antibodies only. WO 2005/046657 relates to the treatment of inflammatory bowel disease by anti-CSF-1-antibodies. US 2002/0141994 relates to inhibitors of colony stimulating factors. WO 2006/096489 relates to the treatment of rheumatoid arthritis by anti-CSF-1-antibodies. WO 2009/026303 and WO 2009/112245 relate to certain anti-CSF-1R antibodies binding to CSF-1R within the first three subdomains (D1 to D3) of the Extracellular Domain (CSF-1R-ECD). WO2011/123381(A1) relates to antibodies against CSF-1R.

Summary ~~of the Invention~~

In a first aspect, the disclosure ~~The invention~~ comprises an antibody binding to human CSF-1R, characterized in binding to the (dimerization) domains D4 to D5 (SEQ ID No: 85) of the extracellular domain of human CSF-1R for use in

a) the inhibition of cell proliferation in CSF-1R ligand-dependent and/or CSF-1 ligand-independent CSF-1R expressing tumor cells;

b) the inhibition of cell proliferation of tumors with CSF-1R ligand-dependent and/or CSF-1R ligand-independent CSF-1R expressing macrophage infiltrate;

c) the inhibition of cell survival (in CSF-1R ligand-dependant and/or CSF-1R ligand-independent) CSF-1R expressing monocytes and macrophages; and/or

d) the inhibition of cell differentiation (in CSF-1R ligand-dependent and/or CSF-1R ligand-independent) CSF-1R expressing monocytes into macrophages,

wherein the anti-CSF-1R antibody is administered in combination with a chemotherapeutic agent, radiation, and/or cancer immunotherapy.

This combination therapy with antibodies binding to human CSF-1R, characterized in binding to the (dimerization) domains D4 to D5, has valuable properties like less activation potential to CSF-1R activation and in consequence reduced toxitcity and no stimulation of CSF-1R receptor (e.g.compared to a combination therapy with antibodies binding to human CSF-1R, characterized in binding to the domains D1 to D3).

The term "ligand dependent" as used herein refers to a ligand-independent signaling through the extracellular ECD (and does not include the ligand independent signaling mediated by activating point mutations in the intracellular kinase domain). In one embodiment CSF-1R ligand in this context refers a CSF-1R ligand selected from human CSF-1 (SEQ ID No: 86) and human IL-34 (SEQ ID No: 87); in one embodiment the CSF-1R ligand is human CSF-1 (SEQ ID No: 86); in one embodiment the CSF-1R ligand is human IL-34 (SEQ ID No: 87)).

In a second aspect, the disclosure comprises an antibody binding to human CSF-1R, characterized in binding to the domains D4 to D5 (SEQ ID No: 85) of the extracellular domain of human CSF-1R for use in the treatment of a patient having a CSF-1R expressing tumor or having a tumor with CSF-1R expressing macrophage infiltrate, wherein the tumor is characterized by an increase of CSF-1R ligand wherein the anti-CSF-1R antibody is administered in combination with a chemotherapeutic agent, radiation and/or cancer immunotherapy.

The ~~invention~~disclosure comprises an antibody binding to human CSF-1R, antibody binding to human CSF-1R, characterized in binding to the (dimerization) domains D4 to D5 (SEQ ID No: 85) of the extracellular domain of human CSF-1R for use in the treatment of a patient having a CSF-1R expressing tumor or having a tumor with CSF-1R expressing macrophage infiltrate, wherein the tumor is characterized by an increase of CSF-1R ligand (in one embodiment the CSF-1R ligand is selected from human CSF-1 (SEQ ID No: 86) and human IL-34 (SEQ ID No: 87); in one embodiment the CSF-1R ligand is human CSF-1 (SEQ ID No: 86); in one embodiment the CSF-1R ligand is human IL-34 (SEQ ID No: 87)) (detectable in serum, urine or tumor biopsies),

wherein the anti-CSF-1R antibody is administered in combination with a chemotherapeutic agent, radiation and/or cancer immunotherapy. The term "increase of CSF-1R ligand" refers to the overexpression of human CSF-1R ligand (in one embodiment the CSF-1R ligand is selected from human CSF-1 (SEQ ID No: 86) and human IL-34 (SEQ ID No: 87); in one embodiment the CSF-1R ligand is human CSF-1 (SEQ ID No: 86); in one embodiment the CSF-1R ligand is human IL-34 (SEQ ID No: 87)) (compared to normal tissue) before treatment or overexpression of human CSF-1R ligand induced by treatment with anti-CSF-1R antibody (and compared to the expression levels before treatment). In certain embodiments, the term "increase" or "above" refers to a level above the reference level or to an overall increase of 5%, 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%,

