ANTIBODIES AGAINST CSF-1R

This application claims the benefit of U.S. Provisional Application No. 61/319,896 which was filed 1 April 2010.

This invention is directed to the fields of immunology and cancer treatment. More specifically, the present invention is directed to human antibodies that bind to human Colony Stimulating Factor-1 Receptor (CSF-1R).

Colony Stimulating Factor-1 Receptor (CSF-1R), also known as M-CSFR or CD-115, (Human CSF-1R variant; SEQ ID NO:15) (Human CSF-1R; SEQ ID NO:16; Uniprot Assession #P07333), encoded by the *c-fms* gene, is a tyrosine kinase receptor expressed selectively on macrophage and granulocyte cell lineages in normal individuals and on tumor cells in cancer. There are two known ligands, Colony Stimulating Factor-1 (CSF-1) (Human CSF-1; SEQ ID NO:17)(Uniprot Assession #P09603), also known as M-CSF, and IL-34 (Human IL-34; SEQ ID NO:18)(Uniprot Assession #Q6ZMJ4), that bind to the extracellular domain of CSF-1R. Upon CSF-1 or IL-34 binding, CSF-1R dimerizes, leading to trans-phosphorylation of the receptor and phosphorylation and activation of downstream signaling molecules such as MAPK and Akt. Phosphorylation of CSF-1R results in: (1) the proliferation and differentiation of macrophages from hematopoietic progenitor stem cells and (2) survival and migration of macrophages to various organs and tissues in the body, particularly the tumor stroma. CSF-1R can also be expressed on the surface of tumor cells.

Antibodies to murine CSF-1R are not cross-reactive in humans and consequently would be ineffective therapeutics for treating cancer in humans.

Human antibodies to CSF-1R are disclosed in PCT Publication WO2009/026303 (Brasel, *et al.*). Such antibodies do not induce Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC) activity against cells bound by the antibodies. ADCC occurs when the antibody binds to a cell expressing the antigen (target cell), making the Fc region of the antibody available for binding to the Fc receptor on natural killer cells, monocytes, neutrophils and dendritic cells (effector cell). Antibodies disclosed in *Brasel*, are incapable of bringing the target cell and effector cell together to initiate the killing of the target cell.

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

One aspect of the present invention is 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 aspect of the present 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 invention may further comprises an amino acid substitution within one of said CDR sequences. In another aspect, the aforementioned CDRs do not have an amino acid substitution in one of the CDR sequences.

Another aspect of the present 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 aspect of the present 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 invention.

The 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 invention further provides methods of purifying the antibodies or fragments thereof. The invention further provides pharmaceutical compositions comprising the antibodies, or fragments thereof, polynucleotides, vectors or host cells of the present invention alone or with a pharmaceutically acceptable carrier, diluent or excipient. The invention provides pharmaceutical compositions comprising the antibodies, or fragments thereof, of the present invention together with a pharmaceutically acceptable carrier, diluent or excipient.

Additionally, the present 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 aspect of the present 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 aspect of the present 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 invention can be used to treat neoplastic diseases, including solid tumors, and for treatment of breast and prostate carcinomas. In another aspect, antibodies of the present invention can be used to treat neoplastic diseases, including solid tumors, and for treatment of breast, endometrial, and prostate carcinomas. In another aspect, antibodies of the present 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 aspect of the present 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 aspect, the previously described antibodies or fragments thereof are for use in treating cancer. The present invention can be used in treating cancers that include but are not limited to leukemia, breast cancer and prostate cancer. In one aspect, the present invention can be

used in treating cancers that include but are not limited to leukemia, breast cancer, endometrial cancer and prostate cancer. In another aspect, the present invention 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 invention also includes the antibody, or fragment thereof, of the present invention 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 invention also provides for use of an antibody of the invention for the manufacture of a medicament for the treatment of cancer. In a one aspect the cancer is leukemia, breast cancer, or prostate cancer. In a one aspect the cancer is leukemia, breast cancer, endometrial cancer, or prostate cancer. In another aspect of the present invention 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 invention 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 invention. The cancer is selected from the group consisting of leukemia, breast cancer, endometrial cancer, and prostate cancer. In another 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 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 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 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 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 invention, or fragments thereof, in patients if the cancer has CSF-1R expressed on the surface of tumor-associated macrophages. The 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 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 invention, or fragments thereof, in patients. The 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 invention if the IL-34 levels are higher than IL-34 levels found in a control population.

One aspect of the present 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 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 aspect of the present 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 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 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 invention possesses properties (a) to (g).

One aspect of the present 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

No. 91-3242 (1991).). Each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4. The portion of an antibody consisting of VL and VH domains is designated Fv (Fragment variable) and constitutes the antigen-binding site. Single chain Fv (scFv) is an antibody fragment containing a VL domain and a VH domain on one polypeptide chain, wherein the N terminus of one domain and the C terminus of the other domain are joined by a flexible linker.

An "isolated antibody" is an antibody that (1) has been partially, substantially, or fully purified from a mixture of components; (2) has been identified and separated and/or recovered from a component of its natural environment; (3) is monoclonal; (4) is free of other proteins from the same species; (5) is expressed by a cell from a different species; or (6) does not occur in nature. Components, as used herein, exclude the antibody of the present invention. Contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. Examples of isolated antibodies include an antibody that has been affinity purified, an antibody that has been made by a hybridoma or other cell line *in vitro*, and a human antibody derived from a transgenic mouse.

The term "monoclonal antibody," as used herein, refers to an antibody obtained from a population of substantially homogeneous antibodies, *e.g.*, the individual antibodies comprising the population are substantially identical except for possible naturally occurring mutations or minor post-translational variations that may be present. Monoclonal antibodies are highly specific, being directed against a single antigenic site (also known as determinant or epitope). Furthermore, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants, each monoclonal antibody is directed against a single determinant on the antigen. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method.

The term "human antibody," as used herein, includes antibodies having variable and constant regions corresponding to human germline immunoglobulin sequences (as described in Kabat *et al.*, *supra*). The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*), for example in the CDRs. The human antibody can have at least one position replaced with an amino acid residue, *e.g.*, an activity enhancing amino acid residue which is not encoded by the human germline immunoglobulin sequence. However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences. Methods of producing a "human antibody", as used herein are not intended to include antibodies produced in a human being.

The phrase "recombinant human antibody" includes human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell, antibodies isolated from a recombinant, combinatorial human antibody library, antibodies isolated from an animal that is transgenic for human immunoglobulin genes, or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline immunoglobulin sequences.

Fc (Fragment, crystallizable region) is the designation for the portion or fragment of an antibody that consists of paired heavy chain constant domains. In an IgG antibody, for example, the Fc consists of heavy chain CH2 and CH3 domains. The Fc of an IgA or an IgM antibody further comprises a CH4 domain. The Fc is associated with Fc receptor binding, activation of antibody-dependent cell-mediated cytotoxicity (ADCC) and/or cell mediated cytotoxicity (CMC). For antibodies such as IgA and IgM, which are complexes of multiple IgG like proteins, complex formation requires Fc constant domains.

Thus, antibodies of the invention include, but are not limited to, isolated antibodies, human antibodies, humanized antibodies, recombinant human antibodies,

monoclonal antibodies, digestion fragments, specified portions and variants thereof, including antibody mimetics or comprising portions of antibodies that mimic the structure and/or function of an antibody or specified fragment or portion thereof; each containing at least one CDR. Functional fragments include antigen binding fragments that bind to a CSF-1R antigen. For example, antibody fragments capable of binding to CSF-1R, or a portion thereof, and which are embraced by the present invention include bivalent fragments such as $(Fab')_2$ with inter-chain disulfide bonds intact, monovalent fragments such as Fab (Fragment, antigen binding) which refers to the fragments of the antibody consisting of VL-CL and VH-CH1 domains and do not retain the heavy chain hinge region (*e.g.*, by papain digestion), Fabs which retain the heavy chain hinge region, Facb (*e.g.*, by plasmin digestion), $F(ab')_2$, Fab' which lack disulfide bonds, pFc' (*e.g.*, by pepsin or plasmin digestion), Fd (*e.g.*, by pepsin digestion, partial reduction and re-aggregation) and Fv or scFv (*e.g.*, by molecular biology techniques). Antibody fragments are also intended to include, *e.g.*, domain deleted antibodies, linear antibodies, single chain antibodies, scFv, single domain antibodies, multivalent single chain antibodies, multi-specific antibodies formed from antibody fragments including diabodies, triabodies, and the like that bind specifically with antigens.

The hinge region separates the Fab and Fc portions of the antibody, providing for mobility of Fabs relative to each other and relative to Fc, as well as including multiple disulfide bonds for covalent linkage of the two heavy chains.

Antibody formats have been developed which retain binding specificity, but have other characteristics that may be desirable, including for example, bispecificity, multivalence (more than two binding sites), and compact size (e.g., binding domains alone). The antibodies of the present invention are specific for CSF-1R. Antibody specificity refers to selective recognition of the antibody for a particular epitope of an antigen. Antibodies of the present invention, for example, can be monospecific or bispecific. Bispecific antibodies (BsAbs) are antibodies that have two different antigen-binding specificities or sites. Where an antibody has more than one specificity, the recognized epitopes can be associated with a single antigen or with more than one antigen. Thus, the present invention provides bispecific antibodies that bind to two

different antigens, with at least one specificity for CSF-1R. As stated above, such antibodies include any fragments thereof.

Specificity of the present antibodies or fragments thereof, for CSF-1R can be determined based on affinity and/or avidity. Affinity, represented by the equilibrium constant for the dissociation of an antigen with an antibody (K_D), measures the binding strength between an antigenic determinant and an antibody-binding site.

The antibodies, or fragments thereof, of the invention bind to an epitope of CSF-1R located on the extracellular domain segments (hereinafter referred simply to as "domains" or "ECD"). The term "epitope" as used herein refers to discrete, three-dimensional sites on an antigen that are recognized by the antibodies of the invention. Epitopes are the immunologically active regions on a complex antigen, the regions that actually bind to a B-cell receptor, and that are actually bound by the resulting antibody molecules that are produced by the B cell. Antigens generally contain at least one epitope and usually more than one epitope. Epitopes on protein antigens can be linear or non-linear. Linear epitopes are those comprised of contiguous amino acid residues in the amino acid sequence of a protein. Linear epitopes may or may not require conformational folding to form the native three-dimensional structure and elicit an immune response that produces antibodies with binding specificity to the antigen. Non-linear epitopes are comprised of non-contiguous amino acid residues. Thus, non-linear epitopes always require some degree of protein folding to bring the requisite amino acid residues into the proximity of one another to form the native three-dimensional structure and elicit an immune response that produces antibodies with binding specificity to the antigen.

Antibodies of the present invention also include those for which binding characteristics have been improved by direct mutation, methods of affinity maturation, phage display, or chain shuffling. Affinity and specificity can be modified or improved by mutating CDR and/or FW residues and screening for antigen binding sites having the desired characteristics (*see e.g.*, Yang *et al.*, J. Mol. Biol., 254: 392-403(1995)). CDRs are mutated in a variety of ways. One way is to randomize individual residues, or combinations of residues, so that in a population of, otherwise identical antigen binding sites, subsets of from two to twenty amino acids are found at particular positions.

Alternatively, mutations can be induced over a range of residues by error prone PCR methods (*see e.g.*, Hawkins *et al.*, J. Mol. Biol., 226: 889-96 (1992)). In another example, phage display vectors containing heavy and light chain variable region genes can be propagated in mutator strains of *E. coli* (*see e.g.*, Low *et al.*, J. Mol. Biol., 250: 359-68 (1996)). These methods of mutagenesis are illustrative of the many methods known to one of skill in the art.

A convenient way for generating substitutional variants is affinity maturation using phage display. Briefly, several CDR region sites are mutated to generate all possible amino acid substitutions at each site. The antibody variants thus generated are displayed in a monovalent fashion from filamentous phage particles as fusions to the gene III product of M13 packaged within each particle. The phage-displayed variants are then screened for their biological activity (e.g., binding affinity (K_D), specificity, EC50) as herein disclosed. In order to identify candidate CDR region sites for modification, alanine scanning mutagenesis can be performed to identify CDR region residues contributing significantly to antigen binding. Alternatively, or in addition, random mutagenesis may be performed on one or more CDR sequences at one or more residue positions, either while the CDR is operably linked to the variable region or while the CDR is independent of other variable region sequence and then the altered CDR returned to a variable region using recombinant DNA technology. Once such variant antibodies are generated and expressed, the panel of variants is subjected to screening as described herein, and antibodies with superior properties in one or more relevant assays may be selected for further development.

In addition to the antibodies specifically described herein, other "substantially homologous" modified antibodies can be readily designed and manufactured utilizing various recombinant DNA techniques well known to those skilled in the art. For example, the framework regions can vary from the native sequences at the primary structure level by several amino acid substitutions, terminal and intermediate additions and deletions, and the like. Moreover, a variety of different human framework regions may be used singly or in combination as a basis for the humanized immunoglobulins of the present invention. In general, modifications of the genes may be readily accomplished by a variety of well-known techniques, such as site-directed mutagenesis.

The present invention includes nucleic acid sequences that encode an anti-CSF-1R antibody heavy chain, comprising any one of the VH regions or a portion thereof, or any one of the VH CDRs, including any variants thereof, as disclosed herein. The invention also includes nucleic acid molecules that encode an anti-CSF-1R antibody light chain comprising any one of the VL regions, or a portion thereof or any one of the VL CDRs, including any variants thereof as disclosed herein. The invention also includes the nucleic acid sequences of Antibody 1, SEQ ID NOs 13 and 14 for heavy chain and light chain respectively. The antibodies of the invention include antibodies comprising the same CDR regions of Antibody 1, and/or the same light chain variable region and/or heavy chain variable region of Antibody 1.

The antibodies of the present invention may be produced by methods known in the art. These methods include the immunological method described by Kohler and Milstein, Nature 256: 495-497 (1975); Laboratory Techniques in Biochemistry and Molecular Biology, Volume 13 (Burdon *et al.* eds., Elsevier Science Publishers, Amsterdam) *in* Monoclonal Antibody Technology, The Production and Characterization of Rodent and Human Hybridomas (Campbell ed., 1984); as well as by the recombinant DNA method described by Huse *et al.*, Science 246: 1275-1281 (1989). The antibodies can also be obtained from libraries bearing combinations of VH and VL domains in the form of scFv or Fab. The VH and VL domains can be encoded by nucleotides that are synthetic, partially synthetic, or naturally derived. The present invention can be made by phage display libraries bearing human antibody fragments. Other sources of human antibodies are transgenic mice engineered to express human immunoglobulin genes.

