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(54) Title: TREATMENT OF BREAST CANCER BY M-CSF ANTAGONIST

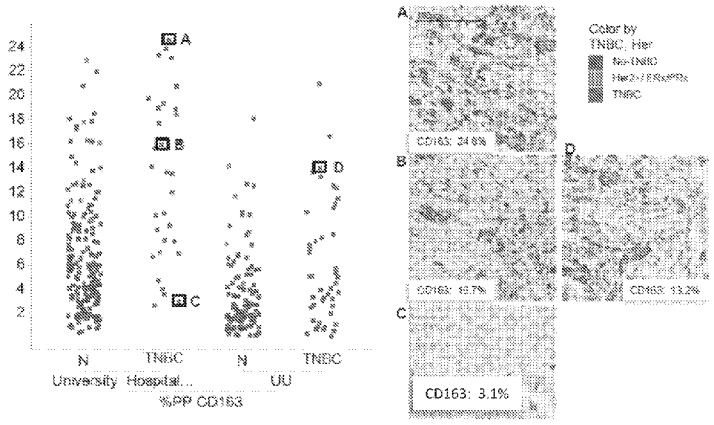

Fig. 1

(57) Abstract: The invention relates, in part, to a method of selectively treating a patient having breast cancer, comprising administering a therapeutically effective amount of a M-CSF antagonist to the patient on the basis of the patient having a level of CD 163 expression that is predictive that the patient will respond to an M-CSF antagonist. The invention relates, in part, to a method of treating a patient having triple negative breast cancer, comprising administering a therapeutically effective amount of an M-CSF antibody or fragment thereof to the patient, wherein the M-CSF antibody or fragment comprises 1, 2, 3, 4, 5, or 6 CDRs as shown in Table 1. The invention relates, in part, to a method of treating a patient having breast cancer, comprising administering a combination of a therapeutically effective amount of an M-CSF antagonist, a therapeutically effective amount of a second agent and a therapeutically effective amount of a third agent.

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TREATMENT OF BREAST CANCER BY M-CSF ANTAGONIST

Field of the Invention

This invention relates to methods of treatment and to methods of selecting patients who have breast cancer for treatment with an M-CSF antagonist.

Background of the Invention

Breast cancer is the most common cancer among women worldwide, with an estimated 1.38 million new cases in 2008, and it is also the most common cause of cancer death in women, with 458,000 deaths. Triple-negative breast cancer (TNBC) accounts for approximately 15% of newly diagnosed breast cancers (25% of metastatic breast cancer) and is characterized by lack of expression of the estrogen (ER) and progesterone (PR) receptors and lack of overexpression of the human epidermal growth factor receptor 2 (HER2).

Currently, there are no targeted therapies for this heterogeneous breast cancer subtype and the only treatment option is chemotherapy. Even though several studies suggest that TNBC disease is sensitive to chemotherapy, prognosis still remains poor with a shorter disease free interval after initial therapy and a more aggressive clinical course than non-TNBC disease in the metastatic setting. Most patients receive anthracycline and taxane-based chemotherapy in the adjuvant setting, and no further standard of care therapy exists for patients with metastatic TNBC. However, emerging data suggest that platinum salts (ie, cisplatin and carboplatin) and gemcitabine and/or combinations thereof are highly active in early and advanced TNBC, and are therefore widely used in the clinical setting. Median survival for metastatic TNBC is approximately 1 year, making TNBC a disease with high unmet medical need.

TNBC, and breast cancer in general, is heterogeneous, and patients do not necessarily respond to a similar drug regimen in a similar manner. Thus, stratification of breast cancer disease patients using markers predictive of drug response will help clinicians select and establish an effective treatment strategy. Moreover, patient stratification will avoid the safety issues and the high financial burden associated with long-term therapy. Thus, there is a need to develop methods of treating breast cancer diseases with therapeutics that first identify those patients most likely to benefit from that therapeutic.

Summary of the Invention

Provided herein are novel methods of treatment for patients having breast cancer. In particular the present invention is directed to the use of an Macrophage Colony Stimulating Factor (M-CSF) antagonist, e.g., H-RX1 antibody as described herein, for the treatment of breast cancer such as triple negative breast cancer (TNBC). The invention further includes personalized therapies that maximize the benefit and minimize the risk of use of M-CSF antagonists, e.g., H-RX1 antibody as described herein, in breast cancer populations by identifying those patients likely to respond favorably prior to treatment with an M-CSF antagonist. Specifically, the present invention includes identifying patients who have breast cancer, such as triple negative breast cancer patients, that have a predetermined level of tumor associated macrophages (TAMs), e.g., by assaying for expression of CD163 that is characteristic of a subset of monocyte-derived TAMs. The level or density of CD163 expression, e.g., mRNA expression and/or protein in a tumor sample obtained from a triple negative breast cancer patient can be used to indicate whether that patient is more likely to respond favorably to M-CSF antagonist treatment.

In one aspect, the invention includes a method of treating a patient having triple negative breast cancer, comprising selectively administering a therapeutically effective amount of an M-CSF antagonist to the patient. In one embodiment, the M-CSF antagonist is an antibody or antigen binding fragment thereof. The M-CSF antibody or antigen binding fragment thereof can comprise a heavy chain variable region that comprises CDR1, CDR2, and CDR3 domains; and a light chain variable region that comprises CDR1, CDR2, and CDR3 domains, wherein the heavy chain variable region CDR3 comprises the amino acids having the sequence set forth in SEQ ID NO:7; and a light chain variable region CDR3 comprises amino acids having the sequence set forth in SEQ ID NO:10. The antibody or antigen-binding portion thereof can bind to human M-CSF with a binding affinity of about 10^{-7} M.

In another aspect the invention includes a method of treating a patient having breast cancer, e.g., triple negative breast cancer, comprising selectively administering a therapeutically effective amount of an M-CSF antagonist, e.g., an M-CSF antibody or antigen binding fragment thereof, e.g., an antibody or fragment thereof having the CDRs as shown in Table 1 or H-RX1.

In another aspect the invention includes a method of treating a patient having breast cancer, e.g., triple negative breast cancer, comprising selectively administering a therapeutically effective amount of an M-CSF antagonist, e.g., an M-CSF antibody or antigen binding fragment thereof, e.g., H-RX1, to the patient on the basis of the patient having a TAMs density that is predictive that the patient is likely to respond to the M-CSF antagonist.

In another aspect the invention includes a method of treating a patient having breast cancer, e.g., triple negative breast cancer, comprising selectively administering a therapeutically effective amount of an M-CSF antagonist, e.g., an M-CSF antibody or antigen binding fragment thereof, e.g., H-RX1, to the patient on the basis of the patient having a level of CD163 expression that is predictive that the patient is likely to respond to the M-CSF antagonist.

In another aspect the invention includes a method of treating a patient having breast cancer, comprising selectively administering a therapeutically effective amount of an M-CSF antagonist, e.g., an M-CSF antibody or antigen binding fragment thereof, e.g., an antibody or binding fragment that includes a heavy chain variable region comprising a CDR1 (SEQ ID NO:5), CDR2 (SEQ ID NO:6) and CDR3 (SEQ ID NO:7) and a light chain variable region comprising a CDR1 (SEQ ID NO:8), CDR2 (SEQ ID NO:9) and CDR3 (SEQ ID NO:10), to the patient on the basis of the patient having a density of CD163 protein that is predictive that the patient is likely to respond to the M-CSF antagonist.

In yet another aspect, the invention includes a method of selectively treating a patient having breast cancer, comprising: selecting the patient for treatment with a therapeutically effective amount of an M-CSF antagonist on the basis of the patient having a level of CD163 expression compared to a control (e.g., a level of CD163 above a cut-off threshold) that is predictive that the patient is likely to respond to M-CSF; and thereafter, administering the therapeutically effective amount of the M-CSF antagonist.

In yet another aspect, the invention includes a method of selecting a patient having breast cancer for treatment with an M-CSF antagonist, comprising: selecting the patient for treatment with a therapeutically effective amount of an M-CSF antagonist on the basis of the patient having a level of CD163 expression compared to a control (e.g., above a cut-off threshold) that is predictive that the patient is likely to respond to the M-CSF antagonist.

In still yet another aspect, the invention includes a method of selectively treating a patient having breast cancer, comprising: assaying a biological sample from the patient for an increased level of CD163 expression; and thereafter, selectively administering to the patient either: a therapeutically effective amount of an M-CSF antagonist on the basis of the patient having a level of CD163 expression (e.g., mRNA or protein) compared to a control that is predictive that the patient is likely to respond to M-CSF, e.g., the sample has an increased level of CD163 expression compared to a control; or a therapeutically effective amount of a therapy other than an M-CSF antagonist on the basis of the biological sample from the patient not having an increased level of CD163 expression compared to a control such that it is predictive that the patient is likely to respond to the M-CSF antagonist.

In yet a further aspect, the invention includes a method of selectively treating a patient having breast cancer, comprising assaying a biological sample from the patient for a level CD163 expression; and thereafter, selecting the patient for treatment with a therapeutically effective amount of an M-CSF antagonist on the basis of the biological sample from the patient having a level of CD163 expression predictive that the patient is likely to respond to M-CSF (e.g., the sample has an increased level of CD163 expression or presence compared to a control); and thereafter, administering the M-CSF antagonist to the selected patient.

In yet another aspect, the invention includes a therapeutically effective amount of an M-CSF antagonist for use in treating a patient having breast cancer, characterized in that the therapeutically effective amount of the M-CSF antagonist is to be administered to the patient on the basis of said patient having a level of CD163 expression compared to a control predictive that the patient is likely to respond to the M-CSF antagonist.

In yet another aspect, the invention includes a therapeutically effective amount of an M-CSF antagonist for use in treating a patient having breast cancer characterized in that the patient is selected for treatment with an M-CSF antagonist on the basis of the patient having a level of CD163 expression (e.g., compared to a control e.g., (a level above a cut-off threshold)) that is predictive that the patient is likely to respond to the M-CSF antagonist; and thereafter, a therapeutically effective amount of the M-CSF antagonist is administered.

In another aspect, the invention includes a method of predicting the likelihood that a patient having breast cancer will respond to treatment with a therapeutically effective amount of a M-CSF antagonist comprising assaying a biological sample from the patient for the level of CD163 expression, wherein the level of CD163 expression compared to a control is indicative

of an increased likelihood that the patient will respond to treatment with the M-CSF antagonist; and the absence of CD163 expression compared to a control is indicative of a decreased likelihood that the patient will respond to treatment with the M-CSF antagonist.

In still yet another aspect, the invention includes a method of selectively treating a patient having breast cancer, e.g., triple negative breast cancer, comprising selectively administering a combination of i) a therapeutically effective amount of a M-CSF antagonist, and ii) a therapeutically effective amount of a second agent (e.g., carboplatin or gemcitabine); to the patient on the basis of the patient having a level of CD163 expression compared to a control predictive that the patient is likely to respond to the M-CSF antagonist. In one embodiment, the M-CSF antagonist is an antibody or fragment thereof comprising 1, 2, 3, 4, 5, or 6 CDRs as shown in Table 1.

In yet another aspect, the invention includes a method of selectively treating a patient having breast cancer, e.g., triple negative breast cancer, comprising: selecting the patient for treatment with a combination of i) a therapeutically effective amount of a M-CSF antagonist, and ii) a therapeutically effective amount of a second agent, e.g., a chemotherapeutic agent such as carboplatin or gemcitabine on the basis of a the patient having a level of CD163 expression compared to a control predictive that the patient is likely to respond to M-CSF; and thereafter, administering the combination of i) the therapeutically effective amount of the M-CSF antagonist, and the therapeutically effective amount of a second agent, simultaneously, separately or sequentially. In one embodiment, the M-CSF antagonist is an antibody or fragment thereof comprising 1, 2, 3, 4, 5, or 6 CDRs as shown in Table 1.

In yet another aspect, the invention includes a method of selectively treating a patient having breast cancer, comprising selectively administering a therapeutically effective amount of a M-CSF antagonist, carboplatin and gemcitabine to the patient on the basis of the patient having a level of CD163 expression compared to a control predictive that the patient is likely to respond to M-CSF. In one embodiment, the M-CSF antagonist is an antibody or fragment thereof comprising 1, 2, 3, 4, 5, or 6 CDRs as shown in Table 1.

In yet another aspect, the invention includes a method of selectively treating a patient having breast cancer, comprising selecting the patient for treatment with a therapeutically effective amount of a M-CSF antagonist, carboplatin and gemcitabine on the basis of the patient having a level of CD163 expression compared to a control predictive that the patient is likely to respond to M-CSF; and thereafter, administering the combination of the therapeutically effective amount of the M-CSF antagonist, carboplatin and gemcitabine, simultaneously,

separately or sequentially. In one embodiment, the M-CSF antagonist is an antibody or fragment thereof comprising 1, 2, 3, 4, 5, or 6 CDRs as shown in Table 1.

In yet another aspect, the invention includes a method of selectively treating a patient having breast cancer, comprising assaying a biological sample from the patient for the level of CD163 protein; and thereafter, selecting the patient for treatment with an M-CSF antagonist on the basis of the biological sample from the patient having a level of CD163 expression compared to a control predictive that the patient is likely to respond to M-CSF; and thereafter, administering the M-CSF antagonist to the selected patient. In one embodiment, the M-CSF antagonist is an antibody or fragment thereof comprising 1, 2, 3, 4, 5, or 6 CDRs as shown in Table 1.

The CD163 can be detected by any known technique, e.g., a technique selected from the group consisting of Northern blot analysis, polymerase chain reaction (PCR), reverse transcription-polymerase chain reaction (RT-PCR), immunoassays, immunohistochemistry (IHC), ELISA and Western blot. In particular CD163 protein can be detected by quantitative IHC, e.g., by immunostaining for CD163 and quantiting CD163 protein expression by using an automated image analysis instrument.

The step of administering of any of the claims above can include administering a dose of between about 5-30 mg/kg, e.g., 10 mg/kg of an M-CSF antagonist to said patient at time 0 ("first administration"); at 7-14 days, e.g., 8 days, following first administration at 10 mg/kg of an M-CSF antagonist ("additional dose") and then administering every three weeks following the first administration, e.g., at 10 mg/kg of an M-CSF antagonist. In a particular embodiment, the M-CSF antagonist is antibody or antigen binding fragment thereof comprising a heavy chain variable region that comprises CDR1, CDR2, and CDR3 domains; and a light chain variable region that comprises CDR1, CDR2, and CDR3 domains, wherein the heavy chain variable region CDR3 comprises the amino acids having the sequence set forth in SEQ ID NO:7; and a light chain variable region CDR3 comprises amino acids having the sequence set forth in SEQ ID NO:10 and wherein the antibody or fragment thereof is administered to the patient at a dose of 10mg/kg at the first administration, an additional dose of 10/mg/kg is administered 7-14 days, e.g., 8 days at a dose of 10mg/kg, after the first administration and then the antibody or fragment thereof is administered every three weeks following the first administration at a dose of 10mg/kg. Additionally, gemcitabine and carboplatin can also be administered to the patient, e.g., on days 1 and every three weeks

thereafter. In one embodiment, the therapeutic agents can be administered by intravenous infusions sequentially, simulataneously or separately.

In another embodiment, in the methods described above, the M-CSF antagonist is an M-CSF antibody or antigen binding fragment thereof. The M-CSF antibody can comprise 1, 2, 3, 4, 5, or 6 CDRs as shown in Table 1.

In another embodiment, in the methods described above, the M-CSF antibody or fragment comprises a VH comprising SEQ ID NO 2: and a VL comprising SEQ ID NO: 4, or an amino acid sequence with 97-99 percent identity thereof.

In yet another aspect, the invention includes a method for producing a transmittable form of information for predicting the responsiveness of a patient having breast cancer to treatment with an M-CSF antagonist, comprising: assaying a biological sample from the patient for the level of CD163 expression; and recording the result of the assaying step on a tangible or intangible media form for use in transmission, wherein if the result is an increase in the level of CD163 expression compared to a control (e.g., the level is above a predetermined threshold level) it is indicative of an increased likelihood that the patient will respond to treatment comprising a M-CSF antagonist. In one embodiment, the M-CSF antagonist is an antibody or fragment thereof comprising 1, 2, 3, 4, 5, or 6 CDRs as shown in Table 1.

In another embodiment, a method of treating a patient having breast cancer, e.g., triple negative breast cancer is provided, comprising: administering a combination of a therapeutically effective amount of an M-CSF antagonist and a therapeutically effective amount of gemcitabine and carboplatin, simultaneously, separately or sequentially. In one embodiment, the M-CSF antagonist is an antibody or fragment thereof comprising 1, 2, 3, 4, 5, or 6 CDRs as shown in Table 1.