80%, 85%, 90%, 95%, 100% or greater, in CSF-1R ligand level detected by the methods described herein, as compared to the CSF-1R ligand level from a reference sample. In certain embodiments, the term increase refers to the increase in CSF-1R ligand level wherein, the increase is at least about 1.5-, 1.75-, 2-, 3-, 4-, 5-, 6-, 7-, 8-, 9-, 10-, 15-, 20-, 25-, 30-, 40-, 50-, 60-, 70-, 75-, 80-, 90-, or 100- fold higher as compared to the CSF-1R ligand level e.g. predetermined from a reference sample. In one preferred embodiment the term increased level relates to a value at or above a reference level.

In one embodiment of the ~~invention~~disclosure the anti-CSF-1R antibody is characterized in that the antibody binds to human CSF-1R Extracellular Domain (SEQ ID NO: 64) (comprising domains D1 to D5) and does not bind to domains D1 to D3 (SEQ ID NO: 66) of the extracellular domain of human CSF-1R.

In one embodiment chemotherapeutic agents, which may be administered with anti-CSF-1R antibody, include, but are not limited to, anti-neoplastic agents including alkylating agents including: nitrogen mustards, such as mechlorethamine, cyclophosphamide, ifosfamide, melphalan and chlorambucil; nitrosoureas, such as carmustine (BCNU), lomustine (CCNU), and semustine (methyl-CCNU); Temodal(TM) (temozolamide), ethylenimines/methylmelamine such as thriethylenemelamine (TEM), triethylene, thiophosphoramide (thiotepa), hexamethylmelamine (HMM, altretamine); alkyl sulfonates such as busulfan; triazines such as dacarbazine (DTIC); antimetabolites including folic acid analogs such as methotrexate and trimetrexate, pyrimidine analogs such as 5-fluorouracil (5FU), fluorodeoxyuridine, gemcitabine, cytosine arabinoside (AraC, cytarabine), 5-azacytidine, 2,2'- difluorodeoxycytidine, purine analogs such as 6-merca.rho.topurine, 6-thioguamne, azathioprine, T- deoxycoformycin (pentostatin), erythrohydroxynonyladenine (EHNA), fludarabine phosphate, and 2-chlorodeoxyadenosine (cladribine, 2-CdA); natural products including antimitotic drugs such as paclitaxel, vinca alkaloids including vinblastine (VLB), vincristine, and vinorelbine, taxotere, estramustine, and estramustine phosphate; pipodophylotoxins such as etoposide and teniposide; antibiotics such as actimomycin D, daunomycin (rubidomycin), doxorubicin, mitoxantrone, idarubicin, bleomycins, plicamycin (mithramycin), mitomycinC, and actinomycin; enzymes such as L-asparaginase; biological response modifiers such as interferon-alpha, IL-2, G-CSF and GM-CSF; miscellaneous agents including platinum coordination complexes such as oxaliplatin, cisplatin and carboplatin, anthracenediones such as mitoxantrone, substituted urea such as hydroxyurea, methylhydrazine derivatives including N- methylhydrazine (MIH) and procarbazine, adrenocortical suppressants such as mitotane (o, p-DDD) and aminoglutethimide; hormones and antagonists including adrenocorticosteroid antagonists such as prednisone and equivalents, dexamethasone and aminoglutethimide; Gemzar(TM) (gemcitabine), progestin such as hydroxyprogesterone caproate, medroxyprogesterone acetate and megestrol acetate; estrogen such as diethylstilbestrol and ethinyl estradiol equivalents; antiestrogen such as tamoxifen; androgens including testosterone propionate and

d) adoptive cell transfer: GVAX(prostate cancer cell line expressing GM-CSF), dendritic cell vaccine, adoptive T cell therapy, adoptive CAR T cell therapy.

In one embodiment the cancer immunotherapy is selected from T cell engaging agents selected from IL-2 (Proleukin), and antagonizing antibodies which bind to human CTLA-4 (e.g. ipilimumab), to PD-1, or to PD-L1.