One embodiment for the preparation of antibodies is the expression of the nucleic acid encoding the antibody according to the invention in a transgenic animal that has a substantial portion of the human antibody producing genome inserted and is rendered deficient in the production of endogenous antibodies. Transgenic animals include but are not limited to mice, goat, and rabbit. The antibodies of the present invention were made with transgenic mice. One further embodiment of the invention includes expression of the antibody-coding gene in, for example, the mammary gland of the animal for secretion of the polypeptide during lactation.

A common method for producing "humanized" antibodies is to graft CDR sequences from a MAb (produced by immunizing a rodent host) onto a human Ig backbone, and transfection of the chimeric genes into Chinese Hamster Ovary (CHO) cells which in turn produce a functional antibody that is secreted by the CHO cells.

It is understood that amino acid residues that are primary determinants of binding of single domain antibodies can be within Kabat or Chothia defined CDRs, but may include other residues as well, such as, for example, residues that would otherwise be buried in the VH-VL interface of a VH-VL heterodimer.

The protein used to identify CSF-1R binding antibodies of the invention is preferably CSF-1R and, more preferably, is the extracellular domain of CSF-1R. The CSF-1R extracellular domain can be free or conjugated to another molecule. The antibodies of this invention can be fused to additional amino acid residues. Such amino acid residues can be a peptide tag, perhaps to facilitate isolation or an IgG FC portion to optimize dimerization. Other amino acid residues for homing of the antibodies to specific organs or tissues are also contemplated.

Antibody fragments can be produced by cleaving a whole antibody, or by expressing DNA that encodes the fragment. Fragments of antibodies may be prepared by methods described by Lamoyi *et al.*, J. Immunol. Methods 56: 235-243 (1983) and by Parham, J. Immunol. 131: 2895-2902 (1983). Such fragments may contain one or both Fab fragments or the $F(ab')_2$ fragment. Such fragments may also contain single-chain fragment variable region antibodies, i.e. scFv, diabodies, or other antibody fragments. Methods of producing such functional equivalents are disclosed in:; European Patent Application Publication No. EP 239,400 (Winter); PCT Publication WO 89/09622 (Hann, *et al.*); European Patent Application Publication No. EP 338,745 (Owens, *et al.*); and European Patent Application Publication No. EP 332,424 (Beldler, *et al.*). Throughout this specification, the term "antibodies" of the invention includes any fragments thereof, whether or not specifically stated.

Preferred host cells for transformation of vectors and expression of the antibodies of the present invention are mammalian cells, e.g., NS0 cells (non-secreting (0) mouse myeloma cells), 293, SP20 and CHO cells and other cell lines of lymphoid origin such as lymphoma, myeloma, or hybridoma cells. Other eukaryotic hosts, such as yeasts, can be alternatively used.

The antibodies of the present invention may be isolated or purified by any method known in the art, including precipitation by ammonium sulfate or sodium sulfate followed by dialysis against saline, ion exchange chromatography, affinity or immuno-affinity chromatography, as well as gel filtration or zone electrophoresis. A preferred method of purification for the antibodies of the current invention is Protein-A affinity chromatography.

DNA encoding human antibodies can be prepared by recombining DNA encoding human constant regions and variable regions, other than the CDRs, derived substantially or exclusively from the corresponding human antibody regions and DNA encoding CDRs derived from a human.

Suitable sources of DNA that encode fragments of antibodies include any cell, such as hybridomas and spleen cells that express the full-length antibody. The fragments may be used by themselves as antibody equivalents, or may be recombined into equivalents, as described above. The DNA recombination and other techniques described herein may be carried out by known methods. Another source of DNA is a phage display library of antibodies, as is known in the art.

Additionally, the present invention provides expression vectors containing the polynucleotide sequences previously described operably linked to a control sequence such as an expression sequence, a promoter and/or an enhancer sequence. A variety of expression vectors for the efficient synthesis of antibody polypeptide in prokaryotic systems, such as bacteria and eukaryotic systems, including but not limited to, yeast and mammalian cell culture systems have been developed. The vectors of the present invention can comprise segments of chromosomal, non-chromosomal and synthetic DNA sequences.

Any suitable expression vector can be used. For example, prokaryotic cloning vectors include plasmids from *E. coli*, such as colE1, pCR1, pBR322, pMB9, pUC, pKSM, and RP4. Prokaryotic vectors also include derivatives of phage DNA such as M13 and other filamentous single-stranded DNA phages. An example of a vector useful in yeast is the 2 μ plasmid. Suitable vectors for expression in mammalian cells include well-known derivatives of SV-40, CMV, adenovirus, retrovirus-derived DNA sequences, and shuttle vectors derived from combination of functional mammalian vectors, such as those described above, and functional plasmids and phage DNA. Additional eukaryotic expression vectors are known in the art (*e.g.,* P.J. Southern and P. Berg, J. Mol. Appl. Genet. 1: 327-41 (1982); Subramani *et al.*, Mol. Cell. Biol. 1: 854-64 (1981); Kaufmann and Sharp, J. Mol. Biol. 159: 601-21 (1982); Scahill *et al.*, Proc. Nat'l Acad. Sci. USA 80: 4654-59 (1983); Urlaub and Chasin, Proc. Nat'l Acad. Sci. USA 77: 4216-20) (1980).

The expression vectors useful in the present invention contain at least one expression control sequence that is operatively linked to the DNA sequence or fragment to be expressed. The control sequence is inserted in the vector in order to control and to regulate the expression of the cloned DNA sequence. Examples of useful expression control sequences are the lac system, the trp system, the tac system, the trc system, major operator and promoter regions of phage lambda, the control region of fd coat protein, the glycolytic promoters of yeast, e.g., the promoter for 3-phosphoglycerate kinase, the promoters of yeast acid phosphatase, e.g., Pho5, the promoters of the yeast alpha-mating factors, and promoters derived from polyoma, adenovirus, retrovirus, and simian virus, e.g., the early and late promoters or SV40, and other sequences known to control the expression of genes of prokaryotic or eukaryotic cells and their viruses or combinations thereof.

Where it is desired to express a gene construct in yeast, a suitable selection gene for use in yeast is the trp1 gene present in the yeast plasmid YRp7. The trp1 gene provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example, ATCC No. 44076. The presence of the trp1 lesion in the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan. Similarly, Leu2-deficient yeast strains (ATCC 20,622 or 38,626) are complemented by known plasmids bearing the Leu2 gene.

The present invention also provides recombinant host cells containing the recombinant vectors previously described. Antibodies of the present invention can be expressed in cell lines other than in hybridomas. Nucleic acids, which comprise a sequence encoding a polypeptide according to the invention, can be used for transformation of a suitable mammalian host cell.

Cell lines of particular preference are selected based on high levels of expression, constitutive expression of protein of interest and minimal contamination from host proteins. Mammalian cell lines available as hosts for expression are well known in the art and include many immortalized cell lines, such as but not limited to, COS-7 cells, Chinese Hamster Ovary (CHO) cells, Baby Hamster Kidney (BHK) cells and many others including cell lines of lymphoid origin such as lymphoma, myeloma, or hybridoma cells. Suitable additional eukaryotic cells include yeast and other fungi. Useful prokaryotic hosts include, for example, *E. coli*, such as *E. coli* SG-936, *E. coli* HB 101, *E. coli* W3110, E. coli X1776, *E. coli* X2282, *E. coli* DHI, and *E. coli* MRCl, *Pseudomonas*, *Bacillus*, such as *Bacillus subtilis*, and *Streptomyces*.

These recombinant host cells can be used to produce an antibody, or fragment thereof, by culturing the cells under conditions permitting expression of the antibody or fragment thereof and purifying the antibody or fragment thereof from the host cell or medium surrounding the host cell. Targeting of the expressed antibody or fragment for secretion in the recombinant host cells can be facilitated by inserting a signal or secretory leader peptide-encoding sequence at the 5' end of the antibody-encoding gene of interest. These secretory leader peptide elements can be derived from either prokaryotic or eukaryotic sequences. Accordingly, suitable secretory leader peptides are used, being amino acids joined to the N-terminal end of a polypeptide to direct movement of the polypeptide out of the host cell cytosol and secretion into the medium.

The transformed host cells are cultured by methods known in the art in a liquid medium containing assimilable sources of carbon (carbohydrates such as glucose or lactose), nitrogen (amino acids, peptides, proteins or their degradation products such as peptones, ammonium salts or the like), and inorganic salts (sulfates, phosphates and/or carbonates of sodium, potassium, magnesium and calcium). The medium furthermore

contains, for example, growth-promoting substances, such as trace elements, for example iron, zinc, manganese and the like.

A method of treating tumor growth in a mammal by administering to the mammal an effective amount of an antibody is also provided by the present invention. Suitable conditions to be treated according to the present invention involve cells preferably expressing CSF-1R. While not intended to be bound to any particular mechanism, the present methods provide for treatment of the growth of cancer cells including for example, those in which neoplastic growth, bone metastases, organ transplant rejection or an immune disorder such as an autoimmune disease which is stimulated by CSF-1R.

"Treatment" or "treat", in the context of the present invention refers to therapeutic treatment including inhibiting, slowing, lessening or reversing the progress of the underlying condition or undesired physiological change associated with a disease or disorder, ameliorating clinical symptoms of a condition or preventing the appearance of clinical symptoms of the condition. Beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of the extent of a disease or disorder, stabilization of a disease or disorder (*i.e.*, where the disease or disorder does not worsen), delay or slowing of the progression of a disease or disorder, amelioration or palliation of the disease or disorder, and remission (whether partial or total) of the disease or disorder, whether detectable or undetectable. Treatment can also mean prolonging survival as compared to expected survival if not receiving treatment. Those in need of treatment include those already with the disease. In one embodiment, the present invention can be used as a medicament.

In the methods of the present invention, a therapeutically effective amount of an antibody of the invention is administered to a mammal or patient in need thereof. Additionally, the pharmaceutical compositions of the invention may include a therapeutically effective amount of an anti-CSF-1R antibody of the invention. A "therapeutically effective amount" or "effective dose" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of the antibody may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the antibody or antibody portion to elicit a desired response in the individual. Other factors include

administration, target site, physiological state of the patient, whether the patient is human or an animal, other medications administered, and whether treatment is prophylactic or therapeutic. Although human antibodies of the invention are particularly useful for administration to humans, they can be administered to other mammals as well. The term mammal as used herein is intended to include, but is not limited to, humans, laboratory animals, domestic pets and farm animals. A therapeutically effective amount is also one in which any toxic or detrimental effects of the antibody or antibody portion are outweighed by the therapeutically beneficial effects.

Dosage regimens may be adjusted to provide the optimum desired response (*e.g.*, a therapeutic or prophylactic response). Treatment dosages may be titrated using routine methods known to those of skill in the art to optimize safety and efficacy. Dosing schedules will typically range from a single bolus dosage or continuous infusion to multiple administrations per day (e.g., every 4-6 hours), or as indicated by the treating physician and the patient's condition. An exemplary, non-limiting range for a therapeutically effective amount of an antibody of the invention is 0.1-50 mg/kg, more preferably 3-35 mg/kg, and more preferably 5-20 mg/kg. Dosing amounts and frequencies will be determined by the physicians treating the patient and may include doses from less than 1 mg/kg to over 100 mg/kg given daily, three times per week, weekly, once every two weeks, or less often. It should be noted, however, that the present invention is not limited to any particular dose.

Anti-CSF-1R antibodies can be administered in combination with one or more other anti-cancer treatments including but not limited to an anti-angiogenesis agent, a chemotherapeutic agent, and an anti-neoplastic agent. Any suitable anti-cancer agent can be used, such as a chemotherapeutic agent, radiation, antibody or combinations thereof.

Anti-cancer agents include but are not limited to anti-neoplastic agents, antibodies, adjuvants, and prodrugs. The anti-neoplastic agents which are presently known in the art, or being evaluated, can be grouped into a variety of classes including, for example, mitotic inhibitors, alkylating agents, anti-metabolites, intercalating antibiotics, growth factor inhibitors, cell cycle inhibitors, enzymes, topoisomerase inhibitors, anti survival agents, biological response modifiers, anti-hormones, and anti-angiogenesis agents. Examples of alkylating agents include, but are not limited to,

cisplatin, cyclophosphamide, melphalan, and dacarbazine. Examples of anti-metabolites include, but are not limited to, doxorubicin, daunorubicin, paclitaxel, gemcitabine, ALIMTA® and topoisomerase inhibitors irinotecan (CPT-11), aminocamptothecin, camptothecin, DX-8951f, topotecan (topoisomerase I), etoposide (VP-16), and teniposide (VM-26) (topoisomerase II). When the anti-neoplastic agent is radiation, the source of the radiation can be either external (external beam radiation therapy – EBRT) or internal (brachytherapy – BT) to the patient being treated. The dose of anti-neoplastic agent administered depends on numerous factors, including, for example, the type of agent, the type and severity tumor being treated, and the route of administration of the agent. It should be emphasized, however, that the present invention is not limited to any particular dose. In one aspect, docetaxel is a preferred anti-neoplastic agent of the invention. In other aspects of the invention paclitaxel, Herceptin® and doxorubicin are the preferred anti-neoplastic agents.

Anti-CSF-1R antibodies of the invention can be administered with antibodies that neutralize other receptors involved in tumor growth or angiogenesis. In an embodiment of the invention, an anti-CSF-1R antibody is used in combination with a receptor antagonist that binds specifically to Her2. Another example of such a receptor is VEGFR. An anti-CSF-1R antibody can be used in combination with a VEGFR antagonist. A CSF-1R antibody can also be administered in combination with one or more suitable adjuvants, such as, for example, cytokines (IL-10, IL-4 and IL-13, for example) or other immune stimulators, such as, but not limited to, chemokine, tumor-associated antigens, and peptides.

