ANTIBODIES TARGETING M-CSF

This disclosure generally relates to antibodies or antibody fragments which specifically bind to M-CSF. In particular antibodies and antibody fragments are disclosed which bind to M-CSF and which inhibit binding of M-CSF to the M-CSF receptor with an IC50 of 10 pM or less. The invention also relates to nucleic acids, vectors and host cells capable of expressing the antibodies or fragments thereof of the invention, pharmaceutical compositions comprising the antibodies or fragments thereof and uses of said antibodies or fragments thereof and compositions for treatment of specific diseases.

BACKGROUND OF THE INVENTION

M-CSF is a secreted cytokine which influences hematopoietic stem cells to differentiate into macrophages or other related cell types. The active form of M-CSF is found extracellularly as a disulfide-linked homodimer. Three different isoforms of M-CSF are found extracellularly: secreted glycosylated M-CSF, secreted proteoglycan M-CSF, and cell-surface M-CSF.

M-CSF is a validated target for therapeutic invention, in particularly for the treatment of inflammatory disorders, such as e.g. rheumatoid arthritis. See e.g. US 8,142,777 which is incorporated by reference. Several molecules are under development which target M-CSF, including antibody approaches. See e.g. WO2005/030124 (Warner-Lambert/Pfizer) WO2005/068503 and (Chiron/Novartis).

The present disclosure provides novel antibodies and antibody fragments which are superior to the anti-M-CSF antibodies known from the prior art. In particular, the antibodies and antibody fragments of the present disclosure specifically bind to M-CSF and inhibit the binding of M-CSF to the M-CSF receptor with an IC50 of 10 pM or less in a receptor binding inhibition assay comprising M-CSF at a final concentration of 12.5 pM. In addition, the antibodies exhibit functional properties which are highly desirable for clinical development and which never have been observed before.

SUMMARY OF THE INVENTION

The present disclosure provides antibodies or antibody fragments which specifically bind to human M-CSF. The present disclosure also provides antibodies or antibody fragments which specifically bind to human M-CSF with a certain affinity, e.g. an affinity of 30 pM or lower. The present disclosure also provides antibodies or antibody fragments which specifically bind to M-CSF and which inhibit binding of M-CSF to the M-CSF receptor with an IC50 of 10 pM or less. The present disclosure also provides antibodies or antibody fragments which specifically binds to M-CSF and wherein said isolated antibody or antibody fragment is able to inhibit the binding of M-CSF to the M-CSF receptor with an IC50 of 10 pM or less in a receptor binding inhibition assay comprising M-CSF at a final concentration of 12.5 pM.

The present disclosure also provides specific antibodies or antibody fragments as defined by way of the amino acid sequences of the six CDR regions. The present disclosure also provides specific antibodies or antibody fragments as defined by way of the amino acid sequences of the variable heavy chain and the variable light chain.

The present disclosure also provides specific antibodies or antibody fragments which compete with the specific antibodies or antibody fragments disclosed herein. The present disclosure also provides specific antibodies or antibody fragments which bind to the same epitope as the specific antibodies or antibody fragments disclosed herein.

The present disclosure also provides the isolated antibody or antibody fragment of the present disclosure use in medicine.

The present disclosure also provides also provides methods for treating patients suffering from a disorder, such as an inflammatory disorder, by administering to said patient an effective amount of the antibodies or antibody fragments of the present disclosure.

The present disclosure also provides pharmaceutical compositions comprising the isolated antibody or antibody fragment of the present disclosure, and a pharmaceutically acceptable carrier.

The present disclosure also provides nucleic acids encoding the antibody or antibody fragment of the present disclosure.

The present disclosure also provides vector comprising nucleic acids encoding the antibodies or antibody fragment antibodies of the present disclosure.

The present disclosure also provides host cell comprising vector or nucleic acids encoding the antibodies or antibody fragments of the present disclosure.

FIGURE LEGENDS

FIGURE 1 demonstrates the ability of M-CSF-specific antibodies of the present disclosure to block the bioactivity of membrane-bound M-CSF isoform in an assay in which proliferation of M-NFS-60 cells was induced by CHO cells stably expressing human membrane-bound M-CSF. With increasing antibody concentration all M-CSF-specific immunoglobulins efficiently inhibit proliferation. In contrast, MOR03207 which is specific for lysozyme failed to inhibit proliferation.

DETAILED DESCRIPTION

The term "isolated" refers to a compound that is substantially free of other cellular materials and/or chemicals. If such compound is an antibody or antibody fragment then the term "isolated" refers to an antibody or antibody fragment that is also free of other antibodies or antigen binding moieties having different antigenic specificities.

The term "antibody" as used herein includes whole antibodies. A naturally occurring "antibody" is a protein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as VH) and a heavy chain constant region. The heavy chain constant region is comprised specific CH domains (e.g. CH1, CH2 and CH3). Each light chain is comprised of a light chain variable region (abbreviated herein as VL) and a light chain constant region. The light chain constant region is comprised of one domain, CL. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementary determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FRs arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (e.g., effector cells) and the first component (C1q) of the classical complement system. The antibodies can be of any isotype (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2), subclass or modified version

thereof (e.g. IgG1 LALA). The antibodies can be of any species, chimeric, humanized or human.

The terms "heavy chain variable region CDR1" and "H-CDR1" are used interchangeably, as are the terms "heavy chain variable region CDR2" and "H-CDR2", the terms "heavy chain variable region CDR3" and "H-CDR3", the terms "light chain variable region CDR1" and "L-CDR1"; the terms "light chain variable region CDR2" and "L-CDR2" and the terms "light chain variable region CDR3" and "L-CDR3"antibody fragment

Antigen binding can be performed by "fragments" "antibody fragments" "antigen binding fragments" of an intact antibody. Herein, both terms are used interchangeably. Examples of binding fragments encompassed within the term "antibody fragment" of an antibody include a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; a F(ab)2 fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; an Fd fragment consisting of the VH and CH1 domains; an Fv fragment consisting of the VL and VH domains of a single arm of an antibody; a single domain antibody (dAb) fragment (Ward et al., (1989) Nature 341:544-546), which consists of a VH domain; and an isolated complementary determining region (CDR).

A "single chain Fragment (scFv)" is a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see, e.g., Bird et al., (1988) Science 242:423-426; and Huston et al., (1988) Proc. Natl. Acad. Sci. 85:5879-5883). Although the two domains VL and VH are coded for by separate genes, they can be joined, using recombinant methods, by an artificial peptide linker that enables them to be made as a single protein chain. Such single chain antibodies include one or more antigen binding moieties. These antibody fragments are obtained using conventional techniques known to those of skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies. In certain aspects the present disclosure provides antibody fragments, wherein said antibody fragment is selected from the group consisting of a Fab, F(ab2)', F(ab)2' and scFV.

In certain aspects the present disclosure provides antibodies or antibody fragments, wherein said antibody or antibody fragment is bispecific. In certains aspects said antibody or antibody fragment is a bispecific antibody-derived scaffold wherein said bispecific antibody-derived scaffold is selected from the group consisting of a bispecific-scFv, a tetravalent bispecific antibody, a cross-linked Fab or a bispecific IgG.

In certain aspects the present disclosure provides to an antibody or antibody fragment, wherein the antibody or antibody fragment is selected from the group consisting of single domain antibodies, maxibodies, minibodies, intrabodies, diabodies, triabodies, tetrabodies, v-NAR, camelid antibodies, ankyrins, domain antibodies, lipocalins, small modular immuno-pharmaceuticals, maxybodies, Protein A and affilins.

The terms "monoclonal antibody" as used herein refer to a preparation of antibody molecules of single molecular composition. A monoclonal antibody composition displays a unique binding site having a unique binding specificity and affinity for particular epitopes. In certain aspects the present disclosure provides monoclonal antibodies or antibody fragments which specifically bind to M-CSF. In certain aspects the present disclosure provides polyclonal antibodies or antibody fragments which specifically bind to M-CSF.

In certain aspect the disclosure provides an isolated antibody or antibody fragment which is cross-reactive to cynomolgus M-CSF. In certain aspect the disclosure provides an isolated antibody or antibody fragment which is cross-reactive to mouse M-CSF. In certain aspect the disclosure provides an isolated antibody or antibody fragment which is cross-reactive to rat M-CSF. In certain aspect the disclosure provides an isolated antibody or antibody fragment which is cross-reactive to cynomolgus and/or mouse and/or rat M-CSF.

The term "human antibody", as used herein, is intended to include antibodies having variable regions in which both the framework and CDR regions are derived from sequences of human origin. As used herein, a human antibody comprises heavy or light chain variable regions or full length heavy or light chains. In certain cases, a human antibody may be at least 60%, 70%, 80%, 90%, or at least 95%, or even at least 96%, 97%, 98%, or 99% identical in amino acid sequence to the amino acid sequence encoded by the germline immunoglobulin gene. Thereby said human antibody can be obtained from technology platforms which comprise antibodies derived from human germline genes either generated by PCR-amplification of VH/VL repertoire isolated from B-cells or are generated synthetically. Technology platforms include library based approaches comprising human immunoglobulin genes displayed on phage, ribosome or yeast. Respective display technologies are standard in the scientific community. Furthermore immunization of a transgenic mouse carrying human immunoglobulin repertoire is another approach to generate human antibodies against an antigen of interest. Antibodies or fragments thereof selected from an antibody library based on the MorphoSys HuCAL® concept (Knappik et al., (2000) J Mol Biol 296:57-86) are considered as fully human.

A "humanized" antibody is an antibody that retains the reactivity of a non-human antibody while being less immunogenic in humans. This can be achieved, for instance, by retaining the non-human CDR regions and replacing the remaining parts of the antibody with their human counterparts (i.e., the constant region as well as the framework portions of the variable region). See, e.g., Morrison et al (1994) Proc. Natl. Acad. Sci. USA, 81:6851-6855; Morrison and Oi (1988) Adv. Immunol., 44:65-92; Verhoeyen et al. (1988) Science, 239:1534-1536; Padlan, Molec (1991) Immun., 28:489-498; and Padlan, Molec (1994) Immun., 31:169-217. Other examples of human engineering technology include, but are not limited to Xoma technology disclosed in US 5,766,886.

The term "chimeric antibody" is an antibody molecule in which (a) the constant region, or a portion thereof, is altered, replaced or exchanged so that the antigen binding site (variable region) is linked to a constant region of a different or altered class, effector function and/or species, or an entirely different molecule which confers new properties to the chimeric antibody, e.g., an enzyme, toxin, hormone, growth factor, drug, etc.; or (b) the variable region, or a portion thereof, is altered, replaced or exchanged with a variable region having a different or altered antigen specificity. For example, a mouse antibody can be modified by replacing its constant region with the constant region from a human immunoglobulin. Due to the replacement with a human constant region, the chimeric antibody can retain its specificity in recognizing the antigen while having reduced antigenicity in human as compared to the original mouse antibody.

In certain aspects the present disclosure provides human antibodies and antibody fragments which specifically bind to M-CSF. In certain aspects the present disclosure provides humanized antibodies and antibody fragments which specifically bind to M-CSF. In certain aspects the present disclosure provides chimeric antibodies and antibody fragments which specifically bind to M-CSF. In certain aspects the present disclosure provides antibodies comprising a human heavy chain constant region and a human light chain constant region.

The term "isotype" refers to the antibody class (e.g., IgM, IgE, IgG such as IgG1 or IgG4) that is provided by the heavy chain constant region genes. Isotype also includes modified versions of one of these classes, where modifications have been made to alter the Fc function, for example, to enhance or reduce effector functions or binding to Fc receptors. For example IgG1 LALA is a modified version of the IgG isotype having significantly reduced effector functions. Specific substitutions of amino acids reduced the binding affinity for Fc gamma RI receptor as compared with unmodified antibody. IgG1 LALA is described in US

serial no. 08/479,752 (SCOTGEN BIOPHARMACEUTICALS INC.) which is incorporated by reference in its entirety. In certain embodiments of the present disclosure the antigen-binding moieties of are antibodies and are of the type IgG, IgM, IgA, IGE or IgD. In specific embodiments the antibodies are of the type IgG. In certain embodiments of the present disclosure the antibodies are of the subtype IgG1, IgG2, IgG3 or IgG4. In specific embodiments the antibodies are of the subtype IgG1 or IgG4. In other specific embodiments the antibodies are of the subtype IgG1 or IgG1 LALA.

In certain specific embodiments of the present disclosure, the antibodies are of a silent isotype. The term "silent" isotype refers to any immunoglobulin with a diminished effector function. Therefore, in certain embodiments of the present disclosure the antibody is of a IgG1 subtype which has an effector function which is diminished compared to the wild type IgG1 subtype. Certain mutations are particularly suited to achieve a diminished effector function. For example the IgG1 LALA subtype is a typical silent isotype. Other silent versions of the IgG1 isotype might be used with the antibodies of the present disclosure as well. One specifically preferred example is the IgG1 isotype harboring a D265A mutation. In this IgG1 version the amino acid aspartic acid at position 265 (numbering according to the EU index; see http://www.imgt.org/IMGTScientificChart/Numbering/Hu_IGHGnber.html) is exchanged for an alanine residue. Therefore, in certain aspects the antibodies of the present disclosure are antibodies of a silent IgG1 subtype. In alternative aspect the antibodies of the present disclosure are antibodies of a mutant IgG1 subtype which has decreased effector as compared to the wildtype IgG1 subtype. In alternative aspects the antibodies of the present disclosure are antibodies of the IgG1 subtype carrying a D265A mutation. In alternative aspect the antibodies of the present disclosure are antibodies of the IgG1 subtype wherein the aspartic acid at position 265 is exchanged for an alanine residue. In alternative aspect the antibodies of the present disclosure are antibodies in which the aspartic acid residue at position 265 (numbering according to the EU index) is exchanged for an alanine residue.The subtypes IgG2 and IgG4 are also known as silent isotypes.

