ANTIBODIES THAT BIND CSF1R

[001] This application claims the benefit of U.S. Provisional Application No. 61/331,177, filed May 4, 2010, which is incorporated by reference herein in its entirety for any purpose.

TECHNICAL FIELD

[002] Antibodies that bind CSF1R are provided. Antibody heavy chains and light chains that are capable of forming antibodies that bind CSF1R are also provided. In addition, antibodies, heavy chains, and light chains comprising one or more particular complementarity determining regions (CDRs) are provided. Polynucleotides encoding antibodies to CSF1R are provided. Polynucleotides encoding antibody heavy chains or lights chains are also provided. Methods of treatment using antibodies to CSF1R are provided. Such methods include, but are not limited to, methods of treating rheumatoid arthritis, bone loss, and multiple sclerosis.

BACKGROUND

[003] Colony stimulating factor 1 receptor (referred to herein as CSF1R; also referred to in the art as FMS, FIM2, C-FMS, and CDl15) is a single-pass transmembrane receptor with an N-terminal extracellular domain (ECD) and a C-terminal intracellular domain with tyrosine kinase activity. Ligand binding of CSF1 or the interleukin 34 ligand (referred to herein as IL34; Lin et al., *Science* 320: 807-11 (2008)) to CSF1R leads to receptor dimerization, upregulation of CSF1R protein tyrosine kinase activity, phosphorylation of CSF1R tyrosine residues, and downstream signaling events. Both CSF1 and IL34 stimulate monocyte survival, proliferation, and differentiation into macrophages.

[004] Many tumor cells have been found to secrete CSF1, which activates monocyte/macrophage cells through CSF1R. The level of CSF1 in tumors has been shown to correlate with the level of tumor-associated macrophages (TAMs) in the tumor. Higher levels of TAMs have been found to correlate with poorer patient prognoses. In addition, CSF1 has been found to promote tumor growth and progression to metastasis in, for example, human breast cancer xenografts in mice. See, e.g., Paulus et al., *Cancer Res.* 66: 4349-56 (2006). Further, CSF1R appears to play a role in osteolytic bone destruction in bone metastasis, as a small molecule inhibitor of receptor tyrosine kinase activity suppresses that destruction. See, e.g., Ohno et al., *Mol. Cancer Ther.* 5: 2634-43 (2006).

[005] CSF1 and its receptor have also been found to be involved in various inflammatory and autoimmune diseases. See, e.g., Hamilton, *Nat. Rev.* 8: 533-44 (2008). For example, synovial endothelial cells from joints afflicted with rheumatoid arthritis have been found to produce CSF1, suggesting a role for CSF1 and its receptor in the disease. Blocking CSF1R activity with an antibody results in positive clinical effects in mouse models of arthritis, including a reduction in the destruction of bone and cartilage and a reduction in macrophage numbers. See, e.g., Kitaura et al., *J. Clin. Invest.* 115: 3418-3427 (2005).

[006] Mature differentiated myeloid lineage cells such as macrophages, microglial cells, and osteoclasts contribute to pathology of various diseases such as rheumatoid arthritis, multiple sclerosis and diseases of bone loss. Differentiated myeloid lineage cells are derived from peripheral blood monocyte intermediates. CSF1R stimulation contributes to development of monocytes from bone marrow precursors, to monocyte proliferation and survival, and to differentiation of peripheral blood monocytes into differentiated myeloid lineage cells such as macrophages, microglial cells, and osteoclasts. CSF1R stimulation thus contributes to proliferation, survival, activation, and maturation of differentiated myeloid lineage cells, and in the pathologic setting, CSF1R stimulation contributes to the ability of differentiated myeloid lineage cells to mediate disease pathology.

[007] Additional antagonists of CSF1R signaling would therefore be useful in the treatment of various CSF1R-related diseases, such as cancer, inflammatory conditions, and autoimmune diseases.

SUMMARY

[008] The present inventors have invented a new set of antibodies, including humanized antibodies, directed against human CSF1R extracellular domain (CSF1R ECD). A Fab phage display library was made from spleens of mice that were immunized with a human CSF1R ECD-Fc fusion protein. 1056 phage clones expressing Fabs that bind to CSF-1R ECD-Fc were isolated through panning of this library. When the 1056 Fabs were expressed as purified protein, 668 were found to bind to CSF1R ECD. Of those 668 binding Fabs, only 121 Fabs blocked binding of CSF1 and/or IL34 to CSF1R. Only 33 of those Fabs were found to block binding of both CSF1 and IL34 to CSF1R. Upon sequencing, the 33 Fabs represented 19 unique sets of sequences. Eleven Fabs with subnanomolar affinity for human CSF1R ECD were chosen to make chimeric antibodies for further study. Based on the human and

cynomolgus monkey CSF1R binding affinities, blocking of CSF1 and IL34 binding to CSF1R, and inhibition of ligand-induced phorphorylation of CSF1R, three chimeric antibodies were selected for humanization, and sixteen humanized antibodies were made based on those three chimeric antibodies.

[009] Fourteen of the sixteen humanized antibodies retained subnanomolar binding affinities for human CSF1R ECD. *See, e.g.*, Table 5. These humanized antibodies block binding of both ligands CSF1 and IL34 to human CSF1R, and many also block binding of both CSF1 and IL34 to cynomolgus monkey CSF1R. *See, e.g.*, Table 4.

[010] For therapeutic drug development, it is beneficial to have antibodies that bind to both human and cynomolgus monkey antigens with similar affinity. The three chimeric antibodies chosen for humanization were selected in part because they had similar binding affinities for human and cynomolgus CSF1R ECD. Most of the humanized versions of one of the chimeric antibodies, 0302, however, lost significant binding affinity for cynomolgus monkey CSF1R ECD upon humanization, although they retained strong human CSF1R ECD binding affinity. *See, e.g.*, Table 3. Humanized versions of 0301 and 0311 retained similarly strong binding to both human and cynomolgus monkey CSF1R ECD, with binding affinity differences for the two species of less than about 2-fold.

[011] Based on CSF1R binding affinities, ligand inhibition, and the potential for immunogenicity, three humanized antibodies were selected for additional studies. The three humanized antibodies were derived from the two chimeric antibodies that did not significantly lose cynomolgus monkey CSF1R binding affinity upon humanization. Those three humanized antibodies inhibit ligand-induced phosphorylation of human CSF1R, and also block ligand-induced proliferation and survival responses in primary human monocytes. *See, e.g.*, Tables 6 and 7, and Figures 10 and 11. Thus, these antibodies are useful for treating diseases involving, for example, ligand-induced proliferation and survival responses in primary human monocytes.

[012] Blocking CSF1R-induced responses with an anti-CSF1R antibody should inhibit proliferation, survival, activation, maturation of differentiated myeloid lineage cells and attenuate their ability to mediate disease pathology. In addition, blocking CSF1R-induced responses with an anti-CSF1R antibody should inhibit differentiation of peripheral blood monocyte intermediates into differentiated myeloid

lineage cells, decreasing the number of pathology-mediating differentiated myeloid lineage cells.

[013] Accordingly, the humanized anti-CSF1R antibodies described herein can be used to treat chronic diseases with extant symptoms by inhibiting the ability of differentiated myeloid lineage cells to mediate disease pathology. The humanized antibodies can also be used to treat chronic diseases that are relapsing and remitting in nature by inhibiting the development of new pathology-mediating myeloid lineage cells differentiated from peripheral blood monocytes during the remitting phase of the disease, thus attenuating the number of and new formation of the pathology-mediating cells.

[014] In some embodiments, an isolated antibody comprising a heavy chain and a light chain is provided, wherein the antibody binds to CSF1R. In some embodiments, the heavy chain and/or light chain have the following structure.

[015] In some embodiments, the heavy chain comprises a sequence that is at least 90%, at least 95%, at least 97%, at least 99%, or 100% identical to a sequence selected from SEQ ID NOs: 9, 11, 13, and 39 to 45. In some embodiments, the light chain comprises a sequence that is at least 90%, at least 95%, at least 97%, at least 99%, or 100% identical to a sequence selected from SEQ ID NOs: 10, 12, 14, and 46 to 52. In some embodiments, the heavy chain comprises a sequence that is at least 90%, at least 95%, at least 97%, at least 99%, or 100% identical to a sequence selected from SEQ ID NOs: 9, 11, 13, and 39 to 45, and the light chain comprises a sequence that is at least 90%, at least 95%, at least 97%, at least 99%, or 100% identical to a sequence selected from SEQ ID NOs: 10, 12, 14, and 46 to 52.

[016] In some embodiments, the HC CDR1, HC CDR2, and HC CDR3 comprise a set of sequences selected from: (a) SEQ ID NOs: 15, 16, and 17; (b) SEQ ID NOs: 21, 22, and 23; and (c) SEQ ID NOs: 27, 28, and 29. In some embodiments, the LC CDR1, LC CDR2, and LC CDR3 comprise a set of sequences selected from: (a) SEQ ID NOs: 18, 19, and 20; (b) SEQ ID NOs: 24, 25, and 26; and (c) SEQ ID NOs: 30, 31, and 32.

[017] In some embodiments, the heavy chain comprises an HC CDR1, HC CDR2, and HC CDR3, wherein the HC CDR1, HC CDR2, and HC CDR3 comprise a set of sequences selected from: (a) SEQ ID NOs: 15, 16, and 17; (b) SEQ ID NOs: 21, 22, and 23; and (c) SEQ ID NOs: 27, 28, and 29; and the light chain comprises an LC CDR1, LC CDR2, and LC CDR3, wherein the LC CDR1, LC CDR2, and LC CDR3

comprise a set of sequences selected from: (a) SEQ ID NOs: 18, 19, and 20; (b) SEQ ID NOs: 24, 25, and 26; and (c) SEQ ID NOs: 30, 31, and 32.

[018] In some embodiments, an isolated antibody is provided, wherein the antibody comprises a heavy chain and a light chain, wherein the antibody comprises: (a) a heavy chain comprising a sequence that is at least 95%, at least 97%, at least 99%, or 100% identical to SEQ ID NO: 9 and a light chain comprising a sequence that is at least 95%, at least 97%, at least 99%, or 100% identical to SEQ ID NO: 10; (b) a heavy chain comprising a sequence that is at least 95%, at least 97%, at least 99%, or 100% identical to SEQ ID NO: 11 and a light chain comprising a sequence that is at least 95%, at least 97%, at least 99%, or 100% identical to SEQ ID NO: 12; (c) a heavy chain comprising a sequence that is at least 95%, at least 97%, at least 99%, or 100% identical to SEQ ID NO: 13 and a light chain comprising a sequence that is at least 95%, at least 97%, at least 99%, or 100% identical to SEQ ID NO: 14; (d) a heavy chain comprising a sequence that is at least 95%, at least 97%, at least 99%, or 100% identical to SEQ ID NO: 39 and a light chain comprising a sequence that is at least 95%, at least 97%, at least 99%, or 100% identical to SEQ ID NO: 46; (e) a heavy chain comprising a sequence that is at least 95%, at least 97%, at least 99%, or 100% identical to SEQ ID NO: 40 and a light chain comprising a sequence that is at least 95%, at least 97%, at least 99%, or 100% identical to SEQ ID NO: 46; (f) a heavy chain comprising a sequence that is at least 95%, at least 97%, at least 99%, or 100% identical to SEQ ID NO: 41 and a light chain comprising a sequence that is at least 95%, at least 97%, at least 99%, or 100% identical to SEQ ID NO: 46; (g) a heavy chain comprising a sequence that is at least 95%, at least 97%, at least 99%, or 100% identical to SEQ ID NO: 39 and a light chain comprising a sequence that is at least 95%, at least 97%, at least 99%, or 100% identical to SEQ ID NO: 47; (h) a heavy chain comprising a sequence that is at least 95%, at least 97%, at least 99%, or 100% identical to SEQ ID NO: 40 and a light chain comprising a sequence that is at least 95%, at least 97%, at least 99%, or 100% identical to SEQ ID NO: 47; (i) a heavy chain comprising a sequence that is at least 95%, at least 97%, at least 99%, or 100% identical to SEQ ID NO: 41 and a light chain comprising a sequence that is at least 95%, at least 97%, at least 99%, or 100% identical to SEQ ID NO: 47; and (j) a heavy chain comprising a sequence that is at least 95%, at least 97%, at least 99%, or 100% identical to SEQ ID NO: 42 and a light chain comprising a sequence that is at least 95%, at least 97%, at least 99%, or 100% identical to SEQ ID NO: 48;

(k) a heavy chain comprising a sequence that is at least 95%, at least 97%, at least 99%, or 100% identical to SEQ ID NO: 42 and a light chain comprising a sequence that is at least 95%, at least 97%, at least 99%, or 100% identical to SEQ ID NO: 49; (l) a heavy chain comprising a sequence that is at least 95%, at least 97%, at least 99%, or 100% identical to SEQ ID NO: 42 and a light chain comprising a sequence that is at least 95%, at least 97%, at least 99%, or 100% identical to SEQ ID NO: 50; (m) a heavy chain comprising a sequence that is at least 95%, at least 97%, at least 99%, or 100% identical to SEQ ID NO: 43 and a light chain comprising a sequence that is at least 95%, at least 97%, at least 99%, or 100% identical to SEQ ID NO: 48; (n) a heavy chain comprising a sequence that is at least 95%, at least 97%, at least 99%, or 100% identical to SEQ ID NO: 43 and a light chain comprising a sequence that is at least 95%, at least 97%, at least 99%, or 100% identical to SEQ ID NO: 49; (o) a heavy chain comprising a sequence that is at least 95%, at least 97%, at least 99%, or 100% identical to SEQ ID NO: 43 and a light chain comprising a sequence that is at least 95%, at least 97%, at least 99%, or 100% identical to SEQ ID NO: 50; (p) a heavy chain comprising a sequence that is at least 95%, at least 97%, at least 99%, or 100% identical to SEQ ID NO: 44 and a light chain comprising a sequence that is at least 95%, at least 97%, at least 99%, or 100% identical to SEQ ID NO: 51; (q) a heavy chain comprising a sequence that is at least 95%, at least 97%, at least 99%, or 100% identical to SEQ ID NO: 44 and a light chain comprising a sequence that is at least 95%, at least 97%, at least 99%, or 100% identical to SEQ ID NO: 52; (r) a heavy chain comprising a sequence that is at least 95%, at least 97%, at least 99%, or 100% identical to SEQ ID NO: 45 and a light chain comprising a sequence that is at least 95%, at least 97%, at least 99%, or 100% identical to SEQ ID NO: 51; or (s) a heavy chain comprising a sequence that is at least 95%, at least 97%, at least 99%, or 100% identical to SEQ ID NO: 45 and a light chain comprising a sequence that is at least 95%, at least 97%, at least 99%, or 100% identical to SEQ ID NO: 52.

[019] In some embodiments, an antibody is provided, wherein the antibody comprises a heavy chain and a light chain, wherein the antibody comprises: (a) a heavy chain comprising a heavy chain (HC) CDR1 having the sequence of SEQ ID NO: 15, an HC CDR2 having the sequence of SEQ ID NO: 16, and an HC CDR3 having the sequence of SEQ ID NO: 17, and a light chain comprising a light chain (LC) CDR1 having the sequence of SEQ ID NO: 18, a LC CDR2 having the sequence of SEQ ID NO: 19, and a LC CDR3 having the sequence of SEQ ID NO: 20; (b) a

heavy chain comprising a heavy chain (HC) CDR1 having the sequence of SEQ ID NO: 21, an HC CDR2 having the sequence of SEQ ID NO: 22, and an HC CDR3 having the sequence of SEQ ID NO: 23, and a light chain comprising a light chain (LC) CDR1 having the sequence of SEQ ID NO: 24, a LC CDR2 having the sequence of SEQ ID NO: 25, and a LC CDR3 having the sequence of SEQ ID NO: 26; or (c) a heavy chain comprising a heavy chain (HC) CDR1 having the sequence of SEQ ID NO: 27, an HC CDR2 having the sequence of SEQ ID NO: 28, and an HC CDR3 having the sequence of SEQ ID NO: 29, and a light chain comprising a light chain (LC) CDR1 having the sequence of SEQ ID NO: 30, a LC CDR2 having the sequence of SEQ ID NO: 31, and a LC CDR3 having the sequence of SEQ ID NO: 32.

[020] In some embodiments, an antibody comprises a heavy chain and a light chain, wherein the antibody comprises: (a) a heavy chain comprising a sequence of SEQ ID NO: 53 and a light chain comprising a sequence of SEQ ID NO: 60; (b) a heavy chain comprising a sequence of SEQ ID NO: 53 and a light chain comprising a sequence of SEQ ID NO: 61; or (c) a heavy chain comprising a sequence of SEQ ID NO: 58 and a light chain comprising a sequence of SEQ ID NO: 65. In some embodiments, an antibody comprises a heavy chain and a light chain, wherein the antibody comprises: (a) a heavy chain consisting of the sequence of SEQ ID NO: 53 and a light chain consisting of the sequence of SEQ ID NO: 60; (b) a heavy chain consisting of the sequence of SEQ ID NO: 53 and a light chain consisting of the sequence of SEQ ID NO: 61; or (c) a heavy chain consisting of the sequence of SEQ ID NO: 58 and a light chain consisting of the sequence of SEQ ID NO: 65.

[021] In some embodiments, an antibody is a humanized antibody. In some embodiments, an antibody is selected from a Fab, an Fv, an scFv, a Fab', and a $(Fab')_2$. In some embodiments, an antibody is a chimeric antibody. In some embodiments, an antibody is selected from an IgA, an IgG, and an IgD. In some embodiments, an antibody is an IgG. In some embodiments, an antibody is an IgG4. In some embodiments, an antibody is an IgG4 comprising an S241P mutation in at least one IgG4 heavy chain constant region.

[022] In some embodiments, an antibody binds to human CSF1R and/or binds to cynomolgus CSF1R. In some embodiments, an antibody blocks ligand binding to CSF1R. In some embodiments, an antibody blocks binding of CSF1 and/or IL34 to CSF1R. In some embodiments, an antibody inhibits ligand-induced CSF1R phosphorylation. In some embodiments, an antibody inhibits CSF1- and/or

IL34-induced CSF1R phosphorylation. In some embodiments, an antibody binds to human CSF1R with an affinity (K_D) of less than 1 nM. In some embodiments, antibody inhibits monocyte proliferation and/or survival responses in the presence of CSF1 or IL34.

[023] In some embodiments, a pharmaceutical composition comprising an antibody that binds CSF1R is provided.

[024] In some embodiments, an isolated nucleic acid is provided, wherein the isolated nucleic acid comprises a polynucleotide sequence that encodes a heavy chain described above. In some embodiments, an isolated nucleic acid encodes a light chain described above. In some embodiments, an isolated nucleic acid encodes a heavy chain described above and a light chain described above. In some embodiments, a composition is provided, wherein the composition comprises a first nucleic acid that comprises a polynucleotide sequence that encodes a heavy chain described above, and a second nucleic acid that comprises a polynucleotide sequence that encodes a light chain described above. In some embodiments, a host cell comprising a nucleic acid or a composition described above is provided. In some embodiments, a host cell is a eukaryotic host cell. In some embodiments, a host cell is a mammalian host cell. In some embodiments, a host cell is selected from a CHO cell, a 293 cell, an NSO cell, and a PER.C6 cell. In some embodiments, a host cell is a 293-6E cell or a DG44 cell.

[025] In some embodiments, methods of treating disease comprising administering to a patient a pharmaceutical composition comprising an antibody that binds CSF1R is provided. In some embodiments, a method of treating multiple sclerosis comprising administering to a patient a pharmaceutical composition comprising an antibody that binds CSF1R is provided. In some embodiments, a method of treating rheumatoid arthritis comprising administering to a patient a pharmaceutical composition comprising an antibody that binds CSF1R is provided. In some embodiments, a method of treating osteolytic bone loss comprising administering to a patient a pharmaceutical composition comprising an antibody that binds CSF1R is provided. In some embodiments, the osteolytic bone loss is selected from osteoporosis, metastasis-induced osteolytic bone loss, and rheumatoid arthritis-induced bone loss. In some embodiments, a method of treating cancer comprising administering to a patient a pharmaceutical composition comprising an antibody that binds CSF1R is provided. In some embodiments, the cancer is selected from breast cancer, prostate cancer, endometrial cancer, bladder cancer, kidney cancer,

esophageal cancer, squamous cell carcinoma, uveal melanoma, follicular lymphoma, renal cell carcinoma, cervical cancer, ovarian cancer, lung cancer, colorectal cancer, brain cancer, pancreatic cancer, head and neck cancer, liver cancer, leukemia, lymphoma, Hodgkin's disease, multiple myeloma, melanoma, astrocytoma, stomach cancer, and pulmonary adenocarcinoma.

[026] In some embodiments, a method of treating an inflammatory condition comprising administering to a patient a pharmaceutical composition comprising an antibody that binds CSF1R is provided.

[027] In some embodiments, antibodies that bind CSF1R and compositions comprising antibodies that bind CSF1R are provided for use in methods of treatment of human or animals. In some embodiments, antibodies that bind CSF1R and compositions comprising antibodies that bind CSF1R are provided for use in a method of treating rheumatoid arthritis in a human or animal. In some embodiments, antibodies that bind CSF1R and compositions comprising antibodies that bind CSF1R are provided for use in a method of treating multiple sclerosis in a human or animal. In some embodiments, antibodies that bind CSF1R and compositions comprising antibodies that bind CSF1R are provided for use in a method of treating cancer in a human or animal. In some embodiments, antibodies that bind CSF1R and compositions comprising antibodies that bind CSF1R are provided for use in a method of treating an inflammatory condition in a human or animal.

BRIEF DESCRIPTION OF THE FIGURES

[028] **FIG. 1** shows an alignment of the humanized heavy chain variable regions for each of humanized antibodies Ab1 to Ab16, as discussed in Example 4. Boxed residues are amino acids in the human acceptor sequence that were changed back to the corresponding mouse residue.

[029] **FIG. 2** shows an alignment of the humanized light chain variable regions for each of humanized antibodies Ab1 to Ab 16, as discussed in Example 4 Boxed amino acids are residues in the human acceptor sequence that were changed back to the corresponding mouse residue.

[030] **FIG. 3** shows binding curves for certain humanized antibodies binding to human CSF1R ECD, as described in Example 5. Figure 3A shows binding curves for parental chimeric antibodies (cAb) 0301 and humanized antibodies (huAb) 0301.1, 0301.2, 0302.3, 0301.4, 0301.5, and 0301.6 (h0301-L0H0, h0301-L0H1, h0301-L0H2, h0301-L1H0, h0301-L1H1, and h0301-L1H2, respectively). Figure 3B

shows binding curves for parental cAb 0302 and humanized antibodies (huAb) 0302.1, 0302.2, 0302.3, 0302.4, 0302.5, and 0302.6 (h0302-L0H1, h0302-L1H1, h0302-L2H1, h0302-L0H2, h0302-L1H2, and h0302-L2H2, respectively). Figure 3C shows binding curves for parental cAb 0311 and humanized antibodies (huAb) 0311.1, 0311.2, 0311.3, and 0311.4 (h0311-L0H1, h0311-L1H1, h0311-L0H2, and h0311-L1H2, respectively).

[031] **FIG. 4** shows binding curves for certain humanized antibodies binding to cynomolgus CSF1R ECD, as described in Example 5. Figure 4A shows binding curves for parental cAb 0301 and humanized antibodies (huAb) 0301.1, 0301.2, 0302.3, 0301.4, 0301.5, and 0301.6 (h0301-L0H0, h0301-L0H1, h0301-L0H2, h0301-L1H0, h0301-L1H1, and h0301-L1H2, respectively). Figure 4B shows binding curves for parental cAb 0302 and humanized antibodies (huAb) 0302.1, 0302.2, 0302.3, 0302.4, 0302.5, and 0302.6 (h0302-L0H1, h0302-L1H1, h0302-L2H1, h0302-L0H2, h0302-L1H2, and h0302-L2H2, respectively). Figure 4C shows binding curves for parental cAb 0311 and humanized antibodies (huAb) 0311.1, 0311.2, 0311.3, and 0311.4 (h0311-L0H1, h0311-L1H1, h0311-L0H2, and h0311-L1H2, respectively).

