoma, ewing sarcoma, fascicular sarcoma, giant cell sarcoma, granulositic sarcoma, immunoblastic sarcoma, juxaccordial osteogenic sarcoma, coppices sarcoma, leukocytic sarcoma (leukemia), lymphatic sarcoma (lympho sarcoma), medullary sarcoma, myeloid sarcoma (granulocitic sarcoma), austiogenci sarcoma, periosteal sarcoma, reticulum cell sarcoma (histiocytic lymphoma), round cell sarcoma, spindle cell sarcoma, synovial sarcoma, telangiectatic audiogenic sarcoma, Burkitt's lymphoma, NPDL, NML, NH and diffuse lymphomas. According to a preferred embodiment, the method according to the invention is directed to the treatment of metastatic cancer to bone, wherein the metastatic cancer is breast, lung, renal, multiple myeloma, thyroid, prostate, adenocarcinoma, blood cell malignancies, including leukemia and lymphoma; head and neck cancers; gastrointestinal cancers, including esophageal cancer, stomach cancer, colon cancer, intestinal cancer, colorectal cancer, rectal cancer, pancreatic cancer, liver cancer, cancer of the bile duct or gall bladder; malignancies of the female genital tract, including ovarian carcinoma, uterine endometrial cancers, vaginal cancer, and cervical cancer; bladder cancer; brain cancer, including neuroblastoma; sarcoma, osteosarcoma; and skin cancer, including malignant melanoma or squamous cell cancer.

[0155] The present invention further concerns a method for improving the treatment of a cancer patient which is undergoing chemotherapeutic treatment with a cancer therapeutic agent, which comprises co-treatment of said patient along with a method as above disclosed.

[0156] The present invention further concerns a method of improving cytotoxic effectiveness of cytotoxic drugs or radiotherapy which comprises co-treating a patient in need of such treatment along with a method as above disclosed.

[0157] The present invention further concerns a method for improving the treatment of a patient with a disease associated to an increased osteoclast activity which is undergoing treatment with a biphosphonate, a selective

oestrogen receptor modulators (SERMs), a parathyroid hormone (PTH) (e.g. teriparatide (Forteo)), strontium ranelate, Denosumab or calcitonin, or a combination thereof, which comprises co-treatment of said patient along with a method as above disclosed.

[0158] In another embodiment use of an antibody of the invention is contemplated in the manufacture of a medicament for preventing or treating metastatic cancer to bone in a patient suffering from metastatic cancer. In a related embodiment, the metastatic cancer is breast, lung, renal, multiple myeloma, thyroid, prostate, adenocarcinoma, blood cell malignancies, including leukemia or lymphoma; head or neck cancers; gastrointestinal cancers, including esophageal cancer, stomach cancer, colon cancer, intestinal cancer, colorectal cancer, rectal cancer, pancreatic cancer, liver cancer, cancer of the bile duct or gall bladder; malignancies of the female genital tract, including ovarian carcinoma, uterine endometrial cancers, vaginal cancer, or cervical cancer; bladder cancer; brain cancer, including neuroblastoma; sarcoma, osteosarcoma; or skin cancer, including malignant melanoma or squamous cell cancer.

[0159] According to another embodiment, the present invention relates to the use of the antibody, the nucleic acid sequence, the vector, the pharmaceutical composition or the kit of parts according to the invention in the manufacture of a medicament.

[0160] According to another embodiment, the present invention relates to the use of the antibody, the nucleic acid sequence, the vector, the pharmaceutical composition or the kit of parts according to the invention in the manufacture of a medicament for treating a patient having cancer.

[0161] According to another embodiment, the present invention relates to the use of the antibody, the nucleic acid sequence, the vector, the pharmaceutical composition or the kit of parts according to the invention in the manufacture of a medicament for treating a patient having a disease associated to an increased osteoclast activity.

[0162] According to another embodiment, the present invention relates to the use of the antibody, the nucleic acid sequence, the vector, the pharmaceutical composition or the kit of parts according to the invention in the

manufacture of a medicament for treating a patient having an inflammatory disease, more specifically an inflammatory bowel disease.

[0163] According to another embodiment, the present invention relates to the use of the antibody, the nucleic acid sequence, the vector, the pharmaceutical composition or the kit of parts according to the invention in the manufacture of a medicament for treating a patient suffering from rheumatoid arthritis.

[0164] Administering the antibody, the nucleic acid sequence, the vector, the pharmaceutical composition or the kit of parts according to the invention may be accomplished by any means known to the skilled artisan. Preferred routes of administration include but are not limited to intradermal, subcutaneous, oral, parenteral, intramuscular, intranasal, sublingual, intratracheal, inhalation, ocular, vaginal, and rectal. According to a preferred embodiment the antibody, the nucleic acid sequence, the vector, the pharmaceutical composition or the kit of parts according to the invention are delivered systemically.

[0165] The administration may take place in a single dose or a dose repeated one or several times after a certain time interval. Desirably, the antibody, the nucleic acid sequence, the vector, the pharmaceutical composition or the kit of parts according to the invention are administered 1 to 10 times at weekly intervals.

[0166] For general guidance, suitable dosage for the antibody is about 2mg/kg to 30mg/kg, 0.1 mg/kg to 30 mg/kg or 0.1 mg/kg to 10 mg/kg body weight. Suitable dosage for the vector according to the invention varies from about 10^4 to 10^{10} pfu (plaque forming units), desirably from about 10^5 and 10^8 pfu for MVA vector whereas it varies from about 10^5 to 10^{13} iu (infectious units), desirably from about 10^7 and 10^{12} iu for adenovirus based vector. A composition based on vector plasmids may be administered in doses of between 10 µg and 20 mg, advantageously between 100 µg and 2 mg.

[0167] When the use or the method according to the invention is for the treatment of cancer, the method or use of the invention can be carried out in conjunction with one or more conventional therapeutic modalities (e.g. radiation, chemotherapy and/or surgery). The use of multiple therapeutic

approaches provides the patient with a broader based intervention. In one embodiment, the method of the invention can be preceded or followed by a surgical intervention. In another embodiment, it can be preceded or followed by radiotherapy (e.g. gamma radiation). Those skilled in the art can readily formulate appropriate radiation therapy protocols and parameters which can be used (see for example PEREZ. Principles and practice of radiation oncology. 2nd edition. LIPPINCOTT, 1992.)

Brief Description of Figures in the Drawings

[0168] Figure 1 depicts the specific staining of CSF-1R-transfected NIH/3T3 cells by mAb CXIIG6.

[0169] Figure 2 shows the inhibition of CSF-1 binding to cell-surface CSF-1R in presence of mAb CXIIG6.