The term "affinity" as used herein refers to the strength of interaction between an antigen binding moiety, like e.g. a monoclonal antibody and an antigen at single antigenic sites. Within each antigenic site, the variable region of the antibody "arm" interacts through weak non-covalent forces with antigen at numerous sites; the more interactions, the stronger the affinity.

The term "specifically binds [to]" an antigen refers to a binding reaction that is determinable in the presence of an antigen in a heterogeneous population of proteins and

other biologics. Thereby the phrases "recognizing an antigen" and "specific for an antigen" are used interchangeably herein with the term "binds specifically to an antigen". Specific binding of an antigen binding moiety, like e.g. a monoclonal antibody, to an antigen can be determined by various established methods known in the art and include ELISA, FACS, Western Blot, Immuno Blot, MSD, BIAcore and SET. In the present disclosure an antigen binding moiety is deemed to be specific for an antigen if the antigen binding moiety is demonstrated to be able to bind to a specific antigen at least 2-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 6-fold, at least 7-fold, at least 8-fold, at least 9-fold, at least 10-fold, at least 20-fold, at least 50-fold, at least 100-fold, at least 500-fold, at least 1000-fold over background. Thereby the background is determined by an antigen binding moiety which is known to be unspecific for the selected antigens or by comparison to binding to an unrelated antigen. The antigen for the inventive antibodies and antibody fragments of the present invention is M-CSF.

The full-length human M-CSF precursor (also known as CSF-1 or macrophage colony-stimulating-factor) has a length of 554 amino acids. The amino acid sequence of human M-CSF precursor is shown in SEQ ID No.: 1 (source: Uniprot, human M-CSF P09603).

SEQ ID NO.:1 (full-length human M-CSF precursor):

MTAPGAAGRCPPTTWLGSLLLLVCLLASRSITEEVSEYCSHMIGSGHLQSLQRLIDS
QMETSCQITFEFVDQEQLKDPVCYLKKAFLLVQDIMEDTMRFRDNTPNAIAIVQLQEL
SLRLKSCFTKDYEEHDKACVRTFYETPLQLLEKVKNVFNETKNLLDKDWNIFSKNCN
NSFAECSSQDVVTKPDCNCLYPKAIPSSDPASVSPHQPLAPSMAPVAGLTWEDSEG
TEGSSLLPGEQPLHTVDPGSAKQRPPRSTCQSFEPPETPVVKDSTIGGSPQPRPSV
GAFNPGMEDILDSAMGTNWVPEEASGEASEIPVPQGTELSPSRPGGGSMQTEPAR
PSNFLSASSPLPASAKGQQPADVTGTALPRVGPVRPTGQDWNHTPQKTDHPSALL
RDPPEPGSPRISSLRPQGLSNPSTLSAQPQLSRSHSSGSVLPLGELEGRRSTRDRR
SPAEPEGGPASEGAARPLPRFNSVPLTDTGHERQSEGSFSPQLQESVFHLLVPSVIL
VLLAVGGLLFYRWRRRSHQEPQRADSPLEQPEGSPLTQDDRQVELPV

Three different extracellular isoforms of M-CSF are expressed as a consequence of splicing and post-translational modifications: secreted glycosylated M-CSF ("sgM-CSF"), secreted proteoglycan M-CSF ("spM-CSF"), and cell-surface M-CSF ("csM-CSF").

The most predominant isoform in human serum is sgM-CSF which is generated by cleavage of the M-CSF precursor at position 255. The amino acid sequence of human sgM-CSF is shown in SEQ ID No.: 2.

SEQ ID NO.: 2 (human secreted glycosylated M-CSF (fragment 33-255 of SEQ. 1)):

EEVSEYCSHMIGSGHLQSLQRLIDSQMETSCQITFEFVDQEQLKDPVCYLKKAFLLV
QDIMEDTMRFRDNTPNAIAIVQLQELSLRLKSCFTKDYEEHDKACVRTFYETPLQLLE
KVKNVFNETKNLLDKDWNIFSKNCNNSFAECSSQDVVTKPDCNCLYPKAIPSSDPAS
VSPHQPLAPSMAPVAGLTWEDSEGTEGSSLLPGEQPLHTVDPGSAKQRPPR

Human secreted proteoglycan M-CSF has a length of 456 amino acids. The amino acid sequence of spM-CSF is shown in SEQ ID NO.: 3.

SEQ ID NO.: 3 (human secreted proteoglycan M-CSF)

EEVSEYCSHMIGSGHLQSLQRLIDSQMETSCQITFEFVDQEQLKDPVCYLKKAFLLV
QDIMEDTMRFRDNTPNAIAIVQLQELSLRLKSCFTKDYEEHDKACVRTFYETPLQLLE
KVKNVFNETKNLLDKDWNIFSKNCNNSFAECSSQDVVTKPDCNCLYPKAIPSSDPAS
VSPHQPLAPSMAPVAGLTWEDSEGTEGSSLLPGEQPLHTVDPGSAKQRPPRSTCQ
SFEPPETPVVKDSTIGGSPQPRPSVGAFNPGMEDILDSAMGTNWVPEEASGEASEI
PVPQGTELSPSRPGGGSMQTEPARPSNFLSASSPLPASAKGQQPADVTGTALPRV
GPVRPTGQDWNHTPQKTDHPSALLRDPPEPGSPRISSLRPQGLSNPSTLSAQPQLS
RSHSSGSVLPLGELEGRRSTRDRRSPAEPEGGPASEGAARPLPRFNSVPLTDTGH
ERQSEGS

The human cell-surface M-CSF is generated by alternative splicing and has a length of 256 amino acids. The amino acid sequence of csM-CSF is shown in SEQ ID NO.: 4.

SEQ ID NO.: 4 (human cell surface M-CSF)

EEVSEYCSHMIGSGHLQSLQRLIDSQMETSCQITFEFVDQEQLKDPVCYLKKAFLLVQDIMED
TMRFRDNTPNAIAIVQLQELSLRLKSCFTKDYEEHDKACVRTFYETPLQLLEKVKNVFNETKNL
LDKDWNIFSKNCNNSFAECSSQGHERQSEGSSSPQLQESVFHLLVPSVILVLLAVGGLLFYR
WRRRSHQEPQRADSPLEQPEGSPLTQDDRQVELPV

All 3 isoforms of M-CSF can bind to the M-CSF receptor and are biologically active. The three isoforms have in common the N-terminal receptor-binding domain (rbdM-CSF), the amino acid sequence of which is shown in SEQ ID NO.: 5.

SEQ ID NO.: 5 (human receptor binding domain M-CSF (fragment 33-190))

EEVSEYCSHMIGSGHLQSLQRLIDSQMETSCQITFEFVDQEQLKDPVCYLKKAFLLV
QDIMEDTMRFRDNTPNAIAIVQLQELSLRLKSCFTKDYEEHDKACVRTFYETPLQLLE
KVKNVFNETKNLLDKDWNIFSKNCNNSFAECSSQDVVTKPDCN

The antibodies and antibody fragments of the present disclosure also bind to M-CSF of cynomolgus monkeys (*Macaca fascicularis)*, which are frequently used in the laboratory for pre-clinical studies. The nucleic acid molecule encoding secreted glycoprotein M-CSF of cynomolgus was cloned by standard PCR techniques from cynomolgus cDNA prepared from breast or pancreas tissues. The sequence of cynomolgus sgM-CSF is shown in SEQ ID NO.: 6.

SEQ ID NO.: 6 (cynomolgus sgM-CSF):

EEVSEYCSHMIGSGHLQSLQRLIDSQMETSCQITFEFVDQEQLKDPVCYLKKAFLLV
QDIMEDTMRFRDNTPNAIAIVQLQELSLRLKSCFTKDYEEHDKACVRTFYETPLQLLE
KVKNVFNETKNLLDKDWNIFSKNCNNSFAECSSQDVVTKPDCNCLYPKAIPSSDPAS
VSPHQPLAPSMAPMAGLTWDDSEGTEGSSLLPGEQPLHTVDPGSAKQRPPR

Human M-CSF receptor (also known as M-CSFR or CSF1R) has a length of 972 amino acids. The amino acid sequence of human M-CSFR is shown in SEQ ID No.: 7 (source: Uniprot, human M-CSFR P07333):

SEQ ID NO.: 7 (human M-CSFR):

MGPGVLLLLLVATAWHGQGIPVIEPSVPELVVKPGATVTLRCVGNGSVEWDGPPSP
HWTLYSDGSSSILSTNNATFQNTGTYRCTEPGDPLGGSAAIHLYVKDPARPWNVLA
QEVVVFEDQDALLPCLLTDPVLEAGVSLVRVRGRPLMRHTNYSFSPWHGFTIHRAK
FIQSQDYQCSALMGGRKVMSISIRLKVQKVIPGPPALTLVPAELVRIRGEAAQIVCSA
SSVDVNFDVFLQHNNTKLAIPQQSDFHNNRYQKVLTLNLDQVDFQHAGNYSCVASN
VQGKHSTSMFFRVVESAYLNLSSEQNLIQEVTVGEGLNLKVMVEAYPGLQGFNWTY
LGPFSDHQPEPKLANATTKDTYRHTFTLSLPRLKPSEAGRYSFLARNPGGWRALTF
ELTLRYPPEVSVIWTFINGSGTLLCAASGYPQPNVTWLQCSGHTDRCDEAQVLQVW
DDPYPEVLSQEPFHKVTVQSLLTVETLEHNQTYECRAHNSVGSGSWAFIPISAGAHT
HPPDEFLFTPVVVACMSIMALLLLLLLLLYKYKQKPKYQVRWKIIESYEGNSYTFIDP
TQLPYNEKWEFPRNNLQFGKTLGAGAFGKVVEATAFGLGKEDAVLKVAVKMLKSTA

HADEKEALMSELKIMSHLGQHENIVNLLGACTHGGPVLVITEYCCYGDLLNFLRRKA
EAMLGPSLSPGQDPEGGVDYKNIHLEKKYVRRDSGFSSQGVDTYVEMRPVSTSSN
DSFSEQDLDKEDGRPLELRDLLHFSSQVAQGMAFLASKNCIHRDVAARNVLLTNGH
VAKIGDFGLARDIMNDSNYIVKGNARLPVKWMAPESIFDCVYTVQSDVWSYGILLWE
IFSLGLNPYPGILVNSKFYKLVKDGYQMAQPAFAPKNIYSIMQACWALEPTHRPTFQ
QICSFLQEQAQEDRRERDYTNLPSSSRSGGSGSSSSELEEESSSEHLTCCEQGDIA
QPLLQPNNYQFC

The antibodies and antibody fragments of the present disclosure also inhibit the binding cynomolgus M-CSF to the cynomolgus M-CSF receptor.

In certain aspects the present disclosure relates to an isolated antibody or antibody fragment which is directed against or specifically binds to the human M-CSF. In alternative aspects the present disclosure relates to an isolated antibody or antibody fragment which is directed against or specifically binds to the polypeptide encoded by SEQ ID NO.: 1.

In certain aspects the present disclosure relates to an isolated antibody or antibody fragment which is directed against or specifically binds to the cynomolgus M-CSF. In alternative aspects the present disclosure relates to an isolated antibody or antibody fragment which is directed against or specifically binds to the polypeptide encoded by SEQ ID NO.: 6.

In certain aspects the present disclosure relates to an isolated antibody or antibody fragment which is directed against or specifically binds to the human M-CSF and which inhibits binding of human M-CSF to the human M-CSF receptor. In alternative aspects the present disclosure relates to an isolated antibody or antibody fragment which is directed against or specifically binds to the polypeptide encoded by SEQ ID NO.: 1 and which inhibits binding of the polypeptide encoded by SEQ ID NO.: 1 to the polypeptide encoded by SEQ ID NO.: 7.

In certain aspects the present disclosure relates to an isolated antibody or antibody fragment which is directed against or specifically binds to the cynomolgus M-CSF and which inhibits binding of cynomolgus M-CSF to the cynomolgus M-CSF receptor. In alternative aspects the present disclosure relates to an isolated antibody or antibody fragment which is directed against or specifically binds to the polypeptide encoded by SEQ ID NO.: 6 and which inhibits binding of the polypeptide encoded by SEQ ID NO.: 6 to the polypeptide encoded by SEQ ID NO.: 7. In alternative aspects the present disclosure relates to an

isolated antibody or antibody fragment which is directed against or specifically binds to the polypeptide encoded by SEQ ID NO.: 6 and which inhibits binding of the polypeptide encoded by SEQ ID NO.: 6 to the cynomolgus M-CSF receptor.

In certain aspects the present disclosure relates to an isolated antibody or antibody fragment which binds to secreted glycosylated M-CSF, secreted proteoglycan M-CSF and cell-surface M-CSF. In certain aspects the present disclosure relates to an isolated antibody or antibody fragment which binds to all isoforms of human M-CSF. In certain aspects the present disclosure relates to an isolated antibody or antibody fragment which binds to a polypeptide encoded by SEQ ID NO.: 2, a polypeptide encoded by SEQ ID NO.: 3 and a polypeptide encoded by SEQ ID NO.: 4.

In certain aspects the present disclosure relates to an isolated antibody or antibody fragment which binds to the N-terminal receptor-binding domain of M-CSF. In certain aspects the present disclosure relates to an isolated antibody or antibody fragment which binds to a polypeptide encoded by SEQ ID NO.: 5.