In still another embodiment, a method of selectively treating a patient having breast cancer is provided, comprising: a) assaying a biological sample from the patient for the level of CD163 protein; b) selecting the patient for treatment with a combination of i) a therapeutically effective amount of a M-CSF antagonist, ii) a therapeutically effective amount of a second agent, and iii) a therapeutically effective amount of a third agent, on the basis of a the patient having a level of CD163 expression compared to a control predictive that the patient is likely to respond to M-CSF; and c) thereafter, administering the combination of i) the therapeutically effective amount of the M-CSF antagonist, ii) the therapeutically effective

amount of a second agent, and iii) a therapeutically effective amount of a third agent, simultaneously, separately or sequentially. In one embodiment, the M-CSF antagonist is an antibody or fragment thereof comprising 1, 2, 3, 4, 5, or 6 CDRs as shown in Table 1.

In yet another embodiment, a method of selectively treating a patient having breast cancer is provided, comprising: a) assaying a biological sample from the patient for the level of tumor associated macrophages (TAMs); b) selecting the patient for treatment with a combination of i) a therapeutically effective amount of a M-CSF antagonist, ii) a therapeutically effective amount of a second agent, and iii) a therapeutically effective amount of a third agent, on the basis of a the patient having a level of TAM content compared to a control predictive that the patient is likely to respond to M-CSF; and c) thereafter, administering the combination of i) the therapeutically effective amount of the M-CSF antagonist, ii) the therapeutically effective amount of a second agent, and iii) a therapeutically effective amount of a third agent, simultaneously, separately or sequentially. In some embodiments, the breast cancer is TNBC. In some embodiments, the level of CD163 protein expression is above a threshold level e.g., the TAM density determined by measuring CD163+ pixel density using quantitative immunoassay is determined to be at least 8%, 10%, 15%, 20%, 30%, or 40% per biopsy section as described herein. In still other embodiments, the threshold or control level of TAM content e.g., the TAM density determined by measuring CD163+ pixel density using quantitative immunoassay, is determined to be less than 8% per biopsy section as described herein. In one embodiment, the M-CSF antagonist is an antibody or fragment thereof comprising 1, 2, 3, 4, 5, or 6 CDRs as shown in Table 1.

In various embodiments, an aforementioned method is provided wherein the M-CSF antagonist is an M-CSF antibody or fragment thereof. In related embodiments, the antibody or fragment thereof comprises 1, 2, 3, 4, 5, or 6 CDRs as shown in Table 1.

In still other ambodiments, an aforementioned method is provided wherein the second agent is carboplatin and the third agent is gemcitabine.

In another embodiment, a method of selectively treating a patient having TNBC is provided, comprising: a) assaying a biological sample from the patient for the level of CD163 protein; b) selecting the patient for treatment with a combination of i) a therapeutically effective amount of a M-CSF antagonist, ii) a therapeutically effective amount of a second agent, and

iii) a therapeutically effective amount of a third agent, on the basis of a the patient having a level of CD163 expression compared to a control predictive that the patient is likely to respond to M-CSF; and c) thereafter, administering the combination of i) the therapeutically effective amount of the M-CSF antagonist, ii) the therapeutically effective amount of a second agent, and iii) a therapeutically effective amount of a third agent, simultaneously, separately or sequentially; wherein the M-CSF antagonist is an M-CSF antibody or fragment thereof that comprises 1, 2, 3, 4, 5, or 6 CDRs as shown in Table 1, and wherein the second agent is carboplatin and the third agent is gemcitabine.

In still another embodiment, a method of selectively treating a patient having TNBC is provided, comprising: a) assaying a biological sample from the patient for TAMs; b) selecting the patient for treatment with a combination of i) a therapeutically effective amount of a M-CSF antagonist, ii) a therapeutically effective amount of a second agent, and iii) a therapeutically effective amount of a third agent, on the basis of a the patient having a level of TAM content compared to a control (e.g., above a cut-off threshold) predictive that the patient is likely to respond to M-CSF; and c) thereafter, administering the combination of i) the therapeutically effective amount of the M-CSF antagonist, ii) the therapeutically effective amount of a second agent, and iii) a therapeutically effective amount of a third agent, simultaneously, separately or sequentially; wherein the M-CSF antagonist is an M-CSF antibody or fragment thereof that comprises 1, 2, 3, 4, 5, or 6 CDRs as shown in Table 1, and wherein the second agent is carboplatin and the third agent is gemcitabine.

In another aspect, the invention includes a pharmaceutical composition comprising a combination of a therapeutically effective amount of an M-CSF antagonist, carboplatin and gemcitabine.

In yet another aspect, the invention includes a pharmaceutical composition comprising an M-CSF antagonist, for simultaneous, separate or sequential administration for the treatment of TNBC. The M-CSF antagonist can be an M-CSF antibody or fragment thereof that comprises 1, 2, 3, 4, 5, or 6 CDRs as shown in Table 1. In one embodiment, the M-CSF antagonist is H-RX1.

In yet another aspect, the invention includes a pharmaceutical composition comprising a combination of a therapeutically effective amount of an M-CSF antagonist, carboplatin and

gemcitabine for simultaneous, separate or sequential administration for the treatment of TNBC. The M-CSF antagonist can be an M-CSF antibody or fragment thereof that comprises 1, 2, 3, 4, 5, or 6 CDRs as shown in Table 1. In one embodiment, the M-CSF antagonist is H-RX1.

In another aspect, the present invention provides a commercial package comprising as therapeutic agents an M-CSF antagonist, carboplatin and gemcitabine together with instructions for the simultaneous, separate or sequential administration thereof in the treatment of TNBC. The M-CSF antagonist can be an M-CSF antibody or fragment thereof that comprises 1, 2, 3, 4, 5, or 6 CDRs as shown in Table 1. In one embodiment, the M-CSF antagonist is H-RX1.

a) Definitions

In order that the present invention may be more readily understood, certain terms are first defined. Additional definitions are set forth throughout the detailed description.

The term "antibody" as used herein refers to whole antibodies that interact with (e.g., by binding, steric hindrance, stabilizing/destabilizing, spatial distribution) a M-CSF epitope and inhibit signal transduction. A naturally occurring "antibody" is a glycoprotein 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 of three domains, 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 V_L regions can be further subdivided into regions of hypervariability, termed complementarity 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 variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. 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 (Clq) of the classical complement system. The term "antibody" includes for example, monoclonal antibodies, human antibodies, humanized antibodies, camelised

antibodies, chimeric antibodies, single-chain Fvs (scFv), disulfide-linked Fvs (sdFv), Fab fragments, F (ab') fragments, and anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies to antibodies of the invention), and epitope-binding fragments of any of the above. 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) or subclass.

Both the light and heavy chains are divided into regions of structural and functional homology. The terms "constant" and "variable" are used functionally. In this regard, it will be appreciated that the variable domains of both the light (VL) and heavy (VH) chain portions determine antigen recognition and specificity. Conversely, the constant domains of the light chain (CL) and the heavy chain (CH1, CH2 or CH3) confer important biological properties such as secretion, transplacental mobility, Fc receptor binding, complement binding, and the like. By convention the numbering of the constant region domains increases as they become more distal from the antigen binding site or amino-terminus of the antibody. The N-terminus is a variable region and at the C-terminus is a constant region; the CH3 and CL domains actually comprise the carboxy-terminus of the heavy and light chain, respectively.

The phrase "antibody fragment", as used herein, refers to one or more portions of an antibody that retain the ability to specifically interact with (e.g., by binding, steric hindrance, stabilizing/destabilizing, spatial distribution) a M-CSF epitope and inhibit signal transduction. Examples of binding fragments include, but are not limited to, 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; a Fd fragment consisting of the VH and CH1 domains; a Fv fragment consisting of the VL and VH domains of a single arm of an antibody; a dAb fragment (Ward *et al.,* (1989) Nature 341:544-546), which consists of a VH domain; and an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as 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). Such single chain antibodies are also intended to be encompassed within the term "antibody fragment". 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.

The phrase "specifically (or selectively) binds" to an antibody (*e.g.*, a M-CSF binding antibody) refers to a binding reaction that is determinative of the presence of a cognate antigen (*e.g.*, a human M-CSF) in a heterogeneous population of proteins and other biologics. In addition to the equilibrium constant (K_A) noted above, a M-CSF binding antibody of the invention typically also has a dissociation rate constant (K_D) (k_{off}/k_{on}) 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^{-10}M, less than $5x10^{-11}$M, 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, and binds to M-CSF with an affinity that is at least two-fold greater than its affinity for binding to a non-specific antigen (*e.g.*, HSA). In one embodiment, the antibody or fragment thereof has dissociation constant (K_d) of less than 3000 pM, less than 2500 pM, less than 2000 pM, less than 1500 pM, less than 1000 pM, less than 750 pM, less than 500 pM, less than 250 pM, less than 200 pM, less than 150 pM, less than 100 pM, less than 75 pM, less than 10 pM, less than 1 pM as assessed using a method described herein or known to one of skill in the art (e.g., a BIAcore assay, ELISA, FACS, SET) (Biacore International AB, Uppsala, Sweden). The term "K_{assoc}" or "K_a", as used herein, refers to the association rate of a particular antibody-antigen interaction, whereas the term "K_{dis}" or "K_d," as used herein, refers to the dissociation rate of a particular antibody-antigen interaction. The term "K_D", as used herein, refers to the dissociation constant, which is obtained from the ratio of K_d to K_a (i.e. K_d/K_a) and is expressed as a molar concentration (M). K_D values for antibodies can be determined using methods well established in the art. A method for determining the K_D of an antibody is by using surface plasmon resonance, or using a biosensor system such as a Biacore® system.

The "M-CSF antagonist" as used herein refers can be an antibody or other antigen-binding protein, a small molecule, a nucleic acid (such as an siRNA), or any other such molecule which interferes with M-CSF activation or function. In one example, the M-CSF antagonist is an antibody which binds to M-CSF. In a particular embodiment, a M-CSF antagonist binds M-CSF and neutralizes the biological activity of M-CSF signaling. An antibody that inhibits M-CSF activity can effect such a statistically significant decrease by at least 10% of the measured parameter, by at least 50%, 80% or 90%, and in certain embodiments an antibody of the invention may inhibit greater than 95%, 98% or 99% of M-CSF functional activity.

As used herein, the phrase "therapeutically effective amount" refers to is meant to refer to an amount of therapeutic or prophylactic M-CSF antagonist such as an antibody that would be appropriate for an embodiment of the present invention that will elicit the desired therapeutic or prophylactic effect or response when administered in accordance with the desired treatment regimen.

Human "M-CSF" as used herein refers to a human polypeptide having substantially the same amino acid sequence as the mature human M-CSFα, M-CSFβ, or M-CSFγ polypeptides described in Kawasaki et al., Science 230:291 (1985), Cerretti et al., Molecular Immunology, 25:761 (1988), or Ladner et al., EMBO Journal 6:2693 (1987), each of which are incorporated herein by reference. Such terminology reflects the understanding that the three mature M-CSFs have different amino acid sequences, as described above, and that the active form of M-CSF is a disulfide bonded dimer; thus, when the term "M-CSF" refers to the biologically active form, the dimeric form is intended. "M-CSF dimer" refers to two M-CSF polypeptide monomers that have dimerized and includes both homodimers (consisting of two of the same type of M-CSF monomer) and heterodimers (consisting of two different monomers). M-CSF monomers may be converted to M-CSF dimers in vitro as described in U.S. Pat. No. 4,929,700, which is incorporated herein by reference.

The phrase "isolated antibody" refers to an antibody that is substantially free of other antibodies having different antigenic specificities (*e.g.*, an isolated antibody that specifically binds M-CSF is substantially free of antibodies that specifically bind antigens other that M-CSF). An isolated antibody that specifically binds M-CSF may, however, have cross-reactivity to other antigens. Moreover, an isolated antibody may be substantially free of other cellular material and/or chemicals.

The phrase "conservatively modified variant" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one species of conservatively modified variations.

Every nucleic acid sequence herein which encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid that encodes a polypeptide is implicit in each described sequence.

The terms "cross-compete" and "cross-competing" are used interchangeably herein to mean the ability of an antibody or other binding agent to interfere with the binding of other antibodies or binding agents to M-CSF in a standard competitive binding assay.

The ability or extent to which an antibody or other binding agent is able to interfere with the binding of another antibody or binding molecule 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. 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-competing uses an ELISA-based approach.

The phrases "percent identical" or "percent identity," in the context of two or more nucleic acids or polypeptide sequences, refers to two or more sequences or subsequences that are the same. Two sequences are "substantially identical" if two sequences have a specified percentage of amino acid residues or nucleotides that are the same (*i.e.*, 60% identity, optionally 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% identity over a specified region, or, when not specified, over the entire sequence), when compared and aligned for maximum correspondence over a comparison window, or designated region as measured using one of the following sequence comparison algorithms or by manual alignment and visual inspection. Optionally, the identity exists over a region that is at least about 50 nucleotides (or 10 amino acids) in length, or more preferably over a region that is 100 to 500 or 1000 or more nucleotides (or 20, 50, 200 or more amino acids) in length.

For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm

then calculates the percent sequence identities for the test sequences compared to the reference sequence, based on the program parameters.

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.

"Tumor" refers to neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues.

The term "about" in relation to a numerical value x means +/-10% unless the cotext dictates otherwise.

The term "assaying" is used to refer to the act of identifying, screening, probing, testing, measuring or determining, which act may be performed by any means. For example, a sample may be assayed for the level of expression of CD163 by using IHC, RT-PCR, ELISA assay, a Northern blot, immunohistochemistry, western blot, mass spectrometry, etc. The term "detecting" (and the like) means the act of extracting particular information from a given source, which may be direct or indirect. The terms "assaying" and "determining" contemplate a transformation of matter, e.g., a transformation of a biological sample, e.g., a blood sample or other tissue sample, from one state to another by means of subjecting that sample to physical testing.

The term "obtaining" means to procure, e.g., to acquire possession of in any way, e.g., by physical intervention (e.g., biopsy, blood draw) or non-physical intervention (e.g, transmittal of information via a server), etc.

The phrase "assaying a biological sample ..." and the like, is used to mean that a sample may be tested (either directly or indirectly) for either the presence, or an increase in the presence, or absence of CD163 nucleic acid expression, protein or activity. It will be understood that, in a situation where the presence or an increase in the presence of a substance denotes one probability and the absence of a substance denotes a different probability, then either the presence or the absence of such substance may be used to guide a therapeutic decision. For example, one may determine if a patient has increased CD163 protein expression by determining protein level of CD163 in the patient sample where the presence of increased

CD163 is indicative of an increased likelihood that the patient will respond to treatment with a M-CSF antagonist or a combination of a M-CSF antagonist and another agent.

The term "derivative", unless otherwise indicated, is used to define amino acid sequence variants, and covalent modifications (e.g., pegylation, deamidation, hydroxylation, phosphorylation, methylation, etc.) of a M-CSF antagonist, e.g., M-CSF antibody or antigen-binding portion thereof. A "functional derivative" includes a molecule having a qualitative biological activity in common with the disclosed M-CSF antagonists, e.g., M-CSF binding molecules. A functional derivative includes fragments and peptide analogs of a M-CSF antagonist as disclosed herein.

The phrase "substantially identical" means that the relevant amino acid or nucleotide sequence (e.g., V_H or V_L domain) will be identical to or have insubstantial differences (e.g., through conserved amino acid substitutions) in comparison to a particular reference sequence. Insubstantial differences include minor amino acid changes, such as 1 or 2 substitutions (e.g., conservative substitutions, such as swapping a serine for a threonine, or substitutions at positions not involved in antibody activity, structural integrity, complement fixation, etc.) in a 5 amino acid sequence of a specified region (e.g., V_H or V_L domain). In the case of antibodies, the second antibody has the same specificity and has at least 50% of the affinity of the same. Sequences substantially identical (e.g., at least about 85% sequence identity) to the sequences disclosed herein are also part of this disclosure. In some embodiments, the sequence identity of a derivative M-CSF antibody can be about 90% or greater, e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or higher compared to the disclosed sequences.

The term "pharmaceutically acceptable" means a nontoxic material that does not interfere with the effectiveness of the biological activity of the active ingredient(s).

The term "administering" in relation to a compound or antibody, e.g., an M-CSF antibody or antigen binding fragment thereof, or another agent, is used to refer to delivery of that compound or antibody to a patient by any route.

The term "treatment" or "treat" refer to both prophylactic or preventative treatment (as the case may be) as well as curative or disease modifying treatment, including treatment of a patient at risk of contracting the disease or suspected to have contracted the disease as well as patients who are ill or have been diagnosed as suffering from a disease or medical condition, and includes suppression of clinical relapse. The treatment may be administered to a patient

having a medical disorder or who ultimately may acquire the disorder, in order to prevent, cure, delay the onset of, reduce the severity of, or ameliorate one or more symptoms of a disorder or recurring disorder, or in order to prolong the survival of a patient beyond that expected in the absence of such treatment.

The phrase "respond to treatment" is used to mean that a patient, upon being delivered a particular treatment, e.g., an M-CSF antibody or fragment there of, shows a clinically meaningful benefit from said treatment. The phrase "respond to treatment" is meant to be construed comparatively, rather than as an absolute response. For example, a breast cancer patient having a level of CD163 marker greater than a control is predicted to have more benefit from treatment with an M-CSF antagonist than an breast cancer patient who does not have increased CD163 levels (nucleic acid, e.g., mRNA or protein). In another example, a breast cancer patient having a level of CD163 marker above a particular threshold, for example IHC analysis reveals a level of CD163 marker above a particular threshold, e.g., above 15%, that cancer patient is predicted to have more benefit from treatment with an M-CSF antagonist than an breast cancer patient who does not a level of CD163 above the threshold (nucleic acid, e.g., mRNA or protein).