In one embodiment the cancer immunotherapy is IL-2 (Proleukin). In one embodiment the cancer immunotherapy is an antagonizing antibody which binds to human CTLA-4 (e.g. ipilimumab).

The disclosure further relates to ~~One further aspect of the invention is~~ the combination therapy of an antibody binding to human CSF-1R (including antibodies binding to domains D1-D3 and antibodies binding to domains D4-D5) with a cancer immunotherapy,

wherein the cancer immunotherapy is selected from the group of:

a) T cell engaging agents selected from agonistic antibodies which bind to human OX40, to GITR, to CD27, or to 4-1BB, und T-cell bispecific antibodies (e.g. T cell-engaging BiTE™ antibodies CD3-CD19, CD3-EpCam, CD3-EGFR), IL-2 (Proleukin), Interferon (IFN) alpha, antagonizing antibodies which bind to human CTLA-4 (e.g. ipilimumab), to PD-1, to PD-L1, to TIM-3, to BTLA, to VISTA, to LAG-3, or to CD25,

b) targeting immunosuppression: antibodies or small molecules targeting STAT3 or NFkB signaling, blocking IL-6, IL-17, IL-23,TNFa function,

c) cancer vaccines/enhance dendritic cell function: OncoVex (oncolytic virus secreting GM-CSF), an agonistic CD40 antibody, Toll-like receptor (TLR) ligands, TLR agonists, recombinant fusion protein encoding MAGE-A3, PROSTVAC; or

d) adoptive cell transfer: GVAX(prostate cancer cell line expressing GM-CSF), dendritic cell vaccine, adoptive T cell therapy, adoptive CAR T cell therapy.

In a third aspect, the disclosure comprises an antibody binding to human CSF-1R, for use in the treatment of a patient having a CSF-1R expressing tumor or having a tumor with CSF-1R expressing macrophage infiltrate, wherein the tumor is characterized by an increase of CSF-1R ligand wherein the anti-CSF-1R antibody is administered in combination with a cancer immunotherapy, wherein the cancer immunotherapy is selected from the group of:

a) T cell engaging agents selected from agonistic antibodies which bind to human OX40, to GITR, to CD27, or to 4-1BB, und T-cell bispecific antibodies (e.g. T cell-engaging BiTE™ antibodies CD3-CD19, CD3-EpCam, CD3-EGFR), IL-2 (Proleukin), Interferon (IFN) alpha, antagonizing antibodies which bind to human CTLA-4 (e.g. ipilimumab), to PD-1, to PD-L1, to TIM-3, to BTLA, to VISTA, to LAG-3, or to CD25,

b) targeting immunosuppression: antibodies or small molecules targeting STAT3 or NFkB signaling, blocking IL-6, IL-17, IL-23,TNFa function,

c) cancer vaccines/enhance dendritic cell function: OncoVex (oncolytic virus secreting GM-CSF), an agonistic CD40 antibody, Toll-like receptor (TLR) ligands, TLR agonists, recombinant fusion protein encoding MAGE-A3, PROSTVAC; or

d) adoptive cell transfer: GVAX(prostate cancer cell line expressing GM-CSF), dendritic cell vaccine, adoptive T cell therapy, adoptive CAR T cell therapy.

The disclosure further relates to ~~One further aspect of the invention is~~ the combination therapy of an antibody binding to human CSF-1R for use in the treatment of cancer (including

antibodies binding to domains D1-D3 and antibodies binding to domains D4-D5) wherein the CSF-1R antibody is administered in combination with a bispecific ANG-2-VEGF antibody (e.g. an ANG2-VEGF antibody as described in WO2010/040508 or WO2011/117329, in one preferred embodiment with the bispecific ANG-2-VEGF antibody XMab1 as described in WO2011/117329). In one embodiment the antibody binding to human CSF-1R for use in the treatment of cancer is characterized in binding to domains D4-D5. In one embodiment such combination therapy comprises an antibody binding to human CSF-1R, is characterized in that the heavy chain variable domain is SEQ ID NO:39 and the light chain variable domain is SEQ ID NO:40 and the bispecific ANG-2-VEGF antibody XMab1 as described in WO2011/117329.