The terms "polypeptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymer. Unless otherwise indicated, a particular polypeptide sequence also implicitly encompasses conservatively modified variants thereof.

The term "nucleic acid" is used herein interchangeably with the term “polynucleotide” and refers to deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-stranded form. The term encompasses nucleic acids containing known nucleotide analogs or modified backbone residues or linkages, which are synthetic, naturally occurring, and non-naturally occurring, which have similar binding properties as the reference nucleic acid, and which are metabolized in a manner similar to the reference nucleotides. Examples of such analogs include, without limitation, phosphorothioates, phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, 2-O-methyl ribonucleotides, peptide-nucleic acids (PNAs). Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions) and complementary sequences, as well as the sequence explicitly indicated. Specifically, as detailed below, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base

and/or deoxyinosine residues (Batzer et al. (1991) Nucleic Acid Res. 19:5081; Ohtsuka et al. (1985) J. Biol. Chem. 260:2605-2608; and Rossolini et al. (1994) Mol. Cell. Probes 8:91-98).

In certain aspects the present disclosure provides a nucleic acid encoding an antibody or antibody fragment according to the present disclosure which specifically binds to M-CSF. In certain aspects the present disclosure provides a nucleic acid encoding an antibody or antibody fragment of Table 1. In certain aspects the present disclosure provides a nucleic acid encoding an antibody or antibody fragment of Table 2.

In certain aspects the present disclosure provides a vector comprising a nucleic acid encoding an antibody or antibody fragment according to the present disclosure which specifically binds to M-CSF. In certain aspects the present disclosure provides a vector comprising a nucleic acid encoding an antibody or antibody fragment of Table 1. In certain aspects the present disclosure provides a vector comprising a nucleic acid encoding an antibody or antibody fragment of Table 2.

In certain aspects the present disclosure provides a host cell comprising a vector comprising a nucleic acid encoding an antibody or antibody fragment of the present disclosure. In certain aspects the present disclosure provides a host cell comprising a nucleic acid encoding an antibody or antibody fragment of the present disclosure.

The term "recombinant host cell" (or simply "host cell") refers to a cell into which a recombinant expression vector has been introduced. It should be understood that such terms are intended to refer not only to the particular subject cell but to the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term "host cell" as used herein.

The term "vector" refers to a polynucleotide molecule capable of transporting another polynucleotide to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of

directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply, "expression vectors"). In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" may be used interchangeably as the plasmid is the most commonly used form of vector. However, the disclosure is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

In certain aspects the present disclosure provides isolated antibodies or antibody fragment, which specifically binds to M-CSF and which inhibits binding of M-CSF to the M-CSF receptor with an IC50 of 10 pM or less. In other aspect said isolated antibodies or antibody fragment inhibits binding of M-CSF to the M-CSF receptor with an IC50 of 20 pM or less, an IC50 of 5.5 pM or less or an IC50 of 5 pM or less.

In certain aspects the present disclosure provides isolated antibody or antibody fragment, which specifically binds to M-CSF and wherein said isolated antibody or antibody fragment is able to inhibit the binding of M-CSF to the M-CSF receptor with an IC50 of 10 pM or less in a receptor binding inhibition assay comprising M-CSF at a final concentration of 12.5 pM.

In certain aspects the present disclosure provides isolated antibody or antibody fragment, which specifically binds to M-CSF and wherein said isolated antibody or antibody fragment is able to inhibit the binding of M-CSF to the M-CSF receptor with an IC50 of 10 pM or less in a receptor binding inhibition assay comprising M-CSF at a final concentration of 0.66 ng/ml and M-CSF receptor at a final concentration of 2 µg/ml.

In certain aspects the present disclosure provides isolated antibody or antibody fragment, which specifically binds to M-CSF and wherein said isolated antibody or antibody fragment is able to inhibit the binding of M-CSF to the M-CSF receptor with an IC50 of 10 pM or less in a receptor binding inhibition assay as described in Example 5.

In certain aspects the present disclosure provides isolated antibodies or antibody fragment, which specifically binds to M-CSF and which inhibits binding of M-CSF to the M-CSF receptor with an IC50 which is at least twice as low as the IC50 of any one of the prior art antibodies HeRX1-10G1 and 8.10.3F. In alternative said isolated antibodies or antibody fragment inhibits binding of M-CSF to the M-CSF receptor with an IC50 which is at least

three times as low as the IC50 of any one of the prior art antibodies HeRX1-10G1 and 8.10.3F.

In certain aspects the present disclosure provides isolated antibodies or antibody fragment, which specifically binds to M-CSF with an EC50 of 10 pM or less as determined in a FACS assay. In alternative aspects said isolated antibodies or antibody fragments specifically bind to M-CSF with an EC50 of 15 pM or less as determined in a FACS assay or with an EC50 of 5 pM or less as determined in a FACS assay.

In certain aspects the present disclosure provides isolated antibodies or antibody fragment, which specifically binds to M-CSF with an EC50 which is at least twice as low as the EC50 of any one of the prior art antibodies HeRX1-10G1 or 8.10.3F as determined in a FACS assay.

In certain aspects the present disclosure provides isolated antibodies or antibody fragment, which specifically binds to M-CSF and which inhibits M-CSF induced proliferation with an IC50 of 10 pM or less. In alternative aspects said isolated antibodies or antibody fragments inhibits M-CSF induced proliferation with an IC50 of 20 pM or less, with an IC50 of 15 pM or less or with an IC50 of 5 pM or less.

In certain aspects the present disclosure provides isolated antibodies or antibody fragment, which specifically binds to M-CSF and which inhibits M-CSF induced proliferation with an IC50 which is at least twice as low as the IC50 of any one of the prior art antibodies HeRX1-10G1 or 8.10.3F.

The term "KD", as used herein, refers to the dissociation constant, which is obtained from the ratio of Kd to Ka (i.e. Kd/Ka) and is expressed as a molar concentration (M). KD values for antigen binding moieties like e.g. monoclonal antibodies can be determined using methods well established in the art. Methods for determining the KD of an antigen binding moiety like e.g. a monoclonal antibody are SET (soluble equilibrium titration) or surface plasmon resonance using a biosensor system such as a Biacore® system. Antibodies of the present disclosure typically have a dissociation rate constant (KD) (koff/kon) of less than $5x10^{-2}$M, less than 10^{-2}M, less than $5x10^{-3}$M, less than 10^{-3}M, less than $5x10^{-4}$M, less than 10^{-4}M, less than $5x10^{-5}$M, less than 10^{-5}M, less than $5x10^{-6}$M, less than 10^{-6}M, less than $5x10^{-7}$M, less than 10^{-7}M, less than $5x10^{-8}$M, less than 10^{-8}M, less than $5x10^{-9}$M, less than 10^{-9}M, less than $5x10^{-10}$M, less than 10-10M, less than 5x10-11M, less than 10^{-11}M, less than $5x10^{-}$

^{12}M, less than 10^{-12}M, less than $5x10^{-13}$M, less than 10^{-13}M, less than $5x10^{-14}$M, less than 10^{-14}M, less than $5x10^{-15}$M, or less than 10^{-15}M or lower.

In certain aspect, the present disclosure provides an isolated antibody or antibody fragment specific for M-CSF, wherein said antibody or antibody fragment binds to a M-CSF, with a dissociation constant (KD) of less than 1 x 10^{7} M^{-1}, 10^{8} M^{-1}, 10^{9} M^{-1}, 10^{10} M^{-1}, 10^{11} M^{-1}, 10^{12} M^{-1} or 10^{13} M^{-1}.

The term "EC50", as used herein, refers to the concentration of an antibody or an antibody fragment which induces a response in an assay half way between the baseline and maximum. It therefore represents the antibody concentration at which 50% of the maximal effect is observed.

The term "IC50", as used herein, refers to the concentration of an inhibitor (e.g. an antibody or antibody fragment) that inhibits a response in an assay half way between the maximal response and the baseline. It represents the antibody concentration that reduces a given response by 50%.

In certain aspects the present disclosure provides isolated antibodies and antibody fragments, wherein said antibody or antibody fragment comprises the HCDR1 region of SEQ ID NO.: 8, the HCDR2 region of SEQ ID NO.: 9, the HCDR3 region of SEQ ID NO.: 10, the LCDR1 region of SEQ ID NO.: 11, the LCDR2 region of SEQ ID NO.: 12 and the LCDR3 region of SEQ ID NO.: 13.

In certain aspects the present disclosure provides isolated antibodies and antibody fragments, wherein said antibody or antibody fragment comprises the HCDR1 region of SEQ ID NO.: 18, the HCDR2 region of SEQ ID NO.: 19, the HCDR3 region of SEQ ID NO.: 20, the LCDR1 region of SEQ ID NO.: 21, the LCDR2 region of SEQ ID NO.: 22 and the LCDR3 region of SEQ ID NO.: 23.

In certain aspects the present disclosure provides isolated antibodies and antibody fragments, wherein said antibody or antibody fragment comprises the HCDR1 region of SEQ ID NO.: 28, the HCDR2 region of SEQ ID NO.: 29, the HCDR3 region of SEQ ID NO.: 30, the LCDR1 region of SEQ ID NO.: 31, the LCDR2 region of SEQ ID NO.: 32 and the LCDR3 region of SEQ ID NO.: 33.

In certain aspects the present disclosure provides isolated antibodies and antibody fragments, wherein said antibody or antibody fragment comprises the HCDR1 region of SEQ ID NO.: 38, the HCDR2 region of SEQ ID NO.: 39, the HCDR3 region of SEQ ID NO.: 40, the LCDR1 region of SEQ ID NO.: 41, the LCDR2 region of SEQ ID NO.: 42 and the LCDR3 region of SEQ ID NO.: 43.

In certain aspects the present disclosure provides isolated antibodies and antibody fragments, wherein said antibody or antibody fragment comprises the HCDR1 region of SEQ ID NO.: 48, the HCDR2 region of SEQ ID NO.: 49, the HCDR3 region of SEQ ID NO.: 50, the LCDR1 region of SEQ ID NO.: 51, the LCDR2 region of SEQ ID NO.: 52 and the LCDR3 region of SEQ ID NO.: 53.

In certain aspects the present disclosure provides isolated antibodies and antibody fragments, wherein said antibody or antibody fragment comprises the HCDR1 region of SEQ ID NO.: 58, the HCDR2 region of SEQ ID NO.: 59, the HCDR3 region of SEQ ID NO.: 60, the LCDR1 region of SEQ ID NO.: 61, the LCDR2 region of SEQ ID NO.: 62 and the LCDR3 region of SEQ ID NO.: 63.

In certain aspects the present disclosure provides isolated antibodies and antibody fragments, wherein said antibody or antibody fragment comprises the HCDR1 region of SEQ ID NO.: 68, the HCDR2 region of SEQ ID NO.: 69, the HCDR3 region of SEQ ID NO.: 70, the LCDR1 region of SEQ ID NO.: 71, the LCDR2 region of SEQ ID NO.: 72 and the LCDR3 region of SEQ ID NO.: 73.

In certain aspects the present disclosure provides isolated antibodies and antibody fragments, wherein said antibody or antibody fragment comprises the HCDR1 region of SEQ ID NO.: 78, the HCDR2 region of SEQ ID NO.: 79, the HCDR3 region of SEQ ID NO.: 80, the LCDR1 region of SEQ ID NO.: 81, the LCDR2 region of SEQ ID NO.: 82 and the LCDR3 region of SEQ ID NO.: 83.

In certain aspects the present disclosure provides isolated antibodies and antibody fragments, wherein said antibody or antibody fragment comprises the HCDR1 region of SEQ ID NO.: 88, the HCDR2 region of SEQ ID NO.: 89, the HCDR3 region of SEQ ID NO.: 90, the LCDR1 region of SEQ ID NO.: 91, the LCDR2 region of SEQ ID NO.: 92 and the LCDR3 region of SEQ ID NO.: 93.

In certain aspects the present disclosure provides isolated antibodies and antibody fragments, wherein said antibody or antibody fragment comprises the HCDR1 region of SEQ ID NO.: 98, the HCDR2 region of SEQ ID NO.: 99, the HCDR3 region of SEQ ID NO.: 100, the LCDR1 region of SEQ ID NO.: 101, the LCDR2 region of SEQ ID NO.: 102 and the LCDR3 region of SEQ ID NO.: 103.

In certain aspects the present disclosure provides isolated antibodies and antibody fragments, wherein said antibody or antibody fragment comprises the HCDR1 region of SEQ ID NO.: 108, the HCDR2 region of SEQ ID NO.: 109, the HCDR3 region of SEQ ID NO.: 110, the LCDR1 region of SEQ ID NO.: 111, the LCDR2 region of SEQ ID NO.: 112 and the LCDR3 region of SEQ ID NO.: 113.

In certain aspects the present disclosure provides isolated antibodies and antibody fragments, wherein said antibody or antibody fragment comprises the variable heavy region of SEQ ID NO.: 14 and the variable light region of SEQ ID NO.: 15.

In certain aspects the present disclosure provides isolated antibodies and antibody fragments, wherein said antibody or antibody fragment comprises the variable heavy region of SEQ ID NO.: 24 and the variable light region of SEQ ID NO.: 25.

In certain aspects the present disclosure provides isolated antibodies and antibody fragments, wherein said antibody or antibody fragment comprises the variable heavy region of SEQ ID NO.: 34 and the variable light region of SEQ ID NO.: 35.

In certain aspects the present disclosure provides isolated antibodies and antibody fragments, wherein said antibody or antibody fragment comprises the variable heavy region of SEQ ID NO.: 44 and the variable light region of SEQ ID NO.: 45.