The phrase "receiving data" is used to mean obtaining possession of information by any available means, e.g., orally, electronically (e.g., by electronic mail, encoded on diskette or other media), written, etc.

As used herein, "selecting" and "selected" in reference to a patient is used to mean that a particular patient is specifically chosen from a larger group of patients on the basis of (due to) the particular patient having a predetermined criteria, e.g., the patient has an increased level of CD163 (e.g., nucleic acid, e.g., mRNA or protein) or a predetermined level above a threshold of CD163. Similarly, "selectively treating" refers to providing treatment to a patient having a particular disease, where that patient is specifically chosen from a larger group of patients on the basis of the particular patient having a predetermined criteria, e.g., a breast cancer patient specifically chosen for treatment due to the patient having an increased level of CD163 protein. Similarly, "selectively administering" refers to administering a drug to a patient that is specifically chosen from a larger group of patients on the basis of (due to) the particular patient having a predetermined criteria, e.g., the patient has an increased level of CD163 protein. By selecting, selectively treating and selectively administering, it is meant that a patient is delivered a personalized therapy based on the patient's particular biology, rather than being delivered a standard treatment regimen based solely on the patient having a

particular disease. Selecting, in reference to a method of treatment as used herein, does not refer to fortuitous treatment of a patient that has an increased level of CD163 expression, but rather refers to the deliberate choice to administer a M-CSF antagonist to a patient based on the patient having a level of CD163 expression compared to a control that is predictive that the patient is likely to respond to M-CSF. Thus, selective treatment differs from standard treatment, which delivers a particular drug to all patients, regardless of the level of expression or protein level of CD163 expression.

In various embodiments, patients are selected for treatment based on inclusion criteria. For example, criteria may include but are not limited to, adult women (≥ 18 years of age) with advanced TNBC; histological or cytological evidence of estrogen-receptor negative (ER-), progesterone receptor negative (PgR-) and human epidermal growth factor-2 receptor negative (HER2-) BC by laboratory testing, based on last available tumor tissue; pre-treatment tumor biopsy demonstrating high TAM content; radiological or objective evidence of recurrence or progression prior to treatment; patients with at least one measurable lesion per RECIST 1.1., including lytic or mixed (lytic + blastic) bone lesions, with an identifiable soft tissue component that meets the measurability criteria) and/or bone lesions (non-measurable lytic or mixed (lytic +blastic) in the absence of measurable disease.

As used herein, "predicting" indicates that the methods described herein provide information to enable a health care provider to determine the likelihood that an individual having breast cancer will respond to or will respond more favorably to treatment with a M-CSF antagonist such as an M-CSF antibody or antigen binding fragment. It does not refer to the ability to predict response with 100% accuracy. Instead, the skilled artisan will understand that it refers to an increased probability.

As used herein, "likelihood" and "likely" is a measurement of how probable an event is to occur. It may be used interchangably with "probability". Likelihood refers to a probability that is more than speculation, but less than certainty. Thus, an event is likely if a reasonable person using common sense, training or experience concludes that, given the circumstances, an event is probable. In some embodiments, once likelihood has been ascertained, the patient may be treated (or treatment continued, or treatment proceed with a dosage increase) with the M-CSF antagonist, e.g., an M-CSF antibody or the patient may not be treated (or treatment discontinued, or treatment proceed with a lowered dose) with the M-CSF antagonist.

The phrase "increased likelihood" refers to an increase in the probability that an event will occur. For example, some methods herein allow prediction of whether a patient will display an increased likelihood of responding to treatment with an M-CSF antagonist, e.g., an M-CSF antibody or an increased likelihood of responding better to treatment with an M-CSF antagonist in comparison to a patient not having a particular level of CD163 expression or having no increase in the level of CD163 expression.

As used herein, the phrase "an increase in the level of CD163" collectively refers to an increase in nucleic acid products e.g., RNA (e.g., pre-mRNA, mRNA, miRNA, etc.) and polypeptide products or fragments thereof.

"TAMs positive" refers to a level of CD163 in a biopsy section that is above a predetermined threshold level which is predictive that the patient will more likely to respond to an M-CSF antagonist as described herein. For example, a TAMs positive patient can refer to a patient having an increase in the density of CD163+ pixels compared to a control. In one embodiment, the predetermined threshold is set to capture around 40-50% of TNBC samples with the highest infiltration of TAMs. In one embodiment, the TAMs positive patient is a patient who has a CD163 pixel density (or TAM density) of greater than 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50% per biopsy section when assayed using quantitative IHC.

The term "specifically binds" in the context of polypeptides is used to mean that a probe binds a given polypeptide target (e.g., CD163) rather than randomly binding undesireable polypeptides. However, "specifically binds" does not exclude some cross reactivity with undesirable polypeptides, as long as that cross reactivity does not interfere with the capability of the probe to provide a a useful measure of the presence of the given polypeptide target.

The term "capable" is used to mean that ability to achieve a given result, e.g., a probe that is capable of detecting the presence of a particular substance means that the probe may be used to detect the particular substance.

An "oliogonucelotide" refers to a short sequence of nucleotides, e.g., 2-100 bases.

Various aspects of the invention are described in further detail in the following sections and subsections.

Brief Description of Figures

Fig. 1 shows a graph showing primary breast cancer samples on 2 tissue microarrays which were independently stained with anti-CD163 antibodies conjugated to DAB, and "TAM density" was determined.

Fig. 2 shows a graph of a predicted population mean serum concentration profiles of free M-CSF antibody H-RX1 following 10 mg/kg Q3W iv infusion with and without a loading dose.

Detailed Description of the Invention

The present invention is directed to novel methods of treatment for patients having breast cancer. In particular the present invention is directed to the use of an M-CSF antagonist such as antibody described herein, for the treatment of breast cancer such as triple negative breast cancer. Currently, there are no targeted therapies for this breast cancer subtype and the only treatment option is chemotherapy. The present invention further provides for the first time personalized therapy that maximizes the benefit and minimizes the risk of use of M-CSF antagonists in breast cancer populations by identifying those patients likely to respond favorably prior to treatment with an M-CSF treatment. The present invention includes identifying patients who have breast cancer, such as triple negative breast cancer patient, that have a level of tumor associated macrophages (TAMs) indicative that the patient is likely to respond to treatment with an M-CSF antagonist. Specifically, the patient's level of TAMs is measured by determining the level of CD163 (e.g., mRNA or protein) in a sample from the patient and the patient's level of CD163 in turn is used to indicate whether that patient is more likely to respond favorably to M-CSF treatment. In one example, if the patient has an increased level of CD163 compared to a control then the patient is identified as a patient more likely to respond to an M-CSF antagonist. In another example, if the patient has an amount of CD163 protein equal or greater than a predetermined CD163 level ("cutoff") then the patient is identified to be a patient more likely to respond to an M-CSF antagonist. The level of CD163 expression assayed in a sample obtained from a breast cancer patient can be, e.g., mRNA expression and/or protein.

Preparation of Samples

Any appropriate sample or test sample of cells taken from an individual having breast cancer can be used. Generally, the test sample of cells or tissue sample will be obtained from the subject with cancer by biopsy or surgical resection. A sample of cells, tissue, or fluid may be

removed by needle aspiration biopsy. For this, a fine needle attached to a syringe is inserted through the skin and into the tissue of interest. The needle is typically guided to the region of interest using ultrasound or computed tomography (CT) imaging. Once the needle is inserted into the tissue, a vacuum is created with the syringe such that cells or fluid may be sucked through the needle and collected in the syringe. A sample of cells or tissue may also be removed by incisional or core biopsy. For this, a cone, a cylinder, or a tiny bit of tissue is removed from the region of interest. CT imaging, ultrasound, or an endoscope is generally used to guide this type of biopsy. More particularly, the entire cancerous lesion may be removed by excisional biopsy or surgical resection. In the present invention, the test sample is typically a sample of cells removed as part of surgical resection.

The test sample of, for example tissue, may also be stored in, e.g., RNAlater (Ambion; Austin Tex.) or flash frozen and stored at -80^{O}C. for later use. The biopsied tissue sample may also be fixed with a fixative, such as formaldehyde, paraformaldehyde, or acetic acid/ethanol. The fixed tissue sample may be embedded in wax (paraffin) or a plastic resin. The embedded tissue sample (or frozen tissue sample) may be cut into thin sections. RNA or protein may also be extracted from a fixed or wax-embedded tissue sample or a frozen tissue sample. Once a sample of cells or sample of tissue is removed from the subject with cancer, it may be processed for the isolation of RNA or protein using techniques well known in the art and as described below.

An example of extraction of RNA from a biopsy taken from a patient with cancers can include, for example, guanidium thiocyanate lysis followed by CsCl centrifugation (Chirgwin, et al., Biochemistry 18:5294-5299, 1979). RNA from single cells may be obtained as described in methods for preparing cDNA libraries from single cells (see, e.g., Dulac, Curr. Top. Dev. Biol. 36:245, 1998; Jena, et al., J. Immunol. Methods 190:199, 1996). In one embodiment, the RNA population may be enriched for CD163. Enrichment may be accomplished, for example, by random hexamers and primer-specific cDNA synthesis, or multiple rounds of linear amplification based on cDNA synthesis and template-directed in vitro transcription (see, e.g., Wang, et al., Proc. Natl. Acad. Sci. USA 86:9717, 1989; Dulac, et al., supra; Jena, et al., supra).

The subject with a tumor or cancer will generally be a mammalian subject such as a primate. In an exemplary embodiment, the subject is a human.

Assaying for TAMs

The presence of TAMs can be assessed by detecting for the presence of CD163.

The methods disclosed herein employ, *inter alia*, determining the level of CD163. The level of CD163 is predictive as to whether that individual is more likely to respond to an M-CSF antagonist. In one example the level of CD163 that is predictive refers to an expression level that is higher than the median level (control) for CD163 expression in breast cancer or greater than a predetermined cut-off value.

In one embodiment, the level of CD163 protein expression is compared against a control, or cut off, for selecting patients for treatment with the M-CSF antagonist, e.g., the level of CD163 expression compared to a control can be predictive that the patient is likely or not likely to respond to M-CSF antagonist such as H-RX1. In one embodiment, patients having a level of expression of CD163 protein expression (also referred to as "TAMs density") above a threshold level are selected for treatment with an M-CSF antagonists. For example, the % TAMS can be determined by quantitative immunohistochemistry by determining the % of CD163 positive pixels in a breast cancer biopsy sample (pixel density = TAM density). In various embodiments, the patient selected for treatment will display level of expression above the threshold level of CD163 expression. In one embodiment, patients having a level of expression of CD163 protein expression ("TAMs density") above a threshold level are selected for treatment with an M-CSF antagonists. TAM density measurements of a biopsy section, for example, may show 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50% or higher CD163+ pixels, where pixel density is equal to TAM density. Such an aforementioned density, in various embodiments, may be deemed TAM positive and the patient will be selected for treatment by the methods described herein. The control level or threshold level of CD163 can be determined essentially contemporaneously with measuring CD163 expression or may have been determined previously.

Detecting CD163 nucleic acid expression

The biological sample from the patient may be assayed for the presence of CD163 expression such as mRNA by any applicable means. Increased levels of CD163 expression may be useful to predict improved response to M-CSF antagonism for patients with breast cancer, e.g., TBNC.

Numerous methods and devices are available to identify the presence of CD163 nucleic acid expression. In some cases, the level of expression of CD163 gene may be determined by

measuring mRNA (or reverse transcribed cDNA) levels using various techniques, e.g., a PCR-based assay, reverse-transcriptase PCR (RT-PCR) assay, Northern blot, etc. Quantitative RT-PCR can also be utilized.

In one example, the method includes determining expression of CD163 by using agents that can be used to specifically detect the gene, for example, RNA transcribed from the gene. The method can include providing a nucleic acid probe comprising a nucleotide sequence, for example, at least 10, 15, 25 or 40 nucleotides, and up to all or nearly all of the coding sequence which is complementary to a portion of the coding sequence of a nucleic acid sequence of CD163; obtaining a tissue sample from the subject having breast cancer; contacting the nucleic acid probe under stringent conditions with RNA obtained from a biopsy taken from a patient with breast cancer (e.g., in a Northern blot, in situ hybridization assay, PCR etc); and determining the amount of hybridization of the probe with RNA. Nucleic acids may be labeled during or after enrichment and/or amplification of RNAs. The biomarker CD163 is intended to also include naturally occurring sequences including allelic variants and other family members. The CD163 biomarker of the invention also include sequences that are complementary to the CD163 sequence resulting from the degeneracy of the code and also sequences that are sufficiently homologous and sequences which hybridize under stringent conditions to CD163.

Conditions for hybridization are known to those skilled in the art and can be found in Current Protocols in Molecular Biology, John Wiley and Sons, N.Y. (1989), 6.3.1-6.3.6. A preferred, non-limiting example of highly stringent hybridization conditions are hybridization in 6 X sodium chloride/sodium citrate (SSC) at about 45 degrees centigrade followed by one or more washes in 0.2 X SSC, 0.1 percent SDS at 50-65 degrees centigrade. By "sufficiently homologous" it is meant an amino acid or nucleotide sequence of a biomarker which contains a sufficient or minimum number of identical or equivalent (e.g., an amino acid residue which has a similar side chain) amino acid residues or nucleotides to a second amino acid or nucleotide sequence such that the first and second amino acid or nucleotide sequences share common structural domains or motifs and/or a common functional activity. For example, amino acid or nucleotide sequences which share common structural domains have at least about 50 percent homology, at least about 60 percent homology, at least about 70 percent, at least about 80 percent, and at least about 90-95 percent homology across the amino acid sequences of the domains are defined herein as sufficiently homologous. Furthermore, amino acid or nucleotide sequences at least about 50 percent homology, at least about 60-70 percent

homology, at least about 70-80 percent, at least about 80-90 percent, and at least about 90-95 percent and share a common functional activity are defined herein as sufficiently homologous.

The comparison of sequences and determination of percent homology between two sequences can be accomplished using a mathematical algorithm. A preferred, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Karlin and Altschul (1990) Proc. Natl. Acad. Sci. USA 87:2264-68, modified as in Karlin and Altschul (1993) Proc. Natl. Acad. Sci. USA 90:5873-77. Such an algorithm is incorporated into the NBLAST and XBLAST programs (version 2.0) of Altschul, et al. (1990) J. MoI. Biol. 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score=100, wordlength=12 to obtain nucleotide sequences homologous to TRL nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score=50, wordlength=3 to obtain amino acid sequences homologous to the CD163 protein sequence. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al., (1997) Nucleic Acids Research 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. See http://www.ncbi.nlm.nih.gov. Another preferred, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the ALIGN algorithm of Myers and Miller, CABIOS (1989). When utilizing the ALIGN program for comparing amino acid sequences, a PAMl 20 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used.

The present invention includes measuring the expression of CD163 in a tumor biopsy taken from a subject suffering from breast cancer. The expression levels can be analyzed and used to generate a score which can be used to differentiate those patients having a tumor that is likely to respond to M-CSF from those that do not. In one example, the level of expression of CD163 is measured and analyzed and used to generate an expression threshold that can be used to select for those individuals who more likely will respond to an M-CSF antagonist. In one embodiment, an individual is selected who has a level of CD163 expression higher than the control, e.g., can be at least 10%, 15%, 20%, 30%, 40% or higher.

It may be necessary to correct for (normalize away) both differences in the amount of RNA assayed and variability in the quality of the RNA used. Therefore, the assay typically

measures and incorporates the expression of certain normalizing genes or housekeeping genes.

The CD163 expression can be measured using any method known in the art such as reverse Transcriptase PCR (RT-PCR). The method includes isolating mRNA using any technique known in the art, e.g., by using a purification kit, buffer set and protease from commercial manufacturers, such as Qiagen. The reverse transcription step is typically primed using specific primers, random hexamers, or oligo-dT primers, depending on the circumstances and the goal of expression profiling and the cDNA derived can then be used as a template in the subsequent PCR reaction. TaqMan(R) RT-PCR can then be performed using , e.g., commercially available equipment.

A more recent variation of the RT-PCR technique is the real time quantitative PCR, which measures PCR product accumulation through a dual-labeled fluorigenic probe (e.g., using TaqMan(R) probe). Real time PCR is compatible both with quantitative competitive PCR, where internal competitor for each target sequence is used for normalization, and with quantitative comparative PCR using a normalization gene contained within the sample, or a housekeeping gene for RT-PCR. For further details see, e.g. Held et al, Genome Research 6:986-994 (1996).

As used herein, the control for comparison can be determined by one skilled in the art. In one aspect, the control is determined by choosing a value that serves as a cut-off value. For example, the value can be a value that differentiates between e.g., those test samples that have increased CD163 expression from those that show minimal or an absence of CD163 expression.