The disclosure further relates to ~~One further aspect of the invention is~~ the combination therapy of an antibody binding to human CSF-1R (including antibodies binding to domains D1-D3 and antibodies binding to domains D4-D5) with a cancer immunotherapy, wherein the cancer immunotherapy is selected from the group of:

cancer vaccines/enhance dendritic cell function: OncoVex (oncolytic virus secreting GM-CSF), an agonistic CD40 antibody, Toll-like receptor (TLR) ligands, TLR agonists, recombinant fusion protein encoding MAGE-A3, PROSTVAC.

One preferred embodiment of the ~~invention~~disclosure is the combination therapy of an antibody binding to human CSF-1R (including antibodies binding to domains D1-D3 and antibodies binding to domains D4-D5, preferably antibodies binding to domains D4-D5 as described herein) with a cancer immunotherapy, wherein the cancer immunotherapy is an agonistic CD40 antibody. CSF-1R antibodies binding to domains D1-D3 of human CSF-1R are described e.g. in WO 2009/026303 and WO 2009/112245 relate to certain anti-CSF-1R antibodies binding to CSF-1R within the first three subdomains (D1 to D3) of the Extracellular Domain (CSF-1R-ECD). WO2011/123381(A1) relates to antibodies against CSF-1R. and Sherr, C.J., et al., Blood 73 (1989) 1786-1793 (typically these antibodies are characterized by inhibiting CSF-1R ligand-dependent but not CSF-1R ligand-independent CSF-1R proliferation and /or signaling).

CSF-1R antibodies binding to domains D4-D5 of human CSF-1R are described e.g. within the present ~~invention~~disclosure, in PCT/EP2012/075241 and Sherr, C.J., et al., Blood

73 (1989) 1786-1793 (typically these antibodies are characterized by inhibiting CSF-1R ligand-dependent and CSF-1R ligand-independent CSF-1R proliferation and/or signaling).

The disclosure further relates to ~~Thus in one aspect of the invention also comprises~~ an antibody binding to human CSF-1R, for use in the treatment of cancer wherein the anti-CSF-1R antibody is administered in combination with a chemotherapeutic agent, radiation, and/or cancer immunotherapy. In one embodiment the cancer immunotherapy is selected the cancer immunotherapy is selected from the group of: a) T cell engaging agents selected from agonistic antibodies, to GITR, to CD27, or to 4-1BB, und T-cell bispecific antibodies (e.g. T cell-engaging BiTE™ antibodies CD3-CD19, CD3-EpCam, CD3-EGFR), IL-2 (Proleukin), Interferon (IFN) alpha, antagonizing antibodies which bind to human CTLA-4 (e.g. ipilimumab), to PD-1, to PD-L1, to TIM-3, to BTLA, to VISTA, to LAG-3, or to CD25, b) targeting immunosuppression: antibodies or small molecules targeting STAT3 or NFkB signaling, blocking IL-1, IL-6, IL-17, IL-23, TNFa function, (e.g antibodies against IL-1, IL-6, IL-17, IL-23, TNFa or against the respective receptor e.g. IL-1R, IL-6R, IL-17R, IL-23R) c) cancer vaccines/enhance dendritic cell function: OncoVex (oncolytic virus secreting GM-CSF), an agonistic CD40 antibody (as described e.g. Beatty et al., Science 331 (2011) 1612-1616, R. H. Vonderheide et al., J Clin Oncol 25, 876 (2007); Khalil, M, et al., Update Cancer Ther. 2007 June 1; 2(2): 61–65, examples in clinical trials are e.g CP-870,893 and dacetuzumab (an agonist CD40 antibody, CAS number 880486-59-9, SGN-40; humanized S2C6 antibody) (Khalil, M, et al, Update Cancer Ther. 2007 June 1; 2(2): 61–65; an agonist CD40 rat anti-mouse IgG2a mAb FGK45 as model antibody is described in S. P. Schoenberger, et al, Nature 393, 480 (1998)) CP-870,893 is a fully human IgG2 CD40 agonist antibody developed by Pfizer. It binds CD40 with a KD of 3.48×10-10 M, but does not block binding of CD40L (see e.g., U.S.7,338,660 or EP1476185 wherein CP-870,893 is described as antibody 21.4.1). CP-870,893 (antibody 21.4.1 of U.S.7,338,660) is characterized by comprising (a) a heavy chain variable domain amino acid sequence of QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWVRQAPGQGLEWMG WINPDSGGTNYAQKFQGRVTMTRDTSISTAYMELNRLRSDDTAVYYCARDQP LGYCTNGVCSYFDYWGQGTLVTVSS (SEQ ID NO: 88) (which corresponds to SEQ ID NO: 42 of US 7,338,660) (b) a light chain variable domain amino acid sequence of DIQMTQSPSSVSASVGDRVTITCRASQGIYSWLAWYQQKPGKAPNLLIYTAST LQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQANIFPLTFGGGTKV

In one embodiment of the ~~invention~~disclosure radiation may be carried out and/or a radiopharmaceutical may be used in addition to the anti-CSF-1R antibody. The source of radiation can be either external or internal to the patient being treated. When the source is external to the patient, the therapy is known as external beam radiation therapy (EBRT). When the source of radiation is internal to the patient, the treatment is called brachytherapy (BT). Radioactive atoms for use in the context of this ~~invention~~disclosure can be selected from the group including, but not limited to, radium, cesium-137, iridium-192, americium-241, gold-198, cobalt-57, copper-67, technetium-99, iodine-123, iodine-131, and indium-111. Is also possible to label the antibody with such radioactive isotopes.

Radiation therapy is a standard treatment for controlling unresectable or inoperable tumors and/or tumor metastases. Improved results have been seen when radiation therapy has been combined with chemotherapy. Radiation therapy is based on the principle that high-dose radiation delivered to a target area will result in the death of reproductive cells in both tumor and normal tissues. The radiation dosage regimen is generally defined in terms of radiation absorbed dose (Gy), time and fractionation, and must be carefully defined by the oncologist. The amount of radiation a patient receives will depend on various considerations, but the two most important are the location of the tumor in relation to other critical structures or organs of the body, and the extent to which the tumor has spread. A typical course of treatment for a patient undergoing radiation therapy will be a treatment schedule over a 1 to 6 week period, with a total dose of between 10 and 80 Gy administered to the patient in a single daily fraction of about 1.8 to 2.0 Gy, 5 days a week. In a preferred embodiment of this ~~invention~~disclosure there is synergy when tumors in human patients are treated with the combination treatment of the ~~invention~~disclosure and radiation. In other words, the inhibition of tumor growth by means of the agents comprising the combination of the ~~invention~~disclosure is enhanced when combined with radiation, optionally with additional chemotherapeutic or anticancer agents. Parameters of adjuvant radiation therapies are, for example, contained in WO 99/60023.

In one embodiment of the ~~invention~~disclosure the anti-CSF-1R antibody is characterized in that the antibody binds to human CSF-1R fragment delD4 (SEQ ID NO: 65) and to human CSF-1R Extracellular Domain (SEQ ID NO: 64) with a ratio of 1:50 or lower.

In one embodiment of the ~~invention~~disclosure the antibody is characterized in that the antibody does not bind to human CSF-1R fragment delD4 (SEQ ID NO: 65).

In one embodiment of the ~~invention~~disclosure the antibody is characterized in that

a) the heavy chain variable domain is SEQ ID NO:7 and the light chain variable domain is SEQ ID NO:8,
b) the heavy chain variable domain is SEQ ID NO:15 and the light chain variable domain is SEQ ID NO:16;
c) the heavy chain variable domain is SEQ ID NO:75 and the light chain variable domain is SEQ ID NO:76;
d) the heavy chain variable domain is SEQ ID NO:83 and the light chain variable domain is SEQ ID NO:84;

or a humanized version thereof.

In one embodiment of the ~~invention~~disclosure the antibody is characterized in that

a) the heavy chain variable domain is SEQ ID NO:7 and the light chain variable domain is SEQ ID NO:8,
b) the heavy chain variable domain is SEQ ID NO:15 and the light chain variable domain is SEQ ID NO:16;

or a humanized version thereof.

In one embodiment of the ~~invention~~disclosure the antibody is characterized in that

a) the heavy chain variable domain is SEQ ID NO:23 and the light chain variable domain is SEQ ID NO:24, or
b) the heavy chain variable domain is SEQ ID NO:31 and the light chain variable domain is SEQ ID NO:32, or
c) the heavy chain variable domain is SEQ ID NO:39 and the light chain variable domain is SEQ ID NO:40, or
d) the heavy chain variable domain is SEQ ID NO:47 and the light chain variable domain is SEQ ID NO:48, or
e) the heavy chain variable domain is SEQ ID NO:55 and the light chain variable domain is SEQ ID NO:56.