[032] **FIG. 5** shows binding curves for certain humanized antibodies binding to mouse CSF1R ECD, as described in Example 5. Figure 5A shows binding curves for parental cAb 0301 and humanized antibodies (huAb) 0301.1, 0301.2, 0302.3, 0301.4, 0301.5, and 0301.6 (h0301-L0H0, h0301-L0H1, h0301-L0H2, h0301-L1H0, h0301-L1H1, and h0301-L1H2, respectively). Figure 5B shows binding curves for parental cAb 0302 and humanized antibodies (huAb) 0302.1, 0302.2, 0302.3, 0302.4, 0302.5, and 0302.6 (h0302-L0H1, h0302-L1H1, h0302-L2H1, h0302-L0H2, h0302-L1H2, and h0302-L2H2, respectively). Figure 5C shows binding curves for parental cAb 0311 and humanized antibodies (huAb) 0311.1, 0311.2, 0311.3, and 0311.4 (h0311-L0H1, h0311-L1H1, h0311-L0H2, and h0311-L1H2, respectively).

[033] **FIG. 6** shows inhibition of CSF1 induced CSF1R phosphorylation by certain humanized antibodies, as described in Example 6. Figure 6A shows blocking curves for parental cAb 0301 and humanized antibodies (huAb) 0301.1, 0301.2, 0302.3, 0301.4, 0301.5, and 0301.6 (h0301-L0H0, h0301-L0H1, h0301-L0H2, h0301-L1H0, h0301-L1H1, and h0301-L1H2, respectively). Figure 6B shows blocking curves for parental cAb 0302 and humanized antibodies (huAb) 0302.1, 0302.2, 0302.3, 0302.4, 0302.5, and 0302.6 (h0302-L0H1, h0302-L1H1, h0302-L2H1, h0302-

L0H2, h0302-L1H2, and h0302-L2H2, respectively). Figure 6C shows blocking curves for parental cAb 0311 and humanized antibodies (huAb) 0311.1, 0311.2, 0311.3, and 0311.4 (h0311-L0H1, h0311-L1H1, h0311-L0H2, and h0311-L1H2, respectively).

[034] **FIG. 7** shows inhibition of IL34 induced CSF1R phosphorylation by certain humanized antibodies, as described in Example 6. Figure 7A shows blocking curves for parental cAb 0301 and humanized antibodies (huAb) 0301.1, 0301.2, 0302.3, 0301.4, 0301.5, and 0301.6 (h0301-L0H0, h0301-L0H1, h0301-L0H2, h0301-L1H0, h0301-L1H1, and h0301-L1H2, respectively). Figure 7B shows blocking curves for parental cAb 0302 and humanized antibodies (huAb) 0302.1, 0302.2, 0302.3, 0302.4, 0302.5, and 0302.6 (h0302-L0H1, h0302-L1H1, h0302-L2H1, h0302-L0H2, h0302-L1H2, and h0302-L2H2, respectively). Figure 7C shows blocking curves for parental cAb 0311 and humanized antibodies (huAb) 0311.1, 0311.2, 0311.3, and 0311.4 (h0311-L0H1, h0311-L1H1, h0311-L0H2, and h0311-L1H2, respectively).

[035] **FIG. 8** shows blocking of human CSF1 binding to cynomolgus CSF1R ECD by certain humanized antibodies, as described in Example 7. Figure 8A shows blocking curves for parental cAb 0301 and humanized antibodies (huAb) 0301.1, 0301.2, 0302.3, 0301.4, 0301.5, and 0301.6 (h0301-L0H0, h0301-L0H1, h0301-L0H2, h0301-L1H0, h0301-L1H1, and h0301-L1H2, respectively). Figure 8B shows blocking curves for parental cAb 0302 and humanized antibodies (huAb) 0302.1, 0302.2, 0302.3, 0302.4, 0302.5, and 0302.6 (h0302-L0H1, h0302-L1H1, h0302-L2H1, h0302-L0H2, h0302-L1H2, and h0302-L2H2, respectively). Figure 8C shows blocking curves for parental cAb 0311 and humanized antibodies (huAb) 0311.1, 0311.2, 0311.3, and 0311.4 (h0311-L0H1, h0311-L1H1, h0311-L0H2, and h0311-L1H2, respectively).

[036] **FIG. 9** shows blocking of human IL34 binding to cynomolgus CSF1R ECD by certain humanized antibodies, as described in Example 7. Figure 9A shows blocking curves for parental cAb 0301 and humanized antibodies (huAb) 0301.1, 0301.2, 0302.3, 0301.4, 0301.5, and 0301.6 (h0301-L0H0, h0301-L0H1, h0301-L0H2, h0301-L1H0, h0301-L1H1, and h0301-L1H2, respectively). Figure 9B shows blocking curves for parental cAb 0302 and humanized antibodies (huAb) 0302.1, 0302.2, 0302.3, 0302.4, 0302.5, and 0302.6 (h0302-L0H1, h0302-L1H1, h0302-L2H1, h0302-L0H2, h0302-L1H2, and h0302-L2H2, respectively). Figure 9C shows

blocking curves for parental cAb 0311 and humanized antibodies (huAb) 0311.1, 0311.2, 0311.3, and 0311.4 (h0311-L0H1, h0311-L1H1, h0311-L0H2, and h0311-L1H2, respectively).

[037] **FIG. 10** shows blocking of CSF1- (A) and IL34- (B) induced CSF1R phosphorylation in CHO cells expressing human CSF1R by humanized antibodies 0301-L0H0, 0301-L1H0, and 0311-L0H1, as described in Example 9.

[038] **FIG. 11** shows blocking of CSF1- (A) and IL34- (B) induced monoclyte proliferation/survival responses by humanized antibodies 0301-L0H0, 0301-L1H0, and 0311-L0H1, as described in Example 10.

[039] **FIG. 12A-C** shows that humanized antibodies 0301-L0H0, 0301-L1H0, and 0311-L0H1 do not stimulate primary monocyte proliferation or survival, using monocytes from three different donors, as described in Example 11.

DETAILED DESCRIPTION

[040] Methods of treating diseases comprising administering novel antibodies to CSF1R are provided. All of the antibodies have binding affinities for human CSF1R ECD of less than 2 nM, and all but two of the humanized antibodies have sub-nanomolar binding affinities for human CSF1R ECD. Further, the new antibodies block binding of both CSF1 and IL34 to human CSF1R, and inhibit ligand-induced phosphorylation of human CSF1R. Many of the new antibodies also block binding of CSF1 and IL34 to cynomolgus CSF1R, which facilitates *in vivo* experiments to support the development of anti-CSF1R antibody therapeutics. The new antibodies are therefore well suited for therapeutic use in human diseases, including, but not limited to, cancer, autoimmune diseases, and inflammatory conditions.

[041] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

Definitions

[042] Unless otherwise defined, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

[043] Exemplary techniques used in connection with recombinant DNA, oligonucleotide synthesis, tissue culture and transformation (e.g., electroporation,

lipofection), enzymatic reactions, and purification techniques are known in the art. Many such techniques and procedures are described, e.g., in Sambrook et al. *Molecular Cloning: A Laboratory Manual* (2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989)), among other places. In addition, exemplary techniques for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients are also known in the art.

[044] In this application, the use of "or" means "and/or" unless stated otherwise. In the context of a multiple dependent claim, the use of "or" refers back to more than one preceding independent or dependent claim in the alternative only. Also, terms such as "element" or "component" encompass both elements and components comprising one unit and elements and components that comprise more than one subunit unless specifically stated otherwise.

[045] As utilized in accordance with the present disclosure, the following terms, unless otherwise indicated, shall be understood to have the following meanings:

[046] The terms "**nucleic acid molecule**" and "**polynucleotide**" may be used interchangeably, and refer to a polymer of nucleotides. Such polymers of nucleotides may contain natural and/or non-natural nucleotides, and include, but are not limited to, DNA, RNA, and PNA. "**Nucleic acid sequence**" refers to the linear sequence of nucleotides that comprise the nucleic acid molecule or polynucleotide.

[047] The terms "**polypeptide**" and "**protein**" are used interchangeably to refer to a polymer of amino acid residues, and are not limited to a minimum length. Such polymers of amino acid residues may contain natural or non-natural amino acid residues, and include, but are not limited to, peptides, oligopeptides, dimers, trimers, and multimers of amino acid residues. Both full-length proteins and fragments thereof are encompassed by the definition. The terms also include post-expression modifications of the polypeptide, for example, glycosylation, sialylation, acetylation, phosphorylation, and the like. Furthermore, for purposes of the present invention, a "polypeptide" refers to a protein which includes modifications, such as deletions, additions, and substitutions (generally conservative in nature), to the native sequence, as long as the protein maintains the desired activity. These modifications may be deliberate, as through site-directed mutagenesis, or may be accidental, such as through mutations of hosts which produce the proteins or errors due to PCR amplification.

[048] The term "**CSF1R**" refers herein to the full-length CSF1R, which includes the N-terminal ECD, the transmembrane domain, and the intracellular tyrosine kinase domain, with or without an N-terminal leader sequence. In some embodiments, the CSF1R is a human CSF1R having the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 2.

[049] The term "**CSF1R extracellular domain**" ("**CSF1R ECD**") as used herein refers to a CSF1R polypeptide that lacks the intracellular and transmembrane domains. CSF1R ECDs include the full-length CSF1R ECD and CSF1R ECD fragments that are capable of binding CSF1R and/or IL34. The human full-length CSF1R ECD is defined herein as comprising either amino acids 1 to 512 (i.e., including the leader sequence) or amino acids 20 to 512 (i.e., lacking the leader sequence) of SEQ ID NO: 2. In some embodiments, a human CSF1R ECD fragment comprises amino acids 20 to 506 of SEQ ID NO: 2 (see SEQ ID NO:5). In some embodiments, a human CSF1R fragment ends at amino acid 507, 508, 509, 510, or 511. In some embodiments, a cynoCSF1R ECD comprises the sequence of SEQ ID NO: 7 (with leader sequence) or amino acids 20 to 506 of SEQ ID NO: 7 (without leader sequence).

[050] The term "**antibody**" as used herein refers to a molecule comprising at least complementarity-determining region (CDR) 1, CDR2, and CDR3 of a heavy chain and at least CDR1, CDR2, and CDR3 of a light chain, wherein the molecule is capable of binding to antigen. The term antibody includes, but is not limited to, fragments that are capable of binding antigen, such as Fv, single-chain Fv (scFv), Fab, Fab', and $(Fab')_2$. The term antibody also includes, but is not limited to, chimeric antibodies, humanized antibodies, and antibodies of various species such as mouse, human, cynomolgus monkey, etc.

[051] In some embodiments, an antibody comprises a heavy chain variable region and a light chain variable region. In some embodiments, an antibody comprises at least one heavy chain comprising a heavy chain variable region and at least a portion of a heavy chain constant region, and at least one light chain comprising a light chain variable region and at least a portion of a light chain constant region. In some embodiments, an antibody comprises two heavy chains, wherein each heavy chain comprises a heavy chain variable region and at least a portion of a heavy chain constant region, and two light chains, wherein each light chain comprises a light chain variable region and at least a portion of a light chain constant region. As

used herein, a single-chain Fv (scFv), or any other antibody that comprises, for example, a single polypeptide chain comprising all six CDRs (three heavy chain CDRs and three light chain CDRs) is considered to have a heavy chain and a light chain. In some such embodiments, the heavy chain is the region of the antibody that comprises the three heavy chain CDRs and the light chain in the region of the antibody that comprises the three light chain CDRs.

[052] The term "**heavy chain variable region**" as used herein refers to a region comprising heavy chain CDR1, framework (FR) 2, CDR2, FR3, and CDR3. In some embodiments, a heavy chain variable region also comprises at least a portion of an FR1 and/or at least a portion of an FR4. In some embodiments, a heavy chain CDR1 corresponds to Kabat residues 26 to 35; a heavy chain CDR2 corresponds to Kabat residues 50 to 65; and a heavy chain CDR3 corresponds to Kabat residues 95 to 102. *See, e.g.*, Kabat Sequences of Proteins of Immunological Interest (1987 and 1991, NIH, Bethesda, Md.); and Figure 1. In some embodiments, a heavy chain CDR1 corresponds to Kabat residues 31 to 35; a heavy chain CDR2 corresponds to Kabat residues 50 to 65; and a heavy chain CDR3 corresponds to Kabat residues 95 to 102. *See id.*

[053] The term "**heavy chain constant region**" as used herein refers to a region comprising at least three heavy chain constant domains, C_H1, C_H2, and C_H3. Nonlimiting exemplary heavy chain constant regions include γ, δ, and α. Nonlimiting exemplary heavy chain constant regions also include ε and μ. Each heavy constant region corresponds to an antibody isotype. For example, an antibody comprising a γ constant region is an IgG antibody, an antibody comprising a δ constant region is an IgD antibody, and an antibody comprising an α constant region is an IgA antibody. Further, an antibody comprising a μ constant region is an IgM antibody, and an antibody comprising an ε constant region is an IgE antibody. Certain isotypes can be further subdivided into subclasses. For example, IgG antibodies include, but are not limited to, IgG1 (comprising a γ_1 constant region), IgG2 (comprising a γ_2 constant region), IgG3 (comprising a γ_3 constant region), and IgG4 (comprising a γ_4 constant region) antibodies; IgA antibodies include, but are not limited to, IgA1 (comprising an α_1 constant region) and IgA2 (comprising an α_2 constant region) antibodies; and IgM antibodies include, but are not limited to, IgM1 and IgM2.

[054] In some embodiments, a heavy chain constant region comprises one or more mutations (or substitutions), additions, or deletions that confer a desired

characteristic on the antibody. A nonlimiting exemplary mutation is the S241P mutation in the IgG4 hinge region (between constant domains C_H1 and C_H2), which alters the IgG4 motif CPSCP to CPPCP, which is similar to the corresponding motif in IgG1. That mutation, in some embodiments, results in a more stable IgG4 antibody. See, e.g., Angal et al., *Mol. Immunol.* 30: 105-108 (1993); Bloom et al., *Prot. Sci.* 6: 407-415 (1997); Schuurman et al., *Mol. Immunol.* 38: 1-8 (2001).

[055] The term "**heavy chain**" as used herein refers to a polypeptide comprising at least a heavy chain variable region, with or without a leader sequence. In some embodiments, a heavy chain comprises at least a portion of a heavy chain constant region. The term "**full-length heavy chain**" as used herein refers to a polypeptide comprising a heavy chain variable region and a heavy chain constant region, with or without a leader sequence.

[056] The term "**light chain variable region**" as used herein refers to a region comprising light chain CDR1, framework (FR) 2, CDR2, FR3, and CDR3. In some embodiments, a light chain variable region also comprises an FR1 and/or an FR4. In some embodiments, a light chain CDR1 corresponds to Kabat residues 24 to 34; a light chain CDR2 corresponds to Kabat residues 50 to 56; and a light chain CDR3 corresponds to Kabat residues 89 to 97. *See, e.g.*, Kabat Sequences of Proteins of Immunological Interest (1987 and 1991, NIH, Bethesda, Md.); and Figure 1.

[057] The term "**light chain constant region**" as used herein refers to a region comprising a light chain constant domain, C_L. Nonlimiting exemplary light chain constant regions include λ and κ.

[058] The term "**light chain**" as used herein refers to a polypeptide comprising at least a light chain variable region, with or without a leader sequence. In some embodiments, a light chain comprises at least a portion of a light chain constant region. The term "**full-length light chain**" as used herein refers to a polypeptide comprising a light chain variable region and a light chain constant region, with or without a leader sequence.

[059] A "**chimeric antibody**" as used herein refers to an antibody comprising at least one variable region from a first species (such as mouse, rat, cynomolgus monkey, etc.) and at least one constant region from a second species (such as human, cynomolgus monkey, etc.). In some embodiments, a chimeric antibody comprises at least one mouse variable region and at least one human constant region. In some embodiments, a chimeric antibody comprises at least one

cynomolgus variable region and at least one human constant region. In some embodiments, all of the variable regions of a chimeric antibody are from a first species and all of the constant regions of the chimeric antibody are from a second species.

[060] A "**humanized antibody**" as used herein refers to an antibody in which at least one amino acid in a framework region of a non-human variable region has been replaced with the corresponding amino acid from a human variable region. In some embodiments, a humanized antibody comprises at least one human constant region or fragment thereof. In some embodiments, a humanized antibody is an Fab, an scFv, a $(Fab')_2$, etc.

[061] A "**CDR-grafted antibody**" as used herein refers to a humanized antibody in which the complementarity determining regions (CDRs) of a first (non-human) species have been grafted onto the framework regions (FRs) of a second (human) species.

[062] A "**human antibody**" as used herein refers to antibodies produced in humans, antibodies produced in non-human animals that comprise human immunoglobulin genes, such as XenoMouse®, and antibodies selected using in vitro methods, such as phage display, wherein the antibody repertoire is based on a human immunoglobulin sequences.

[063] The term "**leader sequence**" refers to a sequence of amino acid residues located at the N terminus of a polypeptide that facilitates secretion of a polypeptide from a mammalian cell. A leader sequence may be cleaved upon export of the polypeptide from the mammalian cell, forming a mature protein. Leader sequences may be natural or synthetic, and they may be heterologous or homologous to the protein to which they are attached. Exemplary leader sequences include, but are not limited to, antibody leader sequences, such as, for example, the amino acid sequences of SEQ ID NOs.: 3 and 4, which correspond to human light and heavy chain leader sequences, respectively. Nonlimiting exemplary leader sequences also include leader sequences from heterologous proteins. In some embodiments, an antibody lacks a leader sequence. In some embodiments, an antibody comprises at least one leader sequence, which may be selected from native antibody leader sequences and heterologous leader sequences.

[064] The term "**vector**" is used to describe a polynucleotide that may be engineered to contain a cloned polynucleotide or polynucleotides that may be

propagated in a host cell. A vector may include one or more of the following elements: an origin of replication, one or more regulatory sequences (such as, for example, promoters and/or enhancers) that regulate the expression of the polypeptide of interest, and/or one or more selectable marker genes (such as, for example, antibiotic resistance genes and genes that may be used in colorimetric assays, e.g., β-galactosidase). The term "**expression vector**" refers to a vector that is used to express a polypeptide of interest in a host cell.

[065] A "**host cell**" refers to a cell that may be or has been a recipient of a vector or isolated polynucleotide. Host cells may be prokaryotic cells or eukaryotic cells. Exemplary eukaryotic cells include mammalian cells, such as primate or non-primate animal cells; fungal cells, such as yeast; plant cells; and insect cells. Nonlimiting exemplary mammalian cells include, but are not limited to, NSO cells, PER.C6® cells (Crucell), and 293 and CHO cells, and their derivatives, such as 293-6E and DG44 cells, respectively.

[066] The term "**isolated**" as used herein refers to a molecule that has been separated from at least some of the components with which it is typically found in nature. For example, a polypeptide is referred to as "isolated" when it is separated from at least some of the components of the cell in which it was produced. Where a polypeptide is secreted by a cell after expression, physically separating the supernatant containing the polypeptide from the cell that produced it is considered to be "isolating" the polypeptide. Similarly, a polynucleotide is referred to as "isolated" when it is not part of the larger polynucleotide (such as, for example, genomic DNA or mitochondrial DNA, in the case of a DNA polynucleotide) in which it is typically found in nature, or is separated from at least some of the components of the cell in which it was produced, e.g., in the case of an RNA polynucleotide. Thus, a DNA polynucleotide that is contained in a vector inside a host cell may be referred to as "isolated" so long as that polynucleotide is not found in that vector in nature.

[067] The terms "**subject**" and "**patient**" are used interchangeably herein to refer to a human. In some embodiments, methods of treating other mammals, including, but not limited to, rodents, simians, felines, canines, equines, bovines, porcines, ovines, caprines, mammalian laboratory animals, mammalian farm animals, mammalian sport animals, and mammalian pets, are also provided.

[068] The term "**rheumatoid arthritis**" ("**RA**") refers to a chronic autoimmune disease characterized primarily by inflammation of the lining (synovium)

of the joints, which can lead to joint damage, resulting in chronic pain, loss of function, and disability. Because RA can affect multiple organs of the body, including skin, lungs, and eyes, it is referred to as a systemic illness.

[069] The term "**multiple sclerosis**" ("**MS**") refers to the chronic, autoimmune, demyelinating disease of the CNS in which the body generates antibodies and white blood cells against the cells that produce the myelin sheath. "**Demyelination**" occurs when the myelin sheath becomes inflamed, injured, and detaches from the nerve fiber.

[070] The term "**cancer**" refers to a proliferative disorder associated with uncontrolled cell proliferation, unrestrained cell growth, and decreased cell deathiapoptosis. Cancer includes, but is not limited to, breast cancer, prostate cancer, lung cancer, kidney cancer, thyroid cancer, esophageal cancer, melanoma, follicular lymphomas, uveal melanoma, brain cancer, head and neck cancer, pulmonary adenocarcinoma, including, but not limited to, colon cancer, cardiac tumors, pancreatic cancer, retinoblastoma, glioblastoma, intestinal cancer, testicular cancer, stomach cancer, neuroblastoma, myxoma, myoma, lymphoma, endothelioma, osteoblastoma, osteoclastoma, osteosarcoma, chondrosarcoma, adenoma, Kaposi's sarcoma, ovarian cancer, leukemia (including acute leukemias (for example, acute lymphocytic leukemia, acute myelocytic leukemia, including myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia)) and chronic leukemias (for example, chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia), myelodysplastic syndrome polycythemia vera, lymphomas (for example, Hodgkin's disease, non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain diseases, and solid tumors including, but not limited to, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, endometrial cancer, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma,

ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, and menangioma. The terms "**metastasis**" and "**cancer metastasis**" are used interchangeably herein to refer to the ability of a cancer cell to spread to other tissues. For example, "**metastasis to bone**" refers to the ability of certain types of cancer including, but not limited to, breast, prostate, lung, kidney, thyroid, and melanoma, to metastasize to bone.

[071] The term "**osteolytic disorders**" is used herein to refer to any condition that is caused by an increase in the activity of osteoclasts, which are cells responsible for bone resorption. The terms "**osteolysis**" and "**osteolytic bone loss**" may be used interchangeably to refer to osteoclast-mediated bone resorption or bone loss associated with an osteolytic disorder. Osteolytic disorders may occur in subjects with a predisposition to develop an osteolytic disorder, or they may occur in subjects with a disease that leads to or contributes to an osteolytic disorder by stimulating osteoclast activity. In exemplary embodiments of the present invention, the osteolytic disorder may include osteolytic bone loss and cancer metastasis-induced osteolytic bone loss. In further exemplary embodiments of the present invention, the osteolytic bone disorder includes metabolic bone disease, including endocrinopathies, such as hypercortisolism, hypogonadism, primary or secondary hyperparathyroidism, and hyperthyroidism; dietary deficiency, including rickets, osteomalacia, scurvy, and malnutrition; osteoporosis; drug use, including glucocorticoids (glucocorticoid-induced osteoporosis), heparin, and alcohol; chronic disease, including malabsorption syndromes; chronic renal failure, including renal osteodystrophy; chronic liver disease, including hepatic osteodystrophy; inherited disease, including osteogenesis imperfecta and homocystinuria; and bone inflammation associated with arthritis, rheumatoid arthritis, psoriatic arthritis, fibrous dysplasia, periodontal disease, and Paget's disease.

[072] The terms "**metastasis-induced osteolytic bone loss**," and "**cancer metastasis-induced osteolytic bone loss**," are used interchangeably herein to refer to osteolysis or osteolytic bone loss resulting from cancer cell metastasis to bone. The term "**cancer metastasis-induced osteoclast activation**" is used herein to refer to the ability of cancer cells that have metastasized to bone to induce the activation of osteoclasts.

[073] The term "**tumor**" is used herein to refer to a group of cells that exhibit abnormally high levels of proliferation and growth. A tumor may be benign, pre-

malignant, or malignant; malignant tumor cells are cancerous. Tumor cells may be solid tumor cells or leukemic tumor cells. The term "**tumor growth**" is used herein to refer to proliferation or growth by a cell or cells that comprise a tumor that leads to a corresponding increase in the size of the tumor. The term "**CSFIR-dependent tumor growth**" is used herein to refer to the requirement of a tumor cell or cells for CSFIR-mediated function(s) in order for the tumor cell or cells to proliferate or grow.

[074] "**Treatment**," as used herein, covers any administration or application of a therapeutic for disease in a mammal, including a human, and includes inhibiting the disease or progression of the disease, inhibiting or slowing the disease or its progression, arresting its development, partially or fully relieving the disease, or curing the disease, for example, by causing regression, or restoring or repairing a lost, missing, or defective function; or stimulating an inefficient process.

[075] The terms "**inhibition**" or "**inhibit**" refer to a decrease or cessation of any phenotypic characteristic or to the decrease or cessation in the incidence, degree, or likelihood of that characteristic.