Detecting CD163 protein

In some cases, the presence of CD163 can be determined by analyzing CD163 polypeptide products. Detection of polypeptide products can be performed using any known method in the art including, but not limited, to immunocytochemical staining, ELISA, flow cytometry, Western blot, spectrophotometry, HPLC, and mass spectrometry.

One method for detecting polypeptide products in a sample is by means of a probe that is a binding protein capable of interacting specifically with a marker protein (e.g., an antibody capable of binding CD163 protein). Preferably, labeled antibodies, binding portions thereof, or other binding partners can be used. The antibodies can be monoclonal or polyclonal in

origin, or may be biosynthetically produced. The binding partners may also be naturally occurring molecules or synthetically produced. The amount of complexed proteins is determined using standard protein detection methodologies described in the art. A detailed review of immunological assay design, theory and protocols can be found in numerous texts in the art, including Practical Immunology, Butt, W. R., ed., Marcel Dekker, New York, 1984. A variety of assays are available for detecting proteins with labeled antibodies. Direct labels include fluorescent or luminescent tags, metals, dyes, radionucleides, and the like, attached to the antibody. Indirect labels include various enzymes well known in the art, such as alkaline phosphatase, hydrogen peroxidase and the like. In a one-step assay, polypeptide products, if present, are immobilized and incubated with a labeled antibody. The labeled antibody binds to the immobilized target molecule. After washing to remove unbound molecules, the sample is assayed for the label.

The use of immobilized antibodies specific for the proteins or polypeptides is also contemplated by the present disclosure. The antibodies can be immobilized onto a variety of solid supports, such as magnetic or chromatographic matrix particles, the surface of an assay place (such as microtiter wells), pieces of a solid substrate material (such as plastic, nylon, paper), and the like. An assay strip can be prepared by coating the antibody or a plurality of antibodies in an array on solid support. This strip can then be dipped into the test sample and then processed quickly through washes and detection steps to generate a measurable signal, such as a colored spot.

In a two-step assay, immobilized polypeptide products of CD163 or CD163 protein may be incubated with an unlabeled antibody. The unlabeled antibody complex, if present, is then bound to a second, labeled antibody that is specific for the unlabeled antibody. The sample is washed and assayed for the presence of the label. The choice of marker used to label the antibodies will vary depending upon the application. However, the choice of the marker is readily determinable to one skilled in the art. The antibodies may be labeled with a radioactive atom, an enzyme, a chromophoric or fluorescent moiety, or a colorimetric tag. The choice of tagging label also will depend on the detection limitations desired. Enzyme assays (ELISAs) typically allow detection of a colored product formed by interaction of the enzyme-tagged complex with an enzyme substrate. Some examples of radioactive atoms include ^{32}P, ^{125}I, ^{3}H, and ^{14}P. Some examples of enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, and glucose-6-phosphate dehydrogenase. Some examples of chromophoric moieties include fluorescein and rhodamine. The antibodies may be conjugated

to these labels by methods known in the art. For example, enzymes and chromophoric molecules may be conjugated to the antibodies by means of coupling agents, such as dialdehydes, carbodiimides, dimaleimides, and the like. Alternatively, conjugation may occur through a ligand-receptor pair. Some suitable ligand-receptor pairs include, for example, biotin-avidin or -streptavidin, and antibody-antigen.

In one aspect, the present disclosure contemplates the use of a sandwich technique for detecting polypeptide products in biological samples. The technique requires two antibodies capable of binding the protein of interest: e.g., one immobilized onto a solid support and one free in solution, but labeled with some easily detectable chemical compound. Examples of chemical labels that may be used for the second antibody include but are not limited to radioisotopes, fluorescent compounds, and enzymes or other molecules which generate colored or electrochemically active products when exposed to a reactant or enzyme substrate. When samples containing polypeptide products are placed in this system, the polypeptide products binds to both the immobilized antibody and the labeled antibody. The result is a "sandwich" immune complex on the support's surface. The complexed protein is detected by washing away nonbound sample components and excess labeled antibody, and measuring the amount of labeled antibody complexed to protein on the support's surface. The sandwich immunoassay is highly specific and very sensitive, provided that labels with good limits of detection are used.

Western blot analysis is well known to the skilled artisan (Sambrook et al., Molecular Cloning, A Laboratory Manual, 1989, Vol. 3, Chapter 18, Cold Spring Harbor Laboratory). In Western blot, the sample is separated by SDS-PAGE. The gel is transferred to a membrane. The membrane is incubated with labeled antibody for detection of the desired protein.

In another embodiment, IHC is used to detect and quantify the CD163 protein in a tumor biopsy as a measure of TAM density in the tumor. In one example, immunostaining for CD163 can be performed on tissue sections of formalin-fixed paraffin-embedded (FFPE) tumor material using a mouse monoclonal anti-human CD163 antibody (clone MRQ-26; Ventana Medical Systems, Tuscon, AZ, USA) on an automated Ventana immunohistochemistry staining instrument. The tumor morphology and its relationship to CD163 staining, which is specific for TAMs, can then be evaluated by a pathologist on digitally scanned slides to determine and define relevant regions of interest (ROI) within the tumor. The area of CD163 staining in the ROI can then be quantified by an automated image analysis algorithm and platform (for review see ref. Hamilton et al. 2014, Methods 70 (2014)

59–73, Mulrane et al. 2008, Expert Rev Mol Diagn, 2008 8(6) 707-725) to determine the percentage of tumor area occupied by TAMs, a measure of "TAM density" in the tumor.

In the methods of the invention described herein, a patient is selected for treatment based on the level of expression of CD163. In one example, the patient is selected when the patient has a particular level of protein expression compared to appropriate control samples.

Control

As used herein, the controls for comparison can be determined by one skilled in the art. In one example, the controls are determined by choosing control samples with TAM density values that define a cut-off value which differentiates between patients who are more likely to respond to an M-CSF antagonist and those that are less likely to respond. In one embodiment, the threshold value is determined to identify TNBC patients who have the highest infiltration of TAMs (this is expected to represent around 40% of TNBC patients). Test samples where the individual has a CD163 level (or TAM density) of equal or greater than 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50 % CD163 pixel density as determined by quantitative IHC are predicted to be more likely to respond to the M-CSF antagonist. In certain embodiments, a cut-off value or threshold value, e.g.,of 8% CD163 pixel density or below as determined by quantitative IHC, is predictive of patients less likely to respond to treatment and are thus not selected for treatment. In another example, the control can be a sample from a healthy volunteer or a sample from a breast cancer patient that is known to have a low CD163 expression (mRNA or protein), and a patient is selected for treatment when the CD163 value determined is greater than the CD163 in these controls.

In yet another example, the control value can be a value predetermined according to historical controls as a basis of a mathematical model. The model, also called a classifier, can be built by using the expression levels (e.g., mRNA or protein) from a collection of breast cancer patients (e.g., TBNCs). The mathematical model, can be, for example, any class prediction method or its variations and the derived control value can be used as the threshold. If the level of expression is at or above the threshold, the patient is more likely to respond to treatment with an M-CSF antagonist. In one embodiment, an individual is selected who has a level of CD163 expression equal or greater than the threshold, e.g., can be 8%, 10%, 15%, 20%, 30%, 40% or higher.

Data analysis

In performing any of the methods described herein that require determining the presence of CD163 expression, e.g., CD163 protein, the information regarding the presence of CD163 can be stored. Typically, once the levels of CD163 expression or level of CD163 protein is determined, physicians or genetic counselors or patients or other researchers may be informed of the result. Specifically the result can be cast in a transmittable form of information that can be communicated or transmitted to other researchers or physicians or genetic counselors or patients. Such a form can vary and can be tangible or intangible. The result in the individual tested can be embodied in descriptive statements, diagrams, photographs, charts, images or any other visual forms. For example, statements regarding levels of CD163 nucleic acid expression or levels of CD163 protein are useful in indicating the testing results. These statements and visual forms can be recorded on a tangible media such as papers, computer readable media such as floppy disks, compact disks, etc., or on an intangible media, e.g., an electronic media in the form of email or website on internet or intranet. In addition, the result can also be recorded in a sound form and transmitted through any suitable media, e.g., analog or digital cable lines, fiber optic cables, etc., via telephone, facsimile, wireless mobile phone, internet phone and the like. All such forms (tangible and intangible) would constitute a "transmittable form of information". Thus, the information and data on a test result can be produced anywhere in the world and transmitted to a different location. For example, when the assay is conducted offshore, the information and data on a test result may be generated and cast in a transmittable form as described above. The test result in a transmittable form thus can be imported into the U.S. Accordingly, the present disclosure also encompasses a method for producing a transmittable form of information containing levels of CD163 nucleic acid expression or levels of CD163 protein. This form of information is useful for predicting the responsiveness of a patient having breast cancer to treatment with a M-CSF antagonist alone or in combination with another agent such as a chemotherapeutic agent, for selecting a course of treatment based upon that information, and for selectively treating a patient based upon that information.

Selection and Treatment of patients with breast cancer

The level of CD163 nucleic acid expression or CD163 protein allows clinicians to provide a personalized therapy for breast cancer patients such as TBNCs, i.e., they allow determination of whether to selectively treat the patient with an M-CSF antagonist. In this way, a clinician can maximize the benefit and minimize the risk of M-CSF antagonism in the entire population of patients afflicted with breast cancer.

Macrophage colony stimulating factor (M-CSF) also known as Colony stimulating factor (CSF-1))

The full-length human M-CSF mRNA encodes a precursor protein of 554 amino acids. Through alternative mRNA splicing and differential post-translational proteolytic processing, M-CSF can either be secreted into the circulation as a glycoprotein or chondroitin sulfate containing proteoglycan or be expressed as a membrane spanning glycoprotein on the surface of M-CSF producing cells. The three-dimensional structure of the bacterially expressed amino terminal 150 amino acids of human M-CSF, the minimal sequence required for full in vitro biological activity, indicates that this protein is a disulfide linked dimer with each monomer consisting of four alpha helical bundles and an anti-parallel beta sheet (Pandit et al., Science 258: 1358-62 (1992)). Three distinct M-CSF species are produced through alternative mRNA splicing. The three polypeptide precursors are M-CFSα of 256 amino acids, M-CSFβ of 554 amino acids, and M-CSFγ of 438 amino acids. M-CSFβ is a secreted protein that does not occur in a membrane-bound form. M-CSFα is expressed as an integral membrane protein that is slowly released by proteolytic cleavage. M-CSFα is cleaved at amino acids 191-197 of the sequence set out in SEQ ID NO 11. The membrane-bound form of M-CSF can interact with receptors on nearby cells and therefore mediates specific cell-to-cell contacts. The term "M-CSF" may also inlcude amino acids 36-438 of SEQ ID NO: 13.

Various forms of M-CSF function by binding to its receptor M-CSFR on target cells. M-CSFR is a membrane spanning molecule with five extracellular immunoglobulin-like domains, a transmembrane domain and an intracellular interrupted Src related tyrosine kinase domain. M-CSFR is encoded by the c-fms proto-oncogene. Binding of M-CSF to the extracellular domain of M-CSFR leads to dimerization of the receptor, which activates the cytoplasmic kinase domain, leading to autophosphorylation and phosphorylation of other cellular proteins (Hamilton J. A., J Leukoc Biol.,62(2):145-55 (1997); Hamilton J, A., Immuno Today., 18(7): 313-7(1997).

SEQ ID NO: 14 is the amino acid sequence of M-CSFα. SEQ ID NO: 15 is the amino acid sequence of M-CSFβ. SEQ ID NO: 16 is the amino acid sequence of M-CSFγ. A number of polymorphisms in the DNA sequence may result in amino acid differences. For example, a common polymorphism provides an Ala rather than Pro at position 104.

M-CSFα	SEQ ID NO: 14	M T A P G A A G R C P P T T W LG S L L L L V C L L A S R S I T E E V S E Y C S H M I G S G H LQ S L Q R L I D S Q M E T S C Q I T F E F V D Q E Q L K D P V C Y L K K A F

		L L V Q D I M E D T M R F R D N T P N A I A I V Q L Q E L S L R L K S C F T K D Y E E H D K A C V R T F Y E T P L Q L L E K V K N V F N E T K N L L D K D W N I F S K N C N N S F A E C S S Q G H E R Q S E G S S S P Q L Q E S V F H L L V P S V I L V L L A V G G L L F Y R W R R R S H Q E P Q R A D S P L E Q P E G S P L T Q D D R Q V E L P V
M-CSFβ	SEQ ID NO: 15	M T A P G A A G R C P P T T W L G S L L L L V C L L A S R S I T E E V S E Y C S H M I G S G H L Q S L Q R L I D S Q M E T S C Q I T F E F V D Q E Q L K D P V C Y L K K A F L L V Q D I M E D T M R F R D N T P N A I A I V Q L Q E L S L R L K S C F T K D Y E E H D K A C V R T F Y E T P L Q L L E K V K N V F N E T K N L L D K D W N I F S K N C N N S F A E C S S Q D V V T K P D C N C L Y P K A I P S S D P A S V S P H Q P L A P S M A P V A G L T W E D S E G T E G S S L L P G E Q P L H T V D P G S A K Q R P P R S T C Q S F E P P E T P V V K D S T I G G S P Q P R P S V G A F N P G M E D I L D S A M G T N W V P E E A S G E A S E I P V P Q G T E L S P S R P G G G S M Q T E P A R P S N F L S A S S P L P A S A K G Q Q P A D V T G T A L P R V G P V R P T G Q D W N H T P Q K T D H P S A L L R D P P E P G S P R I S S L R P Q G L S N P S T L S A Q P Q L S R S H S S G S V L P L G E L E G R R S T R D R R S P A E P E G G P A S E G A A R P L P R F N S V P L T D T G H E R Q S E G S S S P Q L Q E S V F H L L V P S V I L V L L A V G G L L F Y R W R R R S H Q E P Q R A D S P L E Q P E G S P L T Q D D R Q V E L P V
M-CSFγ	SEQ ID NO: 16	M T A P G A A G R C P P T T W L G S L L L L V C L L A S R S I T E E V S E Y C S H M I G S G H L Q S L Q R L I D S Q M E T S C Q I T F E F V D Q E Q L K D P V C Y L K K A F L L V Q D I M E D T M R F R D N T P N A I A I V Q L Q E L S L R L K S C F T K D Y E E H D K A C V R T F Y E T P L Q L L E K V K N V F N E T K N L L D K D W N I F S K N C N N S F A E C S S Q D V V T K P D C N C L Y P K A I P S S D P A S V S P H Q P L A P S M A P V A G L T W E D S E G T E G S S L L P G E Q P L H T V D P G S A K Q R P P R S T C Q S F E P P E T P V V K D S T I G G S P Q P R P S V G A F N P G M E D I L D S A M G T N W V P E E A S G E A S E I P V P Q G T E L S P S R P G G G S M Q T E P A R P S N F L S A S S P L P A S A K G Q Q P A D V T G H E R Q S E G S S S P Q L Q E S V F H L L V P S V I L V L L A V G G L L F Y R W R R R S H Q E P Q R A D S P L E Q P E G S P L T Q D D R Q V E L P V

Examples of M-CSF antagonists

M-CSF antagonists are known in the art. The M-CSF antagonist can be a small molecule, an antibody or other antigen-binding protein, a small molecule, a nucleic acid (such as an siRNA), or any other such molecule which interferes with M-CSF activation or function.

In one example, the M-CSF antagonist useful for treatment in the presently disclosed method includes an antibody or fragment thereof.

M-CSF antibodies that can be useful in the present invention include those M-CSF antibodies disclosed in Int'I Publication No. WO 2005/068503, which is hereby incorporated by reference in its entirety for its teaching with respect to M-CSF antibodies. WO 2005/068503 discloses, for example, antibodies that bind the same epitopes as antibodies RX1, 5H4, MC1, and/or MC3, pharmaceutical formulations including an M-CSF-specific antibody Human Engineered™ versions of the aforementioned antibodies, and methods of preparing the pharmaceutical formulations. The term "antibody" is used in the broadest sense and includes fully assembled antibodies, monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), antibody fragments that can bind antigen (e.g., Fab', F'(ab)2, Fv, single chain antibodies, diabodies), and recombinant peptides comprising the forgoing as long as they exhibit the desired biological activity.

Other M-CSF antibodies that can be useful in the present invention include those M-CSF antibodies disclosed in Int'I Publication No. WO 2003/028752, US2009117103 and US2005059113, each of which is hereby incorporated by reference in its entirety for its teaching with respect to M-CSF antibodies.

In one embodiment in the methods of the invention, the antibody is the Human Engineered™ RX1 antibody or an antigen binding fragment thereof. Below is a table showing the heavy and light variable region and CDRs of RX1 and its CDRs.

In one embodiment, the antibody or fragment thereof useful in the methods of the invention include an antibody or fragment thereof that binds to a linear epitope represented by RFRDNTPN (SEQ ID NO: 11) or RFRDNTAN (SEQ ID NO: 12). Such an antibody is the RX1 antibody. In another embodiment, the antibody can be an antibody or fragment thereof that binds to a linear epitope represented by ITFEFVDQE (SEQ ID NO:13). Such an

antibody is the 5H4 antibody.