In the present invention, any suitable method or route can be used to administer anti-CSF-1R antibodies of the invention, and optionally, to co-administer anti-neoplastic agents and/or antagonists of other receptors. In a combination therapy of the present invention, the anti-CSF-1R antibody can be administered before, during, substantially simultaneous with, or after commencing therapy with another agent, including but not limited to docetaxel, paclitaxel, Herceptin® or doxorubicin, as well as any combination thereof. The anti-neoplastic agent regimens utilized according to the invention, include any regimen believed to be optimally suitable for the treatment of the patient's neoplastic condition. Different malignancies can require use of specific anti-tumor antibodies and

specific anti-neoplastic agents, which will be determined on a patient to patient basis. Routes of administration include, for example, oral, intravenous, intraperitoneal, subcutaneous, or intramuscular administration. The dose of antagonist administered depends on numerous factors, including, for example, the type of antagonists, the type and severity tumor being treated and the route of administration of the antagonists. It should be emphasized, however, that the present invention is not limited to any particular method or route of administration.

The anti-CSF-1R antibodies of the invention, where used in a mammal for the purpose of prophylaxis or treatment, are preferably formulated as pharmaceutical compositions. Such pharmaceutical compositions and processes for preparing the same are well known in the art. *See, e.g.* Remenigton: The Science and Practice of Pharmacy (Gennaro A., *et al.*, eds., 19th ed., Mack Publishing Co., 1995).

In another aspect of the invention, antibodies of the invention can be administered in conjunction with, or chemically or biosynthetically linked to, anti-cancer agents, anti-neoplastic or anti-angiogenic agents or detectable signal-producing agents. Anti-tumor agents linked to an antibody include any agents which destroy or damage neovasculature or a tumor or macrophages to which the antibody has bound or in the environment of the cell to which the antibody has bound. The present invention may be an anti-CSF-1R antibody administered as a conjugate, including but not limited to an immunoconjugate, which binds specifically to the receptor and delivers a toxin following ligand-toxin internalization. The antibody-agent conjugate can be directly linked to each other or via a linker, peptide or non-peptide. For example, an anti-tumor agent or anti-macrophage agent is a toxic agent such as an anti-neoplastic agents or a radioisotope. Suitable anti-neoplastic agents, including chemotherapeutic agents, are known to those skilled in the art and are discussed *infra*. The invention further contemplates anti-CSF-1R antibodies linked to target or reporter moieties, including by way of example only anti-neoplastic agents, other antibodies or reporters, such as radiolabled isotopes, in a diagnostic system where a detectable signal-producing agent is conjugated to the antibody.

In accordance with the invention, a method of inhibiting angiogenesis comprises administering a composition containing an antibody or antibody fragment of the invention to a mammal for a time and in an amount effective to inhibit angiogenesis. Similarly, the antibodies and antibody fragments can be used in methods of inhibiting tumor metastasis in a mammal by administering a composition containing an antibody of the invention to a mammal for a time and in an amount effective to inhibit metastasis of a tumor.

In accordance with the invention, a method of inhibiting macrophage infiltration into the tumor stroma and stimulating tumor growth comprises administering a composition containing an antibody or antibody fragment of the invention to a mammal for a time and in an amount effective to inhibit the effects of macrophages on tumor growth. Similarly, the antibodies and antibody fragments can be used in methods of alleviating bone erosion around a tumor metastasis in a mammal by administering a composition containing an antibody of the invention to a mammal for a time and in an amount effective to inhibit bone erosion.

The invention contemplates using the CSF-1R ligand (CSF-1 or IL-34) as a biomarker in the blood, serum, plasma, tumor cells or circulating tumor cells, of cancer patients who respond to treatment when the cancer has CSF-1R expressed on the surface of tumor-associated macrophages. The invention further contemplates the method of predicting successful treatment of a patient with the antibody or fragment of the present invention by measuring the CSF-1 levels in blood in a sample wherein the sample is selected from the group consisting of serum, plasma, tumor cells or circulating tumor cells. Another aspect of the invention is 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 invention further contemplates the method of predicting successful treatment of a patient with the antibody or fragment of the present invention by measuring the IL-34 levels in blood in a sample wherein the sample is selected from the group consisting of serum, plasma, tumor cells or circulating tumor cells. Another aspect of the invention is 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 invention if the IL-34 levels are higher than IL-34 levels found in a control population.

The present invention also contemplates 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 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.

The present invention further contemplates 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 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 present invention also contemplates 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 invention comprising: (1) *ex vivo* or *in vitro* determining the level of CSF-1, or IL-34, or both, 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, or IL-34, or both, as compared with the level of CSF-1, or IL-34, or both,

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.

CSF-1 or IL-34 levels can be measured in a variety of methods including commercially available kits (R&D Systems for CSF-1 and USCN Life Sciences, Inc. for IL-34). In one such technique, either CSF-1 or IL-34 standards or samples are added to a plate pre-coated with antibodies to human CSF-1 or IL-34 to allow binding of CSF-1 or IL-34 to the antibodies. After washing unbound CSF-1 or IL-34 and other proteins, a secondary antibody is added that recognizes the anti-CSF-1 or IL-34 antibody, respectively. The secondary antibody is coupled to horseradish peroxidase that emits a bluish color when substrate is added to the wells. The intensity of the color correlates to the quantity of CSF-1 or IL-34 found in the plate.

Since CSF-1 and IL-34 are naturally occurring in a healthy human body, a CSF-1 and a IL-34 baseline would need to be determined in a control population. The control population may include a group of individuals that have not been diagnosed as having a cancerous condition or signs of infection. Accordingly, the control population will establish a range of CSF-1 and IL-34 levels which are normal or baseline for healthy individuals. For example, CSF-1 levels are 68% or 77% higher in breast or colorectal cancer patient sera, respectively, than in sera of control groups (Lawicki *et al.*, Clin. Chim. Acta 317: 112-116 (2006); Mroczho *et al.*, Clin. Chim. Acta 380: 208-212 (2007)). In one aspect of the invention, CSF-1R ligand levels are at least 50% higher in cancer patient samples than in samples from the control population. The range of CSF-1 and/or IL-34 levels in the control population will be compared to the CSF-1 and/or IL-34 level identified from the patient's sample or samples to determine if the patient's CSF-1 and/or IL-34 level is higher than the baseline range of the control population.

In one aspect, the antibodies of the present invention are for use in treating cancer wherein the cancer cells are ligand secreting. In another aspect, the antibodies of the present invention are for use in treating cancer wherein the cancer cells are CSF-1 secreting. In yet another aspect, the antibodies of the present invention are for use in treating cancer wherein the cancer cells are IL-34 secreting.

The present invention also includes kits for inhibiting tumor growth and/or angiogenesis comprising a therapeutically effective amount of a human anti-CSF-1R

antibody. The kits can further comprise the antibody or fragment thereof and an additional agent inducing additional anti-cancer agents, anti-neoplastic agents or treatments, including but not limited to docetaxel, paclitaxel, Herceptin® or doxorubicin. Alternatively, or in addition to, the kits can contain any suitable antagonist of, for example, another growth factor receptor involved in tumorigenesis or angiogenesis discussed *infra*. The kits of the present invention can further comprise an adjuvant.

Accordingly, the present receptor antibodies thus can be used *in vivo* and *in vitro* for investigative, diagnostic, prophylactic, or treatment methods, which are well known in the art. Variations in the principles of invention herein disclosed can be made by one skilled in the art and it is intended that such modifications are to be included within the scope of the present invention.

It is to be understood and expected that variations in the principles of invention herein disclosed can be made by one skilled in the art and it is intended that such modifications are to be included within the scope of the present invention.

EXAMPLES

The following examples further illustrate the invention, but should not be construed to limit the scope of the invention in any way; they should in no way be construed as limiting the broad scope of the invention. Detailed descriptions of conventional methods, such as those employed in the construction of vectors and plasmids, the insertion of genes encoding polypeptides into such vectors and plasmids, the introduction of plasmids into host cells, and the expression and determination thereof of genes and gene products can be obtained from numerous publications, including Sambrook, J. *et al.*, Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press (1989) and Coligan, J. *et al.* Current Protocols in Immunology, Wiley & Sons, Incorporated (2007).

Expression and Purification of Human Anti-CSF-1R Antibodies

For each antibody, engineer a suitable heavy chain nucleotide sequence, for example SEQ ID NO. 13 for Antibody 1 into a suitable expression plasmid, for example pGSHC, and engineer a suitable light chain nucleotide sequence, for example SEQ ID NO. 14 for Antibody 1 into a suitable expression plasmid, such as pGSLC, by a suitable method such as PCR cloning. To establish a stable cell line, co-transfect in a suitable host cell line, such as NSO or CHO cells, with linearized heavy and light chain plasmids by electroporation and culture in suitable media such as glutamine free Dulbecco's Modified Eagle Medium with dialyzed fetal calf serum and glutamine synthetase supplement. Screen clones for antibody expression by an enzyme-linked immunosorbent assay (ELISA) and select the highest producer for culture in spinner flasks. Purify antibodies by a suitable method such as protein-A affinity chromatography.

Table 1 provides the amino acid sequences and SEQ ID NOs. of the various CDRs of Antibody 1 of present invention. All CDR sequences are determined using the Kabat convention. Table 2 provides the SEQ ID NOs. of the various sequences related to the present invention. Polynucleic acid sequences that encode the amino acid sequences disclosed below are also included within the scope of the present invention.

Table 1: Amino Acid Sequence of Antibody 1
Heavy and Light Chain Variable Region CDRs

	Heavy Chain	SEQ ID NO.	Light Chain	SEQ ID NO.
CDR1	SYGMH	1	RASQGISNALA	4
CDR2	VIWYDGSNKYYADSVKG	2	DASSLES	5
CDR3	GDYEVDYGMDV	3	QQFNSYPWT	6

Table 2: Amino Acid Sequence SEQ ID NOs. of Antibody 1

	Heavy Chain			Light Chain		
	Variable region	Complete Without signal	Complete With signal	Variable region	Complete Without signal	Complete With signal
Antibody 1	7	9	11	8	10	12

Enzyme-Linked Immunosorbent Assay (ELISA) Binding Assay

For the CSF-1R binding assay, coat a 96-well plate with soluble recombinant human-CSF-1R-Fc fusion protein (R&D Systems) (1 μg/mL x 100 μL) seal plate, and incubate overnight at 4°C. Wash the wells 3 times with PBS (Phosphate Buffered Saline) containing 0.2% TWEEN-20® (PBS/T), then block wells for 1 hour at room temperature (20–25°C) (RT) with PBS containing 3% bovine serum albumin (BSA). Aspirate the BSA-PBS mixture and wash plates 3 times with PBS/T. Make serial dilutions of Antibody 1 or control IgG (starting at 3 μg/mL and diluting 3-fold) and add 100 μL to wells for 1 hour at RT. Wash wells three times with PBS/T. After washing, incubate the plate with 100 μL of an anti-human $F(ab')_2$ fragment specific-HRP conjugate (Jackson ImmunoResearch) in PBS (1:5000 dilution) at RT for 1 hour. Wash the plates 5 times with PBS/T and then incubate with 100 μL of a 1:1 preparation of TMB peroxidase substrate and peroxidase solution B (KPL) for 15 minutes at RT. Stop the colorometric reaction with the addition of 100 μL of 0.1 M H_2SO_4. Collect data using a microplate reader set at 450 nm. Analyze the absorbance data with GraphPad Prism software to calculate the ED_{50}. ED_{50}, or the half maximal effective dose, is the dose necessary to achieve 50% maximal binding.

The ED_{50} of Antibody 1 to human CSF-1R is $8.7x10^{-11}$M, reported as 0.09 nM. Antibody 1 exhibits strong binding to CSF-1R.

Enzyme-Linked Immunosorbent Assay (ELISA) Blocking Assay

ELISA to Show Antibodies Block the CSF-1/CSF-1R Interaction

Coat a 96-well microtiter plates with (0.5 μg/mL x 100 μL) CSF-1 (R&D Systems) at 4°C overnight. Wash the wells 3 times with PBS/T, then block with 100 μL 3% BSA/PBS for 1 hour at RT. Wash again 3 times with PBS/T. Dilute soluble human rh-CSF-1R-Fc fusion protein (R&D Systems) to a final concentration of 0.250 μg/mL in PBS. Concurrently, dilute Antibody 1 in PBS to a final concentration of 200 nM. Serially dilute Antibody 1 in 1:3 increments from the initial 200 nM down to $3x10^{-12}$ M. Combine 100 μL of the rh-CSF-1R-Fc with 100 μL of each dilution of Antibody 1 for 30 minutes at RT. Incubate 100 μL of Antibody 1:CSF-1R-Fc mixtures for 1 hour at RT in

the CSF-1-coated wells. After 1 hour of incubation at RT, wash 3 times with PBS/T and add a 1:5000 dilution of the anti-human IgG-Fc-HRP conjugated antibody to the plates for 1 hour at RT. Wash the plates 5 times with PBS/T and then incubate with 100 µL of a 1:1 preparation of TMB peroxidase substrate and peroxidase solution B (KPL) for 15 minutes at RT. Stop the colorometric reaction with the addition of 100 µL of 0.1 M H_2SO_4. Collect data using a microplate reader set at 450 nm. Analyze the absorbance data with GraphPad Prism software to calculate the IC_{50}. IC_{50}, or the half maximal inhibitory concentration, is the concentration of the antibody causing 50% inhibition of ligand binding to the receptor.

The IC_{50} of Antibody 1 binding to human CSF-1R is $8.1x10^{-10}$ M, reported as 0.81 nM. Antibody 1 inhibits CSF-1 binding to CSF-1R, thereby preventing CSF-1R activation by the CSF-1 ligand.