In one embodiment of the ~~invention~~disclosure the antibody is characterized in that

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

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

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

d) the heavy chain variable domain comprises a CDR3 region of SEQ ID NO: 25, a CDR2 region of SEQ ID NO: 26, and a CDR1 region of SEQ ID NO: 27, and the light chain variable domain comprises a CDR3 region of SEQ ID NO:28, a CDR2 region of SEQ ID NO: 29, and a CDR1 region of SEQ ID NO: 30, or

e) the heavy chain variable domain comprises a CDR3 region of SEQ ID NO: 33, a CDR2 region of SEQ ID NO: 34, and a CDR1 region of SEQ ID NO: 35, and the light chain variable domain comprises a CDR3 region of SEQ ID NO:36, a CDR2 region of SEQ ID NO: 37, and a CDR1 region of SEQ ID NO: 38, or

f) the heavy chain variable domain comprises a CDR3 region of SEQ ID NO:41, a CDR2 region of SEQ ID NO: 42, and a CDR1 region of SEQ ID NO:43, and the light chain variable domain comprises a CDR3 region of SEQ ID NO: 44, a CDR2 region of SEQ ID NO:45, and a CDR1 region of SEQ ID NO:46, or

g) the heavy chain variable domain comprises a CDR3 region of SEQ ID NO: 49, a CDR2 region of SEQ ID NO: 50, and a CDR1 region of SEQ ID NO: 51, and the light chain variable domain comprises a CDR3 region of SEQ ID NO:52, a CDR2 region of SEQ ID NO: 53, and a CDR1 region of SEQ ID NO: 54; or

h) the heavy chain variable domain comprises a CDR3 region of SEQ ID NO:69, a CDR2 region of SEQ ID NO: 70, and a CDR1 region of SEQ ID NO:71, and the light chain variable domain comprises a CDR3 region of SEQ ID NO: 72, a CDR2 region of SEQ ID NO:73, and a CDR1 region of SEQ ID NO:74, or

i) the heavy chain variable domain comprises a CDR3 region of SEQ ID NO: 77, a CDR2 region of SEQ ID NO: 78, and a CDR1 region of SEQ ID NO: 79, and the light chain variable domain comprises a CDR3 region of SEQ ID NO:80, a CDR2 region of SEQ ID NO: 81, and a CDR1 region of SEQ ID NO: 82.

In one embodiment of the ~~invention~~disclosure the antibody is of human IgG1 subclass or of human IgG4 subclass.

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

The ~~invention~~disclosure further comprises the use an of an antibody according to the ~~invention~~disclosure for the manufacture of a medicament for treatment of a CSF-1R mediated disease.

The ~~invention~~disclosure further comprises the use an of an antibody according to the ~~invention~~disclosure for the manufacture of a medicament for treatment of cancer.

The ~~invention~~disclosure further comprises the use an of an antibody according to the ~~invention~~disclosure for the manufacture of a medicament for treatment of bone loss.

The ~~invention~~disclosure further comprises the use an of an antibody according to the ~~invention~~disclosure for the manufacture of a medicament for treatment of metastasis.

The ~~invention~~disclosure further comprises the use ~~an~~ of an antibody according to the ~~invention~~disclosure for the manufacture of a medicament for treatment of inflammatory diseases.

The ~~invention~~disclosure further comprises an antibody according to the ~~invention~~disclosure for treatment of a CSF-1R mediated disease.

The ~~invention~~disclosure further comprises an antibody according to the ~~invention~~disclosure for treatment of cancer.

The ~~invention~~disclosure further comprises an antibody according to the ~~invention~~disclosure for treatment of bone loss.

The ~~invention~~disclosure further comprises an antibody according to the ~~invention~~disclosure for treatment of metastasis.

The ~~invention~~disclosure further comprises an antibody according to the ~~invention~~disclosure for treatment of inflammatory diseases.