In another embodiment useful in the methods of the invention, the antibody is the human engineered RX1 antibody ("H-RX1" as used herein) or an antigen binding fragment thereof. Below is a table showing the heavy and light variable region and CDRs of H-RX1.

(H-RX1) HC coding region	atgggttggtcttgcatcattctctttctcgtcgctaccgcaactggtgtacactcccaagttcaacttcaagaatca ggccccggactcgttaaaccctctcaaactctctctcttacttgcactgtatccgattactctattacttcagactac gcttggaactggatcagacaatttcccggaaaaggactcgaatggatgggatatatctcttactctggctcaacc tcttacaaccctctctcaaatctcgaataacaatctcacgcgatacttctaaaaatcaattctcacttcaacttaact ccgttactgccgccgacactgccgtttactactgtgcttccttcgattacgcccacgctatggattattggggaca aggaactaccgtcactgtcagctcagccagcacaaagggcccatcggtcttcccctggcaccctcctccaag agcacctctgggggcacagcggccctgggctgcctggtcaaggactacttccccgaaccggtgacggtgtcg tggaactcaggcgccctgaccagcggcgtgcacaccttcccggctgtcctacagtcctcaggactctactccct cagcagcgtggtgaccgtgccctccagcagcttgggcacccagacctacatctgcaacgtgaatcacaagcc cagcaacaccaaggtggacaagagagttgagcccaaatcttgtgacaaaactcacacatgtccaccgtgccc agcacctgaactcctgggggggaccgtcagtcttcctcttcccccaaaacccaaggacaccctcatgatctccc ggacccctgaggtcacatgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacg tggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacgtaccgtgt ggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctccaacaa agccctcccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtaca ccctgcccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatc ccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgt gctggactccgacggctccttcttcctctatagcaagctcaccgtggacaagagcaggtggcagcaggggaa cgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtccccgg gtaaatga (SEQ ID NO:1)
(H-RX1) HC derived protein *(incl. leader peptide)*	*MGWSCIILFLVATATGVHS*QVQLQESGPGLVKPSQTLSLTCTVSDYSITS DYAWNWIRQFPGKGLEWMGYISYSGSTSYNPSLKSRITISRDTSKNQF SLQLNSVTAADTAVYYCASFDYAHAMDYWGQGTTVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA VLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSC DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ

	VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 2)
(H-RX1) Light chain (LC) coding region	Atggtctctactccccaattcctcgtctttctgctcttttggatccctgcttctcgcggcgacatagttctcacacaat caccagcattcctctcagttacacccggcgaaaaagtaacctttacctgtcaggcttctcaatctatcggcacttct attcactggtatcaacaaaaaaccgatcaagctcctaaactcctcataaaatacgcatccgaatccatctccggta tcccctccagattttcaggctccggctccggcacagatttcacccttaccattagctcagttgaagccgaagacg cagctgattactactgtcaacaaataaactcatggcccactactttcggcggcggcactaaactcgaaataaaac gtacggtggctgcaccatctgtcttcatcttcccgccatctgatgagcagttgaaatctggaactgcctctgttgtg tgcctgctgaataacttctatcccagagaggccaaagtacagtggaaggtggataacgccctccaatcgggta actcccaggagagtgtcacagagcaggacagcaaggacagcacctacagcctcagcagcaccctgacgct gagcaaagcagactacgagaaacacaaagtctacgcctgcgaagtcacccatcagggcctgagctcgcccg tcacaaagagcttcaacaggggagagtgttag (SEQ ID NO:3)
(H-RX1) LC derived protein *(including leader peptide)*	*MVSTPQFLVFLLFWIPASRG*DIVLTQSPAFLSVTPGEKVTFTCQASQSIG TSIHWYQQKTDQAPKLLIKYASESISGIPSRFSGSGSGTDFTLTISSVEA EDAADYYCQQINSWPTTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGT ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLS STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 4)
Heavy Chain CDR1	SDYAWN (SEQ ID NO: 5)
Heavy Chain CDR2	YISYSGSTSYNPSLKS (SEQ ID NO: 6)
Heavy Chain CDR3	FDYAHAMDY (SEQ ID NO: 7)
Light Chain CDR1	QASQSIGTSIH (SEQ ID NO: 8)
Light Chain CDR2	YASESIS (SEQ ID NO: 9)
Light Chain CDR3	QQINSWPTT (SEQ ID NO: 10)

In one embodiment, the antibody is a humanized antibody or human engineered antibody or fragment thereof comprising the heavy chain variable region sequence set forth in SEQ ID NO: 1 and a light chain variable region sequence set forth in SEQ ID NO: 3.

In yet another embodiment, the antibody or fragment thereof comprises a heavy chain variable region that comprises CDR1, CDR2, and CDR3 domains; and a light chain variable region that comprises CDR1, CDR2, and CDR3 domains, wherein the heavy chain variable region and light chain variable region CDR3 comprises the amino acids having the sequence set forth in SEQ ID NO:7; and a light chain variable region CDR3 comprises amino acids having the sequence set forth in SEQ ID NO:10; and wherein the antibody or antigen-binding portion thereof binds to human M-CSF with a binding affinity of about 10^{-7} M. The antibody or fragment thereof can further include a heavy chain variable region CDR2 comprising amino acids having the sequence set forth in SEQ ID No:6; and a light chain variable region CDR2 comprising amino acids having the sequence set forth in SEQ ID NO:9. The antibody or fragment thereof can further include a heavy chain variable region CDR1 comprising amino acids having the sequence set forth in SEQ ID NO:5; and a light chain variable region CDR1 comprising amino acids having the sequence set forth in SEQ ID NO:8.

In yet another example, the humanized antibody or human engineered antibody or fragment thereof useful in the methods of the invention binds to human M-CSF, wherein said antibody binds an epitope of M-CSF that comprises at least 4 contiguous residues of RFRDNTPN (SEQ ID NO: 11) or RFRDNTAN (SEQ ID NO: 12), wherein said antibody has an affinity Kd (dissociation equilibrium constant) with respect to human M-CSF of at least 10^{-7} M, wherein said antibody comprises all three heavy chain CDRs of SEQ ID NO: 5, SEQ ID NO:6 and SEQ ID NO: 7 and all three light chain CDRs of SEQ ID NO: 8, SEQ ID NO: 9 and SEQ ID NO: 10.

The antibodies disclosed herein can be derivatives of single chain antibodies, diabodies, domain antibodies, nanobodies, and unibodies. For example, the invention provides an isolated monoclonal antibody (or a functional fragment thereof) comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises an amino acid sequence that is at least 80%, at least 90%, or at least 95% identical to an amino acid sequence of SEQ ID NOs: 2; the light chain variable region comprises an amino acid sequence that is at least 80%, at least 90%, or at least 95% identical to an amino acid sequence of SEQ ID NO:4; the antibody binds to M-CSF (*e.g.*, human and/or cynomologus M-CSF) and neutralizes the signaling activity of M-CSF . Also included

within the scope of the invention are variable heavy and light chain parental nucleotide sequences; and full length heavy and light chain sequences optimized for expression in a mammalian cell. Other antibodies of the invention include amino acids or nucleic acids that have been mutated, yet have at least 60, 70, 80, 90, 95, or 98% percent identity to the sequences described above. In some embodiments, it include mutant amino acid sequences wherein no more than 1, 2, 3, 4 or 5 amino acids have been mutated by amino acid deletion, insertion or substitution in the variable regions when compared with the variable regions depicted in the sequence described above.

In other embodiments, the variable heavy chain (VH) and/or variable light chain (VL) amino acid sequences may be 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98% or 99% identical to the sequences set forth in Table 1 above. In other embodiments, the VH and/or VL amino acid sequences may be identical except an amino acid substitution in no more than 1,2,3,4 or 5 amino acid position. An antibody having VH and VL regions having high (*i. e.*, 80% or greater) identity to the VH and VL regions of the antibodies described in Table 1 can be obtained by mutagenesis (*e.g.*, site-directed or PCR-mediated mutagenesis), followed by testing of the encoded altered antibody for retained function using the functional assays described herein.

In other embodiments, the variable regions of heavy chain and/or light chain nucleotide sequences may be 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98% or 99% identical to the sequences set forth above.

In certain embodiments, an antibody of the invention has a heavy chain variable region comprising CDR1, CDR2, and CDR3 sequences and a light chain variable region comprising CDR1, CDR2, and CDR3 sequences, wherein one or more of these CDR sequences have specified amino acid sequences based on the antibodies described herein or conservative modifications thereof, and wherein the antibodies retain the desired functional properties of the M-CSF-binding antibodies described in Table 1.

Accordingly, the invention provides an isolated M-CSF monoclonal antibody, or a fragment thereof, consisting of a heavy chain variable region comprising CDR1, CDR2, and CDR3 sequences and a light chain variable region comprising CDR1, CDR2, and CDR3 sequences, wherein: the heavy chain variable region CDR1 amino acid sequences are selected from the group consisting of SEQ ID NOs: 5, and conservative modifications thereof; the heavy chain variable region CDR2 amino acid sequences are selected from the group consisting of SEQ ID

NOs: 6 and conservative modifications thereof; the heavy chain variable region CDR3 amino acid sequences are selected from the group consisting of SEQ ID NOs: 7 and conservative modifications thereof; the light chain variable regions CDR1 amino acid sequences are selected from the group consisting of SEQ ID NOs: 8 and conservative modifications thereof; the light chain variable regions CDR2 amino acid sequences are selected from the group consisting of SEQ ID NOs: 9, and conservative modifications thereof; the light chain variable regions of CDR3 amino acid sequences are selected from the group consisting of SEQ ID NOs: 10, and conservative modifications thereof; the antibody or fragment thereof specifically binds to M-CSF, and neutralizes M-CSF activity.

The antibodies used in the invention can be a fragment of an antibody that binds to M-CSF selected from the group consisting of; Fab, $F(ab_2)'$, $F(ab)_2'$, scFv, VHH, VH, VL, dAbs.

The present invention also includes antibodies that interacts with (e.g., by binding, steric hindrance, stabilizing/destabilizing, spatial distribution) the same epitope as do the M-CSF - binding antibodies described above.

The antibodies of the invention can encompass various antibody isotypes, or mixtures thereof, such as IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgAsec, IgD, and IgE. Typically, they include IgG1 (e.g., IgG1k) and IgM isotypes. The human sequence antibodies can be full-length (e.g., an IgG1 or IgG4 antibody) or can include only an antigen-binding portion (e.g., a Fab, F(ab').sub.2, Fv or a single chain Fv fragment).

In one embodiment, the invention includes a humanized antibody or fragment thereof comprising a heavy chain comprising a heavy chain variable region sequence set for in SEQ ID NO: 4 and an IgG1 constant region and the light chain comprising a light chain variable region sequence set forth in SEQ ID NO: 2. The light chain can further comprise a human kappa constant region. In preferred embodiments, the invention provides Human Engineered™ antibodies or variants comprising a modified or unmodified IgG1 or IgG4 constant region. In the case of IgG1, modifications to the constant region, particularly the hinge or CH2 region, may increase or decrease effector function, including ADCC and/or CDC activity. In other embodiments, an IgG2 constant region is modified to decrease antibody-antigen aggregate formation. In the case of IgG4, modifications to the constant region, particularly the hinge region, may reduce the formation of half-antibodies. In specific exemplary embodiments, mutating the IgG4 hinge sequence Cys-Pro-Ser-Cys (SEQ ID NO: 17) to the IgG1 hinge sequence Cys-Pro-Pro-Cys (SEQ ID NO: 18) is provided. Human

Engineered™ antibodies containing IgG1 or IgG4 constant regions have been shown to have improved properties compared to Human Engineered™ antibodies containing IgG2 constant regions (WO 2005/068503). Choice of the IgG1 or IgG4 Fc region improved binding affinity, M-CSF neutralization activity, and anti-osteoclast activity. In addition, choice of the IgG1 or IgG4 Fc region provided antigen-antibody complexes that more closely resembled those formed by the parent murine antibody. The mobility at the hinge region thus appears to markedly affect binding of antibody to the dimeric antigen MCSF as well as neutralization activity of the antibody. The invention contemplates generally that preparation of antibodies containing a heavy chain comprising a modified or unmodified IgG1 or IgG4 constant region, particularly the hinge and CH2 domains, or preferably at least the hinge domains, improves binding affinity and/or slows dissociation of antibody from dimeric antigens.

Additional antibody sequences contemplated by the instant disclosure are provided in WO 2005/068503, as described herein. Particular sequences disclosed in WO 2005/068503and contemplated for use in the compositions and methods described herein are as follows.

Additional antibody sequences contemplated by the instant disclosure are provided in WO 2005/068503, as described herein. Particular sequences disclosed in WO 2005/068503 and contemplated for use in the compositions and methods described herein are as follows:

Sequence identifier in WO 2005/068503	Brief description in WO 2005/068503
1	Murine antibody RX1 nucleic acid
2	Murine antibody RX1 amino acid
3	Murine antibody RX1 nucleic acid
4	Murine antibody RX1 amino acid
5	Murine antibody RX1 light chain
6	Murine antibody RX1 heavy chain
7	M-CSFα
8	M-CSFβ
9	M-CSFγ
10	Murine antibody 5H4 amino acid
11	Murine antibody 5H4 amino acid
12	Murine antibody MC1 amino acid
13	Murine antibody MC1 amino acid
14	Murine antibody MC3 amino acid
15	Murine antibody MC3 amino acid
16	Murine antibody CDR
17	Murine antibody CDR
18	Murine antibody CDR
19	Murine antibody CDR
20	Murine antibody CDR
21	Murine antibody CDR

22	Murine antibody CDR
23	Murine antibody CDR
24	Murine antibody CDR
25	Murine antibody CDR
26	Murine antibody CDR
27	Murine antibody CDR
28	Murine antibody CDR
29	Murine antibody CDR
30	Murine antibody CDR
31	Murine antibody CDR
32	Murine antibody CDR
33	Murine antibody CDR
34	Murine antibody CDR
35	Murine antibody CDR
36	Murine antibody CDR
37	Murine antibody CDR
38	Murine antibody CDR
39	Human consensus sequence
40	RX-1-based antibody heavy chain – low risk
41	RX-1-based antibody heavy chain – low risk
42	RX-1-based antibody heavy chain – low + moderate risk
43	RX-1-based antibody heavy chain – low + moderate risk
44	RX-1-based antibody light chain – low risk
45	RX-1-based antibody light chain – low risk
46	RX-1-based antibody light chain – low + moderate risk
47	RX-1-based antibody light chain – low + moderate risk
48	RX-1-based antibody light chain - alternate
49	Human consensus sequence
50	Human consensus germline sequence
51	RX-1-based antibody light chain – low risk
52	RX-1-based antibody light chain – low + moderate risk
53	RX-1-based antibody light chain – low + moderate risk
54	Murine RX1 light chain
55	Human consensus / human germline consensus sequence
56	Human consensus / human germline consensus sequence
57	Human consensus / human germline consensus sequence
58	Human consensus / human germline consensus sequence
59	Human consensus / human germline consensus sequence
60	Human consensus / human germline consensus sequence
61	Human consensus / human germline consensus sequence
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64	Human consensus / human germline consensus sequence
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78	Human consensus / human germline consensus sequence
79	Human consensus / human germline consensus sequence
80	Human consensus / human germline consensus sequence
81	Human consensus / human germline consensus sequence
82	Human consensus / human germline consensus sequence
83	Murine RX1 heavy chain
84	Human consensus / human germline consensus sequence
85	Human consensus / human germline consensus sequence
86	Human consensus / human germline consensus sequence
87	Human consensus / human germline consensus sequence
88	Human consensus / human germline consensus sequence
89	Human consensus / human germline consensus sequence
90	Human consensus / human germline consensus sequence
91	Human consensus / human germline consensus sequence
92	Human consensus / human germline consensus sequence
93	Human consensus / human germline consensus sequence
94	Human consensus / human germline consensus sequence
95	Human consensus / human germline consensus sequence
96	Human consensus / human germline consensus sequence
97	Human consensus / human germline consensus sequence
98	Human consensus / human germline consensus sequence
99	Human consensus / human germline consensus sequence
100	Human consensus / human germline consensus sequence
101	Human consensus / human germline consensus sequence
102	Human consensus / human germline consensus sequence
103	Human consensus / human germline consensus sequence
104	Human consensus / human germline consensus sequence
105	Human consensus / human germline consensus sequence
106	Human consensus / human germline consensus sequence
107	Human consensus / human germline consensus sequence
108	Human consensus / human germline consensus sequence
109	Human consensus / human germline consensus sequence
110	Human consensus / human germline consensus sequence
111	Human consensus / human germline consensus sequence
112	Human consensus / human germline consensus sequence
113	heRX1-1.IgG1 – low risk
114	heRX1-1.IgG1 – low risk
115	heRX1-1.IgG1 – low + moderate risk
116	heRX1-1.IgG1 – low + moderate risk
117	heRX1-1.IgG4 – low risk
118	heRX1-1.IgG4 – low risk
119	heRX1-1.IgG4 – low risk