[0170] Figure 3 shows the specific blockade of soluble human CSF-1R by mAb CXIIG6 ('ctrl' means control; 'neg SN' means negative control hybridoma supernatant).

[0171] Figure 4 shows the inhibition of human osteoclast differentiation and secretion of matrix-metalloprotease-9 (MMP-9) in presence of mAb CXIIG6 ('ctrl' means control; 'CXIIG6 SN' means CXIIG6 hydridoma supernatant; 'neg SN' means negative control hybridoma supernatant).

[0172] Figure 5 shows the non-cross-reactivity of mAb CXIIG6 with other tyrosine kinase receptors having homology to CSF-1R ('SN' means hydridoma supernatant).

[0173] Figure 6 shows the nucleic acid sequence (SEQ ID NO: 1) and deduced amino acid sequence (SEQ ID NO: 2) of the CXIIG6 heavy chain. The primer sequences including restriction sites for cloning added to the nucleotide sequences are underlined. The restriction sites are shown in underlined italic type. The amino acid sequences of the V-domains are highlighted in bold type.

[0174] Figure 7 shows the nucleic acid sequence (SEQ ID NO: 3) and deduced amino acid sequence (SEQ ID NO: 4) of the CXIIG6 light chain. The primer sequences including restriction sites for cloning added to the nucleotide sequences are underlined. The restriction sites are shown in underlined italic type. The amino acid sequences of the V-domains are

highlighted in bold type.

[0175] Figure 8 shows the plasmid construct pTG17753.

[0176] Figure 9 shows the plasmid construct pTG17727.

[0177] Figure 10 shows the plasmid construct pOptiVEC™.

[0178] Figure 11 shows the plasmid construct pTG17895.

[0179] Figure 12 shows the plasmid construct pTG17812.

[0180] Figure 13 shows the plasmid construct pTG17868.

[0181] Figure 14 shows the plasmid construct pTG17869.

[0182] Figure 15 shows humanized CXIIG6 light chain variants.

[0183] Figure 16 shows humanized CXIIG6 IgG1 heavy chain variants.

[0184] Figure 17 shows the specific blockade of soluble human CSF-1R by recombinant murine CXIIG6 and chimeric CXIIG6 IgG1.

[0185] Figure 18 shows the inhibition of human osteoclast differentiation and secretion of matrix-metalloprotease-9 (MMP-9) in presence of recombinant murine CXIIG6 and chimeric CXIIG6 IgG1.

Mode(s) for Carrying Out the Invention

Specific staining of CSF-1R-transfected NIH/3T3 cells by mAb CXIIG6

[0186] The B4-800-5 cell line was generated by stable transfection of NIH/3T3 cells with an expression plasmid encoding the full-length human CSF-1R. Cell-surface CSF-1R expression on B4-800-5 cells was verified by indirect immunostaining with the anti-human CSF-1R mAbs 61701 (mouse IgG_1, R&D Systems) or 2-4A5-4 (rat $IgG_{1,k}$, GeneTex), compared to isotype controls (Figure 1, upper and middle panels). Culture supernatants from hybridoma CXIIG6 or from a negative control hybridoma were used for immunostaining B4-800-5 cells or parental NIH/3T3 cells (Figure 1, lower panels).

[0187] Flow cytometry analysis showed that culture supernatant from hybridoma CXIIG6 selectively stained B4-800-5 cells, demonstrating the mAb specificity for cell-surface CSF-1R.

Inhibition of CSF-1 binding to cell-surface CSF-1R

[0188] 3 x 10^5 THP-1 cells (human CSF-1R-positive monocytic leukemia cell line) were incubated for 30 min at 4°C in the presence of either hybridoma culture supernatants, serum from a naive or an anti-CSF-1R-immunized mouse (dilution 1:1000), mAb anti-CSF-1R 2-4A5-4 (GeneTex) or a control

rat IgG_1 (10 µg/ml), or no reagent. After two washes with cold PBS, cells were incubated with 1 µg/ml biotinylated recombinant human CSF-1 for 30 min. Cells were washed twice and further incubated for 30 min at 4°C with 10 µg/ml streptavidin-Alexa Fluor 488 (Invitrogen). After washing with PBS and fixation with 4% paraformaldehyde, cell staining was analyzed by flow cytometry.

[0189] Decreased fluorescence intensities compared to control samples reflect the inhibition of CSF-1 binding to cell-surface CSF-1R. Serum from a CSF-1R-immunized mouse blocks CSF-1 binding to THP-1 cells (Figure 2). While negative control hybridoma supernatant or an irrelevant mAb show no effect, culture supernatant from hybridoma CXIIG6 inhibits CSF-1 binding to THP-1 cells (Figure 2, lower right panel), as does mAb 2-4A5-4 (lower left panel).

Localization of mAb CXIIG6 binding site.

[0190] To identify the binding site of mAb CXIIG6 on the CSF-1R, a Western blot was performed using soluble forms of the human CSF-1R comprising either the five extracellular immunoglobulin-like domains (Met 1 to Glu 512, R&D Systems) or only the three N-terminal immunoglobulin-like domains of the extracellular region of CSF-1R (Met 1 to Ser 290), both fused at their C-terminal ends to the Fc region of a human IgG_1. A soluble form of the EGFR fused to human IgG_1 Fc (R&D Systems) was used as a negative control.

[0191] Hundred nanograms of each soluble receptor were submitted to electrophoresis in native conditions before transfer to nitrocellulose sheet and probing with either hybridoma supernatants, rabbit pAb c-fms/CSF-1R H300 (Santa Cruz Biotechnology), mouse mAb 61701 (R&D Systems) or serum from naive or CSF-1R-immunized mice.

[0192] Both soluble forms of the CSF-1R were detected as broad bands when probed with pAb c-fms/CSF-1R H300, mAb 61701 or serum from the immunized mouse. No detectable signals were observed with naive mouse serum or a negative control hybridoma supernatant. CXIIG6 hybridoma supernatant recognized $CSF\text{-}1R_{1\text{-}290}$:Fc as well as $CSF\text{-}1R_{1\text{-}512}$:Fc, but not EGFR:Fc, indicating that CXIIG6 binds specifically to an epitope lying

within the three N-terminal immunoglobulin-like domains (between residues 1 to 290) of the human CSF-1R.

Specific blockade of soluble human CSF-1R by mAb CXIIG6

[0193] The CSF-1-dependent murine myeloid leukemia M-NFS-60 cell line (# CRL-1838, ATCC) was used to assess the blocking activity of CXIIG6 hybridoma supernatant on human and murine CSF-1R. Five nanograms of soluble human CSF-1R ($CSF\text{-}1R_{1\text{-}512}$:Fc from R&D Systems) were preincubated in white 96-well microplates with serial dilutions of either hybridoma supernatants, mAb 61701 (R&D Systems) or murine isotype control mAb. 10E4 M-NFS-60 cells cultured overnight in the absence of CSF-1 were then added into the culture wells together with 0.1 ng of human CSF-1 in a final assay volume of 100 µl. Cultures were incubated for 48 h at 37°C and proliferation was quantified by BrdU incorporation using a Cell Proliferation ELISA (Roche).