[076] A "**pharmaceutically acceptable carrier**" refers to a non-toxic solid, semisolid, or liquid filler, diluent, encapsulating material, formulation auxiliary, or carrier conventional in the art for use with a therapeutic agent that together comprise a "**pharmaceutical composition**" for administration to a subject. A pharmaceutically acceptable carrier is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. The pharmaceutically acceptable carrier is appropriate for the formulation employed. For example, if the therapeutic agent is to be administered orally, the carrier may be a gel capsule. If the therapeutic agent is to be administered subcutaneously, the carrier ideally is not irritable to the skin and does not cause injection site reaction.

Anti-CSF1R Antibodies

[077] The present inventors have invented a new set of antibodies directed against CSF1R. Anti-CSF1R antibodies include, but are not limited to, humanized antibodies, chimeric antibodies, mouse antibodies, human antibodies, and antibodies comprising the heavy chain and/or light chain CDRs discussed herein.

Exemplary Humanized Antibodies

[078] In some embodiments, humanized antibodies that bind CSF1R are provided. Humanized antibodies are useful as therapeutic molecules because humanized antibodies reduce or eliminate the human immune response to non-human

antibodies (such as the human anti-mouse antibody (HAMA) response), which can result in an immune response to an antibody therapeutic, and decreased effictiveness of the therapeutic.

[079] Nonlimiting exemplary humanized antibodies include Ab1 through Ab16, described herein. Nonlimiting exemplary humanized antibodies also include antibodies comprising a heavy chain variable region of an antibody selected from Ab1 to Ab16 and/or a light chain variable region of an antibody selected from Ab1 to Ab16. Nonlimiting exemplary humanized antibodies include antibodies comprising a heavy chain variable region selected from SEQ ID NOs: 39 to 45 and/or a light chain variable region selected from SEQ ID NOs: 46 to 52. Exemplary humanized antibodies also include, but are not limited to, humanized antibodies comprising heavy chain CDR1, CDR2, and CDR3, and/or light chain CDR1, CDR2, and CDR3 of an antibody selected from 0301, 0302, and 0311.

[080] In some embodiments, a humanized anti-CSF1R antibody comprises heavy chain CDR1, CDR2, and CDR3 and/or a light chain CDR1, CDR2, and CDR3 of an antibody selected from 0301, 0302, and 0311. Nonlimiting exemplary humanized anti-CSF1R antibodies include antibodies comprising sets of heavy chain CDR1, CDR2, and CDR3 selected from: SEQ ID NOs: 15, 16, and 17; SEQ ID NOs: 21, 22, and 23; and SEQ ID NOs: 27, 28, and 29. Nonlimiting exemplary humanized anti-CSF1R antibodies also include antibodies comprising sets of light chain CDR1, CDR2, and CDR3 selected from: SEQ ID NOs: 18, 19, and 20; SEQ ID NOs: 24, 25, and 26; and SEQ ID NOs: 30, 31, and 32.

[081] Nonlimiting exemplary humanized anti-CSF1R antibodies include antibodies comprising the sets of heavy chain CDR1, CDR2, and CDR3, and light chain CDR1, CDR2, and CDR3 in Table 1 (SEQ ID NOs shown; see Table 8 for sequences). Each row of Table 1 shows the heavy chain CDR1, CDR2, and CDR3, and light chain CDR1, CDR2, and CDR3 of an exemplary antibody.

Table 1: Heavy chain and light chain CDRs

Heavy chain			**Light chain**		
CDR1 SEQ ID	**CDR2 SEQ ID**	**CDR3 SEQ ID**	**CDR1 SEQ ID**	**CDR2 SEQ ID**	**CDR3 SEQ ID**
15	16	17	18	19	20
21	22	23	24	25	26
27	28	29	30	31	32

Further exemplary humanized antibodies

[082] In some embodiments, a humanized anti-CSF1R antibody comprises a heavy chain comprising a variable region sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a sequence selected from SEQ ID NOs: 9, 11, 13, and 39 to 45, and wherein the antibody binds CSF1R. In some embodiments, a humanized anti-CSF1R antibody comprises a light chain comprising a variable region sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a sequence selected from SEQ ID NOs: 10, 12, 14, and 46 to 52, wherein the antibody binds CSF1R. In some embodiments, a humanized anti-CSF1R antibody comprises a heavy chain comprising a variable region sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a sequence selected from SEQ ID NOs: 9, 11, 13, and 39 to 45; and a light chain comprising a variable region sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a sequence selected from SEQ ID NOs: 10, 12, 14, and 46 to 52; wherein the antibody binds CSF1R.

[083] As used herein, whether a particular polypeptide is, for example, at least 95% identical to an amino acid sequence can be determined using, e.g., a computer program. When determining whether a particular sequence is, for example, 95% identical to a reference sequence, the percentage of identity is calculated over the full length of the reference amino acid sequence.

[084] In some embodiments, a humanized anti-CSF1R antibody comprises at least one of the CDRs discussed herein. That is, in some embodiments, a humanized anti-CSF1R antibody comprises at least one CDR selected from a heavy chain CDR1 discussed herein, a heavy chain CDR2 discussed herein, a heavy chain CDR3 discussed herein, a light chain CDR1 discussed herein, a light chain CDR2 discussed herein, and a light chain CDR3 discussed herein. Further, in some embodiments, a humanized anti-CSF1R antibody comprises at least one mutated CDR based on a CDR discussed herein, wherein the mutated CDR comprises 1, 2, 3, or 4 amino acid substitutions relative to the CDR discussed herein. In some embodiments, one or more of the amino acid substitutions are conservative amino acid substitutions. One

skilled in the art can select one or more suitable conservative amino acid substitutions for a particular CDR sequence, wherein the suitable conservative amino acid substitutions are not predicted to significantly alter the binding properties of the antibody comprising the mutated CDR.

[085] Exemplary humanized anti-CSF1R antibodies also include antibodies that compete for binding to CSF1R with an antibody described herein. Thus, in some embodiments, a humanized anti-CSF1R antibody is provided that competes for binding to CSF1R with an antibody selected from Fabs 0301, 0302, and 0311; and bivalent (i.e., having two heavy chains and two light chains) antibody versions of those Fabs.

Exemplary humanized antibody constant regions

[086] In some embodiments, a humanized antibody described herein comprises one or more human constant regions. In some embodiments, the human heavy chain constant region is of an isotype selected from IgA, IgG, and IgD. In some embodiments, the human light chain constant region is of an isotype selected from κ and λ. In some embodiments, a humanized antibody described herein comprises a human IgG constant region. In some embodiments, a humanized antibody described herein comprises a human IgG4 heavy chain constant region. In some such embodiments, a humanized antibody described herein comprises an S241P mutation in the human IgG4 constant region. In some embodiments, a humanized antibody described herein comprises a human IgG4 constant region and a human κ light chain.

[087] The choice of heavy chain constant region can determine whether or not an antibody will have effector function *in vivo*. Such effector function, in some embodiments, includes antibody-dependent cell-mediated cytotoxicity (ADCC) and/or complement-dependent cytotoxicity (CDC), and can result in killing of the cell to which the antibody is bound. In some methods of treatment, including methods of treating some cancers, cell killing may be desirable, for example, when the antibody binds to a cell that supports the maintenance or growth of the tumor. Exemplary cells that may support the maintenance or growth of a tumor include, but are not limited to, tumor cells themselves, cells that aid in the recruitment of vasculature to the tumor, and cells that provide ligands, growth factors, or counter-receptors that support or promote tumour growth or tumour survial. In some embodiments, when effector

function is desirable, an anti-CSF1R antibody comprising a human IgG1 heavy chain or a human IgG3 heavy chain is selected.

[088] In some methods of treatment, effector function may not be desirable. For example, in some embodiments, it may be desirable that antibodies used in the treatment of MS and/or RA and/or osteolysis do not have effector function. Thus, in some embodiments, anti-CSF1R antibodies developed for the treatment of cancer may not be suitable for use in treatment of MS and/or RA and/or osteolysis. Accordingly, in some embodiments, an anti-CSF1R antibody that lacks significant effector function is used in treatment of MS and/or RA and/or osteolysis. In some embodiments, an anti-CSF1R antibody for treatment of MS and/or RA and/or osteolysis comprises a human IgG4 or IgG2 heavy chain constant region. In some embodiments, an IgG4 constant region comprises an S241P mutation.

[089] An antibody may be humanized by any method. Nonlimiting exemplary methods of humanization include methods described, e.g., in U.S. Patent Nos. 5,530,101; 5,585,089; 5,693,761; 5,693,762; 6,180,370; Jones et al., *Nature* 321: 522-525 (1986); Riechmann et al., *Nature* 332: 323-27 (1988); Verhoeyen et al., Science 239: 1534-36 (1988); and U.S. Publication No. US 2009/0136500.

[090] As noted above, a humanized antibody is an antibody in which at least one amino acid in a framework region of a non-human variable region has been replaced with the amino acid from the corresponding location in a human framework region. In some embodiments, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least 10, at least 11, at least 12, at least 15, or at least 20 amino acids in the framework regions of a non-human variable region are replaced with an amino acid from one or more corresponding locations in one or more human framework regions.

[091] In some embodiments, some of the corresponding human amino acids used for substitution are from the framework regions of different human immunoglobulin genes. That is, in some such embodiments, one or more of the non-human amino acids may be replaced with corresponding amino acids from a human framework region of a first human antibody or encoded by a first human immunoglobulin gene, one or more of the non-human amino acids may be replaced with corresponding amino acids from a human framework region of a second human antibody or encoded by a second human immunoglobulin gene, one or more of the non-human amino acids may be replaced with corresponding amino acids from a

human framework region of a third human antibody or encoded by a third human immunoglobulin gene, etc. Further, in some embodiments, all of the corresponding human amino acids being used for substitution in a single framework region, for example, FR2, need not be from the same human framework. In some embodiments, however, all of the corresponding human amino acids being used for substitution are from the same human antibody or encoded by the same human immunoglobulin gene.

[092] In some embodiments, an antibody is humanized by replacing one or more entire framework regions with corresponding human framework regions. In some embodiments, a human framework region is selected that has the highest level of homology to the non-human framework region being replaced. In some embodiments, such a humanized antibody is a CDR-grafted antibody.

[093] In some embodiments, following CDR-grafting, one or more framework amino acids are changed back to the corresponding amino acid in a mouse framework region. Such "back mutations" are made, in some embodiments, to retain one or more mouse framework amino acids that appear to contribute to the structure of one or more of the CDRs and/or that may be involved in antigen contacts and/or appear to be involved in the overall structural integrity of the antibody. In some embodiments, ten or fewer, nine or fewer, eight or fewer, seven or fewer, six or fewer, five or fewer, four or fewer, three or fewer, two or fewer, one, or zero back mutations are made to the framework regions of an antibody following CDR grafting.

[094] In some embodiments, a humanized antibody also comprises a human heavy chain constant region and/or a human light chain constant region.

Exemplary Chimeric Antibodies

[095] In some embodiments, an anti-CSF1R antibody is a chimeric antibody. In some embodiments, an anti-CSF1R antibody comprises at least one non-human variable region and at least one human constant region. In some such embodiments, all of the variable regions of an anti-CSF1R antibody are non-human variable regions, and all of the constant regions of an anti-CSF1R antibody are human constant regions. In some embodiments, one or more variable regions of a chimeric antibody are mouse variable regions. The human constant region of a chimeric antibody need not be of the same isotype as the non-human constant region, if any, it replaces. Chimeric antibodies are discussed, e.g., in U.S. Patent No. 4,816,567; and Morrison et al. *Proc. Natl. Acad. Sci. USA* 81: 6851-55 (1984).

[096] Nonlimiting exemplary chimeric antibodies include chimeric antibodies comprising the heavy and/or light chain variable regions of an antibody selected from 0301, 0302, and 0311. Additional nonlimiting exemplary chimeric antibodies include chimeric antibodies comprising heavy chain CDR1, CDR2, and CDR3, and/or light chain CDR1, CDR2, and CDR3 of an antibody selected from 0301, 0302, and 0311.

[097] Nonlimiting exemplary chimeric anti-CSF1R antibodies include antibodies comprising the following pairs of heavy and light chain variable regions: SEQ ID NOs: 9 and 10; SEQ ID NOs: 11 and 12; and SEQ ID NOs: 13 and 14.

[098] Nonlimiting exemplary anti-CSF1R antibodies include antibodies comprising a set of heavy chain CDR1, CDR2, and CDR3, and light chain CDR1, CDR2, and CDR3 shown above in Table 1.

Further exemplary chimeric antibodies

[099] In some embodiments, a chimeric anti-CSF1R antibody comprises a heavy chain comprising a variable region sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a sequence selected from SEQ ID NOs: 9, 11, 13, and 39 to 45, wherein the antibody binds CSF1R. In some embodiments, a chimeric anti-CSF1R antibody comprises a light chain comprising a variable region sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a sequence selected from SEQ ID NOs: 10, 12, 14, and 46 to 52, wherein the antibody binds CSF1R. In some embodiments, a chimeric anti-CSF1R antibody comprises a heavy chain comprising a variable region sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a sequence selected from SEQ ID NOs: 9, 11, 13, and 39 to 45; and a light chain comprising a variable region sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a sequence selected from SEQ ID NOs: 10, 12, 14, and 46 to 52; wherein the antibody binds CSF1R.

[0100] In some embodiments, a chimeric anti-CSF1R antibody comprises at least one of the CDRs discussed herein. That is, in some embodiments, a chimeric anti-CSF1R antibody comprises at least one CDR selected from a heavy chain CDR1 discussed herein, a heavy chain CDR2 discussed herein, a heavy chain CDR3

discussed herein, a light chain CDR1 discussed herein, a light chain CDR2 discussed herein, and a light chain CDR3 discussed herein. Further, in some embodiments, a chimeric anti-CSF1R antibody comprises at least one mutated CDR based on a CDR discussed herein, wherein the mutated CDR comprises 1, 2, 3, or 4 amino acid substitutions relative to the CDR discussed herein. In some embodiments, one or more of the amino acid substitutions are conservative amino acid substitutions. One skilled in the art can select one or more suitable conservative amino acid substitutions for a particular CDR sequence, wherein the suitable conservative amino acid substitutions are not predicted to significantly alter the binding properties of the antibody comprising the mutated CDR.

[0101] Exemplary chimeric anti-CSF1R antibodies also include chimeric antibodies that compete for binding to CSF1R with an antibody described herein. Thus, in some embodiments, a chimeric anti-CSF1R antibody is provided that competes for binding to CSF1R with an antibody selected from Fabs 0301, 0302, and 0311; and bivalent (i.e., having two heavy chains and two light chains) antibody versions of those Fabs.

Exemplary chimeric antibody constant regions

[0102] In some embodiments, a chimeric antibody described herein comprises one or more human constant regions. In some embodiments, the human heavy chain constant region is of an isotype selected from IgA, IgG, and IgD. In some embodiments, the human light chain constant region is of an isotype selected from κ and λ. In some embodiments, a chimeric antibody described herein comprises a human IgG constant region. In some embodiments, a chimeric antibody described herein comprises a human IgG4 heavy chain constant region. In some such embodiments, a chimeric antibody described herein comprises an S241P mutation in the human IgG4 constant region. In some embodiments, a chimeric antibody described herein comprises a human IgG4 constant region and a human κ light chain.

[0103] As noted above, whether or not effector function is desirable may depend on the particular method of treatment intended for an antibody. Thus, in some embodiments, when effector function is desirable, a chimeric anti-CSF1R antibody comprising a human IgG1 heavy chain constant region or a human IgG3 heavy chain constant region is selected. In some embodiments, when effector function is not desirable, a chimeric anti-CSF1R antibody comprising a human IgG4 or IgG2 heavy chain constant region is selected.

Exemplary Human Antibodies

[0104] Human antibodies can be made by any suitable method. Nonlimiting exemplary methods include making human antibodies in transgenic mice that comprise human immunoglobulin loci. See, e.g., Jakobovits et al., *Proc. Natl. Acad. Sci. USA* 90: 2551-55 (1993); Jakobovits et al., *Nature* 362: 255-8 (1993); Lonberg et al., *Nature* 368: 856-9 (1994); and U.S. Patent Nos. 5,545,807; 6,713,610; 6,673,986; 6,162,963; 5,545,807; 6,300,129; 6,255,458; 5,877,397; 5,874,299; and 5,545,806.

[0105] Nonlimiting exemplary methods also include making human antibodies using phage display libraries. See, e.g., Hoogenboom et al., *J. Mol. Biol.* 227: 381-8 (1992); Marks et al., *J. Mol. Biol.* 222: 581-97 (1991); and PCT Publication No. WO 99/10494.

[0106] In some embodiments, a human anti-CSF1R antibody binds to a polypeptide having the sequence of SEQ ID NO: 1. Exemplary human anti-CSF1R antibodies also include antibodies that compete for binding to CSF1R with an antibody described herein. Thus, in some embodiments, a human anti-CSF1R antibody is provided that competes for binding to CSF1R with an antibody selected from Fabs 0301, 0302, and 0311, and bivalent (i.e., having two heavy chains and two light chains) antibody versions of those Fabs.

[0107] In some embodiments, a human anti-CSF1R antibody comprises one or more human constant regions. In some embodiments, the human heavy chain constant region is of an isotype selected from IgA, IgG, and IgD. In some embodiments, the human light chain constant region is of an isotype selected from κ and λ. In some embodiments, a human antibody described herein comprises a human IgG constant region. In some embodiments, a human antibody described herein comprises a human IgG4 heavy chain constant region. In some such embodiments, a human antibody described herein comprises an S241P mutation in the human IgG4 constant region. In some embodiments, a human antibody described herein comprises a human IgG4 constant region and a human κ light chain.

[0108] In some embodiments, when effector function is desirable, a human anti-CSF1R antibody comprising a human IgG1 heavy chain constant region or a human IgG3 heavy chain constant region is selected. In some embodiments, when effector function is not desirable, a human anti-CSF1R antibody comprising a human IgG4 or IgG2 heavy chain constant region is selected.

Additional Exemplary Anti-CSF1R Antibodies

[0109] Exemplary anti-CSF1R antibodies also include, but are not limited to, mouse, humanized, human, chimeric, and engineered antibodies that comprise, for example, one or more of the CDR sequences described herein. In some embodiments, an anti-CSF1R antibody comprises a heavy chain variable region described herein. In some embodiments, an anti-CSF1R antibody comprises a light chain variable region described herein. In some embodiments, an anti-CSF1R antibody comprises a heavy chain variable region described herein and a light chain variable region described herein. In some embodiments, an anti-CSF1R antibody comprises heavy chain CDR1, CDR2, and CDR3 described herein. In some embodiments, an anti-CSF1R antibody comprises light chain CDR1, CDR2, and CDR3 described herein. In some embodiments, an anti-CSF1R antibody comprises heavy chain CDR1, CDR2, and CDR3 described herein and light chain CDR1, CDR2, and CDR3 described herein.

[0110] In some embodiments, an anti-CSF1R antibody comprises a heavy chain variable region of an antibody selected from Fabs 0301, 0302, and 0311. Nonlimiting exemplary anti-CSF1R antibodies also include antibodies comprising a heavy chain variable region of an antibody selected from humanized antibodies Ab1 to Ab16. Nonlimiting exemplary anti-CSF1R antibodies include antibodies comprising a heavy chain variable region comprising a sequence selected from SEQ ID NOs: 9, 11, 13, and 39 to 45.

[0111] In some embodiments, an anti-CSF1R antibody comprises a light chain variable region of an antibody selected from Fabs 0301, 0302, and 311. Nonlimiting exemplary anti-CSF1R antibodies also include antibodies comprising a light chain variable region of an antibody selected from humanized antibodies Ab1 to Ab16. Nonlimiting exemplary anti-CSF1R antibodies include antibodies comprising a light chain variable region comprising a sequence selected from SEQ ID NOs: 10, 12, 14, and 46 to 52.

[0112] In some embodiments, an anti-CSF1R antibody comprises a heavy chain variable region and a light chain variable region of an antibody selected from Fabs 0301, 0302, and 0311. Nonlimiting exemplary anti-CSF1R antibodies also include antibodies comprising a heavy chain variable region and a light chain variable region of an antibody selected from humanized antibodies Ab1 to Ab16. Nonlimiting exemplary anti-CSF1R antibodies include antibodies comprising the following pairs of heavy and light chain variable regions: SEQ ID NOs: 9 and 10; SEQ ID NOs: 11

and 12; and SEQ ID NOs: 13 and 14; SEQ ID NOs: 39 and 40; SEQ ID NOs: 41 and 42; SEQ ID NOs: 43 and 44; SEQ ID NOs: 45 and 46; SEQ ID NOs: 47 and 48; SEQ ID NOs: 49 and 50; and SEQ ID NOs: 51 and 52. Nonlimiting exemplary anti-CSF1R antibodies also include antibodies comprising the following pairs of heavy and light chains: SEQ ID NOs: 33 and 34; SEQ ID NOs: 35 and 36; and SEQ ID NOs: 37 and 38.

[0113] In some embodiments, an anti-CSF1R antibody comprises heavy chain CDR1, CDR2, and CDR3 of an antibody selected from Fabs 0301, 0302, and 0311. Nonlimiting exemplary anti-CSF1R antibodies include antibodies comprising sets of heavy chain CDR1, CDR2, and CDR3 selected from: SEQ ID NOs: 15, 16, and 17; SEQ ID NOs: 21, 22, and 23; and SEQ ID NOs: 27, 28,and 29.

[0114] In some embodiments, an anti-CSF1R antibody comprises light chain CDR1, CDR2, and CDR3 of an antibody selected from Fabs 0301, 0302, and 0311. Nonlimiting exemplary anti-CSF1R antibodies include antibodies comprising sets of light chain CDR1, CDR2, and CDR3 selected from: SEQ ID NOs: 18, 19, and 20; SEQ ID NOs: 24, 25, and 26; and SEQ ID NOs: 30, 31, and 32.

[0115] In some embodiments, an anti-CSF1R antibody comprises heavy chain CDR1, CDR2, and CDR3, and light chain CDR1, CDR2, and CDR3 of an antibody selected from Fabs 0301, 0302, and 0311.

[0116] Nonlimiting exemplary anti-CSF1R antibodies include antibodies comprising the sets of heavy chain CDR1, CDR2, and CDR3, and light chain CDR1, CDR2, and CDR3 shown above in Table 1.

Further exemplary antibodies

[0117] In some embodiments, an anti-CSF1R antibody comprises a heavy chain comprising a variable region sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a sequence selected from SEQ ID NOs: 9, 11, 13, and 39 to 45, wherein the antibody binds CSF1R. In some embodiments, an anti-CSF1R antibody comprises a light chain comprising a variable region sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a sequence selected from SEQ ID NOs: 10, 12, 14, and 46 to 52, wherein the antibody binds CSF1R. In some embodiments, an anti-CSF1R antibody comprises a heavy chain comprising a variable region sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least

94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a sequence selected from SEQ ID NOs: 9, 11, 13, and 39 to 45; and a light chain comprising a variable region sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a sequence selected from SEQ ID NOs: 10, 12, 14, and 46 to 52; wherein the antibody binds CSF1R.

[0118] In some embodiments, an anti-CSF1R antibody comprises at least one of the CDRs discussed herein. That is, in some embodiments, an anti-CSF1R antibody comprises at least one CDR selected from a heavy chain CDR1 discussed herein, a heavy chain CDR2 discussed herein, a heavy chain CDR3 discussed herein, a light chain CDR1 discussed herein, a light chain CDR2 discussed herein, and a light chain CDR3 discussed herein. Further, in some embodiments, an anti-CSF1R antibody comprises at least one mutated CDR based on a CDR discussed herein, wherein the mutated CDR comprises 1, 2, 3, or 4 amino acid substitutions relative to the CDR discussed herein. In some embodiments, one or more of the amino acid substitutions are conservative amino acid substitutions. One skilled in the art can select one or more suitable conservative amino acid substitutions for a particular CDR sequence, wherein the suitable conservative amino acid substitutions are not predicted to significantly alter the binding properties of the antibody comprising the mutated CDR.

[0119] Exemplary anti-CSF1R antibodies also include antibodies that compete for binding to CSF1R with an antibody described herein. Thus, in some embodiments, an anti-CSF1R antibody is provided that competes for binding to CSF1R with an antibody selected from Fabs 0301, 0302, and 0311, and bivalent (i.e., having two heavy chains and two light chains) antibody versions of those Fabs.

