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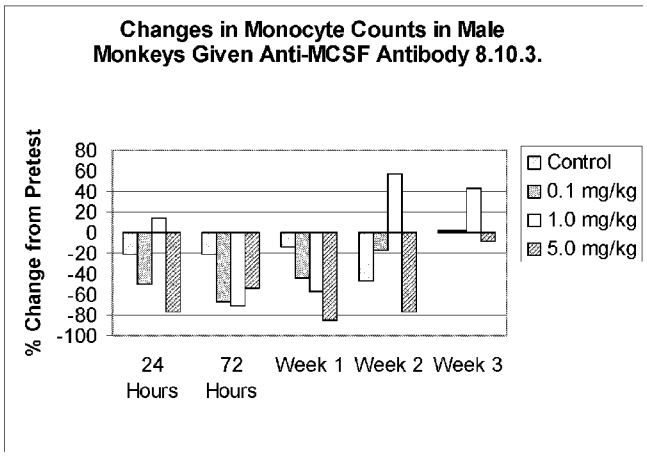
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(54) Title: METHODS OF TREATING INFLAMMATORY DISORDERS USING ANTI-M-CSF ANTIBODIES

FIGURE 1A



(57) Abstract: The present invention relates to antibodies and antigen-binding portions thereof that specifically bind to a M-CSF, preferably human M-CSF, and that function to inhibit a M-CSF. The invention also relates to human anti- M-CSF antibodies and antigen-binding portions thereof. The invention also relates to antibodies that are chimeric, bispecific, derivatized, single chain antibodies or portions of fusion proteins. The invention also relates to isolated heavy and light chain immunoglobulins derived from human anti- M-CSF antibodies and nucleic acid molecules encoding such immunoglobulins. The present invention also relates to methods of making human anti-M-CSF antibodies, compositions comprising these antibodies and methods of using the antibodies and compositions for diagnosis and treatment. The invention also provides gene therapy methods using nucleic acid molecules encoding the heavy and/or light immunoglobulin molecules that comprise the human anti-M-CSF antibodies. The invention also relates to transgenic animals and transgenic plants comprising nucleic acid molecules of the present invention.

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METHODS OF TREATING INFLAMMATORY DISORDERS USING ANTI-M-CSF ANTIBODIES

Related Applications

This application claims the benefit of U.S. Provisional Application No. 61/557,175, filed November 8, 2011, the entire contents of which are hereby incorporated by reference.

Background of the Invention

[0001] Macrophage colony stimulating factor (M-CSF) is a member of the family of proteins referred to as colony stimulating factors (CSFs). M-CSF is a secreted or a cell surface glycoprotein comprised of two subunits that are joined by a disulfide bond with a total molecular mass varying from 40 to 90 kD ((Stanley E.R., *et al.*, *Mol. Reprod. Dev.*, 46:4-10 (1997)). Similar to other CSFs, M-CSF is produced by macrophages, monocytes, and human joint tissue cells, such as chondrocytes and synovial fibroblasts, in response to proteins such as interleukin-1 or tumor necrosis factor-alpha. M-CSF stimulates the formation of macrophage colonies from pluripotent hematopoietic progenitor stem cells (Stanley E.R., *et al.*, *Mol. Reprod. Dev.*, 46:4-10 (1997)).

[0002] M-CSF typically bind to its receptor, *c-fms*, in order to exert a biological effect. *c-fms* contains five extracellular Ig domains, one transmembrane domain, and an intracellular domain with two kinase domains. Upon M-CSF binding to *c-fms*, the receptor homo-dimerizes and

initiates a cascade of signal transduction pathways including the JAK/STAT, PI3K, and ERK pathways.

[0003] M-CSF is an important regulator of the function, activation, and survival of monocytes/macrophages. A number of animal models have confirmed the role of M-CSF in various diseases, including rheumatoid arthritis (RA) and cancer. Macrophages comprise key effector cells in RA. The degree of synovial macrophage infiltration in RA has been shown to closely correlate with the extent of underlying joint destruction. M-CSF, endogenously produced in the rheumatoid joint by monocytes/macrophages, fibroblasts, and endothelial cells, acts on cells of the monocyte/macrophage lineage to promote their survival and differentiation into bone destroying osteoclasts, and enhance pro-inflammatory cellular functions such as cytotoxicity, superoxide production, phagocytosis, chemotaxis and secondary cytokine production. For example, treatment with M-CSF in the rat streptococcus agalactiae sonicate-induced experimental arthritis model lead to enhanced pathology (Abd, A.H., *et al.*, *Lymphokine Cytokine Res.* 10:43-50 (1991)). Similarly, subcutaneous injections of M-CSF in a murine model of collagen-induced arthritis (CIA), which is a model for RA, resulted in a significant exacerbation of the RA disease symptoms (Campbell I.K., *et al., J. Leuk. Biol.* 68:144-150 (2000)). Furthermore, MRL/lpr mice that are highly susceptible to RA and other autoimmune diseases have elevated basal M-CSF serum concentrations (Yui M.A., *et al.*, *Am. J. Pathol.* 139:255-261 (1991)). The requirement for endogenous M-CSF in maintaining CIA was demonstrated by a significant reduction in the severity of established disease by M-CSF neutralizing mouse monoclonal antibody (Campbell I.K., *et al., J. Leuk. Biol.* 68:144-150 (2000)).

[0004] With respect to cancer, inhibition of colony stimulating factors by antisense oligonucleotides suppresses tumor growth in embryonic and colon tumor xenografts in mice by decelerating macrophage-mediated ECM breakdown (Seyedhossein, A., *et al., Cancer Research*, 62:5317-5324 (2002)).

[0005] M-CSF binding to *c-fms* and its subsequent activation of monocyte/macrophages is important in a number of disease states. In addition to RA and cancer, the other examples of M-CSF-related disease states include osteoporosis, destructive arthritis, atherogenesis, glomerulonephritis, Kawasaki disease, and HIV-1 infection, in which monocytes/macrophages and related cell types play a role. For instance, osteoclasts are similar to macrophages and are regulated in part by M-CSF. Growth and differentiation signals induced by M-CSF in the initial stages of osteoclast maturation are essential for their subsequent osteoclastic activity in bone.

[0006] Osteoclast mediated bone loss, in the form of both focal bone erosions and more diffuse juxta-articular osteoporosis, is a major unsolved problem in RA. The consequences of this bone loss include joint deformities, functional disability, increased risk of bone fractures and increased mortality. M-CSF is uniquely essential for osteoclastogenesis and experimental blockade of this cytokine in animal models of arthritis successfully abrogates joint destruction. Similar destructive pathways are known to operate in other forms of destructive arthritis such as psoriatic arthritis, and could represent venues for similar intervention.

[0007] Postmenopausal bone loss results from defective bone remodeling secondary to an uncoupling of bone formation from exuberant osteoclast mediated bone resorption as a consequence of estrogen deficiency. *In-vivo* neutralization of M-CSF using a blocking antibody has been shown in mice to completely prevent the rise in osteoclast numbers, the increase in bone resorption and the resulting bone loss induced by ovariectomy.

[0008] Several lines of evidence point to a central role for M-CSF in atherogenesis, and in proliferative intimal hyperplasia after mechanical trauma to the arterial wall. All the major cell types in atherosclerotic lesions have been shown to express M-CSF, and this is further up-regulated by exposure to oxidized lipoprotein. Blockade of M-CSF signaling with a neutralizing *c-fms* antibody reduces the accumulation of macrophage-

derived foam cells in the aortic root of apolipoprotein E deficient mice maintained on a high fat diet.

[0009] In both experimental and human glomerulonephritis, glomerular M-CSF expression has been found to co-localize with local macrophage accumulation, activation and proliferation and correlate with the extent of glomerular injury and proteinuria. Blockade of M-CSF signaling via an antibody directed against its receptor *c-fms* significantly down-regulates local macrophage accumulation in mice during the renal inflammatory response induced by experimental unilateral ureteric obstruction.

[0010] Kawasaki disease (KD) is an acute, febrile, pediatric vasculitis of unknown cause. Its most common and serious complications involve the coronary vasculature in the form of aneurismal dilatation. Serum M-CSF levels are significantly elevated in acute phase Kawasaki's disease, and normalize following treatment with intravenous immunoglobulin. Giant cell arthritis (GCA) is an inflammatory vasculopathy mainly occurring in the elderly in which T cells and macrophages infiltrate the walls of medium and large arteries leading to clinical consequences that include blindness and stroke secondary to arterial occlusion. The active involvement of macrophages in GCA is evidenced by the presence of elevated levels of macrophage derived inflammatory mediators within vascular lesions.

[0011] M-CSF has been reported to render human monocyte derived macrophages more susceptible to HIV-1 infection *in vitro*. In a recent study, M-CSF increased the frequency with which monocyte-derived macrophages became infected, the amount of HIV mRNA expressed per infected cell, and the level of proviral DNA expressed per infected culture.

[0012] Given the role of M-CSF in various diseases, a method for inhibiting M-CSF activity is desirable.

[0013] There is a critical need for therapeutic anti-M-CSF antibodies.

SUMMARY OF THE INVENTION

[0014] The present invention provides isolated human antibodies or antigen-binding portions thereof that specifically bind human M-CSF and acts as a M-CSF antagonist and compositions comprising said antibody or portion.

[0015] The invention also provides for compositions comprising the heavy and/or light chain, the variable regions thereof, or antigen-binding portions thereof an anti-M-CSF antibody, or nucleic acid molecules encoding an antibody, antibody chain or variable region thereof the invention effective in such treatment and a pharmaceutically acceptable carrier. In certain embodiments, the compositions may further comprise another component, such as a therapeutic agent or a diagnostic agent. Diagnostic and therapeutic methods are also provided by the invention. In certain embodiments, the compositions are used in a therapeutically effective amount necessary to treat or prevent a particular disease or condition.

[0016] The invention also provides methods for treating or preventing a variety of diseases and conditions such as, but not limited to, lupus, inflammation, cancer, atherogenesis, neurological disorders and cardiac disorders with an effective amount of an anti-M-CSF antibody of the invention, or antigen binding portion thereof, nucleic acids encoding said antibody, or heavy and/or light chain, the variable regions, or antigen-binding portions thereof.

[0017] The invention provides isolated cell lines, such as a hybridomas, that produce anti-M-CSF antibodies or antigen-binding portions thereof.

[0018] The invention also provides nucleic acid molecules encoding the heavy and/or light chains of anti-M-CSF antibodies, the variable regions thereof, or the antigen-binding portions thereof.

[0019] The invention provides vectors and host cells comprising the nucleic acid molecules, as well as methods of recombinantly producing the polypeptides encoded by the nucleic acid molecules.

[0020] Non-human transgenic animals or plants that express the heavy and/or light chains, or antigen-binding portions thereof, of anti-M-CSF antibodies are also provided.

[0021] The anti-M-CSF antibodies or antigen binding portions therof, compositions, cell lines, nucleic acid molecules, vectors, host cells, and methods of treating or prevent diseases using the anti-M-CSF antibodies are fully described herein as well as in PCT Application Number PCT/US2004/029390, published as WO 2005/030124, which is incorporated herein by reference in its entirety.

BRIEF DESCRIPTION OF THE DRAWINGS

[0022] Figures 1A and 1B are graphs illustrating that the anti-M-CSF antibodies resulted in a dose-related decrease in total monocyte counts in male and female monkeys over time. The monocyte counts were determined by light scatter using an Abbott Diagnostics Inc. Cell Dyn system. Monocyte counts were monitored from 24 hours through 3 weeks after administration of vehicle or antibody 8.10.3 at 0, 0.1, 1 or 5 mg/kg in a dose volume of 3.79 mL/kg over an approximately 5 minute period.

[0023] Figure 1A male monkeys.

[0024] Figure 1B female monkeys.

[0025] Figures 2A and 2B are graphs illustrating that anti-M-CSF treatment resulted in a reduction in the percentage of CD14+CD16+ monocytes, in male and female monkeys. 0-21 days after administration of vehicle or antibody 8.10.3 at 0, 0.1, 1 or 5 mg/kg in a dose volume of 3.79 mL/kg over an approximately 5 minute period. For each monkey tested, the percentage of monocytes within the CD14+CD16+ subset was determined after each blood draw, on days 1, 3, 7, 14 and 21 after 8.10.3 injection.

[0026] Figure 2A male monkeys.

[0027] Figure 2B female monkeys.

[0028] Figures 3A and 3B are graphs illustrating that anti-M-CSF treatment resulted in a decrease in the percentage change of total

monocytes at all doses of antibody 8.10.3F and antibody 9.14.4I as compared to pre-test levels of monocytes.

[0029] Figure 3A shows data collected from experiments using antibody 8.10.3F.

[0030] Figure 3B shows data collected from experiments using antibody 9.14.4I.

[0031] Figure 4 is a sequence alignment of the predicted amino acid sequences of light and heavy chain variable regions from twenty-six anti-M-CSF antibodies compared with the germline amino acid sequences of the corresponding variable region genes. Differences between the antibody sequences and the germline gene sequences are indicated in bold-faced type. Dashes represent no change from germline. The underlined sequences in each alignment represent, from left to right, the FR1, CDR1, FR2, CDR2, FR3, CDR3 AND FR4 sequences.

[0032] Figure 4A shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 252 (residues 21-127 of SEQ ID NO: 4) to the germline $V_{\kappa}O12$, $J_{\kappa}3$ sequence (SEQ ID NO: 103).

[0033] Figure 4B shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 88 (residues 21-127 of SEQ ID NO: 8) to the germline $V_{\kappa}O12$, $J_{\kappa}3$ sequence (SEQ ID NO: 103).

[0034] Figure 4C shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 100 (residues 21-127 of SEQ ID NO: 12) to the germline $V_{\kappa}L2$, $J_{\kappa}3$ sequence (SEQ ID NO: 107).

[0035] Figure 4D shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 3.8.3 (residues 23-130 of SEQ ID NO: 16) to the germline $V_{\kappa}L5$, $J_{\kappa}3$ sequence (SEQ ID NO: 109).

[0036] Figure 4E shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 2.7.3 (residues 23-

130 of SEQ ID NO: 20) to the germline V_κL5, J_κ4 sequence (SEQ ID NO: 117).

[0037] Figure 4F shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 1.120.1 (residues 21-134 of SEQ ID NO: 24) to the germline V_κB3, J_κ1 sequence (SEQ ID NO: 112).

[0038] Figure 4G shows an alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 252 (residues 20-136 of SEQ ID NO: 2) to the germline V_H3-11, D_H7-27 J_H6 sequence (SEQ ID NO: 106).

[0039] Figure 4H shows an alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 88 (residues 20-138 of SEQ ID NO: 6) to the germline V_H3-7, D_H6-13, J_H4 sequence (SEQ ID NO: 105).

[0040] Figure 4I shows the alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 100 (residues 20-141 of SEQ ID NO: 10) to the germline V_H3-23, D_H1-26, J_H4 sequence (SEQ ID NO: 104).

[0041] Figure 4J shows an alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 3.8.3 (residues 20-135 of SEQ ID NO: 14) to the germline V_H3-11, D_H7-27, J_H4 sequence (SEQ ID NO: 108).

[0042] Figure 4K shows an alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 2.7.3 (residues 20-137 of SEQ ID NO: 18) to the germline V_H3-33, D_H1-26, J_H4 sequence (SEQ ID NO: 110).

[0043] Figure 4L shows an alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 1.120.1 (residues 20-139 of SEQ ID NO: 22) to the germline V_H1-18, D_H4-23, J_H4 sequence (SEQ ID NO: 111).

[0044] Figure 4M shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 8.10.3 (residues 21-

129 of SEQ ID NO: 44) to the germline $V_\kappa A27$, $J_\kappa 4$ sequence (SEQ ID NO: 114).

[0045] Figure 4N shows an alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 8.10.3 (residues 20-141 of SEQ ID NO: 30) to the germline $V_H 3$-48, $D_H 1$-26, $J_H 4b$ sequence (SEQ ID NO: 113).

[0046] Figure 4O shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 9.14.4 (residues 23-130 of SEQ ID NO: 28) to the germline $V_\kappa O12$, $J_\kappa 3$ sequence (SEQ ID NO: 103).

[0047] Figure 4P shows an alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 9.14.4 (residues 20-135 of SEQ ID NO: 38) to the germline $V_H 3$-11, $D_H 7$-27, $J_H 4b$ sequence (SEQ ID NO: 116).

[0048] Figure 4Q shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 9.7.2 (residues 23-130 of SEQ ID NO: 48) to the germline $V_\kappa O12$, $J_\kappa 3$ sequence (SEQ ID NO: 103).

[0049] Figure 4R shows an alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 9.7.2 (residues 20-136 of SEQ ID NO: 46) to the germline $V_H 3$-11, $D_H 6$-13, $J_H 6b$ sequence (SEQ ID NO: 115).

[0050] Figure 4S shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 9.14.4I (residues 23-130 of SEQ ID NO: 28) to the germline $V_\kappa O12$ $J_\kappa 3$ sequence (SEQ ID NO: 103).

[0051] Figure 4T shows an alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 9.14.4I (residues 20-135 of SEQ ID NO: 26) to the germline $V_H 3$-11, $D_H 7$-27, $J_H 4b$ sequence (SEQ ID NO: 116).

[0052] Figure 4U shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 8.10.3F (residues

21-129 of SEQ ID NO: 32) to the germline V_κA27, J_κ4 sequence (SEQ ID NO: 114).

[0053] Figure 4V shows an alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 8.10.3F (residues 20-141 of SEQ ID NO: 30) to the germline V_H3-48, D_H1-26, J_H4b sequence (SEQ ID NO: 113).

[0054] Figure 4W shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 9.7.2IF (residues 23-130 of SEQ ID NO: 36) to the germline V_κO12, J_κ3 sequence (SEQ ID NO: 103).

[0055] Figure 4X shows an alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 9.7.2IF (residues 20-136 of SEQ ID NO: 34) to the germline V_H3-11, D_H6-13, J_H6b sequence (SEQ ID NO: 115).

[0056] Figure 4Y shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 9.7.2C-Ser (residues 23-130 of SEQ ID NO: 52) to the germline V_κO12, J_κ3 sequence (SEQ ID NO: 103).

[0057] Figure 4Z shows an alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 9.7.2C-Ser (residues 20-136 of SEQ ID NO: 50) to the germline V_H3-11, D_H6-13, J_H6b sequence (SEQ ID NO: 115).

[0058] Figure 4AA shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 9.14.4C-Ser (residues 23-130 of SEQ ID NO: 56) to the germline V_κO12, J_κ3 sequence (SEQ ID NO: 103).

[0059] Figure 4BB shows an alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 9.14.4C-Ser (residues 20-135 of SEQ ID NO: 54) to the germline V_H3-11, D_H7-27, J_H4b sequence (SEQ ID NO: 116).