[0194] Soluble human CSF-1R completely inhibited the proliferation of M-NFS-60 cells mediated by human CSF-1, as shown in the presence of negative hybridoma supernatant containing or not a negative control IgG_1 (Figure 3; mean +/- SEM of 3 wells). In contrast, CXIIG6 hybridoma supernatant and positive control mAb 61701 were both able to restore cell proliferation in a dose-dependent manner, showing that they were able to neutralize soluble human CSF-1R.

[0195] In this assay, active M-NFS-60 cell proliferation in the presence of low dilutions of CXIIG6 hybridoma supernatant showed that mAb CXIIG6 was unable to block the murine CSF-1R expressed by M-NFS-60 cells. Moreover, in a murine CSF-1-supported M-NFS-60 proliferation assay performed in the absence of soluble CSF-1R, treatment with mAb AFS98 anti-mouse CSF-1R (eBioscience) resulted in a dramatic concentration-dependent decrease of cell growth (data not shown). CXIIG6 hybridoma supernatant, like negative control antibodies and negative hybridoma supernatant, caused no reduction in cell proliferation. These results demonstrate that mAb CXIIG6 specifically targets human CSF-1R.

Inhibition of human osteoclast differentiation and secretion of matrix-metalloprotease-9 (MMP-9)

[0196] Osteoclasts were generated from human monocytes obtained by

elutriation of PBMCs from a healthy blood donor. In brief, monocytes were seeded at 2 x 10E4 cells per well in 96-well plates and treated for 45 min with either hybridoma culture supernatants, mAbs anti-human CSF-1R 61701 (R&D Systems) or 2-4A5-4 (GeneTex), mAb anti-human CSF-1 26730 (R&D Systems), murine or rat isotype controls, or sera from naive and CSF-1R-immunized mice diluted in hybridoma culture medium. Complete a-MEM medium was added to the culture wells with or without human CSF-1 and RANKL (PeproTech, 25 and 40 ng/ml respectively). Hybridoma supernatants, mAbs or/and medium with or without cytokines were replenished every 3 days for 9 days. Conditioned culture supernatants were harvested on day 9 and assayed for total human MMP-9 using an ELISA assay (R&D Systems). Osteoclast formation was evaluated by staining of tartrate-resistant acid phosphatase (TRAP) using the leukocyte acid phosphatase kit from Sigma-Aldrich.

[0197] CSF-1 + RANKL induced monocytes to differentiate into osteoclasts, defined as large multinucleated TRAP-positive cells, whereas no TRAP-positive osteoclasts were obtained in the absence of cytokines. Addition of 0.5 µg/ml anti-CSF-1 mAb 26730 completely abrogated osteoclast differentiation, as shown by lack of MMP-9 secretion. Anti-CSF-1R mAbs 61701 or 2-4A5-4 at the same concentration and immunized mouse serum (dilution 1:1000) inhibited osteoclast formation only partially (Figure 4; with (+) or without (-) cytokines; mean +/- SEM of 3 wells; *: mean of 2 wells). Treatment with CXIIG6 hybridoma culture supernatant diluted 1:20 or 1:100 significantly reduced the level of MMP-9 production, compared with two negative control hybridoma supernatants (A, B). These results demonstrate that mAb CXIIG6 inhibits the differentiation of osteoclasts from human monocytes by blocking the function of cell surface CSF-1R.

Inhibition of the CSF-1-dependent phosphorylation of CSF-1R

[0198] The B4-800-5 cell line obtained by stable transfection of NIH/3T3 cells with a plasmid expressing human CSF-1R was used to investigate the effect of CXIIG6 hybridoma supernatant on CSF-1-dependent CSF-1R phosphorylation. Cells were seeded at 2 x 10E5 cells per 60-mm Petri dish and cultured for 48 to 72 h. Following serum deprivation for 1 h at 37°C,

cells were treated for 1 h at 37°C with culture medium containing either CXIIG6 hybridoma supernatant, mAb 2-4A5-4 (NeoMarkers) or isotype control mAbs (diluted in negative hybridoma supernatant), and then stimulated with 100 ng/ml hCSF-1 or left unstimulated for 5 min at 37°C. Cell layers were then lysed and total proteins were extracted. Ten µg proteins were analyzed by probing Western blots with either the rabbit pAb c-fms/CSF-1R H300 or the rabbit pAb p-c-fms/CSF-1R (Tyr708)-R (Santa Cruz Biotechnology), followed by goat anti-rabbit immunoglobulins^{HRP}.

[0199] In the absence of CSF-1, neither CXIIG6 hybridoma supernatant nor mAb 2-4A5-4 induced receptor phosphorylation as seen with the antibody specific for CSF-1R phosphorylated at position 708, showing that mAb CXIIG6 alone does not exert an agonistic effect. Upon stimulation with CSF-1, the amount of CSF-1R decreased in isotype control-treated cells compared with unstimulated cells, and CSF-1R was phosphorylated on Tyr708 (data not shown). Pretreatment with CXIIG6 hybridoma supernatant or with mAb 2-4A5-4 did not enhance CSF-1R disappearance. Phosphorylation of CSF-1R was decreased following treatment with CXIIG6 hybridoma supernatant or with mAb 2-4A5-4. These results show that mAb CXIIG6 is able to block the CSF-1-dependent phosporylation of CSF-1R.

Cross-reactivity of mAb CXIIG6

[0200] The cross-reactivity of mAb CXIIG6 was tested by ELISA on a series of purified soluble receptors belonging to the type III subfamily of tyrosine kinase receptors and showing homology to CSF-1R in their extracellular Ig-like domains : soluble VEGFR-1, VEGFR-2, Flt-3 and PDGFRβ (all four expressed as Fc fusion proteins), as well as PDGFRα and SCFR (c-kit) were obtained from R&D Systems and used for coating an ELISA plate. Soluble EGFR (R&D Systems), from the EGFR subfamily of tyrosine kinase receptors, was used as a negative control.

