Combination therapy of antibodies against human CSF-1R and antibodies against human PD-L1

The present invention relates to the combination therapy of specific antibodies which bind human CSF-1R with specific antibodies which bind human PD-L1.

Background of the Invention

CSF-1R and CSF-1R antibodies

The human CSF-1 receptor (CSF-1R; colony stimulating factor 1 receptor; synonyms: M-CSF receptor; Macrophage colony-stimulating factor 1 receptor, Fms proto-oncogene, c-fms, SEQ ID NO: 62) is known since 1986 (Coussens, L., et al., Nature 320 (1986) 277-280). CSF-1R is a growth factor and encoded by the c-fms proto-oncogene (reviewed e.g. in Roth, P., and Stanley, E.R., Curr. Top. Microbiol. Immunol. 181 (1992) 141-167).

CSF-1R is the receptor for CSF-1 (colony stimulating factor 1, also called M-CSF, macrophage colony-stimulating factor) and mediates the biological effects of this cytokine (Sherr, C.J., et al., Cell 41 (1985) 665-676). The cloning of the colony stimulating factor-1 receptor (CSF-1R) (also called c-fms) was described for the first time in Roussel, M.F., et al., Nature 325 (1987) 549-552. In that publication, it was shown that CSF-1R had transforming potential dependent on changes in the C-terminal tail of the protein including the loss of the inhibitory tyrosine 969 phosphorylation which binds Cbl and thereby regulates receptor down regulation (Lee, P.S., et al., Embo J. 18 (1999) 3616-3628). Recently a second ligand for CSF-1R termed interleukin-34 (IL-34) was identified (Lin, H., et al, Science 320 (2008) 807-811).

Currently two CSF-1R ligands that bind to the extracellular domain of CSF-1R are known. The first one is CSF-1 (colony stimulating factor 1, also called M-CSF, macrophage; SEQ ID NO: 86) and is found extracellularly as a disulfide-linked homodimer (Stanley, E.R. et al., Journal of Cellular Biochemistry 21 (1983) 151-159; Stanley, E.R. et al., Stem Cells 12 Suppl. 1 (1995) 15–24). The second one is IL-34 (Human IL-34; SEQ ID NO: 87) (Hume, D. A. , et al, Blood 119 (2012) 1810-1820). The main biological effects of CSF-1R signaling are the differentiation, proliferation, migration, and survival of hematopoietic precursor cells to the macrophage lineage (including osteoclast). Activation of CSF-1R is

mediated by its CSF-1R ligands, CSF-1 (M-CSF) and IL-34. Binding of CSF-1 (M-CSF) to CSF-1R induces the formation of homodimers and activation of the kinase by tyrosine phosphorylation (Li, W. et al, EMBO Journal.10 (1991) 277-288; Stanley, E.R., et al., Mol. Reprod. Dev. 46 (1997) 4-10).

The biologically active homodimer CSF-1 binds to the CSF-1R within the subdomains D1 to D3 of the extracellular domain of the CSF-1 receptor (CSF-1R-ECD). The CSF-1R-ECD comprises five immunoglobulin-like subdomains (designated Dl to D5). The subdomains D4 to D5 of the extracellular domain (CSF-1R-ECD) are not involved in the CSF-1 binding (Wang, Z., et al Molecular and Cellular Biology 13 (1993) 5348-5359). The subdomain D4 is involved in dimerization (Yeung, Y-G., et al Molecular & Cellular Proteomics 2 (2003) 1143–1155; Pixley, F. J., et al., Trends Cell Biol. 14 (2004) 628-638).

Further signaling is mediated by the p85 subunit of PI3K and Grb2 connecting to the PI3K/AKT and Ras/MAPK pathways, respectively. These two important signaling pathways can regulate proliferation, survival and apoptosis. Other signaling molecules that bind the phosphorylated intracellular domain of CSF-1R include STAT1, STAT3, PLCy, and Cbl (Bourette, R.P. and Rohrschneider, L.R., Growth Factors 17 (2000) 155-166).

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

Sherr, C.J., et al., Blood 73 (1989) 1786-1793 relates to some antibodies against CSF-1R that inhibit the CSF-1 activity. Ashmun, R.A., et al., Blood 73 (1989) 827-837 relates to CSF-1R antibodies. Lenda, D., et al., Journal of Immunology 170 (2003) 3254-3262 relates to reduced macrophage recruitment, proliferation, and activation in CSF-1-deficient mice results in decreased tubular apoptosis during renal inflammation. Kitaura, H., et al., Journal of Dental Research 87 (2008) 396-400 refers to an anti-CSF-1 antibody which inhibits orthodontic tooth movement. WO 2001/030381 mentions CSF-1 activity inhibitors including antisense nucleotides and antibodies while disclosing only CSF-1 antisense nucleotides. WO 2004/045532 relates to metastases and bone loss prevention and treatment of

metastatic cancer by a CSF-1 antagonist disclosing as antagonist anti-CSF-1-antibodies only. WO 2005/046657 relates to the treatment of inflammatory bowel disease by anti-CSF-1-antibodies. US 2002/0141994 relates to inhibitors of colony stimulating factors. WO 2006/096489 relates to the treatment of rheumatoid arthritis by anti-CSF-1-antibodies. WO 2009/026303 and WO 2009/112245 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. WO2011/070024 relate to certain anti-CSF-1R antibodies binding to CSF-1R within the dimerization domain (D4 to D5).

PD-L1 and PD-L1 antibodies

Co-stimulation or the provision of two distinct signals to T-cells is a widely accepted model of lymphocyte activation of resting T lymphocytes by antigen-presenting cells (APCs). Lafferty *et al., Aust. J. Exp. Biol. Med. Sci.* 53: 27-42 (1975).

This model further provides for the discrimination of self from non-self and immune tolerance. Bretscher *et al., Science* 169: 1042-1049 (1970); Bretscher, P.A., *P.N.A.S. USA* 96: 185-190 (1999); Jenkins *et al., J. Exp. Med.* 165: 302-319 (1987). The primary signal, or antigen specific signal, is transduced through the T-cell receptor (TCR) following recognition of foreign antigen peptide presented in the context of the major histocompatibility-complex (MHC). The second or co-stimulatory signal is delivered to T-cells by co-stimulatory molecules expressed on antigen-presenting cells (APCs), and induce T-cells to promote clonal expansion, cytokine secretion and effector function. Lenschow *et al., Ann. Rev. Immunol.* 14:233 (1996). In the absence of co-stimulation, T-cells can become refractory to antigen stimulation, do not mount an effective immune response, and further may result in *exhaustion or tolerance* to foreign antigens.

The simple two-signal model can be an oversimplification because the strength of the TCR signal actually has a quantitative influence on T-cell activation and differentiation. Viola *et al., Science* 273: 104-106 (1996); Sloan-Lancaster, *Nature* 363: 156-159 (1993). Moreover, T-cell activation can occur even in the absence of co-stimulatory signal if the TCR signal strength is high. More importantly, T-cells receive both positive and negative secondary co-stimulatory signals. The regulation of such positive and negative signals is critical to maximize the host's protective

immune responses, while maintaining immune tolerance and preventing *autoimmunity*.

Negative secondary signals seem necessary for induction of T-cell tolerance, while positive signals promote T-cell activation. While the simple two-signal model still provides a valid explanation for naive lymphocytes, a host's immune response is a dynamic process, and co-stimulatory signals can also be provided to antigen-exposed T-cells.

The mechanism of co-stimulation is of therapeutic interest because the manipulation of co-stimulatory signals has shown to provide a means to either enhance or terminate cell-based immune response. Recently, it has been discovered that T cell dysfunction or anergy occurs concurrently with an induced and sustained expression of the inhibitory receptor, programmed death 1 polypeptide (PD-1). As a result, therapeutic targeting PD-1 and other molecules which signal through interactions with PD-1, such as programmed death ligand 1 (PD-L1) and programmed death ligand 2 (PD-L2) are an area of intense interest. The inhibition of PD-L1 signaling has been proposed as a means to enhance T cell immunity for the treatment of cancer (*e.g.*, tumor immunity) and infection, including both acute and chronic (*e.g.*, persistent) infection. However, as an optimal therapeutic directed to a target in this pathway has yet to be commercialized, a significant unmet medical need exists. Antibodies against PD-L1 are described e.g. in WO 2010/077634.

Summary

The disclosure comprises the combination therapy of an antibody which binds to human CSF-1R with an antibody which binds to human PD-L1 for use in the treatment of cancer, for use in the prevention or treatment of metastasis, for use in the treatment inflammatory diseases, for use in the treatment of bone loss, for use in treating or delaying progression of an immune related disease such as tumor immunity, or for use in stimulating an immune response or function, such as T cell activity.

The disclosure further comprises the use of antibody which binds to human CSF-1R for the manufacture of a medicament for use in the treatment of cancer, for use in the treatment inflammatory diseases, for use in the treatment of bone loss, for use in treating or delaying progression of an immune related disease such as tumor immunity, or for use in stimulating an immune response or function,

such as T cell

activity, wherein the antibody is administered in combination with an antibody which binds to human PD-L1.

The antibody which binds to human CSF-1R used in the combination therapy is characterized in comprising

a) a heavy chain variable domain VH of SEQ ID NO:23 and a light chain variable domain VL of SEQ ID NO:24, or

b) a heavy chain variable domain VH of SEQ ID NO:31 and a light chain variable domain VL of SEQ ID NO:32, or

c) a heavy chain variable domain VH of SEQ ID NO:39 and a light chain variable domain VL of SEQ ID NO:40, or

d) a heavy chain variable domain VH of SEQ ID NO:47 and a light chain variable domain VL of SEQ ID NO:48, or

e) a heavy chain variable domain VH of SEQ ID NO:55 and a light chain variable domain VL of SEQ ID NO:56;

and the antibody which binds to human PD-L1 used in the combination therapy is characterized in comprising

a) a heavy chain variable domain VH of SEQ ID NO:89 and a light chain variable domain VL of SEQ ID NO:92, or

b) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:93, or

c) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:94, or

d) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:95, or

e) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:96, or

f) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:97, or

g) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:98, or

h) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:99, or

i) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:100, or

j) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:101, or

k) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:102, or

l) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:103, or

m) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:104, or

n) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:105, or

o) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:106, or

p) a heavy chain variable domain VH of SEQ ID NO:91 and a light chain variable domain VL of SEQ ID NO:107.

In one aspect, there is provided a combination of an antibody which binds to human CSF-1R and an antibody which binds to human PD-L1 for use in the treatment of cancer,

wherein the antibody which binds to human CSF-1R used in the combination is characterized in comprising

a) a heavy chain variable domain VH of SEQ ID NO:23 and a light chain variable domain VL of SEQ ID NO:24, or

b) a heavy chain variable domain VH of SEQ ID NO:31 and a light chain variable domain VL of SEQ ID NO:32, or

c) a heavy chain variable domain VH of SEQ ID NO:39 and a light chain variable domain VL of SEQ ID NO:40, or

d) a heavy chain variable domain VH of SEQ ID NO:47 and a light chain variable domain VL of SEQ ID NO:48, or

e) a heavy chain variable domain VH of SEQ ID NO:55 and a light chain variable domain VL of SEQ ID NO:56;

and the antibody which binds to human PD-L1 used in the combination is characterized in comprising

a) a heavy chain variable domain VH of SEQ ID NO:89 and a light chain variable domain VL of SEQ ID NO:92, or

b) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:93, or

c) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:94, or

d) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:95, or

e) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:96, or

f) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:97, or

g) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:98, or

h) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:99, or

i) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:100, or

j) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:101, or

k) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:102, or

l) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:103, or

m) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:104, or

n) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:105, or

o) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:106, or

p) a heavy chain variable domain VH of SEQ ID NO:91 and a light chain variable domain VL of SEQ ID NO:107.

In one embodiment the antibody is for use in the treatment of cancer.

In one embodiment the antibody is for use in the prevention or treatment of metastasis.

In one embodiment the antibody is, for use in the treatment of bone loss.

In one embodiment the antibody is for use in the treatment of inflammatory diseases.

In one embodiment the antibody is for use in treating or delaying progression of an immune related disease such as tumor immunity.

In one embodiment the antibody is for use in stimulating an immune response or function, such as T cell activity.

The disclosure further comprises an antibody which binds to human CSF-1R wherein the antibody is administered in combination with an antibody which binds to human PD-L1 for use in

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

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

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

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

wherein the antibody is administered in combination with an antibody which binds to human PD-L1;

wherein the antibody which binds to human CSF-1R used in the combination therapy is characterized in comprising

a) a heavy chain variable domain VH of SEQ ID NO:23 and a light chain variable domain VL of SEQ ID NO:24, or

b) a heavy chain variable domain VH of SEQ ID NO:31 and a light chain variable domain VL of SEQ ID NO:32, or

c) a heavy chain variable domain VH of SEQ ID NO:39 and a light chain variable domain VL of SEQ ID NO:40, or

d) a heavy chain variable domain VH of SEQ ID NO:47 and a light chain variable domain VL of SEQ ID NO:48, or

e) a heavy chain variable domain VH of SEQ ID NO:55 and a light chain variable domain VL of SEQ ID NO:56;

and the antibody which binds to human PD-L1 used in the combination therapy is characterized in comprising

a) a heavy chain variable domain VH of SEQ ID NO:89 and a light chain variable domain VL of SEQ ID NO:92, or

b) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:93, or

c) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:94, or

d) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:95, or

e) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:96, or

f) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:97, or

g) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:98, or

h) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:99, or

i) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:100, or

j) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:101, or

k) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:102, or

l) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:103, or

m) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:104, or

n) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:105, or

o) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:106, or

p) a heavy chain variable domain VH of SEQ ID NO:91 and a light chain variable domain VL of SEQ ID NO:107.

In another aspect, there is provided a combination comprising an antibody which binds to human CSF-1R and an antibody which binds to human PD-L1, for use in

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

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

wherein the antibody which binds to human CSF-1R used in the combination is characterized in comprising

a) a heavy chain variable domain VH of SEQ ID NO:23 and a light chain variable domain VL of SEQ ID NO:24, or

b) a heavy chain variable domain VH of SEQ ID NO:31 and a light chain variable domain VL of SEQ ID NO:32, or

c) a heavy chain variable domain VH of SEQ ID NO:39 and a light chain variable domain VL of SEQ ID NO:40, or

d) a heavy chain variable domain VH of SEQ ID NO:47 and a light chain variable domain VL of SEQ ID NO:48, or

e) a heavy chain variable domain VH of SEQ ID NO:55 and a light chain variable domain VL of SEQ ID NO:56;

and the antibody which binds to human PD-L1 used in the combination is characterized in comprising

a) a heavy chain variable domain VH of SEQ ID NO:89 and a light chain variable domain VL of SEQ ID NO:92, or

b) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:93, or

c) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:94, or

d) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:95, or

e) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:96, or

f) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:97, or

g) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:98, or

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k) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:102, or

l) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:103, or

m) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:104, or

n) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:105, or

o) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:106, or

p) a heavy chain variable domain VH of SEQ ID NO:91 and a light chain variable domain VL of SEQ ID NO:107.

The disclosure further comprised an antibody which binds 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 and wherein the anti-CSF-1R antibody is administered in combination with an antibody which binds to human PD-L1,

wherein the antibody which binds to human CSF-1R used in the combination therapy is characterized in comprising

a) a heavy chain variable domain VH of SEQ ID NO:23 and a light chain variable domain VL of SEQ ID NO:24, or

b) a heavy chain variable domain VH of SEQ ID NO:31 and a light chain variable domain VL of SEQ ID NO:32, or

c) a heavy chain variable domain VH of SEQ ID NO:39 and a light chain variable domain VL of SEQ ID NO:40, or

d) a heavy chain variable domain VH of SEQ ID NO:47 and a light chain variable domain VL of SEQ ID NO:48, or

e) a heavy chain variable domain VH of SEQ ID NO:55 and a light chain variable domain VL of SEQ ID NO:56;

and the antibody which binds to human PD-L1 used in the combination therapy is characterized in comprising

a) a heavy chain variable domain VH of SEQ ID NO:89 and a light chain variable domain VL of SEQ ID NO:92, or

b) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:93, or

c) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:94, or

d) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:95, or

e) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:96, or

f) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:97, or

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i) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:100, or

j) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:101, or

k) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:102, or

l) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:103, or

m) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:104, or

n) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:105, or

o) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:106, or

p) a heavy chain variable domain VH of SEQ ID NO:91 and a light chain variable domain VL of SEQ ID NO:107.

In another aspect, there is provided a combination comprising an antibody which binds to human CSF-1R and an antibody which binds to human PD-L1, 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 antibody which binds to human CSF-1R used in the combination is characterized in comprising

a) a heavy chain variable domain VH of SEQ ID NO:23 and a light chain variable domain VL of SEQ ID NO:24, or

b) a heavy chain variable domain VH of SEQ ID NO:31 and a light chain variable domain VL of SEQ ID NO:32, or

c) a heavy chain variable domain VH of SEQ ID NO:39 and a light chain variable domain VL of SEQ ID NO:40, or

d) a heavy chain variable domain VH of SEQ ID NO:47 and a light chain variable domain VL of SEQ ID NO:48, or

e) a heavy chain variable domain VH of SEQ ID NO:55 and a light chain variable domain VL of SEQ ID NO:56;

and the antibody which binds to human PD-L1 used in the combination is characterized in comprising

a) a heavy chain variable domain VH of SEQ ID NO:89 and a light chain variable domain VL of SEQ ID NO:92, or

b) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:93, or

c) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:94, or

d) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:95, or

e) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:96, or

f) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:97, or

g) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:98, or

h) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:99, or

i) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:100, or

j) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:101, or

k) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:102, or

l) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:103, or

m) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:104, or

n) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:105, or

o) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:106, or

p) a heavy chain variable domain VH of SEQ ID NO:91 and a light chain variable domain VL of SEQ ID NO:107.

In one embodiment the antibodies are of human IgG1 subclass or human IgG4 subclass.

In another aspect, there is provided use of an antibody which binds to human CSF-1R wherein the antibody is in combination with an antibody which binds to human PD-L1 in the manufacture of a medicament for the treatment of cancer,

wherein the antibody which binds to human CSF-1R used in the combination is characterized in comprising

a) a heavy chain variable domain VH of SEQ ID NO:23 and a light chain variable domain VL of SEQ ID NO:24, or

b) a heavy chain variable domain VH of SEQ ID NO:31 and a light chain variable domain VL of SEQ ID NO:32, or

c) a heavy chain variable domain VH of SEQ ID NO:39 and a light chain variable domain VL of SEQ ID NO:40, or

d) a heavy chain variable domain VH of SEQ ID NO:47 and a light chain variable domain VL of SEQ ID NO:48, or

e) a heavy chain variable domain VH of SEQ ID NO:55 and a light chain variable domain VL of SEQ ID NO:56;

and the antibody which binds to human PD-L1 used in the combination is characterized in comprising

a) a heavy chain variable domain VH of SEQ ID NO:89 and a light chain variable domain VL of SEQ ID NO:92, or

b) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:93, or

c) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:94, or

d) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:95, or

e) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:96, or

f) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:97, or

g) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:98, or

h) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:99, or

i) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:100, or

j) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:101, or

k) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:102, or

l) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:103, or

m) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:104, or

n) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:105, or

o) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:106, or

p) a heavy chain variable domain VH of SEQ ID NO:91 and a light chain variable domain VL of SEQ ID NO:107.

In another aspect, there is provided use of an antibody which binds to human CSF-1R wherein the antibody is in combination with an antibody which binds to human PD-L1, in the manufacture of a medicament for

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

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

wherein the antibody which binds to human CSF-1R used in the combination is characterized in comprising

a) a heavy chain variable domain VH of SEQ ID NO:23 and a light chain variable domain VL of SEQ ID NO:24, or

b) a heavy chain variable domain VH of SEQ ID NO:31 and a light chain variable domain VL of SEQ ID NO:32, or

c) a heavy chain variable domain VH of SEQ ID NO:39 and a light chain variable domain VL of SEQ ID NO:40, or

d) a heavy chain variable domain VH of SEQ ID NO:47 and a light chain variable domain VL of SEQ ID NO:48, or

e) a heavy chain variable domain VH of SEQ ID NO:55 and a light chain variable domain VL of SEQ ID NO:56;

and the antibody which binds to human PD-L1 used in the combination is characterized in comprising

a) a heavy chain variable domain VH of SEQ ID NO:89 and a light chain variable domain VL of SEQ ID NO:92, or

b) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:93, or

c) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:94, or

d) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:95, or

e) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:96, or

f) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:97, or

g) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:98, or

h) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:99, or

i) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:100, or

j) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:101, or

k) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:102, or

l) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:103, or

m) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:104, or

n) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:105, or

o) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:106, or

p) a heavy chain variable domain VH of SEQ ID NO:91 and a light chain variable domain VL of SEQ ID NO:107.

In another aspect, there is provided use of an antibody which binds to human CSF-1R, in the manufacture of a medicament for 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 and wherein the anti-CSF-1R antibody is in combination with an antibody which binds to human PD-L1, wherein the antibody which binds to human CSF-1R used in the combination is characterized in comprising

a) a heavy chain variable domain VH of SEQ ID NO:23 and a light chain variable domain VL of SEQ ID NO:24, or

b) a heavy chain variable domain VH of SEQ ID NO:31 and a light chain variable domain VL of SEQ ID NO:32, or

c) a heavy chain variable domain VH of SEQ ID NO:39 and a light chain variable domain VL of SEQ ID NO:40, or

d) a heavy chain variable domain VH of SEQ ID NO:47 and a light chain variable domain VL of SEQ ID NO:48, or

e) a heavy chain variable domain VH of SEQ ID NO:55 and a light chain variable domain VL of SEQ ID NO:56;

and the antibody which binds to human PD-L1 used in the combination is characterized in comprising

a) a heavy chain variable domain VH of SEQ ID NO:89 and a light chain variable domain VL of SEQ ID NO:92, or

b) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:93, or

c) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:94, or

d) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:95, or

e) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:96, or

f) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:97, or

g) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:98, or

h) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:99, or

i) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:100, or

j) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:101, or

k) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:102, or

l) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:103, or

m) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:104, or

n) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:105, or

o) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:106, or

p) a heavy chain variable domain VH of SEQ ID NO:91 and a light chain variable domain VL of SEQ ID NO:107.

The disclosure further comprises:

A) a method for

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

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

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

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

wherein an antibody which binds to human CSF-1R, is administered in combination with an antibody which binds to human PD-L1,

or

B) a method of 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 and wherein an antibody which binds to human CSF-1R is administered in combination with an antibody which binds to human PD-L1,

wherein the antibody which binds to human CSF-1R used in the combination therapy is characterized in comprising

a) a heavy chain variable domain VH of SEQ ID NO:23 and a light chain variable domain VL of SEQ ID NO:24, or

b) a heavy chain variable domain VH of SEQ ID NO:31 and a light chain variable domain VL of SEQ ID NO:32, or

c) a heavy chain variable domain VH of SEQ ID NO:39 and a light chain variable domain VL of SEQ ID NO:40, or

d) a heavy chain variable domain VH of SEQ ID NO:47 and a light chain variable domain VL of SEQ ID NO:48, or

e) a heavy chain variable domain VH of SEQ ID NO:55 and a light chain variable domain VL of SEQ ID NO:56;

and the antibody which binds to human PD-L1 used in the combination therapy is characterized in comprising

a) a heavy chain variable domain VH of SEQ ID NO:89 and a light chain variable domain VL of SEQ ID NO:92, or

b) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:93, or

c) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:94, or

d) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:95, or

e) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:96, or

f) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:97, or

g) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:98, or

h) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:99, or

i) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:100, or

j) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:101, or

k) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:102, or

l) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:103, or

m) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:104, or

n) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:105, or

o) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:106, or

p) a heavy chain variable domain VH of SEQ ID NO:91 and a light chain variable domain VL of SEQ ID NO:107.

The term “ligand independent” 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)).

The disclosure comprises the combination 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 an antibody which binds to human CSF-1R as described herein is administered in

combination with an anti-PD-L1 antibody as described herein. 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.

The combination therapies of the antibodies described herein show benefits for patients in need of a CSF-1R targeting therapy. The specific anti-CSF-1R antibodies according to the disclosure show efficient antiproliferative activity against ligand–independent and ligand-dependent proliferation and are especially useful inter alia in the treatment of cancer and metastasis in combination with the specific anti-PD-L1 antibodies described herein.

Description of the Figures

Figure 1a-b **1a**: Human Monocytes differentiated into macrophages with coculture of GM-CSF or CSF-1 (100ng/ml ligand). After 6 days differentiation addition of hMab 2F11-e7. Cell viability was measured at day 7 of antibody treatment in a CTG Viability Assay (CellTiterGlo® Promega). Calculation of % cell viability: RLU signals from treated cells divided by RLU signal from untreated control without antibody, (n =4).

1b: Human Monocytes differentiated into macrophages with GM-CSF (M1) or M-CSF (M2) for 7 days. Phenotype analyzed by indirect fluorescence analysis - staining with anti CD163-PE, anti

CD80-PE or anti HLA-DR/DQ/DP-Zenon-Alexa647 labeled. The number in each histogram corresponds to mean ratio fluorescence intensity (MRFI); calculated ratio between mean fluorescence intensity (MFI) of cells stained with the selected antibody (empty histogram) and of corresponding isotype control (negative control; gray filled histogram) (mean ± SD; n ≥ 5).

Figure 2a-d CSF-1 levels in Cynomolgus monkey after application of different dosages of anti-CSF-1R antibody hMab 2F11-e7.

Figure 3 In the presence of TAMs, T cell expansion induced by activation of CD3 and CD28 was suppressed: TAM were isolated from MC38 tumors and co-cultured at the ratios indicated with CFSE-labeled CD8+ T cells in the presence of CD3/CD28 stimulation. T cell proliferation was analyzed after 3 days using bead quantification of CFSElow dividing cells. One representative experiment out of two is depicted as means + SEM of triplicate wells.

Figure 4 Anti tumor Efficacy of <mouse CSF1R> antibody / <PD-L1> antibody combination in the MC38 mouse CRC in vivo model (Kaplan-Meier Plot for Progression of tumor volume > 700 mm3).

Figure 5 Anti tumor Efficacy of <mouse CSF1R> antibody / <PD-L1> antibody combination in the subcutaneous syngeneic CT26.WT colon carcinoma in vivo model (Kaplan-Meier Plot for Progression of tumor volume > 700 mm3).

Detailed Description of the Invention

Many tumors are characterized by a prominent immune cell infiltrate, including macrophages. Initially, the immune cells were thought to be part of a defense mechanism against the tumor, but recent data support the notion that several immune cell populations including macrophages may, in fact, promote tumor progression. Macrophages are characterized by their plasticity. Depending on the cytokine microenvironment, macrophages can exhibit so-called M1 or M2-subtypes. M2 macrophages are engaged in the suppression of tumor immunity. They also play an important role in tissue repair functions such as angiogenesis and tissue remodeling which are coopted by the tumor to support growth. In contrast to tumor promoting M2 macrophages, M1 macrophages exhibit antitumor activity via the secretion of inflammatory cytokines and their engagement in antigen

presentation and phagocytosis (Mantovani, A. et al., Curr. Opin. Immunol. 2 (2010) 231-237).

By secreting various cytokines such as colony stimulating factor 1 (CSF-1) and IL-10, tumor cells are able to recruit and shape macrophages into the M2- subtype, whereas cytokines such as granulocyte macrophage colony stimulating factor (GM-CSF), IFN-gamma program macrophages towards the M1 subtype. Using immunohistochemistry, it is possible to distinguish between a macrophage subpopulation co-expressing CD68 and CD163, which is likely to be enriched for M2 Macrophages, and a subset showing the CD68+/MHC II+, or CD68+/CD80+ immunophenotype, likely to include M1 macrophages. Cell shape, size, and spatial distribution of CD68 and CD163 positive macrophages is consistent with published hypotheses on a tumor-promoting role of M2 macrophages, for example by their preferential location in tumor intersecting stroma, and vital tumor areas. In contrast, CD68+/MHC class II+ macrophages are ubiquitously found. Their hypothetical role in phagocytosis is reflected by clusters of the CD68+/MHC class II+, but CD163- immunophenotype near apoptotic cells and necrotic tumor areas.

The subtype and marker expression of different macrophage subpopulations is linked with their functional state. M2 macrophages can support tumorigenesis by:

a) enhancing angiogenesis via the secretion of angiogenic factors such as VEGF or bFGF,
b) supporting metastasis formation via secretion of matrix metalloproteinases(MMPs), growth factors and migratory factors guiding the tumor cells to the blood stream and setting up the metastatic niche (Wyckoff, J. et al., Cancer Res. 67 (2007) 2649-2656),
c) playing a role in building an immunosuppressive milieu by secreting immunosuppressive cytokines such as IL-4, Il-13, IL-1ra and IL-10, which in turn regulate T regulatory cell function. Conversely CD4 positive T cells have been shown to enhance the activity of tumor promoting macrophages in preclinical models (Mantovani, A. et al., Eur. J. Cancer 40 (2004) 1660-1667; DeNardo, D. et al., Cancer Cell 16 (2009) 91-102).

Accordingly, in several types of cancer (e.g. breast, ovarian, Hodgkin's lymphoma) the prevalence of M2 subtype tumor associated macrophages (TAMs) has been associated with poor prognosis (Bingle, L. et al., J. Pathol. 3 (2002) 254-265; Orre,

M., and Rogers, P.A., Gynecol. Oncol. 1 (1999) 47-50; Steidl, C. et al., N. Engl. J. Med. 10 (2010) 875-885). Recent data show a correlation of CD163 positive macrophage infiltrate in tumors and tumor grade (Kawamura, K. et al., Pathol. Int. 59 (2009) 300-305). TAMs isolated from patient tumors had a tolerant phenotype and were not cytotoxic to tumor cells (Mantovani, A. et al., Eur. J. Cancer 40 (2004) 1660-1667). However, infiltration of TAMs in the presence of cytotoxic T cells correlates with improved survival in non small cell lung cancer and hence reflects a more prominent M1 macrophage infiltrate in this tumor type (Kawai, O. et al., Cancer 6 (2008) 1387-1395).

Recently, a so-called immune signature comprising high numbers of macrophages and CD4 positive T cells, but low numbers of cytotoxic CD8 positive T cells was shown to correlate with reduced overall survival (OS) in breast cancer patients and to represent an independent prognostic factor (DeNardo, D. et al., Cancer Discovery 1 (2011) 54-67).

Consistent with a role for CSF-1 in driving the pro-tumorigenic function of M2 macrophages, high CSF-1 expression in rare sarcomas or locally aggressive connective tissue tumors, such as pigmented villonodular synovitis (PVNS) and tenosynovial giant cell tumor (TGCT) due in part to a translocation of the CSF-1 gene, leads to the accumulation of monocytes and macrophages expressing the receptor for CSF-1, the colony-stimulating factor 1 receptor (CSF-1R) forming the majority of the tumor mass (West, R.B. et al., Proc. Natl. Acad. Sci. USA 3 (2006) 690-695). These tumors were subsequently used to define a CSF-1 dependent macrophage signature by gene expression profiling. In breast cancer and leiomyosarcoma patient tumors this CSF-1 response gene signature predicts poor prognosis (Espinosa, I. et al., Am. J. Pathol. 6 (2009) 2347-2356; Beck, A. et al., Clin. Cancer Res. 3 (2009) 778-787).

CSF-1R belongs to the class III subfamily of receptor tyrosine kinases and is encoded by the c-fms proto-oncogene. Binding of CSF-1 or IL-34 induces receptor dimerization, followed by autophosphorylation and activation of downstream signaling cascades. Activation of CSF-1R regulates the survival, proliferation and differentiation of monocytes and macrophages (Xiong, Y. et al., J. Biol. Chem. 286 (2011) 952-960).

In addition to cells of the monocytic lineage and osteoclasts, which derive from the same hematopoietic precursor as the macrophage, CSF-1R/c-fms has also been

found to be expressed by several human epithelial cancers such as ovarian and breast cancer and in leiomyosarcoma and TGCT/PVNS, albeit at lower expression levels compared to macrophages. As with TGCT/PVNS, elevated levels of CSF-1, the ligand for CSF-1R, in serum as well as ascites of ovarian cancer patients have been correlated with poor prognosis (Scholl, S. et al., Br. J. Cancer 62 (1994) 342-346; Price, F. et al., Am. J. Obstet. Gynecol. 168 (1993) 520-527). Furthermore, a constitutively active mutant form of CSF 1R is able to transform NIH3T3 cells, one of the properties of an oncogene (Chambers, S., Future Oncol 5 (2009) 1429-1440).

Preclinical models provide validation of CSF-1R as an oncology target. Blockade of CSF-1 as well as CSF-1R activity results in reduced recruitment of TAMs. Chemotherapy resulted in elevated CSF-1 expression in tumor cells leading to enhanced TAM recruitment. Blockade of CSF-1R in combination with paclitaxel resulted in activation of CD8 positive cytotoxic T cells leading to reduced tumor growth and metastatic burden in a spontaneous transgenic breast cancer model (DeNardo, D. et al., Cancer Discovery 1 (2011) 54-67).

The human CSF-1R (CSF-1 receptor; synonyms: M-CSF receptor; Macrophage colony-stimulating factor 1 receptor, Fms proto-oncogene, c-fms, SEQ ID NO: 22)) is known since 1986 (Coussens, L., et al., Nature 320 (1986) 277-280). CSF-1R is a growth factor and encoded by the c-fms proto-oncogene (reviewed e.g. in Roth, P. and Stanley, E.R., Curr. Top. Microbiol. Immunol. 181 (1992) 141-167).

CSF-1R is the receptor for the CSF-1R ligands CSF-1 (macrophage colony stimulating factor, also called M-CSF) (SEQ ID No.: 86) and IL-34 (SEQ ID No.: 87) and mediates the biological effects of these cytokines (Sherr, C.J., et al., Cell 41 (1985) 665-676; Lin, H., et al., Science 320 (2008) 807-811). The cloning of the colony stimulating factor-1 receptor (also called c-fms) was described for the first time in Roussel, M.F., et al., Nature 325 (1987) 549-552. In that publication, it was shown that CSF-1R had transforming potential dependent on changes in the C-terminal tail of the protein including the loss of the inhibitory tyrosine 969 phosphorylation which binds Cbl and thereby regulates receptor down regulation (Lee, P.S., et al., Embo J. 18 (1999) 3616-3628).

CSF-1R is a single chain, transmembrane receptor tyrosine kinase (RTK) and a member of the family of immunoglobulin (Ig) motif containing RTKs characterized by 5 repeated Ig-like subdomains D1-D5 in the extracellular domain (ECD) of the receptor (Wang, Z., et al Molecular and Cellular Biology 13 (1993)

5348-5359). The human CSF-1R Extracellular Domain (CSF-1R-ECD) (SEQ ID NO: 64) comprises all five extracellular Ig-like subdomains D1 –D5. The human CSF-1R fragment delD4 (SEQ ID NO: 65) comprises the extracellular Ig-like subdomains D1–D3 and D5, but is missing the D4 subdomain. The human CSF-1R fragment D1-D3 (SEQ ID NO: 66) comprises the respective subdomains D1-D3. The sequences are listed without the signal peptide MGSGPGVLLL LLVATAWHGQ G (SEQ ID NO: 67). The human CSF-1R fragment D4-D3 (SEQ ID NO: 85) comprises the respective subdomains D4-D3.

Currently two CSF-1R ligands that bind to the extracellular domain of CSF-1R are known. The first one is CSF-1 (colony stimulating factor 1, also called M-CSF, macrophage; human CSF-1, SEQ ID NO: 86) and is found extracellularly as a disulfide-linked homodimer (Stanley, E.R. et al., Journal of Cellular Biochemistry 21 (1983) 151-159; Stanley, E.R. et al., Stem Cells 12 Suppl. 1 (1995) 15–24). The second one is IL-34 (human IL-34; SEQ ID NO: 87) (Hume, D. A. , et al, Blood 119 (2012) 1810-1820). Thus in one embodiment the term "CSF-1R ligand" refers to human CSF-1 (SEQ ID NO: 86) and/or human IL-34 (SEQ ID NO: 87).

For experiments often the active 149 amino acid (aa) fragment of human CSF-1 (aa 33-181 of SEQ ID NO: 86) is used. This active 149 aa fragment of human CSF-1 (aa 33-181 of SEQ ID NO: 86) is contained in all 3 major forms of CSF-1 and is sufficient to mediate binding to CSF-1R (Hume, D. A. , et al, Blood 119 (2012) 1810-1820).

The main biological effects of CSF-1R signaling are the differentiation, proliferation, migration, and survival of hematopoietic precursor cells to the macrophage lineage (including osteoclast). Activation of CSF-1R is mediated by its CSF-1R ligands, CSF-1 (M-CSF) and IL-34. Binding of CSF-1 (M-CSF) to CSF-1R induces the formation of homodimers and activation of the kinase by tyrosine phosphorylation (Li, W. et al, EMBO Journal.10 (1991) 277-288; Stanley, E.R., et al., Mol. Reprod. Dev. 46 (1997) 4-10).

The intracellular protein tyrosine kinase domain is interrupted by a unique insert domain that is also present in the other related RTK class III family members that include the platelet derived growth factor receptors (PDGFR), stem cell growth factor receptor (c-Kit) and fins-like cytokine receptor (FLT3). In spite of the structural homology among this family of growth factor receptors, they have distinct tissue-specific functions.

CSF-1R is mainly expressed on cells of the monocytic lineage and in the female reproductive tract and placenta. In addition expression of CSF-1R has been reported in Langerhans cells in skin, a subset of smooth muscle cells (Inaba, T., et al., J. Biol. Chem. 267 (1992) 5693-5699), B cells (Baker, A.H., et al., Oncogene 8 (1993) 371-378) and microglia (Sawada, M., et al., Brain Res. 509 (1990) 119-124). Cells with mutant human CSF-1R ((SEQ ID NO: 23) are known to proliferate independently of ligand stimulation.

As used herein, "binding to human CSF-1R" or "specifically binding to human CSF-1R" or "which binds to human CSF-1R" or "anti-CSF-1R antibody" refers to an antibody specifically binding to the human CSF-1R antigen with a binding affinity of KD-value of 1.0 x 10^{-8} mol/l or lower, in one embodiment of a KD-value of 1.0 x10^{-9} mol/l or lower. The binding affinity is determined with a standard binding assay, such as surface plasmon resonance technique (BIAcore®, GE-Healthcare Uppsala, Sweden). Thus an "antibody binding to human CSF-1R" as used herein refers to an antibody specifically binding to the human CSF-1R antigen with a binding affinity of KD 1.0 x 10^{-8} mol/l or lower (in one embodiment 1.0 x 10^{-8} mol/l - 1.0 x 10^{-13} mol/l), in on embodiment of a KD 1.0 x10^{-9} mol/l or lower (in one embodiment 1.0 x 10^{-9} mol/l - 1.0 x 10^{-13} mol/l).

PD-1/PD-L1/PD-L2 pathway:

An important negative co-stimulatory signal regulating T cell activation is provided by programmed death – 1 receptor (PD-1)(CD279), and its ligand binding partners PD-L1 (B7-H1, CD274; SEQ ID NO: 88) and PD-L2 (B7-DC, CD273). The negative regulatory role of PD-1 was revealed by PD-1 knock outs (Pdcd1-/-), which are prone to autoimmunity. Nishimura et al., Immunity 11: 141-51 (1999); Nishimura et al., Science 291: 319-22 (2001). PD-1 is related to CD28 and CTLA-4, but lacks the membrane proximal cysteine that allows homodimerization. The cytoplasmic domain of PD-1 contains an immunoreceptor tyrosine-based inhibition motif (ITIM, V/IxYxxL/V). PD-1 only binds to PD-L1 and PD-L2. Freeman et al., J. Exp. Med. 192: 1-9 (2000); Dong et al., Nature Med. 5: 1365-1369 (1999); Latchman et al., Nature Immunol. 2: 261-268 (2001); Tseng et al., J. Exp. Med. 193: 839-846 (2001).

PD-1 can be expressed on T cells, B cells, natural killer T cells, activated monocytes and dendritic cells (DCs). PD-1 is expressed by activated, but not by unstimulated human CD4+ and CD8+ T cells, B cells and myeloid cells. This

stands in contrast to the more restricted expression of CD28 and CTLA-4. Nishimura et al., Int. Immunol. 8: 773-80 (1996); Boettler et al., J. Virol. 80: 3532-40 (2006). There are at least 4 variants of PD-1 that have been cloned from activated human T cells, including transcripts lacking (i) exon 2, (ii) exon 3, (iii) exons 2 and 3 or (iv) exons 2 through 4. Nielsen et al., Cell. Immunol. 235: 109-16 (2005). With the exception of PD-1 Δex3, all variants are expressed at similar levels as full length PD-1 in resting peripheral blood mononuclear cells (PBMCs). Expression of all variants is significantly induced upon activation of human T cells with anti-CD3 and anti-CD28. The PD-1 Δex3 variants lacks a transmembrane domain, and resembles soluble CTLA-4, which plays an important role in autoimmunity. Ueda et al., Nature 423: 506-11 (2003). This variant is enriched in the synovial fluid and sera of patients with rheumatoid arthritis. Wan et al., J. Immunol. 177: 8844-50 (2006).

The two PD-1 ligands differ in their expression patterns. PD-L1 is constitutively expressed on mouse T and B cells, CDs, macrophages, mesenchymal stem cells and bone marrow-derived mast cells. Yamazaki et al., J. Immunol. 169: 5538-45 (2002). PD-L1 is expressed on a wide range of nonhematopoietic cells (e.g., cornea, lung, vascular epithelium, liver nonparenchymal cells, mesenchymal stem cells, pancreatic islets, placental synctiotrophoblasts, keratinocytes, etc.) [Keir et al., Annu. Rev. Immunol. 26: 677-704 (2008)], and is upregulated on a number of cell types after activation. Both type I and type II interferons IFN's) upregulate PD-L1. Eppihimer et al., Microcirculation 9: 133-45 (2002); Schreiner et al., J. Neuroimmunol. 155: 172-82 (2004). PD-L1 expression in cell lines is decreased when MyD88, TRAF6 and MEK are inhibited. Liu et al., Blood 110: 296-304 (2007). JAK2 has also been implicated in PD-L1 induction. Lee et al., FEBS Lett. 580: 755-62 (2006); Liu et al., Blood 110: 296-304 (2007). Loss or inhibition of phosphatase and tensin homolog (PTEN), a cellular phosphatase that modified phosphatidylinositol 3-kinase (PI3K) and Akt signaling, increased post-transcriptional PD-L1 expression in cancers. Parsa et al., Nat. Med. 13: 84-88 (2007).

PD-L2 expression is more restricted than PD-L1. PD-L2 is inducibly expressed on DCs, macrophages, and bone marrow-derived mast cells. PD-L2 is also expressed on about half to two-thirds of resting peritoneal B1 cells, but not on conventional B2 B cells. Zhong et al., Eur. J. Immunol. 37: 2405-10 (2007). PD-L2+ B1 cells bind phosphatidylcholine and may be important for innate immune responses against bacterial antigens. Induction of PD-L2 by IFN-gamma is partially

dependent upon NF- κB. Liang et al., Eur. J. Immunol. 33: 2706-16 (2003). PD-L2 can also be induced on monocytes and macrophages by GM-CF, IL-4 and IFN-gamma. Yamazaki et al., J. Immunol. 169: 5538-45 (2002); Loke et al., PNAS 100:5336-41 (2003).

PD-1 signaling typically has a greater effect on cytokine production than on cellular proliferation, with significant effects on IFN-gamma, TNF-alpha and IL-2 production. PD-1 mediated inhibitory signaling also depends on the strength of the TCR signaling, with greater inhibition delivered at low levels of TCR stimulation. This reduction can be overcome by costimulation through CD28 [Freeman et al., J. Exp. Med. 192: 1027-34 (2000)] or the presence of IL-2 [Carter et al., Eur. J. Immunol. 32: 634-43 (2002)].

Evidence is mounting that signaling through PD-L1 and PD-L2 may be bidirectional. That is, in addition to modifying TCR or BCR signaling, signaling may also be delivered back to the cells expressing PD-L1 and PD-L2. While treatment of dendritic cells with a naturally human anti-PD-L2 antibody isolated from a patient with Waldenstrom's macroglobulinemia was not found to upregulate MHC II or B7 costimulatory molecules, such cells did produce greater amount of proinflammatory cytokines, particularly TNF-alpha and IL-6, and stimulated T cell proliferation. Nguyen et al., J. Exp. Med. 196: 1393-98 (2002). Treatment of mice with this antibody also (1) enhanced resistance to transplanted b16 melanoma and rapidly induced tumor-specific CTL. Radhakrishnan et al., J. Immunol. 170: 1830-38 (2003); Radhakrishnan et al., Cancer Res. 64: 4965-72 (2004); Heckman et al., Eur. J. Immunol. 37: 1827-35 (2007); (2) blocked development of airway inflammatory disease in a mouse model of allergic asthma. Radhakrishnan et al., J. Immunol. 173: 1360-65 (2004); Radhakrishnan et al., J. Allergy Clin. Immunol. 116: 668-74 (2005).

Further evidence of reverse signaling into dendritic cells ("DC's") results from studies of bone marrow derived DC's cultured with soluble PD-1 (PD-1 EC domain fused to Ig constant region – "s-PD-1"). Kuipers et al., Eur. J. Immunol. 36: 2472-82 (2006). This sPD-1 inhibited DC activation and increased IL-10 production, in a manner reversible through administration of anti-PD-1.

Additionally, several studies show a receptor for PD-L1 or PD-L2 that is independent of PD-1. B7.1 has already been identified as a binding partner for PD-L1. Butte et al., Immunity 27: 111-22 (2007). Chemical crosslinking studies

suggest that PD-L1 and B7.1 can interact through their IgV-like domains. B7.1:PD-L1 interactions can induce an inhibitory signal into T cells. Ligation of PD-L1 on CD4+ T cells by B7.1 or ligation of B7.1 on CD4+ T cells by PD-L1 delivers an inhibitory signal. T cells lacking CD28 and CTLA-4 show decreased proliferation and cytokine production when stimulated by anti-CD3 plus B7.1 coated beads. In T cells lacking all the receptors for B7.1 (i.e., CD28, CTLA-4 and PD-L1), T cell proliferation and cytokine production were no longer inhibited by anti-CD3 plus B7.1 coated beads. This indicates that B7.1 acts specifically through PD-L1 on the T-cell in the absence of CD28 and CTLA-4. Similarly, T cells lacking PD-1 showed decreased proliferation and cytokine production when stimulated in the presence of anti-CD3 plus PD-L1 coated beads, demonstrating the inhibitory effect of PD-L1 ligation on B7.1 on T cells. When T cells lacking all known receptors for PD-L1 (i.e., no PD-1 and B7.1), T cell proliferation was no longer impaired by anti-CD3 plus PD-L1 coated beads. Thus, PD-L1 can exert an inhibitory effect on T cells either through B7.1 or PD-1.

The direct interaction between B7.1 and PD-L1 suggests that the current understanding of costimulation is incomplete, and underscores the significance to the expression of these molecules on T cells. Studies of PD-L1-/- T cells indicate that PD-L1 on T cells can downregulate T cell cytokine production. Latchman et al., Proc. Natl. Acad. Sci. USA 101: 10691-96 (2004). Because both PD-L1 and B7.1 are expressed on T cells, B cells, DCs and macrophages, there is the potential for directional interactions between B7.1 and PD-L1 on these cells types. Additionally, PD-L1 on non-hematopoietic cells may interact with B7.1 as well as PD-1 on T cells, raising the question of whether PD-L1 is involved in their regulation. One possible explanation for the inhibitory effect of B7.1:PD-L1 interaction is that T cell PD-L1 may trap or segregate away APC B7.1 from interaction with CD28.

As a result, the antagonism of signaling through PD-L1, including blocking PD-L1 from interacting with either PD-1, B7.1 or both, thereby preventing PD-L1 from sending a negative co-stimulatory signal to T-cells and other antigen presenting cells is likely to enhance immunity in response to infection (e.g., acute and chronic) and tumor immunity. In addition, the anti-PD-L1 antibodies of the present invention, may be combined with antagonists of other components of PD-1:PD-L1 signaling, for example, antagonist anti-PD-1 and anti-PD-L2 antibodies.

The term “human PD-L1” refers to the human protein PD-L1 (SEQ ID NO: 88, PD-1 signaling typically). As used herein, "binding to human PD-L1” or "specifically binding to human PD-L1” or “which binds to human PD-L1” or “anti-PD-L1 antibody” refers to an antibody specifically binding to the human PD-L1 antigen with a binding affinity of KD-value of 1.0 x 10^{-8} mol/l or lower, in one embodiment of a KD-value of 1.0 x10^{-9} mol/l or lower. The binding affinity is determined with a standard binding assay, such as surface plasmon resonance technique (BIAcore®, GE-Healthcare Uppsala, Sweden). Thus an “antibody binding to human PD-L1” as used herein refers to an antibody specifically binding to the human PD-L1 antigen with a binding affinity of KD 1.0 x 10^{-8} mol/l or lower (in one embodiment 1.0 x 10^{-8} mol/l - 1.0 x 10^{-13} mol/l), in on embodiment of a KD 1.0 x10^{-9} mol/l or lower (in one embodiment 1.0 x 10^{-9} mol/l - 1.0 x 10^{-13} mol/l).

In one embodiment the antibody which binds to human CSF-1R used in the combination therapy described herein is selected from the group consisting of

hMab 2F11-c11 , hMab 2F11-d8 , hMab 2F11-e7 , hMab 2F11-f12 , and hMab 2F11-g1.

These antibodies are described in WO2011/070024 and are characterized in comprising the following VH and VL sequences as described herein:

Table 1:

anti-CSF-1R antibody	amino acid sequence of the heavy chain variable domain VH, SEQ ID NO:	amino acid sequence of the light chain variable domain VL, SEQ ID NO:
hMab 2F11-c11	23	24
hMab 2F11-d8	31	32
hMab 2F11-e7	39	40
hMab 2F11-f12	47	48
hMab 2F11-g1	55	56

In one embodiment the antibody which binds to human PD-L1 used in the combination therapy described herein is selected from the group consisting of:

243.55.S70, 243.55.H1, 243.55.H12, 243.55.H37, 243.55.H70, 243.55.H89, 243.55.S1, 243.55.5, 243.55.8 , 243.55.30, 243.55.34 , 243.55.S37 , 243.55.49 , 243.55.51, 243.55.62 , and 243.55.84.

These antibodies are described in WO 2010/77634 (sequences are shown in Figure 11 of WO 2010/77634) and are characterized in comprising the following VH and VL sequences as described herein:

Table 2:

anti-PD-L1 antibody	amino acid sequence of the heavy chain variable domain VH, SEQ ID NO:	amino acid sequence of the light chain variable domain VL, SEQ ID NO:
243.55.S70	89	92
243.55.H1	90	93
243.55.H12	90	94
243.55.H37	90	95
243.55.H70	90	96
243.55.H89	90	97
243.55.S1	90	98
243.55.5	90	99
243.55.8	90	100
243.55.30	90	101
243.55.34	90	102
243.55.S37	90	103
243.55.49	90	104
243.55.51	90	105
243.55.62	90	106
243.55.84	91	107

In one embodiment of the invention the antibody which binds to human CSF-1R used in the combination therapy described herein is characterized in comprising

a) a heavy chain variable domain VH of SEQ ID NO:23 and a light chain variable domain VL of SEQ ID NO:24, or

b) a heavy chain variable domain VH of SEQ ID NO:31 and a light chain variable domain VL of SEQ ID NO:32, or

c) a heavy chain variable domain VH of SEQ ID NO:39 and a light chain variable domain VL of SEQ ID NO:40, or

d) a heavy chain variable domain VH of SEQ ID NO:47 and a light chain variable domain VL of SEQ ID NO:48, or

e) a heavy chain variable domain VH of SEQ ID NO:55 and a light chain variable domain VL of SEQ ID NO:56; and

the antibody which binds to human PD-L1 used in the combination therapy is characterized in comprising

a) a heavy chain variable domain VH of SEQ ID NO:89 and a light chain variable domain VL of SEQ ID NO:92, or

b) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:93, or

c) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:94, or

d) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:95, or

e) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:96, or

f) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:97, or

g) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:98, or

h) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:99, or

i) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:100, or

j) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:101, or

k) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:102, or

l) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:103, or

m) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:104, or

n) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:105, or

o) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:106, or

p) a heavy chain variable domain VH of SEQ ID NO:91 and a light chain variable domain VL of SEQ ID NO:107.