In certain aspects the present disclosure provides isolated antibodies and antibody fragments, wherein said antibody or antibody fragment comprises the variable heavy region of SEQ ID NO.: 54 and the variable light region of SEQ ID NO.: 55.

In certain aspects the present disclosure provides isolated antibodies and antibody fragments, wherein said antibody or antibody fragment comprises the variable heavy region of SEQ ID NO.: 64 and the variable light region of SEQ ID NO.: 65.

In certain aspects the present disclosure provides isolated antibodies and antibody fragments, wherein said antibody or antibody fragment comprises the variable heavy region of SEQ ID NO.: 74 and the variable light region of SEQ ID NO.: 75.

In certain aspects the present disclosure provides isolated antibodies and antibody fragments, wherein said antibody or antibody fragment comprises the variable heavy region of SEQ ID NO.: 84 and the variable light region of SEQ ID NO.: 85.

In certain aspects the present disclosure provides isolated antibodies and antibody fragments, wherein said antibody or antibody fragment comprises the variable heavy region of SEQ ID NO.: 94 and the variable light region of SEQ ID NO.: 95.

In certain aspects the present disclosure provides isolated antibodies and antibody fragments, wherein said antibody or antibody fragment comprises the variable heavy region of SEQ ID NO.: 104 and the variable light region of SEQ ID NO.: 105.

In certain aspects the present disclosure provides isolated antibodies and antibody fragments, wherein said antibody or antibody fragment comprises the variable heavy region of SEQ ID NO.: 114 and the variable light region of SEQ ID NO.: 115.

In certain aspects the present disclosure provides isolated antibodies and antibody fragments which compete with the antibodies specifically disclosed herein for binding to M-CSF. In other aspects the present disclosure provides isolated antibodies and antibody fragments which compete with the antibodies specifically disclosed herein for binding to human M-CSF. In other aspects the present disclosure provides isolated antibodies and antibody fragments which compete with the antibodies specifically disclosed herein for binding to a polypeptide encoded by SEQ ID NO.: 1.

The term "competes" or "cross-competes" refers to an antibody or antibody fragment which shares the ability to bind to a specific region of an antigen. In the present disclosure an antibody or antibody fragment that is "cross-competitive" has the ability to interfere with the binding of another antibody or antibody fragment for M-CSF in a standard competitive binding assay. Such an antibody may, according to non-limiting theory, bind to the same or a related or nearby (e.g., a structurally similar or spatially proximal) epitope on M-CSF. Cross-competition studies to find antibodies that competitively bind with one another, e.g., the antibodies compete for binding to the antigen can be performed. The ability or extent to which an antibody or antibody fragment is able to interfere with the binding of another

antibody or antibody fragment to M-CSF and therefore whether it can be said to cross-compete according to the invention, can be determined using standard competition binding assays. Cross-competition is present if antibody A reduces binding of antibody B at least by 50%, at least by 60%, specifically at least by 70% and more specifically at least by 80% and vice versa in comparison to the positive control which lacks one of said antibodies. As the skilled artisan appreciates competition may be assessed in different assay set-ups. One suitable assay involves the use of the Biacore technology (e.g. by using the BIAcore 3000 instrument (Biacore, Uppsala, Sweden)), which can measure the extent of interactions using surface plasmon resonance technology. Another assay for measuring cross-competition uses an ELISA-based approach. Furthermore, a high throughput process for "binning" antibodies based upon their cross-competition is described in International Patent Application No. WO2003/48731. Cross-competition is present if the antibody under investigation reduces the binding of one of the antibodies by 60% or more, specifically by 70% or more and more specifically by 80% or more and if one of the antibodies reduces the binding of said antibody to M-CSF by 60% or more, specifically by 70% or more and more specifically by 80% or more.

In certain aspect the present disclosure pertains to an antibody or antibody fragment specific for M-CSF, that cross-competes with an antibody described in Table 1. In certain aspect the present disclosure pertains to an antibody or antibody fragment specific for M-CSF, that cross-competes with an antibody described in Table 2.

In a certain embodiment, the antibody or antibody fragment that cross-competes with an antibody described in Table 1 reduces the binding of one of the antibodies described in Table 1 to M-CSF, by at least 50%, 60%, 70%, 80% or 90% in an ELISA-based cross-competition assay. In a certain embodiment, the antibody or antibody fragment that cross-competes with an antibody described in Table 2 reduces the binding of one of the antibodies described in Table 2 to M-CSF, by at least 50%, 60%, 70%, 80% or 90% in an ELISA-based cross-competition assay.

In certain aspects the present disclosure provides isolated antibodies and antibody fragments which bind to the same epitope like the antibodies specifically disclosed herein. In certain aspects the present disclosure provides isolated antibodies and antibody fragments which bind to the same epitope like the antibodies described in Table 1. In certain aspects the present disclosure provides isolated antibodies and antibody fragments which bind to the same epitope like the antibodies described in Table 2.

The term "epitope" includes any proteinacious region which is specifically recognized by an immunoglobulin or T-cell receptor or otherwise interacts with a molecule. Generally epitopes are of chemically active surface groupings of molecules such as amino acids or carbohydrate or sugar side chains and generally may have specific three-dimensional structural characteristics, as well as specific charge characteristics. As will be appreciated by one of skill in the art, practically anything to which an antibody can specifically bind could be an epitope.

In certain aspect, the present disclosure pertains to an antibody or antibody fragment specific for M-CSF which interacts with (e.g., by binding, stabilizing, spatial distribution) the same epitope as an antibody described in Table 1. In certain aspect, the disclosure pertains to an antibody or antibody fragment specific for M-CSF which interacts with (e.g., by binding, stabilizing, spatial distribution) the same epitope as an antibody described in Table 2.

In certain aspect, the present disclosure pertains to an antibody or antibody fragment disclosed herein for use in medicine.

In certain aspect the disclosure provides to a pharmaceutical composition comprising an isolated antibody or antibody fragment which is directed against or binds to M-CSF, and a pharmaceutically acceptable carrier. In certain aspects, the present invention provides a pharmaceutical composition comprising an isolated antibody or antibody fragment of the present disclosure, and a pharmaceutically acceptable carrier. In another embodiment the isolated antibody or antibody fragments disclosed herein for use as a drug.

The compositions of the present invention are preferably pharmaceutical compositions comprising an isolated antibody or antibody fragment which is directed against or binds to M-CSF and a pharmaceutically acceptable carrier, diluent or excipient, for the treatment of an inflammatory disorder. Such carriers, diluents and excipients are well known in the art, and the skilled artisan will find a formulation and a route of administration best suited to treat a subject with the anti-MCSF antibodies or antibody fragments of the present invention.

In certain aspects, the present invention provides a method for the treatment or prophylaxis of an inflammatory disorder in a subject, comprising the step of administering to the subject an effective amount of an antibody or antibody fragment, which is directed against or binds to M-CSF. In certain aspects said subject is a human. In alternative aspects said subject is a rodent, such as a rat or a mouse.

"Administration" and "treatment," as it applies to an animal, human, experimental subject, cell, tissue, organ, or biological fluid, refers to contact of an exogenous pharmaceutical, therapeutic, diagnostic agent, or composition to the animal, human, subject, cell, tissue, organ, or biological fluid. "Administration" and "treatment" can refer, e.g., to therapeutic, pharmacokinetic, diagnostic, research, and experimental methods. Treatment of a cell encompasses contact of a reagent to the cell, as well as contact of a reagent to a fluid, where the fluid is in contact with the cell. "Administration" and "treatment" also means *in vitro* and *ex vivo* treatments, e.g., of a cell, by a reagent, diagnostic, binding composition, or by another cell. "Treatment," as it applies to a human, veterinary, or research subject, refers to therapeutic treatment, prophylactic or preventative measures, to research and diagnostic applications. "Treatment" as it applies to a human, veterinary, or research subject, or cell, tissue, or organ, encompasses contact of an agent with animal subject, a cell, tissue, physiological compartment, or physiological fluid. "Treatment of a cell" also encompasses situations where the agent contacts PILR, e.g., in the fluid phase or colloidal phase, but also situations where the agonist or antagonist does not contact the cell or the receptor.

The term “subject” includes human and non-human animals. Non-human animals include all vertebrates, e.g., mammals and non-mammals, such as non-human primates, sheep, dog, cow, chickens, amphibians, and reptiles. Except when noted, the terms “patient” or “subject” are used herein interchangeably.

In another aspect the present disclosure provides to an antibody or antibody fragment specific for M-CSF, comprising 6 CDRs of any of the antibodies in Table 1. In another aspect the present disclosure provides to an antibody or antibody fragment specific for M-CSF, comprising 6 CDRs of any of the antibodies in Table 2.

In another aspect the present disclosure provides to an antibody or antibody fragment specific for M-CSF, comprising the variable heavy chain and the variable light chain of any of the antibodies in Table 1. In another aspect the present disclosure provides to an antibody or antibody fragment specific for M-CSF, comprising the variable heavy chain and the variable light chain of any of the antibodies in Table 2.

In another aspect the present disclosure provides an antibody or antibody fragment specific for M-CSF, encoded by any of the nucleic acid in Table 1. In another aspect the present disclosure provides an antibody or antibody fragment specific for M-CSF, encoded by any of the nucleic acid in Table 2. In another embodiment the present disclosure provides a vector comprising a nucleic acid of Table 1. In another embodiment the present disclosure

provides a vector comprising a nucleic acid of Table 2. In another embodiment the present disclosure provides an isolated host cell comprising a vector comprising a nucleic acid of Table 1. In another embodiment the present disclosure provides an isolated host cell comprising a vector comprising a nucleic acid of Table 2. In a further embodiment said isolated host cell is a mammalian cell.

EXAMPLES

Example 1: Generation of Fab fragments and antibodies that are specific for M-CSF

For the selection of antibodies specifically binding to M-CSF a commercially available phage display library, the MorphoSys HuCAL PLATINUM® library was used. Said antibody library is based on the HuCAL® concept (Knappik et al., (2000) J Mol Biol 296:57-86) and employs the CysDisplay® technology for displaying the Fab on the phage surface (WO2001/05950 to Lohning). However, any other available antibody library would be suitable to identify M-CSF antibodies.

To identify M-CSF-specific antibodies different panning strategies were used. Each panning strategy comprised at least 3 individual rounds of panning against the receptor-binding domain of human M-CSF (rbdM-CSF), the sequence of which is shown in SEQ ID NO.: 5.

The isolated binders identified were maturated, engineered and/or germlined in order to increase affinity and/or functionality of the initial lead molecules. Several hundred binders were screened and rigorously tested for functionality.

A subset of 45 candidate molecules were produced in exploratory scale and characterized in the following *in vitro* assays:

- Binding to human and cynomolgus M-CSF in ELISA.
- Binding to stably membrane M-CSF-transfected CHO cells and endogenously membrane M-CSF-expressing MDA-MB 231 cells (source: ATCC, order number: HTB-26).
- Developability risk ranking
- Functionality in the receptor inhibition assay (RIA) with human M-CSF.

- Functionality in the M-NFS-60 cell (source: ATCC, order number: CTL-1838) viability assay with human M-CSF.

In total led eight preferred lead molecules were identified which are further descried herein below. The amino acid and the nucleic acid sequences of the variable regions and the CDRs of those eight binders are shown in Table 1.

Table 1:

ID#		**Seq. ID:**	**Sequence [amino acid] / [nucleic acid]**
Caline	HCDR1	Seq. ID: 8	SNSAAWN
	HCDR2	Seq. ID: 9	RTYYRSKWKHEYAMSVKS
	HCDR3	Seq. ID: 10	DRYYYSAFDY
	LCDR1	Seq. ID: 11	TGTSSDVGGYNSVS
	LCDR2	Seq. ID: 12	AVSNRPS
	LCDR3	Seq. ID: 13	ASYDERFTRV
	VH	Seq. ID: 14	QVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAWN WIRQSPSRGLEWLGRTYYRSKWKHEYAMSVKSRITIN PDTSKNQFSLQLNSVTPEDTAVYYCARDRYYYSAFDY WGQGTLVTVSS
	VL	Seq. ID: 15	QSALTQPASVSGSPGQSITISCTGTSSDVGGYNSVSW YQQHPGKAPKLMIYAVSNRPSGVSNRFSGSKSGNTA SLTISGLQAEDEADYYCASYDERFTRVFGGGTKLTVL GQ
	VH (DNA)	Seq. ID: 16	caggtgcaattgcagcagagcggtccgggcctggtgaaaccgagccaga ccctgagcctgacctgcgcgatttccggcgatagtgtgagtagcaatagcgc tgcttggaactggattcgtcagagcccgagccgtggcctcgagtggctgggc cgtacctactaccgtagcaaatggaaacatgaatatgccatgagcgtgaaa agccgcattaccattaacccggatacttcgaaaaaccagtttagcctgcaac tgaacagcgtgaccccggaagatacggccgtgtattattgcgcgcgtgacc gttactactactctgctttcgattactggggccaaggcaccctggtgactgttag ctca
	VL (DNA)	Seq. ID: 17	caaagcgcgctgacccagccggcgagcgtgagcggtagcccgggccag agcattaccattagctgcaccggcaccagcagcgatgtgggcggttacaatt ctgtttcttggtaccagcagcatccgggcaaggcgccgaaattgatgatttac gctgtttctaaccgtccgagcggcgtgagcaaccgttttagcggatccaaaa gcggcaacaccgcgagcctgaccattagcggcctgcaagcggaagacg aagcggattattactgcgcttcttacgacgaacgtttcactcgtgtgtttggcgg cggcacgaagttaaccgtcctaggtcag