[0201] Culture supernatants from either hybridoma CXIIG6 (CXIIG6 SN) or a negative control hybridoma, or the anti-CSF-1R mouse IgG_1 61701 (R&D Systems) were incubated on the coated ELISA plate at antibody concentrations of 500 ng/ml. After washing the ELISA plate, bound

antibodies were revealed using peroxidase-conjugated goat anti-mouse Ig (Sigma) and OD $_{(450 - 540\ nm)}$ was measured. Results depicted in Figure 5 show that like mAb 61701, CXIIG6 strongly bound the CSF-1R while no specific signal was detected on any other tyrosine kinase receptor. This shows that among the various type III tyrosine kinase receptors tested, CXIIG6 is specific for CSF-1R.

Construction of expression vectors for mAb CXIIG6

[0202] The OptiCHO™ Antibody Express Kit (Invitrogen, Catalog No.12762-019) was used for the cloning of the genes encoding the CXIIG6 heavy and light chains in order to produce the mAb CXIIG6 in DG44 mammalian cell line. The OptiCHO™ Antibody Express Kit includes: (1) The pOptiVEC™ vector, a bicistronic plasmid that allows the cloning of the gene of interest downstream of CMV promoter. The transcription of the gene of interest is separated from the dihydrofolate reductase (DHFR) auxotrophic selection marker by an internal ribosome entry site (IRES), allowing transcription of the gene of interest and of the selection marker on the same mRNA; (2) The pcDNA™3.3 vector that allows the cloning of the gene of interest downstream of CMV promoter. The pcDNA™3.3 contains a neomycin resistance gene allowing selection using Geneticin®. The pOptiVEC™ and pcDNA™3.3 vectors contain the TK poly-A sequence which directs proper processing of the 3' end of the mRNA of the gene of interest.

[0203] Specific primers (see Table 4) were synthesized and used for the PCR amplification and cloning of the entire CXIIG6 heavy chain and light chain genes (respectively SEQ ID NO:1 and SEQ ID NO:3; see respectively Figure 6 and Figure 7). The backward primers included the Kozak consensus sequence for efficient eukaryotic translation (KOZAK M. An analysis of 5'-noncoding sequences from 699 vertebrate messenger RNAs. *Nucleic Acids Res.* 1987,15(20): 8125–8148.).

Table 4

Primer	Sequence
OTG18929	GCCGCCACCATGTACTTGGGACTGAACTATGTATTC (SEQ ID NO :30)

OTG18930	GGAGATCTTCATTTACCCGGAGTCCGGGA (SEQ ID NO :31)
OTG18931	GCCGCCACCATGAGTGTGCCCACTCAGGTCCTG (SEQ ID NO :32)
OTG18932	GCCCGGGCTAACACTCATTCCTGTTGAAGCTC (SEQ ID NO :33)

[0204] CXIIG6 heavy chain was PCR amplified by using OTG18929 and OTG18930 with plasmid pTG17753 (Figure 8) as template, and cloned into the vector pOptiVEC™-TOPO® (pOptiVEC™-TOPO® TA Cloning® Kit, Invitrogen, Catalog no.12744-017-01) and the pcDNA™3.3-TOPO® vectors (pcDNA™3.3-TOPO® TA Cloning® Kit, Invitrogen, Catalog No.K8300-01) to obtain respectively pTG17786 and pTG17789.

[0205] CXIIG6 light chain was PCR amplified by using OTG18931 and OTG18932 with plasmid pTG17727 (Figure 9) as template, and cloned into the vector pOptiVEC™-TOPO® (pOptiVEC™-TOPO® TA Cloning® Kit, Invitrogen, Catalog no.12744-017-01) and the pcDNA™3.3-TOPO® vectors (pcDNA™3.3-TOPO® TA Cloning® Kit, Invitrogen, Catalog No.K8300-01) to obtain respectively pTG17788 and pTG17787.

[0206] The nucleotide sequence of the whole expression cassette, including CMV promoter and TK polyA signal of pTG17786, pTG17787, pTG17788 and pTG17789 were sequenced and found in compliance with their theoretical sequences.

Generation of chimeric antibodies from mAb CXIIG6

[0207] The variable domains of mAb CXIIG6 were combined with human constant regions.

[0208] To generate the chimeric light chain (named Chimeric CXIIG6 Igκ chain), a theorical sequence was designed by joining the sequence encoding the CXIIG6 VK Domain (from SEQ ID NO:3) to the sequence encoding for the human IGKC region (GenBank accession number: J00241). This XbaI NotI DNA fragment kept the same non translated sequence at 5' end, including Kozak sequence, as the one used in the murine version (as in pTG17787 and pTG17788 described above). The chimeric CXIIG6 light chain

sequence was codon optimized for expression in CHO, assembled from synthetic oligonucleotides, and subcloned into pOptiVEC™ (Figure 10) via XbaI NotI by GeneArt AG. The obtained chimeric CXIIG6 light chain (variable and constant regions) codon optimized nucleic acid sequence is as set forth in SEQ ID NO:34. The obtained plasmid was named pTG17895 (Figure 11).

[0209] To generate the chimeric heavy IgG1 and IgG4 chains (respectively named Chimeric CXIIG6 IgG1 and Chimeric CXIIG6 IgG4 chains), the theorical sequences were designed by joining the sequence encoding the CXIIG6 VH Domain (from SEQ ID NO:1) to the sequences encoding either for the human IGHG1C region (GenBank accession number: J00228) or for the human IGHG4C region (GenBank accession number: K01316). These *XbaI NotI* DNA fragments kept the same non translated sequence at 5' end, including Kozak sequence, as the one used in the murine version (as in pTG17786 and pTG17789 as described above). The chimeric CXIIG6 heavy chains were then codon optimized for expression in CHO, synthesized and cloned into pTG17812 (Figure 12) via *XbaI NotI* by GeneArt AG. The obtained chimeric CXIIG6 IgG1 heavy chain (variable and constant regions) codon optimized nucleic acid sequence is as set forth in SEQ ID NO:35 and the obtained plasmids was named pTG17868 (Figure 13). The obtained chimeric CXIIG6 IgG4 heavy chain (variable and constant regions) codon optimized nucleic acid sequence is as set forth in SEQ ID NO:36 and the obtained plasmids was named pTG17869 (Figure 14).

Generation of human antibodies from mAb CXIIG6

[0210] To generate the humanized light chain variants, amino acid substitutions according to Table 2 were performed within the light-chain variable region as set forth in SEQ ID NO:9.

[0211] DNA sequences were designed by joining modified sequences bearing substitutions of the CXIIG6 VK Domain (from SEQ ID NO:3) to the sequence encoding for the human IGKC region (GenBank accession number: J00241). This *XbaI NotI* DNA fragment kept the same non translated sequence at 5' end including Kozak sequence as the one used

in the murine version (as in pTG17787 and pTG17788 as described above). The humanized CXIIG6 light chain sequences were then codon optimized for expression in CHO, assembled from synthetic oligonucleotides and cloned into pOptiVEC™ (Figure 10) via *XbaI NotI* by GeneArt AG. The obtained humanized CXIIG6 light chain variants and plasmids are listed in Figure 15.