[0060] Figure 4CC shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 8.10.3C-Ser

(residues 21-129 of SEQ ID NO: 60) to the germline V_κA27, J_κ4 sequence (SEQ ID NO: 114).

[0061] Figure 4DD shows an alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 8.10.3C-Ser (residues 20-141 of SEQ ID NO: 58) to the germline V_H3-48, D_H1-26, J_H4b sequence (SEQ ID NO: 113).

[0062] Figure 4EE shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 8.10.3-CG2 (residues 21-129 of SEQ ID NO: 60) to the germline V_κA27, J_κ4 sequence (SEQ ID NO: 114).

[0063] Figure 4FF shows an alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 8.10.3-CG2 (residues 20-141 of SEQ ID NO: 62) to the germline V_H3-48, D_H1-26, J_H4b sequence (SEQ ID NO: 113).

[0064] Figure 4GG shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 9.7.2-CG2 (residues 23-130 of SEQ ID NO: 52) to the germline V_κO12, J_κ3 sequence (SEQ ID NO: 103).

[0065] Figure 4HH shows an alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 9.7.2-CG2 (residues 20-136 of SEQ ID NO: 66) to the germline V_H3-11, D_H6-13, J_H6b sequence (SEQ ID NO: 115).

[0066] Figure 4II shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 9.7.2-CG4 (residues 23-130 of SEQ ID NO: 52) to the germline V_κO12, J_κ3 sequence (SEQ ID NO: 103).

[0067] Figure 4JJ shows an alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 9.7.2-CG4 (residues 20-135 of SEQ ID NO: 70) to the germline V_H3-11, D_H6-13, J_H6b sequence (SEQ ID NO: 115).

[0068] Figure 4KK shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 9.14.4-CG2

(residues 23-130 of SEQ ID NO: 56) to the germline $V_\kappa O12$, $J_\kappa 3$ sequence (SEQ ID NO: 103).

[0069] Figure 4LL shows an alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 9.14.4-CG2 (residues 20-135 of SEQ ID NO: 74) to the germline $V_H 3$-11, $D_H 7$-27, $J_H 4b$ sequence (SEQ ID NO: 116).

[0070] Figure 4MM shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 9.14.4-CG4 (residues 23-130 of SEQ ID NO: 56) to the germline $V_\kappa O12$, $J_\kappa 3$ sequence (SEQ ID NO: 103).

[0071] Figure 4NN shows an alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 9.14.4-CG4 (residues 20-135 of SEQ ID NO: 78) to the germline $V_H 3$-11, $D_H 7$-27, $J_H 4b$ sequence (SEQ ID NO: 116).

[0072] Figure 4OO shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 9.14.4-Ser (residues 23-130 of SEQ ID NO: 28) to the germline $V_\kappa O12$, $J_\kappa 3$ sequence (SEQ ID NO: 103).

[0073] Figure 4PP shows an alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 9.14.4-Ser (residues 20-135 of SEQ ID NO: 82) to the germline $V_H 3$-11, $D_H 7$-27, $J_H 4b$ sequence (SEQ ID NO: 116).

[0074] Figure 4QQ shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 9.7.2-Ser (residues 23-130 of SEQ ID NO: 48) to the germline $V_\kappa O12$, $J_\kappa 3$ sequence (SEQ ID NO: 103).

[0075] Figure 4RR shows an alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 9.7.2-Ser (residues 20-136 of SEQ ID NO: 86) to the germline $V_H 3$-11, $D_H 6$-13, $J_H 6b$ sequence (SEQ ID NO: 115).

[0076] Figure 4SS shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 8.10.3-Ser

(residues 21-129 of SEQ ID NO: 44) to the germline V_κA27, J_κ4 sequence (SEQ ID NO: 114).

[0077] Figure 4TT shows an alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 8.10.3-Ser (residues 20-141 of SEQ ID NO: 90) to the germline V_H3-48, D_H1-26, J_H4b sequence (SEQ ID NO: 113).

[0078] Figure 4UU shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 8.10.3-CG4 (residues 21-129 of SEQ ID NO: 60) to the germline V_κA27, J_κ4 sequence (SEQ ID NO: 114).

[0079] Figure 4VV shows an alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 8.10.3-CG4 (residues 20-141 of SEQ ID NO: 94) to the germline V_H3-48, D_H1-26, J_H4b sequence (SEQ ID NO: 113).

[0080] Figure 4WW shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 9.14.4G1 (residues 23-130 of SEQ ID NO: 28) to the germline V_κO12 J_κ3 sequence (SEQ ID NO: 103).

[0081] Figure 4XX shows an alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 9.14.4G1 (residues 20-135 of SEQ ID NO: 102) to the germline V_H3-11, D_H7-27, J_H4b sequence (SEQ ID NO: 116).

[0082] Figure 4YY shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 8.10.3FG1 (residues 21-129 of SEQ ID NO:32) to the germline V_κA27, J_κ4 sequence (SEQ ID NO: 114).

[0083] Figure 4ZZ shows an alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 8.10.3FG1 (residues 20-141 of SEQ ID NO: 98) to the germline V_H3-48, D_H1-26, J_H4b sequence (SEQ ID NO: 113).

[0084] Figure 5 is a graph depicting the effect of anti-M-CSF antibody on the development of lympadenopathy in the murine MRL-lpr model of lupus.

Mice (n = 10/group) were dosed with saline (diamond), anti-M-CSF Ab, 5A1 (square), CHOCK IgG1 (triangle) or CTLA-4Ig (X) 3x/wk for 12 weeks and scored for lymphadenopathy as described in the Examples. *Anti-M-CSF treated group is significantly different ($p < 0.05$) from saline. **Anti-M-CSF-treated group is significantly different ($p < 0.05$) from saline and CTLA-4Ig. Anti-M-CSF group was not significantly different from CHOCK IgG1 treated group ($p = 0.065$, weeks 6 through 12).

[0085] Figure 6 is a graph depicting the effect of anti-M-CSF antibody on the development of skin lesions in the murine MRL-lpr model of lupus. Mice (n = 10 per group) were dosed with saline (diamond), anti-M-CSF Ab, 5A1 (square), CHOCK IgG1 (triangle) or CTLA-4Ig (X) 3x/week for 12 weeks and scored for skin lesions as described in the Examples. *Anti-M CSF treated group is significantly different ($p < 0.05$) from saline. **Anti-M CSF-treated group is significantly different ($p < 0.05$) from saline and CTLA-4Ig. Anti-M CSF group was not significantly different from CHOCK IgG1 treated group ($p = 0.065$, weeks 6 through 12).

[0086] Figure 7 is a graph depicting the effect of anti-M-CSF antibody on the development of anti-dsDNA autoantibodies in murine MRL-Lpr model of lupus. Anti-dsDNA antibody titers were determined at 4 time points for mice treated with saline (diamond), CTLA-4Ig (square), anti-M CSF Ab 5A1 (triangle) or CHOCK IgG1 isotype control (X). *CTLA-4Ig is significantly different from saline and anti-M CSF ($p < 0.05$). **Anti-M CSF-treated group is significantly different ($p < 0.05$) from CHOCK IgG1.

[0087] Figure 8 is a graph depicting the effect of anti-M-CSF antibody on glomerular nuclear area and C3 deposition in murine MRL-lpr model of lupus. Glomerular nuclear area (left) and immunohistochemical staining for C3 deposition (right) was determined microscopically from kidneys harvested at the termination of study for mice treated with saline, CTLA-4Ig, anti-M CSF Ab 5A1, or CHOCK IgG1 isotype control. Data bars represent average mean score and lines are standard error.

[0088] Figure 9 is a graph depicting the effect of anti-M-CSF antibody on the development of proteinuria in the murine NZBWF1/J lupus model. Mice

treated with saline (square), CHOCK IgG1 isotype control (triangle) or anti-M CSF antibody (circle), were scored for proteinuria using the following scale: 0 = negative; 1 = trace; 2 = >30 mg/dL; 3 = >100 mg/dL; 4 = >300 mg/dL; and 5 = >2000 mg/dL. Figure 9A depicts mean proteinuria scores for each group measured biweekly. Figure 9B depicts individual mouse proteinuria scores at week 10.

[0089] Figure 10 is a graph depicting the effect of anti-M-CSF antibody on the development of anti-dsDNA antibody titres in the murine NZBWF1/J lupus model. Anti-dsDNA antibody levels were determined in mice treated with saline (circle), CHOCK IgG1 isotype control (triangle) or anti-M CSF antibody (diamond) by ELISA as described in the Examples. Figure 10A depicts the antibody titres at the week 6 time point. Figure 10B depicts the antibody titres at the week 10 time point.

[0090] Figure 11 is a graph depicting the effect of anti-M-CSF antibody on serum levels of M-CSF in the murine NZBWF1/J lupus model. Serum levels of M-CSF were determined by specific ELISA from serum collected at the termination of the study from saline (circle), isotype control (square) or anti-M CSF (triangle) treated mice.

[0091] Figure 12 is a graph depicting the effect of anti-M-CSF antibody on immune complex deposition and macrophage infiltration in the kidney of NZBWF1/J mice. Bars represent group mean renal immunohistochemical staining scores for mice treated with saline, CHOCK IgG1 control, or anti-M-CSF antibody, and lines indicate standard errors.

[0092] Figure 13 is a graph depicting mean serum antibody 8.10.3F concentration time profiles following administration of single intravenous solution doses to healthy subjects. Upper and lower panels are linear and semi-logarithmic scales, respectively. Legend is antibody dose in mg. Concentrations after 700 hours were either zero (below limit of quantitation) or represented <3 subjects.

[0093] Figure 14 is a graph depicting antibody 8,10.3F Cmax (Upper Panel) and AUC(0-∞) (Lower Panel) values following administration of single intravenous solution doses to healthy subjects. Left panels show

observed values; right panels show dose-normalized values. Circles are individual subjects, diamonds are arithmetic means

[0094] Figure 15 is a graph depicting mean antibody 8.10.3F (open squares) and M-CSF (X) concentrations following administration of a single intravenous 100-mg dose to healthy subjects.

[0095] Figure 16 is a graph depicting $CD14^{+}16^{+}$ monocyte dose response on Study Day 28 following administration of single intravenous solution doses to healthy subjects.

[0096] Figure 17 is a graph depicting $CD14^{+}16^{+}$ time response following administration of a single, 100-mg intravenous solution dose to healthy subjects.

[0097] Figure 18 is a graph depicting mean antibody 8.10.3F (open squares) concentrations and $CD14^{+}16^{+}$ monocyte counts (X) following administration of single intravenous 100-mg doses to healthy subjects,

[0098] Figure 19 is a graph depicting mean antibody 8.10.3F concentrations (open squares) and uNTX-1 (X) following administration of a single intravenous 100-mg dose to healthy subjects,

DETAILED DESCRIPTION OF THE INVENTION

Definitions and General Techniques

[0099] Unless otherwise defined herein, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclatures used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well known and commonly used in the art.

[0100] The methods and techniques of the present invention are generally performed according to conventional methods well known in the

art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. See, e.g., Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual,* 2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989) and Ausubel *et al.*, *Current Protocols in Molecular Biology*, Greene Publishing Associates (1992), and Harlow and Lane *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1990), which are incorporated herein by reference. Enzymatic reactions and purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. The nomenclatures used in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques are used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

[0101] The following terms, unless otherwise indicated, shall be understood to have the following meanings:

[0102] The term "polypeptide" encompasses native or artificial proteins, protein fragments and polypeptide analogs of a protein sequence. A polypeptide may be monomeric or polymeric.

[0103] The term "isolated protein", "isolated polypeptide" or "isolated antibody" is a protein, polypeptide or antibody that by virtue of its origin or source of derivation has one to four of the following: (1) is not associated with naturally associated components that accompany it in its native state, (2) is free of other proteins from the same species, (3) is expressed by a cell from a different species, or (4) does not occur in nature. Thus, a polypeptide that is chemically synthesized or synthesized in a cellular system different from the cell from which it naturally originates will be "isolated" from its naturally associated components. A protein may also be

rendered substantially free of naturally associated components by isolation, using protein purification techniques well known in the art.

[0104] Examples of isolated antibodies include an anti-M-CSF antibody that has been affinity purified using M-CSF, an anti-M-CSF antibody that has been synthesized by a hybridoma or other cell line *in vitro*, and a human anti-M-CSF antibody derived from a transgenic mouse.

[0105] A protein or polypeptide is "substantially pure," "substantially homogeneous," or "substantially purified" when at least about 60 to 75% of a sample exhibits a single species of polypeptide. The polypeptide or protein may be monomeric or multimeric. A substantially pure polypeptide or protein will typically comprise about 50%, 60%, 70%, 80% or 90% W/W of a protein sample, more usually about 95%, and preferably will be over 99% pure. Protein purity or homogeneity may be indicated by a number of means well known in the art, such as polyacrylamide gel electrophoresis of a protein sample, followed by visualizing a single polypeptide band upon staining the gel with a stain well known in the art. For certain purposes, higher resolution may be provided by using HPLC or other means well known in the art for purification.

[0106] The term "polypeptide fragment" as used herein refers to a polypeptide that has an amino-terminal and/or carboxy-terminal deletion, but where the remaining amino acid sequence is identical to the corresponding positions in the naturally-occurring sequence. In some embodiments, fragments are at least 5, 6, 8 or 10 amino acids long. In other embodiments, the fragments are at least 14, at least 20, at least 50, or at least 70, 80, 90, 100, 150 or 200 amino acids long.

[0107] The term "polypeptide analog" as used herein refers to a polypeptide that comprises a segment that has substantial identity to a portion of an amino acid sequence and that has at least one of the following properties: (1) specific binding to M-CSF under suitable binding conditions, (2) ability to inhibit M-CSF.

[0108] Typically, polypeptide analogs comprise a conservative amino acid substitution (or insertion or deletion) with respect to the

normally-occurring sequence. Analogs typically are at least 20 or 25 amino acids long, preferably at least 50, 60, 70, 80, 90, 100, 150 or 200 amino acids long or longer, and can often be as long as a full-length polypeptide.

[0109] In certain embodiments, amino acid substitutions of the antibody or antigen-binding portion thereof are those which: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter binding affinity for forming protein complexes, or (4) confer or modify other physicochemical or functional properties of such analogs. Analogs can include various muteins of a sequence other than the normally-occurring peptide sequence. For example, single or multiple amino acid substitutions (preferably conservative amino acid substitutions) may be made in the normally-occurring sequence, preferably in the portion of the polypeptide outside the domain(s) forming intermolecular contacts.

[0110] A conservative amino acid substitution should not substantially change the structural characteristics of the parent sequence; e.g., a replacement amino acid should not alter the anti-parallel β-sheet that makes up the immunoglobulin binding domain that occurs in the parent sequence, or disrupt other types of secondary structure that characterizes the parent sequence. In general, glycine and proline analogs would not be used in an anti-parallel β-sheet. Examples of art-recognized polypeptide secondary and tertiary structures are described in *Proteins, Structures and Molecular Principles* (Creighton, Ed., W. H. Freeman and Company, New York (1984)); *Introduction to Protein Structure* (C. Branden and J. Tooze, eds., Garland Publishing, New York, N.Y. (1991)); and Thornton *et al.*, *Nature* 354:105 (1991), which are each incorporated herein by reference.

[0111] Non-peptide analogs are commonly used in the pharmaceutical industry as drugs with properties analogous to those of the template peptide. These types of non-peptide compound are termed "peptide mimetics" or "peptidomimetics." Fauchere, *J. Adv. Drug Res.* 15:29 (1986); Veber and Freidinger, *TINS* p.392 (1985); and Evans *et al.*, *J. Med. Chem.* 30:1229 (1987), which are incorporated herein by reference. Such compounds are often developed with the aid of computerized molecular

modeling. Peptide mimetics that are structurally similar to therapeutically useful peptides may be used to produce an equivalent therapeutic or prophylactic effect. Generally, peptidomimetics are structurally similar to a paradigm polypeptide (i.e., a polypeptide that has a desired biochemical property or pharmacological activity), such as a human antibody, but have one or more peptide linkages optionally replaced by a linkage selected from the group consisting of: --CH_2NH--, --CH_2S--, --CH_2-CH_2--, --CH=CH--(cis and trans), --$COCH_2$--, --$CH(OH)CH_2$--, and –CH_2SO--, by methods well known in the art. Systematic substitution of one or more amino acids of a consensus sequence with a D-amino acid of the same type (e.g., D-lysine in place of L-lysine) may also be used to generate more stable peptides. In addition, constrained peptides comprising a consensus sequence or a substantially identical consensus sequence variation may be generated by methods known in the art (Rizo and Gierasch, *Ann. Rev. Biochem.* 61:387 (1992), incorporated herein by reference); for example, by adding internal cysteine residues capable of forming intramolecular disulfide bridges which cyclize the peptide.

[0112] An "antibody" refers to an intact antibody or an antigen-binding portion that competes with the intact antibody for specific binding. See generally, Fundamental Immunology, Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989)) (incorporated by reference in its entirety for all purposes). Antigen-binding portions may be produced by recombinant DNA techniques or by enzymatic or chemical cleavage of intact antibodies. In some embodiments, antigen-binding portions include Fab, Fab', $F(ab')_2$, Fd, Fv, dAb, and complementarity determining region (CDR) fragments, single-chain antibodies (scFv), chimeric antibodies, diabodies and polypeptides that contain at least a portion of an antibody that is sufficient to confer specific antigen binding to the polypeptide.

[0113] From N-terminus to C-terminus, both the mature light and heavy chain variable domains comprise the regions FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is in accordance with the definitions of Kabat, *Sequences of Proteins of*

Immunological Interest (National Institutes of Health, Bethesda, Md. (1987 and 1991)), Chothia & Lesk, *J. Mol. Biol.* 196:901-917 (1987), or Chothia *et al.*, *Nature* 342:878-883 (1989).

[0114] As used herein, an antibody that is referred to by number is the same as a monoclonal antibody that is obtained from the hybridoma of the same number. For example, monoclonal antibody 3.8.3 is the same antibody as one obtained from hybridoma 3.8.3.

[0115] As used herein, a Fd fragment means an antibody fragment that consists of the V_H and C_H 1 domains; an Fv fragment consists of the V_L and V_H domains of a single arm of an antibody; and a dAb fragment (Ward *et al.*, *Nature* 341:544-546 (1989)) consists of a V_H domain.

[0116] In some embodiments, the antibody is a single-chain antibody (scFv) in which a V_L and a V_H domain are paired to form a monovalent molecule via a synthetic linker that enables them to be made as a single protein chain. (Bird *et al.*, *Science* 242:423-426 (1988) and Huston *et al.*, *Proc. Natl. Acad. Sci. USA* 85:5879-5883 (1988).) In some embodiments, the antibodies are diabodies, i.e., are bivalent antibodies in which V_H and V_L domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites. (See e.g., Holliger P. *et al.*, *Proc. Natl. Acad. Sci. USA* 90:6444-6448 (1993), and Poljak R. J. *et al.*, *Structure* 2:1121-1123 (1994).) In some embodiments, one or more CDRs from an antibody of the invention may be incorporated into a molecule either covalently or noncovalently to make it an immunoadhesin that specifically binds to M-CSF. In such embodiments, the CDR(s) may be incorporated as part of a larger polypeptide chain, may be covalently linked to another polypeptide chain, or may be incorporated noncovalently.