Exemplary antibody constant regions

[0120] In some embodiments, an antibody described herein comprises one or more human constant regions. In some embodiments, the human heavy chain constant region is of an isotype selected from IgA, IgG, and IgD. In some embodiments, the human light chain constant region is of an isotype selected from κ and λ. In some embodiments, an antibody described herein comprises a human IgG constant region. In some embodiments, an antibody described herein comprises a human IgG4 heavy chain constant region. In some such embodiments, an antibody described herein comprises an S241P mutation in the human IgG4 constant region. In

some embodiments, an antibody described herein comprises a human IgG4 constant region and a human κ light chain.

[0121] As noted above, whether or not effector function is desirable may depend on the particular method of treatment intended for an antibody. Thus, in some embodiments, when effector function is desirable, an anti-CSF1R antibody comprising a human IgG1 heavy chain constant region or a human IgG3 heavy chain constant region is selected. In some embodiments, when effector function is not desirable, an anti-CSF1R antibody comprising a human IgG4 or IgG2 heavy chain constant region is selected.

Exemplary Anti-CSF1R Heavy Chain Variable Regions

[0122] In some embodiments, anti-CSF1R antibody heavy chain variable regions are provided. In some embodiments, an anti-CSF1R antibody heavy chain variable region is a mouse variable region, a human variable region, or a humanized variable region.

[0123] An anti-CSF1R antibody heavy chain variable region comprises a heavy chain CDR1, FR2, CDR2, FR3, and CDR3. In some embodiments, an anti-CSF1R antibody heavy chain variable region further comprises a heavy chain FR1 and/or FR4. Nonlimiting exemplary heavy chain variable regions include, but are not limited to, heavy chain variable regions having an amino acid sequence selected from SEQ ID NOs: 9, 11, 13, and 39 to 45.

[0124] In some embodiments, an anti-CSF1R antibody heavy chain variable region comprises a CDR1 comprising a sequence selected from SEQ ID NOs: 15, 21, and 27.

[0125] In some embodiments, an anti-CSF1R antibody heavy chain variable region comprises a CDR2 comprising a sequence selected from SEQ ID NOs: 16, 22, and 28.

[0126] In some embodiments, an anti-CSF1R antibody heavy chain variable region comprises a CDR3 comprising a sequence selected from SEQ ID NOs: 17, 23, and 29.

[0127] Nonlimiting exemplary heavy chain variable regions include, but are not limited to, heavy chain variable regions comprising sets of CDR1, CDR2, and CDR3 selected from: SEQ ID NOs: 15, 16, and 17; SEQ ID NOs: 21, 22, and 23; and SEQ ID NOs: 27, 28, and 29.

[0128] In some embodiments, an anti-CSF1R antibody heavy chain comprises a variable region sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a sequence selected from SEQ ID NOs: 9, 11, 13, and 39 to 45, wherein the heavy chain, together with a light chain, is capable of forming an antibody that binds CSF1R.

[0129] In some embodiments, an anti-CSF1R antibody heavy chain comprises at least one of the CDRs discussed herein. That is, in some embodiments, an anti-CSF1R antibody heavy chain comprises at least one CDR selected from a heavy chain CDR1 discussed herein, a heavy chain CDR2 discussed herein, and a heavy chain CDR3 discussed herein. Further, in some embodiments, an anti-CSF1R antibody heavy chain comprises at least one mutated CDR based on a CDR discussed herein, wherein the mutated CDR comprises 1, 2, 3, or 4 amino acid substitutions relative to the CDR discussed herein. In some embodiments, one or more of the amino acid substitutions are conservative amino acid substitutions. One skilled in the art can select one or more suitable conservative amino acid substitutions for a particular CDR sequence, wherein the suitable conservative amino acid substitutions are not predicted to significantly alter the binding properties of the heavy chain comprising the mutated CDR.

[0130] In some embodiments, a heavy chain comprises a heavy chain constant region. In some embodiments, a heavy chain comprises a human heavy chain constant region. In some embodiments, the human heavy chain constant region is of an isotype selected from IgA, IgG, and IgD. In some embodiments, the human heavy chain constant region is an IgG constant region. In some embodiments, a heavy chain comprises a human igG4 heavy chain constant region. In some such embodiments, the human IgG4 heavy chain constant region comprises an S241P mutation.

[0131] In some embodiments, when effector function is desirable, a heavy chain comprises a human IgG1 or IgG3 heavy chain constant region. In some embodiments, when effector function is less desirable, a heavy chain comprises a human IgG4 or IgG2 heavy chain constant region.

Exemplary Anti-CSF1R Light Chain Variable Regions

[0132] In some embodiments, anti-CSF1R antibody light chain variable regions are provided. In some embodiments, an anti-CSF1R antibody light chain

variable region is a mouse variable region, a human variable region, or a humanized variable region.

[0133] An anti-CSF1R antibody light chain variable region comprises a light chain CDR1, FR2, CDR2, FR3, and CDR3. In some embodiments, an anti-CSF1R antibody light chain variable region further comprises a light chain FR1 and/or FR4. Nonlimiting exemplary light chain variable regions include light chain variable regions having an amino acid sequence selected from SEQ ID NOs: 10, 12, 14, and 46 to 52.

[0134] In some embodiments, an anti-CSF1R antibody light chain variable region comprises a CDR1 comprising a sequence selected from SEQ ID NOs: 18, 24 and 30.

[0135] In some embodiments, an anti-CSF1R antibody light chain variable region comprises a CDR2 comprising a sequence selected from SEQ ID NOs: 19, 25, and 31.

[0136] In some embodiments, an anti-CSF1R antibody light chain variable region comprises a CDR3 comprising a sequence selected from SEQ ID NOs: 20, 26, and 32.

[0137] Nonlimiting exemplary light chain variable regions include, but are not limited to, light chain variable regions comprising sets of CDR1, CDR2, and CDR3 selected from: SEQ ID NOs: 18, 19, and 20; SEQ ID NOs: 24, 25, and 26; and SEQ ID NOs: 30, 31, and 32.

[0138] In some embodiments, an anti-CSF1R antibody light chain comprises a variable region sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a sequence selected from SEQ ID NOs: 10, 12, 14, and 46 to 52, wherein the light chain, together with a heavy chain, is capable of forming an antibody that binds CSF1R.

[0139] In some embodiments, an anti-CSF1R antibody light chain comprises at least one of the CDRs discussed herein. That is, in some embodiments, an anti-CSF1R antibody light chain comprises at least one CDR selected from a light chain CDR1 discussed herein, a light chain CDR2 discussed herein, and a light chain CDR3 discussed herein. Further, in some embodiments, an anti-CSF1R antibody light chain comprises at least one mutated CDR based on a CDR discussed herein, wherein the mutated CDR comprises 1, 2, 3, or 4 amino acid substitutions relative to the CDR

discussed herein. In some embodiments, one or more of the amino acid substitutions are conservative amino acid substitutions. One skilled in the art can select one or more suitable conservative amino acid substitutions for a particular CDR sequence, wherein the suitable conservative amino acid substitutions are not predicted to significantly alter the binding properties of the light chain comprising the mutated CDR.

[0140] In some embodiments, a light chain comprises a human light chain constant region. In some embodiments, a human light chain constant region is selected from a human κ and a human λ light chain constant region.

Exemplary Additional CSF1R Binding Molecules

[0141] In some embodiments, additional molecules that bind CSF1R are provided. Such molecules include, but are not limited to, non-canonical scaffolds, such as anti-calins, adnectins, ankyrin repeats, etc. See, e.g., Hosse et al., *Prot. Sci.* 15:14 (2006); Fiedler, M. and Skerra, A., "Non-Antibody Scaffolds," pp.467-499 in Handbook of Therapeutic Antibodies, Dubel, S., ed., Wiley-VCH, Weinheim, Germany, 2007.

Exemplary Properties of anti-CSF1R antibodies

[0142] In some embodiments, an antibody having a structure described above binds to the CSF1R with a binding affinity (K_D) of less than 1 nM, blocks binding of CSF1 and/or IL34 to CSF1R, and inhibits CSF1R phosphorylation induced by CSF1 and/or IL34.

[0143] In some embodiments, an anti-CSF1R antibody binds to the extracellular domain of CSF1R (CSF1R-ECD). In some embodiments, an anti-CSF1R antibody has a binding affinity (K_D) for CSF1R of less than 1 nM, less than 0.5 nM, less than 0.1 nM, or less than 0.05 nM. In some embodiments, an anti-CSF1R antibody has a K_D of between 0.01 and 1 nM, between 0.01 and 0.5 nM, between 0.01 and 0.1 nM, between 0.01 and 0.05 nM, or between 0.02 and 0.05 nM.

[0144] In some embodiments, an anti-CSF1R antibody blocks ligand binding to CSF1R. In some embodiments, an anti-CSF1R antibody blocks binding of CSF1 to CSF1R. In some embodiments, an anti-CSF1R antibody blocks binding of IL34 to CSF1R. In some embodiments, an anti-CSF1R antibody blocks binding of both CSF1 and IL34 to CSF1R. In some embodiments, an antibody that blocks ligand binding binds to the extracellular domain of CSF1R. An antibody is considered to "block ligand binding to CSF1R" when it reduces the amount of detectable binding of a

ligand to CSF1R by at least 50%, using the assay described in Example 7. In some embodiments, an antibody reduces the amount of detectable binding of a ligand to CSF1R by at least 60%, at least 70%, at least 80%, or at least 90%, using the assay described in Example 7. In some such embodiments, the antibody is said to block ligand binding by at least 50%, at least 60%, at least 70%, etc.

[0145] In some embodiments, an anti-CSF1R antibody inhibits ligand-induced CSF1R phosphorylation. In some embodiments, an anti-CSF1R antibody inhibits CSF1-induced CSF1R phosphorylation. In some embodiments, an anti-CSF1R antibody inhibits IL34-induced CSF1R phosphorylation. In some embodiments, an anti-CSF1R antibody inhibits both CSF1-induced and IL34-induced CSF1R phosphorylation. An antibody is considered to "inhibit ligand-induced CSF1R phosphorylation" when it reduces the amount of detectable ligand-induced CSF1R phosphorylation by at least 50%, using the assay described in Example 6. In some embodiments, an antibody reduces the amount of detectable ligand-induced CSF1R phosphorylation by at least 60%, at least 70%, at least 80%, or at least 90%, using the assay described in Example 6. In some such embodiments, the antibody is said to inhibit ligand-induced CSF1R phosphorylation by at least at least 50%, at least 60%, at least 70%, etc.

[0146] In some embodiments, an antibody inhibits monocyte proliferation and/or survival responses in the presence of CSF1 and/or IL34. An antibody is considered to "inhibit monocyte proliferation and/or survival responses" when it reduces the amount of monocyte proliferation and/or survival responses in the presence of CSF1 and/or IL34 by at least 50%, using the assay described in Example 10. In some embodiments, an antibody reduces the amount of monocyte proliferation and/or survival responses in the presence of CSF1 and/or IL34 by at least 60%, at least 70%, at least 80%, or at least 90%, using the assay described in Example 10. In some such embodiments, the antibody is said to inhibit monocyte proliferation and/or survival responses by at least at least 50%, at least 60%, at least 70%, etc.

Exemplary Antibody Conjugates

[0147] In some embodiments, an anti-CSF1R antibody is conjugated to a label and/or a cytotoxic agent. As used herein, a label is a moiety that facilitates detection of the antibody and/or facilitates detection of a molecule to which the antibody binds. Nonlimiting exemplary labels include, but are not limited to, radioisotopes, fluorescent groups, enzymatic groups, chemiluminescent groups, biotin, epitope tags,

metal-binding tags, etc. One skilled in the art can select a suitable label according to the intended application.

[0148] As used herein, a cytotoxic agent is a moiety that reduces the proliferative capacity of one or more cells. A cell has reduced proliferative capacity when the cell becomes less able to proliferate, for example, because the cell undergoes apoptosis or otherwise dies, the cell fails to proceed through the cell cycle and/or fails to divide, the cell differentiates, etc. Nonlimiting exemplary cytotoxic agents include, but are not limited to, radioisotopes, toxins, and chemotherapeutic agents. One skilled in the art can select a suitable cytotoxic according to the intended application.

[0149] In some embodiments, a label and/or a cytotoxic agent is conjugated to an antibody using chemical methods *in vitro*. Nonlimiting exemplary chemical methods of conjugation are known in the art, and include services, methods and/or reagents commercially available from, e.g., Thermo Scientific Life Science Research Produces (formerly Pierce; Rockford, IL), Prozyme (Hayward, CA), SACRI Antibody Services (Calgary, Canada), AbD Serotec (Raleigh, NC), etc. In some embodiments, when a label and/or cytotoxic agent is a polypeptide, the label and/or cytotoxic agent can be expressed from the same expression vector with at least one antibody chain to produce a polypeptide comprising the label and/or cytotoxic agent fused to an antibody chain. One skilled in the art can select a suitable method for conjugating a label and/or cytotoxic agent to an antibody according to the intended application.

Exemplary Leader Sequences

[0150] In order for some secreted proteins to express and secrete in large quantities, a leader sequence from a heterologous protein may be desirable. In some embodiments, a leader sequence is selected from SEQ ID NOs: 3 and 4, which are light chain and heavy chain leader sequences, respectively. In some embodiments, employing heterologous leader sequences may be advantageous in that a resulting mature polypeptide may remain unaltered as the leader sequence is removed in the ER during the secretion process. The addition of a heterologous leader sequence may be required to express and secrete some proteins.

[0151] Certain exemplary leader sequence sequences are described, e.g., in the online Leader sequence Database maintained by the Department of Biochemistry, National University of Singapore. *See* Choo et al., *BMC* Bioinformatics, 6: 249 (2005); and PCT Publication No. WO 2006/081430.

Nucleic Acid Molecules Encoding Anti-CSF1R Antibodies

[0152] Nucleic acid molecules comprising polynucleotides that encode one or more chains of anti-CSF1R antibodies are provided. In some embodiments, a nucleic acid molecule comprises a polynucleotide that encodes a heavy chain or a light chain of an anti-CSF1R antibody. In some embodiments, a nucleic acid molecule comprises both a polynucleotide that encodes a heavy chain and a polynucleotide that encodes a light chain, of an anti-CSF1R antibody. In some embodiments, a first nucleic acid molecule comprises a first polynucleotide that encodes a heavy chain and a second nucleic acid molecule comprises a second polynucleotide that encodes a light chain.

[0153] In some such embodiments, the heavy chain and the light chain are expressed from one nucleic acid molecule, or from two separate nucleic acid molecules, as two separate polypeptides. In some embodiments, such as when an antibody is an scFv, a single polynucleotide encodes a single polypeptide comprising both a heavy chain and a light chain linked together.

[0154] In some embodiments, a polynucleotide encoding a heavy chain or light chain of an anti-CSF1R antibody comprises a nucleotide sequence that encodes a leader sequence, which, when translated, is located at the N terminus of the heavy chain or light chain. As discussed above, the leader sequence may be the native heavy or light chain leader sequence, or may be another heterologous leader sequence.

[0155] Nucleic acid molecules may be constructed using recombinant DNA techniques conventional in the art. In some embodiments, a nucleic acid molecule is an expression vector that is suitable for expression in a selected host cell.

Anti-CSF1R Antibody Expression and Production

Vectors

[0156] Vectors comprising polynucleotides that encode anti-CSF1R heavy chains and/or anti-CSF1R light chains are provided. Vectors comprising polynucleotides that encode anti-CSF1R heavy chains and/or anti-CSF1R light chains are also provided. Such vectors include, but are not limited to, DNA vectors, phage vectors, viral vectors, retroviral vectors, etc. In some embodiments, a vector comprises a first polynucleotide sequence encoding a heavy chain and a second polynucleotide sequence encoding a light chain. In some embodiments, the heavy chain and light chain are expressed from the vector as two separate polypeptides. In some embodiments, the heavy chain and light chain are expressed as part of a single polypeptide, such as, for example, when the antibody is an scFv.

[0157] In some embodiments, a first vector comprises a polynucleotide that encodes a heavy chain and a second vector comprises a polynucleotide that encodes a light chain. In some embodiments, the first vector and second vector are transfected into host cells in similar amounts (such as similar molar amounts or similar mass amounts). In some embodiments, a mole- or mass-ratio of between 5:1 and 1:5 of the first vector and the second vector is transfected into host cells. In some embodiments, a mass ratio of between 1:1 and 1:5 for the vector encoding the heavy chain and the vector encoding the light chain is used. In some embodiments, a mass ratio of 1:2 for the vector encoding the heavy chain and the vector encoding the light chain is used.

[0158] In some embodiments, a vector is selected that is optimized for expression of polypeptides in CHO or CHO-derived cells, or in NSO cells. Exemplary such vectors are described, e.g., in Running Deer et al., *Biotechnol. Prog.* 20:880-889 (2004).

[0159] In some embodiments, a vector is chosen for *in vivo* expression of anti-CSF1R heavy chains and/or anti-CSF1R light chains in animals, including humans. In some such embodiments, expression of the polypeptide is under the control of a promoter that functions in a tissue-specific manner. For example, liver-specific promoters are described, e.g., in PCT Publication No. WO 2006/076288.

Host Cells

[0160] In various embodiments, anti-CSF1R heavy chains and/or anti-CSF1R light chains may be expressed in prokaryotic cells, such as bacterial cells; or in eukaryotic cells, such as fungal cells (such as yeast), plant cells, insect cells, and mammalian cells. Such expression may be carried out, for example, according to procedures known in the art. Exemplary eukaryotic cells that may be used to express polypeptides include, but are not limited to, COS cells, including COS 7 cells; 293 cells, including 293-6E cells; CHO cells, including CHO-S and DG44 cells; PER.C6® cells (Crucell); and NSO cells. In some embodiments, anti-CSF1R heavy chains and/or anti-CSF1R light chains may be expressed in yeast. See, e.g., U.S. Publication No. US 2006/0270045 A1. In some embodiments, a particular eukaryotic host cell is selected based on its ability to make desired post-translational modifications to the anti-CSF1R heavy chains and/or anti-CSF1R light chains. For example, in some embodiments, CHO cells produce polypeptides that have a higher level of sialylation than the same polypeptide produced in 293 cells.

[0161] Introduction of one or more nucleic acids into a desired host cell may be accomplished by any method, including but not limited to, calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection, etc. Nonlimiting exemplary methods are described, e.g., in Sambrook et al., *Molecular Cloning, A Laboratory Manual,* 3rd ed. Cold Spring Harbor Laboratory Press (2001). Nucleic acids may be transiently or stably transfected in the desired host cells, according to any suitable method.

[0162] In some embodiments, one or more polypeptides may be produced *in vivo* in an animal that has been engineered or transfected with one or more nucleic acid molecules encoding the polypeptides, according to any suitable method.

Purification of Anti-CSF1R Antibodies

[0163] Anti-CSF1R antibodies may be purified by any suitable method. Such methods include, but are not limited to, the use of affinity matrices or hydrophobic interaction chromatography. Suitable affinity ligands include the CSF1R ECD and ligands that bind antibody constant regions. For example, a Protein A, Protein G, Protein A/G, or an antibody affinity column may be used to bind the constant region and to purify an anti-CSF1R antibody. Hydrophobic interactive chromatography, for example, a butyl or phenyl column, may also suitable for purifying some polypeptides. Many methods of purifying polypeptides are known in the art.

Cell-free Production of Anti-CSF1R Antibodies

[0164] In some embodiments, an anti-CSF1R antibody is produced in a cell-free system. Nonlimiting exemplary cell-free systems are described, e.g., in Sitaraman et al., *Methods Mol. Biol.* 498: 229-44 (2009); Spirin, *Trends Biotechnol.* 22: 538-45 (2004); Endo et al., *Biotechnol. Adv.* 21: 695-713 (2003).

Therapeutic Compositions and Methods

Methods of Treating Diseases using Anti-CSF1R Antibodies

[0165] Antibodies of the invention, and compositions comprising antibodies of the invention, are provided for use in methods of treatment for humans or animals. Methods of treating disease comprising administering anti-CSR1R antibodies are also provided. Nonlimiting exemplary diseases that can be treated with anti-CSF1R antibodies include, but are not limited to, RA, MS, cancer, metastasis-induced osteolytic bone loss, osteolytic disorders, and hypercalcemia-induced bone loss.

[0166] In some embodiments, methods of treating inflammatory conditions comprising administering an anti-CSF1R antibody are provided. In some embodiments, an inflammatory condition is selected from psoriasis, SLE (lupus), COPD, atopic dermatitis, and atherosclerosis, macrophage activation syndrome, and histiocytosis X.

[0167] In some embodiments, methods of treating an inflammatory condition comprising administering an anti-CSF1R antibody are provided, wherein the inflammatory condition is selected from: proliferative vascular disease, acute respiratory distress syndrome, cytokine-mediated toxicity, interleukin-2 toxicity, appendicitis, peptic, gastric and duodenal ulcers, peritonitis, pancreatitis, ulcerative, pseudomembranous, acute and ischemic colitis, diverticulitis, epiglottitis, achalasia, cholangitis, cholecystitis, hepatitis, inflammatory bowel disease, Crohn's disease, enteritis, Whipple's disease, asthma, allergy, anaphylactic shock, immune complex disease, organ ischemia, reperfusion injury, organ necrosis, hay fever, sepsis, septicemia, endotoxic shock, cachexia, hyperpyrexia, eosinophilic granuloma, granulomatosis, sarcoidosis, septic abortion, epididymitis, vaginitis, prostatitis, urethritis, bronchitis, emphysema, rhinitis, cystic fibrosis, pneumonitis, alvealitis, bronchiolitis, pharyngitis, pleurisy, sinusitis, influenza, respiratory syncytial virus infection, herpes infection, HIV infection, hepatitis B virus infection, hepatitis C virus infection, disseminated bacteremia, Dengue fever, candidiasis, malaria, filariasis, amebiasis, hydatid cysts, bums, dermatitis, dermatomyositis, sunburn, urticaria, warts, wheals, vasulitis, angiitis, endocarditis, arteritis, atherosclerosis, thrombophlebitis, pericarditis, myocarditis, myocardial ischemia, periarteritis nodosa, rheumatic fever, Alzheimer's disease, celiac disease, congestive heart failure, meningitis, encephalitis, cerebral infarction, cerebral embolism, Guillain-Barre syndrome, neuritis, neuralgia, spinal cord injury, paralysis, uveitis, arthritides, arthralgias, osteomyelitis, fasciitis, Paget's disease, gout, periodontal disease, synovitis, myasthenia gravis, thryoiditis, systemic lupus erythematosus, Goodpasture's syndrome, Behcets's syndrome, allograft rejection, graft-versus-host disease, ankylosing spondylitis, Berger's disease, type I diabetes, type 2 diabetes, Berger's disease, Retier's syndrome, and Hodgkins disease, or in treating inflammation associated with these conditions.

[0168] In some embodiments, methods of treating cancer comprising administering an anti-CSF1R antibody are provided. In some embodiments, the cancer is a CSF1-secreting cancer. In some embodiments, the cancer is one or more

cancers selected from breast cancer, prostate cancer, endometrial cancer, bladder cancer, kidney cancer, esophageal cancer, squamous cell carcinoma, uveal melanoma, follicular lymphoma, renal cell carcinoma, cervical cancer, and ovarian cancer. In some embodiments, an anti-CSF1R antibody is useful for treating one or more cancers selected from lung cancer, colorectal cancer, brain cancer, pancreatic cancer, head and neck cancer, liver cancer, leukemia, lymphoma, Hodgkin's disease, multiple myeloma, melanoma, astrocytoma, stomach cancer, and pulmonary adenocarcinoma.

Routes of Administration and Carriers

[0169] In various embodiments, anti-CSF1R antibodies may be administered *in vivo* by various routes, including, but not limited to, oral, intra-arterial, parenteral, intranasal, intramuscular, intracardiac, intraventricular, intratracheal, buccal, rectal, intraperitoneal, intradermal, topical, transdermal, and intrathecal, or otherwise by implantation or inhalation. The subject compositions may be formulated into preparations in solid, semi-solid, liquid, or gaseous forms; including, but not limited to, tablets, capsules, powders, granules, ointments, solutions, suppositories, enemas, injections, inhalants, and aerosols. A nucleic acid molecule encoding an anti-CSF1R antibody may be coated onto gold microparticles and delivered intradermally by a particle bombardment device, or "gene gun," as described in the literature (see, e.g., Tang et al., *Nature* 356:152-154 (1992)). The appropriate formulation and route of administration may be selected according to the intended application.