ID#		Seq. ID:	Sequence [amino acid] / [nucleic acid]
Camille	HCDR1	Seq. ID: 18	TSSAAWN
	HCDR2	Seq. ID: 19	RTYYRSKWKHEYAVSVKS
	HCDR3	Seq. ID: 20	DRYYYSAFDY
	LCDR1	Seq. ID: 21	TGTSSDVGGYNSVS
	LCDR2	Seq. ID: 22	AVSNRPS
	LCDR3	Seq. ID: 23	ASYDERFTRV
	VH	Seq. ID: 24	QVQLQQSGPGLVKPSQTLSLTCAISGESVSTSSAAWN WIRQSPSRGLEWLGRTYYRSKWKHEYAVSVKSRITIN PDTSKNQFSLQLNSVTPEDTAVYYCARDRYYYSAFDY WGQGTLVTVSS
	VL	Seq. ID: 25	QSALTQPASVSGSPGQSITISCTGTSSDVGGYNSVSW YQQHPGKAPKLLIYAVSNRPSGVSNRFSGSKSGNTAS LTISGLQAEDEADYYCASYDERFTRVFGGGTKLTVLG Q
	VH (DNA)	Seq. ID: 26	caggtgcaattgcagcagagcggtccgggcctggtgaaaccgagccaga ccctgagcctgacctgcgcgatttccggagagagtgtgagcactagtagtgc tgcttggaactggattcgtcagagcccgagccgtggcctcgagtggctgggc cgtacctactaccgtagcaaatggaaacatgaatatgccgtgagcgtgaaa agccgcattaccattaacccggatacttcgaaaaaccagtttagcctgcaac tgaacagcgtgaccccggaagatacggccgtgtattattgcgcgcgtgacc gttactactactctgctttcgattactggggccaaggcaccctggtgactgttag ctca
	VL (DNA)	Seq. ID: 27	caaagcgcgctgacccagccggcgagcgtgagcggtagcccgggccag agcattaccattagctgcaccggcaccagcagcgatgtgggcggttacaac tctgtttcttggtaccagcagcatccgggcaaggcgccgaaactgctgattta cgctgtttctaaccgtccgagcggcgtgagcaaccgttttagcggatccaaa agcggcaacaccgcgagcctgaccattagcggcctgcaagcggaagac gaagcggattattactgcgcttcttacgacgaacgtttcactcgtgtgtttggcg gcggcacgaagttaaccgtcctaggtcag
Celine	HCDR1	Seq. ID: 28	TSSAAWN
	HCDR2	Seq. ID: 29	RTYYRSKWKHEYAVSVKS
	HCDR3	Seq. ID: 30	DRYYYSAFDY
	LCDR1	Seq. ID: 31	TGTSSDVGGYNSVS
	LCDR2	Seq. ID: 32	AVSNRPS
	LCDR3	Seq. ID: 33	ASYDERFTRV
	VH	Seq. ID: 34	QVQLQQSGPGLVKPSQTLSLTCAISGDSVSTSSAAWN WIRQSPSRGLEWLGRTYYRSKWKHEYAVSVKSRITIN PDTSKNQFSLQLNSVTPEDTAVYYCARDRYYYSAFDY WGQGTLVTVSS
	VL	Seq. ID: 35	QSALTQPASVSGSPGQSITISCTGTSSDVGGYNSVSW YQQHPGKAPKLIIYAVSNRPSGVSNRFSGSKSGNTAS

ID#		Seq. ID:	Sequence [amino acid] / [nucleic acid]
			LTISGLQAEDEADYYCASYDERFTRVFGGGTKLTVLG Q
	VH (DNA)	Seq. ID: 36	caggtgcaattgcagcagagcggtccgggcctggtgaaaccgagccaga ccctgagcctgacctgcgcgatttccggagacagcgtgagtaccagtagtg ctgcttggaactggattcgtcagagcccgagccgtggcctcgagtggctggg ccgtacctactaccgtagcaaatggaaacatgaatatgccgtgagcgtgaa aagccgcattaccattaacccggatacttcgaaaaaccagtttagcctgcaa ctgaacagcgtgaccccggaagatacggccgtgtattattgcgcgcgtgac cgttactactactctgctttcgattactggggccaaggcaccctggtgactgtta gctca
	VL (DNA)	Seq. ID: 37	caaagcgcgctgacccagccggcgagcgtgagcggtagcccgggccag agcattaccattagctgcaccggcaccagcagcgatgtgggcggttacaac tctgtttcttggtaccagcagcatccgggcaaggcgccgaaactgatcatcta cgctgtttctaaccgtccgagcggcgtgagcaaccgttttagcggatccaaa agcggcaacaccgcgagcctgaccattagcggcctgcaagcggaagac gaagcggattattactgcgcttcttacgacgaacgtttcactcgtgtgtttggcg gcggcacgaagttaaccgtcctaggtcag
Madeleine	HCDR1	Seq. ID: 38	SNSAAWN
	HCDR2	Seq. ID: 39	RTYYRSKWKKEYAQSVKS
	HCDR3	Seq. ID: 40	DRYYYSAFDY
	LCDR1	Seq. ID: 41	TGTSSDVGGYNSVS
	LCDR2	Seq. ID: 42	AVSNRPS
	LCDR3	Seq. ID: 43	ASYDERFTRV
	VH	Seq. ID: 44	QVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAWN WIRQSPSRGLEWLGRTYYRSKWKKEYAQSVKSRITIN PDTSKNQFSLQLNSVTPEDTAVYYCARDRYYYSAFDY WGQGTLVTVSS
	VL	Seq. ID: 45	QSALTQPASVSGSPGQSITISCTGTSSDVGGYNSVSW YQQHPGKAPKLMIYAVSNRPSGVSNRFSGSKSGNTA SLTISGLQAEDEADYYCASYDERFTRVFGGGTKLTVL GQ
	VH (DNA)	Seq. ID: 46	caggtgcaattgcagcagagcggtccgggcctggtgaaaccgagccaga ccctgagcctgacctgcgcgatttccggagatagcgtgagcagtaactctgc tgcttggaactggattcgtcagagcccgagccgtggcctcgagtggctgggc cgtacctactaccgtagcaaatggaaaaaagaatatgcccagagcgtgaa aagccgcattaccattaacccggatacttcgaaaaaccagtttagcctgcaa ctgaacagcgtgaccccggaagatacggccgtgtattattgcgcgcgtgac cgttactactactctgctttcgattactggggccaaggcaccctggtgactgtta gctca
	VL (DNA)	Seq. ID: 47	caaagcgcgctgacccagccggcgagcgtgagcggtagcccgggccag agcattaccattagctgcaccggcaccagcagcgatgtgggcggttacaatt ctgtttcttggtaccagcagcatccgggcaaggcgccgaaattgatgatttac gctgtttctaaccgtccgagcggcgtgagcaaccgttttagcggatccaaaa gcggcaacaccgcgagcctgaccattagcggcctgcaagcggaagacg

ID#		Seq. ID:	Sequence [amino acid] / [nucleic acid]
			aagcggattattactgcgcttcttacgacgaacgtttcactcgtgtgtttggcgg cggcacgaagttaaccgtcctaggtcag
Maelle	HCDR1	Seq. ID: 48	TSSAAWN
	HCDR2	Seq. ID: 49	RTYYRSKWKKEYAQSVKS
	HCDR3	Seq. ID: 50	DRYYYSAFDY
	LCDR1	Seq. ID: 51	TGTSSDVGGYNSVS
	LCDR2	Seq. ID: 52	AVSNRPS
	LCDR3	Seq. ID: 53	ASYDERFTRV
	VH	Seq. ID: 54	QVQLQQSGPGLVKPSQTLSLTCAISGESVSTSSAAWN WIRQSPSRGLEWLGRTYYRSKWKKEYAQSVKSRITIN PDTSKNQFSLQLNSVTPEDTAVYYCARDRYYYSAFDY WGQGTLVTVSS
	VL	Seq. ID: 55	QSALTQPASVSGSPGQSITISCTGTSSDVGGYNSVSW YQQHPGKAPKLIIYAVSNRPSGVSNRFSGSKSGNTAS LTISGLQAEDEADYYCASYDERFTRVFGGGTKLTVLG Q
	VH (DNA)	Seq. ID: 56	caggtgcaattgcagcagagcggtccgggcctggtgaaaccgagccaga ccctgagcctgacctgcgcgatttccggagaaagcgtgagtaccagcagtg ctgcttggaactggattcgtcagagcccgagccgtggcctcgagtggctggg ccgtacctactaccgtagcaaatggaaaaaagaatatgcccagagcgtga aaagccgcattaccattaacccggatacttcgaaaaaccagtttagcctgca actgaacagcgtgaccccggaagatacggccgtgtattattgcgcgcgtga ccgttactactactctgctttcgattactggggccaaggcaccctggtgactgtt agctca
	VL (DNA)	Seq. ID: 57	caaagcgcgctgacccagccggcgagcgtgagcggtagcccgggccag agcattaccattagctgcaccggcaccagcagcgatgtgggcggttacaac tctgtttcttggtaccagcagcatccgggcaaggcgccgaaactgatcatcta cgctgtttctaaccgtccgagcggcgtgagcaaccgttttagcggatccaaa agcggcaacaccgcgagcctgaccattagcggcctgcaagcggaagac gaagcggattattactgcgcttcttacgacgaacgtttcactcgtgtgtttggcg gcggcacgaagttaaccgtcctaggtcag
Meriem	HCDR1	Seq. ID: 58	TSSAAWN
	HCDR2	Seq. ID: 59	RTYYRSKWKKEYAQSVKS
	HCDR3	Seq. ID: 60	DRYYYSAFDY
	LCDR1	Seq. ID: 61	TGTSSDVGGYNSVS
	LCDR2	Seq. ID: 62	AVSNRPS
	LCDR3	Seq. ID: 63	ASYDERFTRV
	VH	Seq. ID: 64	QVQLQQSGPGLVKPSQTLSLTCAISGDSVSTSSAAWN WIRQSPSRGLEWLGRTYYRSKWKKEYAQSVKSRITIN PDTSKNQFSLQLNSVTPEDTAVYYCARDRYYYSAFDY WGQGTLVTVSS

ID#		Seq. ID:	Sequence [amino acid] / [nucleic acid]
	VL	Seq. ID: 65	QSALTQPASVSGSPGQSITISCTGTSSDVGGYNSVSW YQQHPGKAPKLIIYAVSNRPSGVSNRFSGSKSGNTAS LTISGLQAEDEADYYCASYDERFTRVFGGGTKLTVLG Q
	VH (DNA)	Seq. ID: 66	caggtgcaattgcagcagagcggtccgggcctggtgaaaccgagccaga ccctgagcctgacctgcgcgatttccggcgacagcgtgagcaccagtagtg ctgcttggaactggattcgtcagagcccgagccgtggcctcgagtggctggg ccgtacctactaccgtagcaaatggaaaaaagaatatgcccagagcgtga aaagccgcattaccattaacccggatacttcgaaaaaccagtttagcctgca actgaacagcgtgaccccggaagatacggccgtgtattattgcgcgcgtga ccgttactactactctgctttcgattactggggccaaggcaccctggtgactgtt agctca
	VL (DNA)	Seq. ID: 67	caaagcgcgctgacccagccggcgagcgtgagcggtagcccgggccag agcattaccattagctgcaccggcaccagcagcgatgtgggcggttacaac tctgtttcttggtaccagcagcatccgggcaaggcgccgaaactgatcatcta cgctgtttctaaccgtccgagcggcgtgagcaaccgttttagcggatccaaa agcggcaacaccgcgagcctgaccattagcggcctgcaagcggaagac gaagcggattattactgcgcttcttacgacgaacgtttcactcgtgtgtttggcg gcggcacgaagttaaccgtcctaggtcag
Satine	HCDR1	Seq. ID: 68	IYAMS
	HCDR2	Seq. ID: 69	RIKSNADGGTTEYAAPVKG
	HCDR3	Seq. ID: 70	MRYYSDLYFDP
	LCDR1	Seq. ID: 71	SGDKLGQKYVS
	LCDR2	Seq. ID: 72	QDRKRPS
	LCDR3	Seq. ID: 73	QTWTHLQWV
	VH	Seq. ID: 74	EVQLVESGGGLVKPGGSLRLSCAASGFTFSIYAMSWV RQAPGKGLEWVGRIKSNADGGTTEYAAPVKGRFTISR DDSKNTLYLQMNSLKTEDTAVYYCARMRYYSDLYFDP WGQGTLVTVSS
	VL	Seq. ID: 75	SYELTQPPSVSVSPGQTASITCSGDKLGQKYVSWYQ QKPGQSPVLVISQDRKRPSGIPERFSGSNSGNTATLTI SGTQAEDEADYYCQTWTHLQWVFGGGTKLTVLGQ
	VH (DNA)	Seq. ID: 76	gaagtgcagctggtggaaagcggcggtggcctggtgaaaccaggcggca gcctgcgcctgagctgcgccgccagcggcttcacctttagcatctacgctatg agctgggtgcgccaggccccgggcaaaggtctggaatgggtgggccgtat caaatctaacgctgacggtggtactactgaatatgccgccccagtgaaagg ccgctttaccattagccgcgatgatagcaaaaacaccctgtatctgcaaatg aacagcctgaaaaccgaagatacggccgtgtattattgcgcgcgtatgcgtt actactctgacctgtacttcgatccgtggggtcaaggcaccctggtgactgtct cgagc
	VL (DNA)	Seq. ID: 77	agctatgaactgacccagccgccgagcgttagcgttagcccaggccagac cgccagcattacctgtagcggcgacaaactggggcaaaaatacgtgtcctg gtatcagcagaaaccgggccagagcccggtgctggttatcagtcaggatcg taaacgcccgagcggcattccagaacgctttagcggcagcaacagcggc