120	M-CSF epitope
121	M-CSF epitope
122	M-CSF epitope
123	M-CSF epitope
124	RX-1-based antibody heavy chain
125	RX-1-based antibody heavy chain
126	RX-1-based antibody heavy chain
127	RX-1-based antibody heavy chain
128	RX-1-based antibody light chain
129	RX-1-based antibody light chain
130	RX-1-based antibody light chain
131	RX-1-based antibody light chain
132	RX-1-based antibody light chain
133	M-CSF antibody heavy chain
134	M-CSF antibody light chain
135	RX-1-based antibody light chain - alternate
136	RX-1-based antibody light chain - alternate
137	RX-1-based antibody light chain - alternate

Antibody Combinations

In an another aspect, the invention pertains to M-CSF antagonists, e.g., antibodies, or fragments thereof, in combination with other therapeutic agents which are used in treating cancer. In particular an M-CSF antagonist can be combined a cancer chemotherapeutic agent or with radiation treatment or surgery. Cancer chemotherapeutic agents include, without limitation, alkylating agents, such as carboplatin and cisplatin; nitrogen mustard alkylating agents; nitrosourea alkylating agents, such as carmustine (BCNU); antimetabolites, such as methotrexate; purine analog antimetabolites, mercaptopurine; pyrimidine analog antimetabolites, such as fluorouracil (5-FU) and gemcitabine; hormonal antineoplastics, such as goserelin, leuprolide, and tamoxifen; natural antineoplastics, such as aldesleukin, interleukin-2, docetaxel, etoposide (VP-16), interferon alfa, paclitaxel, and tretinoin (ATRA); antibiotic natural antineoplastics, such as bleomycin, dactinomycin, daunorubicin, doxorubicin, and mitomycin; and vinca alkaloid natural antineoplastics, such as vinblastine, vincristine, vindesine; hydroxyurea; aceglatone, adriamycin, ifosfamide, enocitabine, epitiostanol, aclarubicin, ancitabine, nimustine, procarbazine hydrochloride, carboquone, carboplatin, carmofur, chromomycin A3, antitumor polysaccharides, antitumor platelet factors, cyclophosphamide, Schizophyllan, cytarabine, dacarbazine, thioinosine, thiotepa, tegafur, neocarzinostatin, OK-432, bleomycin, furtulon, broxuridine, busulfan, honvan, peplomycin, Bestatin (ubenimex), interferon-.beta., mepitiostane, mitobronitol, merphalan,

laminin peptides, lentinan, Coriolus versicolor extract, tegafur/uracil, estramustine (estrogen/mechlorethamine).

In one example, the M-CSF antagonist, e.g., M-CSF antibody such as H-RX1 shown in Table 1 above, is administered with the chemotherapeutic agent carboplatin. In another example, the M-CSF antagonist is administered with the chemotherapeutic agent gemcitabine. In yet another embodiment, the M-CSF antagonist, e.g., M-CSF antibody such as H-RX1 shown in Table 1 above, is administered with the chemotherapeutic agent carboplatin and gemcitabine.

Carboplatin is an alkylating agent which acts by producing cross-linking guanine bases in DNA double-helix strands, thereby preventing transcription and replication. The mechanism of action is not cell-cycle specific (McEvoy, GK, ed., American Hospital Formulary Service Drug Information, American Society of Health System (2000)). The major route of elimination of carboplatin is renal excretion. Patients with normal renal function excrete 71% of the dose within 24 hours. Drug interactions with aminoglycosides, phenytoin and warfarin have been reported and concomitant use is cautioned (McEvoy 2000). The main toxicities of carboplatin are myelosuppression, nausea/vomiting, nephrotoxicity and peripheral neuropathy. Hypersensitivity reactions have been reported, particularly when used in combination or after repeated exposure (McEvoy 2000, Markman, M., et al., J. Clin. Oncol., 17(4):1141 (1999)). Carboplatin is approved for use alone or combined with other drugs in ovarian cancer and non-small lung cancer, but widely used also in other malignancies such as head neck cancer, germ cell tumors, breast cancer and others. Numerous dosing schedules exist and depend on disease, response expectations and concomitant therapy (NCCN 2014, ESMO Guidelines 2014).

Gemcitabine is a pyrimidine analog which is metabolized to two active metabolites, gemcitabine diphosphate and gemcitabine triphosphate. The cytotoxic effects of gemcitabine are exerted through incorporation of gemcitabine triphosphate into DNA, resulting in inhibition of DNA synthesis and induction of apoptosis. Gemcitabine is cell-cycle phase specific (S and G1 /S-phases) (Grindey, G., et al., Cancer Invest., 8(2):313 (1990)). Gemcitabine is rapidly metabolized by cytidine deaminases in liver, kidney, blood and other tissue (Gilbert, J., et a., Clin. Cancer. Res., 12:1794-1803 (2006)). Drug elimination is mediated mainly through renal excretion. Within one week of administration 92% to 98% of the dose was recovered, almost entirely in the urine. The main toxicities of gemcitabine are

elevated liver enzymes [aspartate transaminase (ASAT)/ aspartate aminotranferase (ALAT)] and alkaline phospatase (ALK), nausea/vomiting, protein/hematuria, dyspnea, rash and myelosuppression. Gemcitabine is approved for use alone or with other drugs in pancreatic cancer, ovarian cancer, non-small lung cancer and breast cancer. The most commonly used schedule for gemcitabine is administration on days 1 and 8 of each 21-day cycle, but there are several options (NCCN 2014, ESMO Guidelines 2014).

Pharmaceutical Compositions and Administration

To prepare pharmaceutical or sterile compositions including an M-CSF antagonists mixed with a pharmaceutically acceptable carrier or excipient. The compositions can additionally contain one or more other therapeutic agents such as the chemotherapeutic agents described above.

Formulations of therapeutic and diagnostic agents can be prepared by mixing with physiologically acceptable carriers, excipients, or stabilizers in the form of, e.g., lyophilized powders, slurries, aqueous solutions, lotions, or suspensions (see, e.g., Hardman *et al.*, (2001) Goodman and Gilman's The Pharmacological Basis of Therapeutics, McGraw-Hill, New York, N.Y.; Gennaro (2000) Remington: The Science and Practice of Pharmacy, Lippincott, Williams, and Wilkins, New York, N.Y.; Avis, *et al.* (eds.) (1993) Pharmaceutical Dosage Forms: Parenteral Medications, Marcel Dekker, NY; Lieberman, et al. (eds.) (1990) Pharmaceutical Dosage Forms: Tablets, Marcel Dekker, NY; Lieberman, *et al.* (eds.) (1990) Pharmaceutical Dosage Forms: Disperse Systems, Marcel Dekker, NY; Weiner and Kotkoskie (2000) Excipient Toxicity and Safety, Marcel Dekker, Inc., New York, N.Y.).

Selecting an administration regimen for a therapeutic depends on several factors, including the serum or tissue turnover rate of the entity, the level of symptoms, the immunogenicity of the entity, and the accessibility of the target cells in the biological matrix. In certain embodiments, an administration regimen maximizes the amount of therapeutic delivered to the patient consistent with an acceptable level of side effects. Accordingly, the amount of biologic delivered depends in part on the particular entity and the severity of the condition being treated. Guidance in selecting appropriate doses of antibodies, cytokines, and small molecules are available (see, e.g., Wawrzynczak (1996) Antibody Therapy, Bios Scientific Pub. Ltd,

Oxfordshire, UK; Kresina (ed.) (1991) Monoclonal Antibodies, Cytokines and Arthritis, Marcel Dekker, New York, N.Y.; Bach (ed.) (1993) Monoclonal Antibodies and Peptide Therapy in Autoimmune Diseases, Marcel Dekker, New York, N.Y.; Baert *et al.*, (2003) New Engl. J. Med. 348:601-608; Milgrom *et al.*, (1999) New Engl. J. Med. 341:1966-1973; Slamon *et al.*, (2001) New Engl. J. Med. 344:783-792; Beniaminovitz *et al.,* (2000) New Engl. J. Med. 342:613-619; Ghosh *et al.*, (2003) New Engl. J. Med. 348:24-32; Lipsky *et al.*, (2000) New Engl. J. Med. 343:1594-1602).

Determination of the appropriate dose is made by the clinician, e.g., using parameters or factors known or suspected in the art to affect treatment or predicted to affect treatment. Generally, the dose begins with an amount somewhat less than the optimum dose and it is increased by small increments thereafter until the desired or optimum effect is achieved relative to any negative side effects. Important diagnostic measures include those of symptoms of, e.g., the inflammation or level of inflammatory cytokines produced.

Actual dosage levels of the active ingredients in the pharmaceutical compositions of the present invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient. The selected dosage level will depend upon a variety of factors that could affect pharmacokinetics including the activity of the particular compositions of the present invention employed, the route of administration, the time of administration, the rate of disposition of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors known in the medical arts.

Compositions comprising antibodies or fragments thereof of the invention can be provided by continuous infusion, or by doses at intervals of, e.g., one day, one week, or 1-7 times per week. Doses may be provided intravenously, subcutaneously, topically, orally, nasally, rectally, intramuscular, intracerebrally, or by inhalation. A specific dose protocol is one involving the maximal dose or dose frequency that avoids significant undesirable side effects. Every given dose may be at least 0.05 μg/kg, at least 0.2 μg/kg, at least 0.5 μg/kg, at least 1 μ/kg, at least 10 μg/kg, at least 100 μg/kg, at least 0.2 mg/kg, at least 0.3 mg/kg, at least 0.5 mg/kg, at least 1.0 mg/kg, at least 2.0 mg/kg, at least 3.0 mg/kg, at least 5.0 mg/kg, at least 10

mg/kg, at least 15 mg/kg, at least 20 mg/kg, at least 25 mg/kg, or at least 50 mg/kg body weight (or other size descriptor, e.g., ideal body weight (IBW), body mass indesx (BMI), body surface area (BSA), fat-free mass (FFM), lean body weight (LBW), adjusted body weight (ABW), percent of ideal body weight (%IBW) and predicted normal weight (PNWT)) (see, e.g., Yang *et al.*, (2003) New Engl. J. Med. 349:427-434; Herold *et al.*, (2002) New Engl. J. Med. 346:1692-1698; Liu *et al.*, (1999) J. Neurol. Neurosurg. Psych. 67:451-456; Portielji *et al.*, (2003) Cancer Immunol. Immunother. 52:133-144, Green & Duffull, (2004) Br J Clin Pharmacol 58:2 119–133.). The desired dose of antibodies or fragments thereof is about the same as for an antibody or polypeptide, on a moles/kg body weight basis. The desired serum or plasma concentration of the antibodies or fragments thereof is about the same as for an antibody or polypeptide, on a moles/kg body weight basis. The dose may be at least 15 μg at least 20 μg, at least 25 μg, at least 30 μg, at least 35 μg, at least 40 μg, at least 45 μg, at least 50 μg, at least 55 μg, at least 60 μg, at least 65 μg, at least 70 μg, at least 75 μg, at least 80 μgμ, at least 85 μg, at least 90 μg, at least 95 μg, or at least 100 μg. The doses administered to a subject may number at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12, or more.

For antibodies or fragments thereof the invention, the dosage administered to a patient may be 0.01 mg/kg to 100 mg/kg of the patient's body weight (or other size descriptors). The dosage may be between 0.01 mg/kg and 20 mg/kg, 0.01 mg/kg and 10 mg/kg, 0.01 mg/kg and 5 mg/kg, 0.01 and 3 mg/kg, 0.01 and 2 mg/kg, 0.01 and 1 mg/kg, 0.01 mg/kg and 0.75 mg/kg, 0.01 mg/kg and 0.5 mg/kg, 0.01 mg/kg to 0.25 mg/kg, 0.01 to 0.15 mg/kg, or 0.01 to 0.10 mg/kg of the patient's body weight (or other size descriptors). In one example, doses may be delivered based on weight, e.g., 0.01 mg/kg, 0.03 mg/kg, 1 mg/kg, 3 mg/kg, 10 mg/kg, 15 mg/kg, or 20 mg/kg., 30 mg/kg, 40, mg/kg, 50 mg/kg, 60 mg/kg, 70 mg/kg, 80 mg/kg, 90 mg/kg, 100 mg/kg Doses may be delivered as a flat or a fixed amount, e.g., 0.75 mg, 2.5 mg, 5 mg, 10 mg, 25 mg, 75 mg, 150 mg, 300 mg, 500 mg, 700mg, 1000 mg, or 1500mg.

Unit dose of the antibodies or fragments thereof the invention may be 0.1 mg to 20 mg, 0.1 mg to 15 mg, 0.1 mg to 12 mg, 0.1 mg to 10 mg, 0.1 mg to 8 mg, 0.1 mg to 7 mg, 0.1 mg to 5 mg, 0.1 to 2.5 mg, 0.25 mg to 20 mg, 0.25 to 15 mg, 0.25 to 12 mg, 0.25 to 10 mg, 0.25 to 8 mg, 0.25 mg to 7 m g, 0.25 mg to 5 mg, 0.5 mg to 2.5 mg, 1 mg to 20 mg, 1 mg to 15 mg, 1 mg to 12 mg, 1 mg to 10 mg, 1 mg to 8 mg, 1 mg to 7 mg, 1 mg to 5 mg, or 1 mg to 2.5 mg.

The dosage of the antibodies or fragments thereof the invention may achieve a serum titer (or

serum or plasma concentration) of at least 0.1 μg/ml, at least 0.2 μg/ml, at least 0.5 μg/ml, at least 1 μg/ml, at least 2 μg/ml, at least 5 μg/ml, at least 6 μg/ml, at least 10 μg/ml, at least 15 μg/ml, at least 20 μg/ml, at least 25 μg/ml, at least 50 μg/ml, at least 100 μg/ml, at least 125 μg/ml, at least 150 μg/ml, at least 175 μg/ml, at least 200 μg/ml, at least 225 μg/ml, at least 250 μg/ml, at least 275 μg/ml, at least 300 μg/ml, at least 325 μg/ml, at least 350 μg/ml, at least 375 μg/ml, at least 400 μg/ml, at least 425 μg/ml, at least 450 μg/ml, at least 475 μg/ml, or at least 500 μg/ml in a subject. Alternatively, the dosage of the antibodies or fragments thereof the invention may achieve a serum titer of at least 0.1 μg/ml, at least 0.5 μg/ml, at least 1 μg/ml, at least, 2 μg/ml, at least 5 μg/ml, at least 6 μg/ml, at least 10 μg/ml, at least 15 μg/ml, at least 20 μg/ml, at least 25 μg/ml, at least 50 μg/ml, at least 100 μg/ml, at least 125 μg/ml, at least 150 μg/ml, at least 175 μg/ml, at least 200 μg/ml, at least 225 μg/ml, at least 250 μg/ml, at least 275 μg/ml, at least 300 μg/ml, at least 325 μg/ml, at least 350 μg/ml, at least 375 μg/ml, or at least 400 μg/ml in the subject.

Doses of antibodies or fragments thereof the invention may be repeated and the administrations may be separated by at least 1 day, 2 days, 3 days, 5 days, 7 days, 10 days, 14 days, 21 days, 28 days, 30 days, 42 days, 45 days, 56 days, 2 months, 75 days, 3 months, or at least 6 months.

An effective amount for a particular patient may vary depending on factors such as the condition being treated, the overall health of the patient, the method route and dose of administration and the severity of side effects (see, e.g., Maynard *et al.*, (1996) A Handbook of SOPs for Good Clinical Practice, Interpharm Press, Boca Raton, Fla.; Dent (2001) Good Laboratory and Good Clinical Practice, Urch Publ., London, UK).

In one embodiment, the M-CSF antibody is administered at a dose of between about 0.1-20 mg/kg to a patient at time 0 ("first administration"). An additional dose may then optionally be administered between 7-14 days following first administration ("additional dose" or "loading dose"). The M-CSF antibody is then administered every three weeks following the first administration. In another embodiment, the M-CSF antibody, e.g., H-RX1 is administered at a dose of about 10 mg/kg to a patient at time 0 ("first administration"). An additional dose at 10mg/kg is then administered at 8 days following first administration ("additional dose"). The M-CSF antibody is then administered every three weeks following

the first administration. The rationale for adding an additional dose is to reach steady state earlier, thus achieving a faster and more continuous inhibition of M-CSF.

The route of administration may be by, e.g., topical or cutaneous application, injection or infusion by intravenous, intraperitoneal, intracerebral, intramuscular, intraocular, intraarterial, intracerebrospinal, intralesional, or by sustained release systems or an implant (see, e.g., Sidman *et al.*, (1983) Biopolymers 22:547-556; Langer *et al.*, (1981) J. Biomed. Mater. Res. 15:167-277; Langer (1982) Chem. Tech. 12:98-105; Epstein et al., (1985) Proc. Natl. Acad. Sci. USA 82:3688-3692; Hwang *et al.*, (1980) Proc. Natl. Acad. Sci. USA 77:4030-4034; U.S. Pat. Nos. 6,350,466 and 6,316,024). Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lidocaine to ease pain at the site of the injection. In addition, pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent. See, e.g., U.S. Pat. Nos. 6,019,968, 5,985,320, 5,985,309, 5,934,272, 5,874,064, 5,855,913, 5,290,540, and 4,880,078; and PCT Publication Nos. WO 92/19244, WO 97/32572, WO 97/44013, WO 98/31346, and WO 99/66903, each of which is incorporated herein by reference their entirety.

In one embodiment, the M-CSF antibody is administered intravenously at a dose of between about 0.1-20 mg/kg, e.g., 0.1, 0.3, 1, 3, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 mg/kg to a patient at time 0 ("first administration"), an additional dose may then optionally be administered between 7-14 days, 7, 8, 9, 10, 11, 12 days, following first administration ("additional dose") and the M-CSF antibody is then administered every three weeks following the first administration. In another embodiment, the M-CSF antibody is administered at a dose of about 10 mg/kg to a patient at time 0 ("first administration"). An additional dose of about 10 mg/kg is then administered 8 days following first administration ("additional dose"). The M-CSF antibody is then administered of about 10 mg/kg every three weeks following the first administration. The rationale for adding an additional dose is to reach steady state earlier, thus achieving a faster and more continuous inhibition of M-CSF.

A composition of the present invention may also be administered via one or more routes of administration using one or more of a variety of methods known in the art. Parenteral routes of administration may be used for example by injection or infusion. Parenteral administration may represent modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and

intrasternal injection and infusion. Alternatively, a composition of the invention can be administered via a non-parenteral route, such as a topical, epidermal or mucosal route of administration, for example, intranasally, orally, vaginally, rectally, sublingually or topically. In one embodiment, the antibodies or fragments thereof of the invention is administered by infusion. In another embodiment, the multispecific epitope binding protein of the invention is administered subcutaneously.

If the antibodies or fragments thereof of the invention are administered in a controlled release or sustained release system, a pump may be used to achieve controlled or sustained release (see Langer, supra; Sefton, (1987) CRC Crit. Ref Biomed. Eng. 14:20; Buchwald *et al.*, (1980), Surgery 88:507; Saudek *et al.*, (1989) N. Engl. J. Med. 321:574). Polymeric materials can be used to achieve controlled or sustained release of the therapies of the invention (see e.g., Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Fla. (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, (1983) J. Macromol. Sci. Rev. Macromol. Chem. 23:61; see also Levy *et al.*, (1985) Science 228:190; During *et al.*, (1989) Ann. Neurol. 25:351; Howard *et al.*, (1989) J. Neurosurg. 7 1:105); U.S. Pat. No. 5,679,377; U.S. Pat. No. 5,916,597; U.S. Pat. No. 5,912,015; U.S. Pat. No. 5,989,463; U.S. Pat. No. 5,128,326; PCT Publication No. WO 99/15154; and PCT Publication No. WO 99/20253. Examples of polymers used in sustained release formulations include, but are not limited to, poly(2-hydroxy ethyl methacrylate), poly(methyl methacrylate), poly(acrylic acid), poly(ethylene-co-vinyl acetate), poly(methacrylic acid), polyglycolides (PLG), polyanhydrides, poly(N-vinyl pyrrolidone), poly(vinyl alcohol), polyacrylamide, poly(ethylene glycol), polylactides (PLA), poly(lactide-co-glycolides) (PLGA), and polyorthoesters. In one embodiment, the polymer used in a sustained release formulation is inert, free of leachable impurities, stable on storage, sterile, and biodegradable. A controlled or sustained release system can be placed in proximity of the prophylactic or therapeutic target, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138 (1984)).

If the antibodies or fragments thereof of the invention are administered topically, they can be formulated in the form of an ointment, cream, transdermal patch, lotion, gel, shampoo, spray, aerosol, solution, emulsion, or other form well-known to one of skill in the art. See, e.g., Remington's Pharmaceutical Sciences and Introduction to Pharmaceutical Dosage Forms, 19th

ed., Mack Pub. Co., Easton, Pa. (1995). For non-sprayable topical dosage forms, viscous to semi-solid or solid forms comprising a carrier or one or more excipients compatible with topical application and having a dynamic viscosity, in some instances, greater than water are typically employed. Suitable formulations include, without limitation, solutions, suspensions, emulsions, creams, ointments, powders, liniments, salves, and the like, which are, if desired, sterilized or mixed with auxiliary agents (e.g., preservatives, stabilizers, wetting agents, buffers, or salts) for influencing various properties, such as, for example, osmotic pressure. Other suitable topical dosage forms include sprayable aerosol preparations wherein the active ingredient, in some instances, in combination with a solid or liquid inert carrier, is packaged in a mixture with a pressurized volatile (e.g., a gaseous propellant, such as freon) or in a squeeze bottle. Moisturizers or humectants can also be added to pharmaceutical compositions and dosage forms if desired. Examples of such additional ingredients are well-known in the art.

If the compositions comprising antibodies or fragments thereof are administered intranasally, it can be formulated in an aerosol form, spray, mist or in the form of drops. In particular, prophylactic or therapeutic agents for use according to the present invention can be conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebuliser, with the use of a suitable propellant (e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas). In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges (composed of, e.g., gelatin) for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

Methods for co-administration or treatment with a second therapeutic agent are known in the art (see, e.g., Hardman *et al.,* (eds.) (2001) Goodman and Gilman's The Pharmacological Basis of Therapeutics, 10.sup.th ed., McGraw-Hill, New York, N.Y.; Poole and Peterson (eds.) (2001) Pharmacotherapeutics for Advanced Practice:A Practical Approach, Lippincott, Williams & Wilkins, Phila., Pa.; Chabner and Longo (eds.) (2001) Cancer Chemotherapy and Biotherapy, Lippincott, Williams & Wilkins, Phila., Pa.). An effective amount of therapeutic may decrease the symptoms by at least 10%; by at least 20%; at least about 30%; at least 40%, or at least 50%.

In one embodiment, M-CSF is administered with another agents such as a chemotherapeutic agent such as gemcitabine, carboplatin, cisplatin etc.

The M-CSF antagonists and the other inhibitors or agents may be cyclically administered. Cycling therapy involves the administration of a first therapy (e.g., a first prophylactic or therapeutic agent) for a period of time, followed by the administration of a second therapy (e.g., a second prophylactic or therapeutic agent) for a period of time, optionally, followed by the administration of a third therapy (e.g., prophylactic or therapeutic agent) for a period of time and so forth, and repeating this sequential administration, i.e., the cycle in order to reduce the development of resistance to one of the therapies, to avoid or reduce the side effects of one of the therapies, and/or to improve the efficacy of the therapies.

In certain embodiments, the antibodies or fragments thereof the invention can be formulated to ensure proper distribution in vivo. For example, the blood-brain barrier (BBB) excludes many highly hydrophilic compounds. To ensure that the therapeutic compounds of the invention cross the BBB (if desired), they can be formulated, for example, in liposomes. For methods of manufacturing liposomes, see, e.g., U.S. Pat. Nos. 4,522,811; 5,374,548; and 5,399,331. The liposomes may comprise one or more moieties which are selectively transported into specific cells or organs, thus enhance targeted drug delivery (see, e.g., Ranade, (1989) J. Clin. Pharmacol. 29:685). Exemplary targeting moieties include folate or biotin (see, e.g., U.S. Pat. No. 5,416,016 to Low et al); mannosides (Umezawa *et al.,* (1988) Biochem. Biophys. Res. Commun. 153:1038); antibodies (Bloeman *et al.*, (1995) FEBS Lett. 357:140; Owais *et al.*, (1995) Antimicrob. Agents Chemother. 39:180); surfactant protein A receptor (Briscoe *et al.*, (1995) Am. J. Physiol. 1233:134); p 120 (Schreier *et al,* (1994) J. Biol. Chem. 269:9090); see also K. Keinanen; M. L. Laukkanen (1994) FEBS Lett. 346:123; J. J. Killion; I. J. Fidler (1994) Immunomethods 4:273.

The invention provides for the administration of M-CSF antagonists alone or in combination with a other therapies. The therapies (e.g., prophylactic or therapeutic agents) of the combination therapies of the present invention can be administered concomitantly or sequentially to a subject. The therapy of the combination therapies of the present invention can also be cyclically administered. Cycling therapy involves the administration of a first therapy (e.g., a first prophylactic or therapeutic agent) for a period of time, followed by the administration of a second therapy (e.g., a second prophylactic or therapeutic agent) for a

period of time and repeating this sequential administration, i.e., the cycle, in order to reduce the development of resistance to one of the therapies (e.g., agents) to avoid or reduce the side effects of one of the therapies (e.g., agents), and/or to improve, the efficacy of the therapies.

The combination therapies of M-CSF antagonists with other agents can be administered to a subject concurrently. The term "concurrently" is not limited to the administration of therapies (e.g., prophylactic or therapeutic agents) at exactly the same time, but rather it is meant that a pharmaceutical composition comprising antibodies or fragments thereof the invention are administered to a subject in a sequence and within a time interval such that the antibodies of the invention can act together with the other therapy(ies) to provide an increased benefit than if they were administered otherwise. For example, each therapy may be administered to a subject at the same time or sequentially in any order at different points in time; however, if not administered at the same time, they should be administered sufficiently close in time so as to provide the desired therapeutic or prophylactic effect. Each therapy can be administered to a subject separately, in any appropriate form and by any suitable route. In various embodiments, the therapies (e.g., prophylactic or therapeutic agents) are administered to a subject less than 15 minutes, less than 30 minutes, less than 1 hour apart, at about 1 hour apart, at about 1 hour to about 2 hours apart, at about 2 hours to about 3 hours apart, at about 3 hours to about 4 hours apart, at about 4 hours to about 5 hours apart, at about 5 hours to about 6 hours apart, at about 6 hours to about 7 hours apart, at about 7 hours to about 8 hours apart, at about 8 hours to about 9 hours apart, at about 9 hours to about 10 hours apart, at about 10 hours to about 11 hours apart, at about 11 hours to about 12 hours apart, 24 hours apart, 48 hours apart, 72 hours apart, or 1 week apart. In other embodiments, two or more therapies (e.g., prophylactic or therapeutic agents) are administered to a within the same patient visit.

The combination therapies can be administered to a subject in the same pharmaceutical composition. Alternatively, the combination therapies can be administered concurrently to a subject in separate pharmaceutical compositions. The therapeutic agents may be administered to a subject by the same or different routes of administration.

Kits

The invention also encompasses kits for detecting CD163 expression or CD163 protein in a biological sample (a test sample) from a patient and optionally also in a control sample. Such kits can be used to predict if a patient having breast cancer is likely to respond (or have a

higher response) to treatment with a M-CSF antagonist alone or in combination with another agent such as a chemotherapeutic agent. For example, the kit can comprise a probe (e.g., an oligonucleotide, antibody, labeled compound or other agent) capable of detecting the level of CD163 expression, level of CD163 protein or level of CD163 activity, products of those alleles and/or an equivalent genetic marker of those alleles in a biological sample. The kit may also comprise instructions for providing a prediction of the likelihood that the patient will respond to treatment with the M-CSF antagonist alone or in combination with another agent.

The disclosed kits can also comprise, e.g., a buffering agent, a preservative, or a protein stabilizing agent. The kit can also comprise components necessary for detecting the detectable agent (e.g., an enzyme or a substrate). The kit can also contain a control sample or a series of control samples that can be assayed and compared to the test sample contained. Each component of the kit is usually enclosed within an individual container, and all of the various containers are within a single package along with instructions for use.

Such kits may also comprise a M-CSF antagonist, e.g., M-CSF antibody such as H-RX1 (e.g., in liquid or lyophilized form) or a pharmaceutical composition comprising the M-CSF antagonist (described *supra)*, and optionally one or more additional therapeutic agensts described hereing (e.g., a second agent such as carboplatin and a third agent such a gemcitabine) or pharmaceutical compositions comprising such agents. In this way, such kits are useful in the selective treatment of breast cancer patients using an M-CSF antagonist. Additionally, such kits may comprise means for administering the M-CSF antagonist (e.g., a syringe and vial, a prefilled syringe, a prefilled pen) and instructions for use. These kits may contain additional therapeutic agents (described *supra*) for treating an Breast cancer, e.g., for delivery in combination with the enclosed M-CSF antagonist.

The invention having been fully described, it is further illustrated by the following examples and claims, which are illustrative and are not meant to be further limiting.

Examples

Example 1

CMCS110Z2201 is a Phase II, open-label, randomized study of H-RX1 (an antibody having the heavy chain variable region including the amino acids set forth in SEQ ID NO: 2 and the light chain variable region including the amino acids set forth in SEQ ID NO: 4 in combination with carboplatin/gemcitabine (carboplatin/gem) versus carbo/gem alone in

patients with advanced triple negative breast cancer (TNBC). No substantial progress has been made in the treatment of TNBC and the prognosis remains poor. Currently, chemotherapy is the only treatment option. Although TNBC is a chemosensitive disease and a subset of chemo responders has an excellent prognosis, the majority of patients rapidly relapse. Current treatment strategy in TNBC aims at further improving the efficacy of chemotherapy. The targeted study population will include patients whose tumors contain high immunological infiltrates of TAMs ("TAM high"). In this study, only patients with approximately 8% TAM content or higher, as determined by detecting CD163 expression, will be included.

- Detecting TAMs by detecting CD163 to select patients

Recently 6 different subtypes of TNBC were described using gene expression profiling: two basal-like, one mesenchymal, one mesenchymal stem-like, one luminal androgen receptor (LAR) and one immunomodulatory (IM) subtype (Lehmann 2011, J Clin Invest. 121(7):2750-67). Ongoing and emerging treatment approaches include selected targeted therapies for each unique subtype. The IM subtype comprises approximately 25% of all TNBC and is characterized by an elevated expression of genes involved in immune cell signaling such as T-cell function, immune transcription and interferon response (Lehmann 2014). Furthermore, the IM subtype exhibits a substantially higher content of tumor infiltrating lymphocytes (TILs) as well as tumor associated macrophages (TAMs) than other TNBC subtypes (Mahmoud 2012, J Clin Pathol 65:159-163 and Vinayak ASCO 2014, ASCO Annual Meeting J Clin Oncol 32:5s). Forty percent of TNBC have high tumor infiltration of TAMs (TAM high). The TAM high subset is enriched for IM, capturing 75% of all IM (Yuan 2014, OncoTargets and Therapy 7:1475–1480; Lehmann 2011, supra, Jaeger [TCGA] 2014, Novartis's own data analyzing the TCGA database).

M2 macrophages or TAMs have been defined as CD163+, CD68+, but it has been shown by immunofluorescence staining of breast cancer tissue sections that CD163 staining is coincident with CD68 staining. Immunohistochemical staining also showed CD163 co-staining with CD68, however, CD163-DAB conjugated antibodies yielded clearer images with reduced background compared to that of CD68-DAB conjugated antibodies. Based on these data, single immunohistochemical staining using anti-CD163-DAB conjugated antibodies was selected to detect M2 macrophages or TAMs.

Two different tissue microarrays sets containing breast cancer tumor samples were stained using anti-CD163-DAB conjugated antibodies to detect TAMs. IHC was performed using a Ventana Discovery XT autostainer. Sections were deparaffinized, treated with the Ventana Cell Conditioning #1 (CCIS) antigen retrieval reagent and then incubated for 32 minutes at room temperature with the primary antibody (CD163 reagents are ready-to-us). Detection was performed using ChromoMap Kit (Roche/Ventana, cat# 760-159). All stained slides were digitized using an Aperio Scanscope whole slide scanner. Stained tumor sections from each sample were analyzed using an automated image analysis algorithm to determine the percentage of positive pixel from the total pixel covered by the CD163-DAB stain, defined as the measure of "TAM density". TNBC samples showed the widest range in CD163 expression, or "TAM density". For TNBC samples we obtained "TAM density" ranges of ~25% to <1%. Based on the understanding that 40% of TNBC patients with the highest infiltration of TAMs capture 75% of all the IM subtype of TNBC, we set the threshold, or lower boundary, defining the TAM high population at ~12-15% TAM density, thus capturing the 40-50% of TNBC samples with the highest infiltration of TAMs.

Fig. 1 shows primary breast cancer samples on 2 tissue microarrays which were independently stained with anti-CD163 antibodies conjugated to DAB, and "TAM density" was determined as described. Column 1 depicts the TAM density values obtained for breast cancer samples that are not TNBC, and those that are HER2+/ER+/PR+; Column 2 shows the TAM density values obtained for TNBC samples. All samples in Column 1 and Column 2 were obtained from the University Hospital Basel tissue microarray, which were obtained from patients with disease of various stages, including metastatic disease. All samples from Column 3 and Column 4 were obtained from Uppsala University, which were obtained from patients with less advanced disease (T1-2/N0/M0). Column 3 depicts the TAM density values obtained for breast cancer samples that are not TNBC, and those that are HER2+/ER+/PR+; Column 4 shows the TAM density values obtained for TNBC samples. The data shows that more samples with higher TAM density were identified in the University Hospital Basel microarray, consistent with higher number of advanced stage TNBC patient samples included in this array. The images to the right labeled A, B, C, D depict the actual CD163 stained tissue sections for the indicated patient samples; TAM density values based on CD163 staining (in %) are indicated in the inset panel for each sample.

Based on the CD163 staining, TAM infiltration is greatly increased in triple negative breast cancer (TNBC) compared to hormone receptor positive breast cancer samples. These findings indicate that CD163 staining in TNBC patients is a suitable marker for measuring changes in CD163 positive TAM population upon M-CSF signaling blockade using an M-CSF inhibitor.

Example 2

TNBC patients having a TAM density based on CD163 staining received the M-CSF antagonist, H-RX1, at every three weeks at a dose of 10 mg/kg iv. The 3-week cycle was chosen to coordinate with the administration cycle of the chemotherapy (carbo/gem). In addition, an additional dose or loading dose of M-CSF was administered 8 days following the first dosing.

H-RX1 concentrations are predicted to reach steady-state at Week 6 instead of Week 12 with a loading dose on Cycle 1 Day 8, based on the population PK model developed from healthy volunteers. Achieving a faster TAM depletion and, thus enhanced chemotherapy efficacy may be of clinical importance in TNBC given the aggressive clinical course. Fig. 2 shows a graph of a predicted population mean serum concentration profiles of free H-RX1 following 10 mg/kg Q3W iv infusion with and without an additional dose.

The rationale for adding an additional dose is to reach steady state earlier, thus achieving a faster and more continuous inhibition of H-RX1. Based on the population PK model developed from healthy volunteer data, H-RX1concentrations are predicted to reach steady-state at Week 6 instead of Week 12 with an additional dose on Cycle 1 Day 8. Achieving a faster TAM depletion, and thus enhanced chemotherapy efficacy, may be of clinical importance in TNBC given the aggressive clinical course.

Example 3

A 78-year old woman, diagnosed with metastatic TNBC, was found to have high TAM content (above 10% TAMs) in her tumor biopsy and therefore eligible for enrollment into the study. The patient had not received any prior adjuvant or neoadjuvant treatment for her breast cancer; the disease was already metastatic when diagnosed. The patient was randomized to receive study treatment with H-RX1 combined with carboplatin/gemcitabine. After 2 cycles of treatment the tumor status was evaluated and the patient was found to have a 40% reduction of tumor index lesion (i.e., a partial response to the treatment). Particularly for the patient, target 1 (tumor left breast) decreased from 52.9 x 34.8 mm to 47.1 x 32.8 mm. Target

2 (lymph node right axilla) decreased from 16.4 mm to 5 mm. Target 3 (lymph node right axilla) decreased from 18.6 to 5.3 mm. Target 4 (lymph node left groin) decreased from 26 mm to 14 mm. Target 5 (paracaval lymph node) decreased from 16.2 mm to 7.9 mm. Treatment with carboplatin and gemcitabine alone leads to an objective response rate in about 30% of the patients. The present results indicate that the combined therapy with H-RX1 may result in further benefit.

What is claimed is:

1. A method of treating a patient having triple negative breast cancer comprising administering a therapeutically effective amount of an M-CSF antibody or fragment thereof to the patient, wherein the M-CSF antibody or fragment comprises 1, 2, 3, 4, 5, or 6 CDRs as shown in Table 1.

2. A method of treating a patient having breast cancer, comprising selectively administering a therapeutically effective amount of an M-CSF antibody or fragment thereof to the patient on the basis of the patient having a level of CD163 expression predictive that the patient is likely to respond to the M-CSF antibody or fragment thereof, and wherein the antibody or fragment comprises 1, 2, 3, 4, 5, or 6 CDRs as shown in Table 1.

3. A method of selectively treating a patient having breast cancer, comprising:

a) selecting the patient for treatment with a therapeutically effective amount of an M-CSF antagonist on the basis of the patient having a level of CD163 expression compared to a control predictive that the patient is likely to respond to M-CSF; and

b) thereafter, administering the therapeutically effective amount of the M-CSF antagonist.

4. A method of selectively treating a patient having breast cancer, comprising:

a) assaying a biological sample from the patient for an increased level of CD163 expression; and

b) thereafter, selecting the patient for treatment with a therapeutically effective amount of an M-CSF antagonist on the basis of the biological sample from the patient having a level of CD163 expression compared to a control predictive that the patient is likely to respond to M-CSF; and

c) thereafter, administering the M-CSF antagonist to the selected patient.

5. A therapeutically effective amount of an M-CSF antagonist for use in treating a patient having breast cancer characterized in that:

a) the patient is selected for treatment with a M-CSF antagonist on the basis of the patient having a level of CD163 expression compared to a control predictive that the patient is likely to respond to M-CSF; and

b) thereafter, a therapeutically effective amount of the M-CSF antagonist is administered.

6. A method of predicting the likelihood that a patient having breast cancer will respond to treatment with a therapeutically effective amount of a M-CSF antagonist comprising assaying a biological sample from the patient for the level of CD163 expression, wherein:

a) the presence of an increase in level of CD163 expression compared to a control is indicative of an increased likelihood that the patient will respond to treatment with the M-CSF antagonist; and

b) the absence of an increase in level of CD163 expression compared to a control is indicative of a decreased likelihood that the patient will respond to treatment with the M-CSF antagonist.

7. A method of selectively treating a patient having breast cancer, comprising:

a) assaying a biological sample from the patient for the level of CD163 protein; and

b) thereafter, selecting the patient for treatment with an M-CSF antagonist on the basis of the biological sample from the patient having a level of CD163 expression compared to a control predictive that the patient is likely to respond to M-CSF; and

c) thereafter, administering the M-CSF antagonist to the selected patient.

8. The method according to any of claims 6-7 wherein the expression of CD163 is assayed using quantitative immunohistochemistry.

9. The method according to any of claims 1-8, wherein the step of administering comprises administering a dose of between about 5-20 mg/kg of an M-CSF antagonist to said patient at time 0 ("first administration"), administering again a dose at 7-14 days following first administration ("additional dose") and then administering every three weeks following the first administration.

10. The method according to any of the above claims, wherein the M-CSF antagonist is a M-CSF binding molecule.

11. The method according to claim 10, wherein the M-CSF binding molecule is an M-CSF antibody or fragment thereof.

12. The method of claim 11, wherein the antibody or fragment thereof comprises 1, 2, 3, 4, 5, or 6 CDRs as shown in Table 1.

13. The method according to claim 11, wherein the isolated M-CSF antibody or fragment comprises

a VH comprising SEQ ID NO: 2 and a VL comprising SEQ ID NO: 4, or an amino acid sequence with 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99 percent identity thereof.

14. A method for producing a transmittable form of information for predicting the responsiveness of a patient having breast cancer to treatment with a M-CSF antagonist, comprising:

a) assaying a biological sample from the patient for the level of CD163 expression; and

b) recording the result of the assaying step on a tangible or intangible media form for use in transmission, wherein if the result is a level of CD163 expression is greater than a control it is indicative of an increased likelihood that the patient will respond to treatment comprising a M-CSF antagonist.

15. A method of treating a patient having breast cancer, comprising:

a) administering a combination of i) a therapeutically effective amount of an M-CSF antagonist, ii) a therapeutically effective amount of a second agent, simultaneously, separately or sequentially, and iii) a therapeutically effective amount of a third agent, simultaneously, separately or sequentially.

16. A method of selectively treating a patient having breast cancer, comprising:

a) assaying a biological sample from the patient for an increased level of CD163 protein;

b) selecting the patient for treatment with a combination of i) a therapeutically effective amount of a M-CSF antagonist, ii) a therapeutically effective amount of a second agent, and iii) a therapeutically effective amount of a third agent, on the basis of a the patient having a level of CD163 expression compared to a control predictive that the patient is likely to respond to M-CSF; and

c) thereafter, administering the combination of i) the therapeutically effective amount of the M-CSF antagonist, ii) the therapeutically effective amount of a second agent, and iii) a therapeutically effective amount of a third agent, simultaneously, separately or sequentially.

17. The method of any one of claims 15-16 wherein the breast cancer is TNBC.

18. The method of claim 16 wherein the level of CD163 assayed is protein..

19. The method of any one of claims 15-18 wherein the M-CSF antagonist is an M-CSF antibody or fragment thereof.

20. The method of claim 19, wherein the antibody or fragment thereof comprises 1, 2, 3, 4, 5, or 6 CDRs as shown in Table 1.

21. The method of any one of claims 15-20 wherein the second agent is carboplatin and the third agent is gemcitabine.

22. A method of selectively treating a patient having TNBC, comprising:

administering a combination of i) a therapeutically effective amount of an M-CSF antibody or fragment thereof that comprises 1, 2, 3, 4, 5, or 6 CDRs as shown in Table 1, ii) a therapeutically effective amount of carboplatin, and iii) a therapeutically effective amount of gemcitabine, simultaneously, separately or sequentially; to a patient based on that patient having a level of CD163 which is predictive that the patient is more likely to respond to treatment with the combination.

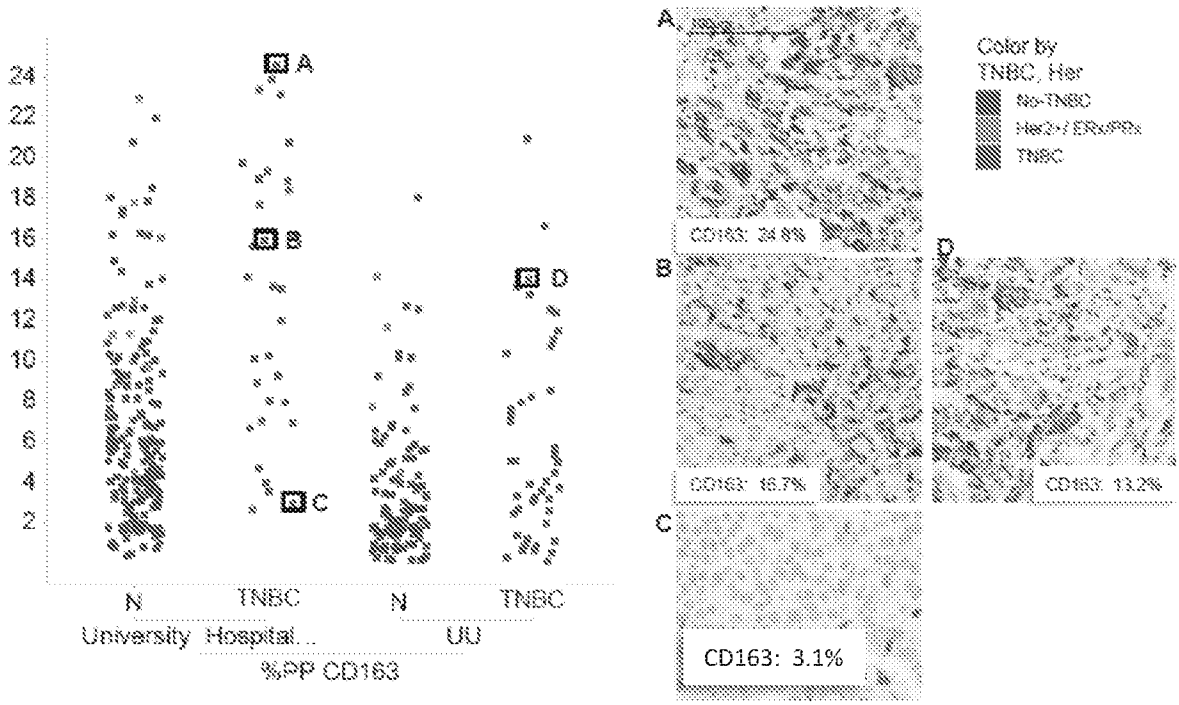
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Fig. 1

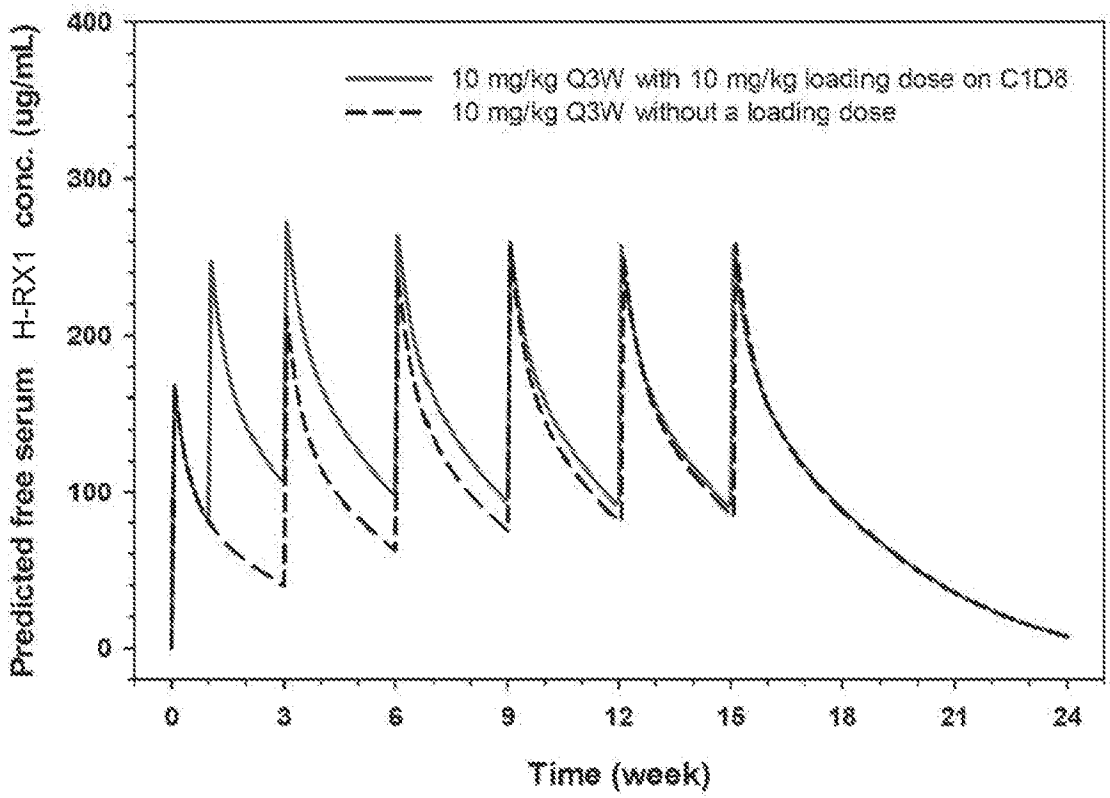
Predicted free serum H-RX1 conc. (ug/mL)
400
300
200
100
0
10 mg/kg Q3W with 10 mg/kg loading dose on C1D8
10 mg/kg Q3W without a loading dose
0
3
6
9
12
15
18
21
24
Time (week)

Fig. 2

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2016/050442

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/395 A61K31/00 C07K16/24 G01N33/574
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2005/068503 A2 (CHIRON CORP [US]; LIU CHENG [US]; ZIMMERMAN DEBORAH LEE [US]; HARROWE) 28 July 2005 (2005-07-28) cited in the application example 2 claim 57 sequences 166,47	1,9-13
T	SWIERCZAK AGNIESZKA ET AL: "The promotion of breast cancer metastasis caused by inhibition of CSF-1R/CSF-1 signaling is blocked by targeting the G-CSF receptor.", CANCER IMMUNOLOGY RESEARCH AUG 2014, vol. 2, no. 8, August 2014 (2014-08), pages 765-776, XP002755689, ISSN: 2326-6074	1,9-13

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 March 2016	13/06/2016

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Bumb, Peter

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2016/050442

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PAULUS PATRICK ET AL: "Colony-stimulating factor-1 antibody reverses chemoresistance in human MCF-7 breast cancer xenografts.", CANCER RESEARCH 15 APR 2006, vol. 66, no. 8, 15 April 2006 (2006-04-15) , pages 4349-4356, XP055149182, ISSN: 0008-5472 the whole document -----	1,9-13
A	CHAVEZ KATHRYN J ET AL: "Triple negative breast cancer cell lines: one tool in the search for better treatment of triple negative breast cancer.", BREAST DISEASE 2010, vol. 32, no. 1-2, 2010, pages 35-48, XP002712389, ISSN: 1558-1551 table 1 -----	1,9-13

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2016/050442

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1(completely); 9-13(partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1(completely); 9-13(partially)

   A method of treating a patient having TNBC (triple negative breast cancer) comprising administering an M-CSF antibody or fragment thereof that comprises 1, 2, 3, 4, 5, or 6 CDRs as shown in Table 1.

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2. claims: 2-8, 14, 16, 22(completely); 9-13, 17-21(partially)

   A method of treating a patient having BC (breast cancer), comprising selecting/assaying the patient on the basis of the patient having a level of CD163 expression.

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3. claims: 15(completely); 17-21(partially)

   A method of treating a patient having BC (breast cancer), comprising administering a combination of an M-CSF antagonist and of a second agent and of a third agent.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2016/050442

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2005068503 A2	28-07-2005	AU 2005205533 A1	28-07-2005
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		WO 2005068503 A2	28-07-2005