[0170] In various embodiments, compositions comprising anti-CSF1R antibodies are provided in formulations with a wide variety of pharmaceutically acceptable carriers (see, e.g., Gennaro, *Remington: The Science and Practice of Pharmacy with Facts and Comparisons: Drugfacts Plus*, 20th ed. (2003); Ansel et al., *Pharmaceutical Dosage Forms and Drug Delivery Systems,* 7th ed., Lippencott Williams and Wilkins (2004); Kibbe et al., *Handbook of Pharmaceutical Excipients,* 3rd ed., Pharmaceutical Press (2000)). Various pharmaceutically acceptable carriers, which include vehicles, adjuvants, and diluents, are available. Moreover, various pharmaceutically acceptable auxiliary substances, such as pH adjusting and buffering agents, tonicity adjusting agents, stabilizers, wetting agents and the like, are also available. Non-limiting exemplary carriers include saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof.

[0171] In various embodiments, compositions comprising anti-CSF1R antibodies may be formulated for injection, including subcutaneous administration, by

dissolving, suspending, or emulsifying them in an aqueous or nonaqueous solvent, such as vegetable or other oils, synthetic aliphatic acid glycerides, esters of higher aliphatic acids, or propylene glycol; and if desired, with conventional additives such as solubilizers, isotonic agents, suspending agents, emulsifying agents, stabilizers and preservatives. In various embodiments, the compositions may be formulated for inhalation, for example, using pressurized acceptable propellants such as dichlorodifluoromethane, propane, nitrogen, and the like. The compositions may also be formulated, in various embodiments, into sustained release microcapsules, such as with biodegradable or non-biodegradable polymers. A non-limiting exemplary biodegradable formulation includes poly lactic acid-glycolic acid polymer. A non-limiting exemplary non-biodegradable formulation includes a polyglycerin fatty acid ester. Certain methods of making such formulations are described, for example, in EP 1 125 584 A1.

[0172] Pharmaceutical packs and kits comprising one or more containers, each containing one or more doses of an anti-CSF1R antibody are also provided. In some embodiments, a unit dosage is provided wherein the unit dosage contains a predetermined amount of a composition comprising an anti-CSF1R antibody, with or without one or more additional agents. In some embodiments, such a unit dosage is supplied in single-use prefilled syringe for injection. In various embodiments, the composition contained in the unit dosage may comprise saline, sucrose, or the like; a buffer, such as phosphate, or the like; and/or be formulated within a stable and effective pH range. Alternatively, in some embodiments, the composition may be provided as a lyophilized powder that may be reconstituted upon addition of an appropriate liquid, for example, sterile water. In some embodiments, the composition comprises one or more substances that inhibit protein aggregation, including, but not limited to, sucrose and arginine. In some embodiments, a composition of the invention comprises heparin and/or a proteoglycan.

[0173] Pharmaceutical compositions are administered in an amount effective for treatment or prophylaxis of the specific indication. The therapeutically effective amount is typically dependent on the weight of the subject being treated, his or her physical or health condition, the extensiveness of the condition to be treated, or the age of the subject being treated. In general, anti-CSF1R antibodies may be administered in an amount in the range of about 10 μg/kg body weight to about 100 mg/kg body weight per dose. In some embodiments, anti-CSF1R antibodies may be

administered in an amount in the range of about 50 μg/kg body weight to about 5 mg/kg body weight per dose. In some embodiments, anti-CSF1R antibodies may be administered in an amount in the range of about 100 μg/kg body weight to about 10 mg/kg body weight per dose. In some embodiments, anti-CSF1R antibodies may be administered in an amount in the range of about 100 μg/kg body weight to about 20 mg/kg body weight per dose. In some embodiments, anti-CSF1R antibodies may be administered in an amount in the range of about 0.5 mg/kg body weight to about 20 mg/kg body weight per dose.

[0174] The anti-CSF1R antibody compositions may be administered as needed to subjects. Determination of the frequency of administration may be made by persons skilled in the art, such as an attending physician based on considerations of the condition being treated, age of the subject being treated, severity of the condition being treated, general state of health of the subject being treated and the like. In some embodiments, an effective dose of an anti-CSF1R antibody is administered to a subject one or more times. In various embodiments, an effective dose of an anti-CSF1R antibody is administered to the subject once a month, more than once a month, such as, for example, every two months or every three months. In other embodiments, an effective dose of an anti-CSF1R antibody is administered less than once a month, such as, for example, every two weeks or every week. An effective dose of an anti-CSF1R antibody is administered to the subject at least once. In some embodiments, the effective dose of an anti-CSF1R antibody may be administered multiple times, including for periods of at least a month, at least six months, or at least a year.

Combination Therapy

[0175] Anti-CSF1R antibodies may be administered alone or with other modes of treatment. They may be provided before, substantially contemporaneous with, or after other modes of treatment, for example, surgery, chemotherapy, radiation therapy, or the administration of a biologic, such as another therapeutic antibody. For treatment of rheumatoid arthritis, anti-CSF1R antibodies may be administered with other therapeutic agents, for example, methotrexate, anti-TNF agents such as Remicade, Humira, Simponi, and Enbrel; glucocorticoids such as prednisone; Leflunomide; Azothioprine; JAK inhibitors such as CP 590690; SYK inhibitors such as R788; anti-IL-6 antibodies; anti-IL-6R antibodies; anti-CD-20 antibodies; anti-CD19 antibodies; anti-GM-CSF antibodies; and anti-GM-CSF-R antibodies. For

treatment of multiple sclerosis, anti-CSF1R antibodies may be administered with other therapeutic agents, for example, interferon alpha; interferon beta; prednisone; anti-alpha4 integrin antibodies such as Tysabri; anti-CD20 antibodies such as Rituxan; FTY720 (Fingolimod); and Cladribine (Leustatin).

EXAMPLES

[0176] The examples discussed below are intended to be purely exemplary of the invention and should not be considered to limit the invention in any way. The examples are not intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (for example, amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

Example 1: Selection of Fabs that bind CSF1R extracellular domain (ECD)

[0177] Mice were immunized with a human CSF1R extracellular domain Fc fusion, hCSF1R ECD.506-Fc (SEQ ID NO: 6). Spleens from immunized mice were isolated, and a Fab phage display library was created from the spenocytes. Fab-expressing phage were selected for binding to human CSF1R ECD. Fabs from positive-binding phage were expressed and purified from bacteria. A total of 1056 Fab clones were selected for further analysis.

[0178] Fabs were screened for the ability to bind human CSF1R ECD, block binding of human CSF1 to human CSF1R ECD, and block binding of human IL34 to human CSF1R ECD. Sequence analysis and clusertering of the Fabs that were selected from that screen was then performed and certain unique Fabs were selected.

[0179] The unique Fabs were further analyzed for the ability to bind human CSF1R ECD, the ability to bind cynomolgus CSF1R ECD, and the ability to bind mouse CSF1R ECD. The Fabs were also analyzed for the ability to block human CSF1 binding to human CSF1R ECD and the ability to block human IL34 binding to human CSF1R ECD, and the ability to inhibit ligand-induced CSF1R phosphorylation in the presence of CSF1 or IL34. (Data not shown.)

Example 2: Reformatting of anti-CSF1R Fabs to make chimeric antibodies

[0180] Following the Fab characterization, eleven of the Fabs were selected for reformatting to chimeric antibodies. Each Fab was reformatted to a chimeric antibody comprising a human IgG4 heavy chain constant region with the S241P mutation, and a human κ light chain constant region. Briefly, Fab VH regions cloned into and expressed from vector pTT5 (Biotechnology Research Institute, Montreal, Canada; and National Research Research Council of Canada, Ottawa, Canada) modified to contain a mouse IgH leader sequence (SEQ ID NO: 4) and a human IgG4 heavy chain constant region with the S241P mutation (SEQ ID NO: 94). Fab VL regions were cloned into and expressed from vector pTT5 modified to contain a mouse Igκ leader sequence (SEQ ID NO: 3) and a human Igκ light chain constant region (SEQ ID NO: 95). Fab V regions were inserted in such a way as not to introduce non-antibody derived amino acid sequences into the final proteins.

Example 3: Expression and characterization of chimeric antibodies

[0181] The chimeric antibodies were transiently expressed and purified substantially as described below in Example 5.

[0182] The 11 chimeric antibodies were assayed for binding to human, cynomolgus, and mouse CSF1R ECD. The chimeric antibodies were also assayed for the ability to block binding of human CSF1 to human CSF1R ECD, the ability to block binding of human IL34 to human CSF1R ECD, the ability to block binding of human CSF1 to cynomolgus CSF1R ECD, and the ability to inhibit ligand-induced CSF1R phosphorylation in the presence of CSF1 or IL34. The chimeric antibodies were further assayed for binding to CSF1R on the surface of cells. Finally, the chimeric antibodies were assayed to confirm that they do not induce CSF1R phosphorylation in the absence of ligand. (Data not shown.)

Example 4: Humanization of anti-CSF1R antibodies

[0183] From the analyses described above, chimeric anti-CSF1R antibodies 0301, 0302, and 0311 were selected for humanization. The antibodies were humanized by changing certain amino acid residues in the framework regions of the heavy and light chain variable regions. The criteria used for humanization were as described previously, e.g., in U.S. Publication No. US 2009/0136500.

[0184] For cAb 0301, three humanized heavy chain variable regions and two humanized light chain variable regions were designed, for a total of six humanized

antibodies, Ab1 to Ab6. For cAb 0302, two humanized heavy chain variable regions and three humanized light chain variable regions were designed, for a total of six humanized antibodies, Ab7 to Ab12. For cAb 0311, two humanized heavy chain variable regions and two humanized light chain variable regions were designed, for a total of four humanized antibodies, Ab13 to Ab16.

[0185] The sequences for each of the humanized heavy chain variable regions and humanized light chain variable regions, aligned with the sequences of the parental chimeric antibody variable regions and the sequences of the human acceptor variable framework regions are shown in Figures 1 (heavy chains) and 2 (light chains). The changes in humanized variable region sequences relative to the human acceptor variable framework region sequences are boxed. Each of the CDRs for each of the variable regions is shown in a boxed region, and labeled as "CDR" above the boxed sequences.

[0186] Table 8, below, shows the full sequences for the humanized heavy chains and humanized light chains of antibodies Ab1 to Ab16. The name and SEQ ID NOs of the humanized heavy chain and humanized light chain of each of those antibodies is shown in Table 2.

Table 2: Humanized heavy chains and light chains of Ab1 to Ab16

Humanized antibody	Humanized HC	SEQ ID NO	Humanized LC	SEQ ID NO
Ab1	h0301-H0	53	h0301-L0	60
Ab2	h0301-H1	54	h0301-L0	60
Ab3	h0301-H2	55	h0301-L0	60
Ab4	h0301-H0	53	h0301-L1	61
Ab5	h0301-H1	54	h0301-L1	61
Ab6	h0301-H2	55	h0301-L1	61
Ab7	h0302-H1	56	h0302-L0	62
Ab8	h0302-H1	56	h0302-L1	63
Ab9	h0302-H1	56	h0302-L2	64
Ab10	h0302-H2	57	h0302-L0	62
Ab11	h0302-H2	57	h0302-L1	63
Ab12	h0302-H2	57	h0302-L2	64
Ab13	h0311-H1	58	h0311-L0	65

Ab14	h0311-H1	58	h0311-L1	66
Ab15	h0311-H2	59	h0311-L0	65
Ab16	h0311-H2	59	h0311-L1	66

Example 5: Humanized anti-CSF1R antibodies bind to human and cynomolgus CSF1R ECD, but not to mouse CSF1R ECD

[0187] The 16 humanized antibodies were transiently expressed in CHO cells, as follows. CHO-3E7 cells were co-transfected with individual heavy and light chain expression plasmids at a mass ratio of 1 heavy chain plasmid to 2 light chain plasmids using polyethyleneinimine (PEI) at a DNA:PEI ratio of 1:5. Total DNA used per transfection was 1.5 μg/ml of cells.

[0188] Humanized antibodies were purified from transfected cell supernatants using HiTrap Protein A HP columns (GE Healthcare) followed by further purification using Phenyl HP columns (GE Healthcare). Antibody containing supernatants were loaded onto HiTrap Protein A HP columns pre-equilibrated with PBS/0.5M NaCl. Antibody loaded columns were washed with 10 column volumes PBS/0.5M NaCl, and eluted with a mixed linear-step gradient of 0.1 M Glycine, pH 2.7/ 0.5 M NaCl directly into 100 ul of 1M Tris buffer, pH 8.0. Antibody containing eluates were dialyzed against PBS, after which 2.4 M $(NH_4)_2SO_4$ (Sigma) was added to achieve a conductivity equal to that of 10mM Potassium Phosphate pH7.0/1.2 M $(NH_4)_2SO_4$. Antibodies were then loaded on 1 ml Phenyl HP columns (GE Healthcare) pre-equilibrated with 10mM Potassium Phosphate pH7.0/1.2 M $(NH_4)_2SO_4$. Antibody loaded columns were washed with 15 column volumes 10mM Potassium Phosphate pH7.0/1.2 M $(NH_4)_2SO_4$, and eluted with a 20 column volume gradient of 10 mM Potassium Phosphate, pH 7.0. Antibody containing fractions were pooled and dialyzed against PBS.

[0189] The humanized antibodies, along with their parental chimeric antibodies (cAbs), were assayed for binding to human, cynomolgus, and mouse CSF1R ECD, as follows.

Human CSF1R Binding Activity

[0190] Ninety-six well clear-bottom ELISA plates were coated overnight with 1 μg/ml recombinant hCSF1R ECD.506-Fc (SEQ ID NO: 6; FivePrime Therapeutics) or Human M-CSF R Fc Chimera (R&D Systems) in PBS. The next morning, wells were washed four times with 0.05% Tween20 in PBS (PBST) and blocked with

Blocker-Blotto (Pierce). Fifty µl of 0.5x serial dilutions of the humanized antibody or parental chimeric antibody, beginning with 2000 ng/ml, diluted 1:1 in Blocker-Blotto were added to the CSF1R-coated wells. After incubation at room temperature (RT) for 90 min, wells were washed four times with PBST, and a 1:5000 dilution of a peroxidase-conjugated Goat anti-Human kappa Light chain antibody (Sigma) in Blocker-Blotto was added to each well. After incubation at RT for 60 min, wells were washed four times with PBST, and 50 µl o-phenylenediamine dihydrochloride peroxidase substrate (Sigma) was added to each well. After incubation at RT for 30 min, A450 values of each well were read directly on a SpectraMaxPlus spectrophotometer with SoftMaxPro software (Molecular Devices).

[0191] The results of that experiment is hown in Figure 3. All of the humanized antibodies bound to human CSF1R ECD within the range of concentrations tested.

Cynomolgus CSF1R Binding Curve

[0192] The binding curve for each humanized antibody binding to cynomolgus CSF1R ECD was determined as described above for human CSF1R, except the wells of the clear-bottom ELISA plates were coated overnight with 2 µg/ml recombinant cynoCSF1R ECD-Fc (FivePrime Therapeutics, SEQ ID NO: 8, but without the 19 amino acid leader sequence).

[0193] The results of that experiment are shown in Figure 4. All of the humanized antibodies bound to cynomolgus CSF1R ECD within the range of concentrations tested.

Mouse CSF1R Binding Curve

[0194] The binding curve for each humanized antibody binding to mouse CSF1R ECD was determined as described above for human CSF1R, except the wells of the clear-bottom ELISA plates were coated overnight with 2 µg/ml recombinant mCSF1R ECD-Fc (FivePrime Therapeutics, SEQ ID NO: 93).

[0195] The results of that experiment are shown in Figure 5. None of the humanized antibodies, or the parental chimeric antibodies, detectably bound to mouse CSF1R ECD over the range of concentrations tested.

Calculation of EC50s

[0196] Table 3 shows the EC50, calculated using the non-linear regression (curve-fit) analysis algorithm of the GraphPad Prism software (GraphPad Software) for each humanized antibody binding to human CSF1R ECD and cynomolgus CSF1R

ECD. Because none of the chimeric antibodies detectably bound to mouse CSF1R ECD, an EC50 could not be calculated from that data. Table 3 also includes the calculated EC50s for the parental chimeric antibodies.

Table 3: Binding activity of humanized anti-CSF1R antibodies

Humanized antibody	Human CSF1R ECD EC50 (ng/ml)	Cynomolgus CSF1R ECD EC50 (ng/ml)
cAb 0301	11.4	15.18
h0301-L0H0	13.4	15.11
h0301-L0H1	14.23	14.39
h0301-L0H2	14.77	13.79
h0301-L1H0	13.35	11.93
h0301-L1H1	16.47	16.66
h0301-L1H2	16.23	16.59
cAb 0302	15.94	17.34
h0302-L0H1	14.64	466.5
h0302-L1H1	21.43	1058
h0302-L2H1	7.741	66.04
h0302-L0H2	17.85	154.9
h0302-L1H2	22.1	172.5
h0302-L2H2	10.15	17.96
cAb 0311	17.65	20.06
h0311-L0H1	13.12	21.65
h0311-L1H1	14.32	30.88
h0311-L0H2	11.54	17.47
h0311-L1H2	13.26	20.27

Example 6: Humanized anti-CSF1R antibodies inhibit ligand-induced CSF1R phosphorylation

[0197] CSF1R is phosphorylated in the presence of ligands CSF1 or IL34. The humanized antibodies, along with their parental chimeric antibodies (cAbs), were tested for their ability to inhibit CSF1R phosphorylation induced by either ligand, as follows.

Inhibition of CSF1-Induced Phosphorylation

[0198] CSF1R (SEQ ID NO: 2)-transfected CHO cells were incubated with serial dilutions of each humanized antibody or a parental chimeric antibody, beginning at 8 μg/ml, for 60 min on ice, after which 3.3 nM of human CSF1 (M-CSF,

R&D Systems) was added to the cells. (For the 0301 series of humanized antibodies, serial dilutions beginning at 2 μg/ml of humanized antibody and parental chimeric antibody was used.) The cells were incubated for 3 minutes at 37°C, and then lysed by addition of 1/10x volume of 10x cell lysis buffer (Cell Signaling Technology). The amount of phosphorylated CSF1R in the cell lysates was quantified using a human phospho-M-CSF R ELISA kit (R&D Systems) according to the manufacturer's instructions.

[0199] The results of that experiment are shown in Figures 6A to 6C. All of the humanized antibodies were able to inhibit human CSF1-induced phosphorylation of human CSF1R ECD within the range of concentrations tested.

Inhibition of IL34-Induced Phosphorylation

[0200] CSF1R (SEQ ID NO: 2)-transfected CHO cells were incubated with 0.002 to 8 μg/ml of each humanized antibody or a parental chimeric antibody for 60 min on ice, after which 3.3 nM of human IL34 (FivePrime Therapeutics; SEQ ID NO: 68) was added to the cells. The cells were incubated for 3 minutes at 37°C, and then lysed by addition of 1/10x volume of 10x cell lysis buffer (Cell Signaling Technology). The amount of phosphorylated CSF1R in the cell lysates was quantified using a human phospho-M-CSF R ELISA kit (R&D Systems) according to the manufacturer's instructions.

[0201] The results of that experiment are shown in Figures 7A to 7C. All of the humanized antibodies were able to inhibit human IL34-induced phosphorylation of human CSF1R within the range of concentrations tested.

Example 7: Humanized anti-CSF1R antibodies block human CSF1 and human IL34 binding to human and cynomolgous CSF1R

Human CSF1/CSF1R Blocking Activity

[0202] The humanized antibodies, along with the parental chimeric antibodies (cAbs), were tested for their ability to block human CSF1 binding to human and cynomolgus CSF1R ECD, as follows.

[0203] Recombinant Human CSF1 (M-CSF; R&D Systems) was biotinylated using an NH2-Biotin Labeling Kit (Dojindo Molecular Technologies). One hundred μl of 1 μg/ml biotinylated CSF1 in PBST/0.1% BSA was added to the wells of Reacti-Bind Streptavidin coated plates (Pierce) pre-blocked with SuperBlock blocking buffer (Pierce) according to the manufacturer's instructions. Fifty μl of 0.5x serial dilutions of the humanized antibody or parental chimeric antibody, beginning with 2000 ng/ml,

was incubated with 50 ng/ml hCSF1R ECD.506-Fc (SEQ ID NO: 6; FivePrime Therapeutics) or 50 ng/ml cynoCSF1R ECD-Fc (FivePrime Therapeutics, SEQ ID NO: 8, but without the 19 amino acid leader sequence) in 100 µl PBST/0.1% BSA for 90 min at RT, after which the admix was transferred to one or more wells of a ligand-coated plate. After 90 min at RT, wells were washed with PBST, and a 1:5000 dilution of an Fc-fragment-specific peroxidase-conjugated goat anti-human IgG (Jackson Immuno Research) in PBST/0.1% BSA was added to each well. After incubation at RT for 60 min, wells were washed with PBST/0.1% BSA, and o-phenylenediamine dihydrochloride peroxidase substrate (Sigma) was added to each well. After incubation at RT for 30 min, A450 values of each well were read directly on a SpectraMaxPlus spectrophotometer with SoftMaxPro software (Molecular Devices).

[0204] The results of that experiment for cynomolgus CSF1R are shown in Figures 8A to 8C. All of the humanized antibodies based on Fabs 0301 and 0311 were able to block human CSF1 binding to cynomolgus CSF1R ECD within the range of concentrations tested. None of the humanized antibodies based on Fab 0302 showed similar blocking activity in that experiment compared to the blocking activity of cAb 0302.

Human IL34/CSF1R Blocking Activity

[0205] The humanized antibodies were tested for their ability to block human IL34 binding to human CSF1R ECD. The blocking activity of each humanized antibody was determined as described above for blocking of CSF1, except recombinant human IL34 (FivePrime Therapeutics; SEQ ID NO: 68) was biotinylated using an NH2-Biotin Labeling Kit (Dojindo Molecular Technologies), and then 100 µl of 1 µg/ml biotinylated recombinant IL34 in PBST/0.1% BSA was added to the wells of Reacti-Bind Streptavidin coated plates (Pierce) pre-blocked with SuperBlock blocking buffer (Pierce) according to the manufacturer's instructions.

[0206] The results of that experiment for cynomolgus CSF1R are shown in Figures 9A to 9C. All of the humanized antibodies based on Fabs 0301 and 0311 were able to block human IL34 binding to cynomolgus CSF1R ECD within the range of concentrations tested. None of the humanized antibodies based on Fab 0302 showed similar blocking activity in that experiment compared to the blocking activity of cAb 0302.

Calculation of IC50s

[0207] Table 4 shows the IC50, calculated using the non-linear regression (curve-fit) analysis algorithm of the GraphPad Prism software (GraphPad Software), for inhibition of ligand-induced CSF1R phosphorylation by each humanized antibody. Table 4 also shows the IC50, calculated using the non-linear regression (curve-fit) analysis algorithm of the GraphPad Prism software (GraphPad Software), for blocking of ligand binding to CSF1R ECD by each humanized antibody. Finally, Table 4 shows the number of amino acids in the framework regions of the light and heavy chain of each humanized antibody that were back-mutated to the corresponding mouse amino acid residue. For example, humanized antibody h0301L1H1 has one amino acid in a light chain framework region that was back-mutated to the mouse amino acid, and one amino acid in the heavy chain framework regions that was back-mutated to the mouse amino acid. Referring to Figures 1 and 2, the back-mutated amino acid in the light chain framework is at position 1 in framework 1, and the back-mutated amino acid in the heavy chain is at position 71 in framework 3 according to Kabat numbering (see Figure 1B).

Table 4: Blocking activity of humanized anti-CSF1R antibodies

Humanized Antibody	Human CSF1 / Human CSF1R ECD IC50 (ng/ml)	Human IL34 / Human CSF1R ECD IC50 (ng/ml)	Human CSF1 / CynoCSF1R ECD IC50 (ng/ml)	Human IL34 / CynoCSF1R ECD IC50 (ng/ml)	Back-mutated mouse residues in FRs (L+H)
cAb0301	307.2	312.2	22.01	29.53	
h0301-L0H0	1031	433	27.64	35.92	0 + 0
h0301-L0H1	778.1	452.6	27.45	36.43	0 + 1
h0301-L0H2	1317	480.9	28.05	37.37	0 + 4
h0301-L1H0	6150	378	25.53	34.84	1 + 0
h0301-L1H1	814.2	384.4	31.07	42.41	1 + 1
h0301-L1H2	682.1	397.1	27.77	36.53	1 +4
cAb0302	263.5	350.8	33.09	49.38	
h0302-L0H1	927.7	615	15.55	2.00E+12	0 + 2
h0302-L1H1	742	363.7	60.49	676.4	1 + 2
h0302-L2H1	384	303.1	89827	509.1	3 + 2
h0302-L0H2	438.2	474.2	none	248.1	0 + 5
h0302-L1H2	597.8	495.3	1085	541.3	1 + 5
h0302-L2H2	354.4	240.1	837.6	278.7	3 + 5
cAb 0311	577	994.2	43.47	52.1	

h0311-L0H1	291.3	343.2	32.47	50.4	0 + 2
h0311-L1H1	507.5	667.4	24.68	53.69	2 + 2
h0311-L0H2	435.5	633.3	25.96	40.79	0 + 5
h0311-L1H2	419	578.2	30.76	48.56	2 + 5

Example 8: Humanized anti-CSF1R antibody binding constants

[0208] The k_a, k_d, and K_D for binding to human CSF1R ECD was determined for each of the humanized antibodies as follows.