ID#		Seq. ID:	Sequence [amino acid] / [nucleic acid]
			aacaccgccaccctgaccattagcggcacccaggccgaagacgaagcc gattattactgccagacttggacccacctgcaatgggtgtttggcggcggtac caagctgaccgtgctgggccag
Servane	HCDR1	Seq. ID: 78	TYAIS
	HCDR2	Seq. ID: 79	FIKSKHNSGTTEYAAPVKG
	HCDR3	Seq. ID: 80	MRYYSDLYFDP
	LCDR1	Seq. ID: 81	SGDKLGQKYVS
	LCDR2	Seq. ID: 82	QDRKRPS
	LCDR3	Seq. ID: 83	QTWTHLQWV
	VH	Seq. ID: 84	EVQLVESGGGLVKPGGSLRLSCAASGFTFSTYAISWV RQAPGKGLEWVGFIKSKHNSGTTEYAAPVKGRFTISR DDSKNTLYLQMNSLKTEDTAVYYCARMRYYSDLYFDP WGQGTLVTVSS
	VL	Seq. ID: 85	SYELTQPPSVSVSPGQTASITCSGDKLGQKYVSWYQ QKPGQSPVLVISQDRKRPSGIPERFSGSNSGNTATLTI SGTQAEDEADYYCQTWTHLQWVFGGGTKLTVLGQ
	VH (DNA)	Seq. ID: 86	gaagtgcaattggtggaaagcggcggtggcctggtgaaaccaggcggca gcctgcgcctgagctgcgccgcctccggattcacctttctacttacgctatctc ttgggtgcgccaggccccgggcaaaggtctcgagtgggtgggcttcatcaa atctaaacataactctggtactactgaatatgccgccccagtgaaaggccgc tttaccattagccgcgatgattcgaaaaacaccctgtatctgcaaatgaacag cctgaaaaccgaagatacggccgtgtattattgcgcgcgtatgcgttactact ctgacctgtacttcgatccgtggggtcaaggcaccctggtgactgtctcgagc
	VL (DNA)	Seq. ID: 87	agctatgaactgacccagccgccgagcgttagcgttagcccaggccagac cgccagcattacctgtagcggcgacaaactggggcaaaaatacgtgtcctg gtatcagcagaaaccgggccagagcccggtgctggttatcagtcaggatcg taaacgcccgagcggcattccagaacgctttagcggcagcaacagcggc aacaccgccaccctgaccattagcggcacccaggccgaagacgaagcc gattattactgccagacttggacccacctgcaatgggtgtttggcggcggtac caagctgaccgtgctgggccag

Antibodies *Caline*, *Camille* and *Celine* are derivatives of parental antibody *Blanche*. Antibodies *Madeleine*, *Maelle* and *Meriem* are derivatives of parental antibody *Laurine*. Antibodies *Satine* and *Servane* are derivatives of parental antibody *Romaine*. The amino acid and the nucleic acid sequences of the variable regions and the CDRs of the parental antibodies are shown in Table 2.

Table 2:

ID#		Seq. ID:	Sequence [amino acid] / [nucleic acid]
Blanche	HCDR1	Seq. ID: 88	SNSAAWN
	HCDR2	Seq. ID: 89	RTYYRSKWKHEYAMSVKS
	HCDR3	Seq. ID: 90	DRYYYSAFDY
	LCDR1	Seq. ID: 91	TGTSSDVGGYNSVS
	LCDR2	Seq. ID: 92	AVSNRPS
	LCDR3	Seq. ID: 93	ASYDERFTRV
	VH	Seq. ID: 94	QVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAWN WIRQSPSRGLEWLGRTYYRSKWKHEYAMSVKSRITIN PDTSKNQFSLQLNSVTPEDTAVYYCARDRYYYSAFDY WGQGTLVTVSS
	VL	Seq. ID: 95	DIALTQPASVSGSPGQSITISCTGTSSDVGGYNSVSWY QQHPGKAPKLMIYAVSNRPSGVSNRFSGSKSGNTASL TISGLQAEDEADYYCASYDERFTRVFGGGTKLTVLGQ
	VH (DNA)	Seq. ID: 96	caggtgcaattgcagcagagcggtccgggcctggtgaaaccgagccaga ccctgagcctgacctgcgcgatttccggagatagcgtgagcagtaactctgc tgcttggaactggattcgtcagagcccgagccgtggcctcgagtggctgggc cgtacctactaccgtagcaaatggaaacatgaatatgccatgagcgtgaaa agccgcattaccattaacccggatacttcgaaaaaccagtttagcctgcaac tgaacagcgtgaccccggaagatacggccgtgtattattgcgcgcgtgacc gttactactactctgctttcgattactggggccaaggcaccctggtgactgttag ctca
	VL (DNA)	Seq. ID: 97	gatatcgcgctgacccagccggcgagcgtgagcggtagcccgggccaga gcattaccattagctgcaccggcaccagcagcgatgtgggcggttacaact ctgtgtcttggtaccagcagcatccgggcaaggcgccgaaactgatgatcta cgctgtttctaaccgtccgagcggcgtgagcaaccgttttagcggatccaaa agcggcaacaccgcgagcctgaccattagcggcctgcaagcggaagac gaagcggattattactgcgcttcttacgacgaacgtttcactcgtgtgtttggcg gcggcacgaagttaaccgtcctaggtcag
Laurine	HCDR1	Seq. ID: 98	SNSAAWN
	HCDR2	Seq. ID: 99	RTYYRSKWKKEYAQSVKS
	HCDR3	Seq. ID: 100	DRYYYSAFDY
	LCDR1	Seq. ID: 101	TGTSSDVGGYNSVS
	LCDR2	Seq. ID: 102	AVSNRPS
	LCDR3	Seq. ID: 103	ASYDERFTRV
	VH	Seq. ID: 104	QVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAWN WIRQSPSRGLEWLGRTYYRSKWKKEYAQSVKSRITIN PDTSKNQFSLQLNSVTPEDTAVYYCARDRYYYSAFDY WGQGTLVTVSS

ID#		Seq. ID:	Sequence [amino acid] / [nucleic acid]
	VL	Seq. ID: 105	DIALTQPASVSGSPGQSITISCTGTSSDVGGYNSVSWY QQHPGKAPKLMIYAVSNRPSGVSNRFSGSKSGNTASL TISGLQAEDEADYYCASYDERFTRVFGGGTKLTVLGQ
	VH (DNA)	Seq. ID: 106	caggtgcaattgcagcagagcggtccgggcctggtgaaaccgagccaga ccctgagcctgacctgcgcgatttccggagatagcgtgagcagtaactctgc tgcttggaactggattcgtcagagcccgagccgtggcctcgagtggctgggc cgtacctactaccgtagcaaatggaaaaaagaatatgcccagagcgtgaa aagccgcattaccattaacccggatacttcgaaaaaccagtttagcctgcaa ctgaacagcgtgaccccggaagatacggccgtgtattattgcgcgcgtgac cgttactactactctgctttcgattactggggccaaggcaccctggtgactgtta gctca
	VL (DNA)	Seq. ID: 107	Gatatcgcgctgacccagccggcgagcgtgagcggtagcccgggccag agcattaccattagctgcaccggcaccagcagcgatgtgggcggttacaac tctgtgtcttggtaccagcagcatccgggcaaggcgccgaaactgatgatct acgctgtttctaaccgtccgagcggcgtgagcaaccgttttagcggatccaa aagcggcaacaccgcgagcctgaccattagcggcctgcaagcggaaga cgaagcggattattactgcgcttcttacgacgaacgtttcactcgtgtgtttggc ggcggcacgaagttaaccgtcctaggtcag
Romaine	HCDR1	Seq. ID: 108	IYAMS
	HCDR2	Seq. ID: 109	RIKSNADGGTTEYAAPVKG
	HCDR3	Seq. ID: 110	MRYYSDLYFDP
	LCDR1	Seq. ID: 111	SGDAIGSKYVH
	LCDR2	Seq. ID: 112	KDNKRPS
	LCDR3	Seq. ID: 113	QTATVSSYWWV
	VH	Seq. ID: 114	EVQLVESGGGLVKPGGSLRLSCAASGFTFSIYAMSWV RQAPGKGLEWVGRIKSNADGGTTEYAAPVKGRFTISR DDSKNTLYLQMNSLKTEDTAVYYCARMRYYSDLYFDP WGQGTLVTVSS
	VL	Seq. ID: 115	DIELTQPPSVSVSPGQTASITCSGDAIGSKYVHWYQQ KPGQAPVLVISKDNKRPSGIPERFSGSNSGNTATLTIS GTQAEDEADYYCQTATVSSYWWVFGGGTKLTVLGQ
	VH (DNA)	Seq. ID: 116	caggtgcaattggtggaaagcggcggtggcctggtgaaaccaggcggca gcctgcgcctgagctgcgccgcctccggattcacctttctatctacgctatgtc ttgggtgcgccaggccccgggcaaaggtctcgagtgggtgggccgtatcaa atctaacgctgacggtggtactactgaatatgccgccccagtgaaaggccg ctttaccattagccgcgatgattcgaaaaacaccctgtatctgcaaatgaaca gcctgaaaaccgaagatacggccgtgtattattgcgcgcgtatgcgttactac tctgacctgtacttcgatccgtggggccaaggcaccctggtgactgttagctc a
	VL (DNA)	Seq. ID: 117	gatatcgaactgacccagccgccgagcgtgagcgtgagcccgggccaga ccgcgagcattacctgtagcggcgatgctatcggttctaaatacgttcattggt accagcagaaaccgggccaggcgccggtgctggtgatctctaaagacaa caaacgtccgagcggcatcccggaacgttttagcggatccaacagcggca acaccgcgaccctgaccattagcggcacccaggcggaagacgaagcgg

ID#		Seq. ID:	Sequence [amino acid] / [nucleic acid]
			attattactgccagactgctactgtttcttcttactggtgggtgtttggcggcggc acgaagttaaccgtcctaggtcag

Example 2: Characterisation and benchmarking of the M-CSF specific antibodies

The eight preferred M-CSF specific antibodies were characterized in depth as described herein below. The antibodies were compared to the respective parental antibodies and to two prior art antibodies which were generated for clinical development. The amino acid sequence of HeRX1-10G1 (Novartis) is for example disclosed in WO 2005/068503 and also shown in Table 3. The amino acid sequence of 8.10.3F (Pfizer) is for example disclosed in WO 2005/030124 and also shown in Table 3. HeRX1-10G1 and 8.10.3F were synthesized using conventional molecular biology techniques.

Table 3:

ID#		Seq. ID:	Sequence [amino acid] / [nucleic acid]
HeRX1-10G1	HCDR1	Seq. ID: 118	SDYAWN
	HCDR2	Seq. ID: 119	YISYSGSTSYNPSLKS
	HCDR3	Seq. ID: 120	FDYAHAMDY
	LCDR1	Seq. ID: 121	QASQSIGTSIH
	LCDR2	Seq. ID: 122	YASESIS
	LCDR3	Seq. ID: 123	QQINSWPT
	VH	Seq. ID: 124	QVQLQESGPGLVKPSQTLSLTCTVSDYSITSDYAWNW IRQFPGKGLEWMGYISYSGSTSYNPSLKSRITISRDTS KNQFSLQLNSVTAADTAVYYCASFDYAHAMDYWGQG TTVTVSS
	VL	Seq. ID: 125	DIVLTQSPAFLSVTPGEKVTFTCQASQSIGTSIHWYQQ KTDQAPKLLIKYASESISGIPSRFSGSGSGTDFTLTISS VEAEDAADYYCQQINSWPTTFGGGTKLEIKRT
	VH (DNA)	Seq. ID: 126	caggtgcaattgcaggaaagcggccctggcctggtcaagcctagccagac cctgagcctgacctgcaccgtgtccgactacagcatcaccagcgactacgc ctggaactggatccggcagttccccggcaagggcctggaatggatgggcta catcagctacagcggcagcaccagctacaaccccagcctgaagtcccgg atcaccatcagccgggacaccagcaagaaccagtttagcctccagctgaa

ID#		Seq. ID:	Sequence [amino acid] / [nucleic acid]
			cagcgtgacagccgccgacaccgccgtgtactactgcgccagcttcgactacgcccacgccatggattactggggccagggcaccaccgtgaccgtcagctca
	VL (DNA)	Seq. ID: 127	gatatcgtgctgacccagagccccgcctttctgagcgtgacccctggcgagaaagtgacattcacatgccaggccagccagagcatcggcaccagcatccactggtatcagcagaaaaccgaccaggcccccaaactcctgatcaagtacgccagcgagtccatcagcggcatccccagcagattcagcggcagcggctccggcaccgacttcaccctgaccatcagcagcgtggaggccgaggacgccgccgactactactgccagcagatcaacagctggcccaccacctttggcggaggcaccaagctggaaatcaagcgtacg
8.10.3F	HCDR1	Seq. ID: 128	SFSMT
	HCDR2	Seq. ID: 129	YISSRSSTISYADSVKG
	HCDR3	Seq. ID: 130	DPLLAGATFFDY
	LCDR1	Seq. ID: 131	RASQSVSSSYLA
	LCDR2	Seq. ID: 132	GASSRAT
	LCDR3	Seq. ID: 133	QQYGSSPL
	VH	Seq. ID: 134	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSFSMTWVRQAPGKGLEWVSYISSRSSTISYADSVKGRFTISRDNAKNSLYLQMNSLRDEDTAVYYCARDPLLAGATFFDYWGQGTLVTVSS
	VL	Seq. ID: 135	DIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPLTFGGGTKVEIKRT
	VH (DNA)	Seq. ID: 136	caggtgcaattggtggagagcggcggaggactggtgcagcctggcggaagcctgagactgtcttgcgccgccagcggcttcaccttcagcagcttcagcatgacatgggtccgccaagcccctggaaagggcctggaatgggtgtcctacatcagcagccggtccagcaccatcagctacgccgacagcgtgaagggccggttcaccatcagccgggacaacgccaagaacagcctgtacctgcagatgaacagcctgcgggacgaggacaccgccgtgtactactgcgccagagatcctctgctggctggcgccaccttcttcgactactggggccagggcaccctggtcacagtcagctca
	VL (DNA)	Seq. ID: 137	gatatcgtgctgacccagtctcctggcaccctgtctctgagccctggcgagagagccaccctgagctgcagagccagccagagcgtgtccagcagctacctggcctggtatcagcagaagcccggccaggcccccagactgctgatctacggcgccagcagcagagccaccggcatccccgacagattcagcggcagcggctccggcaccgacttcaccctgaccatctctcggctggaacccgaggacttcgccgtgtactactgccagcagtacggcagcagccctctgaccttcggcggaggcaccaaggtggagatcaagcgtacg

The antibodies of the present disclosure may be used in any format, i.e. as full lengths immunoglobulins or as antibody fragments as described herein. The full length antibodies

may be used in any immunoglobulin isotype or class. With a few exceptions, the antibodies of the present invention were tested in IgG1 format and in a silenced IgG1 format harboring a D265A mutation in the Fc region. Antibody 8.10.3F was also tested in IgG2 format.

