Antibodies to the ligand are not cross reactive. Therefore antibodies to CSF-1 do not inhibit IL-34 binding to CSF-1R and antibodies to IL-34 do not inhibit CSF-1 binding to CSF-1R. Ligand binding to the receptor may have an effect on cancer growth. Additionally, antibodies to the ligand do not internalize, nor do they induce CSF-1R degradation, nor do they stimulate ADCC activity against cells.

A need exists for multifunctional antibodies which block the binding of ligands to CSF-1R and also induce ADCC activity against cells bound by the antibodies.

Antibodies of the present invention are advantageous over known antibodies because they have a multitude of functions. Antibodies of the invention block CSF-1 *and* IL-34 binding to the receptor, thereby preventing dimerization of the receptor and the resulting phosphorylation of the intracellular tyrosine residues, functions which are critical in preventing macrophage induced tumor growth. Antibodies of the invention internalize and induce CSF-1R degradation. Importantly, in addition to blocking ligand binding, antibodies of the invention enhance ADCC activity by stimulating the killing of tumor cells and tumor-associated macrophages and monocytes. Antibodies of the invention also simultaneously affect macrophage activity, an activity which plays a major role in tumor progression. Because of the multitude of therapeutic functions which they have, the antibodies of the present invention have a significant advantage over the antibodies known in the art.

SUMMARY ~~OF THE INVENTION~~

In o~~O~~ne aspect ~~of the present invention~~, there is provided an antibody, or fragment thereof, that specifically binds human CSF-1R variant (SEQ ID NO:15), comprising a CDRH1 comprising the sequence SYGMH (SEQ ID NO:1), a CDRH2 comprising the sequence VIWYDGSNKYYADSVKG (SEQ ID NO:2), a CDRH3 comprising the sequence GDYEVDYGMDV (SEQ ID NO:3), a CDRL1 comprising the sequence RASQGISNALA (SEQ ID NO:4), a CDRL2 comprising the sequence DASSLES (SEQ ID NO:5), and a CDRL3 comprising the sequence QQFNSYPWT (SEQ ID NO:6). Another embodiment~~aspect~~ of the present disclosure~~invention~~ is an antibody, or fragment thereof, that specifically binds human CSF-1R (SEQ ID NO:16), comprising a CDRH1 comprising the sequence SYGMH (SEQ ID NO:1), a CDRH2 comprising the sequence

VIWYDGSNKYYADSVKG (SEQ ID NO:2), a CDRH3 comprising the sequence GDYEVDYGMDV (SEQ ID NO:3), a CDRL1 comprising the sequence RASQGISNALA (SEQ ID NO:4), a CDRL2 comprising the sequence DASSLES (SEQ ID NO:5), and a CDRL3 comprising the sequence QQFNSYPWT (SEQ ID NO:6). One aspect of the present invention is an antibody, or fragment thereof, that specifically binds human CSF-1R (SEQ ID NO:15), comprising a CDRH1 comprising the sequence SYGMH (SEQ ID NO:1), a CDRH2 comprising the sequence VIWYDGSNKYYADSVKG (SEQ ID NO:2), a CDRH3 comprising the sequence GDYEVDYGMDV (SEQ ID NO:3), a CDRL1 comprising the sequence RASQGISNALA (SEQ ID NO:4), a CDRL2 comprising the sequence DASSLES (SEQ ID NO:5), and a CDRL3 comprising the sequence QQFNSYPWT (SEQ ID NO:6). Antibodies of the present disclosure~~invention~~ may further comprise~~s~~ an amino acid substitution within one of said CDR sequences. In another embodiment~~aspect~~, the aforementioned CDRs do not have an amino acid substitution in one of the CDR sequences.

Another embodiment~~aspect~~ of the present disclosure~~invention~~ is an antibody, or fragment thereof, that binds CSF-1R, and comprises a VH comprising the amino acid sequence:

QDQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGEGLEWV
AVIWYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCA
RGDYEVDYGMDVWGQGTTVTVAS (SEQ ID NO:7),

or a VL comprising the amino acid sequence:

AIQLTQSPSSLSASVGDRVTITCRASQGISNALAWYQQKPGKAPKLLIYDA
SSLESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQFNSYPWTFGQGTK
VEIK (SEQ ID NO:8).