[0212] To generate the humanized heavy chain variants, amino acid substitutions according to Table 1 were performed within the heavy-chain variable region as set forth in SEQ ID NO:6.

[0213] DNA sequences were designed by joining modified sequences bearing substitutions of the CXIIG6 VH Domain (from SEQ ID NO:1) to the sequences encoding for the human IGHG1C region (GenBank accession number: J00228). The *XbaI ApaI* DNA fragments kept the same non translated sequence at 5' end including Kozak sequence as used the one in the murine version (as in pTG17786 and pTG17789 as described above). The DNA sequences were then codon optimized for expression in CHO, synthesized and cloned into pTG17812 (Figure 12) via *XbaI ApaI* by GeneArt AG. The obtained humanized CXIIG6 IgG1 heavy chain variants and plasmids are listed in Figure 16.

In vitro inhibitory activities of recombinant murine CXIIG6 and chimeric CXIIG6 IgG1

[0214] To determine whether purified recombinant murine CXIIG6 (as previously described) and its chimeric IgG1 variant (chimeric CXIIG6 IgG1 as previously described) were able to block soluble human CSF-1R, dose-response studies were performed in the M-NFS-60 cell proliferation and osteoclast differentiation models (as previously described). Purified polyclonal murine IgG2a from Rockland (Rockland, 010-0141) and a chimeric IgG1 produced by the applicant were tested in parallel as control antibodies. Blocking effect was evaluated by exposing cells to concentration ranges of active anti-CSF-1R antibodies, as measured by antigen binding in a SPR biosensor assay. Comparison between mAbs CXIIG6 and their respective control mAb was done by loading equal amounts of total antibody (SPR biosensor assay by Fc binding).

[0215] M-NFS-60 bioassay: In the M-NFS-60 bioassay, cells were treated with

0.23 ng/ml to 0.5 µg/ml active mAbs CXIIG6 (recombinant murine CXIIG6; chimeric CXIIG6 IgG1) or corresponding concentrations of control mAbs in the presence of 50 ng/ml human soluble CSF-1R and 1 ng/ml human CSF-1 for 48 h. Results depicted in Figure 17 show that M-NFS-60 cell growth increased in response to increasing concentrations of both mAbs CXIIG6 (recombinant murine CXIIG6; chimeric CXIIG6 IgG1) demonstrating that they antagonized the binding of soluble CSF-1R to CSF-1 (mean +/- SEM of triplicate wells). Chimeric CXIIG6 IgG1 was as effective as recombinant murine CXIIG6 in restoring cell proliferation. Control murine IgG2a and chimeric IgG1 had no effect on CSF-1 neutralization by soluble CSF-1R over their respective concentration range.

[0216] Osteoclast bioassay: In the osteoclast bioassay, elutriated human monocytes were incubated for 8 days with 0.85 ng/ml to 0.62 µg/ml active mAbs CXIIG6 (recombinant murine CXIIG6; chimeric CXIIG6 IgG1) in the presence of 25 ng/ml CSF-1 (ImmunoTools) and 40 ng/ml RANKL. The medium and all added agents were replenished on day 4 and 6 and total MMP-9 was measured in culture media conditoned from day 6 to 8. Results depicted in Figure 18 show that in comparison with control antibodies, recombinant murine CXIIG6 and its chimeric variant (chimeric CXIIG6 IgG1) each significantly reduced MMP-9 production which parallels osteoclast differentiation, indicating that growth retardation occurred (Figure 18; mean +/- SEM of triplicate wells).

[0217] Taken together, these results further demonstrate that purified recombinant murine CXIIG6 and chimeric CXIIG6 IgG1 inhibit cell-surface as well as soluble human CSF-1R.

References

- WO 01/30381
- WO 03/059395
- WO 2005/068503
- EP 1488792 A
- US 2005059113
- EP 901463 B

- EP 83286 A
- EP 206920 A
- US 5747323
- WO 94/28152
- WO 97/04119
- WO 94/18992
- WO 96/17070
- SHERR. Colony-stimulating factor-1 receptor. *blood.* 1990, vol.75, no.1, p.1-12.
- HUME, et al. Regulation of CSF-1 receptor expression. *Molecular reproduction and development.* 1997, vol.46, no.1, p.46-52.
- KLUGER, et al. Macrophage colony-stimulating factor-1 receptor expression is associated with poor outcome in breast cancer by large cohort tissue microarray analysis. *Clinical cancer research* . 2004, vol.10, no.1, p.173-7.
- SCHOLL, et al. Anti-colony-stimulating factor-1 antibody staining in primary breast adenocarcinomas correlates with marked inflammatory cell infiltrates and prognosis. *Journal of the National Cancer Institute.* 1994, vol.86, no.2, p.120-6.
- CHAMBERS, et al. Overexpression of epithelial macrophage colony-stimulating factor (CSF-1) and CSF-1 receptor: a poor prognostic factor in epithelial ovarian cancer, contrasted with a protective effect of stromal CSF-1. *Clinical Cancer Research.* 1997, vol.3, no.6, p.999-1007.
- BAÏOCCHI, et al. Expression of the macrophage colony-stimulating factor and its receptor in gynecologic malignancies. *Cancer.* 1991, vol.67, no.4, p.990-6.
- KIRMA, et al. Elevated expression of the oncogene c-fms and its ligand, the macrophage colony-stimulating factor-1, in cervical cancer and the role of transforming growth factor-beta1 in inducing c-fms expression. *Cancer res..* 2007, vol.67, no.5, p.1918-26.
- HEMMERLEIN, et al. Expression of acute and late-stage inflammatory antigens, c-fms, CSF-1, and human monocytic serine esterase 1, in

tumor-associated macrophages of renal cell carcinomas. *Cancer immunology, immunotherapy*. 2000, vol.49, no.9, p.485-92.