Example 3: Specificity for M-CSF as determined in an ELISA assay

Specificity for human M-CSF and cynomolgus M-CSF was tested in an ELISA assay. BSA was used as a negative control substrate.

Maxisorp™ 384 well plates were coated with human rbdM-CSF, human sgM-CSF, cyno sgM-CSF or mouse sgM-CSF at a concentration of 2 µg/ml in PBS. After blocking of plates with 5% skim milk powder in PBS, Fab-containing *E. coli* lysates, IgG-containing cell culture supernatants or purified IgG or Fab proteins were added. Binding of Fabs or IgGs was detected by $F(ab)_2$ specific goat anti-human IgG conjugated to alkaline phosphatase (Dianova Cat# 109-055-097; diluted 1:5000) using Attophos fluorescence substrate (Roche, #11681982001). Fluorescence emission at 535 nm was recorded with excitation at 430 nm.

In an alternative experimental set-up, Maxisorp™ 384 well plates were coated with Fd fragment specific sheep anti-human IgG (The Binding Site, #PC075) diluted 1:1000 in PBS. After blocking with 5% skim milk powder in PBS, Fab-containing *E. coli* lysates were added. Subsequently the captured HuCAL®-Fab fragments were allowed to bind to 0.5 µg/ml biotinylated M-CSF (human sgM-CSF, human rbdM-CSF, or mouse sgM-CSF) which was detected by incubation with streptavidin conjugated to alkaline phosphatase followed by addition of AttoPhos fluorescence substrate (Roche, #11681982001). Fluorescence emission at 535 nm was recorded with excitation at 430 nm.

All antibodies shown in Tables 1, 2 and 3 strongly bound to human M-CSF and cynomolgus M-CSF (>35,000 units), whereas none of the antibodies bound to BSA (<1,000 units). All antibodies are therefore highly specific for M-CSF.

Example 4: Specificity for M-CSF as determined in a FACS experiment; EC50s

A FACS study was performed in order to test if the antibodies of the present disclosure also binds to M-CSF expressed on cells. Two cell lines were used for this purpose: CHO cells which were stably transfected with M-CSF, and the cell line MDA-MB-231 which

endogenously expresses M-CSF. Apparent K_D values (EC_{50}) were determined with both cell lines. The results are shown in Table 4. All values are in [nM].

Table 4: EC_{50} values of antibodies as determined in a FACS assay (nM)

Antibody#	Format	CHO cells (n=3)	MDA-MB 231 cells (n=2)
Blanche	IgG1_D265A	7.5	10
Caline	IgG1_D265A	9.8	14
Camille	IgG1_D265A	6.1	14
Celine	IgG1_D265A	9.7	13
Laurine	IgG1_D265A	11.2	9
Madeleine	IgG1_D265A	6.4	10
Maelle	IgG1_D265A	10.2	8
Meriem	IgG1_D265A	12.3	10
Romaine	IgG1_D265A	16.4	19
Satine	IgG1_D265A	11.3	26
Servane	IgG1_D265A	21.3	n.d.
HeRX1-10G1	IgG1	12.6	27
HeRX1-10G1	IgG1_D265A	8.9	23
8.10.3F	IgG2	23.2	310
8.10.3F	IgG1_D265A	8.9	16

All antibodies of the present invention show EC_{50} values which are at least as good as those of the prior art antibodies HeRX1-10G1 and 8.10.3F, and many antibodies show EC_{50} values which are better (lower) that those of the prior art antibodies.

Example 5: Inhibition of receptor binding

In this experiment the ability of the antibodies to block binding of human or cynomolgus M-CSF to recombinant human M-CSF receptor (provided as Fc fusion protein) was assessed.

The following recombinant ligand/receptor-combinations were used:

Table 5: Ligand/receptor combinations used in Receptor inhibition assays

Human M-CSF	
Ligand	Recombinant human sgM-CSF
Receptor	Recombinant human CSF1-R-Fc (R&D Systems Cat.Nr. #329-MR)
Mouse M-CSF	
Ligand	Recombinant murine sgM-CSF
Receptor	Recombinant murine M-CSF receptor CSF-1R-Fc (R&D Systems, Cat.Nr.#3818-MR)
Cyno M-CSF	
Ligand	Recombinant cynomolgus monkey sgM-CSF
Receptor	Recombinant human CSF1-R-Fc (R&D Systems Cat.Nr. #329-MR)

M-CSF at a final concentration of 12.5 pM (0.66 ng/ml) was preincubated with different concentrations of anti-M-CSF antibody (0.1 pM – 100 nM; all dilutions in ECL-buffer) for 1 h at room temperature. Complexes were then transferred to 384 well MSD plates coated with 2 µg/ml of the corresponding receptor. Plates were then washed and incubated with the streptavidin/ECL conjugate for 2 h with shaking at room temperature. 35 µl MSD read buffer T with surfactant was then added per well and subsequently measured using MSD Sector Imager 6000.

IC50 values were calculated for both, human M-CSF and cynomolgus M-CSF. The results are shown in Table 6. All values are in [pM]. n=4, except for HeRX1-10G1 in IgG1 format and 8.10.3F in IgG2 format.

Table 6: IC_{50} values of antibodies as determined in a receptor binding inhibition assay (pM)

Antibody#	**Format**	**Human M-CSF**	**Cynomolgus M-CSF**
Blanche	IgG1_D265A	6.2	6.6
Caline	IgG1_D265A	6.1	6.3
Camille	IgG1_D265A	4.4	5.9
Celine	IgG1_D265A	4.7	6.9
Laurine	IgG1_D265A	4.9	4.3
Madeleine	IgG1_D265A	5.6	5.6
Maelle	IgG1_D265A	5.3	6.4
Meriem	IgG1_D265A	7.0	7.3

Romaine	IgG1_D265A	9.1	9.1
Satine	IgG1_D265A	15.1	13.5
Servane	IgG1_D265A	31.3	11.8
HeRX1-10G1	IgG1	26.5	27.6
HeRX1-10G1	IgG1_D265A	28.0	18.0
8.10.3F	IgG2	31.4	20.7
8.10.3F	IgG1_D265A	19.2	14.6

All candidates blocked the binding of M-CSF to its receptor better than the prior art antibodies HeRX1-10G1 and 8.10.3F. This is particularly true for the antibodies which are derived from the parental antibodies Blanche and Laurine, all of which shown IC50 values of below 10 pM.

Example 6: Inhibition of proliferation induced by recombinant M-CSF

The ability of the antibodies of the present disclosure to block the bioactivity of recombinant M-CSF was assessed in a cell viability assay using the M-CSF-dependent murine myeloid cell line M-NFS-60. Proliferation of this cell line can be induced by human, cynomolgus, rat, and mouse M-CSF. To characterize the anti-M-CSF antibodies, proliferation was induced either by recombinant soluble M-CSF, native soluble M-CSF, or by transfectants expressing cell-surface M-CSF. The following components were tested (Examples 5-7).

Table 7: Origin of the M-CSF tested in the Proliferation Inhibition Assays (Examples 5-7)

Recombinant M-CSF
Recombinant human sgM-CSF [final conc. in assay: 9.5 pM]
Recombinant murine sgM-CSF [final conc. in assay: 9.5 pM]
Recombinant cynomolgus monkey sgM-CSF [final conc. in assay: 9.5 pM]
Recombinant rat M-CSF (PromoKine, Cat #E60442) [final conc. in assay: 9.5 pM]
Secreted M-CSF
Conditioned cell culture medium from MDA-MB-231 cells collected after 3 days of incubation [final conc. in assay: 50%]
Cell-bound M-CSF
CHO cells stably transfected with human M-CSF, fixed with 2.5% glutaraldehyde for

30 min at room temperature
Serum
Human serum (Sigma H45522; heat inactivated 30 min at 56°C) [final conc. in assay: 50%]

M-NFS-60 cells were cultured (96 well plates) in RPMI 1640 containing stabilized glutamine (Pan Biotech, PAN-P04-18500) and supplemented with 10% FCS, 1mM Na-Pyruvate, 10mM HEPES and in the presence of 9.5 pM (0.5 ng/ml) recombinant human or cynomolgus M-CSF and increasing concentration of antibodies. Viability of the cells was determined after 3 days of culture with CellTiter-Glo® reagent (Promega, Cat# G-7571). Luminescence was measured with a standard luminometer to determine cell viability (relative ATP content). IC_{50} values were determined using GraphPad Prism software. The results are shown in Table 8. All values are in [pM].

Table 8: IC_{50} values of antibodies as determined in an assay measuring the inhibition of proliferation induced by recombinant M-CSF [pM]

Antibody#	Format	Human M-CSF (n=2)	Cynomolgus M-CSF (n=2)
Blanche	IgG1_D265A	2.9	3.3
Caline	IgG1_D265A	3.1	4.3
Camille	IgG1_D265A	2.3	2.6
Celine	IgG1_D265A	2.7	3.0
Laurine	IgG1_D265A	2.6	2.8
Madeleine	IgG1_D265A	3.0	2.8
Maelle	IgG1_D265A	2.7	2.3
Meriem	IgG1_D265A	3.1	2.8
Romaine	IgG1_D265A	8.4	8.1
Satine	IgG1_D265A	5.3	5.4
Servane	IgG1_D265A	11.8	13.4
HeRX1-10G1	IgG1	3.6	4.1
HeRX1-10G1	IgG1_D265A	3.0	3.5
8.10.3F	IgG2	71.1	84.6
8.10.3F	IgG1_D265A	14.9	16.5

All antibodies tested blocked the binding of M-CSF to its receptor. In particular, the antibodies which are derived from the parental antibodies Blanche and Laurine are more potent than the prior art antibodies, in particular more potent than 8.10.3F.

Example 7: Inhibition of proliferation induced by native M-CSF

The ability of the antibodies of the present disclosure to block the bioactivity of native human M-CSF was assessed in an M-NFS-60 cell proliferation assay. Here, proliferation was induced either by human serum or MDA-MB-231 conditioned medium. Antibodies were pre-incubated for 30min in serum in a 96 well-plate before 1000 M-NFS-60 cells were added. The final serum concentration was 50% and the cell culture medium was not supplemented with FCS. Cell viability was determined as described above after 3 days. Immunoglobulins were titrated and IC50s were calculated. The results are shown in Table 9. All values are in [pM].

Table 9: IC_{50} values of antibodies as determined in an assay mesruing the inhibition of proliferation by recombinant M-CSF (pM)

Antibody#	Format	Human serum	MDA-MB-231 conditioned medium
Blanche	IgG1_D265A	18.3	120.5
Caline	IgG1_D265A	17.7	106.9
Camille	IgG1_D265A	17.7	98.5
Celine	IgG1_D265A	23.8	72.4
Laurine	IgG1_D265A	15.6	95.0
Madeleine	IgG1_D265A	20.0	75.4
Maelle	IgG1_D265A	16.0	98.9
Meriem	IgG1_D265A	15.1	76.9
Romaine	IgG1_D265A	25.2	83.3
Satine	IgG1_D265A	31.8	70.8
Servane	IgG1_D265A	93.2	107.0
HeRX1-10G1	IgG1	15.4	90.3
HeRX1-10G1	IgG1_D265A	12.4	59.1
8.10.3F	IgG2	349.7	739.0
8.10.3F	IgG1_D265A	201.4	79.5

All antibodies tested efficiently blocked the bioactivity of M-CSF present in MDA-MB-231-conditioned medium. Prior art antibody 8.10.3F was less potent than the other antibodies tested. Similarly, all antibodies inhibited the bioactivity of M-CSF present in human serum. Again, prior art antibody 8.10.3F was less potent than the other immunoglobulins tested.

Example 8: Inhibition of proliferation induced by cell surface M-CSF

The ability of the antibodies of the present disclosure to block the bioactivity of membrane-bound M-CSF isoforms was assessed in an assay in which proliferation of M-NFS-60 cells was induced by CHO cells stably expressing human cell-surface M-CSF (CHO_hM-CSF cells). 2000 CHO_hM-CSF cells per well were cultured overnight at 37°C in cell-culture 96 well plates, washed twice with PBS and fixed with 100 µl 2% glutaraldehyde/PBS per well for 30 min at 37°C. After washing with PBS, fixed cells were incubated with anti-M-CSF antibodies (final IgG concentrations 0.05 nM, 0.5 nM, 5 nM, 50 nM) for 30 min at 37°C. Subsequently, 5000 M-NFS-60 cells per well were added and cultivated for 72 h at 37°C before cell viability was determined as described above. Results are shown in Figure 1.