ELISA to Show Antibodies Block the IL-34/CSF-1R Interaction

Coat a 96-well microtiter plates with (0.5 µg/mL x 100 µL) IL-34 (R&D Systems) at 4°C overnight. Wash the wells 3 times with PBS/T, then block with 100 µL 3% BSA/PBS for 1 hour at RT. Wash again with 3 times PBS/T. Dilute soluble human rh-CSF-1R-Fc fusion protein (R&D Systems) to a final concentration of 0.250 µg/mL in PBS. Concurrently, dilute Antibody 1 in PBS to a final concentration of 200 nM. Serially dilute Antibody 1 in 1:3 increments from the initial 200 nM down to $3x10^{-12}$ M. Combine 100 µL of the rh-CSF-1R-Fc with 100 µL of each dilution of Antibody 1 for 30 minute at RT. Incubate 100 µL of Antibody 1–CSF-1R-Fc mixtures for 1 hour at RT in the IL-34-coated wells. After 1 hour of incubation at RT, wash 3 times with PBS/T and add a 1:5000 dilution of the anti-human IgG-Fc-HRP conjugated antibody to the plates for 1 hour at RT. Wash the plates 5 times with PBS/T and then incubate with 100 µL of a 1:1 preparation of TMB peroxidase substrate and peroxidase solution B (KPL) for 15 minutes at RT. Stop the colorometric reaction with the addition of 100 µL of 0.1 M H_2SO_4. Collect data using a microplate reader set at 450 nm. Analyze the absorbance data with GraphPad Prism software to calculate the IC_{50}. IC_{50}, or the half maximal

inhibitory concentration, is the concentration of the antibody causing 50% inhibition of ligand binding to the receptor.

The IC_{50} of Antibody 1 binding to human CSF-1R is $7.0x10^{-10}$M, reported as 0.71 nM. Antibody 1 inhibits IL-34 binding to CSF-1R, thereby preventing CSF-1R activation by the IL-34 ligand.

Binding Kinetics Analysis by Surface Plasmon Resonance / Biacore® Analysis

Measure the binding kinetics of Antibody 1 to CSF-1R-Fc at 25°C on a Biacore® 2000 SPR Biosensor (GE Healthcare). Immobilized soluble CSF-1R-Fc fusion protein (concentration of 10 µg/mL and pH 5), ranging from 395 to 1200 response units, on a CM5 chip using the standard amine coupling protocol. Use HBS-EP (0.01 M HEPES (pH 7.4), 0.15 mM NaCl, and 3 mM EDTA, 0.005% v/v Surfactant P20) as a running buffer during binding affinity measurements. Perform interaction analyses as the antibodies in solution are injected at concentrations ranging from 1.5-100 nM over the prepared surface of the CM5 sensor chip. Inject the antibodies over 3 minutes for binding and allow to dissociate for 15 minutes. Perform regeneration of the immobilized protein by a 10 µL/min injection of 20 mM HCL. Use BIAevaluation version 4.1 software to determine the K_a (k_{on}) and K_d (k_{off}) of the complex formation by simultaneous global fitting of the data to a 1:1 Langmuir model. Calculate the equilibrium association constant (K_A) from the ratio of $1/K_D$ measured in Molar (1/M). Calculate the equilibrium dissociation constant (K_D) from the ratio of rate constants K_d / K_a measured in Molar (M).

The average K_a, K_d, and K_D values for multiple Biacore® analyses for Antibody 1 with human CSF-1R are summarized in Table 3.

Table 3: Binding Kinetics of Antibody to Recombinant Human CSF-1R

Antibody	K_a (1/Ms) K_{on}	K_d (1/s) K_{off}	K_D (M)
Antibody 1	3.7×10^5	3.3×10^{-4}	8.0×10^{-10}

Antibody 1 demonstrates a high binding affinity to CSF-1R.

Phosphorylation of CSF-1R Detected by Western Blot

Seed NIH3T3 cells stably transfected with CSF-1R cells in a 12-well plate at a density of $5x10^5$ cells/well in 1 mL well of Dulbecco's Modified Eagles Medium (DMEM) containing 10% fetal bovine serum (FBS) and 1 mg/mL geneticin for 5 hours. Wash monolayers twice in PBS and culture overnight in 0.9 mL/well DMEM with 1% FBS. Make serial dilutions of Antibody 1 in DMEM (starting at 1 µg/mL and diluting 3-fold) and add 100 µL to wells for 2 hours at 37°C. Stimulate cells with 100 ng/mL CSF-1 or IL-34 ligand for 10 minutes and then place on ice and wash with ice-cold PBS. Lyse the cells in 100 µL of 50 mM Hepes (pH 7.5 150 mM NaCl, 0.5% Triton X-100, 1 mM Na_3VO_4, 10 mM NaPPi, 50 mM NaF) and tablet of protease inhibitors (Roche) on ice for 10 minutes. Clarify the lysed cells by centrifugation at 4°C. Load 20 µL lysate on a denaturing electrophoresis gel and blot onto a nitrocellulose membrane. Detect tyrosine-phosphorylated CSF-1R on the blot by using an anti-phosphoCSF-1R antibody at 1 µg/mL (Cell Signaling). Determine CSF-1R signaling by probing the blot with either anti-phospho-MAPK (1:500 dilution) or anti-phopho-Akt (1:1000) (Cell Signaling). To insure equal loading of the lanes, probe blots with anti-CSF-1R antibodies (1 µg/mL; R&D Systems) or anti-actin antibodies (1:2000; Sigma). Incubate all primary antibodies with rocking for 1 hour at RT; followed by the corresponding secondary antibody conjugated with HRP, also with rocking for 1 hour at RT. Visualize bands with a chemiluminescence reagent (GE Healthcare).

NIH-3T3 cells stably transfected with CSF-1R show rapid phosphorylation of CSF-1R when stimulated with either CSF-1 or IL-34. The presence of Antibody 1 inhibits CSF-1R phosphorylation, even when levels of Antibodies are at 1 nM, indicating that the binding of Antibody 1 to CSF-1R prevents activation of the receptor by either CSF-1 or IL-34. Inhibition of CSF-1R phosphorylation also leads to diminished phosphorylation of the signaling molecules used by the either the CSF-1 or IL-34 pathway. Both Akt and MAPK, which are downstream of CSF1-R, have decreased phosphorylation levels when cells are incubated with 100 nM to 1 nM of Antibodies 1. Therefore, Antibody 1 prevents CSF-1R activation and the activation of the kinase cascade that follows CSF-1R stimulation. There was no difference in the protein levels of

actin and CSF-1R between lanes, as evidenced by equal chemiluminescent signal in each lane seen when the blots were probed with antibodies against these molecules. Hence, the inhibition of CSF-1R phosphorylation is not due to technical difficulties with uneven loading of protein samples but a true representation of decreased signaling.

Internalization and Degradation Assays

Antibody 1 Induces Internalization of the CSF-1R Receptor

Plate NIH-3T3-CSF-1R cells on 8-well chambered slides (Nunc) and incubate at 37°C until cells cover 50% of the surface area of the wells. Allow the slides to cool to 4°C before the start of the experiment in a water:ice slurry mixture. Incubate cells with 5 µg/mL Antibody 1 and immediately transfer to 37°C for 15 minutes to 4 hours to allow internalization to occur. Continue incubating a separate set of slides in the water:ice slurry mixture for 15 minutes to 4 hours with 5 µg/mL Antibody 1 as a control. The 4°C incubation prevents internalization of the CSF-1 receptor, therefore any signal seen within the cell is an artifact. After incubation, wash cells twice with cold PBS before fixing the cells with ice-cold 1% paraformaldehyde for 15 minutes. After washing three times with cold PBS, permeabilize cells in PBS containing 0.5% saponin and 1% BSA for 5 minutes and then wash again in PBS containing 1% BSA. Label with goat Cy3 anti-human IgG (1:5000 dilution) for 1 hour to fluorescently label Antibody 1. Perform three final washes with PBS before mounting in GelMount (Biomeda) and covering the slides with coverglass. The fluorescently labeled Antibody 1 can be visualized microscopically to determine cellular localization under the various conditions described above.

Upon microscopic inspection, Antibody 1 is seen on the periphery of the cell at 4°C before the start of the experiment. Switching the cells to 37°C allows rapid internalization of the Antibody1/CSF-1R complex, usually seen within 15 minute. After 1 or 2 hours of incubation with Antibody 1, all antibody/CSF-1R complexes are perinuclear with very little visualized on the plasma membrane, indicating that Antibody 1 binding to CSF-1R induces the receptor to internalize rapidly and remain inside the cell. Cells kept at 4°C for up to 2 hours show only peripheral staining of the cell, with no

internalization of Antibody 1. Thus, internalization of the antibody/CSF-1R is an ATP-dependent process that occurs within 15 minutes of antibody addition to the cells.

Antibody 1 Induces Degradation of the CSF-1R Receptor

Seed NIH-3T3-CSF-1R cells at $5x10^5$ cells/well in a 12 well plate in 1 mL DMEM media containing 10% FBS and 1 mg/mL geniticin. After incubating cell at 37°C for 5 hours, add either 100 ng/mL CSF-1 or 15 μg/mL Antibody 1 to the plates. CSF-1 is known to induce internalization and degradation of CSF-1R, thereby acting as a positive control for the experiment. Allow CSF-1 to remain on cells for 1 and 5 hours before collecting cells. In the other wells, incubate for 24 and 48 hours with Antibody 1 before collecting cells. Aspirate medium, lyse cells and run clarified lysates on gels (as described above). Probe transferred proteins with anti-CSF-1R antibodies to determine the amount of receptor for each condition. Probe the same blots with anti-actin antibodies to normalize protein levels. Quantify CSF-1R and actin levels using the Multi-Gauge program to measure band density on the Western blot. The relative total CSF-1R protein level is represented by the total CSF-1R band density divided by the corresponding actin band density.

CSF-1 incubation with NIH-3T3-CSF-1R cells leads to degradation of CSF-1R within 1 hour of treatment and CSF-1R levels are halved by 5 hours. Degradation of CSF-1R when incubated with Antibody 1 is not as pronounced; Antibody 1 decreases CSF-1R levels by one quarter. After 48 hours, degradation levels remain the same, indicating that the degradation rate remains constant. Therefore, Antibody 1 binding to CSF-1R leads to the degradation of the receptor in cells.

Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC) by Antibody 1

Besides inhibiting CSF-1 binding to CSF-1R and inducing internalization and degradation of CSF-1R, Antibody 1 also inhibits CSF-1R by triggering an ADCC response.

NKM-1 human leukemia cells are seeded at $2x10^5$ cells/mL in 25 μL RPMI media containing 10% Ultra-Low IgG FBS and 3 ng/mL of human IL-2 in a 96 well plate. Make 1:3 serial dilutions of 1 μg/mL Antibody 1 in either an IgG1 or IgG2

backbone, adding 25 µL/well. After a 15 minute incubation, seed 25 µL of $3x10^6$ cells/mL human NK cells (Lonza) and incubate for an additional four hours at 37°C. Add 10 µL of Lysis Buffer from aCella-TOX kit (Cell Technology) and bring to RT for 15 minutes. Add 125 µL of low IgG FBS and centrifuge plates for 1 minute at 750 xg. In Optiplates (Perkin Elmer), add 50 µL Enzyme Assay Diluent from aCella-TOX kit and carefully transfer 50 µL of cell supernatant to Optiplates. Prepare enzyme assay reagent and detection reagent according to kit instructions, adding 100 µL enzyme assay reagent immediately followed by 50 µL of detection reagent to Optiplates. Read plates in luminometer 20 minutes after reagents are added.

ADCC activity increases in an Antibody 1 dose-dependent manner with 9% of NKM-1 cells killed when Antibody 1 concentrations reach 1 µg/mL. In contrast, when Antibody 1 is cloned into an IgG2 backbone there is no change in ADCC activity with increasing amounts of Antibody 1. Therefore Antibody 1, an IgG1 molecule, induces ADCC of cells to which it binds.

Epitope Mapping

Previous publications have determined that CSF-1 binds to the first three Ig domains of CSF-1R. Wang *et al.,* Mol. Cell. Biol. 13: 5348 (1993). Antibody 1 binds CSF-1R and inhibits CSF-1 binding to human CSF-1R, thus it must bind on or near the first three Ig domains of CSF-1R. To determine which epitopes Antibody 1 binds to on the CSF-1R molecule, the first three Ig domains of mouse and human are swapped (see Table 4). Antibody 1 does not recognize the mouse CSF-1R therefore, if a mouse Ig domain is inserted in a critical binding region of the human CSF-1R, binding of the antibodies would not occur.

Insert the modifications of the first three Ig domains into a human CSF-1R backbone containing the remaining two most C-terminal Ig domains of the extracellular domain. Fuse these constructs to the Fc portion of an IgG molecule for ease in protein production and stability of the molecules.

Table 4: CSF-1R Ig Domains Defined

Ig Domain	Amino Acid No.
1	21-107
2	108-201
3	202-292
4	293-402
5	401-512

Chimera 1 thus contains mouse Ig domain 1, human Ig domain 2 and human Ig domain 3 (m,h,h), human Ig domains 4 and 5 fused to an Fc tag. Chimera 2 to chimera 7 are as follows: m,m,h (chimera 2), m,m,m (chimera 3), h,m,h (chimera 4), h,m,m (chimera 5), h,h,m (chimera 6) and m,h,m (chimera 7) fused to the last two Ig domains of human CSF-1R and then the Fc tag.

Antibody 1 binding to the chimeric CSF-1R proteins is determined by binding ELISAs and Biacore® as described above. Briefly, coat plates with 100 µL of 200 ng/mL human, mouse or chimera 1-7 proteins overnight. After washing and blocking the plates, add Antibody 1 in replicates at 100 nM concentration. Add anti-human Fab secondary antibodies conjugated to HRP at a concentration of 1:10,000 to detect Antibody 1 binding to the CSF-1R molecules.

As expected, Antibody 1 binds to the human but not mouse CSF-1R in the ELISA binding assays. Antibody 1 binds weakly to chimeras containing the first or second human Ig domains, but not those that contain the first or second mouse Ig domain. Antibody 1 binds strongly to the chimera that contains both the first and second Ig domain of human CSF-1R. All other constructs do not bind Antibody 1. Therefore, Antibody 1 binds to Ig domains one and two, but requires both domains for strong antibody binding to human CSF-1R when evaluated in a binding assay. Biacore® data recapitulates the ELISA data. Any chimeric construct that did not contain a human second Ig domain did not bind Antibody 1 even when antibody levels were at 1 µM, indicating that the second Ig domain is required for Antibody 1 binding. Antibody 1 binding was further enhanced when the first Ig domain was also of human origin. Therefore, Antibody 1 primarily binds to the second Ig domain of CSF-1R but has some beneficial contacts with the first Ig domain.