- IDE, et al. Expression of colony-stimulating factor 1 receptor during prostate development and prostate cancer progression. *Proc. Natl. Acad. Sci. U.S.A.*. 2002, vol.99, no.22, p.14404-9.
- RAMBALDI, et al. Expression of the macrophage colony-stimulating factor and c-fms genes in human acute myeloblastic leukemia cells. *Journal of Clinical Investigation.* 1988, vol.81, no.4, p.1030-5.
- SCHOLL, et al. Anti-colony-stimulating factor-1 antibody staining in primary breast adenocarcinomas correlates with marked inflammatory cell infiltrates and prognosis. *Journal of the National Cancer Institute.* 1994, vol.86, no.2, p.120-6.
- TANG, et al. M-CSF (monocyte colony stimulating factor) and M-CSF receptor expression by breast tumour cells: M-CSF mediated recruitment of tumour infiltrating monocytes?. *Journal of cellular biochemistry.* 1992, vol.50, no.4, p.350-6.
- SCHOLL, et al. Circulating levels of colony-stimulating factor 1 as a prognostic indicator in 82 patients with epithelial ovarian cancer. *British journal of cancer.* 1994, vol.69, no.2, p.342-6.
- SCHOLL. Circulating levels of the macrophage colony stimulating factor CSF-1 in primary and metastatic breast cancer patients. A pilot study. *Breast cancer research and treatment.* 1996, vol.39, no.3, p.275-83.
- DORSCH, et al. Macrophage colony-stimulating factor gene transfer into tumor cells induces macrophage infiltration but not tumor suppression. *European journal of immunology.* 1993, vol.23, no.1, p.186-90.
- WANG, et al. Induction of monocyte migration by recombinant macrophage colony-stimulating factor. *Journal of immunology* . 1988, vol.141, no.2, p.575-9.
- FILDERMAN, et al. Macrophage colony-stimulating factor (CSF-1) enhances invasiveness in CSF-1 receptor-positive carcinoma cell lines. *Cancer res.*. 1992, vol.52, no.13, p.3661-6.

- DORSCH, et al. Macrophage colony-stimulating factor gene transfer into tumor cells induces macrophage infiltration but not tumor suppression. *European journal of immunology.* 1993, vol.23, no.1, p.186-90.
- SCHOLL. Circulating levels of the macrophage colony stimulating factor CSF-1 in primary and metastatic breast cancer patients. A pilot study. *Breast cancer research and treatment.* 1996, vol.39, no.3, p.275-83.
- BARON, et al. Modulation of MHC class II transport and lysosome distribution by macrophage-colony stimulating factor in human dendritic cells derived from monocytes. *Journal of cell science.* 2001, vol.114, no.pt5, p.999-1010.
- CECCHINI, et al. Role of CSF-1 in bone and bone marrow development. *Molecular reproduction and development.* 1997, vol.46, no.1, p.75-83.
- BRUZZANITI, et al. Molecular regulation of osteoclast activity. *Reviews in endocrine* . 2006, vol.7, no.1-2, p.123-39.
- CICEK, et al. Breast cancer bone metastasis and current small therapeutics. *Cancer metastasis reviews.* 2006, vol.25, no.4, p.635-44.
- KITAURA, et al. The journal of clinical investigation. *M-CSF mediates TNF-induced inflammatory osteolysis.* 2005, vol.115, no.12, p.3418-27.
- MARSHALL, et al. Blockade of colony stimulating factor-1 (CSF-I) leads to inhibition of DSS-induced colitis. *Inflammatory bowel diseases.* 2007, vol.13, no.2, p.219-24.
- JOSE, et al. Blockade of macrophage colony-stimulating factor reduces macrophage proliferation and accumulation in renal allograft rejection. *American journal of transplantation* . 2003, vol.3, no.3, p.294-300.
- KUTZA, et al. Macrophage colony-stimulating factor antagonists inhibit replication of HIV-1 in human macrophages. *Journal of immunology* . 2000, no.164, p.4955-4960.
- SMITH, et al. Identification of common molecular subsequences. *Journal of Molecular Biology.* 1981, no.147, p.195-7.

- HARLOW. Antibodies: A Laboratory manual. 2nd edition. Cold Spring Harbor: Laboratory press, 1988.
- HAMMERLING, et al. Monoclonal Antibodies and T Cell Hybridomas. New York: Elsevier, 1981. p.563-681.
- SMITH, et al. Identification of common molecular subsequences. *Journal of Molecular Biology.* 1981, no.147, p.195-7.
- LENNARD. Standard protocols for the construction of scFv libraries. *Methods in molecular biology* . 2002, no.178, p.59-71.
- POLJAK. Production and structure of diabodies. *Structure.* 1994, vol.2, no.12, p.1121-3.
- HUDSON, et al. High avidity scFv multimers; diabodies and triabodies. *Journal of immunological methods.* 1999, vol.231, no.1-2, p.177-89.
- KIPRIYANOV. Generation of bispecific and tandem diabodies. *Methods in molecular biology* . 2002, no.178, p.317-31.
- HOLLIGER, et al. Engineered antibody fragments and the rise of single domains. *Nature biotechnology.* 2005, vol.23, no.9, p.1126-36.
- HOOGENBOOM, et al. Natural and designer binding sites made by phage display technology. *Immunology today.* 2000, vol.21, no.8, p.371-8.
- MARKS, et al. By-passing immunization: building high affinity human antibodies by chain shuffling. *Biotechnology* . 1992, vol.10, no.7, p.779-83.
- BARBAS, et al. In vitro evolution of a neutralizing human antibody to human immunodeficiency virus type 1 to enhance affinity and broaden strain cross-reactivity. *Proceedings of the National Academy of Sciences of the United States of America.* 1994, vol.91, no.9, p.3809-13.
- SCHIER. Identification of functional and structural amino-acid residues by parsimonious mutagenesis. *Gene.* 1996, vol.169, no.2, p.147-55.
- YELTON. Affinity maturation of the BR96 anti-carcinoma antibody by codon-based mutagenesis. *J. immunol..* 1995, vol.155, no.4, p.1994-2004.

- JACKSON, et al. In vitro antibody maturation. Improvement of a high affinity, neutralizing antibody against IL-1 beta. *J. immunol..* 1995, vol.154, no.7, p.3310-9.
- HAWKINS, et al. Selection of phage antibodies by binding affinity. Mimicking affinity maturation. *Journal of molecular biology.* 1992, vol.226, no.3, p.889-96.
- SMITH, et al. Identification of common molecular subsequences. *Journal of Molecular Biology.* 1981, no.147, p.195-7.
- BRENNAN, et al. Preparation of bispecific antibodies by chemical recombination of monoclonal immunoglobulin G1 fragments. *Science.* 1985, vol.229, no.4708, p.81-3.
- SHALABY, et al. Development of humanized bispecific antibodies reactive with cytotoxic lymphocytes and tumor cells overexpressing the HER2 protooncogene. *The Journal of experimental medicine.* 1992, vol.175, no.1, p.217-25.
- KOSTELNY, et al. Formation of a bispecific antibody by the use of leucine zippers. *J. immunol..* 1992, vol.148, no.5, p.1547-33.
- ZAPATA, et al. Engineering linear F(ab')2 fragments for efficient production in Escherichia coli and enhanced antiproliferative activity. *Protein engineering.* 1995, vol.8, no.10, p.1057-62.
- HERMANSON. Bioconjugate techniques. Academic press, 1996.
- HERMANSON. Bioconjugate techniques. Academic press, 1996. p.118-228.
- HERMANSON. Bioconjugate techniques. Academic press, 1996. p.229-285.
- FELGNER, et al. Cationic liposome mediated transfection. *Proceedings of the Western Pharmacology Society.* 1989, vol.32, p.115-21.
- HODGSON, et al. Virosomes: cationic liposomes enhance retroviral transduction. *Nature biotechnology.* 1996, vol.14, no.3, p.339-42.
- REMY, et al. Gene transfer with a series of lipophilic DNA-binding molecules. *Bioconjugate chemistry.* 1994, vol.5, no.6, p.647-54.