Another embodiment~~aspect~~ of the present disclosure~~invention~~ is an antibody, or fragment thereof, that binds CSF-1R, and comprises a light chain comprising the amino acid sequence of SEQ ID NO: 10 and a heavy chain comprising the amino acid sequence of SEQ ID NO: 9. In yet another aspect of the present invention, an antibody comprises two light chains each comprising the amino acid sequence of SEQ ID NO: 10 and two heavy chains each comprising the amino acid sequence of SEQ ID NO: 9.

CSF-1R-binding fragments of such antibodies are part of the disclosure~~invention~~.

The disclosure~~invention~~ also provides isolated DNA and polynucleotides/polynucleic acids encoding the antibodies or fragments thereof described above, expression vectors comprising the polynucleotides, and host cells comprising the polynucleotides. The disclosure~~invention~~ further provides methods of purifying the antibodies or fragments thereof. The disclosure~~invention~~ further provides pharmaceutical compositions comprising the antibodies, or fragments thereof, polynucleotides, vectors or host cells of the present disclosure~~invention~~ alone or with a pharmaceutically acceptable carrier, diluent or excipient. ~~The invention~~In another aspect, there is provideds a pharmaceutical compositions comprising the antibody~~ies~~, or fragments ~~thereof, of the present invention~~as defined above together with a pharmaceutically acceptable carrier, diluent or excipient. In another aspect, there is provided a pharmaceutical composition as defined above further comprising an additional pharmaceutical agent.

Additionally, the present disclosure~~invention~~ is directed to methods of inhibiting growth of a cancer cell, and methods of treating leukemia, breast and prostate carcinomas, in mammals, by administering an effective amount of an antibody. Another embodiment~~aspect~~ of the present disclosure~~invention~~ is directed to methods of inhibiting growth of a cancer cell, and methods of treating leukemia, endometrial, breast and prostate carcinomas, in mammals, by administering an effective amount of an antibody. Yet another embodiment~~aspect~~ of the present disclosure~~invention~~ is directed to methods of inhibiting growth of a cancer cell, and methods of treating leukemia, endometrial, breast and prostate carcinomas, as well as ovarian cancer, colorectal cancer, hepatocellular cancer, renal cancer, multiple myeloma, and hodgkin's lymphoma. Antibodies of the present disclosure~~invention~~ can be used to treat neoplastic diseases, including solid tumors, and for treatment of breast and prostate carcinomas. In another embodiment~~aspect~~, antibodies of the present disclosure~~invention~~ can be used to treat neoplastic diseases, including solid tumors, and for treatment of breast, endometrial, and prostate carcinomas. In another embodiment~~aspect~~, antibodies of the present disclosure~~invention~~ can be used to treat neoplastic diseases, including solid tumors, and for treatment of breast, endometrial, prostate, ovarian, colorectal, hepatocellular, and renal carcinomas.

One embodiment~~aspect~~ of the present disclosure~~invention~~ is the antibody, or fragments thereof, for use in therapy, or for use in treatment or for use as a medicament. In yet another embodiment~~aspect~~, the previously described antibodies or fragments thereof are for use in treating cancer. The present disclosure~~invention~~ can be used in treating cancers that include but are not limited to leukemia, breast cancer and prostate cancer. In one embodiment~~aspect~~, the present disclosure~~invention~~ can be

used in treating cancers that include but are not limited to leukemia, breast cancer, endometrial cancer and prostate cancer. In another embodimentaspect, the present disclosureinvention can be used in treating cancers that include but are not limited to leukemia, breast cancer, endometrial cancer, prostate cancer, ovarian cancer, colorectal cancer, hepatocellular cancer, renal cancer, multiple myeloma, and hodgkin's lymphoma.

The present disclosureinvention also includes the antibody, or fragment thereof, of the present disclosureinvention for use in treating cancer including providing or administering an effective amount of another anti-cancer treatment wherein the anti-cancer treatment includes but is not limited to an anti-angiogenesis agent, a chemotherapeutic agent, or an anti-neoplastic agent. Further, anti-neoplastic agents include but are not limited to docetaxel, paclitaxel, Herceptin® and doxorubicin. The anti-cancer treatment is administered to the patient in addition to the presently disclosed antibody or fragment. The antibody or fragment thereof is administered before, during, substantially simultaneously with, or after commencing therapy with other anti-cancer treatment or another anti-neoplastic agent.