Differentiation and Proliferation Assays

Macrophage Differentiation by CSF-1

Seed monocytes (Lonza) at a density of $4x10^5$ cells/mL in each chamber of an 8-chamber slide in 900 µL Roswell Park Memorial Institute (RPMI) 1640 media containing 10% FBS and 100 ng/mL hCSF-1. Make serial dilutions of Antibody 1 in RPMI (starting at 1 µg/mL and diluting 3-fold) and add 100 µL to the wells. Incubate at 37°C, changing media every three days until monocytes are visibly seen to adhere to the plate and elongate, which is characteristic of macrophage differentiation.

Monocytes treated with 2 nM or higher concentrations of Antibody 1 in the presence of CSF-1 fail to differentiate into macrophages, retaining their rounded morphology characteristic of monocytes. CSF-1 induction of monocyte to macrophage differentiation can be inhibited with Antibody 1 with an IC_{50} of 0.25 nM. In addition, many die during treatment, unable to survive without continual stimulation with CSF-1.

Macrophage Differentiation by IL-34

Seed monocytes (Lonza) at a density of $4x10^5$ cells/mL in each chamber of an 8-chamber slide in 900 µL Roswell Park Memorial Institute (RPMI) 1640 media containing 10% FBS and 100 ng/mL hIL-34. Make serial dilutions of Antibody 1 in RPMI (starting at 1 µg/mL and diluting 3-fold) and add 100 µL to the wells. Incubate at 37°C, changing media every three days until monocytes are visibly seen to adhere to the plate and elongate, which is characteristic of macrophage differentiation.

Monocytes treated with 2 nM or higher concentrations of Antibody 1 in the presence of IL-34 fail to differentiate into macrophages, retaining their rounded morphology characteristic of monocytes. IL-34 induction of monocyte to macrophage differentiation can be inhibited with Antibody 1 with an IC_{50} of 0.3 nM. In addition, many die during treatment, unable to survive without continual stimulation with CSF-1.

Monocyte Proliferation by CSF-1

Seed monocytes (Lonza) at a density of 3000 cells/well in a 96-well plate in the presence of RPMI 1640 media containing 10% FBS and 100 ng/mL CSF-1. The following day, exchange media and add Antibody 1 serially diluted from 20 nM to 0.01 nM (1:3 fold dilutions). Exchange media for fresh media containing 10% FBS, 100 ng/mL CSF-1, and antibody three days later and allow cells to grow an additional 5 days. Upon addition of 100 µL of CellTiter Glo Luminecent buffer and substrate (Promega), shake cells for 10 minutes and read luminescence as an indicator of viability.

The IC_{50} of Antibody 1 inhibiting 50% of monocyte growth is $1.4x10^{-10}M$, reported as 0.1 nM. The IC_{50} for CSF-1 induction of monocyte growth in the presence of Antibody 1 indicates that Antibody 1 inhibits proliferation of monocytes in culture.

Monocyte Proliferation by IL-34

Seed monocytes (Lonza) at a density of 3000 cells/well in a 96-well plate in the presence of RPMI 1640 media containing 10% FBS and 100 ng/mL IL-34. The following day, exchange media and add Antibody 1 serially diluted from 20 nM to 0.01 nM (1:3 fold dilutions). Exchange media for fresh media containing 10% FBS, 100 ng/mL IL-34, and antibody three days later and allow cells to grow an additional 5 days. Upon addition of 100 µL of CellTiter Glo Luminecent buffer and substrate (Promega), shake cells for 10 minutes and read luminescence as an indicator of viability.

The IC_{50} of Antibody 1 inhibiting 50% of monocyte growth is 0.5 nM. The IC_{50} for IL-34 induction of monocyte growth in the presence of Antibody 1 indicates that Antibody 1 inhibits proliferation of monocytes in culture.

Proliferation Assay for Tumor Cell Lines with CSF-1

Seed NKM-1 leukemia cells at $1x10^4$ cells/mL in 100 µL RPMI 1640 media containing 1% FBS in a 96 well plate overnight. Add 20 ng/mL of CSF-1 to cells in RPMI 1640 media containing 1% FBS and 20 nM to 0.01 nM serially diluted Antibody 1 (1:3 fold dilutions). Incubate for an additional 3 days at 37°C. Upon addition of 100 µL

of CellTiter Glo Luminecent buffer and substrate (Promega), shake cells for 10 minutes before reading luminescence (as an indicator of viability).

The IC_{50} for NKM-1 growth in the presence of Antibody 1 is $7.6x10^{-11}$ M, reported as 0.07 nM. This indicates that Antibody 1 inhibits proliferation of NKM-1 cells in culture.

***In Vivo* Tumor Models wherein CSF-1R is Expressed on the Surface of the Tumor**

Prolonged Survival of NKM-1 Human Leukemia-Bearing Mice Treated with Antibody 1

Irradiate Nu/nu mice sublethally with 200 rads/mouse 24 hours before intravenous injection with $2.5x10^6$ NKM-1 leukemia cells. A further 24 hours later, randomly divide mice into 4 groups receiving either 60 mg/kg human IgG, 60 mg/kg, 20 mg/kg or 5 mg/kg Antibody 1 twice weekly. Monitor mice daily for survival. Determine P values by log rank Mantel Cox test.

Table 5: Increased Survival Of Mice Treated With Antibody 1 Bearing NKM-1 Leukemia Cells

Treatment	Median survival(days)	Cohort comparison	P value relative to control
Human IgG	35	N/A	N/A
Antibody 1 (5 mg/kg)	55	Human IgG control	<0.0001
Antibody 1 (20 mg/kg)	46	Human IgG control	<0.0001
Antibody 1 (60 mg/kg)	46	Human IgG control	<0.0001

N/A=Not Applicable

Tumor volumes are calculated by the formula Volume = $[(Pi/6)\ l\ x\ w^2]$, wherein Pi equals 3.14, w represents width and l represents length. Percent of Control or %T/C = 100* (Treatment Volume) / (Control Volume). Statistical significance was ascribed if the p-value was less than or equal to 0.05.

Antibody 1 increases the survival of mice bearing NKM-1 leukemia cells as seen in Table 5.

***In Vivo* Tumor Models wherein CSF-1R is Expressed on the Surface of Tumor-Associated Macrophages**

Anti-CSF-1R Antibody 1 is a fully human antibody that recognizes the human form of CSF-1R but not the murine form of the receptor. Therefore, an anti-mouse antibody is required to conduct proof of concept *in vivo* studies wherein CSF-1R is expressed on the macrophage. These studies address the role of mouse macrophages on the growth of human tumors in mice xenograft models. An anti-mouse antibody would affect the mouse macrophages but not the tumor, allowing the ability to gauge the effect of stromal macrophages on tumor growth. The following experiments are conducted with Antibody 2, a rat-anti-mouse CSF-1R antibody.

Efficacy of the Anti-Mouse CSF-1R mAb in the AN3CA Xenograft Model of Human Endometrial Carcinoma

Subcutaneously inject Nu/nu mice (female, 7-8 weeks of age) with $5x10^6$ AN3CA cells/mouse into the left flank. When tumors reach approximately 200 mm^3, randomize mice into groups of 12 mice/treatment.

Prepare Antibody 2 and Rat IgG in saline at a concentration of 6 mg/mL and administer subcutaneously three times a week at 40 mg/kg. On day 15 record tumor volumes and calculate % T/C. Analyze tumor volumes using RM ANOVA. A T/C% of 66% is calculated for the treatment group with Antibody 2 versus the Rat IgG control group. These results show significant tumor inhibition ($p=0.045$) in animals treated with Antibody 2.

Efficacy of the Anti-Mouse CSF-1R mAb in the HCC1954 Xenograft Model Of Human Breast Carcinoma

Inject Nu/nu (female, 7-8weeks; Charles River Laboratories) mice subcutaneously with $1x10^7$ HCC1954 cells/mouse. When tumors reach 300 mm^3 in size, randomize mice by tumor size, allocating 12 animals to each treatment group. Prepare rat IgG and Antibody 2 in saline at a concentration of 6 mg/mL and administer subcutaneously, three times a week. Dose rat IgG animals at 40 mg/kg and dose Antibody 2 animals with either 40 mg/kg or 10 mg/kg at each injection. Record tumor measurements twice weekly; 37

days after first injection calculate the T/C%. Analyze tumor volumes using RM ANOVA.

As shown in Table 6, a T/C% of 77% was calculated for the treatment group receiving 10 mg/kg and T/C% of 56% for the treatment group receiving 40 mg/kg on day 37 as the ratio of the relative tumor volumes versus the saline control group. The results show significant tumor inhibition (p=0.0014) in animals treated with 40 mg/kg Antibody 2.

Antitumor Effect of an Anti-Mouse CSF-1R mAb in the DU145 Human Prostate Xenograft Model

Subcutaneously inject Nu/nu mice (male, 7-8 weeks of age) with $15x10^6$ DU145 cells/mouse into the left flank. When tumors reach approximately 250 mm^3, randomize mice into groups of 12 mice/treatment.

Prepare Antibody 2 and Rat IgG in saline at a concentration of 6 mg/mL and administer subcutaneously three times a week at 40 and 10 mg/kg. On day 21 record tumor volumes and calculate % T/C for the 10 mg/kg and 40 mg/kg. Analyze tumor volumes using RM ANOVA.

As shown in Table 6, a T/C% of 50 and 43 is calculated treatment groups 10 mg/kg and 40 mg/kg respectively versus the Rat IgG control group. These results show significant tumor inhibition (p=<0.0001 for both concentrations) in animals treated with Antibody 2.

Table 6: Inhibition of Human Tumor Xenografts by Antibody 2

Model Description	Treatment	% T/C	Cohort Comparison	P value relative to control
HCC1954 (breast)	Antibody 2 (10 mg/kg)	77	Rat IgG control	0.4543
HCC1954 (breast)	Antibody 2 (40 mg/kg)	56	Rat IgG control	0.0014
DU145 (prostate)	Antibody 2 (10 mg/kg)	50	Rat IgG control	<0.0001
DU145 (prostate)	Antibody 2 (40 mg/kg)	43	Rat IgG control	<0.0001

Decreased Macrophage Infiltration in HCC1954 Breast and DU145 Prostate Tumors Treated with an Anti-Mouse CSF-1R mAb

Remove tumors from the animals in the HCC1954 (breast) and DU145 (prostate) animal studies described above. Cut tumor along the longest axis and place in 10% formalin overnight at 4°C while rocking. After 24 hours, wash 2 times in PBS over 20 minutes, then add solutions of progressively higher concentrations of ethanol, until the tumor is in 100% ethanol. Exchange the ethanol for xylene by several incubations in 100% xylene and embed the tumor in paraffin. Section paraffin blocks at 4 μm and place on glass slides. Deparaffinize and rehydrate tissue by progressive incubations in xylene followed by increasing concentrations of water in ethanol. Heat tumor slides in target retrieval solution (Dako) for 3 minutes in microwave before blocking endogenous peroxidases with 3% H_2O_2 for 10 minutes at RT. Block non-specific protein binding with Protein Block (Dako) for 10 minutes before adding rat anti-mouse macrophage-specific antibody, F4/80 (2 μg/mL; Serotec) conjugated to biotin and incubate overnight at 4°C. Incubate in HRP-Streptavidin (1:1000 dilution; Jackson ImmunoResearch) for 45 minutes at RT and wash 3 times. Develop in DAB (Dako) per kit instructions, stopping the reaction with two washes in water. Counterstain briefly with Mayer's Hematoxylin (10 minutes; Dako) followed by a water wash, acid alcohol dip, a second water wash and bluing using ammonia water. Dehydrate, clear and coverslip using a permanent mounting medium. Analyze 5 images from three animals for each treatment group. Using ImagePro software, determine the number of macrophages/ area for each treatment group.

Table 7: Macrophage Infiltration in Tumors Treated with an Anti-Mouse CSF-1R mAb Antibody 2

Model Description	Treatment	Avg. Macrophage No./Area	Cohort Comparison	P value relative to control
HCC1954 (breast)	Rat IgG	664	N/A	N/A
HCC1954 (breast)	Antibody 2 (10 mg/kg)	82	Rat IgG control	<0.0001
HCC1954 (breast)	Antibody 2 (40 mg/kg)	76	Rat IgG control	<0.0001
DU145 (prostate)	Rat IgG	84	N/A	N/A
DU145 (prostate)	Antibody 2 (10 mg/kg)	5	Rat IgG control	<0.0001
DU145 (prostate)	Antibody 2 (40 mg/kg)	0.5	Rat IgG control	<0.0001

N/A=Not Applicable

As seen in Table 7, macrophage numbers are decreased in both tumor models, especially along the periphery. Thus, Antibody 2 treatments lead to a decrease in macrophage infiltration of the tumors, indicating that the anti-mouse CSF-1R antibody is responsible for depleting the macrophage population in these tumors.

Significance of Macrophages and CSF-1 Levels on Tumor Progression

In addition to breast and prostate, inhibition of tumor growth, as well as the treatment of many cancers can be beneficially effected by the administration of Antibody 1. Some tumor types such as renal cancer express CSF-1R on their cell surface and could be directly inhibited from growing with Antibody 1 treatment (Soares, *et al.*, Modern Pathol. 22: 744-752 (2009)). Other tumor types such as Hodgkin's lymphoma and multiple myeloma, which have a large tumor-associated macrophage population could benefit from Antibody 1 treatment, where Antibody 1 could eliminate the macrophages that are inducing tumor growth (Steidl, *et al.* New Engl. J. Med. 362: 875-885 (2010); Zheng, *et al.*, Blood 114: 3625-3628 (2009)). Additionally, tumors that secrete CSF-1 are sensitive to anti-CSF-1 or CSF-1R treatment, such as colorectal cancer (Aharinejad, *et al.*, Cancer Res. 62: 5317-5324 (2002)) and would be appropriate for Antibody 1 treatment.

Moreover, ovarian cancer, hepatocellular carcinoma and renal cell carcinomas, whose high CSF-1 expression correlates with poor prognosis would all be candidates for Antibody 1 treatment (Toy, *et al.*, Gyn. Oncol. 80: 194-200 (2001); Zhang, *et al.*, Gyn. Oncol. 107: 526-531 (2007); Zhu, *et al.*, J. Clin. Oncol. 26: 2707-2716 (2008); Gerharz, *et al.*, Urol. 58: 821-827 (2001)).