[0209] Binding kinetics of anti-CSF1R humanized antibodies to CSF1R ECD was determined using Biacore T100 Surface Plasmon Resononance (SPR) (GE Healthcare Life Sciences, Piscataway, NY). Each of the humanized anti-CSF1R antibodies was captured on a CM5 sensor chip immobilized with anti-Human IgG antibody using the Human antibody capture kit (GE Healthcare Life Sciences, Piscataway, NY) at 150RU so that the Rmax value for hCSF1R ECD.506 (SEQ ID NO: 5) binding was 100RU. Rmax values of less than 150RU are recommended for accurately determining kinetic values. 10 mM Hepes buffered saline, pH 7.4, with 0.05% Tween20 (HPS-P; GE Healthcare Life Sciences, Piscataway, NY) was used as the running and dilution buffer. hCSF1R ECD.506 was injected at six concentrations (90 nM, 30 nM, 10 nM, 3.33 nM, 1.11 nM, and 0 nM) for 2 minutes and dissociation was observed for 5 minutes to determine humanized antibody/hCSF1R ECD binding kinetic parameters. The association constant, dissociation constant, affinity, and binding capacity of each of the Fabs for human CSF1R ECD was calculated using the Biacore T100 Evaluation software package using the 1:1 binding model.

[0210] The results of the kinetic determinations are shown in Table 5.

Table 5: Humanised antibody binding affinity for human CSF1R

huAbAb	**k_a ($M^{-1}s^{-1}$)**	**K_d (s^{-1})**	**K_D (nM)**
huAb 0301-L0H0	3.22 x 10^6	1.11 x 10^{-03}	0.35
huAb 0301-L0H1	3.56 x 10^6	1.22 x 10^{-03}	0.34
huAb 0301-L0H2	2.32 x 10^6	6.60 x 10^{-04}	0.28
huAb 0301-L1H0	3.29 x 10^6	1.15 x 10^{-03}	0.35
huAb 0301-L1H1	2.87 x 10^6	9.21 x 10^{-04}	0.32
huAb 0301-L1H2	2.95 x 10^6	7.42 x 10^{-04}	0.25
huAb 0302-L0H1	3.54 x 10^6	3.69 x 10^{-03}	1.04
huAb 0302-L1H1	3.47 x 10^6	4.04 x 10^{-03}	1.17
huAb 0302-L2H1	1.60 x 10^6	9.14 x 10^{-04}	0.57

huAb 0302-L0H2	3.40 x 10^6	1.79 x 10^{-03}	0.53
huAb 0302-L1H2	2.71 x 10^6	1.53 x 10^{-03}	0.56
huAb 0302-L2H2	1.84 x 10^6	8.40 x 10^{-04}	0.46
huAb 0311-L0H1	1.22 x 10^6	5.40 x 10^{-04}	0.44
huAb 0311-L1H1	1.32 x 10^6	6.64 x 10^{-04}	0.50
huAb 0311-L0H2	1.34 x 10^6	4.73 x 10^{-04}	0.35
huAb 0311-L1H2	1.51 x 10^6	6.09 x 10^{-04}	0.40

[0211] All but two of the humanized antibodies showed sub-nanomolar binding affinities for human CSF1R ECD, and the remaining two humanized antibodies showed binding affinities for human CSF1R ECD of less than 2 nM.

Example 9: Humanized anti-CSF1R antibodies block ligand-induced phosphorylation

[0212] Based on the data above, including CSF1R binding and ligand inhibition, and the likelihood of immunogenicity for each humanized antibody, three humanized antibodies were selected for further study: 0301-L0H0, 0301-L1H0, and 0311-L0H1.

[0213] After confirming that 0301-L0H0, 0301-L1H0, and 0311-L0H1 each bind to CSF1R on the surface of cells (data not shown), each of the antibodies was tested for the ability to block ligand-induced CSF1R phosphorylation in CHO cells, as described in Example 6.

[0214] The results of that experiment are shown in Figure 10. All three of the humanized antibodies tested blocked both CSF1-induced (A) and IL34-induced (B) phosphorylation of CSF1R in CHO cells. Table 6 shows the IC50 for blocking of ligand-induced CSF1R phosphorylation for each antibody.

Table 6: Ligand-induced phosphorylation blocking IC50 for humanized antibodies

Humanized antibody	CSF1 blocking IC50 (ng/ml)	IL34 blocking IC50 (ng/ml)
0301-L0H0	305.4	340.8
0301-L1H0	213.2	242.2
0311-L0H1	127.2	337.6

Example 10: Humanized anti-CSF1R antibodies block ligand-induced proliferation/survival responses of primary human monocytes

[0215] Humanized antibodies 0301-L0H0, 0301-L1H0, and 0311-L0H1 were tested for their ability to block ligand-induced monocyte proliferation/survival responses as follows.

[0216] Human peripheral blood mononuclear cells (PBMCs) were isolated from healthy donor blood by centrifugation onto a Ficoll-Paque cushion (GE Healthcare Bio-Sciences) according to the manufacturer's instructions. Peripheral blood monocytes were subsequently isoated from the recovered PBMC fraction by centrifugation onto a 48.5% Percoll™ cushion (GE Healthcare Bio-Sciences). After recovery from the Percoll™ cushion, the purified peripheral blood monocytes were stimuated with 162 pM recombinant human CSF1 or 1.6 nM recombinant human IL34 (both from R&D Systems) in the presence or absence of serial dilutions of humanised antibody 0301-L0H0, humanised antibody 0301-L1H0, or humanised antibody 0311-L0H1. After incubation at 37°C for 48 hours, relative cellular ATP content of each individual culture was assessed using CellTiter-Glo® reagent (Promega) according to the manufacturer's instructions. In this assay, relative cellular ATP content is directly proportional to the number of viable cells in culture, and thus reflects monocyte proliferation/survival responses.

[0217] The results of that experiment are shown in Figure 11. All three of the humanized antibodies tested were able to block monocyte proliferation/survival responses following CSF1 (A) or IL34 (B) stimulation. Table 7 shows the IC50s for blocking of ligand-induced monocyte proliferation/survival responses for each antibody. The values shown in Table 7 represent the range observed from the three different primary donors tested.

Table 7: Monocyte proliferation/survival blocking IC50 for humanized antibodies

Humanized antibody	CSF1 blocking IC50 (ng/ml)	IL34 blocking IC50 (ng/ml)
0301-L0H0	31.9-77.5	12.2-29.9
0301-L1H0	19.0-71.9	10.5-30.6
0311-L0H1	75.9-134.8	26.9-152.2

Example 11: Humanized anti-CSF1R antibodies do not directly stimulate primary human monocyte proliferation or survival responses

[0218] Humanized antibodies 0301-L0H0, 0301-L1H0, and 0311-L0H1 were tested for their ability to directly stimulate primary monocyte proliferation and/or survival, as follows.

[0219] Human peripheral blood monocytes were isolated as described in Example 10. Serial dilutions of humanised antibody 0301-L0H0, humanised antibody 0301-L1H0, or humanised antibody 0311-L0H1 were added to the monocytes in the absence of stimulation either by exogenous CSF1 or by exogenous IL34. After

incubation at 37°C for 48 hours, relative ATP content of each individual cuture was assessed using CellTiter Glo® reagent (Promega) as in Example 10. The experiment was carried out on peripheral blood monocytes from three different donors.

[0220] The results of that experiment are shown in Figure 12. None of the humanized antibodies stimulated primary monocyte proliferation or survival in either of the primary monocyte preparations tested.

TABLE OF SEQUENCES

[0221] Table 8 provides certain sequences discussed herein. All polypeptide and antibody sequences are shown without leader sequences, unless otherwise indicated.

Table 8: Sequences and Descriptions

SEQ ID NO	Description	Sequence
1	hCSF1R (full-length, no leader sequence)	IPVIEPSVPE LVVKPGATVT LRCVGNGSVE WDGPPSPHWT LYSDGSSSIL STNNATFQNT GTYRCTEPGD PLGGSAAIHL YVKDPARPWN VLAQEVVVFE DQDALLPCLL TDPVLEAGVS LVRVRGRPLM RHTNYSFSPW HGFTIHRAKF IQSQDYQCSA LMGGRKVMSI SIRLKVQKVI PGPPALTLVP AELVRIRGEA AQIVCSASSV DVNFDVFLQH NNTKLAIPQQ SDFHNNRYQK VLTLNLDQVD FQHAGNYSCV ASNVQGKHST SMFFRVVESA YLNLSSEQNL IQEVTVGEGL NLKVMVEAYP GLQGFNWTYL GPFSDHQPEP KLANATTKDT YRHTFTLSLP RLKPSEAGRY SFLARNPGGW RALTFELTLR YPPEVSVIWT FINGSGTLLC AASGYPQPNV TWLQCSGHTD RCDEAQVLQV WDDPYPEVLS QEPFHKVTVQ SLLTVETLEH NQTYECRAHN SVGSGSWAFI PISAGAHTHP PDEFLFTPVV VACMSIMALL LLLLLLLLYK YKQKPKYQVR WKIIESYEGN SYTFIDPTQL PYNEKWEFPR NNLQFGKTLG AGAFGKVVEA TAFGLGKEDA VLKVAVKMLK STAHADEKEA LMSELKIMSH LGQHENIVNL LGACTHGGPV LVITEYCCYG DLLNFLRRKA EAMLGPSLSP GQDPEGGVDY KNIHLEKKYV RRDSGFSSQG VDTYVEMRPV STSSNDSFSE QDLDKEDGRP LELRDLLHFS SQVAQGMAFL ASKNCIHRDV AARNVLLTNG HVAKIGDFGL ARDIMNDSNY IVKGNARLPV KWMAPESIFD CVYTVQSDVW SYGILLWEIF SLGLNPYPGI LVNSKFYKLV KDGYQMAQPA FAPKNIYSIM QACWALEPTH RPTFQQICSF LQEQAQEDRR ERDYTNLPSS SRSGGSGSSS SELEEESSSE HLTCCEQGDI AQPLLQPNNY QFC
2	hCSF1R (full-length, + leader sequence)	MGPGVLLLLL VATAWHGQGI PVIEPSVPEL VVKPGATVTL RCVGNGSVEW DGPPSPHWTL YSDGSSSILS TNNATFQNTG TYRCTEPGDP LGGSAAIHLY VKDPARPWNV LAQEVVVFED QDALLPCLLT DPVLEAGVSL VRVRGRPLMR HTNYSFSPWH GFTIHRAKFI QSQDYQCSAL MGGRKVMSIS IRLKVQKVIP GPPALTLVPA ELVRIRGEAA QIVCSASSVD VNFDVFLQHN NTKLAIPQQS DFHNNRYQKV LTLNLDQVDF QHAGNYSCVA SNVQGKHSTS MFFRVVESAY LNLSSEQNLI QEVTVGEGLN LKVMVEAYPG LQGFNWTYLG PFSDHQPEPK LANATTKDTY RHTFTLSLPR LKPSEAGRYS FLARNPGGWR ALTFELTLRY PPEVSVIWTF INGSGTLLCA ASGYPQPNVT WLQCSGHTDR CDEAQVLQVW DDPYPEVLSQ EPFHKVTVQS LLTVETLEHN QTYECRAHNS VGSGSWAFIP ISAGAHTHPP DEFLFTPVVV ACMSIMALLL LLLLLLLYKY KQKPKYQVRW KIIESYEGNS YTFIDPTQLP YNEKWEFPRN NLQFGKTLGA GAFGKVVEAT AFGLGKEDAV LKVAVKMLKS TAHADEKEAL MSELKIMSHL GQHENIVNLL GACTHGGPVL VITEYCCYGD LLNFLRRKAE AMLGPSLSPG QDPEGGVDYK NIHLEKKYVR RDSGFSSQGV DTYVEMRPVS TSSNDSFSEQ DLDKEDGRPL

		ELRDLLHFSS QVAQGMAFLA SKNCIHRDVA ARNVLLTNGH VAKIGDFGLA RDIMNDSNYI VKGNARLPVK WMAPESIFDC VYTVQSDVWS YGILLWEIFS LGLNPYPGIL VNSKFYKLVK DGYQMAQPAF APKNIYSIMQ ACWALEPTHR PTFQQICSFL QEQAQEDRRE RDYTNLPSSS RSGGSGSSSS ELEEESSSEH LTCCEQGDIA QPLLQPNNYQ FC
5	hCSF1R ECD.506	IPVIEPSVPE LVVKPGATVT LRCVGNGSVE WDGPPSPHWT LYSDGSSSIL STNNATFQNT GTYRCTEPGD PLGGSAAIHL YVKDPARPWN VLAQEVVVFE DQDALLPCLL TDPVLEAGVS LVRVRGRPLM RHTNYSFSPW HGFTIHRAKF IQSQDYQCSA LMGGRKVMSI SIRLKVQKVI PGPPALTLVP AELVRIRGEA AQIVCSASSV DVNFDVFLQH NNTKLAIPQQ SDFHNNRYQK VLTLNLDQVD FQHAGNYSCV ASNVQGKHST SMFFRVVESA YLNLSSEQNL IQEVTVGEGL NLKVMVEAYP GLQGFNWTYL GPFSDHQPEP KLANATTKDT YRHTFTLSLP RLKPSEAGRY SFLARNPGGW RALTFELTLR YPPEVSVIWT FINGSGTLLC AASGYPQPNV TWLQCSGHTD RCDEAQVLQV WDDPYPEVLS QEPFHKVTVQ SLLTVETLEH NQTYECRAHN SVGSGSWAFI PISAGAH
6	hCSF1R ECD.506-Fc	IPVIEPSVPE LVVKPGATVT LRCVGNGSVE WDGPPSPHWT LYSDGSSSIL STNNATFQNT GTYRCTEPGD PLGGSAAIHL YVKDPARPWN VLAQEVVVFE DQDALLPCLL TDPVLEAGVS LVRVRGRPLM RHTNYSFSPW HGFTIHRAKF IQSQDYQCSA LMGGRKVMSI SIRLKVQKVI PGPPALTLVP AELVRIRGEA AQIVCSASSV DVNFDVFLQH NNTKLAIPQQ SDFHNNRYQK VLTLNLDQVD FQHAGNYSCV ASNVQGKHST SMFFRVVESA YLNLSSEQNL IQEVTVGEGL NLKVMVEAYP GLQGFNWTYL GPFSDHQPEP KLANATTKDT YRHTFTLSLP RLKPSEAGRY SFLARNPGGW RALTFELTLR YPPEVSVIWT FINGSGTLLC AASGYPQPNV TWLQCSGHTD RCDEAQVLQV WDDPYPEVLS QEPFHKVTVQ SLLTVETLEH NQTYECRAHN SVGSGSWAFI PISAGAHEPK SSDKTHTCPP CPAPELLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK TKPREEQYNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL PAPIEKTISK AKGQPREPQV YTLPPSRDEL TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ KSLSLSPGK
7	cynoCSF1R ECD (with leader sequence)	MGPGVLLLLL VVTAWHGQGI PVIEPSGPEL VVKPGETVTL RCVGNGSVEW DGPISPHWTL YSDGPSSVLT TTNATFQNTR TYRCTEPGDP LGGSAAIHLY VKDPARPWNV LAKEVVVFED QDALLPCLLT DPVLEAGVSL VRLRGRPLLR HTNYSFSPWH GFTIHRAKFI QGQDYQCSAL MGSRKVMSIS IRLKVQKVIP GPPALTLVPA ELVRIRGEAA QIVCSASNID VDFDVFLQHN TTKLAIPQRS DFHDNRYQKV LTLSLGQVDF QHAGNYSCVA SNVQGKHSTS MFFRVVESAY LDLSSEQNLI QEVTVGEGLN LKVMVEAYPG LQGFNWTYLG PFSDHQPEPK LANATTKDTY RHTFTLSLPR LKPSEAGRYS FLARNPGGWR ALTFELTLRY PPEVSVIWTS INGSGTLLCA ASGYPQPNVT WLQCAGHTDR CDEAQVLQVW VDPHPEVLSQ EPFQKVTVQS LLTAETLEHN QTYECRAHNS VGSGSWAFIP ISAGAR
8	cynoCSF1R ECD-Fc (with leader sequence)	MGPGVLLLLL VVTAWHGQGI PVIEPSGPEL VVKPGETVTL RCVGNGSVEW DGPISPHWTL YSDGPSSVLT TTNATFQNTR TYRCTEPGDP LGGSAAIHLY VKDPARPWNV LAKEVVVFED QDALLPCLLT DPVLEAGVSL VRLRGRPLLR HTNYSFSPWH GFTIHRAKFI QGQDYQCSAL MGSRKVMSIS IRLKVQKVIP GPPALTLVPA ELVRIRGEAA QIVCSASNID VDFDVFLQHN TTKLAIPQRS DFHDNRYQKV LTLSLGQVDF QHAGNYSCVA SNVQGKHSTS MFFRVVESAY LDLSSEQNLI QEVTVGEGLN LKVMVEAYPG LQGFNWTYLG PFSDHQPEPK LANATTKDTY RHTFTLSLPR LKPSEAGRYS FLARNPGGWR ALTFELTLRY PPEVSVIWTS INGSGTLLCA ASGYPQPNVT WLQCAGHTDR CDEAQVLQVW VDPHPEVLSQ EPFQKVTVQS LLTAETLEHN QTYECRAHNS VGSGSWAFIP ISAGARGSEP KSSDKTHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSRDE LTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK

3	Light chain leader sequence	METDTLLLWV LLLWVPGSTG
4	Heavy chain leader sequence	MAVLGLLLCL VTFPSCVLS
9	Fab 0301 heavy chain variable region	EVQLQQSGPE LVRPGASVKM SCKASGYTFT DNYMIWVKQS HGKSLEWIGD INPYNGGTTF NQKFKGKATL TVEKSSSTAY MQLNSLTSED SAVYYCARES PYFSNLYVMD YWGQGTSVTV SS
10	Fab 0301 light chain variable region	NIVLTQSPAS LAVSLGQRAT ISCKASQSVD YDGDNYMNWY QQKPGQPPKL LIYAASNLES GIPARFSGSG SGTDFTLNIH PVEEEDAATY YCHLSNEDLS TFGGGTKLEI K
11	Fab 0302 heavy chain variable region	EIQLQQSGPE LVKPGASVKM SCKASGYTFS DFNIHWVKQK PGQGLEWIGY INPYTDVTVY NEKFKGKATL TSDRSSSTAY MDLSSLTSED SAVYYCASYF DGTFDYALDY WGQGTSITVS S
12	Fab 0302 light chain variable region	DVVVTQTPAS LAVSLGQRAT ISCRASESVD NYGLSFMNWF QQKPGQPPKL LIYTASNLES GIPARFSGGG SRTDFTLTID PVEADDAATY FCQQSKELPW TFGGGTRLEI K
13	Fab 0311 heavy chain variable region	EIQLQQSGPD LMKPGASVKM SCKASGYIFT DYNMHWVKQN QGKSLEWMGE INPNNGVVVY NQKFKGTTTL TVDKSSSTAY MDLHSLTSED SAVYYCTRAL YHSNFGWYFD SWGKGTTLTV SS
14	Fab 0311 light chain variable region	DIVLTQSPAS LAVSLGQRAT ISCKASQSVD YDGDSHMNWY QQKPGQPPKL LIYTASNLES GIPARFSGSG SGADFTLTIH PVEEEDAATY YCQQGNEDPW TFGGGTRLEI K
15	0301 heavy chain CDR1	GYTFTDNYMI
16	0301 heavy chain CDR2	DINPYNGGTT FNQKFKG
17	0301 heavy chain CDR3	ESPYFSNLYV MDY
18	0301 light chain CDR1	KASQSVDYDG DNYMN
19	0301 light chain CDR2	AASNLES
20	0301 light chain CDR3	HLSNEDLST
21	0302 heavy chain CDR1	GYTFSDFNIH
22	0302 heavy chain CDR2	YINPYTDVTV YNEKFKG
23	0302 heavy chain CDR3	YFDGTFDYAL DY
24	0302 light chain CDR1	RASESVDNYG LSFMN

25	0302 light chain CDR2	TASNLES
26	0302 light chain CDR3	QQSKELPWT
27	0311 heavy chain CDR1	GYIFTDYNMH
28	0311 heavy chain CDR2	EINPNNGVVV YNQKFKG
29	0311 heavy chain CDR3	ALYHSNFGWY FDS
30	0311 light chain CDR1	KASQSVDYDG DSHMN
31	0311 light chain CDR2	TASNLES
32	0311 light chain CDR3	QQGNEDPWT
33	cAb 0301 heavy chain	EVQLQQSGPE LVRPGASVKM SCKASGYTFT DNYMIWVKQS HGKSLEWIGD INPYNGGTTF NQKFKGKATL TVEKSSSTAY MQLNSLTSED SAVYYCARES PYFSNLYVMD YWGQGTSVTV SSASTKGPSV FPLAPCSRST SESTAALGCL VKDYFPEPVT VSWNSGALTS GVHTFPAVLQ SSGLYSLSSV VTVPSSSLGT KTYTCNVDHK PSNTKVDKRV ESKYGPPCPP CPAPEFLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSQ EDPEVQFNWY VDGVEVHNAK TKPREEQFNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKGL PSSIEKTISK AKGQPREPQV YTLPPSQEEM TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL DSDGSFFLYS RLTVDKSRWQ EGNVFSCSVM HEALHNHYTQ KSLSLSLGK
34	cAb 0301 light chain	NIVLTQSPAS LAVSLGQRAT ISCKASQSVD YDGDNYMNWY QQKPGQPPKL LIYAASNLES GIPARFSGSG SGTDFTLNIH PVEEEDAATY YCHLSNEDLS TFGGGTKLEI KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAKV QWKVDNALQS GNSQESVTEQ DSKDSTYSLS STLTLSKADY EKHKVYACEV THQGLSSPVT KSFNRGEC
35	cAb 0302 heavy chain	EIQLQQSGPE LVKPGASVKM SCKASGYTFS DFNIHWVKQK PGQGLEWIGY INPYTDVTVY NEKFKGKATL TSDRSSSTAY MDLSSLTSED SAVYYCASYF DGTFDYALDY WGQGTSITVS SASTKGPSVF PLAPCSRSTS ESTAALGCLV KDYFPEPVTV SWNSGALTSG VHTFPAVLQS SGLYSLSSVV TVPSSSLGTK TYTCNVDHKP SNTKVDKRVE SKYGPPCPPC PAPEFLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSQE DPEVQFNWYV DGVEVHNAKT KPREEQFNST YRVVSVLTVL HQDWLNGKEY KCKVSNKGLP SSIEKTISKA KGQPREPQVY TLPPSQEEMT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPVLD SDGSFFLYSR LTVDKSRWQE GNVFSCSVMH EALHNHYTQK SLSLSLGK
36	cAb 0302 light chain	DVVVTQTPAS LAVSLGQRAT ISCRASESVD NYGLSFMNWF QQKPGQPPKL LIYTASNLES GIPARFSGGG SRTDFTLTID PVEADDAATY FCQQSKELPW TFGGGTRLEI KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAKV QWKVDNALQS GNSQESVTEQ DSKDSTYSLS STLTLSKADY EKHKVYACEV THQGLSSPVT KSFNRGEC
37	cAb 0311 heavy chain	EIQLQQSGPD LMKPGASVKM SCKASGYIFT DYNMHWVKQN QGKSLEWMGE INPNNGVVVY NQKFKGTTTL TVDKSSSTAY MDLHSLTSED SAVYYCTRAL YHSNFGWYFD SWGKGTTLTV SSASTKGPSV FPLAPCSRST SESTAALGCL VKDYFPEPVT VSWNSGALTS GVHTFPAVLQ SSGLYSLSSV VTVPSSSLGT KTYTCNVDHK PSNTKVDKRV ESKYGPPCPP CPAPEFLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSQ EDPEVQFNWY VDGVEVHNAK TKPREEQFNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKGL PSSIEKTISK AKGQPREPQV YTLPPSQEEM TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL DSDGSFFLYS RLTVDKSRWQ EGNVFSCSVM HEALHNHYTQ KSLSLSLGK