- LATHE, et al. Plasmid and bacteriophage vectors for excision of intact inserts. *Gene.* 1987, vol.57, no.2-3, p.193-201.
- LUPTON, et al. Mapping genetic elements of Epstein-Barr virus that facilitate extrachromosomal persistence of Epstein-Barr virus-derived plasmids in human cells. *Molecular and cellular biology.* 1985, vol.5, no.10, p.2533-42.
- YATES, et al. Stable replication of plasmids derived from Epstein-Barr virus in various mammalian cells. *Nature.* 1985, vol.313, no.6005, p.812-5.
- SUMMERS, et al. Multimerization of high copy number plasmids causes instability: ColE1 encodes a determinant essential for plasmid monomerization and stability. *Cell.* 1984, vol.36, no.4, p.1097-103.
- SUTTER, et al. Nonreplicating vaccinia vector efficiently expresses recombinant genes. *Proc. Natl. Acad. Sci. U.S.A..* 1992, vol.89, no.22, p.10847-51.
- MEYER, et al. Mapping of deletions in the genome of the highly attenuated vaccinia virus MVA and their influence on virulence. *The Journal of general virology.* 1991, vol.72, no.Pt5, p.1031-8.
- SUTTER, et al. A recombinant vector derived from the host range-restricted and highly attenuated MVA strain of vaccinia virus stimulates protective immunity in mice to influenza virus. *Vaccine.* 1994, vol.12, no.11, p.1032-40.
- GRAHAM, et al. Methods in molecular biology. Edited by MURREY. The human press inc, 1991. p.109-128.
- ZAKHARCHUK, et al. Physical mapping and homology studies of egg drop syndrome (EDS-76) adenovirus DNA. *Archives of virology .* 1993, vol.128, no.1-2, p.171-6.
- SPIBEY, et al. Molecular cloning and restriction endonuclease mapping of two strains of canine adenovirus type 2. *The Journal of general virology .* 1989, vol.70, no.Pt 1, p.165-72.
- JOUVENNE, et al. Cloning, physical mapping and cross-hybridization of the canine adenovirus types 1 and 2 genomes. *Gene.* 1987, vol.60, no.1, p.21-8.

- MITTAL, et al. Development of a bovine adenovirus type 3-based expression vector. *The Journal of general virology.* 1995, vol.76, no.Pt 1 , p.93-102.
- BISCHOFF, et al. An adenovirus mutant that replicates selectively in p53-deficient human tumor cells. *Science.* 1996, vol.274, no.5286, p.373-6.
- HEISE, et al. An adenovirus E1A mutant that demonstrates potent and selective systemic anti-tumoral efficacy. *Nature Medicine.* 2000, vol.6, no.10, p.1134-9.
- FUEYO, et al. A mutant oncolytic adenovirus targeting the Rb pathway produces anti-glioma effect in vivo. *Oncogene.* 2000, vol.19, no.1, p.2-12.
- MCIVOR. Human purine nucleoside phosphorylase and adenosine deaminase: gene transfer into cultured cells and murine hematopoietic stem cells by using recombinant amphotropic retroviruses. *Molecular and cellular biology.* 1987, vol.7, no.2, p.838-46.
- TABIN, et al. Adaptation of a retrovirus as a eucaryotic vector transmitting the herpes simplex virus thymidine kinase gene. *Molecular and cellular biology.* 1982, vol.2, no.4, p.426-36.
- TAKEBE, et al. SR alpha promoter: an efficient and versatile mammalian cDNA expression system composed of the simian virus 40 early promoter and the R-U5 segment of human T-cell leukemia virus type 1 long terminal repeat. *Molecular and cellular biology.* 1988, vol.8, no.1, p.466-72.
- CHEN, et al. Breast cancer selective gene expression and therapy mediated by recombinant adenoviruses containing the DF3/MUC1 promoter. *The Journal of clinical investigation.* 1995, vol.96, no.6, p.2775-82.
- SCHREWE, et al. Cloning of the complete gene for carcinoembryonic antigen: analysis of its promoter indicates a region conveying cell type-specific expression. *Molecular and cellular biology.* 1990, vol.10, no.6, p.2738-48.

- VILE, et al. Use of tissue-specific expression of the herpes simplex virus thymidine kinase gene to inhibit growth of established murine melanomas following direct intratumoral injection of DNA. *Cancer res..* 1993, vol.53, no.17, p.3860-4.
- HARRIS, et al. Gene therapy for cancer using tumour-specific prodrug activation. *Gene therapy.* 1994, vol.1, no.3, p.170-5.
- KANAI, et al. In vivo gene therapy for alpha-fetoprotein-producing hepatocellular carcinoma by adenovirus-mediated transfer of cytosine deaminase gene. *Cancer res..* 1997, vol.57, no.3, p.461-5.
- PEREZ. Principles and practice of radiation oncology. 2nd edition. LIPPINCOTT, 1992.
- KOZAK M. An analysis of 5'-noncoding sequences from 699 vertebrate messenger RNAs. *Nucleic Acids Res.* 1987,15(20): 8125–8148.
- CHOTHIA and LESK. Canonical structures for the hypervariable regions of immunoglobulins (1987) J Mol Biol. 1987 Aug 20;196(4):901-17.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS;

1. A recombinant or purified antibody that specifically binds to (as herein defined) CSF-1R comprising the following CDRs:

(a) a CDR comprising at least eight amino acids of the sequence starting in position 45 and finishing in position 54 of SEQ ID NO:2,

(b) a CDR comprising at least eight amino acids of the sequence starting in position 66 and finishing in position 87 of SEQ ID NO:2,

(c) a CDR comprising at least eight amino acids of the sequence starting in position 117 and finishing in position 126 of SEQ ID NO:2,

(d) a CDR comprising at least eight amino acids of the sequence starting in position 44 and finishing in position 56 of SEQ ID NO:4,

(e) a CDR comprising at least eight amino acids of the sequence starting in position 66 and finishing in position 76 of SEQ ID NO:4; and

(f) a CDR comprising at least eight amino acids of the sequence starting in position 109 and finishing in position 117 of SEQ ID NO:4.