The present disclosureinvention also provides for use of an antibody of the disclosureinvention for the manufacture of a medicament for the treatment of cancer. In a one embodimentaspect the cancer is leukemia, breast cancer, or prostate cancer. In a one embodimentaspect the cancer is leukemia, breast cancer, endometrial cancer, or prostate cancer. In another embodimentaspect of the present disclosureinvention the cancer is leukemia, breast cancer, endometrial cancer, prostate cancer, ovarian cancer, colorectal cancer, hepatocellular cancer, renal cancer, multiple myeloma, or hodgkin's lymphoma. The use of the antibody includes providing or administering an effective amount of another anti-cancer treatment wherein the anti-cancer treatment includes but is not limited to an anti-angiogenesis agent, a chemotherapeutic agent, or an anti-neoplastic agent. Further, anti-neoplastic agents include but are not limited to docetaxel, paclitaxel, Herceptin® and doxorubicin. The anti-cancer treatment is administered to the patient in addition to the presently disclosed antibody or fragment. The antibody or fragment thereof is administered before, during, substantially simultaneously with, or after commencing therapy with other anti-cancer treatment or another anti-neoplastic agent.

The disclosureinvention further provides for a method of treating cancer in a mammal, comprising administering to said mammal in need thereof an effective amount of the

antibody or fragment thereof the present disclosure~~invention~~. The cancer is selected from the group consisting of leukemia, breast cancer, endometrial cancer, and prostate cancer. In another embodiment~~aspect~~, the cancer is selected from the group consisting of leukemia, breast cancer, endometrial cancer, prostate cancer, ovarian cancer, colorectal cancer, hepatocellular cancer, renal cancer, pancreatic cancer, multiple myeloma, and hodgkin's lymphoma. Additionally, the method can further comprise administering another anti-cancer treatment to said mammal. The anti-cancer treatment is selected from the group consisting of an anti-angiogenesis agent, a chemotherapeutic agent, and an anti-neoplastic agent. The anti-neoplastic agent may be selected from the group consisting of docetaxel, paclitaxel, Herceptin® and doxorubicin.

The disclosure~~invention~~ further provides methods of using the antibodies, or compositions, to treat a mammal in need thereof, for example, to inhibit angiogenesis or bone metastases, or to inhibit tumor or hyperproliferative growth or to treat inflammatory diseases. The disclosure~~invention~~ further provides antibodies, or compositions, for use in treatment of a mammal in need thereof, for example, to inhibit angiogenesis or bone metastases, or inhibit tumor or hyperproliferative growth or inflammatory diseases.

The present disclosure~~invention~~ is also directed to a product or pharmaceutical composition containing the presently disclosed antibody, or fragment thereof. In addition the product or pharmaceutical composition may also include an additional pharmaceutical agent, anti-neoplastic agent, or anti-cancer agent or treatment, including but not limited to docetaxel, paclitaxel, Herceptin® or doxorubicin, given in combination with the presently disclosed antibody simultaneous, separate or sequential in therapy.

The disclosure~~invention~~ provides using CSF-1 levels in samples of blood, serum, plasma, tumor cells or circulating tumor cells as an indicator of the successful treatment with CSF-1R antibodies of the present disclosure~~invention~~, or fragments thereof, in patients if the cancer has CSF-1R expressed on the surface of tumor-associated macrophages. The disclosure~~invention~~ also provides a method of treating cancer in a patient, comprising the steps: (1) measuring the level of CSF-1 in a sample taken from the patient wherein the sample is selected from the group consisting of blood, serum, plasma, tumor cells and circulating tumor cells, and (2) administering to the patient the antibody or fragment thereof of the present invention if the CSF-1 levels are higher than CSF-1 levels found in a control population.