In one embodiment the antibody which binds to human CSF-1R used in the combination therapy is characterized in comprising

a heavy chain variable domain VH of SEQ ID NO:23 and a light chain variable domain VL of SEQ ID NO:24.

In one embodiment the antibody which binds to human CSF-1R used in the combination therapy is characterized in comprising

a heavy chain variable domain VH of SEQ ID NO:31 and a light chain variable domain VL of SEQ ID NO:32.

In one embodiment the antibody which binds to human CSF-1R used in the combination therapy is characterized in comprising

a heavy chain variable domain VH of SEQ ID NO:39 and a light chain variable domain VL of SEQ ID NO:40.

In one embodiment the antibody which binds to human CSF-1R used in the combination therapy is characterized in comprising

a heavy chain variable domain VH of SEQ ID NO:47 and a light chain variable domain VL of SEQ ID NO:48.

In one embodiment the antibody which binds to human PD-L1 used in the combination therapy is characterized in comprising

a heavy chain variable domain VH of SEQ ID NO:89 and a light chain variable domain VL of SEQ ID NO:92.

In one embodiment the antibody which binds to human PD-L1 used in the combination therapy is characterized in comprising

a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:93.

In one embodiment the antibody which binds to human PD-L1 used in the combination therapy is characterized in comprising

a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:94.

In one embodiment the antibody which binds to human PD-L1 used in the combination therapy is characterized in comprising

a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:95.

In one embodiment the antibody which binds to human PD-L1 used in the combination therapy is characterized in comprising

a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:96.

In one embodiment the antibody which binds to human PD-L1 used in the combination therapy is characterized in comprising

a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:97.

In one embodiment the antibody which binds to human PD-L1 used in the combination therapy is characterized in comprising

a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:98.

In one embodiment the antibody which binds to human PD-L1 used in the combination therapy is characterized in comprising

a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:99.

In one embodiment the antibody which binds to human PD-L1 used in the combination therapy is characterized in comprising

a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:100.

In one embodiment the antibody which binds to human PD-L1 used in the combination therapy is characterized in comprising

a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:101.

In one embodiment the antibody which binds to human PD-L1 used in the combination therapy is characterized in comprising

a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:102.

In one embodiment the antibody which binds to human PD-L1 used in the combination therapy is characterized in comprising

a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:103.

In one embodiment the antibody which binds to human PD-L1 used in the combination therapy is characterized in comprising

a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:104.

In one embodiment the antibody which binds to human PD-L1 used in the combination therapy is characterized in comprising

a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:105.

In one embodiment the antibody which binds to human PD-L1 used in the combination therapy is characterized in comprising

a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:106.

In one embodiment the antibody which binds to human PD-L1 used in the combination therapy is characterized in comprising

a heavy chain variable domain VH of SEQ ID NO:91 and a light chain variable domain VL of SEQ ID NO:107.

In one preferred embodiment of the invention the antibody which binds to human CSF-1R used in the combination therapy described herein is characterized in comprising

a heavy chain variable domain VH of SEQ ID NO:39 and a light chain variable domain VL of SEQ ID NO:40, and

the antibody which binds to human PD-L1 used in the combination therapy is characterized in comprising

a heavy chain variable domain VH of SEQ ID NO:89 and a light chain variable domain VL of SEQ ID NO:92.

The term "epitope" denotes a protein determinant of human CSF-1R or PD-L1 capable of specifically binding to an antibody. Epitopes usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually epitopes have specific three dimensional structural characteristics, as well as specific charge characteristics. Conformational and nonconformational epitopes are distinguished in that the binding to the former but not the latter is lost in the presence of denaturing solvents.

The "variable domain" (light chain variable domain VL, heavy chain variable domain VH) as used herein denotes each of the pair of light and heavy chain domains which are involved directly in binding the antibody to the antigen. The variable light and heavy chain domains have the same general structure and each domain comprises four framework (FR) regions whose sequences are widely conserved, connected by three "hypervariable regions" (or complementary determining regions, CDRs). The framework regions adopt a beta-sheet conformation and the CDRs may form loops connecting the beta-sheet structure. The CDRs in each chain are held in their three-dimensional structure by the framework regions and form together with the CDRs from the other chain the antigen binding site. The antibody's heavy and light chain CDR3 regions play a

particularly important role in the binding specificity/affinity of the antibodies according to the invention and therefore provide a further object of the invention.

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

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

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

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

ADCC (antibody-dependent cell-mediated cytotoxicity) and CDC (complement-dependent cytotoxicity) based on complement activation, C1q binding and Fc receptor binding. Complement activation (CDC) is initiated by binding of complement factor C1q to the Fc part of most IgG antibody subclasses. While the influence of an antibody on the complement system is dependent on certain conditions, binding to C1q is caused by defined binding sites in the Fc part. Such binding sites are known in the state of the art and described e.g. by Boackle, R.J., et al., Nature 282 (1979) 742-743; Lukas, T.J., et al., J. Immunol. 127 (1981) 2555-2560; Brunhouse, R., and Cebra, J.J., Mol. Immunol. 16 (1979) 907-917; Burton, D.R., et al., Nature 288 (1980) 338-344; Thommesen, J.E., et al., Mol. Immunol. 37 (2000) 995-1004; Idusogie, E.E., et al., J. Immunol.164 (2000) 4178-4184; Hezareh, M., et al., J. Virology 75 (2001) 12161-12168; Morgan, A., et al., Immunology 86 (1995) 319-324; EP 0 307 434. Such binding sites are e.g. L234, L235, D270, N297, E318, K320, K322, P331 and P329 (numbering according to EU index of Kabat, E.A., see below). Antibodies of subclass IgG1, IgG2 and IgG3 usually show complement activation and C1q and C3 binding, whereas IgG4 do not activate the complement system and do not bind C1q and C3.

In one embodiment the antibody according to the invention comprises an Fc part derived from human origin and preferably all other parts of the human constant regions. As used herein the term “Fc part derived from human origin” denotes a Fc part which is either a Fc part of a human antibody of the subclass IgG1, IgG2, IgG3 or IgG4, preferably a Fc part from human IgG1 subclass, a mutated Fc part from human IgG1 subclass (in one embodiment with a mutation on L234A + L235A), a Fc part from human IgG4 subclass or a mutated Fc part from human IgG4 subclass (in one embodiment with a mutation on S228P). In one preferred embodiment the human heavy chain constant region is SEQ ID NO: 58 (human IgG1 subclass), in another preferred embodiment the human heavy chain constant region is SEQ ID NO: 59 (human IgG1 subclass with mutations L234A and L235A) , in another preferred embodiment the human heavy chain constant region is SEQ ID NO: 60 (human IgG4 subclass), and in another preferred embodiment the human heavy chain constant region is SEQ ID NO: 61 (human IgG4 subclass with mutation S228P). In one embodiment said antibodies have reduced or minimal effector function. In one embodiment the minimal effector function results from an effectorless Fc mutation. In one embodiment the effectorless Fc mutation is L234A/L235A or L234A/L235A/P329G or N297A or D265A/N297A. In one embodiment the effectorless Fc mutation is selected for each of the antibodies

independently of each other from the group comprising (consisting of) L234A/L235A, L234A/L235A/P329G, N297A and D265A/N297A.

In one embodiment the antibodies described herein are of human IgG class (i.e. of IgG1, IgG2, IgG3 or IgG4 subclass).

In a preferred embodiment the antibodies described herein are of human IgG1 subclass or of human IgG4 subclass. In one embodiment the described herein are of human IgG1 subclass. In one embodiment the antibodies described herein are of human IgG4 subclass.

In one embodiment the antibody described herein is characterized in that the constant chains are of human origin. Such constant chains are well known in the state of the art and e.g. described by Kabat, E.A., (see e.g. Johnson, G. and Wu, T.T., Nucleic Acids Res. 28 (2000) 214-218). For example, a useful human heavy chain constant region comprises an amino acid sequence of SEQ ID NO: 58. For example, a useful human light chain constant region comprises an amino acid sequence of a kappa-light chain constant region of SEQ ID NO: 57.

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

The invention comprises the use of an antibody according to the invention for the described therapy.

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

There are 3 distinct mechanisms by which CSF-1R signaling is likely involved in tumor growth and metastasis. The first is that expression of CSF-ligand and receptor has been found in tumor cells originating in the female reproductive system (breast, ovarian, endometrium, cervical) (Scholl, S.M., et al., J. Natl. Cancer Inst. 86 (1994) 120-126; Kacinski, B.M., Mol. Reprod. Dev. 46 (1997) 71-74; Ngan, H.Y., et al., Eur. J. Cancer 35 (1999) 1546-1550; Kirma, N., et al., Cancer Res 67 (2007) 1918-1926) and the expression has been associated with

breast cancer xenograft growth as well as poor prognosis in breast cancer patients. Two point mutations were seen in CSF-1R in about 10-20% of acute myelocytic leukemia, chronic myelocytic leukemia and myelodysplasia patients tested in one study, and one of the mutations was found to disrupt receptor turnover (Ridge, S.A., et al., Proc. Natl. Acad. Sci USA 87 (1990) 1377-1380). However the incidence of the mutations could not be confirmed in later studies (Abu-Duhier, F.M., et al., Br. J. Haematol. 120 (2003) 464-470). Mutations were also found in some cases of hepatocellular cancer (Yang, D.H., et al., Hepatobiliary Pancreat. Dis. Int. 3 (2004) 86-89) and idiopathic myelofibrosis (Abu-Duhier, F.M., et al., Br. J. Haematol. 120 (2003) 464-470). Recently, in the GDM-1 cell line derived from a patient with myelomonoblastic leukemia the Y571D mutation in CSF-1R was identified (Chase, A., et al., Leukemia 23 (2009) 358-364).

Pigmented villonodular synovitis (PVNS) and Tenosynovial Giant cell tumors (TGCT) can occur as a result of a translocation that fuses the M-CSF gene to a collagen gene COL6A3 and results in overexpression of M-CSF (West, R.B., et al., Proc. Natl. Acad. Sci. USA 103 (2006) 690-695). A landscape effect is proposed to be responsible for the resulting tumor mass that consists of monocytic cells attracted by cells that express M-CSF. TGCTs are smaller tumors that can be relatively easily removed from fingers where they mostly occur. PVNS is more aggressive as it can recur in large joints and is not as easily controlled surgically.

The second mechanism is based on blocking signaling through M-CSF/CSF-1R at metastatic sites in bone which induces osteoclastogenesis, bone resorption and osteolytic bone lesions. Breast, multiple myeloma and lung cancers are examples of cancers that have been found to metastasize to the bone and cause osteolytic bone disease resulting in skeletal complications. M-CSF released by tumor cells and stroma induces the differentiation of hematopoietic myeloid monocyte progenitors to mature osteoclasts in collaboration with the receptor activator of nuclear factor kappa-B ligand-RANKL. During this process, M-CSF acts as a permissive factor by giving the survival signal to osteoclasts (Tanaka, S., et al., J. Clin. Invest. 91 (1993) 257-263). Inhibition of CSF-1R activity during osteoclast differentiation and maturation with an anti-CSF-1R antibody is likely to prevent unbalanced activity of osteoclasts that cause osteolytic disease and the associated skeletal related events in metastatic disease. Whereas breast, lung cancer and multiple myeloma typically result in osteolytic lesions, metastasis to the bone in prostate cancer initially has an osteoblastic appearance in which increased bone forming activity results in 'woven bone' which is different from typical lamellar structure of

normal bone. During disease progression bone lesions display a significant osteolytic component as well as high serum levels of bone resorption and suggests that anti-resorptive therapy may be useful. Bisphosphonates have been shown to inhibit the formation of osteolytic lesions and reduced the number of skeletal-related events only in men with hormone-refractory metastatic prostate cancer but at this point their effect on osteoblastic lesions is controversial and bisphosphonates have not been beneficial in preventing bone metastasis or hormone responsive prostate cancer to date. The effect of anti-resorptive agents in mixed osteolytic/osteoblastic prostate cancer is still being studied in the clinic (Choueiri, M.B., et al., Cancer Metastasis Rev. 25 (2006) 601-609; Vessella, R.L. and Corey, E., Clin. Cancer Res. 12 (20 Pt 2) (2006) 6285s-6290s).

The third mechanism is based on the recent observation that tumor associated macrophages (TAM) found in solid tumors of the breast, prostate, ovarian and cervical cancers correlated with poor prognosis (Bingle, L., et al., J. Pathol. 196 (2002) 254-265; Pollard, J.W., Nat. Rev. Cancer 4 (2004) 71-78). Macrophages are recruited to the tumor by M-CSF and other chemokines. The macrophages can then contribute to tumor progression through the secretion of angiogenic factors, proteases and other growth factors and cytokines and may be blocked by inhibition of CSF-1R signaling. Recently it was shown by Zins et a1 (Zins, K., et al., Cancer Res. 67 (2007) 1038-1045) that expression of siRNA of Tumor necrosis factor alpha (TNF alpha), M-CSF or the combination of both would reduce tumor growth in a mouse xenograft model between 34% and 50% after intratumoral injection of the respective siRNA. SiRNA targeting the TNF alpha secreted by the human SW620 cells reduced mouse M-CSF levels and led to reduction of macrophages in the tumor. In addition treatment of MCF7 tumor xenografts with an antigen binding fragment directed against M-CSF did result in 40% tumor growth inhibition, reversed the resistance to chemotherapeutics and improved survival of the mice when given in combination with chemotherapeutics (Paulus, P., et al., Cancer Res. 66 (2006) 4349-4356).

TAMs are only one example of an emerging link between chronic inflammation and cancer. There is additional evidence for a link between inflammation and cancer as many chronic diseases are associated with an increased risk of cancer, cancers arise at sites of chronic inflammation, chemical mediators of inflammation are found in many cancers; deletion of the cellular or chemical mediators of inflammation inhibits development of experimental cancers and long-term use of anti-inflammatory agents reduce the risk of some cancers. A link to cancer exists

for a number of inflammatory conditions among- those H.pylori induced gastritis for gastric cancer, Schistosomiasis for bladder cancer, HHVX for Kaposi's sarcoma, endometriosis for ovarian cancer and prostatitis for prostate cancer (Balkwill, F., et al., Cancer Cell 7 (2005) 211-217). Macrophages are key cells in chronic inflammation and respond differentially to their microenvironment. There are two types of macrophages that are considered extremes in a continuum of functional states: M1 macrophages are involved in Type 1 reactions. These reactions involve the activation by microbial products and consequent killing of pathogenic microorganisms that result in reactive oxygen intermediates. On the other end of the extreme are M2 macrophages involved in Type 2 reactions that promote cell proliferation, tune inflammation and adaptive immunity and promote tissue remodeling, angiogenesis and repair (Mantovani, A., et al., Trends Immunol. 25 (2004) 677-686). Chronic inflammation resulting in established neoplasia is usually associated with M2 macrophages. A pivotal cytokine that mediates inflammatory reactions is TNF alpha that true to its name can stimulate anti-tumor immunity and hemorrhagic necrosis at high doses but has also recently been found to be expressed by tumor cells and acting as a tumor promoter (Zins, K., et al., Cancer Res. 67 (2007) 1038-1045; Balkwill, F., Cancer Metastasis Rev. 25 (2006) 409-416). The specific role of macrophages with respect to the tumor still needs to be better understood including the potential spatial and temporal dependence on their function and the relevance to specific tumor types.

Thus one embodiment of the invention are the CSF-1R antibodies described herein in for use in the treatment of cancer in combination with an anti-PD-L1 antibody as described herein. The term “cancer” as used herein may be, for example, lung cancer, non small cell lung (NSCL) cancer, bronchioloalviolar cell lung cancer, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, gastric cancer, colon cancer, breast cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, prostate cancer, cancer of the bladder, cancer of the kidney or ureter, renal cell carcinoma, carcinoma of the renal pelvis, mesothelioma, hepatocellular cancer, biliary cancer, neoplasms of the central nervous system (CNS), spinal axis tumors,

brain stem glioma, glioblastoma multiforme, astrocytomas, schwanomas, ependymonas, medulloblastomas, meningiomas, squamous cell carcinomas, pituitary adenoma, lymphoma, lymphocytic leukemia, including refractory versions of any of the above cancers, or a combination of one or more of the above cancers. In one preferred embodiment such cancer is a breast cancer, colorectal cancer, melanoma, head and neck cancer, lung cancer or prostate cancer. In one preferred embodiment such cancer is a breast cancer, ovarian cancer, cervical cancer, lung cancer or prostate cancer. In another preferred embodiment such cancer is breast cancer, lung cancer, colon cancer, ovarian cancer, melanoma cancer, bladder cancer, renal cancer, kidney cancer, liver cancer, head and neck cancer, colorectal cancer, pancreatic cancer, gastric carcinoma cancer, esophageal cancer, mesothelioma, prostate cancer, leukemia, lymphoma, myelomas. In one preferred embodiment such cancers are further characterized by CSF-1 or CSF-1R expression or overexpression. One further embodiment the invention are the CSF-1R antibodies of the present invention for use in the simultaneous treatment of primary tumors and new metastases. Thus another embodiment of the invention are the CSF-1R antibodies of the present invention for use in the treatment of periodontitis, histiocytosis X, osteoporosis, Paget's disease of bone (PDB), bone loss due to cancer therapy, periprosthetic osteolysis, glucocorticoid-induced osteoporosis, rheumatoid arthritis, psiratic arthritis, osteoarthritis, inflammatory arthridities, and inflammation.