38	cAb 0311 light chain	DIVLTQSPAS LAVSLGQRAT ISCKASQSVD YDGDSHMNWY QQKPGQPPKL LIYTASNLES GIPARFSGSG SGADFTLTIH PVEEEDAATY YCQQGNEDPW TFGGGTRLEI KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAKV QWKVDNALQS GNSQESVTEQ DSKDSTYSLS STLTLSKADY EKHKVYACEV THQGLSSPVT KSFNRGEC
39	h0301-H0 heavy chain variable region	QVQLVQSGAE VKKPGSSVKV SCKASGYTFT DNYMIWVRQA PGQGLEWMGD INPYNGGTTF NQKFKGRVTI TADKSTSTAY MELSSLRSED TAVYYCARES PYFSNLYVMD YWGQGTLVTV SS
40	h0301-H1 heavy chain variable region	QVQLVQSGAE VKKPGSSVKV SCKASGYTFT DNYMIWVRQA PGQGLEWMGD INPYNGGTTF NQKFKGRVTI TVDKSTSTAY MELSSLRSED TAVYYCARES PYFSNLYVMD YWGQGTLVTV SS
41	h0301-H2 heavy chain variable region	QVQLVQSGAE VKKPGSSVKV SCKASGYTFT DNYMIWVRQA PGQGLEWIGD INPYNGGTTF NQKFKGRATL TVDKSTSTAY MELSSLRSED TAVYYCARES PYFSNLYVMD YWGQGTLVTV SS
42	H0302-H1 heavy chain variable region	QVQLVQSGAE VKKPGSSVKV SCKASGYTFS DFNIHWVRQA PGQGLEWMGY INPYTDVTVY NEKFKGRVTI TSDKSTSTAY MELSSLRSED TAVYYCASYF DGTFDYALDY WGQGTLVTVS S
43	H0302-H2 heavy chain variable region	QVQLVQSGAE VKKPGSSVKV SCKASGYTFS DFNIHWVRQA PGQGLEWIGY INPYTDVTVY NEKFKGRATL TSDKSTSTAY MELSSLRSED TAVYYCASYF DGTFDYALDY WGQGTLVTVS S
44	H0311-H1 heavy chain variable region	QVQLVQSGAE VKKPGSSVKV SCKASGYIFT DYNMHWVRQA PGQGLEWMGE INPNNGVVVY NQKFKGRVTI TVDKSTSTAY MELSSLRSED TAVYYCTRAL YHSNFGWYFD SWGQGTLVTV SS
45	H0311-H2 heavy chain variable region	QVQLVQSGAE VKKPGSSVKV SCKASGYIFT DYNMHWVRQA PGQGLEWMGE INPNNGVVVY NQKFKGTTTL TVDKSTSTAY MELSSLRSED TAVYYCTRAL YHSNFGWYFD SWGQGTLVTV SS
46	h0301-L0 light chain variable region	EIVLTQSPAT LSLSPGERAT LSCKASQSVD YDGDNYMNWY QQKPGQAPRL LIYAASNLES GIPARFSGSG SGTDFTLTIS SLEPEDFAVY YCHLSNEDLS TFGGGTKVEI K
47	h0301-L1 light chain variable region	NIVLTQSPAT LSLSPGERAT LSCKASQSVD YDGDNYMNWY QQKPGQAPRL LIYAASNLES GIPARFSGSG SGTDFTLTIS SLEPEDFAVY YCHLSNEDLS TFGGGTKVEI K
48	H0302-L0 light chain variable region	EIVLTQSPAT LSLSPGERAT LSCRASESVD NYGLSFMNWY QQKPGQAPRL LIYTASNLES GIPARFSGSG SGTDFTLTIS SLEPEDFAVY YCQQSKELPW TFGQGTKVEI K
49	H0302-L1 light chain variable region	EIVLTQSPAT LSLSPGERAT LSCRASESVD NYGLSFMNWY QQKPGQAPRL LIYTASNLES GIPARFSGSG SRTDFTLTIS SLEPEDFAVY YCQQSKELPW TFGQGTKVEI K
50	H0302-L2 light chain variable region	EIVVTQSPAT LSLSPGERAT LSCRASESVD NYGLSFMNWF QQKPGQAPRL LIYTASNLES GIPARFSGSG SRTDFTLTIS SLEPEDFAVY YCQQSKELPW TFGQGTKVEI K

51	H0311-L0 light chain variable region	EIVLTQSPAT LSLSPGERAT LSCKASQSVD YDGDSHMNWY QQKPGQAPRL LIYTASNLES GIPARFSGSG SGTDFTLTIS SLEPEDFAVY YCQQGNEDPW TFGQGTKVEI K
52	H0311-L1 light chain variable region	DIVLTQSPAT LSLSPGERAT LSCKASQSVD YDGDSHMNWY QQKPGQAPRL LIYTASNLES GIPARFSGSG SGADFTLTIS SLEPEDFAVY YCQQGNEDPW TFGQGTKVEI K
53	h0301-H0 heavy chain	QVQLVQSGAE VKKPGSSVKV SCKASGYTFT DNYMIWVRQA PGQGLEWMGD INPYNGGTTF NQKFKGRVTI TADKSTSTAY MELSSLRSED TAVYYCARES PYFSNLYVMD YWGQGTLVTV SSASTKGPSV FPLAPCSRST SESTAALGCL VKDYFPEPVT VSWNSGALTS GVHTFPAVLQ SSGLYSLSSV VTVPSSSLGT KTYTCNVDHK PSNTKVDKRV ESKYGPPCPP CPAPEFLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSQ EDPEVQFNWY VDGVEVHNAK TKPREEQFNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKGL PSSIEKTISK AKGQPREPQV YTLPPSQEEM TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL DSDGSFFLYS RLTVDKSRWQ EGNVFSCSVM HEALHNHYTQ KSLSLSLGK
54	h0301-H1 heavy chain	QVQLVQSGAE VKKPGSSVKV SCKASGYTFT DNYMIWVRQA PGQGLEWMGD INPYNGGTTF NQKFKGRVTI TVDKSTSTAY MELSSLRSED TAVYYCARES PYFSNLYVMD YWGQGTLVTV SSASTKGPSV FPLAPCSRST SESTAALGCL VKDYFPEPVT VSWNSGALTS GVHTFPAVLQ SSGLYSLSSV VTVPSSSLGT KTYTCNVDHK PSNTKVDKRV ESKYGPPCPP CPAPEFLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSQ EDPEVQFNWY VDGVEVHNAK TKPREEQFNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKGL PSSIEKTISK AKGQPREPQV YTLPPSQEEM TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL DSDGSFFLYS RLTVDKSRWQ EGNVFSCSVM HEALHNHYTQ KSLSLSLGK
55	h0301-H2 heavy chain	QVQLVQSGAE VKKPGSSVKV SCKASGYTFT DNYMIWVRQA PGQGLEWIGD INPYNGGTTF NQKFKGRATL TVDKSTSTAY MELSSLRSED TAVYYCARES PYFSNLYVMD YWGQGTLVTV SSASTKGPSV FPLAPCSRST SESTAALGCL VKDYFPEPVT VSWNSGALTS GVHTFPAVLQ SSGLYSLSSV VTVPSSSLGT KTYTCNVDHK PSNTKVDKRV ESKYGPPCPP CPAPEFLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSQ EDPEVQFNWY VDGVEVHNAK TKPREEQFNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKGL PSSIEKTISK AKGQPREPQV YTLPPSQEEM TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL DSDGSFFLYS RLTVDKSRWQ EGNVFSCSVM HEALHNHYTQ KSLSLSLGK
56	H0302-H1 heavy chain	QVQLVQSGAE VKKPGSSVKV SCKASGYTFS DFNIHWVRQA PGQGLEWMGY INPYTDVTVY NEKFKGRVTI TSDKSTSTAY MELSSLRSED TAVYYCASYF DGTFDYALDY WGQGTLVTVS SASTKGPSVF PLAPCSRSTS ESTAALGCLV KDYFPEPVTV SWNSGALTSG VHTFPAVLQS SGLYSLSSVV TVPSSSLGTK TYTCNVDHKP SNTKVDKRVE SKYGPPCPPC PAPEFLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSQE DPEVQFNWYV DGVEVHNAKT KPREEQFNST YRVVSVLTVL HQDWLNGKEY KCKVSNKGLP SSIEKTISKA KGQPREPQVY TLPPSQEEMT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPVLD SDGSFFLYSR LTVDKSRWQE GNVFSCSVMH EALHNHYTQK SLSLSLGK
57	H0302-H2 heavy chain	QVQLVQSGAE VKKPGSSVKV SCKASGYTFS DFNIHWVRQA PGQGLEWIGY INPYTDVTVY NEKFKGRATL TSDKSTSTAY MELSSLRSED TAVYYCASYF DGTFDYALDY WGQGTLVTVS SASTKGPSVF PLAPCSRSTS ESTAALGCLV KDYFPEPVTV SWNSGALTSG VHTFPAVLQS SGLYSLSSVV TVPSSSLGTK TYTCNVDHKP SNTKVDKRVE SKYGPPCPPC PAPEFLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSQE DPEVQFNWYV DGVEVHNAKT KPREEQFNST YRVVSVLTVL HQDWLNGKEY KCKVSNKGLP SSIEKTISKA KGQPREPQVY TLPPSQEEMT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPVLD SDGSFFLYSR LTVDKSRWQE GNVFSCSVMH EALHNHYTQK SLSLSLGK
58	H0311-H1 heavy chain	QVQLVQSGAE VKKPGSSVKV SCKASGYIFT DYNMHWVRQA PGQGLEWMGE INPNNGVVVY NQKFKGRVTI TVDKSTSTAY MELSSLRSED TAVYYCTRAL YHSNFGWYFD SWGQGTLVTV SSASTKGPSV FPLAPCSRST SESTAALGCL VKDYFPEPVT VSWNSGALTS GVHTFPAVLQ SSGLYSLSSV VTVPSSSLGT

		KTYTCNVDHK PSNTKVDKRV ESKYGPPCPP CPAPEFLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSQ EDPEVQFNWY VDGVEVHNAK TKPREEQFNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKGL PSSIEKTISK AKGQPREPQV YTLPPSQEEM TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL DSDGSFFLYS RLTVDKSRWQ EGNVFSCSVM HEALHNHYTQ KSLSLSLGK
59	H0311-H2 heavy chain	QVQLVQSGAE VKKPGSSVKV SCKASGYIFT DYNMHWVRQA PGQGLEWMGE INPNNGVVVY NQKFKGTTTL TVDKSTSTAY MELSSLRSED TAVYYCTRAL YHSNFGWYFD SWGQGTLVTV SSASTKGPSV FPLAPCSRST SESTAALGCL VKDYFPEPVT VSWNSGALTS GVHTFPAVLQ SSGLYSLSSV VTVPSSSLGT KTYTCNVDHK PSNTKVDKRV ESKYGPPCPP CPAPEFLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSQ EDPEVQFNWY VDGVEVHNAK TKPREEQFNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKGL PSSIEKTISK AKGQPREPQV YTLPPSQEEM TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL DSDGSFFLYS RLTVDKSRWQ EGNVFSCSVM HEALHNHYTQ KSLSLSLGK
60	h0301-L0 light chain	EIVLTQSPAT LSLSPGERAT LSCKASQSVD YDGDNYMNWY QQKPGQAPRL LIYAASNLES GIPARFSGSG SGTDFTLTIS SLEPEDFAVY YCHLSNEDLS TFGGGTKVEI KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAKV QWKVDNALQS GNSQESVTEQ DSKDSTYSLS STLTLSKADY EKHKVYACEV THQGLSSPVT KSFNRGEC
61	h0301-L1 light chain	NIVLTQSPAT LSLSPGERAT LSCKASQSVD YDGDNYMNWY QQKPGQAPRL LIYAASNLES GIPARFSGSG SGTDFTLTIS SLEPEDFAVY YCHLSNEDLS TFGGGTKVEI KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAKV QWKVDNALQS GNSQESVTEQ DSKDSTYSLS STLTLSKADY EKHKVYACEV THQGLSSPVT KSFNRGEC
62	H0302-L0 light chain	EIVLTQSPAT LSLSPGERAT LSCRASESVD NYGLSFMNWY QQKPGQAPRL LIYTASNLES GIPARFSGSG SGTDFTLTIS SLEPEDFAVY YCQQSKELPW TFGQGTKVEI KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAKV QWKVDNALQS GNSQESVTEQ DSKDSTYSLS STLTLSKADY EKHKVYACEV THQGLSSPVT KSFNRGEC
63	H0302-L1 light chain	EIVLTQSPAT LSLSPGERAT LSCRASESVD NYGLSFMNWY QQKPGQAPRL LIYTASNLES GIPARFSGSG SRTDFTLTIS SLEPEDFAVY YCQQSKELPW TFGQGTKVEI KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAKV QWKVDNALQS GNSQESVTEQ DSKDSTYSLS STLTLSKADY EKHKVYACEV THQGLSSPVT KSFNRGEC
64	H0302-L2 light chain	EIVVTQSPAT LSLSPGERAT LSCRASESVD NYGLSFMNWF QQKPGQAPRL LIYTASNLES GIPARFSGSG SRTDFTLTIS SLEPEDFAVY YCQQSKELPW TFGQGTKVEI KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAKV QWKVDNALQS GNSQESVTEQ DSKDSTYSLS STLTLSKADY EKHKVYACEV THQGLSSPVT KSFNRGEC
65	H0311-L0 light chain	EIVLTQSPAT LSLSPGERAT LSCKASQSVD YDGDSHMNWY QQKPGQAPRL LIYTASNLES GIPARFSGSG SGTDFTLTIS SLEPEDFAVY YCQQGNEDPW TFGQGTKVEI KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAKV QWKVDNALQS GNSQESVTEQ DSKDSTYSLS STLTLSKADY EKHKVYACEV THQGLSSPVT KSFNRGEC
66	H0311-L1 light chain	DIVLTQSPAT LSLSPGERAT LSCKASQSVD YDGDSHMNWY QQKPGQAPRL LIYTASNLES GIPARFSGSG SGADFTLTIS SLEPEDFAVY YCQQGNEDPW TFGQGTKVEI KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAKV QWKVDNALQS GNSQESVTEQ DSKDSTYSLS STLTLSKADY EKHKVYACEV THQGLSSPVT KSFNRGEC
67	Human CSF1	EEVSEYCSHM IGSGHLQSLQ RLIDSQMETS CQITFEFVDQ EQLKDPVCYL KKAFLLVQDI MEDTMRFRDN TPNAIAIVQL QELSLRLKSC FTKDYEEHDK ACVRTFYETP LQLLEKVKNV FNETKNLLDK DWNIFSKNCN NSFAECSSQG HERQSEGS
68	Human IL34	NEPLEMWPLT QNEECTVTGF LRDKLQYRSR LQYMKHYFPI NYKISVPYEG VFRIANVTRL QRAQVSEREL RYLWVLVSLSATESVQDVLL EGHPSWKYLQ

		EVQTLLLNVQ QGLTDVEVSP KVESVLSLLN APGPNLKLVR PKALLDNCFR VMELLYCSCC KQSSVLNWQD CEVPSPQSCS PEPSLQYAAT QLYPPPPWSP SSPPHSTGSV RPVRAQGEGL LP
69	Human acceptor A FR1	QVQLVQSGAE VKKPGSSVKV SCKAS
70	Human acceptor A FR2	WVRQAPGQGL EWMG
71	Human acceptor A FR3	RVTITADKST STAYMELSSL RSEDTAVYYC AR
72	Human acceptor A FR4	WGQGTLVTVS S
73	Human acceptor B FR1	QVQLVQSGAE VKKPGSSVKV SCKAS
74	Human acceptor B FR2	WVRQAPGQGL EWMG
75	Human acceptor B FR3	RVTITADKST STAYMELSSL RSEDTAVYYC AR
76	Human acceptor B FR4	WGQGTLVTVSS
77	Human acceptor C FR1	QVQLVQSGAE VKKPGSSVKV SCKAS
78	Human acceptor C FR2	WVRQAPGQGL EWMG
79	Human acceptor C FR3	RVTITADKST STAYMELSSL RSEDTAVYYC AR
80	Human acceptor C FR4	WGQGTLVTVS S
81	Human acceptor D FR1	EIVLTQSPAT LSLSPGERAT LSC
82	Human acceptor D FR2	WYQQKPGQAP RLLIY
83	Human acceptor D FR3	GIPARFSGSG SGTDFTLTIS SLEPEDFAVY YC
84	Human acceptor D FR4	FGGGTKVEIK

85	Human acceptor E FR1	EIVLTQSPAT LSLSPGERAT LSC
86	Human acceptor E FR2	WYQQKPGQAP RLLIY
87	Human acceptor E FR3	GIPARFSGSG SGTDFTLTIS SLEPEDFAVY YC
88	Human acceptor E FR4	FGQGTKVEIK
89	Human acceptor F FR1	EIVLTQSPAT LSLSPGERAT LSC
90	Human acceptor F FR2	WYQQKPGQAP RLLIY
91	Human acceptor F FR3	GIPARFSGSG SGTDFTLTIS SLEPEDFAVY YC
92	Human acceptor F FR4	FGQGTKVEIK
93	mCSF1R ECD-Fc	APVIEPSGPE LVVEPGETVT LRCVSNGSVE WDGPISPYWT LDPESPGSTL TTRNATFKNT GTYRCTELED PMAGSTTIHL YVKDPAHSWN LLAQEVTVVE GQEAVLPCLI TDPALKDSVS LMREGGRQVL RKTVYFFSPW RGFIIRKAKV LDSNTYVCKT MVNGRESTST GIWLKVNRVH PEPPQIKLEP SKLVRIRGEA AQIVCSATNA EVGFNVILKR GDTKLEIPLN SDFQDNYYKK VRALSLNAVD FQDAGIYSCV ASNDVGTRTA TMNFQVVESA YLNLTSEQSL LQEVSVGDSL ILTVHADAYP SIQHYNWTYL GPFFEDQRKL EFITQRAIYR YTFKLFLNRV KASEAGQYFL MAQNKAGWNN LTFELTLRYP PEVSVTWMPV NGSDVLFCDV SGYPQPSVTW MECRGHTDRC DEAQALQVWN DTHPEVLSQK PFDKVIIQSQ LPIGTLKHNM TYFCKTHNSV GNSSQYFRAV SLGQSKQEPK SSDKTHTCPP CPAPELLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK TKPREEQYNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL PAPIEKTISK AKGQPREPQV YTLPPSRDEL TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ KSLSLSPGK
94	Human IgG4 S241P	ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTKT YTCNVDHKPS NTKVDKRVES KYGPPCPPCP APEFLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSQED PEVQFNWYVD GVEVHNAKTK PREEQFNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKGLPS SIEKTISKAK GQPREPQVYT LPPSQEEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSRL TVDKSRWQEG NVFSCSVMHE ALHNHYTQKS LSLSLGK
95	Human Igκ	RTVAAPSVFI FPPSDEQLKS GTASVVCLLN NFYPREAKVQ WKVDNALQSG NSQESVTEQD SKDSTYSLSS TLTLSKADYE KHKVYACEVT HQGLSSPVTK SFNRGEC

CLAIMS

1. An isolated antibody comprising a heavy chain and a light chain, wherein the heavy chain comprises a sequence that is at least 90% identical to a sequence selected from SEQ ID NOs: 9, 11, 13, and 39 to 45, and wherein the antibody binds to colony stimulating factor 1 receptor (CSF1R).

2. The antibody of claim 1, wherein the heavy chain comprises a sequence that is at least 95% identical to a sequence selected from SEQ ID NOs: 9, 11, 13, and 39 to 45.

3. The antibody of claim 1, wherein the heavy chain comprises a sequence that is at least 97% identical to a sequence selected from SEQ ID NOs: 9, 11, 13, and 39 to 45.

4. The antibody of claim 1, wherein the heavy chain comprises a sequence that is at least 99% identical to a sequence selected from SEQ ID NOs: 9, 11, 13, and 39 to 45.

5. The antibody of claim 1, wherein the heavy chain comprises a sequence selected from SEQ ID NOs: 9, 11, 13, and 39 to 45.

6. The antibody of any one of claims 1 to 5, wherein the light chain comprises a sequence that is at least 90% identical to a sequence selected from SEQ ID NOs: 10, 12, 14, and 46 to 52.

7. The antibody of any one of claims 1 to 5, wherein the light chain comprises a sequence that is at least 95% identical to a sequence selected from SEQ ID NOs: 10, 12, 14, and 46 to 52.

8. The antibody of any one of claims 1 to 5, wherein the light chain comprises a sequence that is at least 97% identical to a sequence selected from SEQ ID NOs: 10, 12, 14, and 46 to 52.

9. The antibody of any one of claims 1 to 5, wherein the light chain comprises a sequence that is at least 99% identical to a sequence selected from SEQ ID NOs: 10, 12, 14, and 46 to 52.

10. The antibody of any one of claims 1 to 5, wherein the light chain comprises a sequence selected from SEQ ID NOs: 10, 12, 14, and 46 to 52.

11. An isolated antibody comprising a heavy chain and a light chain, wherein the light chain comprises a sequence that is at least 90% identical to a sequence selected from SEQ ID NOs: 10, 12, 14, and 46 to 52, and wherein the antibody binds to colony stimulating factor 1 receptor (CSF1R).

12. The antibody of claim 11, wherein the light chain comprises a sequence that is at least 95% identical to a sequence selected from SEQ ID NOs: 10, 12, 14, and 46 to 52.

13. The antibody of claim 11, wherein the light chain comprises a sequence that is at least 97% identical to a sequence selected from SEQ ID NOs: 10, 12, 14, and 46 to 52.

14. The antibody of claim 11, wherein the light chain comprises a sequence that is at least 99% identical to a sequence selected from SEQ ID NOs: 10, 12, 14, and 46 to 52.

15. The antibody of claim 11, wherein the light chain comprises a sequence selected from SEQ ID NOs: 10, 12, 14, and 46 to 52.

16. An isolated antibody comprising a heavy chain and a light chain, wherein the heavy chain comprises: (i) a heavy chain (HC) CDR1 comprising a sequence selected from SEQ ID NOs: 15, 21, and 27; (ii) an HC CDR2 comprising a sequence selected from SEQ ID NOs: 16, 22, and 28; and (iii) an HC CDR3 comprising a sequence selected from SEQ ID NOs: 17, 23, and 29, wherein the antibody binds to CSF1R.

17. The antibody of claim 16, wherein the HC CDR1, HC CDR2, and HC CDR3 comprise a set of sequences selected from: (a) SEQ ID NOs: 15, 16, and 17; (b) SEQ ID NOs: 21, 22, and 23; and (c) SEQ ID NOs: 27, 28, and 29.

18. The antibody of claim 16 or claim 17, wherein the light chain comprises (i) a light chain (LC) CDR1 comprising a sequence selected from SEQ ID NOs: 18, 24, and 30; (ii) an LC CDR2 comprising a sequence selected from SEQ ID NOs: 19, 25, and 31; and (iii) an LC CDR3 comprising a sequence selected from SEQ ID NOs: 20, 26, and 32.

19. The antibody of claim 18, wherein the LC CDR1, LC CDR2, and LC CDR3 comprise a set of sequences selected from: (a) SEQ ID NOs: 18, 19, and 20; (b) SEQ ID NOs: 24, 25, and 26; and (c) SEQ ID NOs: 30, 31, and 32.

20. An isolated antibody comprising a heavy chain and a light chain, wherein the light chain comprises: (i) a light chain (LC) CDR1 comprising a sequence selected from SEQ ID NOs: 18, 24, and 30; (ii) an LC CDR2 comprising a sequence selected from SEQ ID NOs: 19, 25, and 31; and (iii) an LC CDR3 comprising a sequence selected from SEQ ID NOs: 20, 26, and 32; and wherein the antibody binds to CSF1R.

21. The antibody of claim 20, wherein the LC CDR1, LC CDR2, and LC CDR3 comprise a set of sequences selected from: (a) SEQ ID NOs: 18, 19, and 20; (b) SEQ ID NOs: 24, 25, and 26; and (c) SEQ ID NOs: 30, 31, and 32.