The disclosure~~invention~~ provides using IL-34 levels in samples of blood, serum, plasma, tumor cells or circulating tumor cells as an indicator of the successful treatment with CSF-1R antibodies of the present disclosure~~invention~~, or fragments thereof, in patients. The disclosure~~invention~~ also provides a method of treating cancer in a patient, comprising the steps: (1) measuring the level of IL-34 in a sample taken from the patient wherein the sample is selected from the group consisting of blood, serum, plasma, tumor cells and circulating tumor cells, and (2) administering to the patient the antibody or fragment thereof of the present disclosure~~invention~~ if the IL-34 levels are higher than IL-34 levels found in a control population.

One embodiment~~aspect~~ of the present disclosure~~invention~~ is a method for determining whether a subject having a cancer is a candidate for an anti-CSF-1R antibody-based cancer treatment regimen, wherein said antibody is the antibody of the present disclosure~~invention~~ comprising: (1) *ex vivo* or *in vitro* determining the level of CSF-1 in a sample of the subject, wherein the sample is selected from the group consisting of blood, serum, plasma, tumor cells and circulating tumor cells; and (2) wherein an increase in the level of CSF-1, as compared with the level of CSF-1 in an individual not suffering from cancer, is indicative that the subject is a candidate for the anti-CSF-1R antibody-based cancer treatment regimen.

Another embodiment~~aspect~~ of the present disclosure~~invention~~ is a method for determining whether a subject having a cancer is a candidate for an anti-CSF-1R antibody-based cancer treatment regimen, wherein said antibody is the antibody of the present disclosure~~invention~~, comprising: (1) *ex vivo* or *in vitro* determining the level of IL-34 in a sample of the subject, wherein the sample is selected from the group consisting of blood, serum, plasma, tumor cells and circulating tumor cells; and (2) wherein an increase in the level of IL-34, as compared with the level of IL-34 in an individual not suffering from cancer, is indicative that the subject is a candidate for the anti-CSF-1R antibody-based cancer treatment regimen.

The disclosure~~invention~~ also provides antibodies which bind specifically to CSF-1R. The antibodies have at least one property selected from (a) inhibit binding of CSF-1 or IL-34 to CSF-1R; (b) inhibit activation of CSF-1R; (c) reduce phosphorylation of CSF-1R; (d) reduce activation of MAPK; (e) reduce activation of Akt; (f) reduce CSF-1R amount; and

(g) induce ADCC. A preferred embodiment of the present disclosure~~invention~~ possesses properties (a) to (g).

One embodiment~~aspect~~ of the present disclosure~~invention~~ is an antibody, or fragment thereof, that specifically binds human CSF-1R variant (SEQ ID NO:15) or human CSF-1R (SEQ ID NO:16), and inhibits signaling of CSF-1R, internalizes and induces CSF-1R degradation, and stimulates Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC) activity against a variety of cells including tumors, macrophages and monocytes. SEQ ID NO:15 and SEQ ID NO:16 differ by one amino acid at position 54 which falls outside the binding region.

As used herein, the term "antibody" includes immunoglobulin molecules comprising four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Individual chains can fold into domains having similar sizes (110-125 amino acids) and structures, but different functions.

The light chain can comprise one variable domain (abbreviated herein as VL) and/or one constant domain (abbreviated herein as CL). The light chains of human antibodies (immunoglobulins) are either kappa (K) light chains or lambda (λ) light chains. The expression VL, as used herein, is intended to include both the variable regions from kappa-type light chains (VK) and from lambda-type light chains (Vλ). The heavy chain can also comprise one variable domain (abbreviated herein as VH) and/or, depending on the class or isotype of antibody, three or four constant domains (CH1, CH2, CH3 and CH4) (abbreviated herein collectively as CH). In humans, the isotypes are IgA, IgD, IgE, IgG, and IgM, with IgA and IgG further subdivided into subclasses or subtypes (IgA1-2 and IgG1-4). The present invention includes antibodies of any of the aforementioned classes or subclasses. Human IgG_1 is the preferred isotype for the antibodies of the present invention.

Three regions, called hypervariable or complementarity-determining regions (CDRs), are found in each of VL and VH, which are supported by less variable regions called frameworks (abbreviated herein as FR). Amino acids are assigned to a particular CDR region or domain in accordance with Kabat convention (Kabat, *et al.*, Ann. NY Acad. Sci. 190:382-93 (1971); Kabat, *et al.*, Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication