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(54) Title: ANTI-CD115 ANTIBODIES

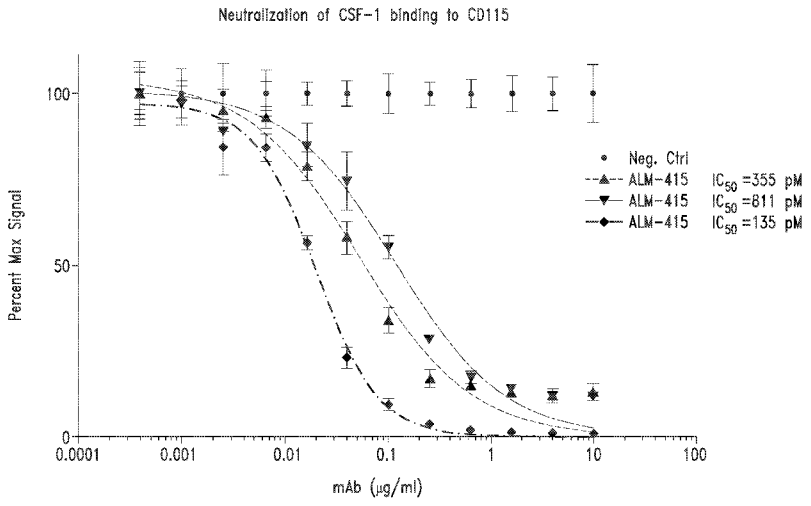

FIG. 10

(57) Abstract: The present invention provides anti-CD115 monoclonal antibodies and related compositions, which may be used in any of a variety of therapeutic and diagnostic methods for the treatment of cancer, autoimmune, and other diseases.

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ANTI-CD115 ANTIBODIES

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit under 35 U.S.C. § 119(e) of U.S. Provisional Application No. 62/220,147, filed September 17, 2015 and U.S. Provisional Application No. 62/219,578, filed September 16, 2015, each of which is incorporated by reference herein in its entirety.

SEQUENCE LISTING

[0002] The Sequence Listing associated with this application is provided in text format in lieu of a paper copy, and is hereby incorporated by reference into the specification. The name of the text file containing the Sequence Listing is ABLX_007_02WO_ST25.txt. The text file is 525 KB, was created on September16, 2016, and is being submitted electronically via EFS-Web.

BACKGROUND

Technical Field

[0003] The present invention relates generally to anti-CD115 antibodies, compositions and methods of using same. Such antibodies are useful, for example, for treating a variety of diseases, such as oncological and immunological diseases.

Description of the Related Art

[0004] CD115, also known as Colony-Stimulating Factor 1 Receptor (CSF1R) and macrophage-colony stimulating factor receptor (M-CSFR), is a cell surface receptor tyrosine kinase belonging to the platelet-derived growth factor family. CD115 has two structurally unrelated ligands, namely CSF-1 (M-CSF) and IL-34. CD115 is expressed by hematopoietic stem cells, myeloid cells, including monocytes, macrophages, osteoclasts, dendritic cells, and microglia, neural progenitor cells, and epithelial cells, including Paneth cells (Stanley and Chitu, Cold Spring Harb Perspect Biol 2014;6:a021857).

[0005] Dysregulation of CD115, and/or its ligands, is associated with proliferative diseases and disorders (e.g., neoplasms, tumors and metastases), as well as immunological and neurological diseases and disorders. The present invention provides chimeric and fully human anti-CD115 antibodies, including CD115 antagonists.

SUMMARY OF THE INVENTION

[0006] The present invention relates to anti-CD115 antibodies. More specifically, it relates to chimeric anti-CD115 antibodies generated from an AlivaMab Mouse, fully human anti-CD115 antibodies produced therefrom, and methods of use thereof.

[0007] One aspect of the invention provides an isolated anti-CD115 antibody, or an antigen-binding fragment thereof, comprising i) a heavy chain variable region comprising a VHCDR1 selected from any of SEQ ID NOs:436-543, a VHCDR2 selected from any of SEQ ID NOs:868-975, and a VHCDR3 selected from any of SEQ ID NOs:1300-1407 and ii) a light chain variable region comprising a VLCDR1 selected from any of SEQ ID NOs:652-759, a VLCDR2 selected from any of SEQ ID NOs:1084-1191, and a VLCDR3 selected from any of SEQ ID NOs:1516-1623.

[0008] In one embodiment, the VHCDR1, VHCDR2, and VHCDR3 of the anti-CD115 antibody, or antigen-binding fragment thereof, comprise SEQ ID NOs:450, 882, and 1314, respectively. In one embodiment, the VLCDR1, VLCDR2, and VLCDR3 comprise SEQ ID NOs:666, 1098, and 1530, respectively. In another embodiment, the VH is selected from any one of SEQ ID NOs:109-216. In yet another embodiment, the VL is selected from any one of SEQ ID NOs:325-432. In one embodiment, the VH comprises SEQ ID NO:123. In another embodiment, the VL comprises SEQ ID NO:339. In another embodiment, the VH comprises SEQ ID NO:123, and the VL comprises SEQ ID NO:339.

[0009] In one embodiment, the anti-CD115 antibody, or antigen-binding fragment thereof, is human. In one embodiment, the antibody is chimeric. In certain embodiments, the antibody is selected from a single-variable domain antibody, single chain antibody, a scFv, a bispecific antibody, a multi-specific antibody, a Fab, a F(ab')2, and a whole antibody.

[0010] One aspect of the invention provides a recombinant polynucleotide encoding the anti-CD115 antibody, or antigen-binding fragment thereof, described above. Another aspect of the invention provides an expression vector comprising the recombinant polynucleotide. In another aspect of the invention provides an isolated host cell that comprises the expression vector. One aspect of the invention provides a composition comprising an anti-CD115 antibody, or antigen-binding fragment thereof, described herein and a physiologically acceptable carrier.

BRIEF DESCRIPTION OF THE FIGURES

[0011] Figure 1 shows ELISA can detect an increase in p-MCSFR. For each grouping of bars, the lysate ratio is neat, 1:2, 1:4, 1:8, 1:16 and 1:32 from left to right.

[0012] Figure 2 shows Bin1 α-CD115 mAbs may block M-CSF and IL34 induced CD115 phosphorylation and Bin 3 35A may block all phosphorylation.

[0013] Figure 3 shows CD115 phosphorylation (p-CD115 or p-MCSFR) measured by ELISA.

[0014] Figures 4A and 4B show inhibition of m-CSF- and IL-34-induced phosphorylation of CD115 (MCSFR).

[0015] Figure 5 shows binding of anti-CD115 IgGκ mAbs to CD115 expressed on OCI-AML5 cells.

[0016] Figure 6 shows an assay for detecting inhibition of m-CSF (CSF-1) induced phosphorylation on CD115 expressing AML5 cells by anti-CD115 IgGκ mAb.

[0017] Figure 7 shows binding of anti-CD115 IgGλ mAbs to OCI-AML5 cells.

[0018] Figure 8 shows anti-CD115 IgGλ mAb inhibition of CSF-1 induced phosphorylation of CD115.

[0019] Figure 9 shows the IC50 of selected anti-CD115 mAbs. For each grouping of bars, no M-CSF, 10, 2, 0.4, 0.08, 0.016, 0.0032, and 0.00064 ng/ml are shown from left to right.

[0020] Figure 10 shows neutralization of CSF-1 binding to CD115 by anti-CD115 mAbs.

[0021] Figure 11 shows neutralization of CSF-1 induced phosphorylation of CD115 by anti-CD115 mAbs.

[0022] Figure 12 shows internalization of anti-CD115 mAbs. From left to right, examples within the panel of anti-CD115 mAbs exhibiting no internalization, weak internalization, mid internalization, strong internalization and very strong internalization are depicted.

[0023] Figure 13 shows conversion of anti-CD115 mAb ALM-423 to fully human antibodies.

[0024] Figure 14 shows neutralization of CSF-1 binding to CD115 by fully human anti-CD115 mAbs.

[0025] Figure 15 shows neutralization of pTyr formation on CD115 by fully human anti-CD115 mAbs.

[0026] Figure 16 shows some anti-CD115 antibodies that are antagonists of CSF-1 induce p-tyr formation on CD115.

BRIEF DESCRIPTION OF THE SEQUENCES

[0027] SEQ ID NOs:1-108 are polynucleotide sequences encoding VH regions of the anti-CD115 antibodies listed in Table 2.

[0028] SEQ ID NOs:109-216 are amino acid sequences of VH regions of the anti-CD115 antibodies listed in Table 2.

[0029] SEQ ID NOs:217-324 are polynucleotide sequences encoding VL regions of the anti-CD115 antibodies listed in Table 2.

[0030] SEQ ID NOs:325-432 are amino acid sequences of VL regions of the anti-CD115 antibodies listed in Table 2.

[0031] SEQ ID NO:433 is an IgG specific primer.

[0032] SEQ ID NO:434 is an Igλ specific primer.

[0033] SEQ ID NO:435 is an Igκ specific primer.

[0034] SEQ ID NOs:436-543 are amino acid sequences of the VHCDR1 of the anti-CD115 antibodies listed in Table 2.

[0035] SEQ ID NOs:544-651 are polynucleotide sequences encoding the VHCDR1 of the anti-CD115 antibodies listed in Table 2.

[0036] SEQ ID NOs:652-759 are amino acid sequences of the VLCDR1 of the anti-CD115 antibodies listed in Table 2.

[0037] SEQ ID NOs:760-867 are polynucleotide sequences encoding the VLCDR1 of the anti-CD115 antibodies listed in Table 2.

[0038] SEQ ID NOs:868-975 are amino acid sequences of the VHCDR2 of the anti-CD115 antibodies listed in Table 2.

[0039] SEQ ID NOs:976-1083 are polynucleotide sequences encoding the VHCDR2 of the anti-CD115 antibodies listed in Table 2.

[0040] SEQ ID NOs:1084-1191 are amino acid sequences of the VLCDR2 of the anti-CD115 antibodies listed in Table 2.

[0041] SEQ ID NOs:1192-1299 are polynucleotide sequences encoding the VLCDR2 of the anti-CD115 antibodies listed in Table 2.

[0042] SEQ ID NOs:1300-1407 are amino acid sequences of the VHCDR3 of the anti-CD115 antibodies listed in Table 2.

[0043] SEQ ID NOs:1408-1515 are polynucleotide sequences encoding the VHCDR3 of the anti-CD115 antibodies listed in Table 2.

[0044] SEQ ID NOs:1516-1623 are amino acid sequences of the VLCDR3 of the anti-CD115 antibodies listed in Table 2.

[0045] SEQ ID NOs:1624-1731 are polynucleotide sequences encoding the VLCDR3 of the anti-CD115 antibodies listed in Table 2.

DETAILED DESCRIPTION

[0046] The present disclosure relates to anti-CD115 antibodies. Ablexis has used its proprietary AlivaMab Mouse technology (*See* WO 2010/039900 and WO 2011/123708, incorporated herein in their entirety) to generate panels of monoclonal antibodies (mAbs) against human CD115. Antibodies that potently neutralize CD115 signaling induced by CSF-1 were identified within the panel of CD115 AlivaMab antibodies. In one embodiment, anti-CD115 AlivaMab antibodies potently neutralize CD115 signaling induced by IL-34. In one embodiment, anti-CD115 AlivaMab antibodies that potently neutralize CD115 signaling induced by both CSF-1 and IL-34. CD115 (colony-stimulating factor 1 receptor, CSF1R, C-FMS) is a member of the receptor tyrosine kinase superfamily. For a review of CD115 biology, refer to Stanley and Chitu, Cold Spring Harb. Perspect. Biol. 2014 Jun 2;6(6).

[0047] Embodiments of the invention pertain to the use of anti-CD115 antibodies, or antigen-binding fragments thereof, for the diagnosis, assessment and treatment of diseases and disorders associated with CD115, CSF-1 and/or IL-34 or aberrant expression thereof. The subject antibodies are used in the treatment or prevention of neoplasms and/or the treatment or prevention of autoimmune and/or inflammatory diseases, among other diseases.

[0048] Portions of variable regions from the AlivaMab antibodies may include all or a combination of the complementarity determining regions (CDRs) of the VH and/or VL. The variable regions may be formatted with constant regions, either native or modified for various desired effector functions, in a standard antibody structure (two heavy chains with two light chains). The variable regions may also be formatted as multi-specific antibodies, e.g., bispecific antibodies binding to two different epitopes on CD115 or to two different antigens, one of which is CD115. The variable regions may also be formatted as antibody fragments, e.g., single-domain antibodies comprising a single VH or VL, Fabs or Fab'2. The antibodies may also be used as antibody-drug conjugates, or carry other additions such as small molecule toxins, biologic toxins, cytokines, oligopeptides, or RNAs to increase therapeutic modality and/or increase safety.

[0049] The practice of the present invention will employ, unless indicated specifically to the contrary, conventional methods of virology, immunology, microbiology, molecular

biology and recombinant DNA techniques within the skill of the art, many of which are described below for the purpose of illustration. Such techniques are explained fully in the literature. See, e.g., Current Protocols in Molecular Biology or Current Protocols in Immunology, John Wiley & Sons, New York, N.Y.(2009); Ausubel et al., Short Protocols in Molecular Biology, 3rd ed., Wiley & Sons, 1995; Sambrook and Russell, Molecular Cloning: A Laboratory Manual (3rd Edition, 2001); Maniatis et al., Molecular Cloning: A Laboratory Manual (1982); DNA Cloning: A Practical Approach, vol. I & II (D. Glover, ed.); Oligonucleotide Synthesis (N. Gait, ed., 1984); Nucleic Acid Hybridization (B. Hames & S. Higgins, eds., 1985); Transcription and Translation (B. Hames & S. Higgins, eds., 1984); Animal Cell Culture (R. Freshney, ed., 1986); Perbal, A Practical Guide to Molecular Cloning (1984) and other like references.

[0050] Before describing certain embodiments in detail, it is to be understood that this invention is not limited to particular compositions or biological systems, which can vary. It is also to be understood that the terminology used herein is for the purpose of describing particular illustrative embodiments only, and is not intended to be limiting. The terms used in this specification generally have their ordinary meaning in the art, within the context of this invention and in the specific context where each term is used. Certain terms are discussed below or elsewhere in the specification, to provide additional guidance to the practitioner in describing the compositions and methods of the invention and how to make and use them. The scope and meaning of any use of a term will be apparent from the specific context in which the term is used. As such, the definitions set forth herein are intended to provide illustrative guidance in ascertaining particular embodiments of the invention, without limitation to particular compositions or biological systems.

[0051] As used in the present disclosure and the appended claims, the singular forms "a," "an" and "the" include plural references unless the content clearly dictates otherwise.

[0052] Throughout the present disclosure and the appended claims, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or group of elements but not the exclusion of any other element or group of elements.

[0053] The terms "antibody" and "immunoglobulin" (Ig) are used interchangeably herein. An antibody may be either membrane bound or secreted. As used herein, the term encompasses not only intact, or "whole", polyclonal or monoclonal antibodies, but also fragments thereof (such as single-variable domain (VH, VL or combination thereof) antibodies, Fab, Fab', F(ab')2, Fv), single chain (ScFv), synthetic variants thereof, naturally

occurring variants, fusion proteins comprising an antibody portion with an antigen-binding fragment of the required specificity, humanized antibodies, chimeric antibodies, and any other modified configuration of the immunoglobulin molecule that comprises an antigen-binding site or fragment (epitope recognition site) of the required specificity.

[0054] Antibody, or Ig, molecules are typically comprised of two identical heavy chains and two identical light chains linked together through disulfide bonds. Both heavy chains (IgH) and light chains (IgL) contain a variable (V) region or domain and a constant (C) region or domain. The portion of the IgH locus encoding the V region comprises multiple copies of variable (V), diversity (D), and joining (J) gene segments. The portion of the IgL loci encoding the V region comprises multiple copies of V and J gene segments. The V region encoding portion of the IgH and IgL loci undergo gene segment rearrangement, e.g., different combinations of a V, (D) and J gene segments arrange to form the IgH and IgL variable regions, to develop diverse antigen specificity in antibodies. Each variable region comprises three complementarity-determining regions (CDRs) interspersed between the less variable framework regions (FRs). The heavy chain comprises VHCDR1, VHCDR2, and VHCDR3. The light chain comprises VLCDR1, VLCDR2, and VLCDR3. The secreted form of the IgH C region is made up of three C domains, CH1, CH2, CH3, optionally CH4 (Cμ), and a hinge region except for Cμ, which lacks a hinge region. The membrane-bound form of the IgH C region also has membrane and intra-cellular domains. The IgH constant region determines the isotype of the antibody, e.g. IgM, IgD, IgG1, IgG2, IgG3, IgG4, IgA and IgE. It will be appreciated that non-human mammals, such as an AlivaMab Mouse, encoding multiple Ig isotypes will be able to undergo isotype class switching. There are two types of human IgL, Igκ and Igλ.

[0055] The term "antigen-binding fragment" as used herein refers to a polypeptide fragment that contains at least one CDR of an immunoglobulin heavy and/or light chain that binds to CD115. In this regard, an antigen-binding fragment of the antibodies may comprise 1, 2, 3, 4, 5, or all 6 CDRs of a VH and VL sequence set forth herein from anti-CD115 antibodies described herein. An antigen-binding fragment of the CD115-specific antibodies described herein is capable of binding to CD115. In certain embodiments, an antigen-binding fragment or an antibody comprising an antigen-binding fragment, prevents or inhibits CSF-1 and/or IL-34 binding to CD115 and subsequent signaling events. In other embodiments, an anti-CD115 antibody, or an antigen-binding fragment thereof, prevents signaling events mediated by CD115 by preventing dimerization of CD115, including dimerization that is

induced by CSF-1 or IL-34 binding or that may happen spontaneously under certain conditions of expression CD115. In certain embodiments, the antigen-binding fragment binds specifically to and/or inhibits or modulates the biological activity of human CD115.

[0056] In certain embodiments, antibodies and antigen-binding fragments thereof as described herein include a heavy chain and a light chain CDR set, respectively interposed between a heavy chain and a light chain framework region (FR) set that provide conformational support to the CDRs and define the spatial relationship of the CDRs relative to each other. As used herein, the term "CDR set" refers to the three hypervariable regions of a heavy or light chain V region. Proceeding from the N terminus of a heavy or light chain, these regions are denoted as "CDR1," "CDR2," and "CDR3" respectively. An antigen-binding site, therefore, includes six CDRs, comprising the CDR set from each of a heavy and a light chain V region.

[0057] A "Fab" domain or fragment comprises the N-terminal portion of the IgH, which includes the V region and the CH1 domain of the IgH, and the entire IgL. A "$F(ab')_2$" domain comprises the Fab domain and a portion of the hinge region, wherein the 2 IgH are linked together via disulfide linkage in the middle hinge region. Both the Fab and $F(ab')_2$ are "antigen-binding fragments." The C-terminal portion of the IgH, comprising the CH2 and CH3 domains, is the "Fc" domain. The Fc domain is the portion of the Ig recognized by cell receptors, such as the FcR, and to which the complement-activating protein, C1q, binds. The lower hinge region, which is encoded in the 5' portion of the CH2 exon, provides flexibility within the antibody for binding to FcR receptors. An "Fv" fragment includes a non-covalent VH::VL heterodimer including an antigen-binding site. In certain embodiments, single chain Fv (scFv) antibodies are contemplated. A scFv is a covalently linked VH::VL heterodimer which is expressed from a gene fusion including VH- and VL-encoding genes linked by a peptide-encoding linker (Huston et al. (1988) Proc. Nat. Acad. Sci. USA 85(16):5879-5883).

[0058] Where bispecific antibodies are to be used, these may be conventional bispecific antibodies, which can be manufactured in a variety of ways (Holliger, P. and Winter G. Current Opinion Biotechnol. 4, 446-449 (1993)), e.g., prepared chemically or from hybrid hybridomas, or may be any of the bispecific antibody fragments mentioned above.

[0059] As used herein "chimeric antibody" refers to an antibody encoded by a polynucleotide sequence containing polynucleotide sequences from two or more species, e.g., human and mouse.

[0060] As used herein "chimeric Ig chain" refers to an Ig heavy chain or an Ig light chain encoded by a polynucleotide sequence containing polynucleotide sequences from two

or more species, e.g., human and mouse. For example, a chimeric Ig heavy chain may comprise a human VH domain, DH domain, JH domain, CH1 domain, and upper hinge region and mouse CH2 and CH3 domains. In one embodiment, the middle hinge region is mouse. In one embodiment, the middle hinge region is human. In one embodiment, the middle hinge region is chimeric.

[0061] "Polypeptide," "peptide" or "protein" are used interchangeably herein to describe a chain of amino acids that are linked together by chemical bonds. A polypeptide or protein may be an IgH, IgL, V domain, C domain, or an antibody.

[0062] The strength, or affinity of immunological binding interactions can be expressed in terms of the dissociation constant (K_D) of the interaction, wherein a smaller K_D represents a greater affinity. Immunological binding properties of selected polypeptides can be quantified using methods well known in the art. One such method entails measuring the rates of antigen-binding site/antigen complex formation and dissociation, wherein those rates depend on the concentrations of the complex partners, the affinity of the interaction, and on geometric parameters that equally influence the rate in both directions. Thus, both the "on rate constant" (K_{on}) and the "off rate constant" (K_{off}) can be determined by calculation of the concentrations and the actual rates of association and dissociation. The ratio of K_{off} /K_{on} enables cancellation of all parameters not related to affinity, and is thus equal to the dissociation constant, K_D. See, generally, Davies et al. (1990) Annual Rev. Biochem. 59:439-473.

[0063] "Polynucleotide" refers to a chain of nucleic acids that are linked together by chemical bonds. Polynucleotides include, but are not limited to, DNA, cDNA, RNA, mRNA, and gene sequences and segments. Polynucleotides may be isolated from a living source such as a eukaryotic cell, prokaryotic cell or virus, or may be derived through in vitro manipulation by using standard techniques of molecular biology, or by DNA synthesis, or by a combination of a number of techniques.

[0064] As used herein, the term "vector" refers to a nucleic acid molecule into which another nucleic acid fragment can be integrated without loss of the vector's ability to replicate. Vectors may originate from a virus, a plasmid or the cell of a higher organism. Vectors are utilized to introduce foreign or recombinant DNA into a host cell, wherein the vector is replicated.

[0065] A polynucleotide agent can be contained in a vector, which can facilitate manipulation of the polynucleotide, including introduction of the polynucleotide into a target cell. The vector can be a cloning vector, which is useful for maintaining the polynucleotide,

or can be an expression vector, which contains, in addition to the polynucleotide, regulatory elements useful for expressing the polynucleotide and, where the polynucleotide encodes an RNA, for expressing the encoded RNA in a particular cell, either for subsequent translation of the RNA into a polypeptide or for subsequent trans regulatory activity by the RNA in the cell. An expression vector can contain the expression elements necessary to achieve, for example, sustained transcription of the encoding polynucleotide, or the regulatory elements can be operatively linked to the polynucleotide prior to its being cloned into the vector.

[0066] An expression vector (or the polynucleotide) generally contains or encodes a promoter sequence, which can provide constitutive or, if desired, inducible or tissue specific or developmental stage specific expression of the encoding polynucleotide, a poly-A recognition sequence, and a ribosome recognition site or internal ribosome entry site, or other regulatory elements such as an enhancer, which can be tissue specific. The vector also can contain elements required for replication in a prokaryotic or eukaryotic host system or both, as desired. Such vectors, which include plasmid vectors and viral vectors such as bacteriophage, baculovirus, retrovirus, lentivirus, adenovirus, vaccinia virus, alpha virus and adeno-associated virus vectors, are well known and can be purchased from a commercial source (Promega, Madison Wis.; Stratagene, La Jolla Calif.; GIBCO/BRL, Gaithersburg Md.) or can be constructed by one skilled in the art (see, for example, Meth. Enzymol., Vol. 185, Goeddel, ed. (Academic Press, Inc., 1990); Jolly, Canc. Gene Ther. 1:51-64, 1994; Flotte, J. Bioenerg. Biomemb 25:37-42, 1993; Kirshenbaum et al., J. Clin. Invest 92:381-387, 1993; each of which is incorporated herein by reference).

[0067] The term "construct" as used herein refers to a sequence of DNA artificially constructed by genetic engineering, recombineering or synthesis. In one embodiment, the DNA constructs are linearized prior to recombination. In another embodiment, the DNA constructs are not linearized prior to recombination.

[0068] The terms "inhibit", "neutralize", and "antagonize" are used interchangeably herein and encompass anti-CD115 antibodies that block, inhibit, and/or decrease the activity of CD115. Examples of CD115 activity include kinase function and ligand binding, e.g., binding to CSF-1 and/or IL-34.

[0069] The term "treating" with regard to a subject, refers to improving at least one symptom of the subject's disease or disorder. Treating includes curing, improving, or at least partially ameliorating the disease or disorder.

[0070] As used herein, the term "disorder" refers to, and is used interchangeably with, the terms disease, condition, or illness.

[0071] The term "pharmaceutically acceptable carrier" refers generally to any material (e.g., carrier, excipient, or stabilizer) that may accompany a therapeutic agent and is nontoxic to the subject or patient being exposed thereto.

[0072] The term "administering," as used herein, refers to any mode of transferring, delivering, introducing, or transporting a pharmaceutical composition or other agent, such as an anti-CD115 antibody, to a subject. Such modes include oral administration, topical contact, intravenous, intraperitoneal, intramuscular, intranasal, or subcutaneous administration.

[0073] The term "inhibit" or "neutralize" or "block" may relate generally to the ability of one or more anti-CD115 antibodies of the invention to decrease a biological activity of CD115, such as intracellular signaling and/or ligand binding. The inhibition/blocking of CSF-1 and/or IL-34 to CD115 preferably reduces or alters the normal level or type of cell signaling that occurs when CSF-1 and/or IL-34 binds to CD115 without inhibition or blocking. Inhibition and blocking are also intended to include any measurable decrease in the binding of CSF-1 and/or IL-34 to CD115 when in contact with an anti CD115 antibody as disclosed herein as compared to the ligand not in contact with an anti CD115 antibody, e.g., the blocking of CSF-1 and/or IL-34 to CD115 by at least about a 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% decrease, including all integers in between. In one embodiment, a neutralizing anti-CD115 antibody inhibits binding of CSF-1 and/or IL-34 to CD115 by at least about a 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% decrease, including all integers in between.

[0074] An antibody, or antigen-binding fragment thereof, is said to "specifically bind," "immunologically bind," and/or is "immunologically reactive" to CD115 if it reacts at a detectable level (within, for example, an ELISA assay) with CD115, and does not react detectably with unrelated polypeptides under similar conditions. Antibodies are considered to specifically bind to a target polypeptide when the binding affinity is at least $1x10^{-7}$ M or, preferably, at least $1x10^{-8}$ M. In one embodiment, the antibody, or antigen-binding fragment thereof, specifically binds human CD115.

[0075] Each embodiment in this specification is to be applied mutatis mutandis to every other embodiment unless expressly stated otherwise.

[0076] Standard techniques may be used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation (e.g., electroporation, lipofection). Enzymatic reactions and purification techniques may be performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein. These and related techniques and procedures may be generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. Unless specific definitions are provided, the nomenclature utilized in connection with, and the laboratory procedures and techniques of, molecular biology, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well-known and commonly used in the art. Standard techniques may be used for recombinant technology, molecular biological, microbiological, chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

CD115

[0077] CD115 is expressed by a variety of cells, including, but not limited to, hematopoietic stem cells (HSCs); myeloid cells, including monocytes, macrophages, osteoclasts, dendritic cells, and microglia; neural progenitor cells; and epithelial cells, including Paneth cells (Stanley and Chitu, Cold Spring Harb Perspect Biol 2014;6:a021857). Dysregulation of CD115, and/or its ligands, is associated with proliferative diseases and disorders (e.g., neoplasms, tumors and metastases), as well as immunological and neurological diseases and disorders, making it an important therapeutic target.

Anti-CD115 Antibodies

[0078] AlivaMab Mouse anti-CD115 antibodies were generated using both AlivaMab Mouse Kappa mice and AlivaMab Mouse Lambda mice (also referred to herein interchangeably as AlivaMab Kappa Mice and AlivaMab Lambda Mice, respectively). Antibodies produced by AlivaMab Kappa Mice comprise a chimeric immunoglobulin heavy (IgH) chain and a human immunoglobulin kappa (Igκ) light chain. Antibodies produced by AlivaMab Lambda Mice comprise a chimeric IgH chain and a human immunoglobulin lambda (Igλ) light chain. The chimeric IgH chain of the AlivaMab Mouse antibodies comprises a human variable region comprising a human variable heavy (VH) domain, a human diversity heavy (DH) domain, and a human joining heavy (JH) domain, a human constant heavy 1 (CH1) domain, a human upper hinge region (except for Cμ, which is

naturally missing an upper hinge region), a mouse middle hinge region, a mouse CH2 domain, and a mouse CH3 domain. Upon identification of a lead candidate antibody, e.g., an anti-CD115 antibody, the human heavy chain variable region is readily appended to a fully human constant region while maintaining the antigen-binding characteristics of the parent chimeric antibody that were developed *in vivo* in the AlivaMab Mouse. In one embodiment, the human heavy chain variable region, CH1 and, optionally, upper hinge region of the chimeric antibody are appended to human hinge, a human CH2 domain and a human CH3 domain in order to produce a fully human antibody.

[0079] Accordingly, in one embodiment, an anti-CD115 antibody, or an antigen-binding fragment thereof, of the invention is chimeric. In one embodiment, the chimeric anti-CD115 antibody, or an antigen-binding fragment thereof, comprises a chimeric IgH chain and a human Igκ chain. In one embodiment, the chimeric anti-CD115 antibody, or an antigen-binding fragment thereof, comprises a chimeric IgH chain and a human Igλ chain. In one embodiment, the chimeric anti-CD115 antibody is human and mouse. In one embodiment, an anti-CD115 antibody, or an antigen-binding fragment thereof, of the invention is human. In one embodiment, the human anti-CD115 antibody, or an antigen-binding fragment thereof, comprises a human IgH chain and a human Igκ chain. In one embodiment, the human anti-CD115 antibody, or an antigen-binding fragment thereof, comprises a human IgH chain and a human Igλ chain. In one embodiment, the isotype of the anti-CD115 antibody is selected from IgM, IgD, IgG1, IgG2, IgG3, IgG4, IgA and IgE. In one embodiment, the isotype of the anti-CD115 antibody is selected from IgG1, IgG2, IgG3, and IgG4.

[0080] In one embodiment, the anti-CD115 antibody binds an Fc receptor (FcR) selected from an FcγR, an FcεR, and an FcαR. In one embodiment, the anti-CD115 antibody binds an FcγR selected from FcγRI (CD64), FcγRII (CD32), and FcγRIII (CD16), including isoforms thereof. In one embodiment, the Fc region of the anti-CD115 antibody comprises a mutation so that it preferentially binds a particular FcγR (see, e.g., U.S. 6,737,056 and U.S. 2015/0031862).

[0081] In one aspect of the invention, the CDRs of an anti-CD115 antibody, or antigen-binding fragment thereof, may be mixed and matched between the CDRs of antibody clones described herein. In one embodiment, an anti-CD115 antibody, or antigen-binding fragment thereof, comprises a VHCDR1 comprising any one of SEQ ID NOs:436-543, a VHCDR2 comprising any one of SEQ ID NOs:868-975, and a VHCDR3 comprising any one of SEQ ID NOs:1300-1407. In one embodiment, the VHCDR1, VHCDR2 and VHCDR3 are

selected from three different anti-CD115 clones disclosed herein. In one embodiment, the VHCDR1, VHCDR2 and VHCDR3 are selected from two different anti-CD115 clones disclosed herein.

[0082] In one embodiment, an anti-CD115 antibody, or an antigen-binding fragment thereof, comprises a VLCDR1 comprising any one of SEQ ID NOs:652-759, a VLCDR2 comprising any one of SEQ ID NOs:1084-1191, and a VLCDR3 comprising any one of SEQ ID NOs:1516-1623. In one embodiment, the VLCDR1, VLCDR2 and VLCDR3 are selected from three different anti-CD115 clones disclosed herein. In one embodiment, the VLCDR1, VLCDR2 and VLCDR3 are selected from two different anti-CD115 clones disclosed herein.

[0083] In one embodiment, an anti-CD115 antibody, or an antigen-binding fragment thereof, comprises 1) comprises a VHCDR1 comprising any one of SEQ ID NOs: 436-543, a VHCDR2 comprising any one of SEQ ID NOs: 868-975, and a VHCDR3 comprising any one of SEQ ID NOs: 300-1407, and 2) a VLCDR1 comprising any one of SEQ ID NOs: 652-759, a VLCDR2 comprising any one of SEQ ID NOs: 1084-1191, and a VLCDR3 comprising any one of SEQ ID NOs: 1516-1623.

[0084] In one aspect of the invention, the CDRs of an anti-CD115 antibody, or antigen-binding fragment thereof, are from the same anti-CD115 antibody clone disclosed herein. In one embodiment, an anti-CD115 antibody, or an antigen-binding fragment thereof, comprises a VHCDR1, a VHCDR2 and a VHCDR3 from the same anti-CD115 clone disclosed herein. In one embodiment, an anti-CD115 antibody, or an antigen-binding fragment thereof, comprises a VHCDR1, a VHCDR2, and a VHCDR3 of a VH selected from any one of SEQ ID NOs:109-216. In one embodiment, an anti-CD115 antibody, or an antigen-binding fragment thereof, comprises a VHCDR1, a VHCDR2, and a VHCDR3 comprising the corresponding sequences listed in Table 3.

[0085] Accordingly, in one embodiment, an anti-CD115 antibody, or an antigen-binding fragment thereof, comprises a VHCDR1, a VHCDR2, and a VHCDR3 selected from: SEQ ID NOs:436, 868, and 1300; SEQ ID NOs:437, 869, and 1301; SEQ ID NOs:438, 870, and 1302; SEQ ID NOs:439, 871, and 1303; SEQ ID NOs:440, 872, and 1304; SEQ ID NOs:441, 873, and 1305; SEQ ID NOs:442, 874, and 1306; SEQ ID NOs:443, 875, and 1307; SEQ ID NOs:444, 876, and 1308; SEQ ID NOs:445, 877, and 1309; SEQ ID NOs:446, 878, and 1310; SEQ ID NOs:447, 879, and 1311; SEQ ID NOs:448, 880, and 1312; SEQ ID NOs:449, 881, and 1313; SEQ ID NOs:450, 882, and 1314; SEQ ID NOs:451, 883, and 1315; SEQ ID NOs:452, 884, and 1316; SEQ ID NOs:453, 885, and 1317; SEQ ID NOs:454, 886, and 1318; SEQ ID NOs:455, 887, and 1319; SEQ ID NOs:456, 888, and 1320; SEQ ID

NOs:457, 889, and 1321; SEQ ID NOs:458, 890, and 1322; SEQ ID NOs:459, 891, and 1323; SEQ ID NOs:460, 892, and 1324; SEQ ID NOs:461, 893, and 1325; SEQ ID NOs:462, 894, and 1326; SEQ ID NOs:463, 895, and 1327; SEQ ID NOs:464, 896, and 1328; SEQ ID NOs:465, 897, and 1329; SEQ ID NOs:466, 898, and 1330; SEQ ID NOs:467, 899, and 1331; SEQ ID NOs:468, 900, and 1332; SEQ ID NOs:469, 901, and 1333; SEQ ID NOs:470, 902, and 1334; SEQ ID NOs:471, 903, and 1335; SEQ ID NOs:472, 904, and 1336; SEQ ID NOs:473, 905, and 1337; SEQ ID NOs:474, 906, and 1338; SEQ ID NOs:475, 907, and 1339; SEQ ID NOs:476, 908, and 1340; SEQ ID NOs:477, 909, and 1341; SEQ ID NOs:478, 910, and 1342; SEQ ID NOs:479, 911, and 1343; SEQ ID NOs:480, 912, and 1344; SEQ ID NOs:481, 913, and 1345; SEQ ID NOs:482, 914, and 1346; SEQ ID NOs:483, 915, and 1347; SEQ ID NOs:484, 916, and 1348; SEQ ID NOs:485, 917, and 1349; SEQ ID NOs:486, 918, and 1350; SEQ ID NOs:487, 919, and 1351; SEQ ID NOs:488, 920, and 1352; SEQ ID NOs:489, 921, and 1353; SEQ ID NOs:490, 922, and 1354; SEQ ID NOs:491, 923, and 1355; SEQ ID NOs:492, 924, and 1356; SEQ ID NOs:493, 925, and 1357; SEQ ID NOs:494, 926, and 1358; SEQ ID NOs:495, 927, and 1359; SEQ ID NOs:496, 928, and 1360; SEQ ID NOs:497, 929, and 1361; SEQ ID NOs:498, 930, and 1362; SEQ ID NOs:499, 931, and 1363; SEQ ID NOs:500, 932, and 1364; SEQ ID NOs:501, 933, and 1365; SEQ ID NOs:502, 934, and 1366; SEQ ID NOs:503, 935, and 1367; SEQ ID NOs:504, 936, and 1368; SEQ ID NOs:505, 937, and 1369; SEQ ID NOs:506, 938, and 1370; SEQ ID NOs:507, 939, and 1371; SEQ ID NOs:508, 940, and 1372; SEQ ID NOs:509, 941, and 1373; SEQ ID NOs:510, 942, and 1374; SEQ ID NOs:511, 943, and 1375; SEQ ID NOs:512, 944, and 1376; SEQ ID NOs:513, 945, and 1377; SEQ ID NOs:514, 946, and 1378; SEQ ID NOs:515, 947, and 1379; SEQ ID NOs:516, 948, and 1380; SEQ ID NOs:517, 949, and 1381; SEQ ID NOs:518, 950, and 1382; SEQ ID NOs:519, 951, and 1383; SEQ ID NOs:520, 952, and 1384; SEQ ID NOs:521, 953, and 1385; SEQ ID NOs:522, 954, and 1386; SEQ ID NOs:523, 955, and 1387; SEQ ID NOs:524, 956, and 1388; SEQ ID NOs:525, 957, and 1389; SEQ ID NOs:526, 958, and 1390; SEQ ID NOs:527, 959, and 1391; SEQ ID NOs:528, 960, and 1392; SEQ ID NOs:529, 961, and 1393; SEQ ID NOs:530, 962, and 1394; SEQ ID NOs:531, 963, and 1395; SEQ ID NOs:532, 964, and 1396; SEQ ID NOs:533, 965, and 1397; SEQ ID NOs:534, 966, and 1398; SEQ ID NOs:535, 967, and 1399; SEQ ID NOs:536, 968, and 1400; SEQ ID NOs:537, 969, and 1401; SEQ ID NOs:538, 970, and 1402; SEQ ID NOs:539, 971, and 1403; SEQ ID NOs:540, 972, and 1404; SEQ ID NOs:541, 973, and 1405; SEQ ID NOs:542, 974, and 1406; and SEQ ID NOs:543, 975, and 1407.

[0086] In one embodiment, an anti-CD115 antibody, or an antigen-binding fragment thereof, comprises a VLCDR1, a VLCDR2 and a VLCDR3 from the same anti-CD115 clone disclosed herein. In one embodiment, an anti-CD115 antibody, or an antigen-binding fragment thereof, comprises a VLCDR1, a VLCDR2, and a VLCDR3 of a VL selected from any one of SEQ ID NOs:325-432. In one embodiment, an anti-CD115 antibody, or an antigen-binding fragment thereof, comprises a VLCDR1, a VLCDR2, and a VLCDR3 comprising the corresponding sequences listed in Table 3.

[0087] Accordingly, in one embodiment, an anti-CD115 antibody, or an antigen-binding fragment thereof, comprises a VLCDR1, a VLCDR2, and a VLCDR3 selected from: SEQ ID NOs:652, 1084, and 1516; SEQ ID NOs:653, 1085, and 1517; SEQ ID NOs:654, 1086, and 1518; SEQ ID NOs:655, 1087, and 1519; SEQ ID NOs:656, 1088, and 1520; SEQ ID NOs:657, 1089, and 1521; SEQ ID NOs:658, 1090, and 1522; SEQ ID NOs:659, 1091, and 1523; SEQ ID NOs:660, 1092, and 1524; SEQ ID NOs:661, 1093, and 1525; SEQ ID NOs:662, 1094, and 1526; SEQ ID NOs:663, 1095, and 1527; SEQ ID NOs:664, 1096, and 1528; SEQ ID NOs:665, 1097, and 1529; SEQ ID NOs:666, 1098, and 1530; SEQ ID NOs:667, 1099, and 1531; SEQ ID NOs:668, 1100, and 1532; SEQ ID NOs:669, 1101, and 1533; SEQ ID NOs:670, 1102, and 1534; SEQ ID NOs:671, 1103, and 1535; SEQ ID NOs:672, 1104, and 1536; SEQ ID NOs:673, 1105, and 1537; SEQ ID NOs:674, 1106, and 1538; SEQ ID NOs:675, 1107, and 1539; SEQ ID NOs:676, 1108, and 1540; SEQ ID NOs:677, 1109, and 1541; SEQ ID NOs:678, 1110, and 1542; SEQ ID NOs:679, 1111, and 1543; SEQ ID NOs:680, 1112, and 1544; SEQ ID NOs:681, 1113, and 1545; SEQ ID NOs:682, 1114, and 1546; SEQ ID NOs:683, 1115, and 1547; SEQ ID NOs:684, 1116, and 1548; SEQ ID NOs:685, 1117, and 1549; SEQ ID NOs:686, 1118, and 1550; SEQ ID NOs:687, 1119, and 1551; SEQ ID NOs:688, 1120, and 1552; SEQ ID NOs:689, 1121, and 1553; SEQ ID NOs:690, 1122, and 1554; SEQ ID NOs:691, 1123, and 1555; SEQ ID NOs:692, 1124, and 1556; SEQ ID NOs:693, 1125, and 1557; SEQ ID NOs:694, 1126, and 1558; SEQ ID NOs:695, 1127, and 1559; SEQ ID NOs:696, 1128, and 1560; SEQ ID NOs:697, 1129, and 1561; SEQ ID NOs:698, 1130, and 1562; SEQ ID NOs:699, 1131, and 1563; SEQ ID NOs:700, 1132, and 1564; SEQ ID NOs:701, 1133, and 1565; SEQ ID NOs:702, 1134, and 1566; SEQ ID NOs:703, 1135, and 1567; SEQ ID NOs:704, 1136, and 1568; SEQ ID NOs:705, 1137, and 1569; SEQ ID NOs:706, 1138, and 1570; SEQ ID NOs:707, 1139, and 1571; SEQ ID NOs:708, 1140, and 1572; SEQ ID NOs:709, 1141, and 1573; SEQ ID NOs:710, 1142, and 1574; SEQ ID NOs:711, 1143, and 1575; SEQ ID NOs:712, 1144, and 1576; SEQ ID NOs:713, 1145, and 1577; SEQ ID NOs:714, 1146, and

1578; SEQ ID NOs:715, 1147, and 1579; SEQ ID NOs:716, 1148, and 1580; SEQ ID NOs:717, 1149, and 1581; SEQ ID NOs:718, 1150, and 1582; SEQ ID NOs:719, 1151, and 1583; SEQ ID NOs:720, 1152, and 1584; SEQ ID NOs:721, 1153, and 1585; SEQ ID NOs:722, 1154, and 1586; SEQ ID NOs:723, 1155, and 1587; SEQ ID NOs:724, 1156, and 1588; SEQ ID NOs:725, 1157, and 1589; SEQ ID NOs:726, 1158, and 1590; SEQ ID NOs:727, 1159, and 1591; SEQ ID NOs:728, 1160, and 1592; SEQ ID NOs:729, 1161, and 1593; SEQ ID NOs:730, 1162, and 1594; SEQ ID NOs:731, 1163, and 1595; SEQ ID NOs:732, 1164, and 1596; SEQ ID NOs:733, 1165, and 1597; SEQ ID NOs:734, 1166, and 1598; SEQ ID NOs:735, 1167, and 1599; SEQ ID NOs:736, 1168, and 1600; SEQ ID NOs:737, 1169, and 1601; SEQ ID NOs:738, 1170, and 1602; SEQ ID NOs:739, 1171, and 1603; SEQ ID NOs:740, 1172, and 1604; SEQ ID NOs:741, 1173, and 1605; SEQ ID NOs:742, 1174, and 1606; SEQ ID NOs:743, 1175, and 1607; SEQ ID NOs:744, 1176, and 1608; SEQ ID NOs:745, 1177, and 1609; SEQ ID NOs:746, 1178, and 1610; SEQ ID NOs:747, 1179, and 1611; SEQ ID NOs:748, 1180, and 1612; SEQ ID NOs:749, 1181, and 1613; SEQ ID NOs:750, 1182, and 1614; SEQ ID NOs:751, 1183, and 1615; SEQ ID NOs:752, 1184, and 1616; SEQ ID NOs:753, 1185, and 1617; SEQ ID NOs:754, 1186, and 1618; SEQ ID NOs:755, 1187, and 1619; SEQ ID NOs:756, 1188, and 1620; SEQ ID NOs:757, 1189, and 1621; SEQ ID NOs:758, 1190, and 1622; and SEQ ID NOs:759, 1191, and 1623.