Non-CSF-1R Expressing Tumors that Possess Tumor-Associated Macrophages are Growth Inhibited by Anti-CSF-1R Antibodies Only if CSF-1 Secreting

Seed tumor cell lines HCC1954 (breast), DU145 (prostate) and Pc3 (prostate) at $5x10^5$ cells/mL for 48 hours in growth medium containing 1% FBS. Collect, clarify, and analyze the medium using a R&D Quantikine Human M-CSF Assay Kit (R&D Systems). Determine CSF-1 levels at time 0 and 48 h as pg/mL.

At 0 hours, as expected, there was no discernable CSF-1 in the media. However, within 48 hours, the HCC1954 cells secreted 3613 pg/mL of CSF-1 while the DU145 cells producde 4019 pg/mL. In contrast, the Pc3 cells media only contained 39 pg/mL of CSF-1.

The Prostate Cell Line Pc3 is Grown in Mice to Verify if Lack of CSF-1 Expression Is Correlated with Resistance to Anti-CSF-1R Treatment

Inject $5x10^6$ Pc3 prostate cells subcutaneously into nude mice (male, 7-8 weeks of age) and allow tumors to reach 300 mm^3 before subdividing into treatment groups of 12 mice each. Dose animals with either 40 mg/kg of Rat IgG, 40 mg/kg Antibody 2 or 10 mg/kg Antibody 2 three times a week until control tumors reach 2000 mm^3. Measure tumor volumes and calculate the T/C% for each treatment group on day 18 as the ratio of the relative tumor volumes versus the Rat IgG control group. Analyze tumor volumes using RM ANOVA.

Table 8: Correlation of CSF-1 and Resistance to Anti-CSF-1R Treatment

Treatment	T/C%	Cohort Comparison	P value relative to control
Antibody 2 (10 mg/kg)	101	Rat IgG control	0.95
Antibody2 (40 mg/kg)	102	Rat IgG control	0.53

There is no difference in growth between the Rat IgG treated control groups versus the Antibody 2 treated groups, indicating that elevated CSF-1 levels may be a biomarker for treatment with anti-CSF-1R antibodies.

CSF-1 Secretion is Correlated to Sensitivity of Anti-CSF-1R Treatment when CSF-1R is Expressed on the Surface of Tumor-Associated Macrophages

Inject cells, as detailed in Tables 9 and 10 below, subcutaneously into nude mice and allow tumors to reach 300 mm^3 before subdividing into treatment groups of 12 mice each. Dose animals according to the treatment regiment detailed in Tables 9 and 10 below three times a week until control tumors reach 2000 mm^3. Measure tumor volumes and calculate the T/C% for each treatment group as the ratio of the relative tumor volumes versus the Rat IgG control group. Analyze tumor volumes using RM ANOVA.

Table 9: Sensitivity in CSF-1 Secreting Tumor Models

Model Description	Tumor Type	Species	Treatment	% T/C	Cohort Comparison	P value relative to control
MDA-MB-231 LS-OP-PT	Breast	Human	40 mg/kg TIW	51	Rat IgG control	0.0002
HCC-1954	Breast	Human	40 mg/kg TIW	56	Rat IgG control	0.0014
DU145	Prostate	Human	40 mg/kg TIW	43	Rat IgG control	<0.0001
4T1	Breast	Mouse	40 mg/kg TIW	54	Rat IgG control	0.0001
EMT6	Breast	Mouse	40 mg/kg TIW	42	Rat IgG control	<0.0001

TIW=Three time a week.

Table 10: Sensitivity in Non-CSF-1 Secreting Tumor Models

Model Description	Tumor Type	Species	Treatment	% T/C	Cohort Comparison	P value relative to control
MCF-7	Breast	Human	60 mg/kg BIW	70	Rat IgG control	0.0166
JimT1	Breast	Human	40 mg/kg TIW	93	Rat IgG control	0.23
PC3	Prostate	Human	40 mg/kg TIW	102	Rat IgG control	0.53

TIW=Three time a week.

CSF-1 secreting tumors (Table 9) all respond to anti-CSF-1R treatment with Antibody 2 while those tumors that do not secrete CSF-1 (Table 10) did not respond or only poorly to anti-CSF-1R Antibody 2. CSF-1 secretion is correlated to sensitivity of anti-CSF-1R treatments in tumor models wherein CSF-1R is expressed on the surface of tumor-associated macrophages. Accordingly, elevated CSF-1 levels function as a potential sensitivity indicator or biomarker when CSF-1R is expressed on the surface of tumor-associated macrophages.

CSF-1R can also be expressed on the surface of tumor cells. CSF-1 secretion is not correlated to sensitivity of anti-CSF-1R treatments for CSF-1R tumor cell surface expression.

IL-34 Levels in Tumors and Patient Sera Samples

In addition to CSF-1, IL-34 can also bind to CSF-1R, induce phosphorylation of the receptor, and activate downstream signaling molecules, which lead to macrophage differentiation and survival. Both CSF-1 and IL-34 bind to the IgG domains in the extracellular region of CSF-1R (Lin, *et al.,* Science, 320: 807-11 (2008)) and both ligands can be inhibited from binding to CSF-1R by Antibody 1 (IC_{50}= 0.81 nM and IC_{50}=0.71 nM for inhibition of CSF-1 and IL-34 binding by Antibody 1, respectively as discussed *supra*). Treatment of NIH-3T3 cells stably transfected with CSF-1R with Antibody 1 inhibits phosphorylation of CSF-1R by IL-34 (as discussed *supra*). Furthermore, IL-34 induction of monocyte to macrophage differentiation and macrophage proliferation can be inhibited with Antibody 1 with IC_{50}s of 0.3 nM and 0.5nM, respectively (as discussed *supra*), which is comparable to the results seen with CSF-1. Therefore, IL-34 activity and its inhibition by Antibody 1 are similar to that seen with CSF-1.

IL-34 levels are elevated in many tumor cells including breast, prostate and endometrial tumor cell lines. Additionally, a subpopulation of prostate and breast patients have high IL-34 protein levels in their sera. Accordingly, since IL-34 functions nearly identically to CSF-1 and CSF-1 secretion is correlated to sensitivity of anti-CSF-1R treatments, elevated IL-34 levels also function as a sensitivity indicator or biomarker.

Breast Tumor Combination Studies

Inject Nu/nu mice (female, 7-8 weeks of age) subcutaneously with $1x10^7$ HCC-1954 breast cells/mouse and allow tumors to reach 300 mm^3 before treatment. Randomize mice into groups of 12 and treat as follows:

Table 11: Breast Combination Studies

Treatment	Dose	% T/C	Cohort Comparison	P value relative to control
40 mpk Human IgG + 40 mpk Antibody 2	TIW	44	40 mpk Human IgG + 40 mpk Rat IgG control	0.0002
40 mpk Herceptin® + 40 mpk Rat IgG	TIW	46	40 mpk Human IgG + 40 mpk Rat IgG control	<0.0001
40 mpk Herceptin® + 40 mpk Antibody 2	TIW	26	40 mpk Human IgG + 40 mpk Rat IgG control	<0.0001
40 mg/kg Antibody 2	TIW	42	saline control	0.006
8 mg/kg IP Doxorubicin	Q7Dx2	50	saline control	0.008
40 mg/kg Antibody 2 8 mg/kg Doxorubicin	TIW, Q7Dx2	28	saline control	0.0005
40 mg/kg Antibody 2	TIW	38	saline control	<0.0001
10 mg/kg IP Paclitaxel	Q7Dx3	60	saline control	<0.01
40 mg/kg Antibody 2 10 mg/kg Paclitaxel	TIW, Q7Dx3	23	saline control	<0.0001

TIW=Three time a week; Q7Dx2=once every seven days, twice; Q7Dx3=once every seven days, three times.

Measure tumor volumes and calculate the T/C% for each treatment group as the ratio of the relative tumor volumes versus the control group. Analyze tumor volumes using RM ANOVA.

In all the studies shown in Table 11, Antibody 2, Herceptin®, Doxorubicin and Paclitaxel inhibit tumor growth as single agents. However, combining Antibody 2 with a tumor-targeting agent has an additive effect indicating that a combination of an anti-CSF-1R antibody with chemotherapeutic or other agents that affect the tumor will be more efficacious than these reagents alone.

Prostate Tumor Combination Studies

Inject Nu/nu mice (male, 7-8 weeks of age) subcutaneously with $1.5x10^7$ DU145 prostate cells/mouse and allow tumors to reach 300 mm^3 before treatment. Randomize mice into groups of 10 and treat as follows:

Table 12: Prostate Combination Study

Treatment	Dose	% T/C	Cohort Comparison	P value relative to control
40 mg/kg Antibody 2	TIW	50	saline control	0.03
12 mg/kg IP Docetaxel	Q7Dx3	59	saline control	0.15
40 mg/kg Antibody 2 12 mg/kg Docetaxel	TIW, Q7Dx3	29	saline control	<0.0001

TIW=Three time a week; Q7Dx3=once every seven days, three times.

Measure tumor volumes and calculate the T/C% for each treatment as the ratio of the relative tumor volumes versus the control group. Analyze tumor volumes using RM ANOVA.

Combining Antibody 2 with docetaxel has an additive effect indicating that combination with chemotherapeutics in prostate cancer will be more efficacious than chemotherapeutic reagents alone.

Additional Sequences

SEQ ID NO. 9

Gln Asp Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30
Gly Met His Trp Val Arg Gln Ala Pro Gly Glu Gly Leu Glu Trp Val
35 40 45
Ala Val Ile Trp Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Gly Asp Tyr Glu Val Asp Tyr Gly Met Asp Val Trp Gly Gln

100 105 110
Gly Thr Thr Val Thr Val Ala Ser Ala Ser Thr Lys Gly Pro Ser Val
115 120 125
Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
130 135 140
Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145 150 155 160
Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
165 170 175
Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
180 185 190
Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
195 200 205
Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp
210 215 220
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
225 230 235 240
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
245 250 255
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
260 265 270
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
275 280 285
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
290 295 300
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
305 310 315 320
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
325 330 335
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
340 345 350
Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
355 360 365
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
370 375 380
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
385 390 395 400
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
405 410 415
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
420 425 430
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
435 440 445
Gly Lys
450

SEQ ID NO. 10

Ala Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Asn Ala
20 25 30
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45
Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Tyr Pro Trp
85 90 95
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
100 105 110
Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125
Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130 135 140
Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160
Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165 170 175
Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180 185 190
Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195 200 205
Phe Asn Arg Gly Glu Cys
210

SEQ ID NO. 11

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
1 5 10 15
Val His Ser Gln Asp Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln
20 25 30
Pro Gly Arg Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
35 40 45
Ser Ser Tyr Gly Met His Trp Val Arg Gln Ala Pro Gly Glu Gly Leu
50 55 60
Glu Trp Val Ala Val Ile Trp Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala
65 70 75 80
Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn
85 90 95
Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val
100 105 110
Tyr Tyr Cys Ala Arg Gly Asp Tyr Glu Val Asp Tyr Gly Met Asp Val

115 120 125
Trp Gly Gln Gly Thr Thr Val Thr Val Ala Ser Ala Ser Thr Lys Gly
130 135 140
Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly
145 150 155 160
Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
165 170 175
Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
180 185 190
Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
195 200 205
Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val
210 215 220
Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys
225 230 235 240
Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
245 250 255
Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
260 265 270
Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
275 280 285
Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
290 295 300
Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
305 310 315 320
Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
325 330 335
Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
340 345 350
Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
355 360 365
Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln
370 375 380
Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
385 390 395 400
Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
405 410 415
Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
420 425 430
Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
435 440 445
Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
450 455 460
Leu Ser Pro Gly Lys
465

SEQ ID NO. 12

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
1 5 10 15
Val His Ser Ala Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala
20 25 30
Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile
35 40 45
Ser Asn Ala Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
50 55 60
Leu Leu Ile Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg
65 70 75 80
Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser
85 90 95
Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser
100 105 110
Tyr Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr
115 120 125
Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu
130 135 140
Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro
145 150 155 160
Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly
165 170 175
Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr
180 185 190
Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His
195 200 205
Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val
210 215 220
Thr Lys Ser Phe Asn Arg Gly Glu Cys
225 230

SEQ ID NO. 13

atgggatggt catgtatcat ccttttttctg gtagcaactg caactggagt acattcacag 60
gaccagctgg tggagtctgg gggaggcgtg gtccagcctg ggaggtccct gagactctcc 120
tgtgcagcgt ctggattcac cttcagtagc tatggcatgc actgggtccg ccaggctcca 180
ggcgaggggc tggagtgggt ggcagttata tggtatgatg gaagtaataa atactatgca 240
gactccgtga agggccgatt caccatctcc agagacaatt ccaagaacac actgtatctg 300
caaatgaaca gcctgagagc cgaggacacg gctgtgtatt actgtgcgag aggtgactac 360
gaggtcgact acggaatgga cgtctggggc caagggacca cggtcaccgt cgcctcagct 420
agcaccaagg gcccatcggt cttccccctg gcaccctcct ccaagagcac ctctgggggc 480
acagcggccc tgggctgcct ggtcaaggac tacttccccg aaccggtgac ggtgtcgtgg 540
aactcaggcg ccctgaccag cggcgtgcac accttcccgg ctgtcctaca gtcctcagga 600
ctctactccc tcagcagcgt ggtgaccgtg ccctccagca gcttgggcac ccagacctac 660
atctgcaacg tgaatcacaa gcccagcaac accaaggtgg acaagagagt tgagcccaaa 720
tcttgtgaca aaactcacac atgcccaccg tgcccagcac ctgaactcct gggggggaccg 780

tcagtcttcc tcttcccccc aaaacccaag gacaccctca tgatctcccg gacccctgag 840
gtcacatgcg tggtggtgga cgtgagccac gaagaccctg aggtcaagtt caactggtat 900
gtggacggcg tggaggtgca taatgccaag acaaagccgc gggaggagca gtacaacagc 960
acgtaccgtg tggtcagcgt cctcaccgtc ctgcaccaag actggctgaa tggcaaggag 1020
tacaagtgca aggtctccaa caaagccctc ccagccccca tcgagaaaac catctccaaa 1080
gccaaagggc agccccgaga accacaggtg tacaccctgc ccccatcccg ggaggagatg 1140
accaagaacc aagtcagcct gacctgcctg gtcaaaggct tctatcccag cgacatcgcc 1200
gtggagtggg agagcaatgg gcagccggag aacaactaca agaccacgcc tcccgtgctg 1260
gactccgacg gctccttctt cctctattcc aagctcaccg tggacaagag caggtggcag 1320
caggggaacg tcttctcatg ctccgtgatg catgaggctc tgcacaacca ctacacgcag 1380
aagagcctct ccctgtctcc gggcaaa 1407

SEQ ID NO. 14

atgggatggt catgtatcat ccttttttcta gtagcaactg caactggagt acattcagcc 60
atccagttga cccagtctcc atcctccctg tctgcatctg taggagacag agtcaccatc 120
acttgccggg caagtcaggg cattagcaat gctttagcct ggtatcagca gaaaccaggg 180
aaagctccta agctcctgat ctatgatgcc tccagtttgg aaagtggggt cccatcaagg 240
ttcagcggca gtggatctgg gacagatttc actctcacca tcagcagcct gcagcctgaa 300
gattttgcaa cttattactg tcaacagttt aatagttacc cgtggacgtt cggccaaggg 360
accaaggtgg aaatcaaacg tgagttctag aggatccatc tgggataagc atgctgtttt 420
ctgtctgtcc ctaacatgcc ctgtgattat ccgcaaacaa cacacccaag ggcagaactt 480
tgttacttaa acaccatcct gtttgcttct ttcctcagga actgtggctg caccatctgt 540
cttcatcttc ccgccatctg atgagcagtt gaaatctgga actgcctctg ttgtgtgcct 600
gctgaataac ttctatccca gagaggccaa agtacagtgg aaggtggata acgccctcca 660
atcgggtaac tcccaggaga gtgtcacaga gcaggacagc aaggacagca cctacagcct 720
cagcagcacc ctgacgctga gcaaagcaga ctacgagaaa cacaaagtct acgcctgcga 780
agtcacccat cagggcctga gctcgcccgt cacaaagagc ttcaacaggg gagagtgt 838

SEQ ID NO. 15

Met Gly Pro Gly Val Leu Leu Leu Leu Leu Val Ala Thr Ala Trp His
1 5 10 15
Gly Gln Gly Ile Pro Val Ile Glu Pro Ser Val Pro Glu Leu Val Val
20 25 30
Lys Pro Gly Ala Thr Val Thr Leu Arg Cys Val Gly Asn Gly Ser Val
35 40 45
Glu Trp Asp Gly Pro Ala Ser Pro His Trp Thr Leu Tyr Ser Asp Gly
50 55 60
Ser Ser Ser Ile Leu Ser Thr Asn Asn Ala Thr Phe Gln Asn Thr Gly
65 70 75 80
Thr Tyr Arg Cys Thr Glu Pro Gly Asp Pro Leu Gly Gly Ser Ala Ala
85 90 95
Ile His Leu Tyr Val Lys Asp Pro Ala Arg Pro Trp Asn Val Leu Ala
100 105 110
Gln Glu Val Val Val Phe Glu Asp Gln Asp Ala Leu Leu Pro Cys Leu
115 120 125

Leu Thr Asp Pro Val Leu Glu Ala Gly Val Ser Leu Val Arg Val Arg
130 135 140
Gly Arg Pro Leu Met Arg His Thr Asn Tyr Ser Phe Ser Pro Trp His
145 150 155 160
Gly Phe Thr Ile His Arg Ala Lys Phe Ile Gln Ser Gln Asp Tyr Gln
165 170 175
Cys Ser Ala Leu Met Gly Gly Arg Lys Val Met Ser Ile Ser Ile Arg
180 185 190
Leu Lys Val Gln Lys Val Ile Pro Gly Pro Pro Ala Leu Thr Leu Val
195 200 205
Pro Ala Glu Leu Val Arg Ile Arg Gly Glu Ala Ala Gln Ile Val Cys
210 215 220
Ser Ala Ser Ser Val Asp Val Asn Phe Asp Val Phe Leu Gln His Asn
225 230 235 240
Asn Thr Lys Leu Ala Ile Pro Gln Gln Ser Asp Phe His Asn Asn Arg
245 250 255
Tyr Gln Lys Val Leu Thr Leu Asn Leu Asp Gln Val Asp Phe Gln His
260 265 270
Ala Gly Asn Tyr Ser Cys Val Ala Ser Asn Val Gln Gly Lys His Ser
275 280 285
Thr Ser Met Phe Phe Arg Val Val Glu Ser Ala Tyr Leu Asn Leu Ser
290 295 300
Ser Glu Gln Asn Leu Ile Gln Glu Val Thr Val Gly Glu Gly Leu Asn
305 310 315 320
Leu Lys Val Met Val Glu Ala Tyr Pro Gly Leu Gln Gly Phe Asn Trp
325 330 335
Thr Tyr Leu Gly Pro Phe Ser Asp His Gln Pro Glu Pro Lys Leu Ala
340 345 350
Asn Ala Thr Thr Lys Asp Thr Tyr Arg His Thr Phe Thr Leu Ser Leu
355 360 365
Pro Arg Leu Lys Pro Ser Glu Ala Gly Arg Tyr Ser Phe Leu Ala Arg
370 375 380
Asn Pro Gly Gly Trp Arg Ala Leu Thr Phe Glu Leu Thr Leu Arg Tyr
385 390 395 400
Pro Pro Glu Val Ser Val Ile Trp Thr Phe Ile Asn Gly Ser Gly Thr
405 410 415
Leu Leu Cys Ala Ala Ser Gly Tyr Pro Gln Pro Asn Val Thr Trp Leu
420 425 430
Gln Cys Ser Gly His Thr Asp Arg Cys Asp Glu Ala Gln Val Leu Gln
435 440 445
Val Trp Asp Asp Pro Tyr Pro Glu Val Leu Ser Gln Glu Pro Phe His
450 455 460
Lys Val Thr Val Gln Ser Leu Leu Thr Val Glu Thr Leu Glu His Asn
465 470 475 480
Gln Thr Tyr Glu Cys Arg Ala His Asn Ser Val Gly Ser Gly Ser Trp
485 490 495

Ala Phe Ile Pro Ile Ser Ala Gly Ala His Thr His Pro Pro Asp Glu
500 505 510
Phe Leu Phe Thr Pro Val Val Val Ala Cys Met Ser Ile Met Ala Leu
515 520 525
Leu Leu Leu Leu Leu Leu Leu Leu Leu Tyr Lys Tyr Lys Gln Lys Pro
530 535 540
Lys Tyr Gln Val Arg Trp Lys Ile Ile Glu Ser Tyr Glu Gly Asn Ser
545 550 555 560
Tyr Thr Phe Ile Asp Pro Thr Gln Leu Pro Tyr Asn Glu Lys Trp Glu
565 570 575
Phe Pro Arg Asn Asn Leu Gln Phe Gly Lys Thr Leu Gly Ala Gly Ala
580 585 590
Phe Gly Lys Val Val Glu Ala Thr Ala Phe Gly Leu Gly Lys Glu Asp
595 600 605
Ala Val Leu Lys Val Ala Val Lys Met Leu Lys Ser Thr Ala His Ala
610 615 620
Asp Glu Lys Glu Ala Leu Met Ser Glu Leu Lys Ile Met Ser His Leu
625 630 635 640
Gly Gln His Glu Asn Ile Val Asn Leu Leu Gly Ala Cys Thr His Gly
645 650 655
Gly Pro Val Leu Val Ile Thr Glu Tyr Cys Cys Tyr Gly Asp Leu Leu
660 665 670
Asn Phe Leu Arg Arg Lys Ala Glu Ala Met Leu Gly Pro Ser Leu Ser
675 680 685
Pro Gly Gln Asp Pro Glu Gly Gly Val Asp Tyr Lys Asn Ile His Leu
690 695 700
Glu Lys Lys Tyr Val Arg Arg Asp Ser Gly Phe Ser Ser Gln Gly Val
705 710 715 720
Asp Thr Tyr Val Glu Met Arg Pro Val Ser Thr Ser Ser Asn Asp Ser
725 730 735
Phe Ser Glu Gln Asp Leu Asp Lys Glu Asp Gly Arg Pro Leu Glu Leu
740 745 750
Arg Asp Leu Leu His Phe Ser Ser Gln Val Ala Gln Gly Met Ala Phe
755 760 765
Leu Ala Ser Lys Asn Cys Ile His Arg Asp Val Ala Ala Arg Asn Val
770 775 780
Leu Leu Thr Asn Gly His Val Ala Lys Ile Gly Asp Phe Gly Leu Ala
785 790 795 800
Arg Asp Ile Met Asn Asp Ser Asn Tyr Ile Val Lys Gly Asn Ala Arg
805 810 815
Leu Pro Val Lys Trp Met Ala Pro Glu Ser Ile Phe Asp Cys Val Tyr
820 825 830
Thr Val Gln Ser Asp Val Trp Ser Tyr Gly Ile Leu Leu Trp Glu Ile
835 840 845
Phe Ser Leu Gly Leu Asn Pro Tyr Pro Gly Ile Leu Val Asn Ser Lys
850 855 860

Phe Tyr Lys Leu Val Lys Asp Gly Tyr Gln Met Ala Gln Pro Ala Phe
865 870 875 880
Ala Pro Lys Asn Ile Tyr Ser Ile Met Gln Ala Cys Trp Ala Leu Glu
885 890 895
Pro Thr His Arg Pro Thr Phe Gln Gln Ile Cys Ser Phe Leu Gln Glu
900 905 910
Gln Ala Gln Glu Asp Arg Arg Glu Arg Asp Tyr Thr Asn Leu Pro Ser
915 920 925
Ser Ser Arg Ser Gly Gly Ser Gly Ser Ser Ser Ser Glu Leu Glu Glu
930 935 940
Glu Ser Ser Ser Glu His Leu Thr Cys Cys Glu Gln Gly Asp Ile Ala
945 950 955 960
Gln Pro Leu Leu Gln Pro Asn Asn Tyr Gln Phe Cys
965 970

SEQ ID NO. 16
Met Gly Pro Gly Val Leu Leu Leu Leu Leu Val Ala Thr Ala Trp His
1 5 10 15
Gly Gln Gly Ile Pro Val Ile Glu Pro Ser Val Pro Glu Leu Val Val
20 25 30
Lys Pro Gly Ala Thr Val Thr Leu Arg Cys Val Gly Asn Gly Ser Val
35 40 45
Glu Trp Asp Gly Pro Pro Ser Pro His Trp Thr Leu Tyr Ser Asp Gly
50 55 60
Ser Ser Ser Ile Leu Ser Thr Asn Asn Ala Thr Phe Gln Asn Thr Gly
65 70 75 80
Thr Tyr Arg Cys Thr Glu Pro Gly Asp Pro Leu Gly Gly Ser Ala Ala
85 90 95
Ile His Leu Tyr Val Lys Asp Pro Ala Arg Pro Trp Asn Val Leu Ala
100 105 110
Gln Glu Val Val Val Phe Glu Asp Gln Asp Ala Leu Leu Pro Cys Leu
115 120 125
Leu Thr Asp Pro Val Leu Glu Ala Gly Val Ser Leu Val Arg Val Arg
130 135 140
Gly Arg Pro Leu Met Arg His Thr Asn Tyr Ser Phe Ser Pro Trp His
145 150 155 160
Gly Phe Thr Ile His Arg Ala Lys Phe Ile Gln Ser Gln Asp Tyr Gln
165 170 175
Cys Ser Ala Leu Met Gly Gly Arg Lys Val Met Ser Ile Ser Ile Arg
180 185 190
Leu Lys Val Gln Lys Val Ile Pro Gly Pro Pro Ala Leu Thr Leu Val
195 200 205
Pro Ala Glu Leu Val Arg Ile Arg Gly Glu Ala Ala Gln Ile Val Cys
210 215 220
Ser Ala Ser Ser Val Asp Val Asn Phe Asp Val Phe Leu Gln His Asn
225 230 235 240

Asn Thr Lys Leu Ala Ile Pro Gln Gln Ser Asp Phe His Asn Asn Arg
245 250 255
Tyr Gln Lys Val Leu Thr Leu Asn Leu Asp Gln Val Asp Phe Gln His
260 265 270
Ala Gly Asn Tyr Ser Cys Val Ala Ser Asn Val Gln Gly Lys His Ser
275 280 285
Thr Ser Met Phe Phe Arg Val Val Glu Ser Ala Tyr Leu Asn Leu Ser
290 295 300
Ser Glu Gln Asn Leu Ile Gln Glu Val Thr Val Gly Glu Gly Leu Asn
305 310 315 320
Leu Lys Val Met Val Glu Ala Tyr Pro Gly Leu Gln Gly Phe Asn Trp
325 330 335
Thr Tyr Leu Gly Pro Phe Ser Asp His Gln Pro Glu Pro Lys Leu Ala
340 345 350
Asn Ala Thr Thr Lys Asp Thr Tyr Arg His Thr Phe Thr Leu Ser Leu
355 360 365
Pro Arg Leu Lys Pro Ser Glu Ala Gly Arg Tyr Ser Phe Leu Ala Arg
370 375 380
Asn Pro Gly Gly Trp Arg Ala Leu Thr Phe Glu Leu Thr Leu Arg Tyr
385 390 395 400
Pro Pro Glu Val Ser Val Ile Trp Thr Phe Ile Asn Gly Ser Gly Thr
405 410 415
Leu Leu Cys Ala Ala Ser Gly Tyr Pro Gln Pro Asn Val Thr Trp Leu
420 425 430
Gln Cys Ser Gly His Thr Asp Arg Cys Asp Glu Ala Gln Val Leu Gln
435 440 445
Val Trp Asp Asp Pro Tyr Pro Glu Val Leu Ser Gln Glu Pro Phe His
450 455 460
Lys Val Thr Val Gln Ser Leu Leu Thr Val Glu Thr Leu Glu His Asn
465 470 475 480
Gln Thr Tyr Glu Cys Arg Ala His Asn Ser Val Gly Ser Gly Ser Trp
485 490 495
Ala Phe Ile Pro Ile Ser Ala Gly Ala His Thr His Pro Pro Asp Glu
500 505 510
Phe Leu Phe Thr Pro Val Val Val Ala Cys Met Ser Ile Met Ala Leu
515 520 525
Leu Leu Leu Leu Leu Leu Leu Leu Leu Tyr Lys Tyr Lys Gln Lys Pro
530 535 540
Lys Tyr Gln Val Arg Trp Lys Ile Ile Glu Ser Tyr Glu Gly Asn Ser
545 550 555 560
Tyr Thr Phe Ile Asp Pro Thr Gln Leu Pro Tyr Asn Glu Lys Trp Glu
565 570 575
Phe Pro Arg Asn Asn Leu Gln Phe Gly Lys Thr Leu Gly Ala Gly Ala
580 585 590
Phe Gly Lys Val Val Glu Ala Thr Ala Phe Gly Leu Gly Lys Glu Asp
595 600 605

Ala Val Leu Lys Val Ala Val Lys Met Leu Lys Ser Thr Ala His Ala
610 615 620
Asp Glu Lys Glu Ala Leu Met Ser Glu Leu Lys Ile Met Ser His Leu
625 630 635 640
Gly Gln His Glu Asn Ile Val Asn Leu Leu Gly Ala Cys Thr His Gly
645 650 655
Gly Pro Val Leu Val Ile Thr Glu Tyr Cys Cys Tyr Gly Asp Leu Leu
660 665 670
Asn Phe Leu Arg Arg Lys Ala Glu Ala Met Leu Gly Pro Ser Leu Ser
675 680 685
Pro Gly Gln Asp Pro Glu Gly Gly Val Asp Tyr Lys Asn Ile His Leu
690 695 700
Glu Lys Lys Tyr Val Arg Arg Asp Ser Gly Phe Ser Ser Gln Gly Val
705 710 715 720
Asp Thr Tyr Val Glu Met Arg Pro Val Ser Thr Ser Ser Asn Asp Ser
725 730 735
Phe Ser Glu Gln Asp Leu Asp Lys Glu Asp Gly Arg Pro Leu Glu Leu
740 745 750
Arg Asp Leu Leu His Phe Ser Ser Gln Val Ala Gln Gly Met Ala Phe
755 760 765
Leu Ala Ser Lys Asn Cys Ile His Arg Asp Val Ala Ala Arg Asn Val
770 775 780
Leu Leu Thr Asn Gly His Val Ala Lys Ile Gly Asp Phe Gly Leu Ala
785 790 795 800
Arg Asp Ile Met Asn Asp Ser Asn Tyr Ile Val Lys Gly Asn Ala Arg
805 810 815
Leu Pro Val Lys Trp Met Ala Pro Glu Ser Ile Phe Asp Cys Val Tyr
820 825 830
Thr Val Gln Ser Asp Val Trp Ser Tyr Gly Ile Leu Leu Trp Glu Ile
835 840 845
Phe Ser Leu Gly Leu Asn Pro Tyr Pro Gly Ile Leu Val Asn Ser Lys
850 855 860
Phe Tyr Lys Leu Val Lys Asp Gly Tyr Gln Met Ala Gln Pro Ala Phe
865 870 875 880
Ala Pro Lys Asn Ile Tyr Ser Ile Met Gln Ala Cys Trp Ala Leu Glu
885 890 895
Pro Thr His Arg Pro Thr Phe Gln Gln Ile Cys Ser Phe Leu Gln Glu
900 905 910
Gln Ala Gln Glu Asp Arg Arg Glu Arg Asp Tyr Thr Asn Leu Pro Ser
915 920 925
Ser Ser Arg Ser Gly Gly Ser Gly Ser Ser Ser Ser Glu Leu Glu Glu
930 935 940
Glu Ser Ser Ser Glu His Leu Thr Cys Cys Glu Gln Gly Asp Ile Ala
945 950 955 960
Gln Pro Leu Leu Gln Pro Asn Asn Tyr Gln Phe Cys
965 970

SEQ ID NO. 17

Met Thr Ala Pro Gly Ala Ala Gly Arg Cys Pro Pro Thr Thr Trp Leu
1 5 10 15
Gly Ser Leu Leu Leu Leu Val Cys Leu Leu Ala Ser Arg Ser Ile Thr
20 25 30
Glu Glu Val Ser Glu Tyr Cys Ser His Met Ile Gly Ser Gly His Leu
35 40 45
Gln Ser Leu Gln Arg Leu Ile Asp Ser Gln Met Glu Thr Ser Cys Gln
50 55 60
Ile Thr Phe Glu Phe Val Asp Gln Glu Gln Leu Lys Asp Pro Val Cys
65 70 75 80
Tyr Leu Lys Lys Ala Phe Leu Leu Val Gln Asp Ile Met Glu Asp Thr
85 90 95
Met Arg Phe Arg Asp Asn Thr Pro Asn Ala Ile Ala Ile Val Gln Leu
100 105 110
Gln Glu Leu Ser Leu Arg Leu Lys Ser Cys Phe Thr Lys Asp Tyr Glu
115 120 125
Glu His Asp Lys Ala Cys Val Arg Thr Phe Tyr Glu Thr Pro Leu Gln
130 135 140
Leu Leu Glu Lys Val Lys Asn Val Phe Asn Glu Thr Lys Asn Leu Leu
145 150 155 160
Asp Lys Asp Trp Asn Ile Phe Ser Lys Asn Cys Asn Asn Ser Phe Ala
165 170 175
Glu Cys Ser Ser Gln Asp Val Val Thr Lys Pro Asp Cys Asn Cys Leu
180 185 190
Tyr Pro Lys Ala Ile Pro Ser Ser Asp Pro Ala Ser Val Ser Pro His
195 200 205
Gln Pro Leu Ala Pro Ser Met Ala Pro Val Ala Gly Leu Thr Trp Glu
210 215 220
Asp Ser Glu Gly Thr Glu Gly Ser Ser Leu Leu Pro Gly Glu Gln Pro
225 230 235 240
Leu His Thr Val Asp Pro Gly Ser Ala Lys Gln Arg Pro Pro Arg Ser
245 250 255
Thr Cys Gln Ser Phe Glu Pro Pro Glu Thr Pro Val Val Lys Asp Ser
260 265 270
Thr Ile Gly Gly Ser Pro Gln Pro Arg Pro Ser Val Gly Ala Phe Asn
275 280 285
Pro Gly Met Glu Asp Ile Leu Asp Ser Ala Met Gly Thr Asn Trp Val
290 295 300
Pro Glu Glu Ala Ser Gly Glu Ala Ser Glu Ile Pro Val Pro Gln Gly
305 310 315 320
Thr Glu Leu Ser Pro Ser Arg Pro Gly Gly Gly Ser Met Gln Thr Glu
325 330 335
Pro Ala Arg Pro Ser Asn Phe Leu Ser Ala Ser Ser Pro Leu Pro Ala
340 345 350

Ser Ala Lys Gly Gln Gln Pro Ala Asp Val Thr Gly Thr Ala Leu Pro
355 360 365
Arg Val Gly Pro Val Arg Pro Thr Gly Gln Asp Trp Asn His Thr Pro
370 375 380
Gln Lys Thr Asp His Pro Ser Ala Leu Leu Arg Asp Pro Pro Glu Pro
385 390 395 400
Gly Ser Pro Arg Ile Ser Ser Leu Arg Pro Gln Gly Leu Ser Asn Pro
405 410 415
Ser Thr Leu Ser Ala Gln Pro Gln Leu Ser Arg Ser His Ser Ser Gly
420 425 430
Ser Val Leu Pro Leu Gly Glu Leu Glu Gly Arg Arg Ser Thr Arg Asp
435 440 445
Arg Arg Ser Pro Ala Glu Pro Glu Gly Gly Pro Ala Ser Glu Gly Ala
450 455 460
Ala Arg Pro Leu Pro Arg Phe Asn Ser Val Pro Leu Thr Asp Thr Gly
465 470 475 480
His Glu Arg Gln Ser Glu Gly Ser Phe Ser Pro Gln Leu Gln Glu Ser
485 490 495
Val Phe His Leu Leu Val Pro Ser Val Ile Leu Val Leu Leu Ala Val
500 505 510
Gly Gly Leu Leu Phe Tyr Arg Trp Arg Arg Arg Ser His Gln Glu Pro
515 520 525
Gln Arg Ala Asp Ser Pro Leu Glu Gln Pro Glu Gly Ser Pro Leu Thr
530 535 540
Gln Asp Asp Arg Gln Val Glu Leu Pro Val
545 550

SEQ ID NO. 18
Met Pro Arg Gly Phe Thr Trp Leu Arg Tyr Leu Gly Ile Phe Leu Gly
1 5 10 15
Val Ala Leu Gly Asn Glu Pro Leu Glu Met Trp Pro Leu Thr Gln Asn
20 25 30
Glu Glu Cys Thr Val Thr Gly Phe Leu Arg Asp Lys Leu Gln Tyr Arg
35 40 45
Ser Arg Leu Gln Tyr Met Lys His Tyr Phe Pro Ile Asn Tyr Lys Ile
50 55 60
Ser Val Pro Tyr Glu Gly Val Phe Arg Ile Ala Asn Val Thr Arg Leu
65 70 75 80
Gln Arg Ala Gln Val Ser Glu Arg Glu Leu Arg Tyr Leu Trp Val Leu
85 90 95
Val Ser Leu Ser Ala Thr Glu Ser Val Gln Asp Val Leu Leu Glu Gly
100 105 110
His Pro Ser Trp Lys Tyr Leu Gln Glu Val Glu Thr Leu Leu Leu Asn
115 120 125
Val Gln Gln Gly Leu Thr Asp Val Glu Val Ser Pro Lys Val Glu Ser
130 135 140

Val Leu Ser Leu Leu Asn Ala Pro Gly Pro Asn Leu Lys Leu Val Arg
145 150 155 160
Pro Lys Ala Leu Leu Asp Asn Cys Phe Arg Val Met Glu Leu Leu Tyr
165 170 175
Cys Ser Cys Cys Lys Gln Ser Ser Val Leu Asn Trp Gln Asp Cys Glu
180 185 190
Val Pro Ser Pro Gln Ser Cys Ser Pro Glu Pro Ser Leu Gln Tyr Ala
195 200 205
Ala Thr Gln Leu Tyr Pro Pro Pro Pro Trp Ser Pro Ser Ser Pro Pro
210 215 220
His Ser Thr Gly Ser Val Arg Pro Val Arg Ala Gln Gly Glu Gly Leu
225 230 235 240
Leu Pro

WE CLAIM:

1. 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).

2. 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).

3. The antibody, or fragment thereof, of Claims 1 or 2, comprising a VL comprising the amino acid sequence:
AIQLTQSPSSLSASVGDRVTITCRASQGISNALAWYQQKPGKAPKLLIYDASSLES GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQFNSYPWTFGQGTKVEIK (SEQ ID NO:8),
and a VH comprising the amino acid sequence:
QDQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGEGLEWVAVIWY DGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGDYEVDYG MDVWGQGTTVTVAS (SEQ ID NO:7).

4. The antibody, or fragment thereof, of any one of Claims 1-3, comprising a heavy chain comprising the amino acid sequence of SEQ ID NO:9 and a light chain comprising the amino acid sequence of SEQ ID NO:10.

5. The antibody, or fragment thereof, of any one of Claims 1-4 comprising two heavy chains, each comprising the amino acid sequence of SEQ ID NO:9 and two light chains, each comprising the amino acid sequence of SEQ ID NO:10.

6. A pharmaceutical composition comprising the antibody or fragment of any one of Claims 1-5 together with a pharmaceutically acceptable carrier, diluent or excipient.

7. A pharmaceutical composition of Claim 6 further comprising an additional pharmaceutical agent.

8. The antibody or fragment of any one of Claims 1-5 for use in therapy.

9. The antibody or fragment thereof of any one of Claims 1-5 for use in treating cancer.

10. The antibody or fragment thereof for use according to Claim 9, wherein the cancer is leukemia, breast cancer, endometrial cancer, prostate cancer, ovarian cancer, colorectal cancer, hepatocellular cancer, renal cancer, multiple myeloma, or hodgkin's lymphoma.

11. The antibody or fragment thereof for use according to Claim 10, wherein the cancer is leukemia, breast cancer, endometrial cancer, or prostate cancer.

12. The antibody or fragment thereof for use according to Claim 11, wherein the cancer is leukemia, breast cancer, or prostate cancer.

13. The antibody or fragment thereof for use according to Claims 8-12, wherein the antibody or fragment thereof is administered before, during, substantially simultaneously with, or after commencing therapy with another anti-cancer treatment.

14. The antibody or fragment thereof for use according to Claim 13, wherein said anti-cancer treatment is selected from the group consisting of an anti-angiogenesis agent, a chemotherapeutic agent, and an anti-neoplastic agent.

15. The antibody or fragment thereof for use according to Claim 14, wherein said anti-neoplastic agent is selected from the group consisting of docetaxel, paclitaxel, Herceptin® and doxorubicin.

16. 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 of Claims 1-5 wherein the cancer is selected from the group consisting of leukemia, breast cancer, endometrial cancer, prostate cancer, ovarian cancer, colorectal cancer, hepatocellular cancer, renal cancer, multiple myeloma, and hodgkin's lymphoma.

17. The method of Claim 16, wherein the cancer is selected from the group consisting of leukemia, breast cancer, endometrial cancer, and prostate cancer.

18. The method of Claim 17, wherein the cancer is selected from the group consisting of leukemia, breast cancer, and prostate cancer.

19. The method of Claim 16, further comprising administering another anti-cancer treatment to said mammal wherein said anti-cancer treatment is selected from the group consisting of an anti-angiogenesis agent, a chemotherapeutic agent, and an anti-neoplastic agent.

20. The method of Claim 19, wherein said anti-neoplastic agent is selected from the group consisting of docetaxel, paclitaxel, Herceptin® and doxorubicin.

21. 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 according to any one of the Claims 1-5 if the CSF-1 levels are higher than CSF-1 levels found in a control population.

22. 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 according to any one of the Claims 1-5 if the IL-34 levels are higher than IL-34 levels found in a control population.

23. 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 Claims 1-5, comprising:

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,

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.

24. 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 Claims 1-5, comprising:

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,

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.