The combination therapies of the antibodies described herein show benefits for patients in need of a CSF-1R targeting therapy. The antibodies according to the ~~invention~~disclosure show efficient antiproliferative activity against ligand–independent and ligand-dependent proliferation and are therefore especially useful in the treatment of cancer and metastasis in combination with a chemotherapeutic agent, radiation and/or cancer immunotherapy.

The ~~invention~~disclosure further provides a method for treating a patient suffering from cancer, comprising administering to a patient diagnosed as having such a disease (and therefore being in need of such a therapy) an effective amount of an antibody according to the ~~invention~~disclosure in combination with a chemotherapeutic agent, radiation and/or cancer immunotherapy. The antibody is administered preferably in a pharmaceutical composition.

Surprisingly it has been found that, using a human CSF-1R fragment delD4 in which the D4 subdomain of human CSF-1R-ECD was deleted (SEQ ID NO:65), the anti-CSF-1R antibodies could be selected. These antibodies show valuable properties like excellent ligand-dependent cell growth inhibition and at the same time ligand independent cell growth inhibition of NIH 3T3 cell, retrovirally infected with either an expression vector for full-length wildtype CSF-1R (SEQ ID NO:62) or mutant CSF-1R L301S Y969F (SEQ ID NO:63) whereby mutant

CSF-1R recombinant cells are able to form spheroids independent of the CSF-1 ligand. Furthermore these antibodies inhibit (both) human and cynomolgous macrophage differentiation, as they inhibit survival of human and cynomolgous monocytes.

Further antibodies binding to the binding to the (dimerization) domains D4 to D5 can be selected by screening for antibodies that bind to the complete extracellular domain of human CSF-1R (SEQ ID NO: 64) (including domains D1 to D5), and not binding to the domains D1 to D3 (SEQ ID NO: 66) of the extracellular domain of human CSF-1R.

In a fourth aspect, the disclosure relates to a method for determining whether a subject having a cancer is a candidate for an anti-CSF-1R antibody-based cancer treatment regimen, the method comprising:

- ex vivo or in vitro determining in vitro the level of one or more of the following markers: CSF-1R, CD68/CD163, CD68/MHC class II, CD31 (microvessel density), and Ki67 and other markers like e.g. immuninfiltrates; in a sample of the subject, wherein the sample is selected from the group consisting of tissue, blood, serum, plasma, tumor cells and circulating tumor cells; and

- wherein a change in the level of one or more of CSF-1R, CD68/CD163, CD68/MHC class II, CD31 (microvessel density) and Ki67 and other markers like e.g. immuninfiltrates (e.g. T cells (e.g. CD4- and/or CD8-T cells), as compared with to the corresponding level in an individual not suffering from cancer, is indicative that the subject is a candidate for the anti -CSF -1 R antibody -based cancer treatment regimen.

In a fifth aspect, the disclosure relates to a method for determining whether a subject having a cancer is a candidate for an anti-CSF-1R antibody-based cancer treatment regimen, the method comprising:

- ex vivo or in vitro determining in vitro the level of one or more of the following markers: CSF-1, Trap5b, sCD163, IL-34; in a sample of the subject, wherein the sample is selected from the group consisting of tissue, blood, serum, plasma, tumor cells and circulating tumor cells; and

- wherein a change in the level of one or more of CSF-1, Trap5b, sCD163, IL-34, as compared with to the corresponding level in an individual not suffering from

cancer, is indicative that the subject is a candidate for the anti -CSF -1 R antibody -based cancer treatment regimen.

In a sixth aspect, the disclosure relates to a method for determining whether a subject having a cancer is a candidate for an anti-CSF-1R antibody-based cancer treatment regimen, the method comprising:

- ex vivo or in vitro determining in vitro the level of one or more of the following markers: IFNγ, TNFα , IL-1β, IL-4, IL-6 , IL-8 , IL-10, IL-13, GM-CSF, VEGF, MCP-1, CCL18, CCL22, MIP-1 , Galectin 3, IL1Ra, TGF alpha; in a sample of the subject, wherein the sample is selected from the group consisting of tissue, blood, serum, plasma, tumor cells and circulating tumor cells; and

- wherein a change in the level of one or more of IFNγ, TNFα , IL-1β, IL-4, IL-6 , IL-8 , IL-10, IL-13, GM-CSF, VEGF, MCP-1, CCL18, CCL22, MIP-1, Galectin 3, IL1Ra, TGF alpha, as compared with to the corresponding level in an individual not suffering from cancer, is indicative that the subject is a candidate for the anti -CSF -1 R antibody -based cancer treatment regimen.