[0117] In embodiments having one or more binding sites, the binding sites may be identical to one another or may be different.

[0118] As used herein, the term "human antibody" means any antibody in which the variable and constant domain sequences are human sequences.

The term encompasses antibodies with sequences derived from human genes, but which have been changed, e.g. to decrease possible immunogenicity, increase affinity, eliminate cysteines that might cause undesirable folding, etc. The term emcompasses such antibodies produced recombinantly in non-human cells, which might impart glycosylation not typical of human cells. These antibodies may be prepared in a variety of ways, as described below.

[0119] The term "chimeric antibody" as used herein means an antibody that comprises regions from two or more different antibodies. In one embodiment, one or more of the CDRs are derived from a human anti-M-CSF antibody. In another embodiment, all of the CDRs are derived from a human anti-M-CSF antibody. In another embodiment, the CDRs from more than one human anti-M-CSF antibodies are combined in a chimeric antibody. For instance, a chimeric antibody may comprise a CDR1 from the light chain of a first human anti-M-CSF antibody, a CDR2 from the light chain of a second human anti-M-CSF antibody and a CDR3 from the light chain of a third human anti-M-CSF antibody, and the CDRs from the heavy chain may be derived from one or more other anti-M-CSF antibodies. Further, the framework regions may be derived from one of the anti-M-CSF antibodies from which one or more of the CDRs are taken or from one or more different human antibodies.

[0120] Fragments or analogs of antibodies or immunoglobulin molecules can be readily prepared by those of ordinary skill in the art following the teachings of this specification. Preferred amino- and carboxy-termini of fragments or analogs occur near boundaries of functional domains. Structural and functional domains can be identified by comparison of the nucleotide and/or amino acid sequence data to public or proprietary sequence databases. Preferably, computerized comparison methods are used to identify sequence motifs or predicted protein conformation domains that occur in other proteins of known structure and/or function. Methods to identify protein sequences that fold into a known three-dimensional structure are known. See Bowie *et al.*, *Science* 253:164 (1991).

[0121] The term "surface plasmon resonance", as used herein, refers to an optical phenomenon that allows for the analysis of real-time biospecific interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIACORE™ system (Pharmacia Biosensor AB, Uppsala, Sweden and Piscataway, N.J.). For further descriptions, see Jonsson U. *et al.*, *Ann. Biol. Clin.* 51:19-26 (1993); Jonsson U. *et al.*, *Biotechniques* 11:620-627 (1991); Jonsson B. *et al.*, *J. Mol. Recognit.* 8:125-131 (1995); and Johnsson B. *et al.*, *Anal. Biochem.* 198:268-277 (1991).

[0122] The term "K_D" refers to the equilibrium dissociation constant of a particular antibody-antigen interaction.

[0123] The term "epitope" includes any protein determinant capable of specific binding to an immunoglobulin or T-cell receptor or otherwise interacting with a molecule. Epitopic determinants generally consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and generally have specific three dimensional structural characteristics, as well as specific charge characteristics. An epitope may be "linear" or "conformational." In a linear epitope, all of the points of interaction between the protein and the interacting molecule (such as an antibody) occur linearally along the primary amino acid sequence of the protein. In a conformational epitope, the points of interaction occur across amino acid residues on the protein that are separated from one another. An antibody is said to specifically bind an antigen when the dissociation constant is ≤ 1 mM, preferably ≤ 100 nM and most preferably ≤ 10 nM. In certain embodiments, the K_D is 1 pM to 500 pM. In other embodiments, the K_D is between 500 pM to 1 μM. In other embodiments, the K_D is between 1 μM to 100 nM. In other embodiments, the K_D is between 100 mM to 10 nM. Once a desired epitope on an antigen is determined, it is possible to generate antibodies to that epitope, e.g., using the techniques described in the present invention. Alternatively, during the discovery process, the generation and characterization of antibodies may elucidate information about desirable epitopes. From this information, it is then possible to

competitively screen antibodies for binding to the same epitope. An approach to achieve this is to conduct cross-competition studies to find antibodies that competitively bind with one another, e.g., the antibodies compete for binding to the antigen. A high throughout process for "binning" antibodies based upon their cross-competition is described in International Patent Application No. WO 03/48731.

[0124] As used herein, the twenty conventional amino acids and their abbreviations follow conventional usage. See *Immunology - A Synthesis* (2nd Edition, E.S. Golub and D.R. Gren, Eds., Sinauer Associates, Sunderland, Mass. (1991)), which is incorporated herein by reference.

[0125] The term "polynucleotide" as referred to herein means a polymeric form of nucleotides of at least 10 bases in length, either ribonucleotides or deoxynucleotides or a modified form of either type of nucleotide. The term includes single and double stranded forms.

[0126] The term "isolated polynucleotide" as used herein means a polynucleotide of genomic, cDNA, or synthetic origin or some combination thereof, which by virtue of its origin or source of derivation, the "isolated polynucleotide" has one to three of the following: (1) is not associated with all or a portion of a polynucleotides with which the "isolated polynucleotide" is found in nature, (2) is operably linked to a polynucleotide to which it is not linked in nature, or (3) does not occur in nature as part of a larger sequence.

[0127] The term "oligonucleotide" as used herein includes naturally occurring, and modified nucleotides linked together by naturally occurring and non-naturally occurring oligonucleotide linkages. Oligonucleotides are a polynucleotide subset generally comprising a length of 200 bases or fewer. Preferably oligonucleotides are 10 to 60 bases in length and most preferably 12, 13, 14, 15, 16, 17, 18, 19, or 20 to 40 bases in length. Oligonucleotides are usually single stranded, e.g. for primers and probes; although oligonucleotides may be double stranded, e.g. for use in the construction of a gene mutant. Oligonucleotides of the invention can be either sense or antisense oligonucleotides.

[0128] The term "naturally occurring nucleotides" as used herein includes deoxyribonucleotides and ribonucleotides. The term "modified nucleotides" as used herein includes nucleotides with modified or substituted sugar groups and the like. The term "oligonucleotide linkages" referred to herein includes oligonucleotides linkages such as phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phoshoraniladate, phosphoroamidate, and the like. See e.g., LaPlanche *et al.*, *Nucl. Acids Res.* 14:9081 (1986); Stec *et al.*, *J. Am. Chem. Soc.* 106:6077 (1984); Stein *et al.*, *Nucl. Acids Res.* 16:3209 (1988); Zon *et al.*, *Anti-Cancer Drug Design* 6:539 (1991); Zon *et al.*, *Oligonucleotides and Analogues: A Practical Approach*, pp. 87-108 (F. Eckstein, Ed., Oxford University Press, Oxford England (1991)); U.S. Patent No. 5,151,510; Uhlmann and Peyman, *Chemical Reviews* 90:543 (1990), the disclosures of which are hereby incorporated by reference. An oligonucleotide can include a label for detection, if desired.

[0129] "Operably linked" sequences include both expression control sequences that are contiguous with the gene of interest and expression control sequences that act in *trans* or at a distance to control the gene of interest. The term "expression control sequence" as used herein means polynucleotide sequences that are necessary to effect the expression and processing of coding sequences to which they are ligated. Expression control sequences include appropriate transcription initiation, termination, promoter and enhancer sequences; efficient RNA processing signals such as splicing and polyadenylation signals; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (i.e., Kozak consensus sequence); sequences that enhance protein stability; and when desired, sequences that enhance protein secretion. The nature of such control sequences differs depending upon the host organism; in prokaryotes, such control sequences generally include promoter, ribosomal binding site, and transcription termination sequence; in eukaryotes, generally, such control sequences include promoters and transcription termination sequence. The term "control sequences" is intended to include,

at a minimum, all components whose presence is essential for expression and processing, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences.

[0130] The term "vector", as used herein, means a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. In some embodiments, the vector is a plasmid, i.e., a circular double stranded DNA loop into which additional DNA segments may be ligated. In some embodiments, the vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. In some embodiments, the vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). In other embodiments, the vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply, "expression vectors").

[0131] The term "recombinant host cell" (or simply "host cell"), as used herein, means a cell into which a recombinant expression vector has been introduced. It should be understood that "recombinant host cell" and "host cell" mean not only the particular subject cell but also the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term "host cell" as used herein.

[0132] The term "selectively hybridize" referred to herein means to detectably and specifically bind. Polynucleotides, oligonucleotides and fragments thereof in accordance with the invention selectively hybridize to nucleic acid strands under hybridization and wash conditions that minimize appreciable amounts of detectable binding to nonspecific nucleic acids.

"High stringency" or "highly stringent" conditions can be used to achieve selective hybridization conditions as known in the art and discussed herein. One example of "high stringency" or "highly stringent" conditions is the incubation of a polynucleotide with another polynucleotide, wherein one polynucleotide may be affixed to a solid surface such as a membrane, in a hybridization buffer of 6X SSPE or SSC, 50% formamide, 5X Denhardt's reagent, 0.5% SDS, 100 µg/ml denatured, fragmented salmon sperm DNA at a hybridization temperature of 42°C for 12-16 hours, followed by twice washing at 55°C using a wash buffer of 1X SSC, 0.5% SDS. See also Sambrook *et al.*, *supra*, pp. 9.50-9.55.

[0133] The term "percent sequence identity" in the context of nucleic acid sequences means the percent of residues when a first contiguous sequence is compared and aligned for maximum correspondence to a second contiguous sequence. The length of sequence identity comparison may be over a stretch of at least about nine nucleotides, usually at least about 18 nucleotides, more usually at least about 24 nucleotides, typically at least about 28 nucleotides, more typically at least about 32 nucleotides, and preferably at least about 36, 48 or more nucleotides. There are a number of different algorithms known in the art which can be used to measure nucleotide sequence identity. For instance, polynucleotide sequences can be compared using FASTA, Gap or Bestfit, which are programs in Wisconsin Package Version 10.0, Genetics Computer Group (GCG), Madison, Wisconsin. FASTA, which includes, e.g., the programs FASTA2 and FASTA3, provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson, *Methods Enzymol.* 183:63-98 (1990); Pearson, *Methods Mol. Biol.* 132:185-219 (2000); Pearson, *Methods Enzymol.* 266:227-258 (1996); Pearson, *J. Mol. Biol.* 276:71-84 (1998); herein incorporated by reference). Unless otherwise specified, default parameters for a particular program or algorithm are used. For instance, percent sequence identity between nucleic acid sequences can be determined using FASTA with its default parameters (a word size of 6 and the NOPAM factor for the scoring matrix)

or using Gap with its default parameters as provided in GCG Version 6.1, herein incorporated by reference.

[0134] A reference to a nucleotide sequence encompasses its complement unless otherwise specified. Thus, a reference to a nucleic acid having a particular sequence should be understood to encompass its complementary strand, with its complementary sequence.

[0135] The term "percent sequence identity" means a ratio, expressed as a percent of the number of identical residues over the number of residues compared.

[0136] The term "substantial similarity" or "substantial sequence similarity," when referring to a nucleic acid or fragment thereof, means that when optimally aligned with appropriate nucleotide insertions or deletions with another nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 85%, preferably at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% of the nucleotide bases, as measured by any well-known algorithm of sequence identity, such as FASTA, BLAST or Gap, as discussed above.

[0137] As applied to polypeptides, the term "substantial identity" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, as supplied with the programs, share at least 70%, 75%, 80% or 85% sequence identity, preferably at least 90%, 91%, 92%, 93%, 94% 95%, 96%, 97%, 98% or 99% sequence identity. In certain embodiments, residue positions that are not identical differ by conservative amino acid substitutions. A "conservative amino acid substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain R group with similar chemical properties (e.g., charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment

are well-known to those of skill in the art. See, e.g., Pearson, *Methods Mol. Biol.* 243:307-31 (1994). Examples of groups of amino acids that have side chains with similar chemical properties include 1) aliphatic side chains: glycine, alanine, valine, leucine, and isoleucine; 2) aliphatic-hydroxyl side chains: serine and threonine; 3) amide-containing side chains: asparagine and glutamine; 4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; 5) basic side chains: lysine, arginine, and histidine; 6) acidic side chains: aspartic acid and glutamic acid; and 7) sulfur-containing side chains: cysteine and methionine. Conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine.

[0138] Alternatively, a conservative replacement is any change having a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet *et al.*, *Science* 256:1443-45 (1992), herein incorporated by reference. A "moderately conservative" replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix.

[0139] Sequence identity for polypeptides, is typically measured using sequence analysis software. Protein analysis software matches sequences using measures of similarity assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For instance, GCG contains programs such as "Gap" and "Bestfit" which can be used with default parameters, as specified with the programs, to determine sequence homology or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild type protein and a mutein thereof. See, e.g., GCG Version 6.1. Polypeptide sequences also can be compared using FASTA using default or recommended parameters, see GCG Version 6.1. (University of Wisconsin WI) FASTA (e.g., FASTA2 and FASTA3) provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson, *Methods Enzymol.* 183:63-98 (1990); Pearson, *Methods Mol. Biol.*

132:185-219 (2000)). Another preferred algorithm when comparing a sequence of the invention to a database containing a large number of sequences from different organisms is the computer program BLAST, especially blastp or tblastn, using default parameters, as supplied with the programs. See, e.g., Altschul *et al.*, *J. Mol. Biol.* 215:403-410 (1990); Altschul *et al.*, *Nucleic Acids Res.* 25:3389-402 (1997).

[0140] The length of polypeptide sequences compared for homology will generally be at least about 16 amino acid residues, usually at least about 20 residues, more usually at least about 24 residues, typically at least about 28 residues, and preferably more than about 35 residues. When searching a database containing sequences from a large number of different organisms, it is preferable to compare amino acid sequences.

[0141] As used herein, the terms "label" or "labeled" refers to incorporation of another molecule in the antibody. In one embodiment, the label is a detectable marker, e.g., incorporation of a radiolabeled amino acid or attachment to a polypeptide of biotinyl moieties that can be detected by marked avidin (e.g., streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or colorimetric methods). In another embodiment, the label or marker can be therapeutic, e.g., a drug conjugate or toxin. Various methods of labeling polypeptides and glycoproteins are known in the art and may be used. Examples of labels for polypeptides include, but are not limited to, the following: radioisotopes or radionuclides (e.g., ^{3}H, ^{14}C, ^{15}N, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I), fluorescent labels (*e.g.*, FITC, rhodamine, lanthanide phosphors), enzymatic labels (*e.g.*, horseradish peroxidase, β-galactosidase, luciferase, alkaline phosphatase), chemiluminescent markers, biotinyl groups, predetermined polypeptide epitopes recognized by a secondary reporter (e.g., leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags), magnetic agents, such as gadolinium chelates, toxins such as pertussis toxin, taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione,

mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. In some embodiments, labels are attached by spacer arms of various lengths to reduce potential steric hindrance.

[0142] Throughout this specification and claims, the word "comprise," or variations such as "comprises" or "comprising," will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

Human Anti-M-CSF Antibodies and Characterization Thereof

[0143] In one embodiment, the invention provides humanized anti-M-CSF antibodies. In another embodiment, the invention provides human anti-M-CSF antibodies. In some embodiments, human anti-M-CSF antibodies are produced by immunizing a non-human transgenic animal, e.g., a rodent, whose genome comprises human immunoglobulin genes so that the rodent produces human antibodies.

[0144] An anti-M-CSF antibody of the invention can comprise a human kappa or a human lamda light chain or an amino acid sequence derived therefrom. In some embodiments comprising a kappa light chain, the light chain variable domain (V_L) is encoded in part by a human $V_\kappa O12$, $V_\kappa L2$, $V_\kappa L5$, $V_\kappa A27$ or $V_\kappa B3$ gene and a $J_\kappa 1$, $J_\kappa 2$, $J_\kappa 3$, or $J_\kappa 4$ gene. In particular embodiments of the invention, the light chain variable domain is encoded by $V_\kappa O12/J\kappa 3$, $V_\kappa L2/J\kappa 3$, $V_\kappa L5/J\kappa 3$, $V_\kappa L5/J\kappa 4$, $V_\kappa A27/ J\kappa 4$ or $V_\kappa B3/J\kappa 1$ gene.

[0145] In some embodiments, the V_L of the M-CSF antibody comprises one or more amino acid substitutions relative to the germline amino acid sequence. In some embodiments, the V_L of the anti-M-CSF antibody comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid substitutions relative to the germline amino acid sequence. In some embodiments, one or more of those substitutions from germline is in the CDR regions of the light chain. In some embodiments, the amino acid substitutions relative to germline are at one or more of the same positions as the substitutions relative to

germline in any one or more of the V_L of antibodies 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 or 9.14.4G1. For example, the V_L of the anti-M-CSF antibody may contain one or more amino acid substitutions compared to germline found in the V_L of antibody 88, and other amino acid substitutions compared to germline found in the V_L of antibody 252 which utilizes the same V_K gene as antibody 88. In some embodiments, the amino acid changes are at one or more of the same positions but involve a different mutation than in the reference antibody.

[0146] In some embodiments, amino acid changes relative to germline occur at one or more of the same positions as in any of the V_L of antibodies 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 or 9.14.4G1, but the changes may represent conservative amino acid substitutions at such position(s) relative to the amino acid in the reference antibody. For example, if a particular position in one of these antibodies is changed relative to germline and is glutamate, one may substitute aspartate at that position. Similarly, if an amino acid substitution compared to germline is serine, one may substitute threonine for serine at that position. Conservative amino acid substitutions are discussed *supra*.

[0147] In some embodiments, the light chain of the human anti-M-CSF antibody comprises the amino acid sequence that is the same as the amino acid sequence of the V_L of antibody 252 (SEQ ID NO: 4), 88 (SEQ ID NO: 8), 100 (SEQ ID NO: 12), 3.8.3 (SEQ ID NO: 16), 2.7.3 (SEQ ID NO: 20), 1.120.1 (SEQ ID NO: 24), 9.14.4I (SEQ ID NO: 28), 8.10.3F (SEQ ID NO: 32), 9.7.2IF (SEQ ID NO: 36), 9.14.4 (SEQ ID NO: 28), 8.10.3 (SEQ ID NO: 44), 9.7.2 (SEQ ID NO: 48), 9.7.2C-Ser (SEQ ID NO: 52), 9.14.4C-Ser (SEQ ID NO: 56), 8.10.3C-Ser (SEQ ID NO: 60), 8.10.3-CG2 (SEQ ID NO:

60), 9.7.2-CG2 (SEQ ID NO: 52), 9.7.2-CG4 (SEQ ID NO: 52), 9.14.4-CG2 (SEQ ID NO: 56), 9.14.4-CG4 (SEQ ID NO: 56), 9.14.4-Ser (SEQ ID NO: 28), 9.7.2-Ser (SEQ ID NO: 48), 8.10.3-Ser (SEQ ID NO: 44), 8.10.3-CG4 (SEQ ID NO: 60) 8.10.3FG1 (SEQ ID NO: 32) or 9.14.4G1 (SEQ ID NO: 28), or said amino acid sequence having up to 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 conservative amino acid substitutions and/or a total of up to 3 non-conservative amino acid substitutions. In some embodiments, the light chain comprises the amino acid sequence from the beginning of the CDR1 to the end of the CDR3 of any one of the foregoing antibodies.