[0088] In another aspect of the invention, the CDRs of an anti-CD115 antibody, or antigen-binding fragment thereof, are selected from the corresponding VH and VL of a single clone described herein. In one embodiment, an anti-CD115 antibody, or an antigen-binding fragment thereof, comprises 1) a VHCDR1, a VHCDR2, and a VHCDR3 selected from the VHCDR1, VHCDR2 and VHCDR3 of one VH selected from any one of SEQ ID NOs: 109-216 and 2) a VLCDR1, a VLCDR2, and a VLCDR3 selected from the VLCDR1, VLCDR2 and VLCDR3 of one VL selected from any one of SEQ ID NOs:325-432. In one embodiment, an anti-CD115 antibody, or antigen-binding fragment thereof, comprises a VHCDR1, a VHCDR2, a VHCDR3, a VLCDR1, a VLCDR2, and a VLCDR3 within the corresponding VH and VL amino acid sequences of a single clone as set forth in Table 3.

[0089] Accordingly, in one embodiment, an anti-CD115 antibody, or an antigen-binding fragment thereof, comprises a VHCDR1, a VHCDR2, a VHCDR3, a VLCDR1, a VLCDR2, and a VLCDR3 selected from: SEQ ID NOs:436, 868, 1300, 652, 1084, and 1516; SEQ ID NOs:437, 869, 1301, 653, 1085, and 1517; SEQ ID NOs:438, 870, 1302, 654, 1086, and 1518; SEQ ID NOs:439, 871, 1303, 655, 1087, and 1519; SEQ ID NOs:440, 872, 1304,

656, 1088, and 1520; SEQ ID NOs:441, 873, 1305, 657, 1089, and 1521; SEQ ID NOs:442, 874, 1306, 658, 1090, and 1522; SEQ ID NOs:443, 875, 1307, 659, 1091, and 1523; SEQ ID NOs:444, 876, 1308, 660, 1092, and 1524; SEQ ID NOs:445, 877, 1309, 661, 1093, and 1525; SEQ ID NOs:446, 878, 1310, 662, 1094, and 1526; SEQ ID NOs:447, 879, 1311, 663, 1095, and 1527; SEQ ID NOs:448, 880, 1312, 664, 1096, and 1528; SEQ ID NOs:449, 881, 1313, 665, 1097, and 1529; SEQ ID NOs:450, 882, 1314, 666, 1098, and 1530; SEQ ID NOs:451, 883, 1315, 667, 1099, and 1531; SEQ ID NOs:452, 884, 1316, 668, 1100, and 1532; SEQ ID NOs:453, 885, 1317, 669, 1101, and 1533; SEQ ID NOs:454, 886, 1318, 670, 1102, and 1534; SEQ ID NOs:455, 887, 1319, 671, 1103, and 1535; SEQ ID NOs:456, 888, 1320, 672, 1104, and 1536; SEQ ID NOs:457, 889, 1321, 673, 1105, and 1537; SEQ ID NOs:458, 890, 1322, 674, 1106, and 1538; SEQ ID NOs:459, 891, 1323, 675, 1107, and 1539; SEQ ID NOs:460, 892, 1324, 676, 1108, and 1540; SEQ ID NOs:461, 893, 1325, 677, 1109, and 1541; SEQ ID NOs:462, 894, 1326, 678, 1110, and 1542; SEQ ID NOs:463, 895, 1327, 679, 1111, and 1543; SEQ ID NOs:464, 896, 1328, 680, 1112, and 1544; SEQ ID NOs:465, 897, 1329, 681, 1113, and 1545; SEQ ID NOs:466, 898, 1330, 682, 1114, and 1546; SEQ ID NOs:467, 899, 1331, 683, 1115, and 1547; SEQ ID NOs:468, 900, 1332, 684, 1116, and 1548; SEQ ID NOs:469, 901, 1333, 685, 1117, and 1549; SEQ ID NOs:470, 902, 1334, 686, 1118, and 1550; SEQ ID NOs:471, 903, 1335, 687, 1119, and 1551; SEQ ID NOs:472, 904, 1336, 688, 1120, and 1552; SEQ ID NOs:473, 905, 1337, 689, 1121, and 1553; SEQ ID NOs:474, 906, 1338, 690, 1122, and 1554; SEQ ID NOs:475, 907, 1339, 691, 1123, and 1555; SEQ ID NOs:476, 908, 1340, 692, 1124, and 1556; SEQ ID NOs:477, 909, 1341, 693, 1125, and 1557; SEQ ID NOs:478, 910, 1342, 694, 1126, and 1558; SEQ ID NOs:479, 911, 1343, 695, 1127, and 1559; SEQ ID NOs:480, 912, 1344, 696, 1128, and 1560; SEQ ID NOs:481, 913, 1345, 697, 1129, and 1561; SEQ ID NOs:482, 914, 1346, 698, 1130, and 1562; SEQ ID NOs:483, 915, 1347, 699, 1131, and 1563; SEQ ID NOs:484, 916, 1348, 700, 1132, and 1564; SEQ ID NOs:485, 917, 1349, 701, 1133, and 1565; SEQ ID NOs:486, 918, 1350, 702, 1134, and 1566; SEQ ID NOs:487, 919, 1351, 703, 1135, and 1567; SEQ ID NOs:488, 920, 1352, 704, 1136, and 1568; SEQ ID NOs:489, 921, 1353, 705, 1137, and 1569; SEQ ID NOs:490, 922, 1354, 706, 1138, and 1570; SEQ ID NOs:491, 923, 1355, 707, 1139, and 1571; SEQ ID NOs:492, 924, 1356, 708, 1140, and 1572; SEQ ID NOs:493, 925, 1357, 709, 1141, and 1573; SEQ ID NOs:494, 926, 1358, 710, 1142, and 1574; SEQ ID NOs:495, 927, 1359, 711, 1143, and 1575; SEQ ID NOs:496, 928, 1360, 712, 1144, and 1576; SEQ ID NOs:497, 929, 1361, 713, 1145, and 1577; SEQ ID NOs:498, 930, 1362, 714, 1146, and 1578; SEQ ID NOs:499, 931, 1363, 715, 1147, and 1579; SEQ ID

NOs:500, 932, 1364, 716, 1148, and 1580; SEQ ID NOs:501, 933, 1365, 717, 1149, and 1581; SEQ ID NOs:502, 934, 1366, 718, 1150, and 1582; SEQ ID NOs:503, 935, 1367, 719, 1151, and 1583; SEQ ID NOs:504, 936, 1368, 720, 1152, and 1584; SEQ ID NOs:505, 937, 1369, 721, 1153, and 1585; SEQ ID NOs:506, 938, 1370, 722, 1154, and 1586; SEQ ID NOs:507, 939, 1371, 723, 1155, and 1587; SEQ ID NOs:508, 940, 1372, 724, 1156, and 1588; SEQ ID NOs:509, 941, 1373, 725, 1157, and 1589; SEQ ID NOs:510, 942, 1374, 726, 1158, and 1590; SEQ ID NOs:511, 943, 1375, 727, 1159, and 1591; SEQ ID NOs:512, 944, 1376, 728, 1160, and 1592; SEQ ID NOs:513, 945, 1377, 729, 1161, and 1593; SEQ ID NOs:514, 946, 1378, 730, 1162, and 1594; SEQ ID NOs:515, 947, 1379, 731, 1163, and 1595; SEQ ID NOs:516, 948, 1380, 732, 1164, and 1596; SEQ ID NOs:517, 949, 1381, 733, 1165, and 1597; SEQ ID NOs:518, 950, 1382, 734, 1166, and 1598; SEQ ID NOs:519, 951, 1383, 735, 1167, and 1599; SEQ ID NOs:520, 952, 1384, 736, 1168, and 1600; SEQ ID NOs:521, 953, 1385, 737, 1169, and 1601; SEQ ID NOs:522, 954, 1386, 738, 1170, and 1602; SEQ ID NOs:523, 955, 1387, 739, 1171, and 1603; SEQ ID NOs:524, 956, 1388, 740, 1172, and 1604; SEQ ID NOs:525, 957, 1389, 741, 1173, and 1605; SEQ ID NOs:526, 958, 1390, 742, 1174, and 1606; SEQ ID NOs:527, 959, 1391, 743, 1175, and 1607; SEQ ID NOs:528, 960, 1392, 744, 1176, and 1608; SEQ ID NOs:529, 961, 1393, 745, 1177, and 1609; SEQ ID NOs:530, 962, 1394, 746, 1178, and 1610; SEQ ID NOs:531, 963, 1395, 747, 1179, and 1611; SEQ ID NOs:532, 964, 1396, 748, 1180, and 1612; SEQ ID NOs:533, 965, 1397, 749, 1181, and 1613; SEQ ID NOs:534, 966, 1398, 750, 1182, and 1614; SEQ ID NOs:535, 967, 1399, 751, 1183, and 1615; SEQ ID NOs:536, 968, 1400, 752, 1184, and 1616; SEQ ID NOs:537, 969, 1401, 753, 1185, and 1617; SEQ ID NOs:538, 970, 1402, 754, 1186, and 1618; SEQ ID NOs:539, 971, 1403, 755, 1187, and 1619; SEQ ID NOs:540, 972, 1404, 756, 1188, and 1620; SEQ ID NOs:541, 973, 1405, 757, 1189, and 1621; SEQ ID NOs:542, 974, 1406, 758, 1190, and 1622; and SEQ ID NOs:543, 975, 1407, 759, 1191, and 1623.

[0090] In one embodiment, an anti-CD115 antibody, or antigen-binding fragment thereof, comprises a VH comprising any one of SEQ ID NOs: 109-216. In one embodiment, an anti-CD115 antibody, or antigen-binding fragment thereof, comprises a VL comprising any one of SEQ ID NOs:325-432. In one embodiment, an anti-CD115 antibody, or an antigen-binding fragment thereof, comprises a corresponding VH and VL of a single clone as set forth in Table 3.

[0091] Accordingly, in one embodiment, an anti-CD115 antibody, or an antigen-binding fragment thereof, comprises a VH and a VL selected from: SEQ ID NOs:109 and

325; SEQ ID NOs:110 and 326; SEQ ID NOs:111 and 327; SEQ ID NOs:112 and 328; SEQ ID NOs:113 and 329; SEQ ID NOs:114 and 330; SEQ ID NOs:115 and 331; SEQ ID NOs:116 and 332; SEQ ID NOs:117 and 333; SEQ ID NOs:118 and 334; SEQ ID NOs:119 and 335; SEQ ID NOs:120 and 336; SEQ ID NOs:121 and 337; SEQ ID NOs:122 and 338; SEQ ID NOs:123 and 339; SEQ ID NOs:124 and 340; SEQ ID NOs:125 and 341; SEQ ID NOs:126 and 342; SEQ ID NOs:127 and 343; SEQ ID NOs:128 and 344; SEQ ID NOs:129 and 345; SEQ ID NOs:130 and 346; SEQ ID NOs:131 and 347; SEQ ID NOs:132 and 348; SEQ ID NOs:133 and 349; SEQ ID NOs:134 and 350; SEQ ID NOs:135 and 351; SEQ ID NOs:136 and 352; SEQ ID NOs:137 and 353; SEQ ID NOs:138 and 354; SEQ ID NOs:139 and 355; SEQ ID NOs:140 and 356; SEQ ID NOs:141 and 357; SEQ ID NOs:142 and 358; SEQ ID NOs:143 and 359; SEQ ID NOs:144 and 360; SEQ ID NOs:145 and 361; SEQ ID NOs:146 and 362; SEQ ID NOs:147 and 363; SEQ ID NOs:148 and 364; SEQ ID NOs:149 and 365; SEQ ID NOs:150 and 366; SEQ ID NOs:151 and 367; SEQ ID NOs:152 and 368; SEQ ID NOs:153 and 369; SEQ ID NOs:154 and 370; SEQ ID NOs:155 and 371; SEQ ID NOs:156 and 372; SEQ ID NOs:157 and 373; SEQ ID NOs:158 and 374; SEQ ID NOs:159 and 375; SEQ ID NOs:160 and 376; SEQ ID NOs:161 and 377; SEQ ID NOs:162 and 378; SEQ ID NOs:163 and 379; SEQ ID NOs:164 and 380; SEQ ID NOs:165 and 381; SEQ ID NOs:166 and 382; SEQ ID NOs:167 and 383; SEQ ID NOs:168 and 384; SEQ ID NOs:169 and 385; SEQ ID NOs:170 and 386; SEQ ID NOs:171 and 387; SEQ ID NOs:172 and 388; SEQ ID NOs:173 and 389; SEQ ID NOs:174 and 390; SEQ ID NOs:175 and 391; SEQ ID NOs:176 and 392; SEQ ID NOs:177 and 393; SEQ ID NOs:178 and 394; SEQ ID NOs:179 and 395; SEQ ID NOs:180 and 396; SEQ ID NOs:181 and 397; SEQ ID NOs:182 and 398; SEQ ID NOs:183 and 399; SEQ ID NOs:184 and 400; SEQ ID NOs:185 and 401; SEQ ID NOs:186 and 402; SEQ ID NOs:187 and 403; SEQ ID NOs:188 and 404; SEQ ID NOs:189 and 405; SEQ ID NOs:190 and 406; SEQ ID NOs:191 and 407; SEQ ID NOs:192 and 408;SEQ ID NOs:193 and 409; SEQ ID NOs:194 and 410; SEQ ID NOs:195 and 411; SEQ ID NOs:196 and 412; SEQ ID NOs:197 and 413; SEQ ID NOs:198 and 414; SEQ ID NOs:199 and 415; SEQ ID NOs:200 and 416; SEQ ID NOs:201 and 417; SEQ ID NOs:202 and 418; SEQ ID NOs:203 and 419; SEQ ID NOs:204 and 420; SEQ ID NOs:205 and 421; SEQ ID NOs:206 and 422; SEQ ID NOs:207 and 423; SEQ ID NOs:208 and 424; SEQ ID NOs:209 and 425; SEQ ID NOs:210 and 426; SEQ ID NOs:211 and 427; SEQ ID NOs:212 and 428; SEQ ID NOs:213 and 429; SEQ ID NOs:214 and 430; SEQ ID NOs:215 and 431; and SEQ ID NOs:216 and 432.

[0092] In one embodiment, an anti-CD115 antibody is a whole antibody. In one embodiment, an anti-CD115 antibody is a single chain antibody. In one embodiment, an anti-CD115 antibody is a scFv. In one embodiment, an anti-CD115 antibody is a Fab. In one embodiment, an anti-CD115 antibody is a $F(ab')_2$. In one embodiment, an anti-CD115 antibody is a Fv.

[0093] In one embodiment, an anti-CD115 antibody is a bispecific antibody. In one embodiment, a bispecific anti-CD115 antibody specifically recognizes two different epitopes of CD115. In one embodiment, a bispecific anti-CD115 comprises a first CDR set comprising the VHCDR1, VHCDR2, VHCDR3, VLCDR1, VLCDR2, and VLCDR3 from a first anti-CD115 antibody clone disclosed herein and a second CDR set comprising the VHCDR1, VHCDR2, VHCDR3, VLCDR1, VLCDR2, and VLCDR3 of a second anti-CD115 antibody clone disclosed herein. In one embodiment, a bispecific anti-CD115 comprises a corresponding first VH and first VL of a first anti-CD115 antibody clone disclosed herein and a corresponding second VH and second VL of a second anti-CD115 antibody clone disclosed herein. In one embodiment, a bispecific anti-CD115 antibody specifically recognizes CD115 and another antigen.

Neutralizing Anti-CD115 Antibodies

[0094] One aspect of the present invention provides anti-CD115 antibodies, and antigen-binding fragments thereof, that are CD115 antagonists. In one embodiment, an antagonist anti-CD115 antibody, or antigen-binding fragment thereof, neutralizes or inhibits one or more ligands of CD115 from binding CD115. In one embodiment, an antagonist anti-CD115 antibody, or antigen-binding fragment thereof, inhibits CSF-1 from binding CD115. In one embodiment, an antagonist anti-CD115 antibody, or antigen-binding fragment thereof, inhibits IL-34 from binding CD115. In one embodiment, an antagonist anti-CD115 antibody, or antigen-binding fragment thereof, inhibits CSF-1 and IL-34 from binding CD115. In one embodiment, an antagonist anti-CD115 antibody, or antigen-binding fragment thereof, prevents dimerization of CD115, including dimerization that is induced by CSF-1 or IL-34 binding or that may happen spontaneously under certain conditions of expression CD115. In one embodiment, an antagonist anti-CD115 antibody, or antigen-binding fragment thereof, inhibits CD115 signaling. In one embodiment, an antagonist anti-CD115 antibody, or antigen-binding fragment thereof, inhibits ligand-induced phosphorylation of CD115.

Polynucleotides

[0095] One aspect of the present invention provides a polynucleotide sequence that encodes an anti-CD115 antibody, or antigen-binding fragment thereof, disclosed herein. In one embodiment, the polynucleotide is a recombinant polynucleotide. In one embodiment, the polynucleotide is cDNA.

[0096] In one embodiment, a polynucleotide sequence encodes a CDR of an anti-CD115 antibody disclosed herein. In one embodiment, the polynucleotide comprises a VHCDR1 polynucleotide sequence selected from any one of SEQ ID NOs:544-651. In one embodiment, the polynucleotide comprises a VHCDR2 polynucleotide sequence selected from any one of SEQ ID NOs:976-1083. In one embodiment, the polynucleotide comprises a VHCDR3 polynucleotide sequence selected from any one of SEQ ID NOs:1408-1515. In one embodiment, the polynucleotide comprises a VLCDR1 polynucleotide sequence selected from any one of SEQ ID NOs:760-867. In one embodiment, the polynucleotide comprises a VLCDR2 polynucleotide sequence selected from any one of SEQ ID NOs:1192-1299. In one embodiment, the polynucleotide comprises a VLCDR3 polynucleotide sequence selected from any one of SEQ ID NOs:1624-1731.

[0097] In one embodiment, a polynucleotide sequence encodes a VH of an anti-CD115 antibody disclosed herein. In one embodiment, the polynucleotide comprises a VH polynucleotide sequence selected from any one of SEQ ID NOs:1-108. In one embodiment, a polynucleotide sequence encodes a VL of an anti-CD115 antibody disclosed herein. In one embodiment, the polynucleotide comprises a VL polynucleotide sequence selected from any one of SEQ ID NOs:217-324. In one embodiment, a polynucleotide sequence encodes a VH and a VL of an anti-CD115 antibody disclosed herein.

[0098] One embodiment of the invention provides a vector comprising a polynucleotide sequence encoding an anti-CD115 antibody, or an antigen-binding fragment thereof, disclosed herein. In one embodiment, the vector is an expression vector. In one embodiment, the vector is a cloning vector. One embodiment of the invention provides a host cell comprising the vector.

Methods of Use

[0099] The AlivaMab antibodies against CD115, and in particular fully human antibodies incorporating all or portions of the heavy chain and light chain variable regions from the AlivaMab antibodies, may have utility for the treatment of human disease including, but not limited to, diseases in oncology and autoimmunity and inflammation. As the understanding of CD115 biology and disease association becomes better known, it is

expected that opportunities for human clinical therapeutic indications may expand. In particular, oncological, immunological, and neurological diseases and disorders are contemplated.

[00100] An anti-CD115 antibody, or antigen-binding fragment thereof, disclosed herein may be used in research, diagnostic, and/or therapeutic methods. In one embodiment, an anti-CD115 antibody, or antigen-binding fragment thereof, disclosed herein is used to treat diseases and disorders associated with CD115, CSF-1 and/or IL-34. In one embodiment, an anti-CD115 antibody, or antigen-binding fragment thereof, disclosed herein is used to treat diseases and disorders associated with CD115 overexpression. In one embodiment, an anti-CD115 antibody, or antigen-binding fragment thereof, disclosed herein is used to treat diseases and disorders associated with CSF-1 overexpression. In one embodiment, an anti-CD115 antibody, or antigen-binding fragment thereof, disclosed herein is used to treat diseases and disorders associated with IL-34 overexpression. In one embodiment, an anti-CD115 antibody, or antigen-binding fragment thereof, disclosed herein is used to treat diseases and disorders associated with aberrant CD115 signaling.

[00101] Embodiments of the invention pertain to the use of anti-CD115 antibodies, or antigen-binding fragments thereof, for the diagnosis and prognosis of diseases and disorders associated with CD115, CSF-1 and/or IL-34 or aberrant expression thereof.

Modified Anti-CD115 Antibodies and Compositions

[00102] Anti-CD115 antibodies of the present invention, and antigen-binding fragments and variants thereof, may also be conjugated or operably linked to another compound (e.g., therapeutic agent, label, or tag), referred to herein as a conjugate. The conjugate may be a cytotoxic agent, a chemotherapeutic agent, a cytokine, an anti-angiogenic agent, a tyrosine kinase inhibitor, a toxin, a radioisotope, or other therapeutically active agent. Chemotherapeutic agents, cytokines, anti-angiogenic agents, tyrosine kinase inhibitors, and other therapeutic agents are contemplated. In one embodiment, the antibody is conjugated or operably linked to a toxin, including but not limited to small molecule toxins and enzymatically active toxins of bacterial, fungal, plant, animal or synthetic origin, including fragments and/or variants thereof.

[00103] There are many linking groups known in the art for making antibody conjugates, including, for example, those disclosed in U.S. Pat. No. 5,208,020 or EP Patent 0425235 B1, and Chari et al., Cancer Research 52: 127-131 (1992). The linking groups include disulfide groups, thioether groups, acid labile groups, photolabile groups, peptidase

labile groups, or esterase labile groups, as disclosed in the above-identified patents, disulfide and thioether groups being preferred.

[00104] The present invention further relates to pharmaceutical compositions and methods of use. The pharmaceutical compositions of the present invention include an antibody, or fragment thereof, in a pharmaceutically acceptable carrier. Pharmaceutical compositions may be administered *in vivo* for the treatment or prevention of a disease or disorder. Furthermore, pharmaceutical compositions comprising an antibody, or a fragment thereof, of the present invention may include one or more agents for use in combination, or may be administered in conjunction with one or more agents. Agents for use in combination with an anti-CD115 antibody disclosed herein include, but are not limited to cytotoxic agents, chemotherapeutic agents, cytokines, anti-angiogenic agents, tyrosine kinase inhibitors, toxins, and radioisotopes.

[00105] The present invention also provides kits relating to any of the antibodies, or fragments thereof, and/or methods described herein. Kits of the present invention may include diagnostic or therapeutic agents. A kit of the present invention may further provide instructions for use of a composition or antibody and packaging. A kit of the present invention may include devices, reagents, containers or other components. Furthermore, a kit of the present invention may also require the use of an apparatus, instrument or device, including a computer.

EXAMPLES

[00106] The Examples below utilize a CD115 phosphorylation assay in order to detect phospho-CD115. SR cells (confirmed CD115 expression by FACS) were serum starved overnight (1% FBS). Cells were treated with M-CSF or IL-34 in the presence of CD115 mAbs for 30 minutes on ice. Cells were lysed in buffer containing phosphatase and protease inhibitors. Lysates were run on R&D systems p-MCSFR DUOSET, which is an ELISA comprising validated phospho-CD115-specific antibody pairs. Exemplary results from a p-CD115 (MCSFR) ELISA using SR cells are shown in Figures 1 and 3.

EXAMPLE 1

PREPARATION OF MONOCLONAL ANTIBODIES TO CD115

[00107] Monoclonal antibodies were prepared in accordance with a general method as described in "Antibodies: A Laboratory Manual" (Harlow and Lane 1988 CSH Press). Eight-

week old AlivaMab Kappa Mice and eight-week old AlivaMab Lambda Mice mice were immunized using a RIMMS protocol. 50 ug of human CD115 extracellular domain (Sino Biological, China 10161-H08H) was mixed with 40 ul (first immunization), 20 ul (immunizations 2-4) or 0 ul (final immunization) Gerbu MM adjuvant (C-C Biotech, Valley Center, CA #3001-6030) and PBS was added to a final volume of 100 ul. The 50 ug mixture was injected in 20 ul portions in 5 locations per mouse; right and left flanks and right and left shoulder/armpit subcutaneously, and the remaining 20 ul intraperitoneally. This was done 5 times per mouse on days, 1, 4, 7, 9, and 11. On Day 14 mice were sacrificed and terminal materials were collected. Spleens and lymph nodes were prepared and fused with CRL-2016 myeloma cells (ATCC) using a PEG based method as generally described in "Antibodies: A Laboratory Manual" (Harlow and Lane 1988 CSH Press) to establish hybridomas.

[00108] Hybridomas were grown in 384-well tissue culture plates and supernatants from individual wells were screened by ELISA for production of antibodies recognizing huCD115. Positive wells were then transferred to 48-well plates, expanded, and supernatants were collected for huCD115 binding confirmation by ELISA. Positive supernatants were also counter-screened against a non-related histidine-tagged protein. Fifty to sixty hybridoma lines each from AlivaMab Kappa Mice and AlivaMab Lambda Mice confirmed to bind CD115 specifically by ELISA were picked at random and single-cell cloned into 96-well plates. One hundred and eight (108) hybridoma lines were cloned. They were grown into colonies and the supernatant from these individual colonies was screened by ELISA to re-confirm monoclonal antibody binding to huCD115. These supernatants were then screened by FACS to confirm binding to native CD115 on OCI-AML5 cells (DSMZ #ACC-247, Table 1 shows results for select antibodies, and Figure 7). Seventy-five hybridoma clones were confirmed to produce mAb that bound to CD115-expressing OCI-AML5 cells (Figure 5).

Table 1. Summary of Screening for Binding to CD115 on cell Surface

HYBRIDOMA	FACS Binding
CCL-247A	+
CCK-423A	+
CCK-415A	+
CCK-416A	+
CCK-541A	+
CCK-424A	+
CCK-507A	+
CCK-461A	+

CCK-421A	+
CCL-331A	+
CCK-422A	+
CCK-437A	+
CCL-327A	+
CCK-522A	+
CCL-309A	+
CCL-321A	+
CCL-332A	+
CCL-217A	+
CCL-328A	+
CCL-221A	+
CCK-402A	+
CCL-238A	+
CCL-245A	+
CCK-417A	+
CCL-215A	+
CCL-346A	+
CCL-213A	+
CCL-205A	+
CCL-216A	+
CCL-211A	+
CCL-204A	+
CCL-325A	+
CCL-337A	+
CCL-249A	+

EXAMPLE 2

SEQUENCES OF ANTI-CD115 VH AND VL

[0109] Total RNA was extracted from hybridomas producing anti-CD115 monoclonal antibodies using the Qiagen RNeasy Mini kit (Cat No. 74104), followed by 5' RACE, using the 5' RACE system kit (Life Technologies, US cat # 18734-058) with the following 3' gene specific primers IgG 5'- GGTTCGGGGAAGTAGTCCTTGACC -3' (SEQ ID NO:433) IgL 5' – CTGTAGCTTCTGTGGGACTTCCACTGCTC -3' (SEQ ID NO:434) IgK 5' – CCGATTGGAGGGCGTTATCCAC -3' (SEQ ID NO:435). RACE products were gel purified and cloned into pCR4-TOPO using TOPO TA cloning kit for sequencing with One Shot Top 10 chemically competent *E. coli* (Life Technologies, US Cat # K4575-01). Sequencing of vector containing colonies was performed by Sequetech (Mountain View, CA)

using M13F or M13R sequencing primers. The reported nucleotide sequences start at the first nucleotide in the first codon for the amino terminal amino acid in framework 1. The reported polypeptide sequences are based on an *in silico* translation of the nucleic acid sequence and start at the first amino acid at the amino terminus of framework 1.

Table 2. Anti-CD115 mAb Amino Acid (aa) and Polynucleotide (nt) Sequences

	VH		VHCDR1		VHCDR2		VHCDR3		VL		VLCDR1		VLCDR2		VLCDR3	
Clone	aa SEQ ID NO:	nt SEQ ID NO:	aa SEQ ID NO:	nt SEQ ID NO:	aa SEQ ID NO:	nt SEQ ID NO:	aa SEQ ID NO:	nt SEQ ID NO:	aa SEQ ID NO:	nt SEQ ID NO:	aa SEQ ID NO:	nt SEQ ID NO:	aa SEQ ID NO:	nt SEQ ID NO:	aa SEQ ID NO:	nt SEQ ID NO:
CCK-401A	109	1	436	544	868	976	1300	1408	325	217	652	760	1084	1192	1516	1624
CCK-402A	110	2	437	545	869	977	1301	1409	326	218	653	761	1085	1193	1517	1625
CCK-406A	111	3	438	546	870	978	1302	1410	327	219	654	762	1086	1194	1518	1626
CCK-407A	112	4	439	547	871	979	1303	1411	328	220	655	763	1087	1195	1519	1627
CCK-408A	113	5	440	548	872	980	1304	1412	329	221	656	764	1088	1196	1520	1628
CCK-410A	114	6	441	549	873	981	1305	1413	330	222	657	765	1089	1197	1521	1629
CCK-412A	115	7	442	550	874	982	1306	1414	331	223	658	766	1090	1198	1522	1630
CCK-414A	116	8	443	551	875	983	1307	1415	332	224	659	767	1091	1199	1523	1631
CCK-415A	117	9	444	552	876	984	1308	1416	333	225	660	768	1092	1200	1524	1632
CCK-416A	118	10	445	553	877	985	1309	1417	334	226	661	769	1093	1201	1525	1633
CCK-417A	119	11	446	554	878	986	1310	1418	335	227	662	770	1094	1202	1526	1634
CCK-418A	120	12	447	555	879	987	1311	1419	336	228	663	771	1095	1203	1527	1635
CCK-421A	121	13	448	556	880	988	1312	1420	337	229	664	772	1096	1204	1528	1636
CCK-422A	122	14	449	557	881	989	1313	1421	338	230	665	773	1097	1205	1529	1637
CCK-423A	123	15	450	558	882	990	1314	1422	339	231	666	774	1098	1206	1530	1638
CCK-424A	124	16	451	559	883	991	1315	1423	340	232	667	775	1099	1207	1531	1639
CCK-425A	125	17	452	560	884	992	1316	1424	341	233	668	776	1100	1208	1532	1640
CCK-434A	126	18	453	561	885	993	1317	1425	342	234	669	777	1101	1209	1533	1641
CCK-435A	127	19	454	562	886	994	1318	1426	343	235	670	778	1102	1210	1534	1642
CCK-436A	128	20	455	563	887	995	1319	1427	344	236	671	779	1103	1211	1535	1643
CCK-437A	129	21	456	564	888	996	1320	1428	345	237	672	780	1104	1212	1536	1644

CCK-455A	130	22	457	565	889	997	1321	1429	346	238	673	781	1105	1213	1537	1645
CCK-456A	131	23	458	566	890	998	1322	1430	347	239	674	782	1106	1214	1538	1646
CCK-458A	132	24	459	567	891	999	1323	1431	348	240	675	783	1107	1215	1539	1647
CCK-459A	133	25	460	568	892	1000	1324	1432	349	241	676	784	1108	1216	1540	1648
CCK-460A	134	26	461	569	893	1001	1325	1433	350	242	677	785	1109	1217	1541	1649
CCK-461A	135	27	462	570	894	1002	1326	1434	351	243	678	786	1110	1218	1542	1650
CCK-464A	136	28	463	571	895	1003	1327	1435	352	244	679	787	1111	1219	1543	1651
CCK-465A	137	29	464	572	896	1004	1328	1436	353	245	680	788	1112	1220	1544	1652
CCK-467A	138	30	465	573	897	1005	1329	1437	354	246	681	789	1113	1221	1545	1653
CCK-468A	139	31	466	574	898	1006	1330	1438	355	247	682	790	1114	1222	1546	1654
CCK-501A	140	32	467	575	899	1007	1331	1439	356	248	683	791	1115	1223	1547	1655
CCK-503A	141	33	468	576	900	1008	1332	1440	357	249	684	792	1116	1224	1548	1656
CCK-505A	142	34	469	577	901	1009	1333	1441	358	250	685	793	1117	1225	1549	1657
CCK-507A	143	35	470	578	902	1010	1334	1442	359	251	686	794	1118	1226	1550	1658
CCK-511A	144	36	471	579	903	1011	1335	1443	360	252	687	795	1119	1227	1551	1659
CCK-513A	145	37	472	580	904	1012	1336	1444	361	253	688	796	1120	1228	1552	1660
CCK-514A	146	38	473	581	905	1013	1337	1445	362	254	689	797	1121	1229	1553	1661
CCK-516A	147	39	474	582	906	1014	1338	1446	363	255	690	798	1122	1230	1554	1662
CCK-519A	148	40	475	583	907	1015	1339	1447	364	256	691	799	1123	1231	1555	1663
CCK-522A	149	41	476	584	908	1016	1340	1448	365	257	692	800	1124	1232	1556	1664
CCK-525A	150	42	477	585	909	1017	1341	1449	366	258	693	801	1125	1233	1557	1665
CCK-526A	151	43	478	586	910	1018	1342	1450	367	259	694	802	1126	1234	1558	1666
CCK-533A	152	44	479	587	911	1019	1343	1451	368	260	695	803	1127	1235	1559	1667
CCK-539A	153	45	480	588	912	1020	1344	1452	369	261	696	804	1128	1236	1560	1668
CCK-541A	154	46	481	589	913	1021	1345	1453	370	262	697	805	1129	1237	1561	1669
CCK-542A	155	47	482	590	914	1022	1346	1454	371	263	698	806	1130	1238	1562	1670
CCK-543A	156	48	483	591	915	1023	1347	1455	372	264	699	807	1131	1239	1563	1671

CCL-201A	157	49	484	592	916	1024	1348	1456	373	265	700	808	1132	1240	1564	1672
CCL-203A	158	50	485	593	917	1025	1349	1457	374	266	701	809	1133	1241	1565	1673
CCL-204A	159	51	486	594	918	1026	1350	1458	375	267	702	810	1134	1242	1566	1674
CCL-205A	160	52	487	595	919	1027	1351	1459	376	268	703	811	1135	1243	1567	1675
CCL-206A	161	53	488	596	920	1028	1352	1460	377	269	704	812	1136	1244	1568	1676
CCL-207A	162	54	489	597	921	1029	1353	1461	378	270	705	813	1137	1245	1569	1677
CCL-208A	163	55	490	598	922	1030	1354	1462	379	271	706	814	1138	1246	1570	1678
CCL-209A	164	56	491	599	923	1031	1355	1463	380	272	707	815	1139	1247	1571	1679
CCL-211A	165	57	492	600	924	1032	1356	1464	381	273	708	816	1140	1248	1572	1680
CCL-212A	166	58	493	601	925	1033	1357	1465	382	274	709	817	1141	1249	1573	1681
CCL-213A	167	59	494	602	926	1034	1358	1466	383	275	710	818	1142	1250	1574	1682
CCL-215A	168	60	495	603	927	1035	1359	1467	384	276	711	819	1143	1251	1575	1683
CCL-216A	169	61	496	604	928	1036	1360	1468	385	277	712	820	1144	1252	1576	1684
CCL-217A	170	62	497	605	929	1037	1361	1469	386	278	713	821	1145	1253	1577	1685
CCL-218A	171	63	498	606	930	1038	1362	1470	387	279	714	822	1146	1254	1578	1686
CCL-220A	172	64	499	607	931	1039	1363	1471	388	280	715	823	1147	1255	1579	1687
CCL-221A	173	65	500	608	932	1040	1364	1472	389	281	716	824	1148	1256	1580	1688
CCL-223A	174	66	501	609	933	1041	1365	1473	390	282	717	825	1149	1257	1581	1689
CCL-225A	175	67	502	610	934	1042	1366	1474	391	283	718	826	1150	1258	1582	1690
CCL-226A	176	68	503	611	935	1043	1367	1475	392	284	719	827	1151	1259	1583	1691
CCL-229A	177	69	504	612	936	1044	1368	1476	393	285	720	828	1152	1260	1584	1692
CCL-231A	178	70	505	613	937	1045	1369	1477	394	286	721	829	1153	1261	1585	1693
CCL-235A	179	71	506	614	938	1046	1370	1478	395	287	722	830	1154	1262	1586	1694
CCL-238A	180	72	507	615	939	1047	1371	1479	396	288	723	831	1155	1263	1587	1695
CCL-245A	181	73	508	616	940	1048	1372	1480	397	289	724	832	1156	1264	1588	1696
CCL-247A	182	74	509	617	941	1049	1373	1481	398	290	725	833	1157	1265	1589	1697
CCL-249A	183	75	510	618	942	1050	1374	1482	399	291	726	834	1158	1266	1590	1698

CCL-252A	184	76	511	619	943	1051	1375	1483	400	292	727	835	1159	1267	1591	1699
CCL-253A	185	77	512	620	944	1052	1376	1484	401	293	728	836	1160	1268	1592	1700
CCL-255A	186	78	513	621	945	1053	1377	1485	402	294	729	837	1161	1269	1593	1701
CCL-301A	187	79	514	622	946	1054	1378	1486	403	295	730	838	1162	1270	1594	1702
CCL-303A	188	80	515	623	947	1055	1379	1487	404	296	731	839	1163	1271	1595	1703
CCL-305A	189	81	516	624	948	1056	1380	1488	405	297	732	840	1164	1272	1596	1704
CCL-309A	190	82	517	625	949	1057	1381	1489	406	298	733	841	1165	1273	1597	1705
CCL-310A	191	83	518	626	950	1058	1382	1490	407	299	734	842	1166	1274	1598	1706
CCL-311A	192	84	519	627	951	1059	1383	1491	408	300	735	843	1167	1275	1599	1707
CCL-312A	193	85	520	628	952	1060	1384	1492	409	301	736	844	1168	1276	1600	1708
CCL-313A	194	86	521	629	953	1061	1385	1493	410	302	737	845	1169	1277	1601	1709
CCL-314A	195	87	522	630	954	1062	1386	1494	411	303	738	846	1170	1278	1602	1710
CCL-315A	196	88	523	631	955	1063	1387	1495	412	304	739	847	1171	1279	1603	1711
CCL-320A	197	89	524	632	956	1064	1388	1496	413	305	740	848	1172	1280	1604	1712
CCL-321A	198	90	525	633	957	1065	1389	1497	414	306	741	849	1173	1281	1605	1713
CCL-322A	199	91	526	634	958	1066	1390	1498	415	307	742	850	1174	1282	1606	1714
CCL-324A	200	92	527	635	959	1067	1391	1499	416	308	743	851	1175	1283	1607	1715
CCL-325A	201	93	528	636	960	1068	1392	1500	417	309	744	852	1176	1284	1608	1716
CCL-327A	202	94	529	637	961	1069	1393	1501	418	310	745	853	1177	1285	1609	1717
CCL-328A	203	95	530	638	962	1070	1394	1502	419	311	746	854	1178	1286	1610	1718
CCL-329A	204	96	531	639	963	1071	1395	1503	420	312	747	855	1179	1287	1611	1719
CCL-331A	205	97	532	640	964	1072	1396	1504	421	313	748	856	1180	1288	1612	1720
CCL-332A	206	98	533	641	965	1073	1397	1505	422	314	749	857	1181	1289	1613	1721
CCL-333A	207	99	534	642	966	1074	1398	1506	423	315	750	858	1182	1290	1614	1722
CCL-335A	208	100	535	643	967	1075	1399	1507	424	316	751	859	1183	1291	1615	1723
CCL-337A	209	101	536	644	968	1076	1400	1508	425	317	752	860	1184	1292	1616	1724
CCL-338A	210	102	537	645	969	1077	1401	1509	426	318	753	861	1185	1293	1617	1725

CCL-339A	211	103	538	646	970	1078	1402	1510	427	319	754	862	1186	1294	1618	1726
CCL-340A	212	104	539	647	971	1079	1403	1511	428	320	755	863	1187	1295	1619	1727
CCL-341A	213	105	540	648	972	1080	1404	1512	429	321	756	864	1188	1296	1620	1728
CCL-344A	214	106	541	649	973	1081	1405	1513	430	322	757	865	1189	1297	1621	1729
CCL-346A	215	107	542	650	974	1082	1406	1514	431	323	758	866	1190	1298	1622	1730
CCL-349A	216	108	543	651	975	1083	1407	1515	432	324	759	867	1191	1299	1623	1731

EXAMPLE 3

EPITOPE BINNING

[0110] A competition ELISA was performed to establish competitive binding bins. ELISA plates were coated with 1 ug/ml huCD115 protein (Sino Biological, China 10161-H08H) and blocked with Superblock (Thermo Scientific #37518). After washing, wells were incubated with a mouse monoclonal antibody representing one of six unique competition bins and for some of which, exhibit different activities in blocking CSF-1 and/or IL-34 binding to CD115 (Table 7) (these mouse mAbs were generated by hybridoma by immunizing wild-type mice as described in Example 1 as part of a comparator CD115 antibody generation program as part of the first tests of the newly-created AlivaMab Mouse technology). After 1 hour the wells were washed and incubated with individual clonal anti-huCD115 AlivaMab hybridoma supernatants. After another hour the wells were washed and incubated with a specific secondary antibody that either recognized human kappa LC or human lambda LC depending on which AlivaMab Mouse supernatants were being detected (Southern Biotech Goat X hu kappa LC #2061-05 or Bethyl Goat X hu lambda LC #A80-116P) and detected with Supersignal ELISA Pico Chemiluminescent substrate (Thermo Scientific - Product# 37069) (Tables 5 and 6). Individual AlivaMab Mouse antibodies that were able to bind in the presence of a mouse antibody are considered to be in a unique epitope bin from that particular mouse antibody. Individual AlivaMab Mouse antibodies that were unable to bind in the presence of a mouse antibody are considered to be in the same epitope bin as that particular mouse antibody. In this way multiple epitope bins were defined for huCD115 binding antibodies (Tables 3 and 6).

Table 3. Multiple Epitope Bins

	IgGκ	IgGλ	TOTAL
BIN 1	4	0	4
BIN 2	18	16	34
BIN 3	6	28	34
BIN 4	6	8	14
BIN 5	1	5	6
BIN 6	7	2	9
BIN 7	2	0	2
BIN 8	6	0	6

Table 4. Binning of AlivaMab Kappa Mouse Anti-CD115 mAbs

		1A		1B		1C		1D		1E	3		4		5	
		522	505	417	462	511	526	542	519	412	402	503	435	437	401	406
Bin 1	TMR 95A	7	1	3	3	3	9	23	36	3	**131**	**82**	**86**	**84**	**94**	**87**
Bin 1.5	TMR 44B	24	10	19	23	2	3	10	12	**110**	**126**	**89**	**75**	**78**	**93**	**88**
Bin 1.7	TMR 24A	33	0	6	12	**99**	**68**	**94**	**110**	13	**131**	**97**	**79**	**86**	**96**	**96**
Bin 2	TMR 20A	34	53	**110**	**93**	**77**	**92**	7	6	70	**145**	**95**	**85**	**95**	**104**	**103**
Bin 3	TMR 35A	**83**	**110**	**110**	**77**	**76**	**82**	**111**	**110**	**78**	11	23	**96**	**101**	**98**	**99**
Bin 4	TMR 100A	**67**	**111**	**113**	**89**	**91**	**84**	**76**	**118**	**91**	**147**	**96**	17	51	**106**	**109**
	NO COMP	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

Table 4. (continued)

	1A				1B																	
	455	458	522	585	417	462	422	424	461	421	525	415	418	599	516	454	416	456	514	467	466	464
TMR 95A	0	6	7	1	3	3	6	16	15	9	11	11	10	19	20	29	21	27	42	46	34	57
TMR 44B	28	17	24	10	19	23	31	53	41	55	56	46	3	21	10	23	53	27	4	22	13	0
TMR 24A	-1	2	33	0	6	12	11	29	27	18	13	31	35	51	39	32	37	42	53	58	58	39
TMR 20A	58	22	34	53	**110**	**93**	**107**	**98**	**110**	**104**	**80**	**97**	**89**	**82**	**70**	**115**	**94**	**73**	**84**	**96**	**86**	**87**
TMR 35A	54	66	**80**	**110**	**110**	**77**	**111**	**102**	**110**	**100**	**88**	**96**	**82**	**85**	**82**	**110**	**107**	**64**	**75**	**83**	**100**	**85**
TMR 100A	**79**	**79**	**67**	**111**	**113**	**89**	**111**	**98**	**110**	**99**	**100**	**93**	**106**	**85**	**87**	**89**	**104**	**96**	**103**	**102**	**98**	**96**
NO COMP	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

Table 4. (continued)

1C						1D						1E
511	526	460	465	429	418	425	542	519	541	549	468	412

TMR 95A	3	9	23	41	38	35	21	23	36	42	1	**61**	3
TMR 44B	2	3	48	8	45	60	6	10	12	15	2	24	**110**
TMR 24A	**99**	**68**	**61**	**81**	**75**	**75**	**78**	**94**	**110**	**109**	**111**	**85**	13
TMR 20A	77	**92**	**85**	**78**	**84**	**91**	4	7	6	34	59	53	**70**
TMR 35A	**76**	**82**	**86**	**88**	**112**	**86**	**88**	**111**	**110**	**111**	**89**	**104**	**78**
TMR 100A	**91**	**84**	**94**	**92**	**107**	**94**	**98**	**76**	**118**	**93**	**100**	**95**	**91**
NO COM	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

Table 4. (continued)

	3							4		5						
	482	589	587	459	599	487	488	495	497	481	486	414	496	581	519
TMR 95A	**131**	**82**	**74**	**77**	**97**	**84**	**68**	**86**	**84**	**94**	**87**	**86**	**81**	**91**	**77**
TMR 44B	**126**	**89**	**87**	**84**	**92**	**94**	**80**	**75**	**78**	**93**	**88**	**87**	**91**	**106**	**86**
TMR 24A	**131**	**97**	**106**	**86**	**112**	**86**	**74**	**79**	**86**	**96**	**96**	**92**	**87**	**95**	**90**
TMR 20A	**145**	**95**	**112**	**90**	**102**	**97**	**100**	**85**	**95**	**104**	**103**	**106**	**96**	**114**	**92**
TMR 35A	11	23	2	3	4	6	34	**96**	**101**	**98**	**99**	**91**	**92**	**106**	**89**
TMR 100A	**147**	**96**	**123**	**95**	**109**	**103**	**101**	17	51	**106**	**109**	**101**	**100**	**116**	**93**
NO COM	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

Table 5. Epitope Competition Bins in Panels of anti-CD115 mAbs

	BIN 1		BIN 2		BIN 3		BIN 4		BIN 5		BIN 6		BIN 7		BIN 8	
	CCK 522A	CCK 505A	CCK 417A	CCK 462A	CCK 511A	CCK 526A	CCK 542A	CCK 519A	CCK 412A	CCL 321A	CCK 402A	CCK 503A	CCK 435A	CCK 437A	CCK 401A	CCK 406A
TMR	0	0	0	0	0	0	0	0	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**

44B																
TMR 24A	0	0	0	0	1	1	1	1	1	0	1	1	1	1	1	1
TMR 20A	0	0	1	1	1	1	0	0	1	1	1	1	1	1	1	1
TMR 35A	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1
TMR 100A	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1
NO COMP	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1

Table 6. Anti-CD115 mAb Epitope Bins

HYBRIDOMA	BIN	HYBRIDOMA	BIN
CCK-401A	5	CCL-201A	1C
CCK-402A	3	CCL-203A	1C
CCK-406A	5	CCL-204A	1C
CCK-407A	3	CCL-205A	1C
CCK-408A	3	CCL-206A	1B
CCK-410A	1C	CCL-207A	1C
CCK-412A	1E	CCL-208A	1D
CCK-414A	5	CCL-209A	1D
CCK-415A	1B	CCL-211A	1C
CCK-416A	1B	CCL-212A	1B
CCK-417A	1B	CCL-213A	1B
CCK-418A	1B	CCL-215A	1C
CCK-421A	1B	CCL-216A	1C
CCK-422A	1B	CCL-217A	1C
CCK-423A	1C	CCL-218A	1B
CCK-424A	1B	CCL-220A	1C
CCK-425A	1D	CCL-221A	1E
CCK-434A	1B	CCL-223A	1C
CCK-435A	4	CCL-225A	1B
CCK-436A	5	CCL-226A	1C
CCK-437A	4	CCL-229A	1B
CCK-455A	1A	CCL-231A	1E
CCK-456A	1B	CCL-235A	1B
CCK-458A	1A	CCL-238A	1C
CCK-459A	3	CCL-245A	1C
CCK-460A	1D	CCL-247A	1C
CCK-461A	1B	CCL-249A	1C
CCK-464A	1B	CCL-252A	1B
CCK-465A	1C	CCL-253A	1C
CCK-467A	1B	CCL-255A	1C
CCK-468A	1C	CCL-301A	1D
CCK-501A	5	CCL-303A	1C
CCK-503A	3	CCL-305A	1D
CCK-505A	1A	CCL-309A	1E
CCK-507A	3	CCL-310A	1C
CCK-511A	1C	CCL-311A	1B
CCK-513A	5	CCL-312A	3
CCK-514A	1B	CCL-313A	3
CCK-516A	1B	CCL-314A	1B
CCK-519A	1D	CCL-315A	1B
CCK-522A	1A	CCL-320A	1B
CCK-525A	1B	CCL-321A	1E
CCK-526A	1C	CCL-322A	1C
CCK-533A	3	CCL-324A	1B
CCK-539A	1B	CCL-325A	1C
CCK-541A	1D	CCL-327A	1D

CCK-542A	1D	CCL-328A	1D
CCK-543A	1D	CCL-329A	1C
		CCL-331A	1E
		CCL-332A	1C
		CCL-333A	1C
		CCL-335A	1B
		CCL-337A	1C
		CCL-338A	1B
		CCL-339A	1C
		CCL-340A	1C
		CCL-341A	1D
		CCL-344A	1D
		CCL-346A	1C
		CCL-349A	1B

[0111] Based on functional characterization of the bin-defining mouse mAbs, some antibodies within epitope bins 1A or 1C (defined by dual IL-34- and CSF-1-neutralizing mouse mAb, TMR24A) or bin 3 (defined by dual IL-34- and CSF-1-neutralizing mouse mAb, TMR35A) may neutralize P-TYR formation induced by both CSF-1 and IL-34 (Figure 2). Some antibodies within epitope bin 1C (defined by only CSF-1-neutralizing and IL-34 non-neutralizing mouse mAb, TMR20A) may neutralize only CSF-1-induced P-TYR formation on CD115. However, some bin 3 mAbs may neutralize both IL-34 and CSF-1 induced P-TYR formation on CD115. As summarized in Table 7 below, some of the reference wild-type mouse mAbs can block both cytokines from different locations on the receptor (e.g., bin 1 and bin3), some mAbs block M-CSF while not blocking IL-34, none of the mAbs were agonists on their own, and the bin 3 epitope region appears to contain functional diversity (e.g., all 3 mAbs listed below exhibited different activity). Also of note is that mAb 20A slightly increased/enhanced the M-CSF signal (Figures 4A and 4B).

Table 7. Summary of anti-CD115 mAb Activity

mAb	**Bin**	**Blocks M-CSF**	**Blocks IL-34**
24A	1	+++	+++
29A	1	+++	+++
32B	1	+++	+++
20A	2	Slight agonist	NO
10B	3	+++	NO
35A	3	+++	+++

47B	3	NO	NO
40A	4	+	NO
27A	4	+	NO
19B	5	+++	NO
7B	6	NO	NO
44B	6	NO	NO

EXAMPLE 4

AFFINITY DETERMINATION

[0112] Affinity was determined for 24 selected monoclonal hybridoma supernatants (Biosensor Tools, Salt Lake City, Utah). Binding kinetics were measured using a BioRad ProteOn XPR36 optical biosensor equipped with an anti-mouse IgG-Coated GLC sensor chip. Hybridoma supernatants were diluted 10-fold into running buffer and captured for 4 minutes on the anti-mouse IgG surface. Hu CD115 (Sino Biological, China #10161-H08H) was tested in duplicate using a 3-fold dilution series starting at 150 nM. The processed data were fit using a 1:1 interaction model that includes a mass-transport parameter (Scrubber2, Canberra Australia). Within the panel of AlivaMab Mouse anti-CD115 antibodies, there are antibodies with KD values below a nanomolar and KD values in the low nanomolar range, and with fast k_{on} and slow k_{off} rates (Tables 8 and 9).

Table 8. Binding Kinetics of Anti-CD115 IgGλ mAbs

mAb	**ka ($M^{-1}s^{-1}$)**	**kd (s^{-1})**	**K_D (pM)**
329	1.18×10^{5}	4.46×10^{-6}	38
310	5.06×10^{4}	4.35×10^{-6}	79
331	1.86×10^{5}	2.20×10^{-5}	118
215	8.53×10^{4}	1.83×10^{-5}	215
225	5.00×10^{4}	1.38×10^{-5}	277
340	7.41×10^{4}	2.72×10^{-5}	367
312	1.39×10^{5}	6.03×10^{-5}	435
206	5.90×10^{4}	2.92×10^{-5}	495
231	8.10×10^{4}	4.71×10^{-5}	578
249	1.49×10^{5}	8.91×10^{-5}	599
217	9.81×10^{4}	1.20×10^{-4}	1,220
313	7.36×10^{4}	9.09×10^{-5}	1,240
327	1.33×10^{5}	1.89×10^{-4}	1,410

Table 9. Binding Kinetics of Anti-CD115 IgGκ mAbs

mAb	ka ($M^{-1}s^{-1}$)	kd (s^{-1})	K_D (pM)
418	1.33×10^{5}	2.11×10^{-5}	158
533	1.24×10^{5}	3.92×10^{-5}	317
412	1.58×10^{5}	5.94×10^{-4}	376
460	8.92×10^{5}	5.51×10^{-4}	618
467	2.30×10^{4}	1.48×10^{-5}	650
459	5.00×10^{4}	3.38×10^{-5}	680
519	9.00×10^{4}	7.71×10^{-5}	860
407	6.90×10^{4}	6.54×10^{-5}	950
541	2.67×10^{5}	3.74×10^{-4}	1,400
465	5.31×10^{5}	7.49×10^{-4}	1,410
456	2.70×10^{4}	5.30×10^{-5}	1,970
539	3.20×10^{4}	8.50×10^{-5}	2,700

EXAMPLE 5

INTERNALIZATION OF ANTI-CD115 ANTIBODIES

[0113] Anti-CD115 antibodies were tested for their ability to internalize upon binding to native CD115 on the surface of OCI-AML5 cells (DSMZ #ACC-247). OCI-AML5 cells were treated with individual AlivaMab Mouse anti-CD115 supernatants for 1 hour at 37°C. The cells were then transferred to ice and stained with a fluorescently labeled anti-CD115 mAb known to be able to bind CD115 in the presence of bound test antibody (either Biolegend Rat x Hu-CD115-PE #6393 or CCK533A conjugated with Dylight488 Pierce #46403). Detection of fluorescent signal was then measured using a BD FACScalibur instrument. Cells that gave a strong fluorescent signal are considered to be non-internalizers for that individual test anti-CD115 mAb. Cells that are measured to have weak or no fluorescent signal are considered to be strong internalizers for that individual test anti-CD115 mAb. This procedure was repeated with several purified AlivaMab Mouse anti-CD115 mAbs that showed internalization as a supernatant at 20 ug/ml. Other AlivaMab Mouse anti-CD115 mAbs are also shown to exhibit various levels of internalization of CD115 induced by mAb binding (Figure 12 and Table 10).

Table 10. Internalization of Anti-CD115 mAbs

mAb	Internalization

CCK-423A	-
CCK-543A	+
CCK-416A	++
CCL-252A	+++
CCL-331A	++++

(- = no internalization, 1-4 + = strength of internalization)

EXAMPLE 6

NEUTRALIZATION OF CSF-1 BINDING TO CD115

[0114] Anti-CD115 antibodies were tested for their ability to block binding of recombinant CSF-1 to recombinant CD115. ELISA plates were coated with recombinant hu-CD115 (Sino Biological, China #10161-H08H) at 0.5 ug/ml and blocked with Superblock (Thermo Scientific #37518). Wells were incubated for 15 min with anti-CD115 mAbs, then biotinylated Hu-CSF-1 (R&D Systems, #216-MC-005) (biotinylation using NHS-Peg4-biotin, Life Technologies, #21330) was added to a final concentration of 0.25 ug/ml for an additional 15'. After a 4X wash, CSF-1-biotin was detected using 1:10,000 SAV-poly HRP (Life Technologies, #N200). Other AlivaMab Mouse anti-CD115 mAbs are also shown to exhibit various abilities and IC50 values in blocking CSF-1 binding to CD115 (Figure 10 and Table 11).

Table 11. AlivaMab Mouse anti-CD115 mAbs block CSF-1 binding to CD115

mAb	IC_{50} (pM)
CCK-415A	355
CCK-416A	811
CCK-423A	135

EXAMPLE 7

INHIBITION OF P-TYR FORMATION ON CD115 INDUCED BY CSF-1

[0115] Anti-CD115 antibodies were tested for their ability to block hu-CSF1 induced phosphorylation of native CD115. OCI-AML5 cells (DSMZ, # ACC-247) were serum starved (1% FBS) overnight, then harvested and washed twice in PBS with 0.1% BSA. 250,000 cells were plated per well into a 96-well v-bottom polypropylene plate. Anti-CD115 supernatants were added neat for 15 min while incubating the plate on ice. Hu-CSF-1 (R&D Systems, #216-MC-005) was added to each well at a final concentration of 100 ng/ml and incubated for 30' on ice. Cells were then spun down at 1500 RPM for 5' at 4°C and supernatant was removed. Cells were then resuspended in lysis buffer (Cell Signaling, #9803 with 1X HALT protease inhibitors, Pierce, #78430) and incubated on ice for 15 min. Lysates

were then measured for tyrosine phosphorylated CD115 using a p-MCSFR validated DUOSET assay (R&D Systems #CYC3268E) and detected using Supersignal Pico ELISA Substrate (Pierce, #37069) (Figures 6 and 8).

[0116] Unpurified anti-CD115 IgGs (as identified by ELISA as described above) secreted from hybridomas into the tissue culture supernatant was assessed for neutralization of P-TYR formation induced by CSF-1. Neutralization using these unpurified, non-quantified antibodies was rank ordered. From this assessment, sets of the better neutralizing mAbs were identified, one set of IgGκ mAbs and one set of IgGλ mAbs. The hybridomas making these mAbs were grown and mAb purified using a commercially-available kit.

The P-TYR neutralization assay was repeated with several purified anti-CD115 mAbs using a dilution series enabling an IC50 calculation, first in an eight-point dilution curve (Figure 9) and then with a further subset of best neutralizing mAbs in a twelve-point dilution curve to better calculate IC50 values. Of the antibodies tested, CCK423 was identified as having the best IC50 for neutralizing CSF-1 P-TYR formation on CD115 (Figures 11 and 16; Table 12).

Table 12. Anti-CD115 mAbs inhibit CSF-1 induced phosphorylation of CD115

mAb	IC_{50} (pM)
CCK-415A	570
CCK-416A	1350
CCK-423A	45

EXAMPLE 8

CONVERSION OF ALIVAMAB MOUSE ANTI-CD115 MABS TO FULLY HUMAN

[0117] The AlivaMab Mouse anti-CD115 mAbs are easily converted, expressed recombinantly and purified as fully-human antibodies of any isotype. The recombinant fully-human antibody retains all of the characteristics of the parental AlivaMab Mouse antibody. For example, the nucleotide sequences of the heavy and light chain variable region of CCK423A were transmitted to and synthesized into DNA by Lake Pharma (Belmont CA) and then, using vectors for recombinant expression in mammalian cells, the VH cloned in-frame with coding sequences for human IgG1, IgG2, or IgG4 constant regions and the Vκ cloned in-frame with coding sequences for the human Cκ region. Vectors were then transformed into HEK293 cells for expression of recombinant fully human antibody. Fully human IgG1κ, IgG2κ and IgG4κ mAb versions of CCK423A were purified from tissue culture supernatants using protein A (Figure 13).

EXAMPLE 9

AFFINITY OF FULLY HUMAN MABS

[0118] Affinity was determined for AlivaMab CCK423A as well as for the 3 human variants CCK423A-I IgG1κ, CCK423A- IgG2κ, and CCK423A- IgG4κ (Biosensor Tools, Salt Lake City, Utah). Binding kinetics were measured using a BioRad ProteOn XPR36 optical biosensor equipped with a GLM sensor chip. Purified mAbs were amine coupled to the GLM sensor chip. Hu CD115 (Sino Biological, China #10161-H08H) was tested in triplicate using a 3 fold dilution series starting at 10 nM. The processed data were fit using a 1:1 interaction model that includes a mass-transport parameter (Scrubber2, Canberra Australia). All 4 constructs were found to bind hu-CD115 with the same kinetics and affinity (Table 13).

Table 13.

mAb	ka ($M^{-1}s^{-1}$)	kd (s^{-1})	KD (nM)
AlivaMab CCK423A	1.3×10^{7}	1.5×10^{-2}	1.2
Human IgG1κ	1.2×10^{7}	1.7×10^{-2}	1.2
Human IgG2κ	1.3×10^{7}	1.4×10^{-2}	1.3
Human IgG4κ	1.1×10^{7}	1.5×10^{-2}	1.3

EXAMPLE 10

CSF-1 BINDING NEUTRALIZATION WITH FULLY HUMAN MABS

[0119] AlivaMab Mouse CCK423A as well as the 3 human variants CCK423A-IgG1, CCK423A-IgG2, and CCK423A-IgG4 antibodies were tested for their ability to block binding of recombinant CSF-1 to recombinant CD115. ELISA plates were coated with recombinant hu-CD115 (Sino Biological, China #10161-H08H) at 0.5 ug/ml and blocked with Superblock (Thermo Scientific #37518). Wells were incubated for 15 min with anti-CD115 mAbs, then biotinylated Hu-CSF-1 (R&D Systems, #216-MC-005) (biotinylation using NHS-Peg4-biotin, Life Technologies, #21330) was added to a final concentration of 0.25 ug/ml for an additional 15'. After a 4X wash, CSF-1-biotin was detected using 1:10,000 SAV-poly HRP (Life Technologies, #N200). The fully human variants exhibited identical potency as the parental AlivaMab antibody (Figure 14 and Table 14).

Table 14.

mAb	IC_{50} (pM)

AlivaMab CCK423A	191
Human IgG1κ	146
Human IgG2κ	253
Human IgG4κ	133

EXAMPLE 11

INHIBITION OF CSF-1 INDUCED P-TYR WITH FULLY HUMAN MABS

[0120] AlivaMab Mouse CCK423A as well as the 3 human variants CCK423A-IgG1, CCK423A-IgG2, and CCK423A-IgG4 were tested for their ability to block hu-CSF1 induced phosphorylation of native CD115. OCI-AML5 cells (DSMZ, # ACC-247) were serum starved (1% FBS) overnight, then harvested and washed twice in PBS with 0.1% BSA. 250,000 cells were plated per well into a 96-well v-bottom polypropylene plate. Anti-CD115 mAbs were added in a dilution series for 15 min while incubating the plate on ice. Hu-CSF-1 (R&D Systems, #216-MC-005) was added to each well at a final concentration of 100 ng/ml and incubated for 30 min on ice. Cells were then spun down at 1500 RPM for 5 min at 4°C and supernatant was removed. Cells were then resuspended in lysis buffer (Cell Signaling, #9803 with 1X HALT protease inhibitors, Pierce, #78430) and incubated on ice for 15 min. Lysates were then measured for tyrosine phosphorylated-CD115 using a p-MCSFR validated duoset assay (R&D Systems #CYC3268E) and detected using Supersignal Pico ELISA Substrate (Pierce, #37069). The fully human variants exhibited identical potency as the parental AlivaMab antibody (Figure 15 and Table 15).

Table 15.

mAb	IC_{50} (pM)
AlivaMab CCK423A	58
Human IgG1κ	68
Human IgG2κ	59
Human IgG4κ	60

EXAMPLE 12

INHIBITION OF P-TYR FORMATION ON CD115 INDUCED BY IL-34

[0121] Anti-CD115 antibodies of the invention were found to neutralize P-TYR formation on CD115 induced by interleukin-34 (IL-34). Some antibodies that block CSF-1 induced P-TYR formation on CD115 are found to also block IL-34 induced P-TYR formation on CD115. Other antibodies are found that block only CSF-1 induced P-TYR formation on

CD115 and do not block IL-34 induced P-TYR formation on CD115. Antibodies are tested for their ability to block IL-34 induced phosphorylation of native CD115.

[0122] In an example assay, SR cells or other CD115+ IL-34 responsive cell line(s) are serum starved (1% FBS) overnight, then harvested and washed twice in PBS with 0.1% BSA. Cells are plated into a 96-well v-bottom polypropylene plate. Anti-CD115 antibodies, either in hybridoma supernatants, purified antibody, or in purified fully-human recombinant antibody format, are added for 15 min while incubating the plate on ice. Human IL-34 is added to each well at a final concentration sufficient and necessary to trigger P-TYR formation on CD115 and incubated for 30' on ice. Cells were then spun down at 1500 RPM for 5' at 4°C and supernatant was removed. Cells were then resuspended in lysis buffer (Cell Signaling, #9803 with 1X HALT protease inhibitors, Pierce, #78430) and incubated on ice for 15 min. Lysates were then measured for tyrosine phosphorylated CD115 using a p-MCSFR validated DUOSET assay (R&D Systems #CYC3268E) and detected using Supersignal Pico ELISA Substrate (Pierce, #37069).

EXAMPLE 13
NEUTRALIZATION OF IL-34 BINDING TO CD115

[0123] AlivaMab Mouse anti-CD115 antibodies block binding of IL-34 to CD115. For example, anti-CD115 antibodies were tested for their ability to block binding of recombinant human IL-34 to recombinant CD115. ELISA plates were coated with recombinant hu-CD115 (Sino Biological, China #10161-H08H) at 0.5 ug/ml and blocked with Superblock (Thermo Scientific #37518). Wells were incubated for 15 min with anti-CD115 mAbs, then biotinylated Hu-IL-34 (biotinylation using NHS-Peg4-biotin, Life Technologies, #21330) is added for an additional 15 minutes. After a 4X wash, HU-IL-34-biotin was detected using 1:10,000 SAV-poly HRP (Life Technologies, #N200). Other AlivaMab Mouse anti-CD115 mAbs are also shown to exhibit various abilities and IC50 values in blocking HU-IL-34 binding to CD115.

EXAMPLE 14
NEUTRALIZATION OF P-TYR FORMATION ON CD115 WITHOUT NEUTRALIZATION OF BINDING OF CSF-1 AND IL-34

[0124] AlivaMab Mouse anti-CD115 mAbs were found that neutralize p-Tyr formation in cells exposed to either CSF-1 or IL-34. However, these mAbs still allow

binding of CSF-1 and IL-34 to CD115. This set of mAbs block p-Tyr formation through inhibition of dimerization of CD115.

EXAMPLE 15

AlivaMab Mouse ANTI-CD115 MABS AND THEIR FULLY HUMAN DERVATIVES BIND TO AND NEUTRALIZE CD115 FROM CYNOMOLGUS MONKEY

[0125] CD115 is cloned and expressed from cynomolgus monkey using standard molecular biological techniques. The recombinant CD115 may be tagged (histidine, Fc) to support efficient purification. The recombinant cynomolgus CD115 may also be transiently or stably expressed on cell lines. The AlivaMab Mouse anti-CD115 mAbs and their human variants are shown to bind to cynomolgus monkey CD115. The AlivaMab Mouse anti-CD115 mAbs and their human variants are shown to neutralize cynomolgus monkey CD115 in assays as described above for human CD115.

[0126] The various embodiments described above can be combined to provide further embodiments. All of the U.S. patents, U.S. patent application publications, U.S. patent application, foreign patents, foreign patent application and non-patent publications referred to in this specification and/or listed in the Application Data Sheet are incorporated herein by reference, in their entirety. Aspects of the embodiments can be modified, if necessary to employ concepts of the various patents, application and publications to provide yet further embodiments.

[0127] These and other changes can be made to the embodiments in light of the above-detailed description. In general, in the following claims, the terms used should not be construed to limit the claims to the specific embodiments disclosed in the specification and the claims, but should be construed to include all possible embodiments along with the full scope of equivalents to which such claims are entitled. Accordingly, the claims are not limited by the disclosure.

CLAIMS

What is claimed is:

1. An isolated anti-CD115 antibody, or an antigen-binding fragment thereof, comprising i) a heavy chain variable region comprising a VHCDR1 selected from any of SEQ ID NOs:436-543, a VHCDR2 selected from any of SEQ ID NOs:868-975, and a VHCDR3 selected from any of SEQ ID NOs:1300-1407 and ii) a light chain variable region comprising a VLCDR1 selected from any of SEQ ID NOs:652-759, a VLCDR2 selected from any of SEQ ID NOs:1084-1191, and a VLCDR3 selected from any of SEQ ID NOs:1516-1623.

2. The antibody, or antigen-binding fragment thereof, of claim 1, wherein the VHCDR1, VHCDR2, and VHCDR3 comprise SEQ ID NOs:450, 882, and 1314, respectively.

3. The antibody, or antigen-binding fragment thereof, of claim 1 or claim 2, wherein the VLCDR1, VLCDR2, and VLCDR3 comprise SEQ ID NOs:666, 1098, and 1530, respectively.

4. The antibody, or antigen-binding fragment thereof, of claim 1, wherein the VH is selected from any one of SEQ ID NOs:109-216.

5. The antibody, or antigen-binding fragment thereof, of claim 1, wherein the VL is selected from any one of SEQ ID NOs:325-432.

6. The antibody, or antigen-binding fragment thereof, of claim 4, wherein the VH comprises SEQ ID NO:123.

7. The antibody, or antigen-binding fragment thereof, of claim 5 or claim 6, wherein the VL comprises SEQ ID NO:339.

8. The antibody, or antigen-binding fragment thereof, of claim 1, wherein the antibody is human.

9. The antibody, or antigen-binding fragment thereof, of claim 1, wherein the antibody is chimeric.

10. The antibody, or antigen-binding fragment thereof, of claim 1, wherein the antibody is selected from a single-variable domain antibody, single chain antibody, a scFv, a bispecific antibody, a multi-specific antibody, a Fab, a F(ab')2, and a whole antibody.

11. A recombinant polynucleotide encoding the antibody, or antigen-binding fragment thereof, of claim 1.

12. An expression vector comprising the recombinant polynucleotide of claim 11.

13. An isolated host cell comprising the expression vector of claim 12.

14. A composition comprising the antibody, or antigen-binding fragment thereof, of claim 1 and a physiologically acceptable carrier.

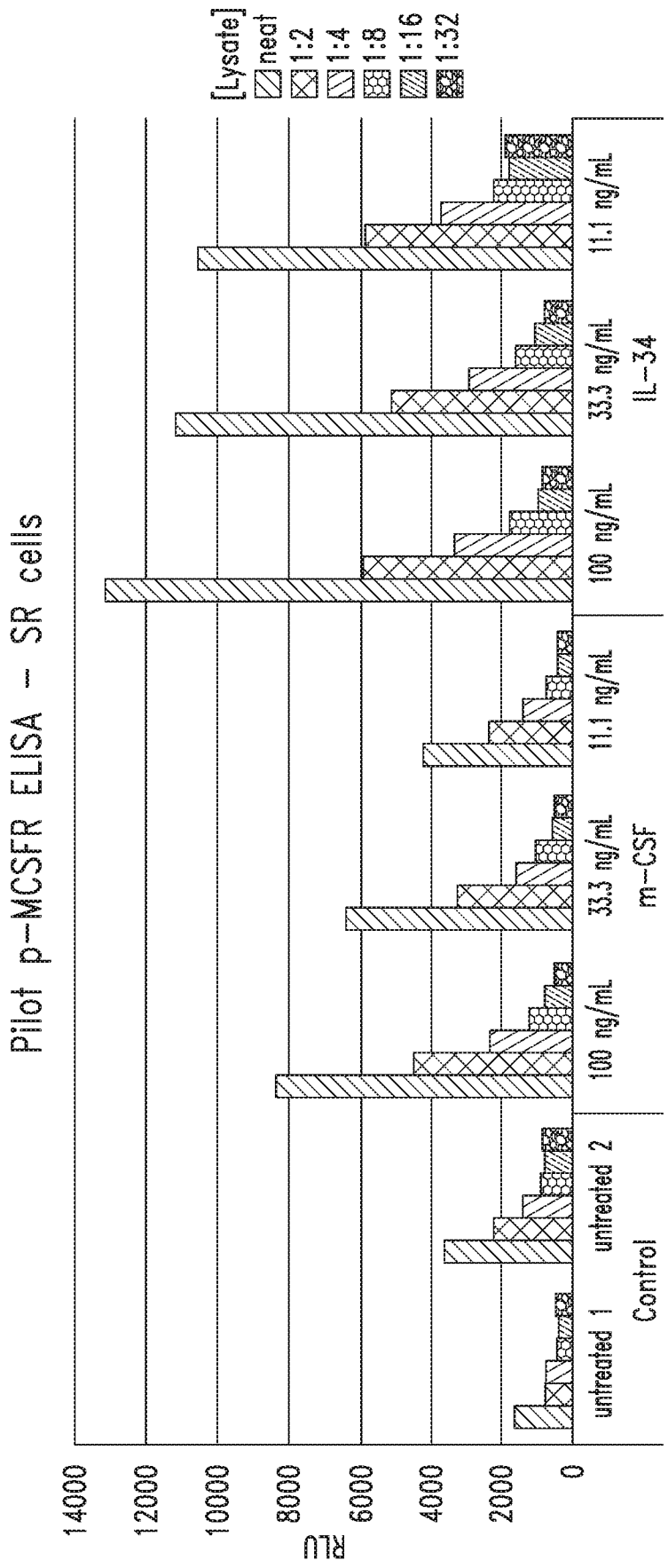

Pilot p-MCSFR ELISA - SR cells
RLU
14000
12000
10000
8000
6000
4000
2000
0
[Lysate]
neat
1:2
1:4
1:8
1:16
1:32
untreated 1
untreated 2
Control
100 ng/mL
33.3 ng/mL
11.1 ng/mL
m-CSF
100 ng/mL
33.3 ng/mL
11.1 ng/mL
IL-34

FIG. 1

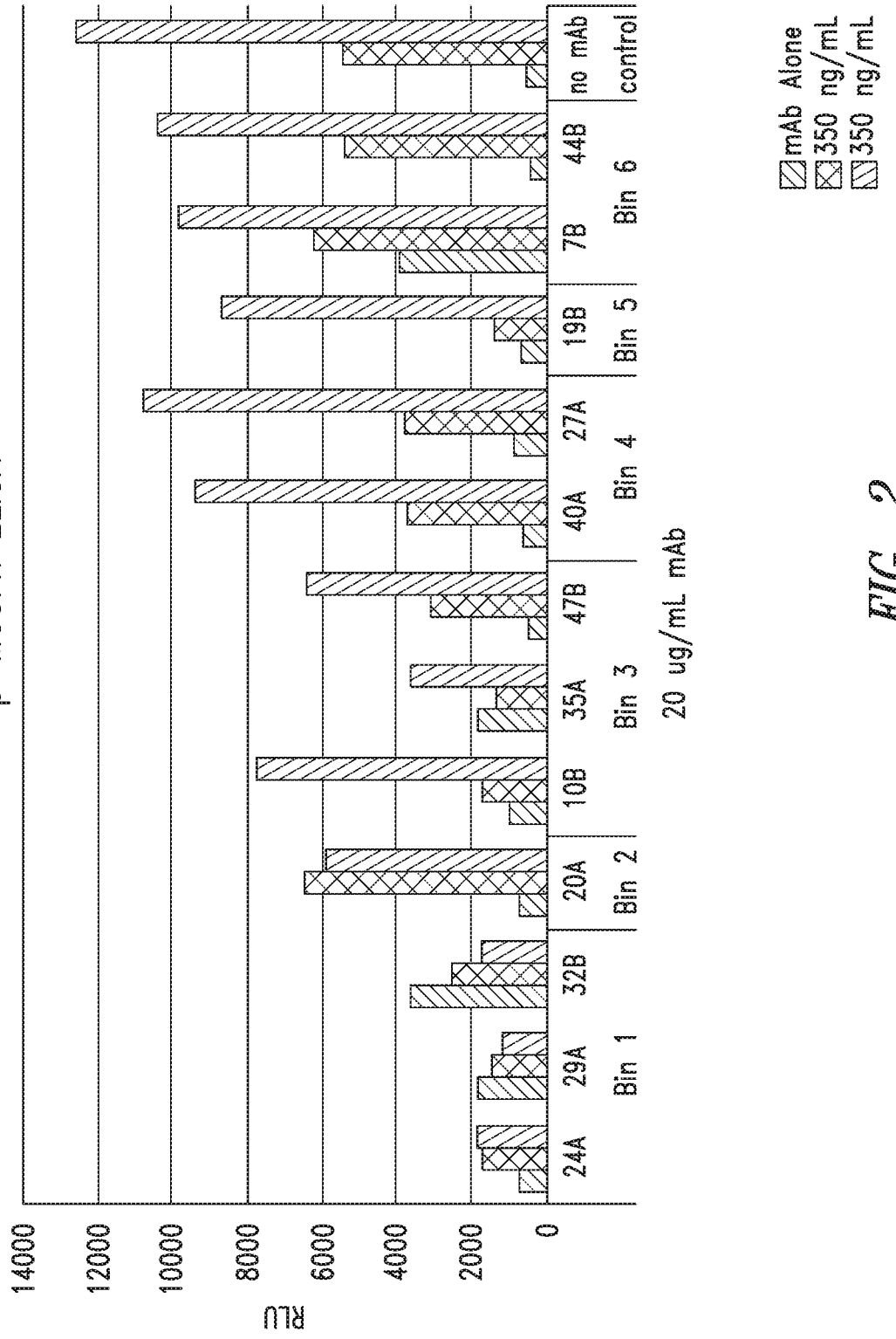
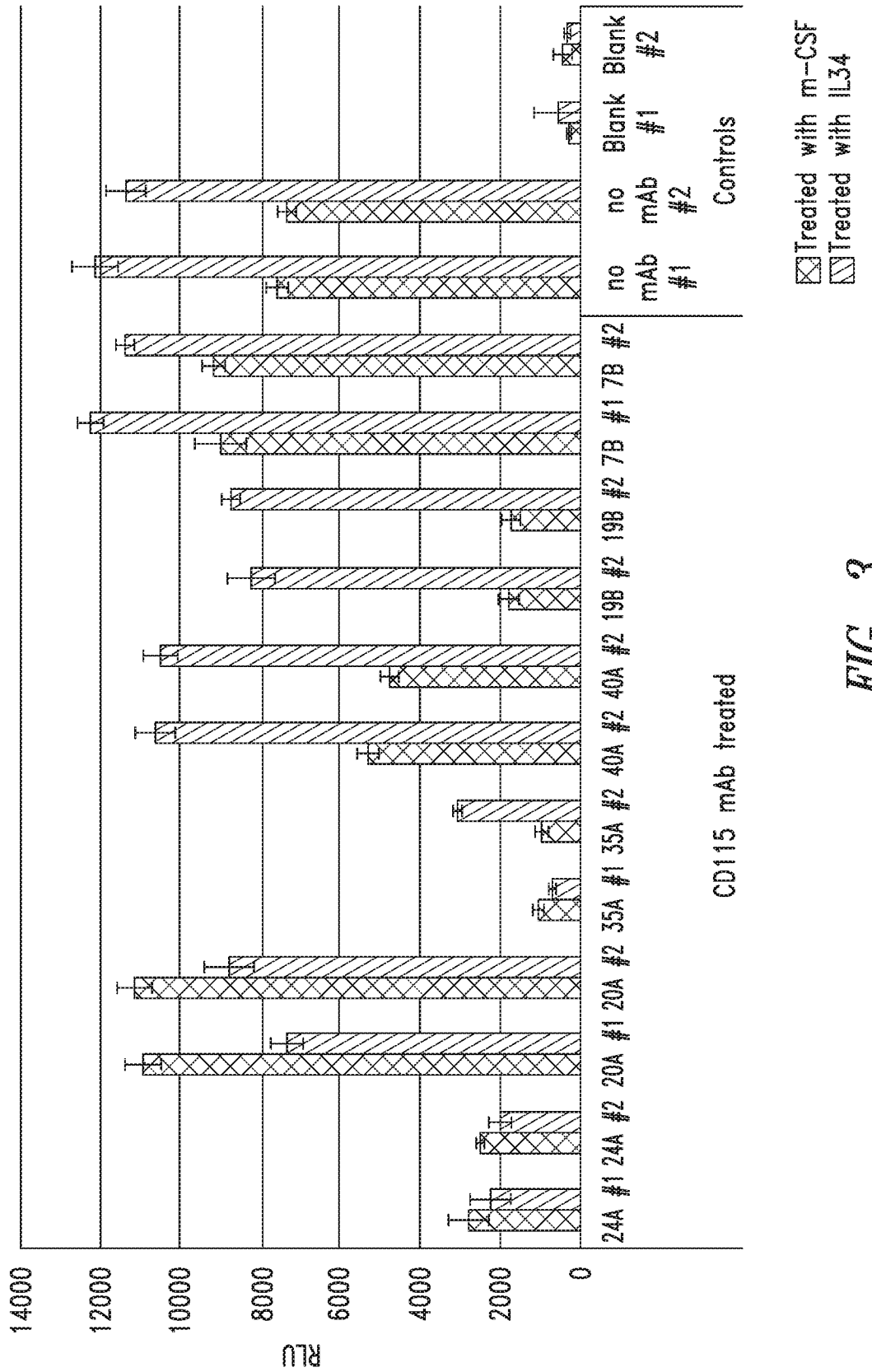

p-MCSFR ELISA
RLU
14000
12000
10000
8000
6000
4000
2000
0
24A
29A
32B
20A
10B
35A
47B
40A
27A
19B
7B
44B
no mAb
control
Bin 1
Bin 2
Bin 3
Bin 4
Bin 5
Bin 6
20 ug/mL mAb
mAb Alone
350 ng/mL ligand m-CSF
350 ng/mL ligand IL34

FIG. 2


p-MCSFR ELISA - mAb inhibition of CD115 phosphorylation
RLU
14000
12000
10000
8000
6000
4000
2000
0
24A #1
24A #2
20A #1
20A #2
35A #1
35A #2
40A #2
40A #2
19B #2
19B #2
7B #2
7B #1
7B #2
CD115 mAb treated
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no mAb #2
Blank #1
Blank #2
Controls
Treated with m-CSF
Treated with IL34

FIG. 3

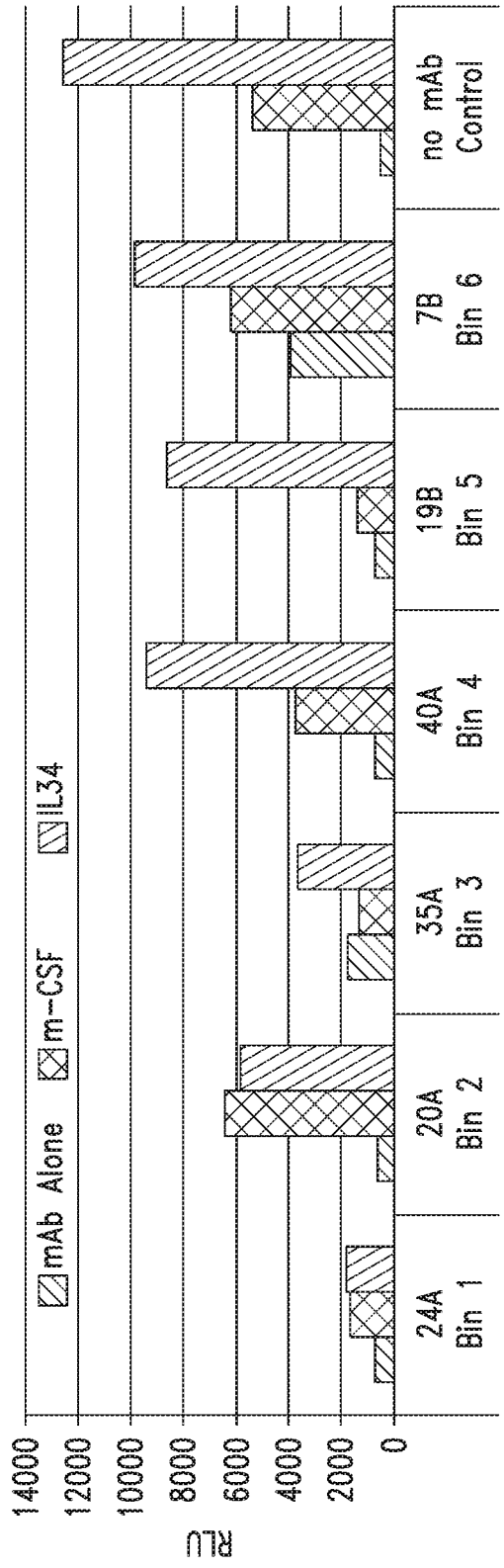

p-MCSFR ELISA - mAb inhibition initial screen
mAb Alone
m-CSF
IL34
RLU
14000
12000
10000
8000
6000
4000
2000
0
24A
Bin 1
20A
Bin 2
35A
Bin 3
40A
Bin 4
19B
Bin 5
7B
Bin 6
no mAb
Control

FIG. 4A

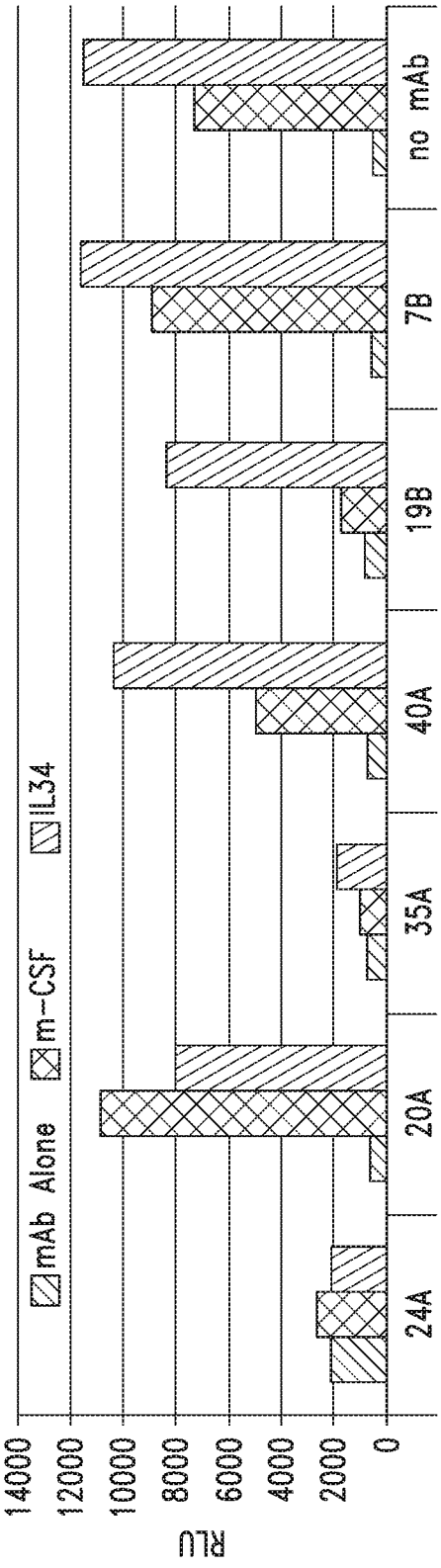
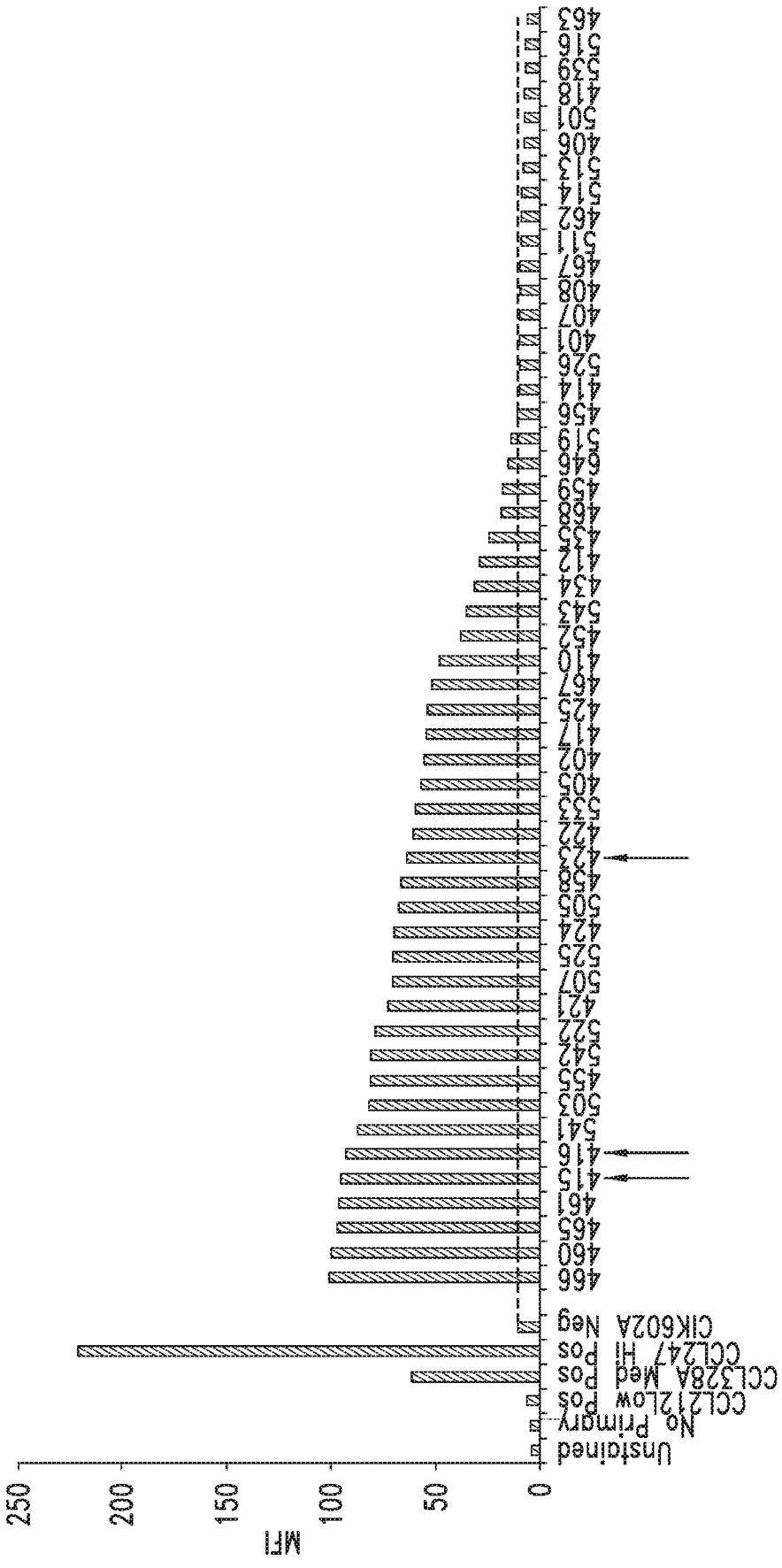

p-MCSFR ELISA - mAb inhibition confirmation screen
mAb Alone
m-CSF
IL34
RLU
14000
12000
10000
8000
6000
4000
2000
0
24A
20A
35A
40A
19B
7B
no mAb

FIG. 4B


MFI
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200
150
100
50
0
Unstained
No Primary
CCL21Low Pos
CCL328A Med Pos
CCL247 Hi Pos
CIK602A Neg

FIG. 5

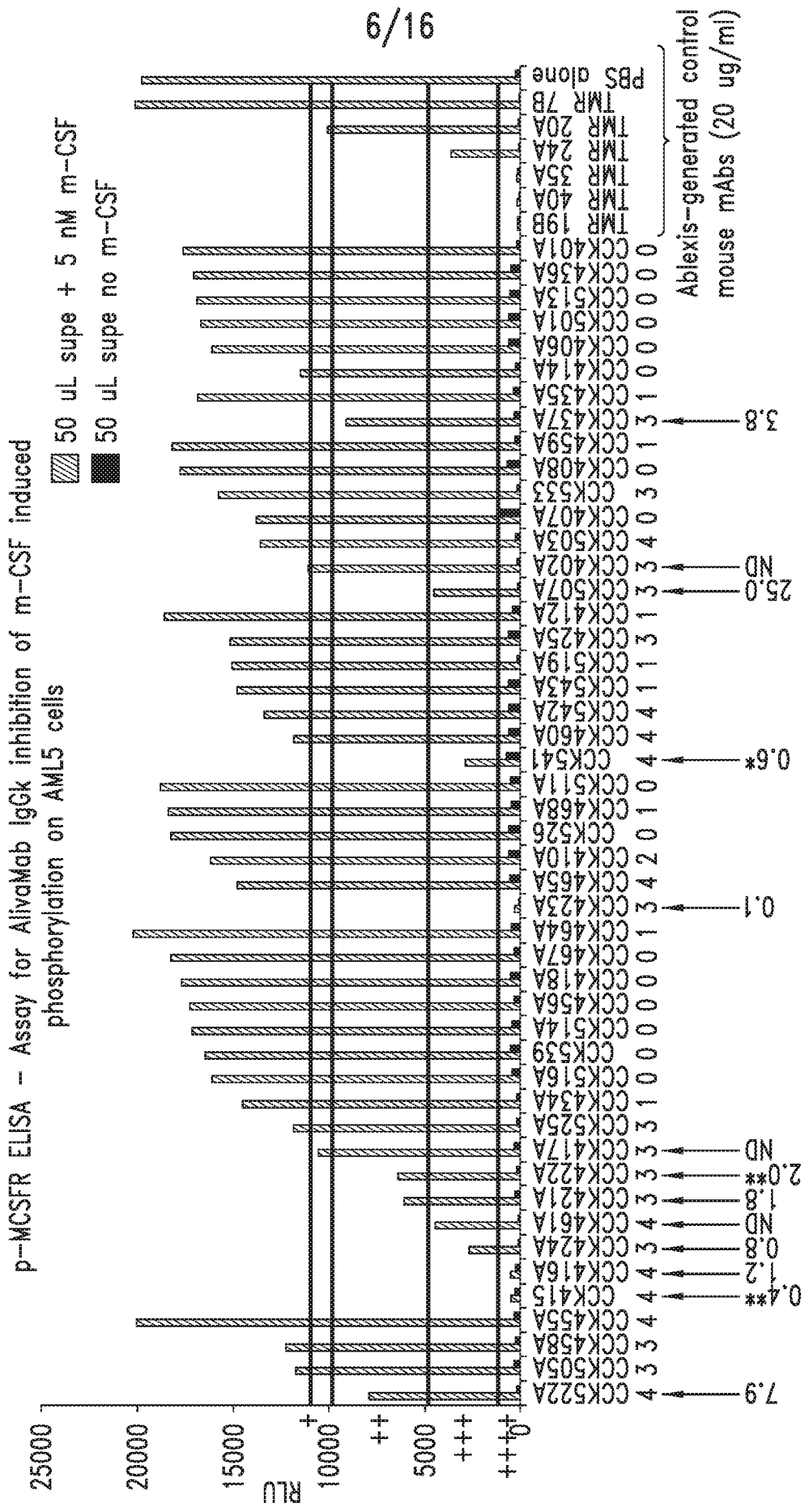
p-MCSFR ELISA - Assay for AlivaMab IgGk inhibition of m-CSF induced phosphorylation on AML5 cells
50 uL supe + 5 nM m-CSF
50 uL supe no m-CSF
RLU
25000
20000
15000
10000
5000
0
++++
+++
++
+
CCK522A
CCK505A
CCK458A
CCK455A
CCK415
CCK416A
CCK424A
CCK461A
CCK421A
CCK422A
CCK417A
CCK525A
CCK434A
CCK516A
CCK539
CCK514A
CCK456A
CCK418A
CCK467A
CCK464A
CCK423A
CCK465A
CCK410A
CCK526
CCK468A
CCK511A
CCK541
CCK460A
CCK542A
CCK543A
CCK519A
CCK425A
CCK412A
CCK507A
CCK402A
CCK503A
CCK407A
CCK533
CCK408A
CCK459A
CCK437A
CCK435A
CCK414A
CCK406A
CCK501A
CCK513A
CCK436A
CCK401A
TMR 19B
TMR 40A
TMR 35A
TMR 24A
TMR 20A
TMR 7B
PBS alone
Ablexis-generated control mouse mAbs (20 ug/ml)
7.9
0.4**
1.2
0.8
ND
1.8
2.0**
ND
0.1
0.6*
25.0
ND
3.8

FIG. 6

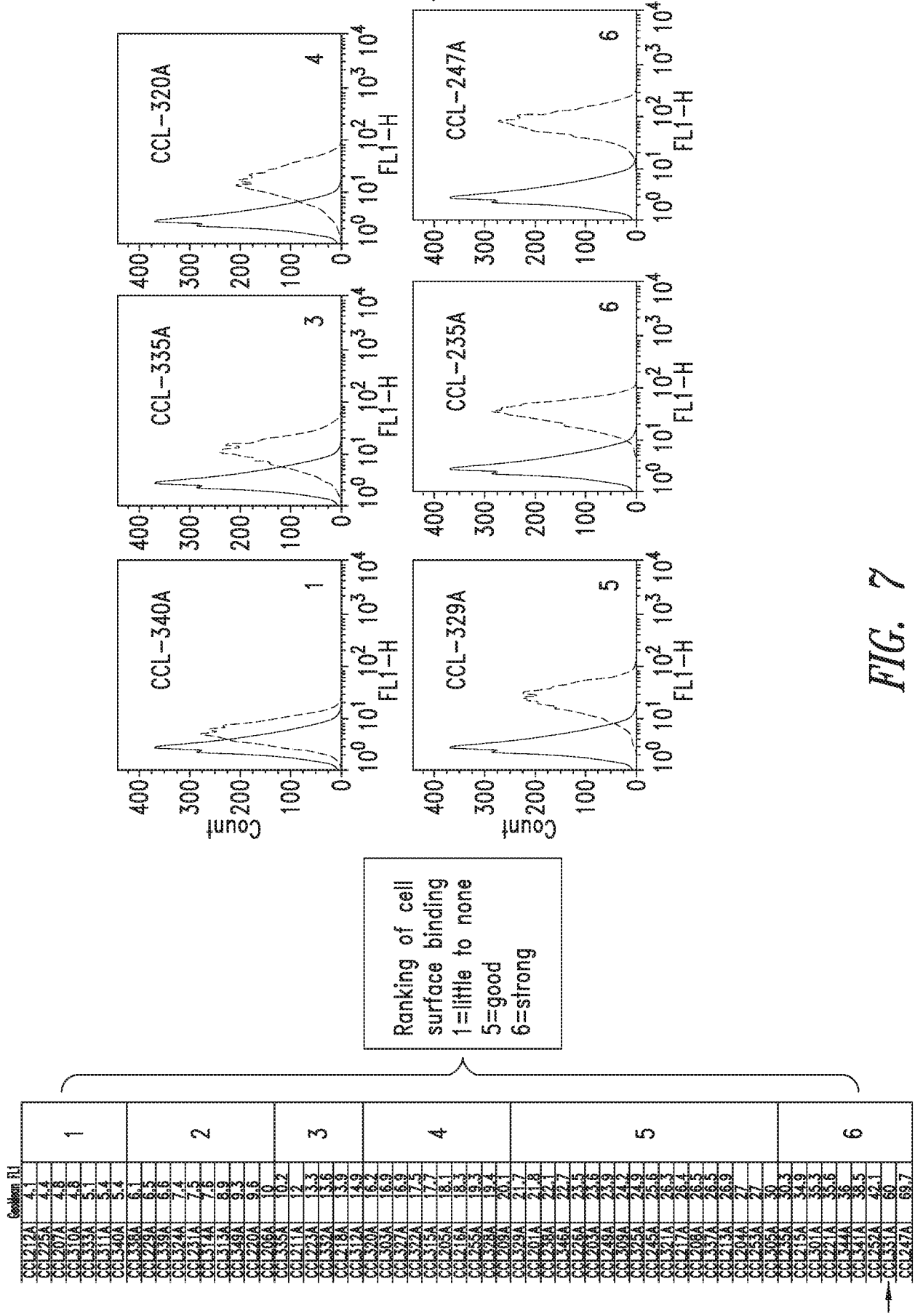
CCL-340A
1
CCL-335A
3
CCL-320A
4
CCL-329A
5
CCL-235A
6
CCL-247A
6
Count
FL1-H
Ranking of cell surface binding
1=little to none
5=good
6=strong
Geoklean RI
1
2
3
4
5
6

FIG. 7

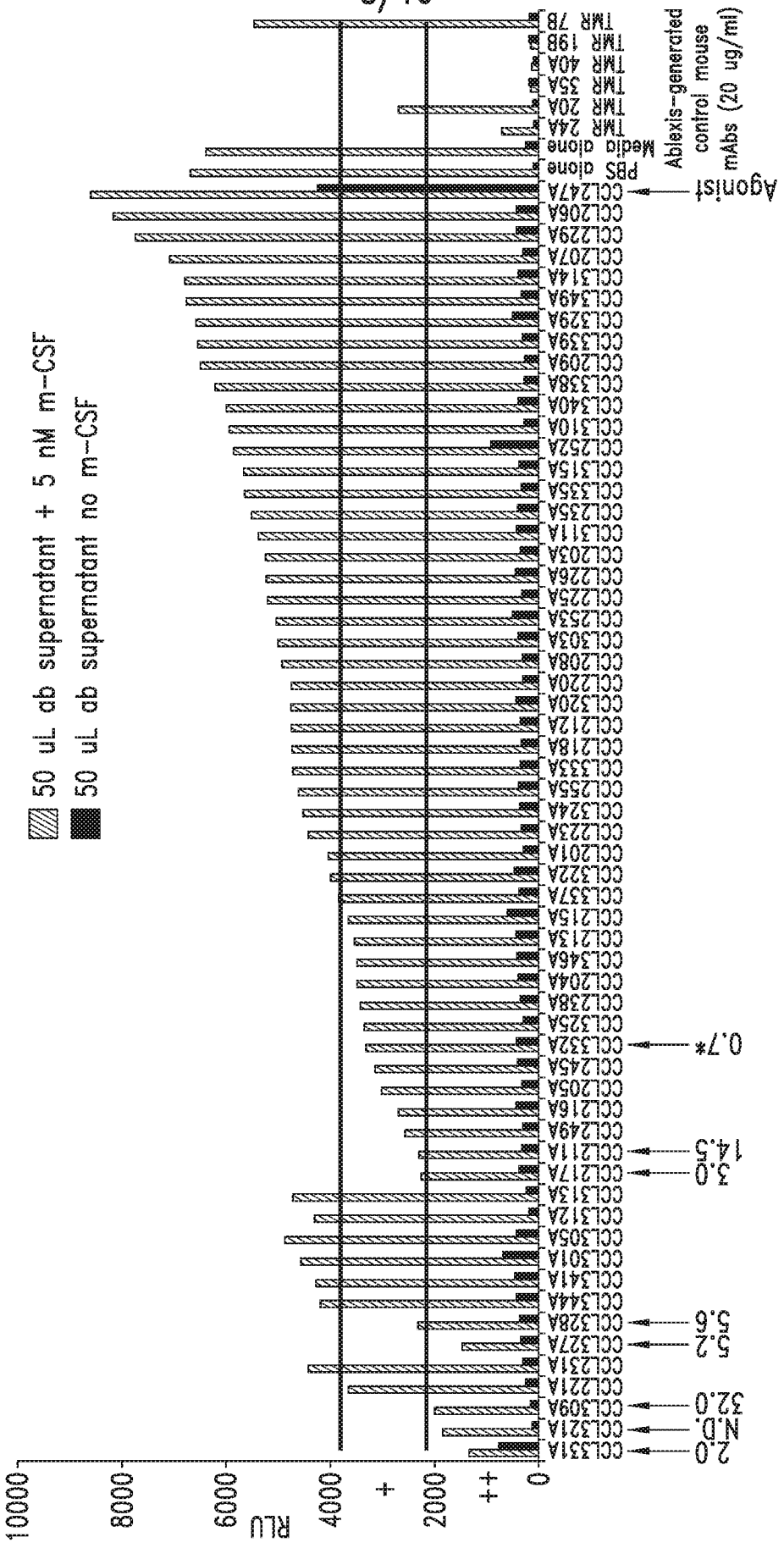
50 uL ab supernatant + 5 nM m-CSF
50 uL ab supernatant no m-CSF
RLU
10000
8000
6000
4000
2000
0
+
++
Agonist
Ablexis-generated control mouse mAbs (20 ug/ml)
PBS alone
Media alone
TMR 24A
TMR 20A
TMR 35A
TMR 40A
TMR 19B
TMR 7B
CCL247A
CCL206A
CCL229A
CCL207A
CCL314A
CCL349A
CCL329A
CCL339A
CCL209A
CCL338A
CCL340A
CCL310A
CCL252A
CCL315A
CCL335A
CCL235A
CCL311A
CCL203A
CCL226A
CCL225A
CCL253A
CCL303A
CCL208A
CCL220A
CCL320A
CCL212A
CCL218A
CCL333A
CCL255A
CCL324A
CCL223A
CCL201A
CCL322A
CCL337A
CCL215A
CCL213A
CCL346A
CCL204A
CCL238A
CCL325A
CCL332A
CCL245A
CCL205A
CCL216A
CCL249A
CCL211A
CCL217A
CCL313A
CCL312A
CCL305A
CCL301A
CCL341A
CCL344A
CCL328A
CCL327A
CCL231A
CCL222A
CCL309A
CCL321A
CCL331A
0.7*
14.5
3.0
5.6
5.2
32.0
N.D.
2.0

FIG. 8

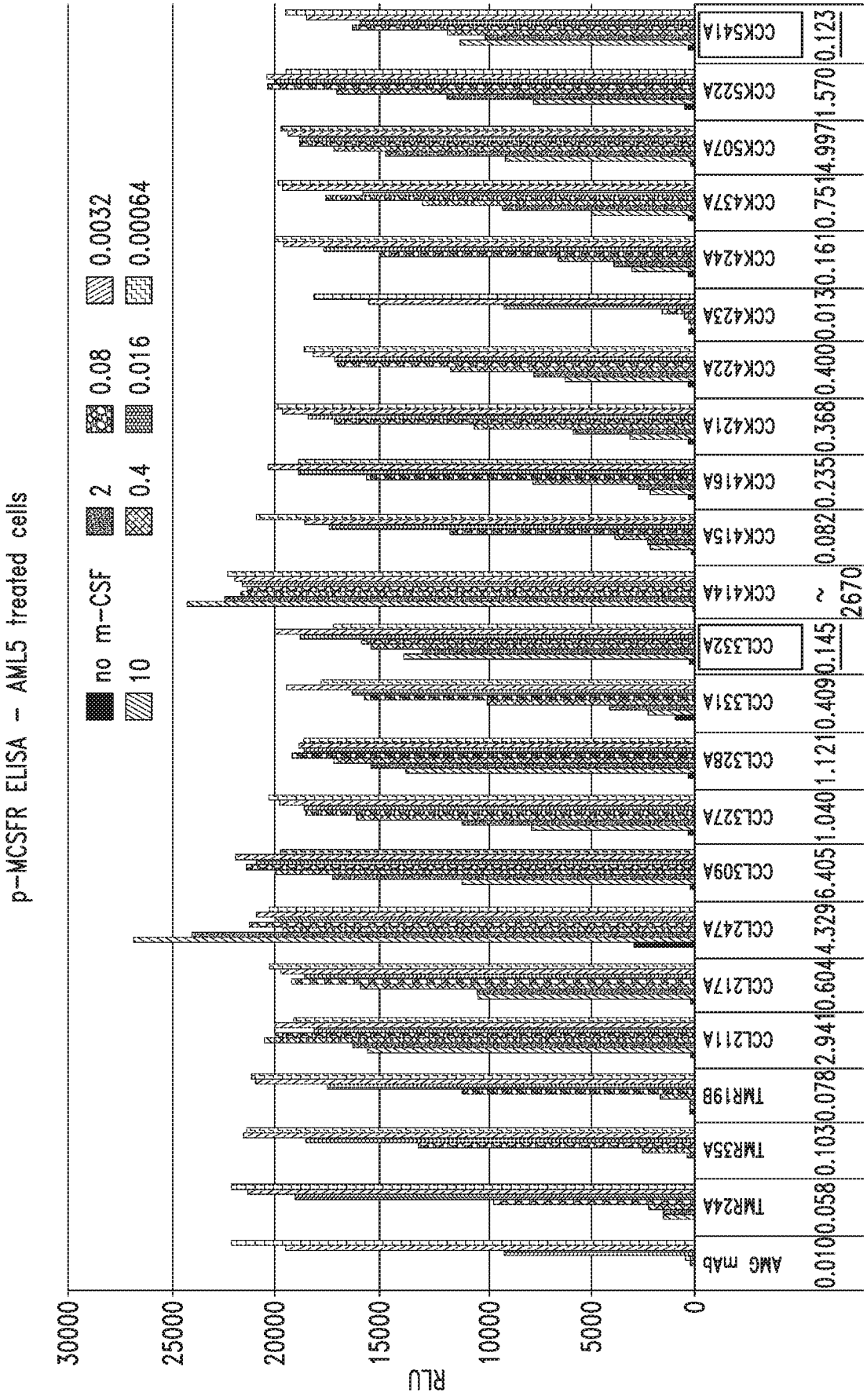
p-MCSFR ELISA - AML5 treated cells
no m-CSF
2
0.08
0.0032
10
0.4
0.016
0.00064
RLU
30000
25000
20000
15000
10000
5000
0
AMG mAb
TMR24A
TMR35A
TMR19B
CCL211A
CCL217A
CCL247A
CCL309A
CCL327A
CCL328A
CCL331A
CCL332A
CCK414A
CCK415A
CCK416A
CCK421A
CCK422A
CCK423A
CCK424A
CCK437A
CCK507A
CCK522A
CCK541A
0.0100
0.058
0.103
0.078
2.941
0.604
4.329
6.405
1.040
1.121
0.409
0.145
2670
0.082
0.235
0.368
0.400
0.013
0.161
0.751
4.997
1.570
0.123

FIG. 9

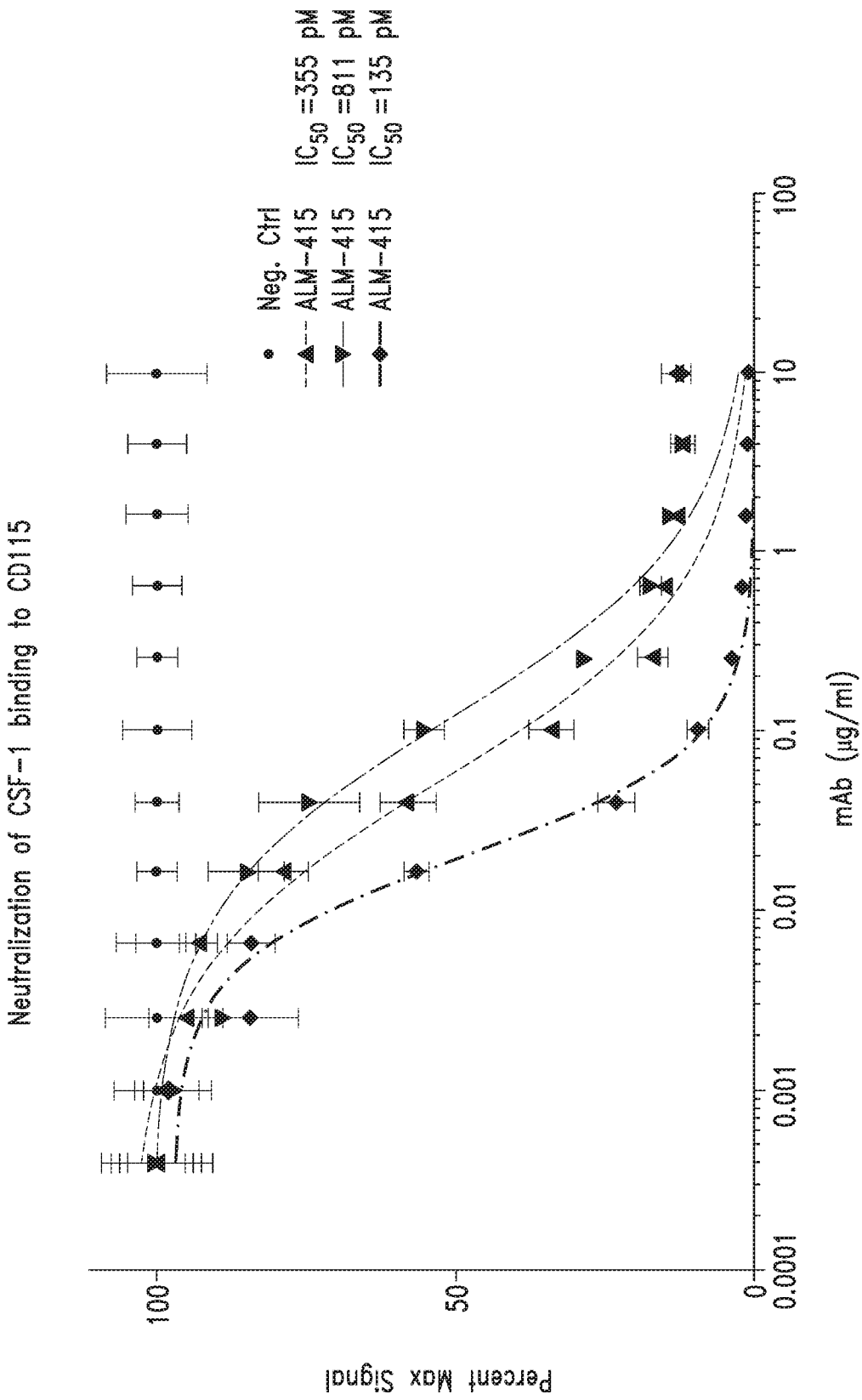
Neutralization of CSF-1 binding to CD115
Neg. Ctrl
ALM-415 IC50 =355 pM
ALM-415 IC50 =811 pM
ALM-415 IC50 =135 pM
Percent Max Signal
100
50
0
0.0001
0.001
0.01
0.1
1
10
100
mAb (µg/ml)

FIG. 10

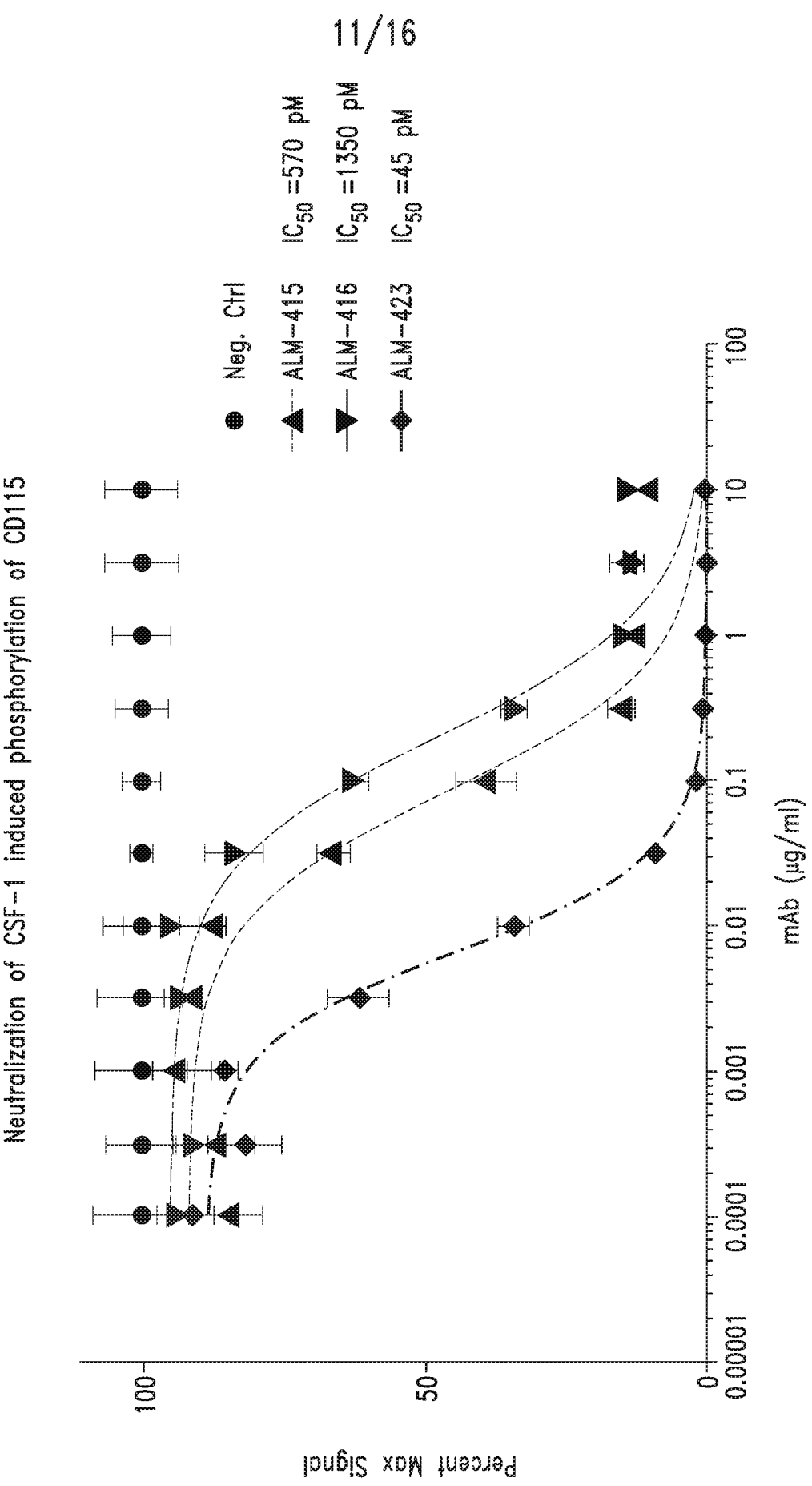
Neutralization of CSF-1 induced phosphorylation of CD115
Neg. Ctrl
ALM-415 IC50 =570 pM
ALM-416 IC50 =1350 pM
ALM-423 IC50 =45 pM
Percent Max Signal
100
50
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
mAb (µg/ml)

FIG. 11

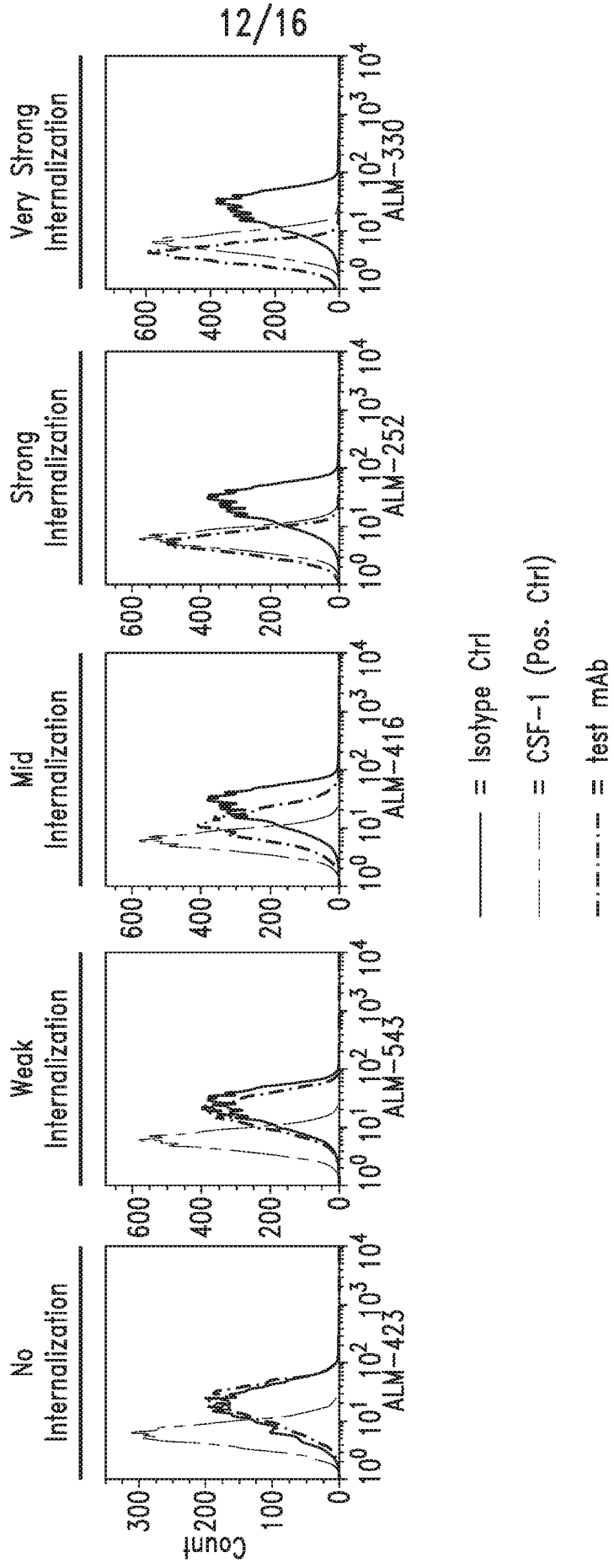
No Internalization
Weak Internalization
Mid Internalization
Strong Internalization
Very Strong Internalization
Count
ALM-423
ALM-543
ALM-416
ALM-252
ALM-330
= Isotype Ctrl
= CSF-1 (Pos. Ctrl)
= test mAb

FIG. 12

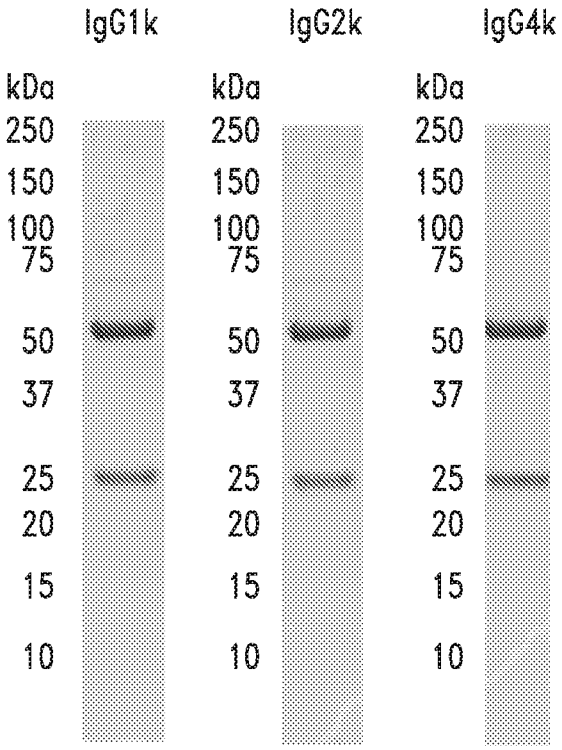

FIG. 13

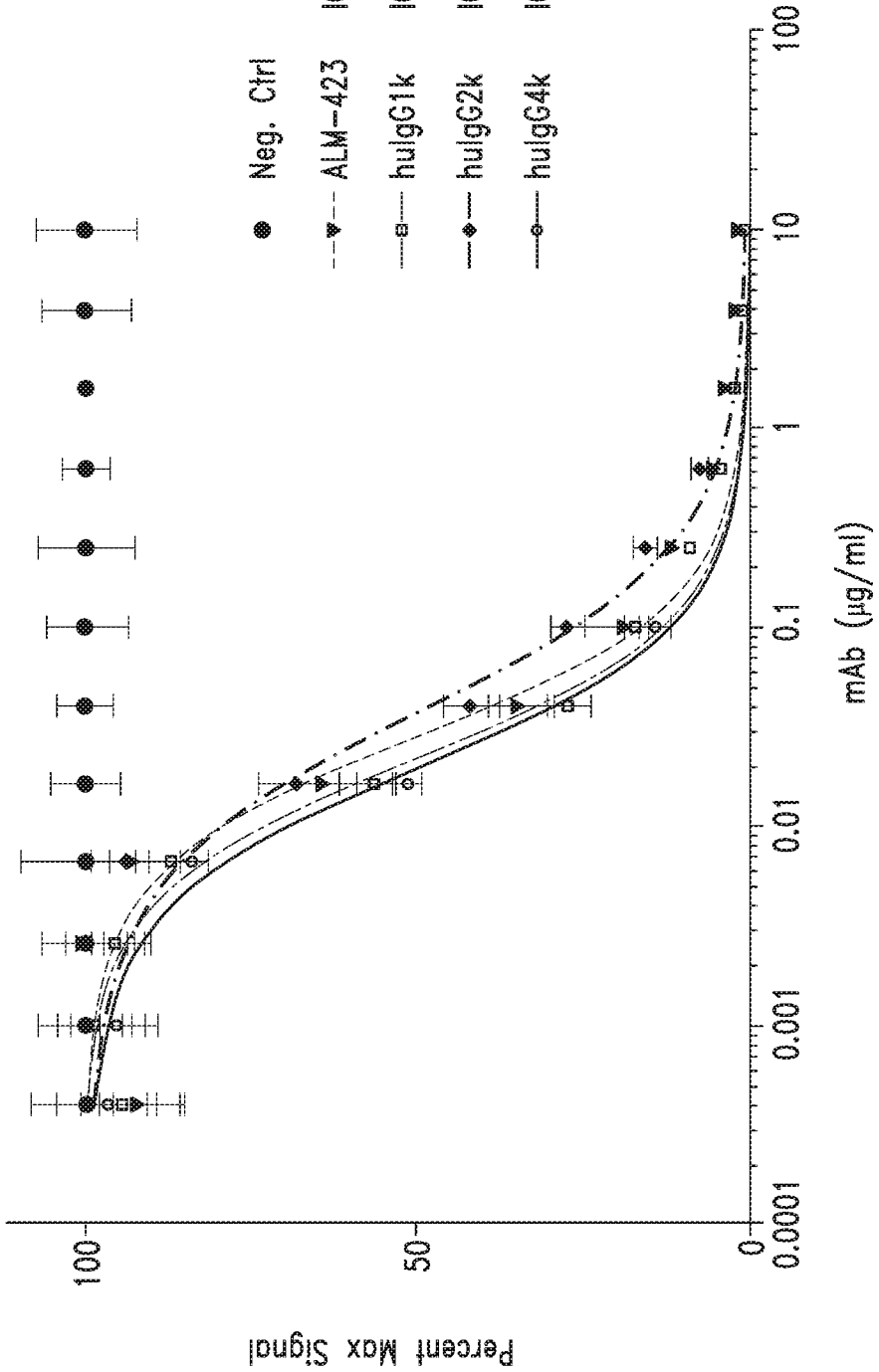
Neg. Ctrl
ALM-423 IC50 =191 pM
huIgG1k IC50 =146 pM
huIgG2k IC50 =253 pM
huIgG4k IC50 =133 pM
Percent Max Signal
100
50
0
0.0001
0.001
0.01
0.1
1
10
100
mAb (µg/ml)

FIG. 14

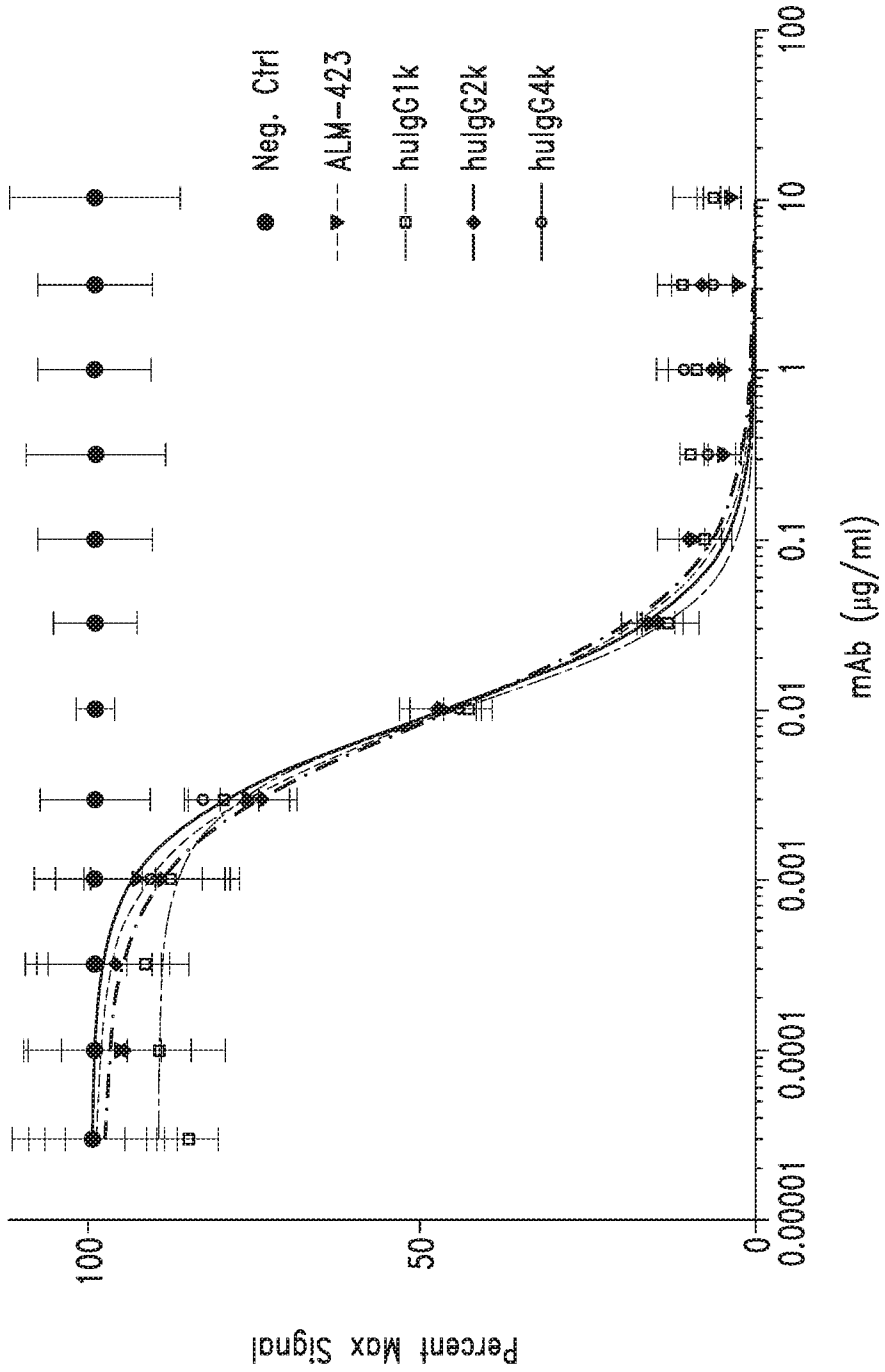
Neg. Ctrl
ALM-423 IC50 =58 pM
huIgG1k IC50 =68 pM
huIgG2k IC50 =59 pM
huIgG4k IC50 =60 pM
Percent Max Signal
100
50
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
mAb (µg/ml)

FIG. 15

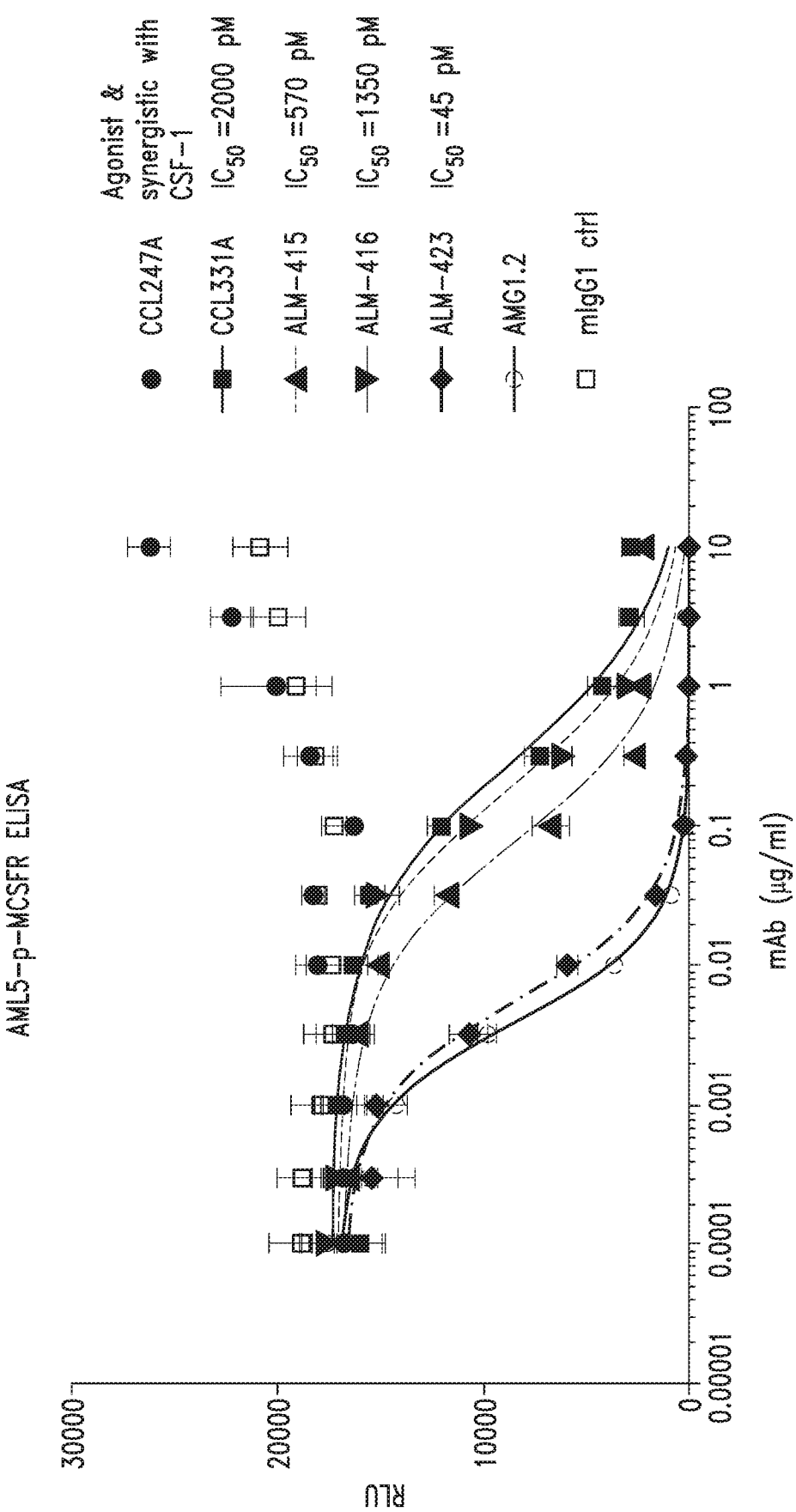
AML5-p-MCSFR ELISA
RLU
30000
20000
10000
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
mAb (µg/ml)
Agonist & synergistic with CSF-1
CCL247A
CCL331A IC50 =2000 pM
ALM-415 IC50 =570 pM
ALM-416 IC50 =1350 pM
ALM-423 IC50 =45 pM
AMG1.2
mIgG1 ctrl

FIG. 16