In a seventh aspect, the disclosure relates to an antibody binding to human CSF-1R for use in the treatment of cancer wherein the antibody is administered in combination with a bispecific ANG-2-VEGF antibody

In a eighth aspect, the disclosure relates to an antibody binding to human CSF-1R for use in the treatment of cancer wherein the anti-CSF-1R antibody is administered in combination with an agonistic CD40 antibody.

Description of the Figures

Figure 1 Growth inhibition of BeWo tumor cells in 3D culture under treatment with different anti-CSF-1R monoclonal antibodies at a concentration of 10µg/ml.
X axis: viability normalized mean relative light units (RLU) corresponding to the ATP-content of the cells (CellTiterGlo assay).
Y axis: tested probes: Minimal Medium (0.5% FBS), mouse IgG1 (mIgG1, 10µg/ml), mouse IgG2a (mIgG2a 10µg/ml), CSF-1 only, Mab 2F11, Mab 2E10, Mab2H7, Mab1G10 and SC 2-4A5. Highest inhibition of CSF-1 induced growth was observed with the anti-CSF-1R antibodies according to the invention.

Figure 2a Biacore sensogram of binding of different anti-CSF-1R antibodies to immobilized human CSF-1R fragment delD4 (comprising the extracellular subdomains D1 –D3 and D5) (SEQ ID NO: 65) (y-axis: binding signal in Response Units (RU), baseline = 0 RU, x-axis: time in seconds (s)): While the antibodies Mab 3291 and sc 2-4A5 clearly show binding to this delD4 fragment, the antibodies according to the invention e.g. Mab 2F11, and Mab 2E10, did not bind to the CSF-1R fragment delD4. The control anti-CCR5 antibody m<CCR5>Pz03.1C5 did also not bind to the CSF-1R fragment delD4.

Figure 2b Biacore sensogram of binding of different anti-CSF-1R antibodies to immobilized human CSF-1R Extracellular Domain (CSF-1R-ECD) (comprising the extracellular subdomains D1 – D5) (SEQ ID NO: 64) (y-axis: binding signal in Response Units

17. An antibody binding to human CSF-1R, for use in

the treatment of a patient having a CSF-1R expressing tumor or having a tumor with CSF-1R expressing macrophage infiltrate, wherein the tumor is characterized by an increase of CSF-1R ligand

wherein the anti-CSF-1R antibody is administered in combination with a cancer immunotherapy,

wherein the cancer immunotherapy is selected from the group of:

a) T cell engaging agents selected from agonistic antibodies which bind to human OX40, to GITR, to CD27, or to 4-1BB, und T-cell bispecific antibodies (e.g. T cell-engaging BiTE™ antibodies CD3-CD19, CD3-EpCam, CD3-EGFR), IL-2 (Proleukin), Interferon (IFN) alpha, antagonizing antibodies which bind to human CTLA-4 (e.g. ipilimumab), to PD-1, to PD-L1, to TIM-3, to BTLA, to VISTA, to LAG-3, or to CD25,

b) targeting immunosuppression: antibodies or small molecules targeting STAT3 or NFkB signaling, blocking IL-6, IL-17, IL-23,TNFa function,

c) cancer vaccines/enhance dendritic cell function: OncoVex (oncolytic virus secreting GM-CSF), an agonistic CD40 antibody, Toll-like receptor (TLR) ligands, TLR agonists, recombinant fusion protein encoding MAGE-A3, PROSTVAC; or

d) adoptive cell transfer: GVAX(prostate cancer cell line expressing GM-CSF), dendritic cell vaccine, adoptive T cell therapy, adoptive CAR T cell therapy.

18. The antibody according to claim 17

wherein the cancer immunotherapy is selected from the group of:

cancer vaccines/enhance dendritic cell function: OncoVex (oncolytic virus secreting GM-CSF), an agonistic CD40 antibody, Toll-like receptor (TLR) ligands, TLR agonists, recombinant fusion protein encoding MAGE-A3, PROSTVAC.

19. The antibody or use according to claim 17, wherein the cancer immunotherapy is an agonistic CD40 antibody.