[0148] In some embodiments, the light chain of the anti-M-CSF antibody comprises at least the light chain CDR1, CDR2 or CDR3 of a germline or antibody sequence, as described herein. In another embodiment, the light chain may comprise a CDR1, CDR2 or CDR3 regions of an antibody independently selected from 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 or 9.14.4G1, or CDR regions each having less than 4 or less than 3 conservative amino acid substitutions and/or a total of three or fewer non-conservative amino acid substitutions. In other embodiments, the light chain of the anti-M-CSF antibody comprises the light chain CDR1, CDR2 or CDR3, each of which are independently selected from the CDR1, CDR2 and CDR3 regions of an antibody having a light chain variable region comprising the amino acid sequence of the V_L region selected from SEQ ID NOS: 4, 8, 12, 16, 20, 24, 28, 32, 36, 44, 48, 52, 56 or 60, or encoded by a nucleic acid molecule encoding the V_L region selected from SEQ ID NOS: 3, 7, 11, 27, 31, 35, 43 or 47. The light chain of the anti-M-CSF antibody may comprise the CDR1, CDR2 and CDR3 regions of an antibody comprising the amino acid sequence of the V_L region selected from 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4,

9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 or 9.14.4G1 or SEQ ID NOS: 4, 8, 12, 16, 20, 24, 28, 32, 36, 44, 48, 52, 56 or 60.

[0149] In some embodiments, the light chain comprises the CDR1, CDR2 and CDR3 regions of antibody 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 or 9.14.4G1, or said CDR regions each having less than 4 or less than 3 conservative amino acid substitutions and/or a total of three or fewer non-conservative amino acid substitutions.

[0150] With regard to the heavy chain, in some embodiments, the variable region of the heavy chain amino acid sequence is encoded in part by a human V_H3-11, V_H3-23, V_H3-7, V_H1-18, V_H3-33, V_H3-48 gene and a J_H4, J_H6, J_H4b, or J_H6b gene. In a particular embodiment of the invention, the heavy chain variable region is encoded by V_H3-11/D_H7-27/J_H6, V_H3-7/D_H6-13/J_H4, V_H3-23/D_H1-26/J_H4, V_H3-11/D_H7-27/J_H4, V_H3-33/D_H1-26/J_H4, V_H1-18/D_H4-23/J_H4, V_H3-11/D_H7-27/J_H4b, V_H3-48/D_H1-26/J_H4b, V_H3-11/D_H6-13/J_H6b, V_H3-11/D_H7-27/J_H4b, V_H3-48/D_H1-6/J_H4b, or V_H3-11/D_H6-13/J_H6b gene. In some embodiments, the V_H of the anti-M-CSF antibody contains one or more amino acid substitutions, deletions or insertions (additions) relative to the germline amino acid sequence. In some embodiments, the variable domain of the heavy chain comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or 18 mutations from the germline amino acid sequence. In some embodiments, the mutation(s) are non-conservative substitutions compared to the germline amino acid sequence. In some embodiments, the mutations are in the CDR regions of the heavy chain. In some embodiments, the amino acid changes are made at one or more of the same positions as the mutations from germline in any one or more of the V_H of antibodies 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 or 9.14.4G1. In

other embodiments, the amino acid changes are at one or more of the same positions but involve a different mutation than in the reference antibody.

[0151] In some embodiments, the heavy chain comprises an amino acid sequence of the variable domain (V_H) of antibody 252 (SEQ ID NO: 2), 88 (SEQ ID NO: 6), 100 (SEQ ID NO: 10), 3.8.3 (SEQ ID NO: 14), 2.7.3 (SEQ. ID NO: 18), 1.120.1 (SEQ. ID NO: 22), 9.14.4I (SEQ ID NO: 26), 8.10.3F (SEQ ID NO: 30), 9.7.2IF (SEQ ID NO: 34), 9.14.4 (SEQ ID NO: 38), 8.10.3 (SEQ ID NO: 30), 9.7.2 (SEQ ID NO: 46), 9.7.2C-Ser (SEQ ID NO: 50), 9.14.4C-Ser (SEQ ID NO: 54), 8.10.3C-Ser (SEQ ID NO: 58), 8.10.3-CG2 (SEQ ID NO: 62), 9.7.2-CG2 (SEQ ID NO: 66), 9.7.2-CG4 (SEQ ID NO: 70), 9.14.4-CG2 (SEQ ID NO: 74), 9.14.4-CG4 (SEQ ID NO: 78), 9.14.4-Ser (SEQ ID NO: 82), 9.7.2-Ser (SEQ ID NO: 86), 8.10.3-Ser (SEQ ID NO: 90) 8.10.3-CG4 (SEQ ID NO: 94), 8.10.3FG1 (SEQ ID NO: 98) or 9.14.4G1 (SEQ ID NO: 102), or said amino acid sequence having up to 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 conservative amino acid substitutions and/or a total of up to 3 non-conservative amino acid substitutions. In some embodiments, the heavy chain comprises the amino acid sequence from the beginning of the CDR1 to the end of the CDR3 of any one of the foregoing antibodies.

[0152] In some embodiments, the heavy chain comprises the heavy chain CDR1, CDR2 and CDR3 regions of antibody 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 or 9.14.4G1, or said CDR regions each having less than 8, less than 6, less than 4, or less than 3 conservative amino acid substitutions and/or a total of three or fewer non-conservative amino acid substitutions.

[0153] In some embodiments, the heavy chain comprises a germline or antibody CDR3, as described above, of an antibody sequence as described herein, and may also comprise the CDR1 and CDR2 regions of a germline sequence, or may comprise a CDR1 and CDR2 of an antibody sequence, each of which are independently selected from an antibody comprising a

heavy chain of an antibody selected from 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 or 9.14.4G1. In another embodiment, the heavy chain comprises a CDR3 of an antibody sequence as described herein, and may also comprise the CDR1 and CDR2 regions, each of which are independently selected from a CDR1 and CDR2 region of a heavy chain variable region comprising an amino acid sequence of the V_H region selected from SEQ ID NOS: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 46, 50, 54, 58, 62, 66, 70, 74, 78, 82, 86, 90, 94, 98 or 102, or encoded by a nucleic acid sequence encoding the V_H region selected from SEQ ID NOS: 1, 5, 9, 25, 29, 33, 37, 45, 97 or 101. In another embodiment, the antibody comprises a light chain as disclosed above and a heavy chain as disclosed above.

[0154] One type of amino acid substitution that may be made is to change one or more cysteines in the antibody, which may be chemically reactive, to another residue, such as, without limitation, alanine or serine. In one embodiment, there is a substitution of a non-canonical cysteine. The substitution can be in a framework region of a variable domain or in the constant domain of an antibody. In another embodiment, the cysteine is in a non-canonical region of the antibody.

[0155] Another type of amino acid substitution that may be made is to remove any potential proteolytic sites in the antibody, particularly those that are in a CDR or framework region of a variable domain or in the constant domain of an antibody. Substitution of cysteine residues and removal of proteolytic sites may decrease the risk of any heterogeneity in the antibody product and thus increase its homogeneity. Another type of amino acid substitution is elimination of asparagine-glycine pairs, which form potential deamidation sites, by altering one or both of the residues.

[0156] In some embodiments, the C-terminal lysine of the heavy chain of the anti-M-CSF antibody of the invention is not present (Lewis D.A., *et al., Anal. Chem*, 66(5): 585-95 (1994)). In various embodiments of the

invention, the heavy and light chains of the anti-M-CSF antibodies may optionally include a signal sequence.

[0157] In one aspect, the invention relates to human anti-M-CSF monoclonal antibodies and the cell lines engineered to produce them. Table 1A lists the sequence identifiers (SEQ ID NOS) of the nucleic acids that encode the variable region of the heavy and light chains and the corresponding predicted amino acid sequences for the monoclonal antibodies: 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3 and 9.7.2. Additional variant antibodies 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4 8.10.3FG1 or 9.14.4G1 could be made by methods known to one skilled in the art. Table 1B lists the percent amino acid identity of the heavy chain, light chains, and both heavy and light chains of the antibodies 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.7.2, 9.14.4, 8.10.3, 8.10.3C-Ser, 8.10.3-CG2, 8.10.3-Ser, 8.10.3-CG4, and 8.10.3FG1 as compared to the heavy chain, light chains, and heavy and light chains, respectively of antibody 8.10.3F.

[0158] In another aspect, the invention relates to anti-M-CSF monoclonal antibodies that are substantially similar to the monoclonal antibody 8,10.3F, wherein the amino acid sequence of the heavy chain, or the light chain, or both the heavy chain and light chain of the antibodies share at least about 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity with the heavy chain, or light chain, or both the heavy chain and light chain of the antibody 8.10.3F respectively.

Table 1A

HUMAN ANTI-M-CSF ANTIBODIES				
MAb	SEQUENCE IDENTIFIER (SEQ ID NOS:)			
	Full Length			
	Heavy		Light	
	DNA	Protein	DNA	Protein
252	1	2	3	4
88	5	6	7	8
100	9	10	11	12
3.8.3		14		16
2.7.3		18		20
1.120.1		22		24
9.14.4I	25	26	27	28
9.14.4	37	38	27	28
9.14.4C-Ser		54		56
9.14.4-CG2		74		56
9.14.4-CG4		78		56
9.14.4-Ser		82	27	28
9.14.4-G1	101	102	27	28
8.10.3F	29	30	31	32
8.10.3	29	30	43	44
8.10.3C-Ser		58		60
8.10.3-CG2		62		60
8.10.3-Ser		90	43	44
8.10.3-CG4		94		60
8.10.3FG1	97	98	31	32
9.7.2IF	33	34	35	36
9.7.2	45	46	47	48
9.7.2C-Ser		50		52
9.7.2-CG2		66		52
9.7.2-CG4		70		52
9.7.2-Ser		86	47	48

Table 1B

MAb	Percentage Identity with Heavy Chain, Light Chain, or Both Heavy and Light Chains of Antibody 8.10.3F		
	Heavy Chain	Light Chain	Heavy & Light Chain Combined
8.10.3-CG2	100	99	99
8.10.3-CG4	94	99	96
8.10.3-Ser	95	99	96
8.10.3C-Ser	95	99	96
8.10.3FG1	94	100	96
8.10.3	100	99	99
1.120.1	87	85	86
100	94	90	93
2.7.3	89	84	87
252	95	84	91
3.8.3	96	83	92
88	95	84	91
9.14.4	91	83	88
9.7.2	90	83	88

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Class and Subclass of Anti-M-CSF Antibodies

[0159] The class and subclass of anti-M-CSF antibodies may be determined by any method known in the art. In general, the class and subclass of an antibody may be determined using antibodies that are specific for a particular class and subclass of antibody. Such antibodies are commercially available. The class and subclass can be determined by ELISA, or Western Blot as well as other techniques. Alternatively, the class and subclass may be determined by sequencing all or a portion of the constant domains of the heavy and/or light chains of the antibodies, comparing their amino acid sequences to the known amino acid sequences of various class and subclasses of immunoglobulins, and determining the class and subclass of the antibodies.

[0160] In some embodiments, the anti-M-CSF antibody is a monoclonal antibody. The anti-M-CSF antibody can be an IgG, an IgM, an IgE, an IgA, or an IgD molecule. In preferred embodiments, the anti-M-CSF antibody is an IgG and is an IgG1, IgG2, IgG3 or IgG4 subclass. In other preferred embodiments, the antibody is subclass IgG2 or IgG4. In another preferred embodiment, the antibody is subclass IgG1.

Species and Molecular Selectivity

[0161] In another aspect of the invention, the anti-M-CSF antibodies demonstrate both species and molecule selectivity. In some embodiments, the anti-M-CSF antibody binds to human, cynomologus monkey and mouse M-CSF. Following the teachings of the specification, one may determine the species selectivity for the anti-M-CSF antibody using methods well known in the art. For instance, one may determine the species selectivity using Western blot, FACS, ELISA, RIA, a cell proliferation assay, or a M-CSF receptor binding assay. In a preferred embodiment, one may determine the species selectivity using a cell proliferation assay or ELISA.

[0162] In another embodiment, the anti-M-CSF antibody has a selectivity for M-CSF that is at least 100 times greater than its selectivity for GM-/G-CSF. In some embodiments, the anti-M-CSF antibody does not exhibit any appreciable specific binding to any other protein other than M-CSF. One

can determine the selectivity of the anti-M-CSF antibody for M-CSF using methods well known in the art following the teachings of the specification. For instance one can determine the selectivity using Western blot, FACS, ELISA, or RIA.

Identification of M-CSF Epitopes Recognized by Anti- M-CSF Antibodies

[0163] The invention provides a human anti-M-CSF monoclonal antibody that binds to M-CSF and competes with, cross-competes with and/or binds the same epitope and/or binds to M-CSF with the same K_D as (a) an antibody selected from 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 or 9.14.4G1; (b) an antibody that comprises a heavy chain variable region having an amino acid sequence of SEQ ID NO: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 46, 50, 54, 58, 62, 66, 70, 74, 78, 82, 86, 90, 94, 98 or 102; (c) an antibody that comprises a light chain variable region having an amino acid sequence of SEQ ID NO: 4, 8, 12, 16, 20, 24, 28, 32, 36, 44, 48, 52, 56 or 60; (d) an antibody that comprises both a heavy chain variable region as defined in (b) and a light chain variable region as defined in (c).

[0164] One can determine whether an antibody binds to the same epitope, competes for binding with, cross competes for binding with or has the same K_D an anti-M-CSF antibody by using methods known in the art. In one embodiment, one allows the anti-M-CSF antibody of the invention to bind to M-CSF under saturating conditions and then measures the ability of the test antibody to bind to M-CSF. If the test antibody is able to bind to M-CSF at the same time as the anti-M-CSF antibody, then the test antibody binds to a different epitope as the anti-M-CSF antibody. However, if the test antibody is not able to bind to M-CSF at the same time, then the test antibody binds to the same epitope, an overlapping epitope, or an epitope that is in close proximity to the epitope bound by the human anti-M-CSF antibody. This experiment can be performed using ELISA, RIA, or FACS.

In a preferred embodiment, the experiment is performed using BIACORE™.

Binding Affinity of Anti-M-CSF Antibodies to M-CSF

[0165] In some embodiments of the invention, the anti-M-CSF antibodies bind to M-CSF with high affinity. In some embodiments, the anti-M-CSF antibody binds to M-CSF with a K_D of 1x 10^{-7} M or less. In other preferred embodiments, the antibody binds to M-CSF with a K_D of 1 x10^{-8} M, 1 x 10^{-9} M, 1 x 10^{-10} M, 1 x 10^{-11} M, 1 x 10^{-12} M or less. In certain embodiments, the K_D is 1 pM to 500 pM. In other embodiments, the K_D is between 500 pM to 1 μM. In other embodiments, the K_D is between 1 μM to 100 nM. In other embodiments, the K_D is between 100 mM to 10 nM. In an even more preferred embodiment, the antibody binds to M-CSF with substantially the same K_D as an antibody selected from 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 or 9.14.4G1. In another preferred embodiment, the antibody binds to M-CSF with substantially the same K_D as an antibody that comprises a CDR2 of a light chain, and/or a CDR3 of a heavy chain from an antibody selected from 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 or 9.14.4G1. In still another preferred embodiment, the antibody binds to M-CSF with substantially the same K_D as an antibody that comprises a heavy chain variable region having an amino acid sequence of SEQ ID NO: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 46, 50, 54, 58, 62, 66, 70, 74, 78, 82, 86, 90, 94, 98 or 102, or that comprises a light chain variable region having an amino acid sequence of SEQ ID NO: 4, 8, 12, 16, 20, 24, 28, 32, 36, 44, 48, 52, 56 or 60. In another preferred embodiment, the antibody binds to M-CSF with substantially the same K_D as an antibody that comprises a CDR2, and may optionally comprise a CDR1 and/or CDR3, of a light chain variable region

having an amino acid sequence of the V_L region of SEQ ID NO: 4, 8, 12, 16, 20, 24, 28, 32, 36, 44, 48, 52, 56 or 60, or that comprises a CDR3, and may optionally comprise a CDR1 and/or CDR2, of a heavy chain variable region having an amino acid sequence of the V_H region of SEQ ID NO: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 46, 50, 54, 58, 62, 66, 70, 74, 78, 82, 86, 90, 94, 98 or 102.

[0166] In some embodiments, the anti-M-CSF antibody has a low dissociation rate. In some embodiments, the anti-M-CSF antibody has an k_{off} of 2.0×10^{-4} s^{-1} or lower. In other preferred embodiments, the antibody binds to M-CSF with a k_{off} of 2.0×10^{-5} or a k_{off} 2.0×10^{-6} s^{-1} or lower. In some embodiments, the k_{off} is substantially the same as an antibody described herein, such as an antibody selected from 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 or 9.14.4G1. In some embodiments, the antibody binds to M-CSF with substantially the same k_{off} as an antibody that comprises (a) a CDR3, and may optionally comprise a CDR1 and/or CDR2, of a heavy chain of an antibody selected from 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 or 9.14.4G1; or (b) a CDR2, and may optionally comprise a CDR1 and/or CDR3, of a light chain from an antibody selected from 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 or 9.14.4G1. In some embodiments, the antibody binds to M-CSF with substantially the same k_{off} as an antibody that comprises a heavy chain variable region having an amino acid sequence of SEQ ID NO: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 46, 50, 54, 58, 62, 66, 70, 74, 78, 82, 86, 90, 94, 98 or 102; or that comprises a light chain variable region having an amino acid sequence of

SEQ ID NO: 4, 8, 12, 16, 20, 24, 28, 32, 36, 44, 48, 52, 56 or 60; In another preferred embodiment, the antibody binds to M-CSF with substantially the same k_{off} as an antibody that comprises a CDR2, and may optionally comprise a CDR1 and/or CDR3, of a light chain variable region having an amino acid sequence of SEQ ID NO: 4, 8, 12, 16, 20, 24, 28, 32, 36, 44, 48, 52, 56 or 60; or a CDR3, and may optionally comprise a CDR1 and/or CDR2, of a heavy chain variable region having an amino acid sequence of SEQ ID NO: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 46, 50, 54, 58, 62, 66, 70, 74, 78, 82, 86, 90, 94, 98 or 102.