Rabello, D., et al., Biochem. Biophys. Res. Commun. 347 (2006) 791-796 has demonstrated that SNPs in the CSF1 gene exhibited a positive association with aggressive periodontitis: an inflammatory disease of the periodontal tissues that causes tooth loss due to resorption of the alveolar bone.

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

The pathophysiology of osteoporosis is mediated by loss of bone forming osteoblasts and increased osteoclast dependent bone resorption. Supporting data

has been described by Cenci et al showing that an anti-M-CSF antibody injection preserves bone density and inhibits bone resorption in ovariectomized mice (Cenci, S., et al., J. Clin. Invest. 105 (2000) 1279-1287). Recently a potential link between postmenopausal bone loss due to estrogen deficiency was identified and found that the presence of TNF alpha producing T-cell affected bone metabolism (Roggia, C., et al., Minerva Med. 95 (2004) 125-132). A possible mechanism could be the induction of M-CSF by TNF alpha in vivo. An important role for M-CSF in TNF-alpha-induced osteoclastogenesis was confirmed by the effect of an antibody directed against M-CSF that blocked the TNF alpha induced osteolysis in mice and thereby making inhibitors of CSF-1R signaling potential targets for inflammatory arthritis (Kitaura, H., et al., J. Clin. Invest. 115 (2005) 3418-3427).

Paget's disease of bone (PDB) is the second most common bone metabolism disorder after osteoporosis in which focal abnormalities of increased bone turnover lead to complications such as bone pain, deformity, pathological fractures and deafness. Mutations in four genes have been identified that regulate normal osteoclast function and predispose individuals to PDB and related disorders: insertion mutations in TNFRSF11A, which encodes receptor activator of nuclear factor (NF) kappaB (RANK)-a critical regulator of osteoclast function, inactivating mutations of TNFRSF11B which encodes osteoprotegerin (a decoy receptor for RANK ligand), mutations of the sequestosome 1 gene (SQSTM1), which encodes an important scaffold protein in the NFkappaB pathway and mutations in the valosin-containing protein (VCP) gene. This gene encodes VCP, which has a role in targeting the inhibitor of NFkappaB for degradation by the proteasome (Daroszewska, A. and Ralston, S.H., Nat. Clin. Pract. Rheumatol. 2 (2006) 270-277). Targeted CSF-1R inhibitors provide an opportunity to block the deregulation of the RANKL signaling indirectly and add an additional treatment option to the currently used bisphosphonates.

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

ablation therapy in prostate cancer which leads to loss of bone mineral density and significantly increases the risk of osteoporosis-related fractures (Stoch, S.A., et al., J. Clin. Endocrinol. Metab. 86 (2001) 2787-2791).

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

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

Rheumatoid arthritis, psioratic arthritis and inflammatory arthridities are in itself potential indications for CSF-1R signaling inhibitors in that they consist of a macrophage component and to a varying degree bone destruction (Ritchlin, C.T., et al., J. Clin. Invest. 111 (2003) 821-831). Osteoarthritis and rheumatoid arthritis are inflammatory autoimmune disease caused by the accumulation of macrophages in the connective tissue and infiltration of macrophages into the synovial fluid, which is at least partially mediated by M-CSF. Campbell, I., K., et al., J. Leukoc. Biol. 68 (2000) 144-150, demonstrated that M-CSF is produced by human-joint tissue cells (chondrocytes, synovial fibroblasts) in vitro and is found in synovial fluid of patients with rheumatoid arthritis, suggesting that it contributes to the synovial tissue proliferation and macrophage infiltration which is associated with the pathogenesis of the disease. Inhibition of CSF-1R signaling is likely to control the number of macrophages in the joint and alleviate the pain from the associated bone destruction. In order to minimize adverse effects and to further understand the impact of the CSF-1R signaling in these indications, one method is to specifically inhibit CSF-1R without targeting a myriad other kinases, such as Raf kinase.

Recent literature reports correlate increased circulating M-CSF with poor prognosis and atherosclerotic progression in chronic coronary artery disease (Saitoh, T., et al., J. Am. Coll. Cardiol. 35 (2000) 655-665; Ikonomidis, I., et al., Eur. Heart. J. 26 (2005) p. 1618-1624); M-CSF influences the atherosclerotic process by aiding the formation of foam cells (macrophages with ingested oxidized LDL) that express CSF-1R and represent the initial plaque (Murayama, T., et al., Circulation 99 (1999) 1740-1746).

Expression and signaling of M-CSF and CSF-1R is found in activated microglia. Microglia, which are resident macrophages of the central nervous system, can be activated by various insults, including infection and traumatic injury. M-CSF is considered a key regulator of inflammatory responses in the brain and M-CSF levels increase in HIV-1, encephalitis, Alzheimer's disease (AD) and brain tumors. Microgliosis as a consequence of autocrine signaling by M-CSF/CSF-1R results in induction of inflammatory cytokines and nitric oxides being released as demonstrated by e.g. using an experimental neuronal damage model (Hao, A.J., et al., Neuroscience 112 (2002) 889-900; Murphy, G.M., Jr., et al., J. Biol. Chem. 273 (1998) 20967-20971). Microglia that have increased expression of CSF-1R are found to surround plaques in AD and in the amyloid precursor protein V717F transgenic mouse model of AD (Murphy, G.M., Jr., et al., Am. J. Pathol. 157 (2000) 895-904). On the other hand op/op mice with fewer microglia in the brain resulted in fibrillar deposition of A-beta and neuronal loss compared to normal control suggesting that microglia do have a neuroprotective function in the development of AD lacking in the op/op mice (Kaku, M., et al., Brain Res. Brain Res. Protoc. 12 (2003) 104-108).

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

Thus another embodiment of the invention are the CSF-1R antibodies being characterized by the above mentioned amino acid sequences and amino acid sequence in combination with an anti-PD-L1 antibody being characterized by the above mentioned amino acid sequences and amino acid sequence for use in the treatment of periodontitis, histiocytosis X, osteoporosis, Paget's disease of bone (PDB), bone loss due to cancer therapy, periprosthetic osteolysis, glucocorticoid-

induced osteoporosis, rheumatoid arthritis, psiratic arthritis, osteoarthritis, inflammatory arthridities, and inflammation.

The invention comprises the combination therapy with an antibody binding to human CSF-1R being characterized by the above mentioned amino acid sequences and amino acid sequence fragments with an anti-PD-L1 antibody being characterized by the above mentioned amino acid sequences and amino acid sequence fragments for the treatment of cancer.

The invention comprises the combination therapy with an antibody binding to human CSF-1R being characterized by the above mentioned amino acid sequences and amino acid sequence fragments with an anti-PD-L1 antibody being characterized by the above mentioned amino acid sequences and amino acid sequence fragments for the treatment of bone loss.

The invention comprises the combination therapy with an antibody binding to human CSF-1R being characterized by the above mentioned amino acid sequences and amino acid sequence fragments with an anti-PD-L1 antibody being characterized by the above mentioned amino acid sequences and amino acid sequence fragments for the prevention or treatment of metastasis.

The invention comprises the combination therapy of antibody binding to human CSF-1R being characterized by the above mentioned amino acid sequences and amino acid sequence fragments with an anti-PD-L1 antibody being characterized by the above mentioned amino acid sequences and amino acid sequence fragments for treatment of inflammatory diseases.

The invention comprises the combination therapy of antibody binding to human CSF-1R being characterized by the above mentioned amino acid sequences and amino acid sequence fragments with an anti-PD-L1 antibody being characterized by the above mentioned amino acid sequences and amino acid sequence fragments for use in treating or delaying progression of an immune related disease such as tumor immunity.

The invention comprises the combination therapy of antibody binding to human CSF-1R being characterized by the above mentioned amino acid sequences and amino acid sequence fragments with an anti-PD-L1 antibody being characterized by the above mentioned amino acid sequences and amino acid

sequence fragments for use in stimulating an immune response or function, such as T cell activity.

The invention comprises the use of an antibody characterized in comprising the antibody binding to human CSF-1R being characterized by the above mentioned amino acid sequences and amino acid sequence fragments for the combination treatment of cancer with an anti-PD-L1 antibody or alternatively for the manufacture of a medicament for the combination treatment of cancer with an anti-PD-L1 antibody as described herein.

The invention comprises the use of an antibody characterized in comprising the antibody binding to human CSF-1R being characterized by the above mentioned amino acid sequences and amino acid sequence fragments for the combination treatment of bone loss with an anti-PD-L1 antibody as described herein or alternatively for the manufacture of a medicament for the combination treatment of bone loss with an anti-PD-L1 antibody as described herein.

The invention comprises the use of an antibody characterized in comprising the antibody binding to human CSF-1R being characterized by the above mentioned amino acid sequences and amino acid sequence fragments for the prevention or treatment of metastasis in the combination with an anti-PD-L1 antibody as described herein or alternatively for the manufacture of a medicament for the prevention or treatment of metastasis in the combination with an anti-PD-L1 antibody as described herein.

The invention comprises the use of an antibody characterized in comprising the antibody binding to human CSF-1R being characterized by the above mentioned amino acid sequences and amino acid sequence fragments for combination treatment of inflammatory diseases with an anti-PD-L1 antibody as described herein or alternatively for the manufacture of a medicament for the combination treatment of inflammatory diseases with an anti-PD-L1 antibody as described herein.

The invention comprises the use of an antibody characterized in comprising the antibody binding to human CSF-1R being characterized by the above mentioned amino acid sequences and amino acid sequence fragments for use in treating or delaying progression of an immune related disease such as tumor immunity in combination with an anti-PD-L1 antibody as described

herein or alternatively for the manufacture of a medicament for use in treating or delaying progression of an immune related disease such as tumor immunity in combination with an anti-PD-L1 antibody as described herein.

The invention comprises the use of an antibody characterized in comprising the antibody binding to human CSF-1R being characterized by the above mentioned amino acid sequences and amino acid sequence fragments for use in stimulating an immune response or function, such as T cell activity in combination with an anti-PD-L1 antibody as described herein or alternatively for the manufacture of a medicament for use in stimulating an immune response or function, such as T cell activity in combination with an anti-PD-L1 antibody as described herein.

In one preferred embodiment of the invention the antibody which binds to human CSF-1R used in the above described combination treatments and medical uses of different diseases is characterized in comprising

a heavy chain variable domain VH of SEQ ID NO:39 and a light chain variable domain VL of SEQ ID NO:40, and

the antibody which binds to human PD-L1 used in such combination treatments is characterized in comprising

a heavy chain variable domain VH of SEQ ID NO:89 and a light chain variable domain VL of SEQ ID NO:92.

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

Recombinant production of antibodies is well-known in the state of the art and described, for example, in the review articles of Makrides, S.C., Protein Expr.

Purif. 17 (1999) 183-202; Geisse, S., et al., Protein Expr. Purif. 8 (1996) 271-282; Kaufman, R.J., Mol. Biotechnol. 16 (2000) 151-161; Werner, R.G., Drug Res. 48 (1998) 870-880.

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

Expression in NS0 cells is described by, e.g., Barnes, L.M., et al., Cytotechnology 32 (2000) 109-123; Barnes, L.M., et al., Biotech. Bioeng. 73 (2001) 261-270. Transient expression is described by, e.g., Durocher, Y., et al., Nucl. Acids. Res. 30 (2002) E9. Cloning of variable domains is described by Orlandi, R., et al., Proc. Natl. Acad. Sci. USA 86 (1989) 3833-3837; Carter, P., et al., Proc. Natl. Acad. Sci. USA 89 (1992) 4285-4289; Norderhaug, L., et al., J. Immunol. Methods 204 (1997) 77-87. A preferred transient expression system (HEK 293) is described by Schlaeger, E.-J. and Christensen, K., in Cytotechnology 30 (1999) 71-83, and by Schlaeger, E.-J., in J. Immunol. Methods 194 (1996) 191-199.

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

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

Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the

sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading frame. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

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

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

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

As used herein, “pharmaceutically acceptable carrier” includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption/resorption delaying agents, and the like that are physiologically compatible. Preferably, the carrier is suitable for injection or infusion.

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

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

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

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

The term "a method of treating" or its equivalent, when applied to, for example, cancer refers to a procedure or course of action that is designed to reduce or eliminate the number of cancer cells in a patient, or to alleviate the symptoms of a cancer. "A method of treating" cancer or another proliferative disorder does not necessarily mean that the cancer cells or other disorder will, in fact, be eliminated, that the number of cells or disorder will, in fact, be reduced, or that the symptoms of a cancer or other disorder will, in fact, be alleviated. Often, a method of treating cancer will be performed even with a low likelihood of success, but which, given

the medical history and estimated survival expectancy of a patient, is nevertheless deemed to induce an overall beneficial course of action.

The terms "administered in combination with" or "co-administration", "co-administering", "combination therapy" or "combination treatment" refer to the administration of the anti-CSF-1R as described herein, and the anti-PD-L1 antibody as described herein e.g. as separate formulations/applications (or as one single formulation/application). The co-administration can be simultaneous or sequential in either order, wherein preferably there is a time period while both (or all) active agents simultaneously exert their biological activities. Said antibody and said further agent are co-administered either simultaneously or sequentially (e.g. intravenous (i.v.) through a continuous infusion. When both therapeutic agents are co-administered sequentially the dose is administered either on the same day in two separate administrations, or one of the agents is administered on day 1 and the second is co-administered on day 2 to day 7, preferably on day 2 to 4. Thus in one embodiment the term "sequentially" means within 7 days after the dose of the first component, preferably within 4 days after the dose of the first component; and the term "simultaneously" means at the same time. The terms "co-administration" with respect to the maintenance doses of anti-CSF-1R antibody and/or anti-PD-L1 antibody mean that the maintenance doses can be either co-administered simultaneously, if the treatment cycle is appropriate for both drugs, e.g. every week. Or the further agent is e.g. administered e.g. every first to third day and said antibody is administered every week. Or the maintenance doses are co-administered sequentially, either within one or within several days.

It is self-evident that the antibodies are administered to the patient in a "therapeutically effective amount" (or simply "effective amount") which is the amount of the respective compound or combination that will elicit the biological or medical response of a tissue, system, animal or human that is being sought by the researcher, veterinarian, medical doctor or other clinician.

The amount of co-administration and the timing of co-administration will depend on the type (species, gender, age, weight, etc.) and condition of the patient being treated and the severity of the disease or condition being treated. Said anti-CSF-1R antibody and further agent are suitably co-administered to the patient at one time or over a series of treatments e.g. on the same day or on the day after.

Depending on the type and severity of the disease, about 0.1 mg /kg to 50 mg/kg (e.g. 0.1-20 mg/kg) of said anti-CSF-1R antibody and/or anti-PD-L1 antibody; is an initial candidate dosage for co-administration of both drugs to the patient The invention comprises the use of the antibodies according to the invention for the treatment of a patient suffering from cancer, especially from colon, lung or pancreas cancer.

Depending on the type and severity of the disease, about 0.1 mg /kg to 50 mg/kg (e.g. 0.1-20 mg/kg) of said anti-CSF-1R antibody and/or anti-PD-L1 antibody; is an initial candidate dosage for co-administration of both drugs to the patient The invention comprises the use of the antibodies according to the invention for the treatment of a patient suffering from cancer, especially from colon, lung or pancreas cancer.

In addition to the anti-CSF-1R antibody in combination with the anti-PD-L1 antibody also a chemotherapeutic agent can be administered.

In one embodiment such additional chemotherapeutic agents, which may be administered with anti-CSF-1R antibody as described herein and the anti-PD-L1 antibody as described herein , 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 actinomycin D, daunomycin (rubidomycin), doxorubicin, mitoxantrone, idarubicin, bleomycins, plicamycin (mithramycin), mitomycin C, 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 fluoxymesterone/equivalents; antiandrogens such as flutamide, gonadotropin-releasing hormone analogs and leuprolide; and non-steroidal antiandrogens such as flutamide. Therapies targeting epigenetic mechanism including, but not limited to, histone deacetylase inhibitors, demethylating agents (e.g., Vidaza) and release of transcriptional repression (ATRA) therapies can also be combined with the antigen binding proteins. In one embodiment the chemotherapeutic agent is selected from the group consisting of taxanes (like e.g. paclitaxel (Taxol), docetaxel (Taxotere), modified paclitaxel (e.g., Abraxane and Opaxio), doxorubicin, sunitinib (Sutent), sorafenib (Nexavar), and other multikinase inhibitors, oxaliplatin, cisplatin and carboplatin, etoposide, gemcitabine, and vinblastine. In one embodiment the chemotherapeutic agent is selected from the group consisting of taxanes (like e.g. taxol (paclitaxel), docetaxel (Taxotere), modified paclitaxel (e.g. Abraxane and Opaxio). In one embodiment, the additional chemotherapeutic agent is selected from 5-fluorouracil (5-FU), leucovorin, irinotecan, or oxaliplatin. In one embodiment the chemotherapeutic agent is 5-fluorouracil, leucovorin and irinotecan (FOLFIRI). In one embodiment the chemotherapeutic agent is 5-fluorouracil, and oxaliplatin (FOLFOX).

Specific examples of combination therapies with additional chemotherapeutic agents include, for instance, therapies taxanes (e.g., docetaxel or paclitaxel) or a modified paclitaxel (e.g., Abraxane or Opaxio), doxorubicin), capecitabine and/or bevacizumab (Avastin) for the treatment of breast cancer; therapies with carboplatin, oxaliplatin, cisplatin, paclitaxel, doxorubicin (or modified doxorubicin (Caelyx or Doxil)), or topotecan (Hycamtin) for ovarian cancer, the therapies with a multi-kinase inhibitor, MKI, (Sutent, Nexavar, or 706) and/or doxorubicin for treatment of kidney cancer; therapies with oxaliplatin, cisplatin and/or radiation for

the treatment of squamous cell carcinoma; therapies with taxol and/or carboplatin for the treatment of lung cancer.

Therefore, in one embodiment the additional chemotherapeutic agent is selected from the group of taxanes (docetaxel or paclitaxel or a modified paclitaxel (Abraxane or Opaxio), doxorubicin, capecitabine and/or bevacizumab for the treatment of breast cancer.

In one embodiment the CSF-1R antibody/PD-L1 antibody combination therapy is no chemotherapeutic agents are administered.

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

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

The invention further provides the use of an antibody according to the invention in an effective amount for the manufacture of a pharmaceutical agent, preferably together with a pharmaceutically acceptable carrier, for the treatment of a patient suffering from cancer.

The invention also provides the use of an antibody according to the invention in an effective amount for the manufacture of a pharmaceutical agent, preferably together with a pharmaceutically acceptable carrier, for the treatment of a patient suffering from cancer.

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

Description of the Sequences

SEQ ID NO: 1	heavy chain CDR3, Mab 2F11
SEQ ID NO: 2	heavy chain CDR2, Mab 2F11
SEQ ID NO: 3	heavy chain CDR1, Mab 2F11
SEQ ID NO: 4	light chain CDR3, Mab 2F11

SEQ ID NO: 5	light chain CDR2, Mab 2F11
SEQ ID NO: 6	light chain CDR1, Mab 2F11
SEQ ID NO: 7	heavy chain variable domain, Mab 2F11
SEQ ID NO: 8	light chain variable domain, Mab 2F11
SEQ ID NO: 9	heavy chain CDR3, Mab 2E10
SEQ ID NO: 10	heavy chain CDR2, Mab 2E10
SEQ ID NO: 11	heavy chain CDR1, Mab 2E10
SEQ ID NO: 12	light chain CDR3, Mab 2E10
SEQ ID NO: 13	light chain CDR2, Mab 2E10
SEQ ID NO: 14	light chain CDR1, Mab 2E10
SEQ ID NO: 15	heavy chain variable domain, Mab 2E10
SEQ ID NO: 16	light chain variable domain, Mab 2E10
SEQ ID NO: 17	heavy chain CDR3, hMab 2F11-c11
SEQ ID NO: 18	heavy chain CDR2, hMab 2F11-c11
SEQ ID NO: 19	heavy chain CDR1, hMab 2F11-c11
SEQ ID NO: 20	light chain CDR3, hMab 2F11-c11
SEQ ID NO: 21	light chain CDR2, hMab 2F11-c11
SEQ ID NO: 22	light chain CDR1, hMab 2F11-c11
SEQ ID NO: 23	heavy chain variable domain, hMab 2F11-c11
SEQ ID NO: 24	light chain variable domain, hMab 2F11-c11
SEQ ID NO: 25	heavy chain CDR3, hMab 2F11-d8
SEQ ID NO: 26	heavy chain CDR2, hMab 2F11-d8
SEQ ID NO: 27	heavy chain CDR1, hMab 2F11-d8
SEQ ID NO: 28	light chain CDR3, hMab 2F11-d8
SEQ ID NO: 29	light chain CDR2, hMab 2F11-d8
SEQ ID NO: 30	light chain CDR1, hMab 2F11-d8
SEQ ID NO: 31	heavy chain variable domain, hMab 2F11-d8
SEQ ID NO: 32	light chain variable domain, hMab 2F11-d8
SEQ ID NO: 33	heavy chain CDR3, hMab 2F11-e7
SEQ ID NO: 34	heavy chain CDR2, hMab 2F11-e7
SEQ ID NO: 35	heavy chain CDR1, hMab 2F11-e7
SEQ ID NO: 36	light chain CDR3, hMab 2F11-e7
SEQ ID NO: 37	light chain CDR2, hMab 2F11-e7
SEQ ID NO: 38	light chain CDR1, hMab 2F11-e7
SEQ ID NO: 39	heavy chain variable domain, hMab 2F11-e7
SEQ ID NO: 40	light chain variable domain, hMab 2F11-e7
SEQ ID NO: 41	heavy chain CDR3, hMab 2F11-f12

SEQ ID NO: 42	heavy chain CDR2, hMab 2F11-f12
SEQ ID NO: 43	heavy chain CDR1, hMab 2F11-f12
SEQ ID NO: 44	light chain CDR3, hMab 2F11-f12
SEQ ID NO: 45	light chain CDR2, hMab 2F11-f12
SEQ ID NO: 46	light chain CDR1, hMab 2F11-f12
SEQ ID NO: 47	heavy chain variable domain, hMab 2F11-f12
SEQ ID NO: 48	light chain variable domain, hMab 2F11-f12
SEQ ID NO: 49	heavy chain CDR3, hMab 2F11-g1
SEQ ID NO: 50	heavy chain CDR2, hMab 2F11-g1
SEQ ID NO: 51	heavy chain CDR1, hMab 2F11-g1
SEQ ID NO: 52	light chain CDR3, hMab 2F11-g1
SEQ ID NO: 53	light chain CDR2, hMab 2F11-g1
SEQ ID NO: 54	light chain CDR1, hMab 2F11-g1
SEQ ID NO: 55	heavy chain variable domain, hMab 2F11-g1
SEQ ID NO: 56	light chain variable domain, hMab 2F11-g1
SEQ ID NO: 57	human kappa light chain constant region
SEQ ID NO: 58	human heavy chain constant region derived from IgG1
SEQ ID NO: 59	human heavy chain constant region derived from IgG1 mutated on L234A and L235A
SEQ ID NO: 60	human heavy chain constant region derived from IgG4
SEQ ID NO: 61	human heavy chain constant region derived from IgG4 mutated on S228P
SEQ ID NO: 62	human wildtype CSF-1R (wt CSF-1R) (including signal sequence)
SEQ ID NO: 63	human mutant CSF-1R L301S Y969F (including signal sequence)
SEQ ID NO: 64	human CSF-1R Extracellular Domain (domains D1-D5)
SEQ ID NO: 65	human CSF-1R fragment delD4
SEQ ID NO: 66	human CSF-1R fragment domains D1-D3
SEQ ID NO: 67	signal peptide
SEQ ID NO: 68	Primer
SEQ ID NO: 69	heavy chain CDR3, Mab 1G10
SEQ ID NO: 70	heavy chain CDR2, Mab 1G10
SEQ ID NO: 71	heavy chain CDR1, Mab 1G10
SEQ ID NO: 72	light chain CDR3, Mab 1G10
SEQ ID NO: 73	light chain CDR2, Mab 1G10
SEQ ID NO: 74	light chain CDR1, Mab 1G10

SEQ ID NO: 75	heavy chain variable domain, Mab 1G10
SEQ ID NO: 76	light chain variable domain, Mab 1G10
SEQ ID NO: 77	heavy chain CDR3, Mab 2H7
SEQ ID NO: 78	heavy chain CDR2, Mab 2H7
SEQ ID NO: 79	heavy chain CDR1, Mab 2H7
SEQ ID NO: 80	light chain CDR3, Mab 2H7
SEQ ID NO: 81	light chain CDR2, Mab 2H7
SEQ ID NO: 82	light chain CDR1, Mab 2H7
SEQ ID NO: 83	heavy chain variable domain, Mab 2H7
SEQ ID NO: 84	light chain variable domain, Mab 2H7
SEQ ID NO: 85	human CSF-1R fragment domains D4-D5
SEQ ID NO: 86	human CSF-1 (including signal sequence)
SEQ ID NO: 87	human IL-34 (including signal sequence)
SEQ ID NO: 88	human PD-L1 (including signal sequence)
SEQ ID NO: 89	heavy chain variable domain VH variant 1, anti-PD-L1 243.55
SEQ ID NO: 90	heavy chain variable domain VH variant 2, anti-PD-L1 243.55
SEQ ID NO: 91	heavy chain variable domain VH variant 3, anti-PD-L1 243.55
SEQ ID NO: 92	light chain variable domain VL variant 1, anti-PD-L1 243.55
SEQ ID NO: 93	light chain variable domain VL variant 2, anti-PD-L1 243.55
SEQ ID NO: 94	light chain variable domain VL variant 3, anti-PD-L1 243.55
SEQ ID NO: 95	light chain variable domain VL variant 4, anti-PD-L1 243.55
SEQ ID NO: 96	light chain variable domain VL variant 5, anti-PD-L1 243.55
SEQ ID NO: 97	light chain variable domain VL variant 6, anti-PD-L1 243.55
SEQ ID NO: 98	light chain variable domain VL variant 7, anti-PD-L1 243.55
SEQ ID NO: 99	light chain variable domain VL variant 8, anti-PD-L1 243.55
SEQ ID NO: 100	light chain variable domain VL variant 9, anti-PD-L1 243.55
SEQ ID NO: 101	light chain variable domain VL variant 10, anti-PD-L1 243.55
SEQ ID NO: 102	light chain variable domain VL variant 11, anti-PD-L1 243.55
SEQ ID NO: 103	light chain variable domain VL variant 12, anti-PD-L1 243.55
SEQ ID NO: 104	light chain variable domain VL variant 13, anti-PD-L1 243.55

SEQ ID NO: 105	light chain variable domain VL variant 14, anti-PD-L1 243.55
SEQ ID NO: 106	light chain variable domain VL variant 15, anti-PD-L1 243.55
SEQ ID NO: 107	light chain variable domain VL variant 16, anti-PD-L1 243.55

In the following embodiment of the invention are described:

1. A) An antibody which binds to human CSF-1R wherein the antibody is administered in combination with an antibody which binds to human PD-L1 for use in the treatment of cancer, for use in the prevention or treatment of metastasis, for use in the treatment inflammatory diseases, for use in the treatment of bone loss, for use in treating or delaying progression of an immune related disease such as tumor immunity, or for use in stimulating an immune response or function, such as T cell activity; or

B) the use of an antibody which binds to human CSF-1R for the manufacture of a medicament for use in the treatment of cancer, for use in the prevention or treatment of metastasis, for use in the treatment inflammatory diseases, for use in the treatment of bone loss, for use in treating or delaying progression of an immune related disease such as tumor immunity, or for use in stimulating an immune response or function, such as T cell activity, wherein the antibody is administered in combination with an antibody which binds to human PD-L1;

wherein the antibody which binds to human CSF-1R used in the combination therapy is characterized in comprising

a) a heavy chain variable domain VH of SEQ ID NO:23 and a light chain variable domain VL of SEQ ID NO:24, or

b) a heavy chain variable domain VH of SEQ ID NO:31 and a light chain variable domain VL of SEQ ID NO:32, or

c) a heavy chain variable domain VH of SEQ ID NO:39 and a light chain variable domain VL of SEQ ID NO:40, or

d) a heavy chain variable domain VH of SEQ ID NO:47 and a light chain variable domain VL of SEQ ID NO:48, or

e) a heavy chain variable domain VH of SEQ ID NO:55 and a light chain variable domain VL of SEQ ID NO:56;

and the antibody which binds to human PD-L1 used in the combination therapy is characterized in comprising

a) a heavy chain variable domain VH of SEQ ID NO:89 and a light chain variable domain VL of SEQ ID NO:92, or

b) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:93, or

c) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:94, or

d) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:95, or

e) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:96, or

f) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:97, or

g) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:98, or

h) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:99, or

i) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:100, or

j) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:101, or

k) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:102, or

l) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:103, or

m) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:104, or

n) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:105, or

o) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:106, or

p) a heavy chain variable domain VH of SEQ ID NO:91 and a light chain variable domain VL of SEQ ID NO:107.

2. Use of a combination of

A) an antibody which binds to human CSF-1R, comprising

a) a heavy chain variable domain VH of SEQ ID NO:23 and a light chain variable domain VL of SEQ ID NO:24, or

b) a heavy chain variable domain VH of SEQ ID NO:31 and a light chain variable domain VL of SEQ ID NO:32, or

c) a heavy chain variable domain VH of SEQ ID NO:39 and a light chain variable domain VL of SEQ ID NO:40, or

d) a heavy chain variable domain VH of SEQ ID NO:47 and a light chain variable domain VL of SEQ ID NO:48, or

e) a heavy chain variable domain VH of SEQ ID NO:55 and a light chain variable domain VL of SEQ ID NO:56;

and

B) an antibody which binds to human PD-L1 comprising

a) a heavy chain variable domain VH of SEQ ID NO:89 and a light chain variable domain VL of SEQ ID NO:92, or

b) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:93, or

c) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:94, or

d) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:95, or

e) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:96, or

f) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:97, or

g) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:98, or

h) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:99, or

i) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:100, or

j) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:101, or

k) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:102, or

l) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:103, or

m) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:104, or

n) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:105, or

o) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:106, or

p) a heavy chain variable domain VH of SEQ ID NO:91 and a light chain variable domain VL of SEQ ID NO:107.

for the manufacture of a medicament for use in the treatment of cancer, for use in the prevention or treatment of metastasis, for use in the treatment inflammatory diseases, for use in the treatment of bone loss, for use in treating or delaying progression of an immune related disease such as tumor immunity, or for use in stimulating an immune response or function, such as T cell activity, wherein the antibody is administered in combination with an antibody which binds to human PD-L1

3. The antibody or use according to any one of embodiments 1 or 2, for use in the treatment of cancer.

4. The antibody or use according to embodiment 3, for use in the treatment of breast cancer, lung cancer, colon cancer, ovarian cancer, melanoma cancer, bladder cancer, renal cancer, kidney cancer, liver cancer, head and neck cancer, colorectal cancer , pancreatic cancer, gastric carcinoma cancer, esophageal cancer, mesotheliioma, prostate cancer, leukemia, lymphomas, myelomas.

5. The antibody or use according to any one of embodiments 1 or 2, for use in the prevention or treatment of metastasis.

6. The antibody or use according to any one of embodiments 1 or 2, for use in the treatment of bone loss.

7. The antibody or use according to any one of embodiments 1 or 2, for use in the treatment of inflammatory diseases.

8. The antibody or use according to any one of embodiments 1 or 2, for use in treating or delaying progression of an immune related disease such as tumor immunity.

9. The antibody or use according to any one of embodiments 1 or 2 for use in stimulating an immune response or function, such as T cell activity.

10. A) An antibody which binds to human CSF-1R wherein the antibody is administered in combination with an antibody which binds to human PD-L1 for use in

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

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

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

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

or

B) use of an antibody which binds to human CSF-1R for the manufacture of a medicament for use in

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

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

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

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

wherein the antibody is administered in combination with an antibody which binds to human PD-L1;

wherein the antibody which binds to human CSF-1R used in the combination therapy is characterized in comprising

a) a heavy chain variable domain VH of SEQ ID NO:23 and a light chain variable domain VL of SEQ ID NO:24, or

b) a heavy chain variable domain VH of SEQ ID NO:31 and a light chain variable domain VL of SEQ ID NO:32, or

c) a heavy chain variable domain VH of SEQ ID NO:39 and a light chain variable domain VL of SEQ ID NO:40, or

d) a heavy chain variable domain VH of SEQ ID NO:47 and a light chain variable domain VL of SEQ ID NO:48, or

e) a heavy chain variable domain VH of SEQ ID NO:55 and a light chain variable domain VL of SEQ ID NO:56;

and the antibody which binds to human PD-L1 used in the combination therapy is characterized in comprising

a) a heavy chain variable domain VH of SEQ ID NO:89 and a light chain variable domain VL of SEQ ID NO:92, or

b) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:93, or

c) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:94, or

d) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:95, or

e) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:96, or

f) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:97, or

g) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:98, or

h) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:99, or

i) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:100, or

j) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:101, or

k) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:102, or

l) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:103, or

m) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:104, or

n) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:105, or

o) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:106, or

p) a heavy chain variable domain VH of SEQ ID NO:91 and a light chain variable domain VL of SEQ ID NO:107.

11. A) An antibody which binds 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 and wherein the anti-CSF-1R antibody is administered in combination with an antibody which binds to human PD-L1, or

B) use of an antibody which binds to human CSF-1R, for the manufacture of a medicament 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 and wherein the anti-CSF-1R antibody is administered in combination with an antibody which binds to human PD-L1,

wherein the antibody which binds to human CSF-1R used in the combination therapy is characterized in comprising

a) a heavy chain variable domain VH of SEQ ID NO:23 and a light chain variable domain VL of SEQ ID NO:24, or

b) a heavy chain variable domain VH of SEQ ID NO:31 and a light chain variable domain VL of SEQ ID NO:32, or

c) a heavy chain variable domain VH of SEQ ID NO:39 and a light chain variable domain VL of SEQ ID NO:40, or

d) a heavy chain variable domain VH of SEQ ID NO:47 and a light chain variable domain VL of SEQ ID NO:48, or

e) a heavy chain variable domain VH of SEQ ID NO:55 and a light chain variable domain VL of SEQ ID NO:56;

and the antibody which binds to human PD-L1 used in the combination therapy is characterized in comprising

a) a heavy chain variable domain VH of SEQ ID NO:89 and a light chain variable domain VL of SEQ ID NO:92, or

b) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:93, or

c) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:94, or

d) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:95, or

e) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:96, or

f) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:97, or

g) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:98, or

h) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:99, or

i) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:100, or

j) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:101, or

k) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:102, or

l) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:103, or

m) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:104, or

n) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:105, or

o) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:106, or

p) a heavy chain variable domain VH of SEQ ID NO:91 and a light chain variable domain VL of SEQ ID NO:107.

12. The antibody or use according any one of the preceding embodiments,

wherein the antibody which binds to human CSF-1R used in the combination therapy is characterized in comprising

c) a heavy chain variable domain VH of SEQ ID NO:39 and a light chain variable domain VL of SEQ ID NO:40, or

and wherein the antibody which binds to human PD-L1 used in the combination therapy is characterized in comprising

a) a heavy chain variable domain VH of SEQ ID NO:89 and a light chain variable domain VL of SEQ ID NO:92.

13. The antibody or use according any one of the preceding embodiments, characterized in that said antibodies are of human IgG1 subclass or human IgG4 subclass.

14. The antibody or use according to any one of the preceding embodiments, characterized in that said antibodies have reduced or minimal effector function.

15. The antibody or use according to any one of the preceding embodiments, wherein the minimal effector function results from an effectorless Fc mutation.

16. The antibody or use according to any one of the preceding embodiments, wherein the effectorless Fc mutation is L234A/L235A or L234A/L235A/P329G or N297A or D265A/N297A.

17. A) A method for

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

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

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

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

wherein an antibody which binds to human CSF-1R, is administered in combination with an antibody which binds to human PD-L1,

or

B) a method of 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 and wherein an antibody which binds to human CSF-1R is administered in combination with an antibody which binds to human PD-L1,

wherein the antibody which binds to human CSF-1R used in the combination therapy is characterized in comprising

a) a heavy chain variable domain VH of SEQ ID NO:23 and a light chain variable domain VL of SEQ ID NO:24, or

b) a heavy chain variable domain VH of SEQ ID NO:31 and a light chain variable domain VL of SEQ ID NO:32, or

c) a heavy chain variable domain VH of SEQ ID NO:39 and a light chain variable domain VL of SEQ ID NO:40, or

d) a heavy chain variable domain VH of SEQ ID NO:47 and a light chain variable domain VL of SEQ ID NO:48, or

e) a heavy chain variable domain VH of SEQ ID NO:55 and a light chain variable domain VL of SEQ ID NO:56;

and the antibody which binds to human PD-L1 used in the combination therapy is characterized in comprising

a) a heavy chain variable domain VH of SEQ ID NO:89 and a light chain variable domain VL of SEQ ID NO:92, or

b) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:93, or

c) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:94, or

d) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:95, or

e) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:96, or

f) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:97, or

g) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:98, or

h) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:99, or

i) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:100, or

j) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:101, or

k) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:102, or

l) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:103, or

m) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:104, or

n) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:105, or

o) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:106, or

p) a heavy chain variable domain VH of SEQ ID NO:91 and a light chain variable domain VL of SEQ ID NO:107.

Examples

Example 1

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

$4.5x10^3$ NIH 3T3 cells, retrovirally infected with an expression vector for full-length CSF-1R, were cultured in DMEM (PAA Cat. No.E15-011), 2mM L-glutamine (Sigma, Cat.No.G7513, 2mM Sodium pyruvate, 1x nonessential amino acids, 10% FKS (PAA, Cat.No.A15-649) and 100µg/ml PenStrep (Sigma, Cat.No. P4333 [10mg/ml]) until they reached confluency. Thereafter cells were washed with serum-free DMEM media (PAA Cat.No.E15-011) supplemented with sodium selenite [5ng/ml] (Sigma, Cat.No. S9133), transferrin [10µg/ml] (Sigma,

Cat.No. T8158), BSA [400µg/ml] (Roche Diagnostics GmbH, Cat.No. 10735078), 4mM L-glutamine (Sigma, Cat.No.G7513), 2mM sodium pyruvate (Gibco, Cat.No. 11360), 1x nonessential amino acids (Gibco, Cat: 11140-035), 2-mercaptoethanol [0,05mM] (Merck, Cat.No. M7522), 100µg/ml and PenStrep (Sigma, Cat. No. P4333) and incubated in 30 µl of the same medium for 16 hours to allow for receptor up-regulation. 10 µl of diluted anti-CSR-1R antibodies were added to the cells for 1.5 h. Then cells were stimulated with 10 µl of 100 ng/ml hu CSF-1 (active 149 aa fragment of human CSF-1 (aa 33-181 of SEQ ID NO: 86) ;Biomol, DE, Cat.No.60530)for 5 min. After the incubation, supernatant was removed, cells were washed twice with 80 µl of ice-cold PBS and 50 µl of freshly prepared ice-cold lysis buffer (150mM NaCl/ 20mM Tris pH 7.5 / 1mM EDTA/ 1mM EGTA/ 1% Triton X-100 /1 protease inhibitor tablet (Roche Diagnostics GmbH Cat.No.1 836 170) per 10 ml buffer/10µl/ml phosphatase inhibitor cocktail 1 (Sigma Cat.No. P-2850, 100x Stock)/ 10µl/ml protease inhibitor 1 (Sigma Cat.No.P-5726, 100x Stock) /10µl/ml 1 M NaF) was added. After 30 minutes on ice the plates were shaken vigorously on a plateshaker for 3 minutes and then centrifuged 10 minutes at 2200 rpm (Heraeus Megafuge 10).

The presence of phosphorylated and total CSF-1 receptor in the cell lysate was analyzed with Elisa. For detection of the phosphorylated receptor the kit from R&D Systems (Cat. No. DYC3268-2) was used according to the instructions of the supplier. For detection of total CSF-1R 10 µl of the lysate was immobilized on plate by use of the capture antibody contained in the kit. Thereafter 1:750 diluted biotinylated anti CSF-1R antibody BAF329 (R&D Systems) and 1:1000 diluted streptavidin-HRP conjugate was added. After 60 minutes plates were developed with freshly prepared ABTS® solution and the absorbance was detected. Data were calculated as % of positive control without antibody and the ratio value phospho/total receptor expressed. The negative control was defined without addition of M-CSF-1. Anti CSF-1R SC 2-4A5 (Santa Cruz Biotechnology, US, see also Sherr, C.J. et al., Blood 73 (1989) 1786-1793), which inhibits the ligand-receptor interaction, was used as reference control.

Table 3 :

Calculated IC50 values for the inhibition of CSF-1 receptor phosphorylation.

CSF-1R Mab	IC50 CSF-1R Phosphorylation [ng/ml]
Mab 2F11	219.4
Mab 2E10	752.0
Mab 2H7	703.4
Mab 1G10	56.6
SC-2-4A5	1006.6

Example 2

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

NIH 3T3 cells, 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), were cultured in DMEM high glucose media (PAA, Pasching, Austria) supplemented with 2mM L-glutamine, 2mM sodium pyruvate and non-essential amino acids and 10% fetal bovine serum (Sigma, Taufkirchen, Germany) on poly-HEMA (poly(2-hydroxyethylmethacrylate)) (Polysciences, Warrington, PA, USA)) coated dishes to prevent adherence to the plastic surface. Cells are seeded in medium replacing serum with 5ng/ml sodium selenite, 10mg/ml transferrin, 400µg/ml BSA and 0.05 mM 2-mercaptoethanol. When treated with 100ng/ml hu CSF-1 (active 149 aa fragment of human CSF-1 (aa 33-181 of SEQ ID NO: 86); Biomol, DE, Cat.No.60530) wtCSF-1R (expressing cells form dense spheroids that grow three dimensionally, a property that is called anchorage independence. These spheroids resemble closely the three dimensional architecture and organization of solid tumors in situ. Mutant CSF-1R recombinant cells are able to form spheroids independent of the CSF-1 ligand. The anti-CSF-1R antibody according to the invention hMab 2F11-e7 and the anti-CSF-1R antibodies 1.2.SM (ligand displacing CSF-1R antibody described in WO 2009/026303), CXIIG6 (ligand displacing CSF-1R antibody described in WO 2009/112245), the goat polyclonal anti-CSF-1R antibody ab10676 (abcam), and SC 2-4A5 (Santa Cruz Biotechnology, US- see also Sherr, C.J. et al., Blood 73 (1989) 1786-1793) and Mab R&D-Systems 3291 were investigated. Reference control Mab R&D-Systems 3291 did not show inhibition of mutant CSF-1R recombinant cell proliferation.

Spheroid cultures were incubated for 3 days in the presence of different concentrations of antibody in order to determine an IC30 (concentration with 30 percent inhibition of cell viability). Maximum concentration was 20 µg/ml The CellTiterGlo assay was used to detect cell viability by measuring the ATP-content of the cells.

Table 4 :

CSF-1R Mab	wtCSF-1R IC_{30} [µg/ml]	Mutant CSF-1R IC_{30} [µg/ml]
hMab 2F11-e7	4.91	0.54
1.2.SM	1.19	> 20 µg/ml (-19% inhibition at 20 µg/ml = 19% stimulation)
CXIIG6	> 20 µg/ml (21% inhibition at 20 µg/ml)	> 20 µg/ml (-36% inhibition at 20 µg/ml = 36% stimulation)
ab10676	14.15	> 20 µg/ml (0% inhibition at 20 µg/ml)
SC 2-4A5	16.62	2.56

Example 3

Inhibition of human macrophage differentiation under treatment with anti-CSF-1R monoclonal antibodies (CellTiterGlo-assay)

Human monocytes were isolated from peripheral blood using the RosetteSep™ Human Monocyte Enrichment Cocktail (StemCell Tech. - Cat. No.15028). Enriched monocyte populations were seeded into 96 well microtiterplates ($2.5x10^4$ cells/well) in 100 µl RPMI 1640 (Gibco - Cat. No.31870) supplemented with 10% FCS (GIBCO - Cat. No.011-090014M), 4 mM L-glutamine (GIBCO - Cat. No.25030) and 1x PenStrep (Roche Cat. No.1 074 440) at 37°C and 5% CO_2 in a humidified atmosphere. When 150 ng/ml huCSF-1 was added to the medium, a clear differentiation into adherent macrophages could be observed. This differentiation could be inhibited by addition of anti-CSF-1R antibodies. Furthermore, the monocyte survival is affected and could be analyzed by CellTiterGlo (CTG) analysis. From the concentration dependent inhibition of the

survival of monocytes by antibody treatment, an IC_{50} was calculated (see Table below).

Table 5 :

CSF-1R Mab	IC_{50} [µg/ml]
Mab 2F11	0.08
Mab 2E10	0.06
Mab 2H7	0.03
Mab 1G10	0.06
SC 2-4A5	0.36

In a separate test series humanized versions of Mab 2 F11, e.g. hMab 2F11-c11, hMab 2F11-d8, hMab 2F11-e7, hMab 2F11-f12, showed IC50 values of 0.07 µg/ml (hMab 2F11-c11), 0.07 µg/ml (hMab 2F11-d8), 0.04 µg/ml (hMab 2F11-e7) and 0.09 µg/ml (hMab 2F11-f12).

Example 4

Inhibition of human macrophage differentiation under treatment with anti-CSF-1R monoclonal antibodies (CellTiterGlo-assay)

Human monocytes were isolated from peripheral blood using the RosetteSep™ Human Monocyte Enrichment Cocktail (StemCell Tech. - Cat. No.15028). Enriched monocyte populations were seeded into 96 well microtiterplates ($2.5x10^4$ cells/well) in 100 µl RPMI 1640 (Gibco - Cat. No.31870) supplemented with 10% FCS (GIBCO - Cat. No.011-090014M), 4 mM L-glutamine (GIBCO - Cat. No.25030) and 1x PenStrep (Roche Cat. No.1 074 440) at 37°C and 5% CO_2 in a humidified atmosphere. When 150 ng/ml huCSF-1 was added to the medium, a clear differentiation into adherent macrophages could be observed. This differentiation could be inhibited by addition of anti-CSF-1R antibodies. Furthermore, the monocyte survival is affected and could be analyzed by CellTiterGlo (CTG) analysis. From the concentration dependent inhibition of the survival of monocytes by antibody treatment, an IC_{50} was calculated. Humanized versions of Mab 2 F11, e.g. hMab 2F11-c11, hMab 2F11-d8, hMab 2F11-e7, hMab 2F11-f12, showed IC50 values of 0.07 µg/ml (hMab 2F11-c11), 0.07 µg/ml (hMab 2F11-d8), 0.04 µg/ml (hMab 2F11-e7) and 0.09 µg/ml (hMab 2F11-f12).

Example 5

Inhibition of human M1 and M2 macrophage differentiation under treatment with anti-CSF-1R monoclonal antibodies (CellTiterGlo-assay)

Human monocytes were isolated from peripheral blood using the RosetteSep™ Human Monocyte Enrichment Cocktail (StemCell Tech. - Cat. No.15028). Enriched monocyte populations were seeded into 96 well microtiterplates ($2.5x10^4$ cells/well) in 100 µl RPMI 1640 (Gibco - Cat. No.31870) supplemented with 10% FCS (GIBCO - Cat. No.011-090014M), 4 mM L-glutamine (GIBCO - Cat. No.25030) and 1x PenStrep (Roche Cat. No.1 074 440) at 37°C and 5% CO_2 in a humidified atmosphere. When 100 ng/ml huCSF-1 was added for 6 days to the medium, a clear differentiation into adherent, M2 macrophages with elongated morphology could be observed. When 100 ng/ml huGM-CSF was added to the medium for 6 days, a clear differentiation into adherent, M1 macrophages with round morphology could be observed. This differentiation was associated with the expression of certain markers such as CD163 for M2 macrophages and CD80 or high MHC class II for M1 macrophages as assessed by flow cytometry. Cells were washed with PBS and, if adherent, detached using a 5mM EDTA solution in PBS (20min at 37°C). Cells were then well resuspended, washed with staining buffer (5% FCS in PBS) and centrifuged at 300xg for 5min. Pellets were resuspended in 1ml staining buffer and cells counted in a Neubauer chamber. Approximately 1x10e5 cells were transferred in each FACS tube, centrifuged at 300xg for 5min and resuspended in staining buffer. Fcγ receptors were blocked by incubation with 1µg human IgG/2,5x10e4 cells (JIR Cat.No.009-000-003) in staining buffer for 20 min on ice. Cells were then mixed with 1,5µl antibody/2,5x10e4 cells for CD80 and CD163 detection whereas 5 µl antibody/2,5x10e4 cells for MHC class II detection was used: PE labeled mouse anti human CD163 (BD Bioscience Cat.No.556018), PE labeled mouse anti human CD80 (BD Bioscience Cat.No. 557227) and Alexa 647 labeled mouse anti human MHC class II (Dako-Cat.No. M0775). The Alexa 647 label was conjugated to the antibody by using the Zenon Alexa 647 mouse IgG labeling kit (Invitrogen Cat.No. Z25008) After a 1-hour incubation on ice cells were washed twice with staining buffer, resuspended and measured at a FACS Canto II.

Exclusively M2 macrophage differentiation which is characterized by the expression of CD163, absence of CD80 and low MHC class II expression could be inhibited by addition of humanized anti-CSF-1R antibody hMab 2F11-e7. Furthermore, the M2 but not M1 macrophage survival is affected and could be

analyzed by CellTiterGlo (CTG) analysis. Concentration dependent inhibition of the survival of macrophages by antibody treatment for 7 days is depicted in Figure 1a. Expression of M1 and M2 macrophage markers assessed by flow cytometry is shown in Figure 1b.

Example 6

CSF-1 level increase during CSF-1R inhibition in Cynomolgus monkey

Serum CSF-1 levels provide a pharmacodynamic marker of CSF-1R neutralizing activity of anti-human CSF-1R dimerization inhibitor hMab 2F11-e7. One male and one female cynomolgus monkey per dosage group (1 and 10 mg/kg) were intravenously administered anti-CSF1R antibody hMab 2F11-e7. Blood samples for analysis of CSF-1 levels were collected 1 week before treatment (pre-dose), 2, 24, 48, 72, 96, 168 hours post-dose and weekly for two additional weeks. CSF-1 levels were determined using a commercially available ELISA kit (Quantikine® human M-CSF) according to the manufacturer's instructions (R&D Systems, UK). Monkey CSF-1 level were determined by comparison with CSF-1 standard curve samples provided in the kit.

Administration of hMab 2F11-e7 induced a dramatic increase in CSF-1 by ~ 1000-fold, which depending on the dose administered lasted for 48 hr (1mg/kg) or 15 days (10mg/kg). Hence, a dimerization inhibitor for CSF-1R offers the advantage to not directly compete with the dramatically upregulated ligand for binding to the receptor in contrast to a ligand displacing antibody. (Results are shown in Figure 2)

Example 7

Relationship between M2 subtype tumor associated macrophages (TAMs) and T cells - Rationale for combining anti-CSF-R1 antibody and a T cell engaging agents

To investigate the functional relationship between TAMs and T cells we isolated TAMs from the MC38 tumor and cocultured them with CD8+ T cells.

TAM suppression assay

TAMs were enriched from single cell suspensions of MC38 tumors after enzymatic digest using a two-step protocol: Single cells were stained with CD11b-FITC (clone M1/70) and positively enriched over MACS columns by anti-FITC beads (Miltenyi). Upon removal from the column, anti-FITC beads were detached using

release buffer protocol as provided the manufacturer. Finally, TAM were isolated by adding anti-Ly6G and anti-Ly6C positive selection beads in order to remove granulocytic and monocytic cells from TAM preparations. Final cell purity was analyzed and was usually > 90%. Subsequently, TAM were titrated in the indicated ratios to total CD3+ T cells labeled with CFSE in U-bottom plates coated with anti-CD3 and soluble anti- CD28 was added. Cell proliferation was determined from CFSElow cells using blank Sphero beads as previously described after 3 days of incubation (Hoves, S. et al. Monocyte-derived human macrophages mediate anergy in allogeneic T cells and induce regulatory T cells. J. Immunol. 177, 2691–2698 (2006)). In the presence of TAMs, T cell expansion induced by activation of CD3 and CD28 was suppressed. (see Figure 3).

Example 8

Inhibition of tumor growth under treatment with anti-CSF-1R monoclonal antibody in combination with PD-L1 antibody in subcutaneous syngeneic MC38 colon carcinoma model

Cells of the murine colorectal adenocarcinoma cell line MC38 (obtained from Beckman Research Institute of the City of Hope, California, USA) were cultured in Dulbecco's Modified Eagle Medium (DMEM, PAN Biotech) supplemented with 10% FCS and 2mM L-glutamine at 37°C in a water saturated atmosphere at 5% CO2. At the day of inoculation, MC38 tumor cells were harvested with PBS from culture flasks and transferred into culture medium, centrifuged, washed once and re-suspended in PBS. For injection of cells, the final titer was adjusted to 1×10^7 cells/ml. Subsequently 100 µl of this suspension (1×10^6 cells) were inoculated subcutaneously into 7-9 weeks old female C57BL/6N mice (obtained from Charles River, Sulzfeld, Germany). Treatment with control antibody (MOPC-21; Bio X Cell, West Lebanon), anti-murine CSF-1R mAb <mouse CSF1R> antibody at a weekly dose of 30 mg/kg i.p. alone or in combination with a mouse crossreactive anti PD-L1 antibody (10 mg/kg i.p., 6x q3d) started after tumors were established and had reached an average size of 100 mm^3. Tumor volume was measured twice a week and animal weights were monitored in parallel.

In first experiment monotherapy with <mouse CSF1R> antibody did not inhibit primary tumor growth when compared to control antibody treatment (TGI: 0%, TCR: 1.07 CI: 0.80-1.43, median time to progression > 700 mm^3: 21 days). Anti-PD-L1 monotherapy had an effect on MC38 primary tumor growth (TGI: 83%, TCR: 0.27 CI: 0.09-0.49, median time to progression > 700 mm^3: 32 days).

Addition of <mouse CSF1R> antibody to anti-PD-L1 therapy led to a slightly improved anti-tumor efficacy compared to anti-PD-L1 treatment alone (TGI: 83%, TCR: 0.28 CI: 0.09-0.51 median time to progression > 700 mm^3: 37 days) (see table below).

Table 6:

Anti tumor Efficacy of <mouse anti-CSF1R> antibody / <anti-PD-L1> antibody combination in the MC38 mouse CRC in vivo model

Group	TGI (day 21)	TCR (day 12)	95% CI vs. group 1	Median time to progression TV > 700 mm^3
Control (Mouse IgG1)	-	-	-	21
<mouse CSF1R>	0%	1.07	(1.43-0.80)	21
<anti-PD-L1>	83%	0.27	(0.49-0.09)	32
<mouse CSF1R> / <anti-PD-L1>	83%	0.28	(0.51-0.09)	37

Median time of progression > 700 mm^3 was 21 days for control (mouse IgG1) treated animals. Monotherapy with <mouse CSF1R> antibody did not inhibit primary tumor growth when compared to control antibody treatment (median time to progression > 700 mm^3: 21 days). Anti-PD-L1 monotherapy had an effect on MC38 primary tumor growth (median time to progression > 700 mm^3: 32 days). Addition of <mouse CSF1R> antibody to anti-PD-L1 therapy led to a slightly improved anti-tumor efficacy compared to PD-L1 treatment alone (median time to progression > 700 mm^3: 37 days) (see table below and Figure 4).

Table 7:

Anti tumor Efficacy of <mouse anti-CSF1R> antibody / <anti-PD-L1> antibody combination in the MC38 mouse CRC in vivo model (Median time to progression > 700 mm^3)

Group	Median time to progression TV > 700 mm^3
Control (Mouse IgG1)	21
<mouse CSF1R>	21
<anti-PD-L1>	32
<mouse CSF1R> / <anti-PD-L1>	37

In analogous experiments, but starting treatment at different tumor sizes (e.g. starting treatment when the tumor has reached a volume above and below 100 mm^3 (different groups are evaluated) and in a further experiment also using different anti PD-L1 antibodies described in table 2, the inhibition of tumor growth under treatment with anti-CSF-1R monoclonal antibody in combination with anti-PD-L1 antibody in subcutaneous syngeneic MC38 colon carcinoma model is evaluated.

Example 9

Inhibition of tumor growth under treatment with anti-CSF-1R monoclonal antibody in combination with PD-L1 antibody in subcutaneous syngeneic CT26.WT colon carcinoma model

Cells of the murine colorectal adenocarcinoma cell line CT26.WT tumor cells (obtained from ATCC) were cultured in Dulbecco's Modified Eagle Medium (DMEM, PAN Biotech) supplemented with 10% FCS and 2mM L-glutamine at 37°C in a water saturated atmosphere at 5% CO2. At the day of inoculation, CT26.WT tumor cells were harvested with PBS from culture flasks and transferred

into culture medium, centrifuged, washed once and re-suspended in PBS. For injection of cells, the final titer was adjusted to 1×10^7 cells/ml. Subsequently 100 µl of this suspension (1×10^6 cells) were inoculated subcutaneously into 11-13 weeks old female Balb/c mice (obtained from Charles River, Sulzfeld, Germany). Treatment with control antibody (MOPC-21; Bio X Cell, West Lebanon), anti-murine CSF-1R mAb <mouse CSF1R> antibody at a weekly dose of 30 mg/kg i.p. alone or in combination with a mouse crossreactive anti PD-L1 antibody (10 mg/kg i.p., 6x q3d) started after tumors were established and had reached an average size of 150 mm^3. While treatment in monotherapy groups started on day 9 after tumor cell inoculation, treatment in combination group was sequential (day 9: start of treatment with anti-murine CSF-1R mAb; day 11: start of treatment with anti PD-L1 antibody). Tumor volume was measured twice a week and animal weights were monitored in parallel. Results are shown in Figure

Median time to progression ≥ 700 mm3 was 17 days for IgG control treatment group, 16 days for <mouse anti-CSF1R> antibody monotherapy group, 18 days for <anti-PD-L1> antibody monotherapy group and 18 days for <mouse anti-CSF1R>/<anti-PD-L1> antibody combination group.

While all animals in control or monotherapy groups needed to be terminated due to progressive tumor burden one animal of the <mouse anti-CSF1R>/<anti-PD-L1> antibody combination group experienced tumor shrinkage and remained tumor-free until study termination on day 79 after tumor inoculation.

Patent Claims

1. A combination of an antibody which binds to human CSF-1R and an antibody which binds to human PD-L1 for use in the treatment of cancer

wherein the antibody which binds to human CSF-1R used in the combination is characterized in comprising

a) a heavy chain variable domain VH of SEQ ID NO:23 and a light chain variable domain VL of SEQ ID NO:24, or

b) a heavy chain variable domain VH of SEQ ID NO:31 and a light chain variable domain VL of SEQ ID NO:32, or

c) a heavy chain variable domain VH of SEQ ID NO:39 and a light chain variable domain VL of SEQ ID NO:40, or

d) a heavy chain variable domain VH of SEQ ID NO:47 and a light chain variable domain VL of SEQ ID NO:48, or

e) a heavy chain variable domain VH of SEQ ID NO:55 and a light chain variable domain VL of SEQ ID NO:56;

and the antibody which binds to human PD-L1 used in the combination is characterized in comprising

a) a heavy chain variable domain VH of SEQ ID NO:89 and a light chain variable domain VL of SEQ ID NO:92, or

b) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:93, or

c) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:94, or

d) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:95, or

e) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:96, or

f) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:97, or

g) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:98, or

h) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:99, or

i) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:100, or

j) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:101, or

k) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:102, or

l) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:103, or

m) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:104, or

n) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:105, or

o) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:106, or

p) a heavy chain variable domain VH of SEQ ID NO:91 and a light chain variable domain VL of SEQ ID NO:107.

2. The combination according to claim 1, for use in the treatment of breast cancer, lung cancer, colon cancer, ovarian cancer, melanoma cancer, bladder cancer, renal cancer, kidney cancer, liver cancer, head and neck cancer, colorectal cancer , pancreatic cancer, gastric carcinoma cancer, esophageal cancer, mesotheliioma, prostate cancer, leukemia, lymphomas, myelomas.

3. A combination comprising an antibody which binds to human CSF-1R and an antibody which binds to human PD-L1, for use in

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

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

wherein the antibody which binds to human CSF-1R used in the combination is characterized in comprising

a) a heavy chain variable domain VH of SEQ ID NO:23 and a light chain variable domain VL of SEQ ID NO:24, or

b) a heavy chain variable domain VH of SEQ ID NO:31 and a light chain variable domain VL of SEQ ID NO:32, or

c) a heavy chain variable domain VH of SEQ ID NO:39 and a light chain variable domain VL of SEQ ID NO:40, or

d) a heavy chain variable domain VH of SEQ ID NO:47 and a light chain variable domain VL of SEQ ID NO:48, or

e) a heavy chain variable domain VH of SEQ ID NO:55 and a light chain variable domain VL of SEQ ID NO:56;

and the antibody which binds to human PD-L1 used in the combination is characterized in comprising

a) a heavy chain variable domain VH of SEQ ID NO:89 and a light chain variable domain VL of SEQ ID NO:92, or

b) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:93, or

c) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:94, or

d) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:95, or

e) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:96, or

f) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:97, or

g) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:98, or

h) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:99, or

i) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:100, or

j) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:101, or

k) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:102, or

l) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:103, or

m) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:104, or

n) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:105, or

o) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:106, or

p) a heavy chain variable domain VH of SEQ ID NO:91 and a light chain variable domain VL of SEQ ID NO:107.

4. A combination comprising an antibody which binds to human CSF-1R and an antibody which binds to human PD-L1, 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 antibody which binds to human CSF-1R used in the combination is characterized in comprising

a) a heavy chain variable domain VH of SEQ ID NO:23 and a light chain variable domain VL of SEQ ID NO:24, or

b) a heavy chain variable domain VH of SEQ ID NO:31 and a light chain variable domain VL of SEQ ID NO:32, or

c) a heavy chain variable domain VH of SEQ ID NO:39 and a light chain variable domain VL of SEQ ID NO:40, or

d) a heavy chain variable domain VH of SEQ ID NO:47 and a light chain variable domain VL of SEQ ID NO:48, or

e) a heavy chain variable domain VH of SEQ ID NO:55 and a light chain variable domain VL of SEQ ID NO:56;

and the antibody which binds to human PD-L1 used in the combination is characterized in comprising

a) a heavy chain variable domain VH of SEQ ID NO:89 and a light chain variable domain VL of SEQ ID NO:92, or

b) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:93, or

c) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:94, or

d) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:95, or

e) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:96, or

f) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:97, or

g) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:98, or

h) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:99, or

i) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:100, or

j) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:101, or

k) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:102, or

l) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:103, or

m) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:104, or

n) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:105, or

o) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:106, or

p) a heavy chain variable domain VH of SEQ ID NO:91 and a light chain variable domain VL of SEQ ID NO:107.

5. The combination according to any one of the preceding claims, characterized in that said antibodies are of human IgG1 subclass or human IgG4 subclass.

6. The combination according to any one of the preceding claims, characterized in that said antibodies comprise a mutation selected from the group consisting of L234A/L235A, L234A/L235A/P329G, N297A and D265A/N297A.

7. Use of an antibody which binds to human CSF-1R wherein the antibody is in combination with an antibody which binds to human PD-L1 in the manufacture of a medicament for the treatment of cancer,

wherein the antibody which binds to human CSF-1R used in the combination is characterized in comprising

a) a heavy chain variable domain VH of SEQ ID NO:23 and a light chain variable domain VL of SEQ ID NO:24, or

b) a heavy chain variable domain VH of SEQ ID NO:31 and a light chain variable domain VL of SEQ ID NO:32, or

c) a heavy chain variable domain VH of SEQ ID NO:39 and a light chain variable domain VL of SEQ ID NO:40, or

d) a heavy chain variable domain VH of SEQ ID NO:47 and a light chain variable domain VL of SEQ ID NO:48, or

e) a heavy chain variable domain VH of SEQ ID NO:55 and a light chain variable domain VL of SEQ ID NO:56;

and the antibody which binds to human PD-L1 used in the combination is characterized in comprising

a) a heavy chain variable domain VH of SEQ ID NO:89 and a light chain variable domain VL of SEQ ID NO:92, or

b) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:93, or

c) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:94, or

d) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:95, or

e) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:96, or

f) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:97, or

g) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:98, or

h) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:99, or

i) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:100, or

j) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:101, or

k) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:102, or

l) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:103, or

m) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:104, or

n) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:105, or

o) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:106, or

p) a heavy chain variable domain VH of SEQ ID NO:91 and a light chain variable domain VL of SEQ ID NO:107.

8. The use according to claim 7, for the treatment of breast cancer, lung cancer, colon cancer, ovarian cancer, melanoma cancer, bladder cancer, renal cancer, kidney cancer, liver cancer, head and neck cancer, colorectal cancer , pancreatic cancer, gastric carcinoma cancer, esophageal cancer, mesotheliioma, prostate cancer, leukemia, lymphomas, myelomas.

9. Use of an antibody which binds to human CSF-1R wherein the antibody is in combination with an antibody which binds to human PD-L1, in the manufacture of a medicament for

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

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

wherein the antibody which binds to human CSF-1R used in the combination is characterized in comprising

a) a heavy chain variable domain VH of SEQ ID NO:23 and a light chain variable domain VL of SEQ ID NO:24, or

b) a heavy chain variable domain VH of SEQ ID NO:31 and a light chain variable domain VL of SEQ ID NO:32, or

c) a heavy chain variable domain VH of SEQ ID NO:39 and a light chain variable domain VL of SEQ ID NO:40, or

d) a heavy chain variable domain VH of SEQ ID NO:47 and a light chain variable domain VL of SEQ ID NO:48, or

e) a heavy chain variable domain VH of SEQ ID NO:55 and a light chain variable domain VL of SEQ ID NO:56;

and the antibody which binds to human PD-L1 used in the combination is characterized in comprising

a) a heavy chain variable domain VH of SEQ ID NO:89 and a light chain variable domain VL of SEQ ID NO:92, or

b) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:93, or

c) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:94, or

d) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:95, or

e) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:96, or

f) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:97, or

g) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:98, or

h) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:99, or

i) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:100, or

j) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:101, or

k) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:102, or

l) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:103, or

m) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:104, or

n) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:105, or

o) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:106, or

p) a heavy chain variable domain VH of SEQ ID NO:91 and a light chain variable domain VL of SEQ ID NO:107.

10. Use of an antibody which binds to human CSF-1R, in the manufacture of a medicament for 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 and wherein the anti-CSF-1R antibody is in combination with an antibody which binds to human PD-L1,

wherein the antibody which binds to human CSF-1R used in the combination is characterized in comprising

a) a heavy chain variable domain VH of SEQ ID NO:23 and a light chain variable domain VL of SEQ ID NO:24, or

b) a heavy chain variable domain VH of SEQ ID NO:31 and a light chain variable domain VL of SEQ ID NO:32, or

c) a heavy chain variable domain VH of SEQ ID NO:39 and a light chain variable domain VL of SEQ ID NO:40, or

d) a heavy chain variable domain VH of SEQ ID NO:47 and a light chain variable domain VL of SEQ ID NO:48, or

e) a heavy chain variable domain VH of SEQ ID NO:55 and a light chain variable domain VL of SEQ ID NO:56;

and the antibody which binds to human PD-L1 used in the combination is characterized in comprising

a) a heavy chain variable domain VH of SEQ ID NO:89 and a light chain variable domain VL of SEQ ID NO:92, or

b) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:93, or

c) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:94, or

d) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:95, or

e) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:96, or

f) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:97, or

g) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:98, or

h) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:99, or

i) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:100, or

j) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:101, or

k) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:102, or

l) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:103, or

m) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:104, or

n) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:105, or

o) a heavy chain variable domain VH of SEQ ID NO:90 and a light chain variable domain VL of SEQ ID NO:106, or

p) a heavy chain variable domain VH of SEQ ID NO:91 and a light chain variable domain VL of SEQ ID NO:107.

11. The use according to any one of claims 7 to 10, wherein said antibodies are of human IgG1 subclass or human IgG4 subclass.

12. The use according to any one of claims 7 to 11, wherein said antibodies comprise a mutation selected from the group consisting of is L234A/L235A, L234A/L235A/P329G, N297A and D265A/N297A.