2. The antibody of claim 1 which comprises at least one CDR selected from the group consisting of the following CDRs:

(a) the CDR set forth in the sequence starting in position 45 and finishing in position 54 of SEQ ID NO:2,

(b) the CDR set forth the sequence starting in position 66 and finishing in position 87 of SEQ ID NO:2;

(c) the CDR set forth in the sequence starting in position 117 and finishing in position 126 of SEQ ID NO:2;

(d) the CDR set forth in the sequence starting in position 44 and finishing in position 56 of SEQ ID NO:4,

(e) the CDR set forth in the sequence starting in position 66 and finishing in position 76 of SEQ ID NO:4; and

(f) the CDR set forth in the sequence starting in position 109 and finishing in

position 117 of SEQ ID NO:4.

3. The antibody of claim 1 comprising at least one CDR comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 11, 12, or 13; or at least one CDR comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 14, 15 or 22.

4. The antibody of claim 3 which comprises at least 2, 3, 4, 5 and even more preferably 6 CDRs comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 11, 12, 13, 14, 15 or 22.

5. The antibody of claim 1 comprising at least one CDR comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 17, 18 or 19; or at least one CDR comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 20, 21 or 22.

6. The antibody of claim 5 comprising at least 2, 3, 4, or 5 CDRs comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 17, 18, 19, 20, 21 or 22.

7. The antibody of claim 5 comprising 6 CDRs comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 17, 18, 19, 20, 21 or 22.

8. The antibody of claim 1 comprising at least one CDR comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 23, 24 or 19; or at least one CDR comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 20, 21 or 22.

9. The antibody of claim 8 comprising at least 2, 3, 4, or 5 CDRs comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 23, 24, 19, 20, 21 or 22.

10. The antibody of claim 8 comprising 6 CDRs comprising an amino acid sequence as set forth in any one of SEQ ID NOs: 23, 24, 19, 20, 21 or 22.

11. The antibody of claim 1 comprising a variable region, wherein the variable

region comprises the CDR set forth in the sequence starting in position 45 and finishing in position 54 of SEQ ID NO:2, the CDR set forth in the sequence starting in position 66 and finishing in position 87 of SEQ ID NO:2 and the CDR set forth in the sequence starting in position 117 and finishing in position 126 of SEQ ID NO:2.

12. The antibody of claim 1 comprising a variable region, wherein the variable region comprises the CDR set forth in the sequence starting in position 44 and finishing in position 56 of SEQ ID NO:4, the CDR set forth in the sequence starting in position 66 and finishing in position 76 of SEQ ID NO:4 and the CDR set forth in the sequence starting in position 109 and finishing in position 117 of SEQ ID NO:4.

13. The antibody of claim 3 or 4 comprising a variable region, wherein the variable region comprises the three CDRs set forth in SEQ ID NOs: 11, 12, and 13.

14. The antibody of claim 3 or 4 comprising a variable region, wherein the variable region comprises the three CDRs set forth in SEQ ID NOs: 14, 15, and 22.

15. The antibody of claim 5 or 6 comprising a variable region, wherein the variable region comprises the three CDRs set forth in SEQ ID NOs: 17, 18, and 19.

16. The antibody of claim 5 or 6 comprising a variable region, wherein the variable region comprises the three CDRs set forth in SEQ ID NOs: 20, 21, and 22.

17. The antibody of any one of claims 7 to 10 comprising a variable region, wherein the variable region comprises the three CDRs set forth in SEQ ID NOs: 23, 24, and 19.

18. The antibody of any one of claims 7 to 10 comprising a variable region, wherein the variable region comprises the three CDRs set forth in SEQ ID NOs: 20, 21, and 22.

19. The antibody of any one of claims 11 to 18, wherein the variable region comprises one, two, three or four human FRs (as herein defined).

20. The antibody of any one of claims 9 to 18 wherein the variable region comprises four human FRs (as herein defined).

21. The antibody of any one of claims 11 to 20, wherein the variable region comprises an amino acid sequence as set forth in SEQ ID NO:6.

22. The antibody of claim 21, wherein the variable region is as set forth in SEQ ID NO:6.

23. The antibody of any one of claims 11 to 20, wherein the variable region comprises an amino acid sequence as set forth in SEQ ID NO:9.

24. The antibody of claim 23, wherein the variable region is as set forth in SEQ ID NO:9.

25. The antibody of claim 13 or 14 comprising a variable region comprising the three CDRs set forth in SEQ ID NOs: 11, 12, and 13, and a variable region comprising the three CDRs set forth in SEQ ID NOs: 14, 15, and 22.

26. The antibody of claim 15 or 16 comprising a variable region comprising the three CDRs set forth in SEQ ID NOs: 17, 18, and 19, and a variable region comprising the three CDRs set forth in SEQ ID NOs: 20, 21, and 22.

27. The antibody of claim 17 or 18 comprising a variable region comprising the three CDRs set forth in SEQ ID NOs: 23, 24, and 19, and a variable region comprising the three CDRs set forth in SEQ ID NOs: 20, 21, and 22.

28. The antibody of any one of claims 21 to 24 comprising a variable region as set forth in SEQ ID NO:6 and a variable region as set forth in SEQ ID NO:9.

29. The antibody of any one of claims 1 to 28 comprising two heavy chains set forth in SEQ ID NO:2 and two light chain set forth in SEQ ID NO:4.

30. A pharmaceutical composition comprising the antibody of any one of claims 1 to 29 and a pharmaceutically acceptable carrier.

31. A kit of part comprising (i) a pharmaceutical composition of claim 30 or an

antibody of any one of claims 1 to 29 and, (ii) a therapeutic compound.

32. Use of an antibody of any one of claims 1 to 29, a pharmaceutical composition of claim 30 or a kit of parts of claim 31 for the treatment of diseases associated with an increased osteoclast activity.

33. Use of an antibody of any one of claims 1 to 29, a pharmaceutical composition of claim 30 or a kit of parts of claim 31 for the treatment of cancer.

34. Use of an antibody of any one of claims 1 to 29, a pharmaceutical composition of claim 30 or a kit of parts of claim 31 for preventing or treating metastatic cancer to bone in a patient suffering from metastatic cancer.

35. Use of an antibody of any one of claims 1 to 29, a pharmaceutical composition according to claim 30 or a kit of parts of claim 31 in the manufacture of a medicament for treating a patient having an inflammatory bowel disease.

36. Use of an antibody of any one of claims 1 to 29, a pharmaceutical composition of claim 30 or a kit of parts of claim 31 in the manufacture of a medicament for treating a patient suffering from rheumatoid arthritis.

37. An antibody according to claim 1, substantially as hereinbefore described.