[0167] The binding affinity and dissociation rate of an anti-M-CSF antibody to a M-CSF can be determined by methods known in the art. The binding affinity can be measured by competitive ELISAs, RIAs, surface plasmon resonance (e.g., by using BIACORE™ technology). The dissociation rate can be measured by surface plasmon resonance. Preferably, the binding affinity and dissociation rate is measured by surface plasmon resonance. More preferably, the binding affinity and dissociation rate are measured using BIACORE™ technology. Example VI exemplifies a method for determining affinity constants of anti-M-CSF monoclonal antibodies by BIACORE™ technology.

Inhibition of M-CSF Activity by Anti-M-CSF Antibody

Inhibition of M-CSF binding to c-fms

[0168] In another embodiment, the invention provides an anti-M-CSF antibody that inhibits the binding of a M-CSF to *c-fms* receptor and blocks or prevents activation of *c-fms*. In an preferred embodiment, the M-CSF is human. In another preferred embodiment, the anti-M-CSF antibody is a human antibody. The IC_{50} can be measured by ELISA, RIA, and cell based assays such as a cell proliferation assay, a whole blood monocyte shape change assay, or a receptor binding inhibition assay. In one embodiment, the antibody or portion thereof inhibits cell proliferation with an IC_{50} of no more than 8.0×10^{-7} M, preferably no more than 3×10^{-7} M, or more preferably no more than 8×10^{-8} M as measured by a cell proliferation assay. In another embodiment, the IC_{50} as measured by a monocyte

shape change assay is no more than 2 x 10^{-6} M, preferably no more than 9.0 x 10^{-7} M, or more preferably no more than 9 x 10^{-8} M. In another preferred embodiment, the IC_{50} as measured by a receptor binding assay is no more than 2 x 10^{-6} M, preferably no more than 8.0 x 10^{-7} M, or more preferably no more than 7.0 x 10^{-8} M. Examples III, IV, and V exemplify various types of assays.

[0169] In another aspect anti-M-CSF antibodies of the invention inhibit monocyte/macrophage cell proliferation in response to a M-CSF by at least 20%, more preferably 40%, 45%, 50%, 55%, 60%, 65%, 70%, 80%, 85%, 90%, 95% or 100% compared to the proliferation of cell in the absence of antibody.

Methods of Producing Antibodies and Antibody Producing Cell Lines

Immunization

[0170] In some embodiments, human antibodies are produced by immunizing a non-human animal comprising in its genome some or all of human immunoglobulin heavy chain and light chain loci with a M-CSF antigen. In a preferred embodiment, the non-human animal is a XENOMOUSE™ animal (Abgenix Inc., Fremont, CA). Another non-human animal that may be used is a transgenic mouse produced by Medarex (Medarex, Inc., Princeton, NJ).

[0171] XENOMOUSE™ mice are engineered mouse strains that comprise large fragments of human immunoglobulin heavy chain and light chain loci and are deficient in mouse antibody production. See, e.g., Green *et al.*, *Nature Genetics* 7:13-21 (1994) and U.S. Patents 5,916,771, 5,939,598, 5,985,615, 5,998,209, 6,075,181, 6,091,001, 6,114,598, 6,130,364, 6,162,963 and 6,150,584. See also WO 91/10741, WO 94/02602, WO 96/34096, WO 96/33735, WO 98/16654, WO 98/24893, WO 98/50433, WO 99/45031, WO 99/53049, WO 00/09560, and WO 00/037504.

[0172] In another aspect, the invention provides a method for making anti-M-CSF antibodies from non-human, non-mouse animals by immunizing non-human transgenic animals that comprise human

immunoglobulin loci with a M-CSF antigen. One can produce such animals using the methods described in the above-cited documents. The methods disclosed in these documents can be modified as described in U.S. Patent 5,994,619. U.S. Patent 5,994,619 describes methods for producing novel cultural inner cell mass (CICM) cells and cell lines, derived from pigs and cows, and transgenic CICM cells into which heterologous DNA has been inserted. CICM transgenic cells can be used to produce cloned transgenic embryos, fetuses, and offspring. The '619 patent also describes the methods of producing the transgenic animals, that are capable of transmitting the heterologous DNA to their progeny. In preferred embodiments, the non-human animals are rats, sheep, pigs, goats, cattle or horses.

[0173] XENOMOUSE™ mice produce an adult-like human repertoire of fully human antibodies and generate antigen-specific human antibodies. In some embodiments, the XENOMOUSE™ mice contain approximately 80% of the human antibody V gene repertoire through introduction of megabase sized, germline configuration yeast artificial chromosome (YAC) fragments of the human heavy chain loci and kappa light chain loci. In other embodiments, XENOMOUSE™ mice further contain approximately all of the lambda light chain locus. See Mendez *et al.*, *Nature Genetics* 15:146-156 (1997), Green and Jakobovits, *J. Exp. Med.* 188:483-495 (1998), and WO 98/24893, the disclosures of which are hereby incorporated by reference.

[0174] In some embodiments, the non-human animal comprising human immunoglobulin genes are animals that have a human immunoglobulin "minilocus". In the minilocus approach, an exogenous Ig locus is mimicked through the inclusion of individual genes from the Ig locus. Thus, one or more V_H genes, one or more D_H genes, one or more J_H genes, a mu constant domain, and a second constant domain (preferably a gamma constant domain) are formed into a construct for insertion into an animal. This approach is described, *inter alia*, in U.S. Patent Nos. 5,545,807, 5,545,806, 5,569,825, 5,625,126, 5,633,425, 5,661,016, 5,770,429,

5,789,650, 5,814,318, 5,591,669, 5,612,205, 5,721,367, 5,789,215, and 5,643,763, hereby incorporated by reference.

[0175] In another aspect, the invention provides a method for making humanized anti-M-CSF antibodies. In some embodiments, non-human animals are immunized with a M-CSF antigen as described below under conditions that permit antibody production. Antibody-producing cells are isolated from the animals, fused with myelomas to produce hybridomas, and nucleic acids encoding the heavy and light chains of an anti-M-CSF antibody of interest are isolated. These nucleic acids are subsequently engineered using techniques known to those of skill in the art and as described further below to reduce the amount of non-human sequence, i.e., to humanize the antibody to reduce the immune response in humans

[0176] In some embodiments, the M-CSF antigen is isolated and/or purified M-CSF. In a preferred embodiment, the M-CSF antigen is human M-CSF. In some embodiments, the M-CSF antigen is a fragment of M-CSF. In some embodiments, the M-CSF fragment is the extracellular domain of M-CSF. In some embodiments, the M-CSF fragment comprises at least one epitope of M-CSF. In other embodiments, the M-CSF antigen is a cell that expresses or overexpresses M-CSF or an immunogenic fragment thereof on its surface. In some embodiments, the M-CSF antigen is a M-CSF fusion protein. M-CSF can be purified from natural sources using known techniques. Recombinant M-CSF is commercially available.

[0177] Immunization of animals can be by any method known in the art. See, e.g., Harlow and Lane, *Antibodies: A Laboratory Manual*, New York: Cold Spring Harbor Press, 1990. Methods for immunizing non-human animals such as mice, rats, sheep, goats, pigs, cattle and horses are well known in the art. See, e.g., Harlow and Lane, *supra*, and U.S. Patent 5,994,619. In a preferred embodiment, the M-CSF antigen is administered with an adjuvant to stimulate the immune response. Exemplary adjuvants include complete or incomplete Freund's adjuvant, RIBI (muramyl dipeptides) or ISCOM (immunostimulating complexes). Such adjuvants may protect the polypeptide from rapid dispersal by sequestering it in a

local deposit, or they may contain substances that stimulate the host to secrete factors that are chemotactic for macrophages and other components of the immune system. Preferably, if a polypeptide is being administered, the immunization schedule will involve two or more administrations of the polypeptide, spread out over several weeks. Example I exemplifies a method for producing anti-M-CSF monoclonal antibodies in XENOMOUSE™ mice.

Production of Antibodies and Antibody-Producing Cell Lines

[0178] After immunization of an animal with a M-CSF antigen, antibodies and/or antibody-producing cells can be obtained from the animal. In some embodiments, anti-M-CSF antibody-containing serum is obtained from the animal by bleeding or sacrificing the animal. The serum may be used as it is obtained from the animal, an immunoglobulin fraction may be obtained from the serum, or the anti-M-CSF antibodies may be purified from the serum.

[0179] In some embodiments, antibody-producing immortalized cell lines are prepared from cells isolated from the immunized animal. After immunization, the animal is sacrificed and lymph node and/or splenic B cells are immortalized. Methods of immortalizing cells include, but are not limited to, transfecting them with oncogenes, infecting them with an oncogenic virus, cultivating them under conditions that select for immortalized cells, subjecting them to carcinogenic or mutating compounds, fusing them with an immortalized cell, e.g., a myeloma cell, and inactivating a tumor suppressor gene. See, e.g., Harlow and Lane, *supra*. If fusion with myeloma cells is used, the myeloma cells preferably do not secrete immunoglobulin polypeptides (a non-secretory cell line). Immortalized cells are screened using M-CSF, a portion thereof, or a cell expressing M-CSF. In a preferred embodiment, the initial screening is performed using an enzyme-linked immunoassay (ELISA) or a radioimmunoassay. An example of ELISA screening is provided in WO 00/37504, incorporated herein by reference.

[0180] Anti-M-CSF antibody-producing cells, e.g., hybridomas, are selected, cloned and further screened for desirable characteristics, including robust growth, high antibody production and desirable antibody characteristics, as discussed further below. Hybridomas can be expanded *in vivo* in syngeneic animals, in animals that lack an immune system, e.g., nude mice, or in cell culture *in vitro*. Methods of selecting, cloning and expanding hybridomas are well known to those of ordinary skill in the art.

[0181] In a preferred embodiment, the immunized animal is a non-human animal that expresses human immunoglobulin genes and the splenic B cells are fused to a myeloma cell line from the same species as the non-human animal. In a more preferred embodiment, the immunized animal is a XENOMOUSE™ animal and the myeloma cell line is a non-secretory mouse myeloma. In an even more preferred embodiment, the myeloma cell line is P3-X63-AG8-653. See, e.g., Example I.

[0182] Thus, in one embodiment, the invention provides methods of producing a cell line that produces a human monoclonal antibody or a fragment thereof directed to M-CSF comprising (a) immunizing a non-human transgenic animal described herein with M-CSF, a portion of M-CSF or a cell or tissue expressing M-CSF; (b) allowing the transgenic animal to mount an immune response to M-CSF; (c) isolating B lymphocytes from a transgenic animal; (d) immortalizing the B lymphocytes; (e) creating individual monoclonal populations of the immortalized B lymphocytes; and (f) screening the immortalized B lymphocytes to identify an antibody directed to M-CSF.

[0183] In another aspect, the invention provides hybridomas that produce an human anti-M-CSF antibody. In a preferred embodiment, the hybridomas are mouse hybridomas, as described above. In other embodiments, the hybridomas are produced in a non-human, non-mouse species such as rats, sheep, pigs, goats, cattle or horses. In another embodiment, the hybridomas are human hybridomas.

[0184] In another preferred embodiment, a transgenic animal is immunized with M-CSF, primary cells, e.g., spleen or peripheral blood cells,

are isolated from an immunized transgenic animal and individual cells producing antibodies specific for the desired antigen are identified. Polyadenylated mRNA from each individual cell is isolated and reverse transcription polymerase chain reaction (RT-PCR) is performed using sense primers that anneal to variable region sequences, e.g., degenerate primers that recognize most or all of the FR1 regions of human heavy and light chain variable region genes and antisense primers that anneal to constant or joining region sequences. cDNAs of the heavy and light chain variable regions are then cloned and expressed in any suitable host cell, e.g., a myeloma cell, as chimeric antibodies with respective immunoglobulin constant regions, such as the heavy chain and κ or λ constant domains. See Babcook, J.S. *et al.*, *Proc. Natl. Acad. Sci.* USA 93:7843-48, 1996, herein incorporated by reference. Anti M-CSF antibodies may then be identified and isolated as described herein.

[0185] In another embodiment, phage display techniques can be used to provide libraries containing a repertoire of antibodies with varying affinities for M-CSF. For production of such repertoires, it is unnecessary to immortalize the B cells from the immunized animal. Rather, the primary B cells can be used directly as a source of DNA. The mixture of cDNAs obtained from B cell, e.g., derived from spleens, is used to prepare an expression library, for example, a phage display library transfected into *E.coli*. The resulting cells are tested for immunoreactivity to M-CSF. Techniques for the identification of high affinity human antibodies from such libraries are described by Griffiths *et al.*, *EMBO J.*, 13:3245-3260 (1994); Nissim *et al.*, *ibid*, pp. 692-698 and by Griffiths *et al.*, *ibid*, 12:725-734. Ultimately, clones from the library are identified which produce binding affinities of a desired magnitude for the antigen and the DNA encoding the product responsible for such binding is recovered and manipulated for standard recombinant expression. Phage display libraries may also be constructed using previously manipulated nucleotide sequences and screened in a similar fashion. In general, the cDNAs encoding heavy and

light chains are independently supplied or linked to form Fv analogs for production in the phage library.

[0186] The phage library is then screened for the antibodies with the highest affinities for M-CSF and the genetic material recovered from the appropriate clone. Further rounds of screening can increase affinity of the original antibody isolated.

[0187] In another aspect, the invention provides hybridomas that produce an human anti-M-CSF antibody. In a preferred embodiment, the hybridomas are mouse hybridomas, as described above. In other embodiments, the hybridomas are produced in a non-human, non-mouse species such as rats, sheep, pigs, goats, cattle or horses. In another embodiment, the hybridomas are human hybridomas.

Nucleic Acids, Vectors, Host Cells, and
Recombinant Methods of Making Antibodies

Nucleic Acids

[0188] The present invention also encompasses nucleic acid molecules encoding anti-M-CSF antibodies. In some embodiments, different nucleic acid molecules encode a heavy chain and a light chain of an anti-M-CSF immunoglobulin. In other embodiments, the same nucleic acid molecule encodes a heavy chain an a light chain of an anti-M-CSF immunoglobulin. In one embodiment, the nucleic acid encodes a M-CSF antibody of the invention.

[0189] In some embodiments, the nucleic acid molecule encoding the variable domain of the light chain comprises a human V_κ L5, O12, L2, B3, A27 gene and a Jκ1, Jκ2, Jκ3, or Jκ4 gene.

[0190] In some embodiments, the nucleic acid molecule encoding the light chain, encodes an amino acid sequence comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 mutations from the germline amino acid sequence. In some embodiments, the nucleic acid molecule comprises a nucleotide sequence that encodes a V_L amino acid sequence comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 non-conservative amino acid substitutions and/or 1, 2, or 3 non-conservative substitutions compared to germline sequence. Substitutions

may be in the CDR regions, the framework regions, or in the constant domain.

[0191] In some embodiments, the nucleic acid molecule encodes a V_L amino acid sequence comprising one or more variants compared to germline sequence that are identical to the variations found in the V_L of one of the antibodies 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 or 9.14.4G1.

[0192] In some embodiments, the nucleic acid molecule encodes at least three amino acid mutations compared to the germline sequence found in the V_L of one of the antibodies 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4, 8.10.3, or 9.7.2.

[0193] In some embodiments, the nucleic acid molecule comprises a nucleotide sequence that encodes the V_L amino acid sequence of monoclonal antibody 252 (SEQ ID NO: 4), 88 (SEQ ID NO: 8), 100 (SEQ ID NO: 12), 3.8.3 (SEQ ID NO: 16), 2.7.3 (SEQ ID NO: 20), 1.120.1 (SEQ ID NO: 24), 9.14.4I (SEQ ID NO: 28), 8.10.3F (SEQ ID NO: 32), 9.7.2IF (SEQ ID NO: 36), 9.14.4 (SEQ ID NO: 28), 8.10.3 (SEQ ID NO: 44), 9.7.2 (SEQ ID NO: 48), 9.7.2C-Ser (SEQ ID NO: 52), 9.14.4C-Ser (SEQ ID NO: 56), 8.10.3C-Ser (SEQ ID NO: 60), 8.10.3-CG2 (SEQ ID NO: 60), 9.7.2-CG2 (SEQ ID NO: 52), 9.7.2-CG4 (SEQ ID NO: 52), 9.14.4-CG2 (SEQ ID NO: 56), 9.14.4-CG4 (SEQ ID NO: 56), 9.14.4-Ser (SEQ ID NO: 28), 9.7.2-Ser (SEQ ID NO: 48), 8.10.3-Ser (SEQ ID NO: 44), 8.10.3-CG4 (SEQ ID NO: 60) 8.10.3FG1 (SEQ ID NO: 32) or 9.14.4G1 (SEQ ID NO: 28), or a portion thereof. In some embodiments, said portion comprises at least the CDR2 region. In some embodiments, the nucleic acid encodes the amino acid sequence of the light chain CDRs of said antibody. In some embodiments, said portion is a contiguous portion comprising CDR1-CDR3.

[0194] In some embodiments, the nucleic acid molecule comprises a nucleotide sequence that encodes the light chain amino acid sequence of

one of SEQ ID NOS: 4, 8, 12, 16, 20, 24, 28, 32, 36, 44, 48, 52, 56 or 60. In some preferred embodiments, the nucleic acid molecule comprises the light chain nucleotide sequence of SEQ ID NOS: 3, 7, 11, 27, 31, 35, 43 or 47, or a portion thereof.

[0195] In some embodiments, the nucleic acid molecule encodes a V_L amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a V_L amino acid sequence shown in Figure 1 or to a V_L amino acid sequences of any one of antibodies 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 or 9.14.4G1, or an amino acid sequence of any one of SEQ ID NOS: 4, 8, 12, 16, 20, 24, 28, 32, 36, 44, 48, 52, 56 or 60. Nucleic acid molecules of the invention include nucleic acids that hybridize under highly stringent conditions, such as those described above, to a nucleic acid sequence encoding the light chain amino acid sequence of SEQ ID NOS: 4, 8, 12, 16, 20, 24, 28, 32, 36, 44, 48, 52, 56 or 60, or that has the light chain nucleic acid sequence of SEQ ID NOS: 3, 7, 11, 27, 31, 35, 43 or 47.

[0196] In another embodiment, the nucleic acid encodes a full-length light chain of an antibody selected from 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 or 9.14.4G1, or a light chain comprising the amino acid sequence of SEQ ID NOS: 4, 8, 12, 16, 20, 24, 28, 32, 36, 44, 48, 52, 56 or 60 and a constant region of a light chain, or a light chain comprising a mutation. Further, the nucleic acid may comprise the light chain nucleotide sequence of SEQ ID NOS: 3, 7, 11, 27, 31, 35, 43 or 47 and the nucleotide sequence encoding a constant region of a light chain, or a nucleic acid molecule encoding a light chain comprise a mutation.