All M-CSF-specific immunoglobulins efficiently inhibited proliferation induced by CHO cells expressing M-CSF. The degree of inhibition increase with an increasing concentration of IgG. Inhibition was almost 100% at the highest IgG concentration tested (50 nM). MOR3207, an antibody with specificity to lysozyme, did not inhibit proliferation.

Example 9: Affinity determination

The monovalent affinity of the antibodies of the present disclosure was determined by soluble equilibrium titration (Haenel et al. (2005) Anal Biochem 339, 182-4). The antibodies were purified in Fab format and K_D to human and cynomolgus M-CSF was determined. The results are shown in Table 10. All values are in [pM].

Table 10: Monovalent affinities (K_D values) of antibodies as determined by SET

Antibody#	Human M-CSF	Cynomolgus M-CSF
Blanche	96	130
Caline	14	16
Camille	18	25
Celine	n.d.	n.d.

Laurine	96	150
Madeleine	28	30
Maelle	13	27
Meriem	11	13
Romaine	13	7
Satine	<2	n.d.
Servane	n.d.	n.d.
HeRX1-10G1	>1,000	n.d.
8.10.3F	38	n.d.

As shown in Table 10 all Fabs tested bound to human and cynomolgus M-CSF. Intriguingly, most antibodies demonstrated K_D values of 30 pM or lower, i.e. affinities which are higher than those of prior art antibodies HeRX1-10G1 and 8.10.3F. There was no significant difference observed in affinity between human and cynomolgus M-CSF.

Example 10: Specificity of the antibodies

The specificity of binding of the antibodies of the present invention was exemplary tested with antibody *Camille* as described in Frese et al. (2013) MAbs, Feb 14;5(2) [Epub ahead of print]. For this specificity profiling test the different proteins and controls were coated on two 384-well MSD plates with a concentration of 1 µg/ml at 4 °C over night. Plates were blocked with BSA and washed three times with PBS with 0.05% (v/v) Tween 20. Antibody samples were diluted to 100 nM and 10 nM in assay buffer (PBS with 0.5% (w/v) BSA, 0.05% (v/v) Tween 20). As controls, an unspecific antibody (MOR03207; anti-lysozym) and assay buffer were used. Samples and controls were incubated for three hours at room temperature. The plates were washed three times and 30 µl detection antibody (ECL-labeled anti-human Fab) were added per well and incubated for 1 h. After washing, MSD Read Buffer T with surfactant was added and electrochemiluminescence signals were detected using a Sector Imager 6000 (Meso Scale Discovery, Gaithersburg, MD, USA).

For evaluation, signals of the antibody sample on a certain protein were normalized to the reference antibody MOR03027. Results are shown in Table 11.

Table 11: Specificity of antibody *Camille*

Target antigen	**Antibody *Camille***	
	100 nM	10 nM
Blank	1	1
Protein A (Staphylococcus aureus)	1	1
Serum albumin (human)	1	1
Fibrinogen (bovine)	1	1
Haemoglobin (human)	1	1
Transferrin (bovine)	1	1
Antitrypsin (human)	1	1
Lysozyme (chicken)	0	0
Cell surface receptor 1 (human)	1	1
Cytokine 1 (human)	1	1
Cytokine 2 (human)	2	1
Cell surface receptor 2 (human)	1	1
M-CSF (human)	24	244
Cell surface receptor 3 (human)	1	1
Blank	2	1
Pepsinogen (pork)	1	1
Aminogylcosidase (Aspergillus niger)	1	1
Trypsin inhibitor (Soybean)	1	1
Cytochrome c (cow)	1	1
Myoglobin (horse)	1	1
Lectin (Lens culinaris)	1	1
Ovalbumin (chicken)	1	1
Trypsinogen (cow)	1	1
Milk powder (cow)	1	1
RNase B (cow)	1	1
RNase A (cow)	1	1
Anti-human Fab (Dianova, # 109-005-097)	1	1
Anti-human Fc (Dianova, #109-005-098)	1	1
blank	1	1

Exemplary antibody *Camille* was highly specific for M-CSF and did not show unspecific binding to any of the unrelated proteins tested in this assay.

Example 11: Comparison of exemplary antibody *Camille* with the prior art antibodies

In the following Table the key features of antibody *Camille* are compared to the prior art antibodies HeRX1.10G1 and 8.10.3F.

Table 12: Comaprison of antibody *Camille* to the antibodies in the prior art

Criterion			***Camille***	**HeRX1.10G1**	**8.10.F3**
Binding	Human M-CSF		18 pM	>1,000 pM	38 pM
	Cynomolgus M-CSF		25 pM	n.d.	n.d.
	IL-34, GM-CSF, SCF		no	no	no
	Membrane-bound M-CSF		yes	yes	yes
Functionality	Receptor binding assay (IC50)	Human M-CSF	4 pM	27 pM	31 pM
		Cynomolgus M-CSF	6 pM	28 pM	21 pM
	Cell viability assay (IC50)	Human M-CSF	2 pM	4 pM	71 pM
		Cynomolgus M-CSF	3 pM	4 pM	85 pM
		Membrane-bound M-CSF	yes	yes	yes
		MDA-MB-231	99 pM	90 pM	739 pM
		Human serum M-CSF	18 pM	15 pM	350 pM

In summary, antibody *Camille*, as well as the other antibodies of the present disclosure, shows a binding affinity which is superior to all the prior art antibodies. This is also reflected in the functional assays, in which *Camille* performs at least as good as, but in most assays better than, the prior art antibodies HeRX1.10G1 and 8.10.F3.

Example 12: Efficacy of the antibodies in a clinical trial

A multi-center, randomized, double-blinded, placebo-controlled study to evaluate the safety, preliminary clinical activity and immunogenicity of multiple doses of the antibodies of the present disclosure administered intravenously to patients with active rheumatoid arthritis will be conducted.

Primary outcome measures are the adverse event rate and the safety profile. Secondary outcome measures included DAS28 scores, ACR scores and EULAR28 response criteria.

The clinical trial comprises three treatment arms. In each treatment arm patients receive either placebo or the antibodies of the present disclosure (0.3 mg/kg body weight for treatment arm 1, 1.0 mg/kg body weight for treatment arm 2 and 1.5 mg/kg body weight for treatment arm 3). Antibodies and placebo are administered intravenously, weekly with 4 doses in total.

Prior to administration the disease activity of all patients is measured according to accepted guidelines by calculating the DAS28 score, a 28-joint Disease Activity Score (see e.g. Ann Rheum Dis (2009) 68, 954-60). DAS28 score is a validated and commonly used tool to quantify the disease status of RA patients. The average DAS28 score is comparable for all treatment arms.

The antibodies of the present disclosure show a favorable safety profile among all doses tested and the treatment is safe.

4 weeks and 8 weeks after the first administration of the antibodies or placebo the DAS28 scores of all patients is determined. A decrease in DAS28 scores correlates to diminished disease severity.

All patients treated with the antibodies of the present disclosure show a decrease in DAS28 scores, indicating less severity of the disease of effectiveness of the treatment. In contrast, patients treated with placebo showed do not show any benefit from treatment.

As another measure of efficacy the ACR20 criteria were used. ACR criteria measure improvement in tender or swollen joint counts and improvement in certain other parameters. The procedure to measure ACR scores is highly standardized. The present clinical trial applied the respective guidelines of the EMEA.

In line with the results of the DAS28 scores, also the ACR scores show a strong clinical improvement of patients' condition upon treatment with the antibodies of the present disclosure. The improvement after 4 weeks is highly significant.

Claims

1. An isolated antibody or antibody fragment of the IgG1 subtype, which specifically binds to M-CSF and wherein said isolated antibody or antibody fragment is able to inhibit the binding of M-CSF to the M-CSF receptor with an IC50 of 10 pM or less in a receptor binding inhibition assay comprising M-CSF at a final concentration of 12.5 pM wherein said antibody or antibody fragment comprises

(a) the HCDR1 region of SEQ ID NO.: 8, the HCDR2 region of SEQ ID NO.: 9, the HCDR3 region of SEQ ID NO.: 10, the LCDR1 region of SEQ ID NO.: 11, the LCDR2 region of SEQ ID NO.: 12 and the LCDR3 region of SEQ ID NO.: 13,

(b) the HCDR1 region of SEQ ID NO.: 18, the HCDR2 region of SEQ ID NO.: 19, the HCDR3 region of SEQ ID NO.: 20, the LCDR1 region of SEQ ID NO.: 21, the LCDR2 region of SEQ ID NO.: 22 and the LCDR3 region of SEQ ID NO.: 23,

(c) the HCDR1 region of SEQ ID NO.: 28, the HCDR2 region of SEQ ID NO.: 29, the HCDR3 region of SEQ ID NO.: 30, the LCDR1 region of SEQ ID NO.: 31, the LCDR2 region of SEQ ID NO.: 32 and the LCDR3 region of SEQ ID NO.: 33,

(d) the HCDR1 region of SEQ ID NO.: 38, the HCDR2 region of SEQ ID NO.: 39, the HCDR3 region of SEQ ID NO.: 40, the LCDR1 region of SEQ ID NO.: 41, the LCDR2 region of SEQ ID NO.: 42 and the LCDR3 region of SEQ ID NO.: 43,

(e) the HCDR1 region of SEQ ID NO.: 48, the HCDR2 region of SEQ ID NO.: 49, the HCDR3 region of SEQ ID NO.: 50, the LCDR1 region of SEQ ID NO.: 51, the LCDR2 region of SEQ ID NO.: 52 and the LCDR3 region of SEQ ID NO.: 53,

(f) the HCDR1 region of SEQ ID NO.: 58, the HCDR2 region of SEQ ID NO.: 59, the HCDR3 region of SEQ ID NO.: 60, the LCDR1 region of SEQ ID NO.: 61, the LCDR2 region of SEQ ID NO.: 62 and the LCDR3 region of SEQ ID NO.: 63,

(g) the HCDR1 region of SEQ ID NO.: 88, the HCDR2 region of SEQ ID NO.: 89, the HCDR3 region of SEQ ID NO.: 90, the LCDR1 region of SEQ ID NO.: 91, the LCDR2 region of SEQ ID NO.: 92 and the LCDR3 region of SEQ ID NO.: 93,

(h) the HCDR1 region of SEQ ID NO.: 98, the HCDR2 region of SEQ ID NO.: 99, the HCDR3 region of SEQ ID NO.: 100, the LCDR1 region of SEQ ID NO.: 101, the LCDR2 region of SEQ ID NO.: 102 and the LCDR3 region of SEQ ID NO.: 103, or

(i) the HCDR1 region of SEQ ID NO.: 108, the HCDR2 region of SEQ ID NO.: 109, the HCDR3 region of SEQ ID NO.: 110, the LCDR1 region of SEQ ID NO.: 111, the LCDR2 region of SEQ ID NO.: 112 and the LCDR3 region of SEQ ID NO.: 113.

2. An isolated antibody or antibody fragment according to claim 1, wherein said antibody or antibody fragment comprises

(a) the variable heavy region of SEQ ID NO.: 14 and the variable light region of SEQ ID NO.: 15,

(b) the variable heavy region of SEQ ID NO.: 24 and the variable light region of SEQ ID NO.: 25,

(c) the variable heavy region of SEQ ID NO.: 34 and the variable light region of SEQ ID NO.: 35,

(d) the variable heavy region of SEQ ID NO.: 44 and the variable light region of SEQ ID NO.: 45,

(e) the variable heavy region of SEQ ID NO.: 54 and the variable light region of SEQ ID NO.: 55,

(f) the variable heavy region of SEQ ID NO.: 64 and the variable light region of SEQ ID NO.: 65,

(g) the variable heavy region of SEQ ID NO.: 94 and the variable light region of SEQ ID NO.: 95,

(h) the variable heavy region of SEQ ID NO.: 104 and the variable light region of SEQ ID NO.: 105, or

(i) the variable heavy region of SEQ ID NO.: 114 and the variable light region of SEQ ID NO.: 115.

3. An isolated antibody according to any one of the preceding claims, wherein said antibody is of a IgG1 subtype which has an effector function which is diminished compared to the wild type IgG1 subtype.

4. The isolated antibody of claim 3, wherein the aspartic acid residue at position 265 of said antibody (numbering according to the EU index) is exchanged for an alanine residue.

5. The isolated antibody or antibody fragment of any of the preceding claims, wherein said antibody or antibody fragment is a monoclonal antibody or a polyclonal antibody.

6. The antibody or antibody fragment of any of the preceding claims, wherein said antibody is a human, humanized or chimeric antibody.

7. The antibody or antibody fragment of any of the preceding claims, wherein said antibody is cross-reactive to cynomolgus M-CSF, mouse M-CSF and/or rat M-CSF.

8. The isolated antibody or antibody fragment of any of the preceding claims for use in medicine.

9. A pharmaceutical composition comprising an isolated antibody or antibody fragment of any one of claims 1-7, and a pharmaceutically acceptable carrier.

10. A nucleic acid encoding an antibody or antibody fragment of any one of claims 1-7.

11. A vector comprising a nucleic acid of claim 10 or encoding any of the antibodies of claims 1-7.

12. An isolated host cell comprising a vector according to claim 11, a nucleic acid according to claim 10 or a nucleic acid encoding an antibody or antibody fragment according to any one of claims 1-7.