22. An isolated antibody comprising a heavy chain and a light chain, wherein the antibody comprises:

(a) a heavy chain comprising a sequence that is at least 95% identical to SEQ ID NO: 9 and a light chain comprising a sequence that is at least 95% identical to SEQ ID NO: 10;

(b) a heavy chain comprising a sequence that is at least 95% identical to SEQ ID NO: 11 and a light chain comprising a sequence that is at least 95% identical to SEQ ID NO: 12;

(c) a heavy chain comprising a sequence that is at least 95% identical to SEQ ID NO: 13 and a light chain comprising a sequence that is at least 95% identical to SEQ ID NO: 14;

(d) a heavy chain comprising a sequence that is at least 95% identical to SEQ ID NO: 39 and a light chain comprising a sequence that is at least 95% identical to SEQ ID NO: 46;

(e) a heavy chain comprising a sequence that is at least 95% identical to SEQ ID NO: 40 and a light chain comprising a sequence that is at least 95% identical to SEQ ID NO: 46;

(f) a heavy chain comprising a sequence that is at least 95% identical to SEQ ID NO: 41 and a light chain comprising a sequence that is at least 95% identical to SEQ ID NO: 46;

(g) a heavy chain comprising a sequence that is at least 95% identical to SEQ ID NO: 39 and a light chain comprising a sequence that is at least 95% identical to SEQ ID NO: 47;

(h) a heavy chain comprising a sequence that is at least 95% identical to SEQ ID NO: 40 and a light chain comprising a sequence that is at least 95% identical to SEQ ID NO: 47;

(i) a heavy chain comprising a sequence that is at least 95% identical to SEQ ID NO: 41 and a light chain comprising a sequence that is at least 95% identical to SEQ ID NO: 47; and

(j) a heavy chain comprising a sequence that is at least 95% identical to SEQ ID NO: 42 and a light chain comprising a sequence that is at least

95% identical to SEQ ID NO: 48;

(k) a heavy chain comprising a sequence that is at least 95% identical to SEQ ID NO: 42 and a light chain comprising a sequence that is at least 95% identical to SEQ ID NO: 49;

(l) a heavy chain comprising a sequence that is at least 95% identical to SEQ ID NO: 42 and a light chain comprising a sequence that is at least 95% identical to SEQ ID NO: 50;

(m) a heavy chain comprising a sequence that is at least 95% identical to SEQ ID NO: 43 and a light chain comprising a sequence that is at least 95% identical to SEQ ID NO: 48;

(n) a heavy chain comprising a sequence that is at least 95% identical to SEQ ID NO: 43 and a light chain comprising a sequence that is at least 95% identical to SEQ ID NO: 49;

(o) a heavy chain comprising a sequence that is at least 95% identical to SEQ ID NO: 43 and a light chain comprising a sequence that is at least 95% identical to SEQ ID NO: 50;

(p) a heavy chain comprising a sequence that is at least 95% identical to SEQ ID NO: 44 and a light chain comprising a sequence that is at least 95% identical to SEQ ID NO: 51;

(q) a heavy chain comprising a sequence that is at least 95% identical to SEQ ID NO: 44 and a light chain comprising a sequence that is at least 95% identical to SEQ ID NO: 52;

(r) a heavy chain comprising a sequence that is at least 95% identical to SEQ ID NO: 45 and a light chain comprising a sequence that is at least 95% identical to SEQ ID NO: 51; or

(s) a heavy chain comprising a sequence that is at least 95% identical to SEQ ID NO: 45 and a light chain comprising a sequence that is at least 95% identical to SEQ ID NO: 52.

23. An isolated antibody comprising a heavy chain and a light chain, wherein the antibody comprises:

(a) a heavy chain comprising a sequence that is at least 97% identical to SEQ ID NO: 9 and a light chain comprising a sequence that is at least 97% identical to SEQ ID NO: 10;

(b) a heavy chain comprising a sequence that is at least 97% identical to

SEQ ID NO: 11 and a light chain comprising a sequence that is at least 97% identical to SEQ ID NO: 12;

(c) a heavy chain comprising a sequence that is at least 97% identical to SEQ ID NO: 13 and a light chain comprising a sequence that is at least 97% identical to SEQ ID NO: 14;

(d) a heavy chain comprising a sequence that is at least 97% identical to SEQ ID NO: 39 and a light chain comprising a sequence that is at least 97% identical to SEQ ID NO: 46;

(e) a heavy chain comprising a sequence that is at least 97% identical to SEQ ID NO: 40 and a light chain comprising a sequence that is at least 97% identical to SEQ ID NO: 46;

(f) a heavy chain comprising a sequence that is at least 97% identical to SEQ ID NO: 41 and a light chain comprising a sequence that is at least 97% identical to SEQ ID NO: 46;

(g) a heavy chain comprising a sequence that is at least 97% identical to SEQ ID NO: 39 and a light chain comprising a sequence that is at least 97% identical to SEQ ID NO: 47;

(h) a heavy chain comprising a sequence that is at least 97% identical to SEQ ID NO: 40 and a light chain comprising a sequence that is at least 97% identical to SEQ ID NO: 47;

(i) a heavy chain comprising a sequence that is at least 97% identical to SEQ ID NO: 41 and a light chain comprising a sequence that is at least 97% identical to SEQ ID NO: 47; and

(j) a heavy chain comprising a sequence that is at least 97% identical to SEQ ID NO: 42 and a light chain comprising a sequence that is at least 97% identical to SEQ ID NO: 48;

(k) a heavy chain comprising a sequence that is at least 97% identical to SEQ ID NO: 42 and a light chain comprising a sequence that is at least 97% identical to SEQ ID NO: 49;

(l) a heavy chain comprising a sequence that is at least 97% identical to SEQ ID NO: 42 and a light chain comprising a sequence that is at least 97% identical to SEQ ID NO: 50;

(m) a heavy chain comprising a sequence that is at least 97% identical to SEQ ID NO: 43 and a light chain comprising a sequence that is at least

97% identical to SEQ ID NO: 48;

(n) a heavy chain comprising a sequence that is at least 97% identical to SEQ ID NO: 43 and a light chain comprising a sequence that is at least 97% identical to SEQ ID NO: 49;

(o) a heavy chain comprising a sequence that is at least 97% identical to SEQ ID NO: 43 and a light chain comprising a sequence that is at least 97% identical to SEQ ID NO: 50;

(p) a heavy chain comprising a sequence that is at least 97% identical to SEQ ID NO: 44 and a light chain comprising a sequence that is at least 97% identical to SEQ ID NO: 51;

(q) a heavy chain comprising a sequence that is at least 97% identical to SEQ ID NO: 44 and a light chain comprising a sequence that is at least 97% identical to SEQ ID NO: 52;

(r) a heavy chain comprising a sequence that is at least 97% identical to SEQ ID NO: 45 and a light chain comprising a sequence that is at least 97% identical to SEQ ID NO: 51; or

(s) a heavy chain comprising a sequence that is at least 97% identical to SEQ ID NO: 45 and a light chain comprising a sequence that is at least 97% identical to SEQ ID NO: 52.

24. An isolated antibody comprising a heavy chain and a light chain, wherein the antibody comprises:

(a) a heavy chain comprising a sequence that is at least 99% identical to SEQ ID NO: 9 and a light chain comprising a sequence that is at least 99% identical to SEQ ID NO: 10;

(b) a heavy chain comprising a sequence that is at least 99% identical to SEQ ID NO: 11 and a light chain comprising a sequence that is at least 99% identical to SEQ ID NO: 12;

(c) a heavy chain comprising a sequence that is at least 99% identical to SEQ ID NO: 13 and a light chain comprising a sequence that is at least 99% identical to SEQ ID NO: 14;

(d) a heavy chain comprising a sequence that is at least 99% identical to SEQ ID NO: 39 and a light chain comprising a sequence that is at least 99% identical to SEQ ID NO: 46;

(e) a heavy chain comprising a sequence that is at least 99% identical to

SEQ ID NO: 40 and a light chain comprising a sequence that is at least 99% identical to SEQ ID NO: 46;

(f) a heavy chain comprising a sequence that is at least 99% identical to SEQ ID NO: 41 and a light chain comprising a sequence that is at least 99% identical to SEQ ID NO: 46;

(g) a heavy chain comprising a sequence that is at least 99% identical to SEQ ID NO: 39 and a light chain comprising a sequence that is at least 99% identical to SEQ ID NO: 47;

(h) a heavy chain comprising a sequence that is at least 99% identical to SEQ ID NO: 40 and a light chain comprising a sequence that is at least 99% identical to SEQ ID NO: 47;

(i) a heavy chain comprising a sequence that is at least 99% identical to SEQ ID NO: 41 and a light chain comprising a sequence that is at least 99% identical to SEQ ID NO: 47;

(j) a heavy chain comprising a sequence that is at least 99% identical to SEQ ID NO: 42 and a light chain comprising a sequence that is at least 99% identical to SEQ ID NO: 48;

(k) a heavy chain comprising a sequence that is at least 99% identical to SEQ ID NO: 42 and a light chain comprising a sequence that is at least 99% identical to SEQ ID NO: 49;

(l) a heavy chain comprising a sequence that is at least 99% identical to SEQ ID NO: 42 and a light chain comprising a sequence that is at least 99% identical to SEQ ID NO: 50;

(m) a heavy chain comprising a sequence that is at least 99% identical to SEQ ID NO: 43 and a light chain comprising a sequence that is at least 99% identical to SEQ ID NO: 48;

(n) a heavy chain comprising a sequence that is at least 99% identical to SEQ ID NO: 43 and a light chain comprising a sequence that is at least 99% identical to SEQ ID NO: 49;

(o) a heavy chain comprising a sequence that is at least 99% identical to SEQ ID NO: 43 and a light chain comprising a sequence that is at least 99% identical to SEQ ID NO: 50;

(p) a heavy chain comprising a sequence that is at least 99% identical to SEQ ID NO: 44 and a light chain comprising a sequence that is at least

99% identical to SEQ ID NO: 51;

(q) a heavy chain comprising a sequence that is at least 99% identical to SEQ ID NO: 44 and a light chain comprising a sequence that is at least 99% identical to SEQ ID NO: 52;

(r) a heavy chain comprising a sequence that is at least 99% identical to SEQ ID NO: 45 and a light chain comprising a sequence that is at least 99% identical to SEQ ID NO: 51; or

(s) a heavy chain comprising a sequence that is at least 99% identical to SEQ ID NO: 45 and a light chain comprising a sequence that is at least 99% identical to SEQ ID NO: 52.

25. An isolated antibody comprising a heavy chain and a light chain, wherein the antibody comprises:

(a) a heavy chain comprising a sequence of SEQ ID NO: 9 and a light chain comprising a sequence of SEQ ID NO: 10;

(b) a heavy chain comprising a sequence of SEQ ID NO: 11 and a light chain comprising a sequence of SEQ ID NO: 12;

(c) a heavy chain comprising a sequence of SEQ ID NO: 13 and a light chain comprising a sequence of SEQ ID NO: 14;

(d) a heavy chain comprising a sequence of SEQ ID NO: 39 and a light chain comprising a sequence of SEQ ID NO: 46;

(e) a heavy chain comprising a sequence of SEQ ID NO: 40 and a light chain comprising a sequence of SEQ ID NO: 46;

(f) a heavy chain comprising a sequence of SEQ ID NO: 41 and a light chain comprising a sequence of SEQ ID NO: 46;

(g) a heavy chain comprising a sequence of SEQ ID NO: 39 and a light chain comprising a sequence of SEQ ID NO: 47;

(h) a heavy chain comprising a sequence of SEQ ID NO: 40 and a light chain comprising a sequence of SEQ ID NO: 47;

(i) a heavy chain comprising a sequence of SEQ ID NO: 41 and a light chain comprising a sequence of SEQ ID NO: 47; and

(j) a heavy chain comprising a sequence of SEQ ID NO: 42 and a light chain comprising a sequence of SEQ ID NO: 48;

(k) a heavy chain comprising a sequence of SEQ ID NO: 42 and a light chain comprising a sequence of SEQ ID NO: 49;

(l) a heavy chain comprising a sequence of SEQ ID NO: 42 and a light chain comprising a sequence of SEQ ID NO: 50;

(m) a heavy chain comprising a sequence of SEQ ID NO: 43 and a light chain comprising a sequence of SEQ ID NO: 48;

(n) a heavy chain comprising a sequence of SEQ ID NO: 43 and a light chain comprising a sequence of SEQ ID NO: 49;

(o) a heavy chain comprising a sequence of SEQ ID NO: 43 and a light chain comprising a sequence of SEQ ID NO: 50;

(p) a heavy chain comprising a sequence of SEQ ID NO: 44 and a light chain comprising a sequence of SEQ ID NO: 51;

(q) a heavy chain comprising a sequence of SEQ ID NO: 44 and a light chain comprising a sequence of SEQ ID NO: 52;

(r) a heavy chain comprising a sequence of SEQ ID NO: 45 and a light chain comprising a sequence of SEQ ID NO: 51; or

(s) a heavy chain comprising a sequence of SEQ ID NO: 45 and a light chain comprising a sequence of SEQ ID NO: 52.

26. An isolated antibody comprising a heavy chain and a light chain, wherein the antibody comprises:

(a) a heavy chain comprising a heavy chain (HC) CDR1 having the sequence of SEQ ID NO: 15, an HC CDR2 having the sequence of SEQ ID NO: 16, and an HC CDR3 having the sequence of SEQ ID NO: 17, and a light chain comprising a light chain (LC) CDR1 having the sequence of SEQ ID NO: 18, a LC CDR2 having the sequence of SEQ ID NO: 19, and a LC CDR3 having the sequence of SEQ ID NO: 20;

(b) a heavy chain comprising a heavy chain (HC) CDR1 having the sequence of SEQ ID NO: 21, an HC CDR2 having the sequence of SEQ ID NO: 22, and an HC CDR3 having the sequence of SEQ ID NO: 23, and a light chain comprising a light chain (LC) CDR1 having the sequence of SEQ ID NO: 24, a LC CDR2 having the sequence of SEQ ID NO: 25, and a LC CDR3 having the sequence of SEQ ID NO: 26; or

(c) a heavy chain comprising a heavy chain (HC) CDR1 having the sequence of SEQ ID NO: 27, an HC CDR2 having the sequence of

SEQ ID NO: 28, and an HC CDR3 having the sequence of SEQ ID NO: 29, and a light chain comprising a light chain (LC) CDR1 having the sequence of SEQ ID NO: 30, a LC CDR2 having the sequence of SEQ ID NO: 31, and a LC CDR3 having the sequence of SEQ ID NO: 32.

27. An isolated antibody comprising a heavy chain and a light chain, wherein the antibody comprises:

(a) a heavy chain comprising a sequence of SEQ ID NO: 53 and a light chain comprising a sequence of SEQ ID NO: 60;

(b) a heavy chain comprising a sequence of SEQ ID NO: 53 and a light chain comprising a sequence of SEQ ID NO: 61; or

(c) a heavy chain comprising a sequence of SEQ ID NO: 58 and a light chain comprising a sequence of SEQ ID NO: 65.

28. An isolated antibody comprising a heavy chain and a light chain, wherein the antibody comprises:

(a) a heavy chain consisting of the sequence of SEQ ID NO: 53 and a light chain consisting of the sequence of SEQ ID NO: 60;

(b) a heavy chain consisting of the sequence of SEQ ID NO: 53 and a light chain consisting of the sequence of SEQ ID NO: 61; or

(c) a heavy chain consisting of the sequence of SEQ ID NO: 58 and a light chain consisting of the sequence of SEQ ID NO: 65.

29. The antibody of any one of claims 1 to 28, wherein the antibody binds to human CSF1R.

30. The antibody of any one of claims 1 to 29, wherein the antibody binds to cynomolgus CSF1R.

31. The antibody of any one of claims 1 to 30, wherein the antibody blocks ligand binding to CSF1R.

32. The antibody of any one of claims 1 to 31, wherein the antibody blocks CSF1 binding to CSF1R.

33. The antibody of any one of claims 1 to 32, wherein the antibody blocks IL34 binding to CSF1R.

34. The antibody of any one of claims 1 to 33, wherein the antibody inhibits ligand-induced CSF1R phosphorylation.

35. The antibody of any one of claims 1 to 34, wherein the antibody

inhibits CSF1-induced CSF1R phosphorylation.

36. The antibody of any one of claims 1 to 35, wherein the antibody inhibits IL34-induced CSF1R phosphorylation.

37. The antibody of any one of claim 1 to 36, wherein the antibody is a chimeric antibody.

38. The antibody of any one of claims 1 to 36, wherein the antibody is a humanized antibody.

39. The antibody of any one of claims 1 to 38, wherein the antibody is selected from a Fab, an Fv, an scFv, a Fab', and a $(Fab')_2$.

40. The antibody of any one of claims 1 to 38, wherein the antibody is selected from an IgA, an IgG, and an IgD.

41. The antibody of claim 40, wherein the antibody is an IgG.

42. The antibody of claim 41, wherein the antibody is an IgG4.

43. The antibody of claim 42, wherein the antibody comprises an S241P mutation in at least one IgG4 heavy chain constant region.

44. The antibody of any one of claims 1 to 43, wherein the antibody binds to human CSF1R with an affinity (K_D) of less than 1 nM.

45. The antibody of any one of claims 1 to 44, wherein the antibody inhibits monocyte proliferation and/or survival responses in the presence of CSF1 or IL34.

46. A pharmaceutical composition comprising an antibody of any one of claims 1 to 45.

47. An isolated nucleic acid comprising a polynucleotide sequence that encodes an amino acid sequence that is at least 90% identical to a sequence selected from SEQ ID NOs: 9, 11, 13, and 39 to 45.

48. The nucleic acid of claim 47, wherein the amino acid sequence is at least 95% identical to a sequence selected from SEQ ID NOs: 9, 11, 13, and 39 to 45.

49. The nucleic acid of claim 47, wherein the amino acid sequence is at least 97% identical to a sequence selected from SEQ ID NOs: 9, 11, 13, and 39 to 45.

50. The nucleic acid of claim 47, wherein the amino acid sequence is at least 99% identical to a sequence selected from SEQ ID NOs: 9, 11, 13, and 39 to 45.

51. The nucleic acid of claim 47, wherein the amino acid sequence is selected from SEQ ID NOs: 9, 11, 13, and 39 to 45.

52. The nucleic acid of any one of claims 45 to 51, wherein the nucleic

acid further comprises a polynucleotide sequence that encodes an amino acid sequence that is at least 90% identical to a sequence selected from SEQ ID NOs: 10, 12, 14, and 46 to 52.

53. The nucleic acid of claim 52, wherein the amino acid sequence is at least 95% identical to a sequence selected from SEQ ID NOs: 10, 12, 14, and 46 to 52.

54. The nucleic acid of claim 52, wherein the amino acid sequence is at least 97% identical to a sequence selected from SEQ ID NOs: 10, 12, 14, and 46 to 52.

55. The nucleic acid of claim 52, wherein the amino acid sequence is at least 99% identical to a sequence selected from SEQ ID NOs: 10, 12, 14, and 46 to 52.

56. The nucleic acid of claim 52, wherein the amino acid sequence is selected from SEQ ID NOs: 10, 12, 14, and 46 to 52.

57. An isolated nucleic acid comprising a polynucleotide sequence that encodes an amino acid sequence that is at least 90% identical to a sequence selected from 10, 12, 14, and 46 to 52.

58. The nucleic acid of claim 57, wherein the amino acid sequence is at least 95% identical to a sequence selected from 10, 12, 14, and 46 to 52.

59. The nucleic acid of claim 57, wherein the amino acid sequence is at least 97% identical to a sequence selected from 10, 12, 14, and 46 to 52.

60. The nucleic acid of claim 57, wherein the amino acid sequence is at least 99% identical to a sequence selected from 10, 12, 14, and 46 to 52.

61. The nucleic acid of claim 57, wherein the amino acid sequence is selected from 10, 12, 14, and 46 to 52.

62. A composition comprising a first nucleic acid and a second nucleic acid, wherein the first nucleic acid is selected from the nucleic acids of claims 47 to 51, and wherein the second nucleic acid is selected from the nucleic acids of claims 57 to 61.

63. An isolated nucleic acid comprising a polynucleotide sequence that encodes a heavy chain, wherein the heavy chain comprises : (i) a heavy chain (HC) CDR1 comprising a sequence selected from SEQ ID NOs: 15, 21, and 27; (ii) an HC CDR2 comprising a sequence selected from SEQ ID NOs: 16, 22, and 28; and (iii) an HC CDR3 comprising a sequence selected from SEQ ID NOs: 17, 23, and 29.

64. The nucleic acid of claim 63, wherein the HC CDR1, HC CDR2, and HC CDR3 comprise a set of sequences selected from: (a) SEQ ID NOs: 15, 16, and 17; (b) SEQ ID NOs: 21, 22, and 23; and (c) SEQ ID NOs: 27, 28, and 29.

65. The nucleic acid of claim 63 or claim 64, wherein the nucleic acid further comprises a polynucleotide sequence that encodes a light chain, wherein the light chain comprises (i) a light chain (LC) CDR1 comprising a sequence selected from SEQ ID NOs: 18, 24, and 30; (ii) an LC CDR2 comprising a sequence selected from SEQ ID NOs: 19, 25, and 31; and (iii) an LC CDR3 comprising a sequence selected from SEQ ID NOs: 20, 26, and 32.

66. The nucleic acid of claim 65, wherein the LC CDR1, LC CDR2, and LC CDR3 comprise a set of sequences selected from: (a) SEQ ID NOs: 18, 19, and 20; (b) SEQ ID NOs: 24, 25, and 26; and (c) SEQ ID NOs: 30, 31, and 32.

67. An isolated nucleic acid comprising a polynucleotide sequence that encodes a light chain, wherein the light chain comprises: (i) a light chain (LC) CDR1 comprising a sequence selected from SEQ ID NOs: 18, 24, and 30; (ii) an LC CDR2 comprising a sequence selected from SEQ ID NOs: 19, 25, and 31; and (iii) an LC CDR3 comprising a sequence selected from SEQ ID NOs: 20, 26, and 32.

68. The nucleic acid of claim 67, wherein the LC CDR1, LC CDR2, and LC CDR3 comprise a set of sequences selected from: (a) SEQ ID NOs: 18, 19, and 20; (b) SEQ ID NOs: 24, 25, and 26; and (c) SEQ ID NOs: 30, 31, and 32.

69. A composition comprising a first nucleic acid and a second nucleic acid, wherein the first nucleic acid is selected from the nucleic acids of claims 63 and 64, and wherein the second nucleic acid is selected from the nucleic acids of claims 67 and 68.

70. An isolated nucleic acid comprising a first polynucleotide sequence and a second polynucleotide sequence, wherein the first polynucleotide sequence and the second polynucleotide sequence encode a heavy chain and a light chain, respectively, of an antibody of any one of claims 22 to 28.

71. A composition comprising a first nucleic acid and a second nucleic acid, wherein the first nucleic acid comprises a first polynucleotide sequence and the second nucleic acid comprises a second polynucleotide sequence, wherein the first polynucleotide sequence and the second polynucleotide sequence encode a heavy chain and a light chain, respectively, of an antibody of any one of claims 22 to 28.

72. A host cell comprising a nucleic acid of any one of claims 47 to 61, 63

to 68, and 70.

73. A host cell comprising a composition of claim 60 or claim 69 or claim 71.

74. The host cell of claim 72 or 73, wherein the host cell is a eukaryotic host cell.

75. The host cell of claim 74, wherein the host cell is a mammalian host cell.

76. The host cell of claim 75, wherein the host cell is selected from a CHO cell, a 293 cell, an NSO cell, and a PER.C6 cell.

77. The host cell of claim 76, wherein the host cell is a 293-6E cell or a DG44 cell.

78. A method of treating multiple sclerosis comprising administering to a patient a pharmaceutical composition of claim 46.

79. A method of treating rheumatoid arthritis comprising administering to a patient a pharmaceutical composition of claim 46.

80. A method of treating osteolytic bone loss comprising administering to a patient a pharmaceutical composition of claim 46.

81. The method of claim 80, wherein the osteolytic bone loss is selected from osteoporosis, metastasis-induced osteolytic bone loss, and rheumatoid arthritis-induced bone loss.

82. A method of treating cancer comprising administering to a patient a pharmaceutical composition of claim 46.

83. The method of claim 82, wherein the cancer is selected from breast cancer, prostate cancer, endometrial cancer, bladder cancer, kidney cancer, esophageal cancer, squamous cell carcinoma, uveal melanoma, follicular lymphoma, renal cell carcinoma, cervical cancer, ovarian cancer, lung cancer, colorectal cancer, brain cancer, pancreatic cancer, head and neck cancer, liver cancer, leukemia, lymphoma, Hodgkin's disease, multiple myeloma, melanoma, astrocytoma, stomach cancer, and pulmonary adenocarcinoma.