[0197] In another preferred embodiment, the nucleic acid molecule encodes the variable domain of the heavy chain (V_H) that comprises a human V_H 1-18, 3-33, 3-11, 3-23, 3-48, or 3-7 gene sequence or a sequence derived therefrom. In various embodiments, the nucleic acid molecule comprises a human V_H 1-18 gene, a D_H4-23 gene and a human J_H4 gene; a human V_H 3-33 gene, a human D_H1-26 gene and a human J_H4 gene; a human V_H 3-11 gene, a human D_H7-27 gene and a human J_H4 gene; a human V_H 3-11 gene, a human D_H 7-27 gene and a human J_H6 gene; a human V_H 3-23 gene, a human D_H1-26 gene and a human J_H4 gene; a human V_H 3-7 gene, a human D_H6-13 gene and a human J_H4 gene; a human V_H3-11 gene, a human D_H7-27 gene, and a human J_H4b gene; a human V_H3-48 gene, a human D_H1-26 gene, and a human J_H4b gene; a human V_H3-11 gene, a human D_H6-13 gene, and a human J_H6b gene, or a sequence derived from the human genes.

[0198] In some embodiments, the nucleic acid molecule encodes an amino acid sequence comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or 18 mutations compared to the germline amino acid sequence of the human V, D or J genes. In some embodiments, said mutations are in the V_H region. In some embodiments, said mutations are in the CDR regions.

[0199] In some embodiments, the nucleic acid molecule encodes one or more amino acid mutations compared to the germline sequence that are identical to amino acid mutations found in the V_H of monoclonal antibody 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 or 9.14.4G1. In some embodiments, the nucleic acid encodes at least three amino acid mutations compared to the germline sequences that are identical to at least three amino acid mutations found in one of the above-listed monoclonal antibodies.

[0200] In some embodiments, the nucleic acid molecule comprises a nucleotide sequence that encodes at least a portion of the V_H amino acid

sequence of antibody 252 (SEQ ID NO: 4), 88 (SEQ ID NO: 8), 100 (SEQ ID NO: 12), 3.8.3 (SEQ ID NO: 16), 2.7.3 (SEQ ID NO: 20), 1.120.1 (SEQ ID NO: 24), 9.14.4I (SEQ ID NO: 28), 8.10.3F (SEQ ID NO: 32), 9.7.2IF (SEQ ID NO: 36), 9.14.4 (SEQ ID NO: 28), 8.10.3 (SEQ ID NO: 44), 9.7.2 (SEQ ID NO: 48), 9.7.2C-Ser (SEQ ID NO: 52), 9.14.4C-Ser (SEQ ID NO: 56), 8.10.3C-Ser (SEQ ID NO: 60), 8.10.3-CG2 (SEQ ID NO: 60), 9.7.2-CG2 (SEQ ID NO: 52), 9.7.2-CG4 (SEQ ID NO: 52), 9.14.4-CG2 (SEQ ID NO: 56), 9.14.4-CG4 (SEQ ID NO: 56), 9.14.4-Ser (SEQ ID NO: 28), 9.7.2-Ser (SEQ ID NO: 48), 8.10.3-Ser (SEQ ID NO: 44), 8.10.3-CG4 (SEQ ID NO: 60) 8.10.3FG1 (SEQ ID NO: 32) or 9.14.4G1 (SEQ ID NO: 28), or said sequence having conservative amino acid mutations and/or a total of three or fewer non-conservative amino acid substitutions. In various embodiments the sequence encodes one or more CDR regions, preferably a CDR3 region, all three CDR regions, a contiguous portion including CDR1-CDR3, or the entire V_H region.

[0201] In some embodiments, the nucleic acid molecule comprises a heavy chain nucleotide sequence that encodes the amino acid sequence of one of SEQ ID NOS: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 46, 50, 54, 58, 62, 66, 70, 74, 78, 82, 86, 90, 94, 98 or 102. In some preferred embodiments, the nucleic acid molecule comprises at least a portion of the heavy chain nucleotide sequence of SEQ ID NO: 1, 5, 9, 25, 29, 33, 37, 45, 97 or 101. In some embodiments, said portion encodes the V_H region, a CDR3 region, all three CDR regions, or a contiguous region including CDR1-CDR3.

[0202] In some embodiments, the nucleic acid molecule encodes a V_H amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%,95%, 96%, 97%, 98% or 99% identical to the V_H amino acid sequences shown in Figure 4 or to a V_H amino acid sequence of any one of SEQ ID NOS: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 46, 50, 54, 58, 62, 66, 70, 74, 78, 82, 86, 90, 94, 98 or 102. Nucleic acid molecules of the invention include nucleic acids that hybridize under highly stringent conditions, such as those described above, to a nucleotide sequence encoding the heavy chain amino acid sequence of SEQ ID NOS: 2, 6, 10,

14, 18, 22, 26, 30, 34, 38, 46, 50, 54, 58, 62, 66, 70, 74, 78, 82, 86, 90, 94, 98 or 102 or that has the nucleotide sequence of SEQ ID NOS: 1, 5, 9, 25, 29, 33, 37, 45, 97 or 101.

[0203] In another embodiment, the nucleic acid encodes a full-length heavy chain of an antibody selected from 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 or 9.14.4G1, or a heavy chain having the amino acid sequence of SEQ ID NOS: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 46, 50, 54, 58, 62, 66, 70, 74, 78, 82, 86, 90, 94, 98 or 102 and a constant region of a heavy chain, or a heavy chain comprising a mutation. Further, the nucleic acid may comprise the heavy chain nucleotide sequence of SEQ ID NOS: 1, 5, 9, 25, 29, 33, 37, 45, 97 or 101 and a nucleotide sequence encoding a constant region of a light chain, or a nucleic acid molecule encoding a heavy chain comprising a mutation.

[0204] A nucleic acid molecule encoding the heavy or entire light chain of an anti-M-CSF antibody or portions thereof can be isolated from any source that produces such antibody. In various embodiments, the nucleic acid molecules are isolated from a B cell isolated from an animal immunized with M-CSF or from an immortalized cell derived from such a B cell that expresses an anti-M-CSF antibody. Methods of isolating mRNA encoding an antibody are well-known in the art. See, e.g., Sambrook *et al.* The mRNA may be used to produce cDNA for use in the polymerase chain reaction (PCR) or cDNA cloning of antibody genes. In a preferred embodiment, the nucleic acid molecule is isolated from a hybridoma that has as one of its fusion partners a human immunoglobulin-producing cell from a non-human transgenic animal. In an even more preferred embodiment, the human immunoglobulin producing cell is isolated from a XENOMOUSE™ animal. In another embodiment, the human immunoglobulin-producing cell is from a non-human, non-mouse transgenic animal, as described above. In another embodiment, the nucleic acid is

isolated from a non-human, non-transgenic animal. The nucleic acid molecules isolated from a non-human, non-transgenic animal may be used, e.g., for humanized antibodies.

[0205] In some embodiments, a nucleic acid encoding a heavy chain of an anti-M-CSF antibody of the invention can comprise a nucleotide sequence encoding a V_H domain of the invention joined in-frame to a nucleotide sequence encoding a heavy chain constant domain from any source. Similarly, a nucleic acid molecule encoding a light chain of an anti-M-CSF antibody of the invention can comprise a nucleotide sequence encoding a V_L domain of the invention joined in-frame to a nucleotide sequence encoding a light chain constant domain from any source.

[0206] In a further aspect of the invention, nucleic acid molecules encoding the variable domain of the heavy (V_H) and light (V_L) chains are "converted" to full-length antibody genes. In one embodiment, nucleic acid molecules encoding the V_H or V_L domains are converted to full-length antibody genes by insertion into an expression vector already encoding heavy chain constant (C_H) or light chain (C_L) constant domains, respectively, such that the V_H segment is operatively linked to the C_H segment(s) within the vector, and the V_L segment is operatively linked to the C_L segment within the vector. In another embodiment, nucleic acid molecules encoding the V_H and/or V_L domains are converted into full-length antibody genes by linking, e.g., ligating, a nucleic acid molecule encoding a V_H and/or V_L domains to a nucleic acid molecule encoding a C_H and/or C_L domain using standard molecular biological techniques. Nucleic acid sequences of human heavy and light chain immunoglobulin constant domain genes are known in the art. See, e.g., Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed., NIH Publ. No. 91-3242, 1991. Nucleic acid molecules encoding the full-length heavy and/or light chains may then be expressed from a cell into which they have been introduced and the anti-M-CSF antibody isolated.

[0207] The nucleic acid molecules may be used to recombinantly express large quantities of anti-M-CSF antibodies. The nucleic acid molecules also

may be used to produce chimeric antibodies, bispecific antibodies, single chain antibodies, immunoadhesins, diabodies, mutated antibodies and antibody derivatives, as described further below. If the nucleic acid molecules are derived from a non-human, non-transgenic animal, the nucleic acid molecules may be used for antibody humanization, also as described below.

[0208] In another embodiment, a nucleic acid molecule of the invention is used as a probe or PCR primer for a specific antibody sequence. For instance, the nucleic acid can be used as a probe in diagnostic methods or as a PCR primer to amplify regions of DNA that could be used, inter alia, to isolate additional nucleic acid molecules encoding variable domains of anti-M-CSF antibodies. In some embodiments, the nucleic acid molecules are oligonucleotides. In some embodiments, the oligonucleotides are from highly variable regions of the heavy and light chains of the antibody of interest. In some embodiments, the oligonucleotides encode all or a part of one or more of the CDRs of antibody 252, 88, 100, 3.8.3, 2.7.3, or 1.120.1, or variants thereof described herein.

Vectors

[0209] The invention provides vectors comprising nucleic acid molecules that encode the heavy chain of an anti-M-CSF antibody of the invention or an antigen-binding portion thereof. The invention also provides vectors comprising nucleic acid molecules that encode the light chain of such antibodies or antigen-binding portion thereof. The invention further provides vectors comprising nucleic acid molecules encoding fusion proteins, modified antibodies, antibody fragments, and probes thereof.

[0210] In some embodiments, the anti-M-CSF antibodies, or antigen-binding portions of the invention are expressed by inserting DNAs encoding partial or full-length light and heavy chains, obtained as described above, into expression vectors such that the genes are operatively linked to necessary expression control sequences such as transcriptional and transnational control sequences. Expression vectors include plasmids,

retroviruses, adenoviruses, adeno-associated viruses (AAV), plant viruses such as cauliflower mosaic virus, tobacco mosaic virus, cosmids, YACs, EBV derived episomes, and the like. The antibody gene is ligated into a vector such that transcriptional and transnational control sequences within the vector serve their intended function of regulating the transcription and translation of the antibody gene. The expression vector and expression control sequences are chosen to be compatible with the expression host cell used. The antibody light chain gene and the antibody heavy chain gene can be inserted into separate vectors. In a preferred embodiment, both genes are inserted into the same expression vector. The antibody genes are inserted into the expression vector by standard methods (e.g., ligation of complementary restriction sites on the antibody gene fragment and vector, or blunt end ligation if no restriction sites are present).

[0211] A convenient vector is one that encodes a functionally complete human C_H or C_L immunoglobulin sequence, with appropriate restriction sites engineered so that any V_H or V_L sequence can easily be inserted and expressed, as described above. In such vectors, splicing usually occurs between the splice donor site in the inserted J region and the splice acceptor site preceding the human C domain, and also at the splice regions that occur within the human C_H exons. Polyadenylation and transcription termination occur at native chromosomal sites downstream of the coding regions. The recombinant expression vector also can encode a signal peptide that facilitates secretion of the antibody chain from a host cell. The antibody chain gene may be cloned into the vector such that the signal peptide is linked in-frame to the amino terminus of the immunoglobulin chain. The signal peptide can be an immunoglobulin signal peptide or a heterologous signal peptide (i.e., a signal peptide from a non-immunoglobulin protein).

[0212] In addition to the antibody chain genes, the recombinant expression vectors of the invention carry regulatory sequences that control the expression of the antibody chain genes in a host cell. It will be appreciated by those skilled in the art that the design of the expression

vector, including the selection of regulatory sequences may depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. Preferred regulatory sequences for mammalian host cell expression include viral elements that direct high levels of protein expression in mammalian cells, such as promoters and/or enhancers derived from retroviral LTRs, cytomegalovirus (CMV) (such as the CMV promoter/enhancer), Simian Virus 40 (SV40) (such as the SV40 promoter/enhancer), adenovirus, (e.g., the adenovirus major late promoter (AdMLP)), polyoma and strong mammalian promoters such as native immunoglobulin and actin promoters. For further description of viral regulatory elements, and sequences thereof, see e.g., U.S. Patent No. 5,168,062, U.S. Patent No. 4,510,245 and U.S. Patent No. 4,968,615. Methods for expressing antibodies in plants, including a description of promoters and vectors, as well as transformation of plants is known in the art. See, e.g., United States Patents 6,517,529, herein incorporated by reference. Methods of expressing polypeptides in bacterial cells or fungal cells, e.g., yeast cells, are also well known in the art.

[0213] In addition to the antibody chain genes and regulatory sequences, the recombinant expression vectors of the invention may carry additional sequences, such as sequences that regulate replication of the vector in host cells (e.g., origins of replication) and selectable marker genes. The selectable marker gene facilitates selection of host cells into which the vector has been introduced (see e.g., U.S. Patent Nos. 4,399,216, 4,634,665 and 5,179,017). For example, typically the selectable marker gene confers resistance to drugs, such as G418, hygromycin or methotrexate, on a host cell into which the vector has been introduced. Preferred selectable marker genes include the dihydrofolate reductase (DHFR) gene (for use in dhfr-host cells with methotrexate selection/amplification), the neomycin resistance gene (for G418 selection), and the glutamate synthetase gene.

Non-Hybridoma Host Cells and Methods of Recombinantly Producing Protein

[0214] Nucleic acid molecules encoding anti-M-CSF antibodies and vectors comprising these nucleic acid molecules can be used for transfection of a suitable mammalian, plant, bacterial or yeast host cell. Transformation can be by any known method for introducing polynucleotides into a host cell. Methods for introduction of heterologous polynucleotides into mammalian cells are well known in the art and include dextran-mediated transfection, calcium phosphate precipitation, polybrene-mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei. In addition, nucleic acid molecules may be introduced into mammalian cells by viral vectors. Methods of transforming cells are well known in the art. See, e.g., U.S. Patent Nos. 4,399,216, 4,912,040, 4,740,461, and 4,959,455 (which patents are hereby incorporated herein by reference). Methods of transforming plant cells are well known in the art, including, e.g., Agrobacterium-mediated transformation, biolistic transformation, direct injection, electroporation and viral transformation. Methods of transforming bacterial and yeast cells are also well known in the art.

[0215] Mammalian cell lines available as hosts for expression are well known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC). These include, *inter alia*, Chinese hamster ovary (CHO) cells, NSO, SP2 cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g., Hep G2), A549 cells, and a number of other cell lines. Cell lines of particular preference are selected through determining which cell lines have high expression levels. Other cell lines that may be used are insect cell lines, such as Sf9 cells. When recombinant expression vectors encoding antibody genes are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the

antibody in the host cells or, more preferably, secretion of the antibody into the culture medium in which the host cells are grown. Antibodies can be recovered from the culture medium using standard protein purification methods. Plant host cells include, e.g., Nicotiana, Arabidopsis, duckweed, corn, wheat, potato, etc. Bacterial host cells include *E. coli* and *Streptomyces* species. Yeast host cells include *Schizosaccharomyces pombe*, *Saccharomyces cerevisiae* and *Pichia pastoris*.

[0216] Further, expression of antibodies of the invention (or other moieties therefrom) from production cell lines can be enhanced using a number of known techniques. For example, the glutamine synthetase gene expression system (the GS system) is a common approach for enhancing expression under certain conditions. The GS system is discussed in whole or part in connection with European Patent Nos. 0 216 846, 0 256 055, and 0 323 997 and European Patent Application No. 89303964.4.

[0217] It is possible that antibodies expressed by different cell lines or in transgenic animals will have different glycosylation from each other. However, all antibodies encoded by the nucleic acid molecules provided herein, or comprising the amino acid sequences provided herein are part of the instant invention, regardless of the glycosylation state or pattern or modification of the antibodies.

Transgenic Animals and Plants

[0218] Anti-M-CSF antibodies of the invention also can be produced transgenically through the generation of a mammal or plant that is transgenic for the immunoglobulin heavy and light chain sequences of interest and production of the antibody in a recoverable form therefrom. In connection with the transgenic production in mammals, anti-M-CSF antibodies can be produced in, and recovered from, the milk of goats, cows, or other mammals. See, e.g., U.S. Patent Nos. 5,827,690, 5,756,687, 5,750,172, and 5,741,957. In some embodiments, non-human transgenic animals that comprise human immunoglobulin loci are immunized with M-CSF or an immunogenic portion thereof, as described

above. Methods for making antibodies in plants, yeast or fungi/algae are described, e.g., in US patents 6,046,037 and US 5,959,177.

[0219] In some embodiments, non-human transgenic animals or plants are produced by introducing one or more nucleic acid molecules encoding an anti-M-CSF antibody of the invention into the animal or plant by standard transgenic techniques. See Hogan and United States Patent 6,417,429, *supra*. The transgenic cells used for making the transgenic animal can be embryonic stem cells or somatic cells. The transgenic non-human organisms can be chimeric, nonchimeric heterozygotes, and nonchimeric homozygotes. See, e.g., Hogan *et al.*, *Manipulating the Mouse Embryo: A Laboratory Manual* 2ed., Cold Spring Harbor Press (1999); Jackson *et al.*, *Mouse Genetics and Transgenics: A Practical Approach*, Oxford University Press (2000); and Pinkert, *Transgenic Animal Technology: A Laboratory Handbook*, Academic Press (1999). In some embodiments, the transgenic non-human animals have a targeted disruption and replacement by a targeting construct that encodes a heavy chain and/or a light chain of interest. In a preferred embodiment, the transgenic animals comprise and express nucleic acid molecules encoding heavy and light chains that specifically bind to M-CSF, preferably human M-CSF. In some embodiments, the transgenic animals comprise nucleic acid molecules encoding a modified antibody such as a single-chain antibody, a chimeric antibody or a humanized antibody. The anti-M-CSF antibodies may be made in any transgenic animal. In a preferred embodiment, the non-human animals are mice, rats, sheep, pigs, goats, cattle or horses. The non-human transgenic animal expresses said encoded polypeptides in blood, milk, urine, saliva, tears, mucus and other bodily fluids.

Phage Display Libraries

[0220] The invention provides a method for producing an anti-M-CSF antibody or antigen-binding portion thereof comprising the steps of synthesizing a library of human antibodies on phage, screening the library

with M-CSF or a portion thereof, isolating phage that bind M-CSF, and obtaining the antibody from the phage. By way of example, one method for preparing the library of antibodies for use in phage display techniques comprises the steps of immunizing a non-human animal comprising human immunoglobulin loci with M-CSF or an antigenic portion thereof to create an immune response, extracting antibody producing cells from the immunized animal; isolating RNA from the extracted cells, reverse transcribing the RNA to produce cDNA, amplifying the cDNA using a primer, and inserting the cDNA into a phage display vector such that antibodies are expressed on the phage. Recombinant anti-M-CSF antibodies of the invention may be obtained in this way.

[0221] Recombinant anti-M-CSF human antibodies of the invention can be isolated by screening a recombinant combinatorial antibody library. Preferably the library is a scFv phage display library, generated using human V_L and V_H cDNAs prepared from mRNA isolated from B cells. Methodologies for preparing and screening such libraries are known in the art. There are commercially available kits for generating phage display libraries (e.g., the Pharmacia Recombinant Phage Antibody System, catalog no. 27-9400-01; and the Stratagene SurfZAP™ phage display kit, catalog no. 240612). There also are other methods and reagents that can be used in generating and screening antibody display libraries (see, e.g., U.S. Patent No. 5,223,409; PCT Publication Nos. WO 92/18619, WO 91/17271, WO 92/20791, WO 92/15679, WO 93/01288, WO 92/01047, WO 92/09690; Fuchs *et al.*, *Bio/Technology* 9:1370-1372 (1991); Hay *et al.*, *Hum. Antibod. Hybridomas* 3:81-85 (1992); Huse *et al.*, *Science* 246:1275-1281 (1989); McCafferty *et al.*, *Nature* 348:552-554 (1990); Griffiths *et al.*, *EMBO J.* 12:725-734 (1993); Hawkins *et al.*, *J. Mol. Biol.* 226:889-896 (1992); Clackson *et al.*, *Nature* 352:624-628 (1991); Gram *et al.*, *Proc. Natl. Acad. Sci. USA* 89:3576-3580 (1992); Garrad *et al.*, *Bio/Technology* 9:1373-1377 (1991); Hoogenboom *et al.*, *Nuc. Acid Res.* 19:4133-4137 (1991); and Barbas *et al.*, *Proc. Natl. Acad. Sci. USA* 88:7978-7982 (1991).

[0222] In one embodiment, to isolate a human anti-M-CSF antibodies with the desired characteristics, a human anti-M-CSF antibody as described herein is first used to select human heavy and light chain sequences having similar binding activity toward M-CSF, using the epitope imprinting methods described in PCT Publication No. WO 93/06213. The antibody libraries used in this method are preferably scFv libraries prepared and screened as described in PCT Publication No. WO 92/01047, McCafferty *et al.*, *Nature* 348:552-554 (1990); and Griffiths *et al.*, *EMBO J.* 12:725-734 (1993). The scFv antibody libraries preferably are screened using human M-CSF as the antigen.

[0223] Once initial human V_L and V_H domains are selected, "mix and match" experiments are performed, in which different pairs of the initially selected V_L and V_H segments are screened for M-CSF binding to select preferred V_L/V_H pair combinations. Additionally, to further improve the quality of the antibody, the V_L and V_H segments of the preferred V_L/V_H pair(s) can be randomly mutated, preferably within the CDR3 region of V_H and/or V_L, in a process analogous to the *in vivo* somatic mutation process responsible for affinity maturation of antibodies during a natural immune response. This *in vitro* affinity maturation can be accomplished by amplifying V_H and V_L domains using PCR primers complimentary to the V_H CDR3 or V_L CDR3, respectively, which primers have been "spiked" with a random mixture of the four nucleotide bases at certain positions such that the resultant PCR products encode V_H and V_L segments into which random mutations have been introduced into the V_H and/or V_L CDR3 regions. These randomly mutated V_H and V_L segments can be re-screened for binding to M-CSF.

[0224] Following screening and isolation of an anti-M-CSF antibody of the invention from a recombinant immunoglobulin display library, nucleic acids encoding the selected antibody can be recovered from the display package (e.g., from the phage genome) and subcloned into other expression vectors by standard recombinant DNA techniques. If desired, the nucleic acid can further be manipulated to create other antibody forms

of the invention, as described below. To express a recombinant human antibody isolated by screening of a combinatorial library, the DNA encoding the antibody is cloned into a recombinant expression vector and introduced into a mammalian host cells, as described above.

Class switching

[0225] Another aspect of the invention provides a method for converting the class or subclass of an anti-M-CSF antibody to another class or subclass. In some embodiments, a nucleic acid molecule encoding a V_L or V_H that does not include any nucleic acid sequences encoding C_L or C_H is isolated using methods well-known in the art. The nucleic acid molecule then is operatively linked to a nucleic acid sequence encoding a C_L or C_H from a desired immunoglobulin class or subclass. This can be achieved using a vector or nucleic acid molecule that comprises a C_L or C_H chain, as described above. For example, an anti-M-CSF antibody that was originally IgM can be class switched to an IgG. Further, the class switching may be used to convert one IgG subclass to another, e.g., from IgG1 to IgG2. Another method for producing an antibody of the invention comprising a desired isotype comprises the steps of isolating a nucleic acid encoding a heavy chain of an anti-M-CSF antibody and a nucleic acid encoding a light chain of an anti-M-CSF antibody, isolating the sequence encoding the V_H region, ligating the V_H sequence to a sequence encoding a heavy chain constant domain of the desired isotype, expressing the light chain gene and the heavy chain construct in a cell, and collecting the anti-M-CSF antibody with the desired isotype.

[0226] In some embodiments, anti-M-CSF antibodies of the invention have the serine at position 228 (according to the EU-numbering convention) of the heavy chain changed to a proline. Accordingly, the CPSC sub-sequence in the F_C region of IgG4 becomes CPPC, which is the sub-sequence in IgG1. (Aalberse, R.C. and Schuurman, J., *Immunology*, 105:9-19 (2002)). For example, the serine at residue 243 SEQ ID NO: 46 (which corresponds to reside 228 in the EU-numbering convention) would

become proline. Similarly, the serine at residue 242 of SEQ ID NO: 38 (which corresponds to reside 228 in the EU-numbering convention) would become proline. In some embodiments, the framework region of the IgG4 antibody can be back-mutated to the germline framework sequence. Some embodiments comprise both the back-mutates framework region and the serine to proline change in the F_C region. See, e.g., SEQ ID NO: 54 (antibody 9.14.4C-Ser) and SEQ ID NO: 58 (antibody 8.10.3C-Ser) in Table 1A.

Deimmunized Antibodies

[0227] Another way of producing antibodies with reduced immunogenicity is the deimmunization of antibodies. In another aspect of the invention, the antibody may be deimmunized using the techniques described in, e.g., PCT Publication Nos. WO98/52976 and WO00/34317 (which incorporated herein by reference in their entirety).

Mutated Antibodies

[0228] In another embodiment, the nucleic acid molecules, vectors and host cells may be used to make mutated anti-M-CSF antibodies. The antibodies may be mutated in the variable domains of the heavy and/or light chains, e.g., to alter a binding property of the antibody. For example, a mutation may be made in one or more of the CDR regions to increase or decrease the K_D of the antibody for M-CSF, to increase or decrease k_{off}, or to alter the binding specificity of the antibody. Techniques in site-directed mutagenesis are well-known in the art. See, e.g., Sambrook *et al.* and Ausubel *et al.*, *supra*. In a preferred embodiment, mutations are made at an amino acid residue that is known to be changed compared to germline in a variable domain of an anti-M-CSF antibody. In another embodiment, one or more mutations are made at an amino acid residue that is known to be changed compared to the germline in a CDR region or framework region of a variable domain, or in a constant domain of a monoclonal antibody 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3,

9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 or 9.14.4G1. In another embodiment, one or more mutations are made at an amino acid residue that is known to be changed compared to the germline in a CDR region or framework region of a variable domain of a heavy chain amino acid sequence selected from SEQ ID NOS: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 46, 50, 54, 58, 62, 66, 70, 74, 78, 82, 86, 90, 94, 98 or 102, or whose heavy chain nucleotide sequence is presented in SEQ ID NOS: 1, 5, 9, 25, 29, 33, 37, 45, 97 or 101. In another embodiment, one or more mutations are made at an amino acid residue that is known to be changed compared to the germline in a CDR region or framework region of a variable domain of a light chain amino acid sequence selected from SEQ ID NOS: 4, 8, 12, 16, 20, 24, 28, 32, 36, 44, 48, 52, 56 or 60, or whose light chain nucleotide sequence is presented in SEQ ID NOS: 3, 7, 11, 27, 31, 35, 43 or 47.

[0229] In one embodiment, the framework region is mutated so that the resulting framework region(s) have the amino acid sequence of the corresponding germline gene. A mutation may be made in a framework region or constant domain to increase the half-life of the anti-M-CSF antibody. See, e.g., PCT Publication No. WO 00/09560, herein incorporated by reference. A mutation in a framework region or constant domain also can be made to alter the immunogenicity of the antibody, to provide a site for covalent or non-covalent binding to another molecule, or to alter such properties as complement fixation, FcR binding and antibody-dependent cell-mediated cytotoxicity (ADCC). According to the invention, a single antibody may have mutations in any one or more of the CDRs or framework regions of the variable domain or in the constant domain.

[0230] In some embodiments, there are from 1 to 8 including any number in between, amino acid mutations in either the V_H or V_L domains of the mutated anti-M-CSF antibody compared to the anti-M-CSF antibody prior to mutation. In any of the above, the mutations may occur in one or more CDR regions. Further, any of the mutations can be conservative amino

acid substitutions. In some embodiments, there are no more than 5, 4, 3, 2, or 1 amino acid changes in the constant domains.

Modified Antibodies

[0231] In another embodiment, a fusion antibody or immunoadhesin may be made that comprises all or a portion of an anti-M-CSF antibody of the invention linked to another polypeptide. In a preferred embodiment, only the variable domains of the anti-M-CSF antibody are linked to the polypeptide. In another preferred embodiment, the V_H domain of an anti-M-CSF antibody is linked to a first polypeptide, while the V_L domain of an anti-M-CSF antibody is linked to a second polypeptide that associates with the first polypeptide in a manner such that the V_H and V_L domains can interact with one another to form an antibody binding site. In another preferred embodiment, the V_H domain is separated from the V_L domain by a linker such that the V_H and V_L domains can interact with one another (see below under Single Chain Antibodies). The V_H-linker-V_L antibody is then linked to the polypeptide of interest. The fusion antibody is useful for directing a polypeptide to a M-CSF-expressing cell or tissue. The polypeptide may be a therapeutic agent, such as a toxin, growth factor or other regulatory protein, or may be a diagnostic agent, such as an enzyme that may be easily visualized, such as horseradish peroxidase. In addition, fusion antibodies can be created in which two (or more) single-chain antibodies are linked to one another. This is useful if one wants to create a divalent or polyvalent antibody on a single polypeptide chain, or if one wants to create a bispecific antibody.

[0232] To create a single chain antibody, (scFv) the V_H- and V_L-encoding DNA fragments are operatively linked to another fragment encoding a flexible linker, e.g., encoding the amino acid sequence $(Gly_4\text{-}Ser)_3$, such that the V_H and V_L sequences can be expressed as a contiguous single-chain protein, with the V_L and V_H domains joined by the flexible linker. See, e.g., Bird *et al.*, *Science* 242:423-426 (1988); Huston *et al.*, *Proc. Natl. Acad. Sci. USA* 85:5879-5883 (1988); McCafferty *et al.*, *Nature*

348:552-554 (1990). The single chain antibody may be monovalent, if only a single V_H and V_L are used, bivalent, if two V_H and V_L are used, or polyvalent, if more than two V_H and V_L are used. Bispecific or polyvalent antibodies may be generated that bind specifically to M-CSF and to another molecule.

[0233] In other embodiments, other modified antibodies may be prepared using anti-M-CSF antibody-encoding nucleic acid molecules. For instance, "Kappa bodies" (Ill *et al.*, *Protein Eng.* 10: 949-57 (1997)), "Minibodies" (Martin *et al.*, *EMBO J.* 13: 5303-9 (1994)), "Diabodies" (Holliger *et al.*, *Proc. Natl. Acad. Sci. USA* 90: 6444-6448 (1993)), or "Janusins" (Traunecker *et al.*, *EMBO J.* 10:3655-3659 (1991) and Traunecker *et al.*, *Int. J. Cancer* (Suppl.) 7:51-52 (1992)) may be prepared using standard molecular biological techniques following the teachings of the specification.

[0234] Bispecific antibodies or antigen-binding fragments can be produced by a variety of methods including fusion of hybridomas or linking of Fab' fragments. See, e.g., Songsivilai & Lachmann, *Clin. Exp. Immunol.* 79: 315-321 (1990), Kostelny *et al.*, *J. Immunol.* 148:1547-1553 (1992). In addition, bispecific antibodies may be formed as "diabodies" or "Janusins." In some embodiments, the bispecific antibody binds to two different epitopes of M-CSF. In some embodiments, the bispecific antibody has a first heavy chain and a first light chain from monoclonal antibody 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, or 9.7.2 and an additional antibody heavy chain and light chain. In some embodiments, the additional light chain and heavy chain also are from one of the above-identified monoclonal antibodies, but are different from the first heavy and light chains.

[0235] In some embodiments, the modified antibodies described above are prepared using one or more of the variable domains or CDR regions from a human anti-M-CSF monoclonal antibody provided herein, from an amino acid sequence of said monoclonal antibody, or from a heavy chain or light chain encoded by a nucleic acid sequence encoding said monoclonal antibody.

Derivatized and Labeled Antibodies

[0236] An anti-M-CSF antibody or antigen-binding portion of the invention can be derivatized or linked to another molecule (e.g., another peptide or protein). In general, the antibodies or portion thereof is derivatized such that the M-CSF binding is not affected adversely by the derivatization or labeling. Accordingly, the antibodies and antibody portions of the invention are intended to include both intact and modified forms of the human anti-M-CSF antibodies described herein. For example, an antibody or antibody portion of the invention can be functionally linked (by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody (e.g., a bispecific antibody or a diabody), a detection agent, a cytotoxic agent, a pharmaceutical agent, and/or a protein or peptide that can mediate associate of the antibody or antibody portion with another molecule (such as a streptavidin core region or a polyhistidine tag).

[0237] One type of derivatized antibody is produced by crosslinking two or more antibodies (of the same type or of different types, e.g., to create bispecific antibodies). Suitable crosslinkers include those that are heterobifunctional, having two distinctly reactive groups separated by an appropriate spacer (e.g., m-maleimidobenzoyl-N-hydroxysuccinimide ester) or homobifunctional (e.g., disuccinimidyl suberate). Such linkers are available from Pierce Chemical Company, Rockford, Ill.

[0238] Another type of derivatized antibody is a labeled antibody. Useful detection agents with which an antibody or antigen-binding portion of the invention may be derivatized include fluorescent compounds, including fluorescein, fluorescein isothiocyanate, rhodamine, 5-dimethylamine-1-napthalenesulfonyl chloride, phycoerythrin, lanthanide phosphors and the like. An antibody can also be labeled with enzymes that are useful for detection, such as horseradish peroxidase, β-galactosidase, luciferase, alkaline phosphatase, glucose oxidase and the like. When an antibody is labeled with a detectable enzyme, it is detected by adding

additional reagents that the enzyme uses to produce a reaction product that can be discerned. For example, when the agent horseradish peroxidase is present, the addition of hydrogen peroxide and diaminobenzidine leads to a colored reaction product, which is detectable. An antibody can also be labeled with biotin, and detected through indirect measurement of avidin or streptavidin binding. An antibody can also be labeled with a predetermined polypeptide epitope recognized by a secondary reporter (e.g., leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags). In some embodiments, labels are attached by spacer arms of various lengths to reduce potential steric hindrance.

[0239] An anti-M-CSF antibody can also be labeled with a radiolabeled amino acid. The radiolabeled anti-M-CSF antibody can be used for both diagnostic and therapeutic purposes. For instance, the radiolabeled anti-M-CSF antibody can be used to detect M-CSF-expressing tumors by x-ray or other diagnostic techniques. Further, the radiolabeled anti-M-CSF antibody can be used therapeutically as a toxin for cancerous cells or tumors. Examples of labels for polypeptides include, but are not limited to, the following radioisotopes or radionuclides – ^{3}H, ^{14}C, ^{15}N, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, and ^{131}I.

[0240] An anti-M-CSF antibody can also be derivatized with a chemical group such as polyethylene glycol (PEG), a methyl or ethyl group, or a carbohydrate group. These groups are useful to improve the biological characteristics of the antibody, e.g., to increase serum half-life or to increase tissue binding.

Pharmaceutical Compositions and Kits

[0241] The invention also relates to compositions comprising a human anti-M-CSF antagonist antibody for the treatment of subjects in need of treatment for rheumatoid arthritis, osteoporosis, or atherosclerosis. In some embodiments, the subject of treatment is a human. In other embodiments, the subject is a veterinary subject. Hyperproliferative disorders where monocytes play a role that may be treated by an

antagonist anti-M-CSF antibody of the invention can involve any tissue or organ and include but are not limited to brain, lung, squamous cell, bladder, gastric, pancreatic, breast, head, neck, liver, renal, ovarian, prostate, colorectal, esophageal, gynecological, nasopharynx, or thyroid cancers, melanomas, lymphomas, leukemias or multiple myelomas. In particular, human antagonist anti-M-CSF antibodies of the invention are useful to treat or prevent carcinomas of the breast, prostate, colon and lung.

[0242] This invention also encompasses compositions for the treatment of a condition selected from the group consisting of lupus, including systemic lupus erythematosus, lupus nephritis, and cutaneous lupus,arthritis, psoriatic arthritis, Reiter's syndrome, gout, traumatic arthritis, rubella arthritis and acute synovitis, rheumatoid arthritis, rheumatoid spondylitis, ankylosing spondylitis, osteoarthritis, gouty arthritis and other arthritic conditions, sepsis, septic shock, endotoxic shock, gram negative sepsis, toxic shock syndrome, Alzheimer's disease, stroke, neurotrauma, asthma, adult respiratory distress syndrome, cerebral malaria, chronic pulmonary inflammatory disease, silicosis, pulmonary sarcoidosis, bone resorption disease, osteoporosis, restenosis, cardiac and renal reperfusion injury, thrombosis, glomerularonephritis, diabetes, graft vs. host reaction, allograft rejection, inflammatory bowel disease, Crohn's disease, ulcerative colitis, multiple sclerosis, muscle degeneration, eczema, contact dermatitis, psoriasis, sunburn, or conjunctivitis in a mammal, including a human, comprising an amount of a human anti-M-CSF monoclonal antibody of the invention effective in such treatment and a pharmaceutically acceptable carrier.

[0243] Treatment may involve administration of one or more antagonist anti-M-CSF monoclonal antibodies of the invention, or antigen-binding fragments thereof, alone or with a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" means any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Some examples of pharmaceutically

acceptable carriers are water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Additional examples of pharmaceutically acceptable substances are wetting agents or minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the antibody.

[0244] Anti-M-CSF antibodies of the invention and compositions comprising them, can be administered in combination with one or more other therapeutic, diagnostic or prophylactic agents. Additional therapeutic agents include other anti-neoplastic, anti-tumor, anti-angiogenic or chemotherapeutic agents. Such additional agents may be included in the same composition or administered separately. In some embodiments, one or more inhibitory anti-M-CSF antibodies of the invention can be used as a vaccine or as adjuvants to a vaccine.

[0245] The compositions of this invention may be in a variety of forms, for example, liquid, semi-solid and solid dosage forms, such as liquid solutions (e.g., injectable and infusible solutions), dispersions or suspensions, tablets, pills, powders, liposomes and suppositories. The preferred form depends on the intended mode of administration and therapeutic application. Typical preferred compositions are in the form of injectable or infusible solutions, such as compositions similar to those used for passive immunization of humans. The preferred mode of administration is parenteral (e.g., intravenous, subcutaneous, intraperitoneal, intramuscular). In a preferred embodiment, the antibody is administered by intravenous infusion or injection. In another preferred embodiment, the antibody is administered by intramuscular or subcutaneous injection. In another embodiment, the invention includes a method of treating a subject in need thereof with an antibody or an antigen-binding portion thereof that specifically binds to M-CSF comprising the steps of : (a) administering an effective amount of an isolated nucleic acid molecule encoding the heavy

chain or the antigen-binding portion thereof, an isolated nucleic acid molecule encoding the light chain or the antigen-binding portion thereof, or both the nucleic acid molecules encoding the light chain and the heavy chain or antigen-binding portions thereof; and (b) expressing the nucleic acid molecule.

[0246] Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable to high drug concentration. Sterile injectable solutions can be prepared by incorporating the anti-M-CSF antibody in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

[0247] The antibodies of the present invention can be administered by a variety of methods known in the art, although for many therapeutic applications, the preferred route/mode of administration is subcutaneous, intramuscular, or intravenous infusion. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results.

[0248] In certain embodiments, the antibody compositions active compound may be prepared with a carrier that will protect the antibody against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art. See, e.g., *Sustained and Controlled Release Drug Delivery Systems* (J. R. Robinson, ed., Marcel Dekker, Inc., New York, 1978).

[0249] In certain embodiments, an anti-M-CSF antibody of the invention can be orally administered, for example, with an inert diluent or an assimilable edible carrier. The compound (and other ingredients, if desired) can also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into the subject's diet. For oral therapeutic administration, the anti-M-CSF antibodies can be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. To administer a compound of the invention by other than parenteral administration, it may be necessary to coat the compound with, or co-administer the compound with, a material to prevent its inactivation.

[0250] Additional active compounds also can be incorporated into the compositions. In certain embodiments, an anti-M-CSF antibody of the invention is co-formulated with and/or co-administered with one or more additional therapeutic agents. These agents include antibodies that bind other targets, antineoplastic agents, antitumor agents, chemotherapeutic agents, peptide analogues that inhibit M-CSF, soluble *c-fms* that can bind M-CSF, one or more chemical agents that inhibit M-CSF, anti-inflammatory agents, anti-coagulants, agents that lower blood pressure (i.e, angiotensin-converting enzyme (ACE) inhibitors). Such combination therapies may require lower dosages of the anti-M-CSF antibody as well as the co-

administered agents, thus avoiding possible toxicities or complications associated with the various monotherapies.

[0251] Inhibitory anti-M-CSF antibodies of the invention and compositions comprising them also may be administered in combination with other therapeutic regimens, in particular in combination with radiation treatment for cancer. The compounds of the present invention may also be used in combination with anticancer agents such as endostatin and angiostatin or cytotoxic drugs such as adriamycin, daunomycin, cis-platinum, etoposide, taxol, taxotere and alkaloids, such as vincristine, farnesyl transferase inhibitors, VEGF inhibitors, and antimetabolites such as methotrexate.

[0252] The compounds of the invention may also be used in combination with antiviral agents such as Viracept, AZT, aciclovir and famciclovir, and antisepsis compounds such as Valant.

[0253] The compositions of the invention may include a "therapeutically effective amount" or a "prophylactically effective amount" of an antibody or antigen-binding portion of the invention. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of the antibody or antibody portion may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the antibody or antibody portion to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the antibody or antibody portion are outweighed by the therapeutically beneficial effects. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

[0254] Dosage regimens can be adjusted to provide the optimum desired response (e.g., a therapeutic or prophylactic response). For example, a single bolus can be administered, several divided doses can be

administered over time or the dose can be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the anti-M-CSF antibody or portion and the particular therapeutic or prophylactic effect to be achieved, and (b) the limitations inherent in the art of compounding such an antibody for the treatment of sensitivity in individuals.

[0255] An exemplary, non-limiting range for a therapeutically or prophylactically effective amount of an antibody or antibody portion of the invention is 0.025 to 50 mg/kg, more preferably 0.1 to 50 mg/kg, more preferably 0.1-25, 0.1 to 10 or 0.1 to 3 mg/kg. It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

[0256] Another aspect of the present invention provides kits comprising an anti-M-CSF antibody or antigen-binding portion of the invention or a composition comprising such an antibody or portion. A kit may include, in addition to the antibody or composition, diagnostic or therapeutic agents. A kit also can include instructions for use in a diagnostic or therapeutic method. In a preferred embodiment, the kit includes the antibody or a composition comprising it and a diagnostic agent that can be used in a

method described below. In another preferred embodiment, the kit includes the antibody or a composition comprising it and one or more therapeutic agents that can be used in a method described below. One embodiment of the invention is a kit comprising a container, instructions on the administration of an anti-M-CSF antibody to a human suffering from an inflammatory disease, or instructions for measuring the number of CD14+CD16+ monocytes in a biological sample and an anti-M-CSF antibody.

[0257] This invention also relates to compositions for inhibiting abnormal cell growth in a mammal comprising an amount of an antibody of the invention in combination with an amount of a chemotherapeutic agent, wherein the amounts of the compound, salt, solvate, or prodrug, and of the chemotherapeutic agent are together effective in inhibiting abnormal cell growth. Many chemotherapeutic agents are known in the art. In some embodiments, the chemotherapeutic agent is selected from the group consisting of mitotic inhibitors, alkylating agents, anti-metabolites, intercalating antibiotics, growth factor inhibitors, cell cycle inhibitors, enzymes, topoisomerase inhibitors, biological response modifiers, anti-hormones, e.g. anti-androgens, and anti-angiogenesis agents.

[0258] Anti-angiogenic agents, such as MMP-2 (matrix-metalloproteinase 2) inhibitors, MMP-9 (matrix-metalloproteinase 9) inhibitors, and COX-II (cyclooxygenase II) inhibitors, can be used in conjunction with an anti-M-CSF antibody of the invention. Examples of useful COX-II inhibitors include CELEBREX™ (celecoxib), valdecoxib, and rofecoxib. Examples of useful matrix metalloproteinase inhibitors are described in WO 96/33172 (published October 24, 1996), WO 96/27583 (published March 7, 1996), European Patent Application No. 97304971.1 (filed July 8, 1997), European Patent Application No. 99308617.2 (filed October 29, 1999), WO 98/07697 (published February 26, 1998), WO 98/03516 (published January 29, 1998), WO 98/34918 (published August 13, 1998), WO 98/34915 (published August 13, 1998), WO 98/33768 (published August 6, 1998), WO 98/30566 (published July 16, 1998), European Patent Publication

606,046 (published July 13, 1994), European Patent Publication 931,788 (published July 28, 1999), WO 90/05719 (published May 31, 1990), WO 99/52910 (published October 21, 1999), WO 99/52889 (published October 21, 1999), WO 99/29667 (published June 17, 1999), PCT International Application No. PCT/IB98/01113 (filed July 21, 1998), European Patent Application No. 99302232.1 (filed March 25, 1999), Great Britain patent application number 9912961.1 (filed June 3, 1999), U.S. Provisional Application No. 60/148,464 (filed August 12, 1999), U.S. Patent 5,863,949 (issued January 26, 1999), U.S. Patent 5,861,510 (issued January 19, 1999), and European Patent Publication 780,386 (published June 25, 1997), all of which are incorporated herein in their entireties by reference. Preferred MMP inhibitors are those that do not demonstrate arthralgia. More preferred, are those that selectively inhibit MMP-2 and/or MMP-9 relative to the other matrix-metalloproteinases (i.e. MMP-1, MMP-3, MMP-4, MMP-5, MMP-6, MMP-7, MMP-8, MMP-10, MMP-11, MMP-12, and MMP-13). Some specific examples of MMP inhibitors useful in the present invention are AG-3340, RO 32-3555, RS 13-0830, and the compounds recited in the following list: 3-[[4-(4-fluoro-phenoxy)-benzenesulfonyl]-(1-hydroxycarbamoyl-cyclopentyl)-amino]-propionic acid; 3-exo-3-[4-(4-fluoro-phenoxy)-benzenesulfonylamino]-8-oxa-bicyclo[3.2.1]octane-3-carboxylic acid hydroxyamide; (2R, 3R) 1-[4-(2-chloro-4-fluoro-benzyloxy)-benzenesulfonyl]-3-hydroxy-3-methyl-piperidine-2-carboxylic acid hydroxyamide; 4-[4-(4-fluoro-phenoxy)-benzenesulfonylamino]-tetrahydro-pyran-4-carboxylic acid hydroxyamide; 3-[[4-(4-fluoro-phenoxy)-benzenesulfonyl]-(1-hydroxycarbamoyl-cyclobutyl)-amino]-propionic acid; 4-[4-(4-chloro-phenoxy)-benzenesulfonylamino]-tetrahydro-pyran-4-carboxylic acid hydroxyamide; (R) 3-[4-(4-chloro-phenoxy)-benzenesulfonylamino]-tetrahydro-pyran-3-carboxylic acid hydroxyamide; (2R, 3R) 1-[4-(4-fluoro-2-methyl-benzyloxy)-benzenesulfonyl]-3-hydroxy-3-methyl-piperidine-2-carboxylic acid hydroxyamide; 3-[[4-(4-fluoro-phenoxy)-benzenesulfonyl]-(1-hydroxycarbamoyl-1-methyl-ethyl)-amino]-propionic acid; 3-[[4-(4-fluoro-phenoxy)-benzenesulfonyl]-(4-hydroxycarbamoyl-

tetrahydro-pyran-4-yl)-amino]-propionic acid; 3-exo-3-[4-(4-chloro-phenoxy)-benzenesulfonylamino]-8-oxa-bicyclo[3.2.1]octane-3-carboxylic acid hydroxyamide; 3-endo-3-[4-(4-fluoro-phenoxy)-benzenesulfonylamino]-8-oxa-bicyclo[3.2.1]octane-3-carboxylic acid hydroxyamide; and (R) 3-[4-(4-fluoro-phenoxy)-benzenesulfonylamino]-tetrahydro-furan-3-carboxylic acid hydroxyamide; and pharmaceutically acceptable salts and solvates of said compounds.

[0259] A compound comprising a human anti-M-CSF monoclonal antibody of the invention can also be used with signal transduction inhibitors, such as agents that can inhibit EGF-R (epidermal growth factor receptor) responses, such as EGF-R antibodies, EGF antibodies, and molecules that are EGF-R inhibitors; VEGF (vascular endothelial growth factor) inhibitors, such as VEGF receptors and molecules that can inhibit VEGF; and erbB2 receptor inhibitors, such as organic molecules or antibodies that bind to the erbB2 receptor, for example, HERCEPTIN™ (Genentech, Inc.). EGF-R inhibitors are described in, for example in WO 95/19970 (published July 27, 1995), WO 98/14451 (published April 9, 1998), WO 98/02434 (published January 22, 1998), and United States Patent 5,747,498 (issued May 5, 1998), and such substances can be used in the present invention as described herein. EGFR-inhibiting agents include, but are not limited to, the monoclonal antibodies C225 and anti-EGFR 22Mab (ImClone Systems Incorporated), ABX-EGF (Abgenix/Cell Genesys), EMD-7200 (Merck KgaA), EMD-5590 (Merck KgaA), MDX-447/H-477 (Medarex Inc. and Merck KgaA), and the compounds ZD-1834, ZD-1838 and ZD-1839 (AstraZeneca), PKI-166 (Novartis), PKI-166/CGP-75166 (Novartis), PTK 787 (Novartis), CP 701 (Cephalon), leflunomide (Pharmacia/Sugen), CI-1033 (Warner Lambert Parke Davis), CI-1033/PD 183,805 (Warner Lambert Parke Davis), CL-387,785 (Wyeth-Ayerst), BBR-1611 (Boehringer Mannheim GmbH/Roche), Naamidine A (Bristol Myers Squibb), RC-3940-II (Pharmacia), BIBX-1382 (Boehringer Ingelheim), OLX-103 (Merck & Co.), VRCTC-310 (Ventech Research), EGF fusion toxin (Seragen Inc.), DAB-389 (Seragen/Lilgand), ZM-252808 (Imperial Cancer

Research Fund), RG-50864 (INSERM), LFM-A12 (Parker Hughes Cancer Center), WHI-P97 (Parker Hughes Cancer Center), GW-282974 (Glaxo), KT-8391 (Kyowa Hakko) and EGF-R Vaccine (York Medical/Centro de Immunologia Molecular (CIM)). These and other EGF-R-inhibiting agents can be used in the present invention.

[0260] VEGF inhibitors, for example SU-5416 and SU-6668 (Sugen Inc.), AVASTIN™ (Genentech), SH-268 (Schering), and NX-1838 (NeXstar) can also be combined with the compound of the present invention. VEGF inhibitors are described in, for example in WO 99/24440 (published May 20, 1999), PCT International Application PCT/IB99/00797 (filed May 3, 1999), in WO 95/21613 (published August 17, 1995), WO 99/61422 (published December 2, 1999), United States Patent 5,834,504 (issued November 10, 1998), WO 98/50356 (published November 12, 1998), United States Patent 5,883,113 (issued March 16, 1999), United States Patent 5,886,020 (issued March 23, 1999), United States Patent 5,792,783 (issued August 11, 1998), WO 99/10349 (published March 4, 1999), WO 97/32856 (published September 12, 1997), WO 97/22596 (published June 26, 1997), WO 98/54093 (published December 3, 1998), WO 98/02438 (published January 22, 1998), WO 99/16755 (published April 8, 1999), and WO 98/02437 (published January 22, 1998), all of which are incorporated herein in their entireties by reference. Other examples of some specific VEGF inhibitors useful in the present invention are IM862 (Cytran Inc.); anti-VEGF monoclonal antibody of Genentech, Inc.; and angiozyme, a synthetic ribozyme from Ribozyme and Chiron. These and other VEGF inhibitors can be used in the present invention as described herein. ErbB2 receptor inhibitors, such as GW-282974 (Glaxo Wellcome plc), and the monoclonal antibodies AR-209 (Aronex Pharmaceuticals Inc.) and 2B-1 (Chiron), can furthermore be combined with the compound of the invention, for example those indicated in WO 98/02434 (published January 22, 1998), WO 99/35146 (published July 15, 1999), WO 99/35132 (published July 15, 1999), WO 98/02437 (published January 22, 1998), WO 97/13760 (published April 17, 1997), WO 95/19970 (published July 27, 1995), United

States Patent 5,587,458 (issued December 24, 1996), and United States Patent 5,877,305 (issued March 2, 1999), which are all hereby incorporated herein in their entireties by reference. ErbB2 receptor inhibitors useful in the present invention are also described in United States Patent 6,465,449 (issued October 15, 2002), and in United States Patent 6,284,764 (issued September 4, 2001), both of which are incorporated in their entireties herein by reference. The erbB2 receptor inhibitor compounds and substance described in the aforementioned PCT applications, U.S. patents, and U.S. provisional applications, as well as other compounds and substances that inhibit the erbB2 receptor, can be used with the compound of the present invention in accordance with the present invention.

[0261] Anti-survival agents include anti-IGF-IR antibodies and anti-integrin agents, such as anti-integrin antibodies.

[0262] Anti-inflammatory agents can be used in conjunction with an anti-M-CSF antibody of the invention. For the treatment of rheumatoid arthritis, the human anti-M-CSF antibodies of the invention may be combined with agents such as TNF-∀ inhibitors such as TNF drugs (such as REMICADE™, CDP-870 and HUMIRA™) and TNF receptor immunoglobulin molecules (such as ENBREL™), IL-1 inhibitors, receptor antagonists or soluble IL-1ra (e.g. Kineret or ICE inhibitors), COX-2 inhibitors (such as celecoxib , rofecoxib, valdecoxib and etoricoxib), metalloprotease inhibitors (preferably MMP-13 selective inhibitors), p2X7 inhibitors, ∀2δ ligands (such as NEUROTIN™ AND PREGABALIN™), low dose methotrexate, leflunomide, hydroxychloroquine, d-penicillamine, auranofin or parenteral or oral gold. The compounds of the invention can also be used in combination with existing therapeutic agents for the treatment of osteoarthritis. Suitable agents to be used in combination include standard non-steroidal anti-inflammatory agents (hereinafter NSAID's) such as piroxicam, diclofenac, propionic acids such as naproxen, flurbiprofen, fenoprofen, ketoprofen and ibuprofen, fenamates such as mefenamic acid, indomethacin, sulindac, apazone, pyrazolones such as phenylbutazone, salicylates such as aspirin, COX-2 inhibitors such as

celecoxib, valdecoxib, rofecoxib and etoricoxib, analgesics and intraarticular therapies such as corticosteroids and hyaluronic acids such as hyalgan and synvisc.

[0263] Anti-coagulant agents can be used in conjunction with an anti-M-CSF antibody of the invention. Examples of anti-coagulant agents include, but are not limited to, warfarin (COUMADIN™), heparin, and enoxaparin (LOVENOX™).

[0264] The human anti-M-CSF antibodies of the present invention may also be used in combination with cardiovascular agents such as calcium channel blockers, lipid lowering agents such as statins, fibrates, beta-blockers, Ace inhibitors, Angiotensin-2 receptor antagonists and platelet aggregation inhibitors. The compounds of the present invention may also be used in combination with CNS agents such as antidepressants (such as sertraline), anti-Parkinsonian drugs (such as deprenyl, L-dopa, REQUIP™, MIRAPEX™, MAOB inhibitors such as selegine and rasagiline, comP inhibitors such as Tasmar, A-2 inhibitors, dopamine reuptake inhibitors, NMDA antagonists, Nicotine agonists, Dopamine agonists and inhibitors of neuronal nitric oxide synthase), and anti-Alzheimer's drugs such as donepezil, tacrine, ∀2δ LIGANDS (such NEUROTIN™ and PREGABALIN™) inhibitors, COX-2 inhibitors, propentofylline or metryfonate.

[0265] The human anti-M-CSF antibodies of the present invention may also be used in combination with osteoporosis agents such as roloxifene, droloxifene, lasofoxifene or fosomax and immunosuppressant agents such as FK-506 and rapamycin.

Diagnostic Methods of Use

[0266] In another aspect, the invention provides diagnostic methods. The anti-M-CSF antibodies can be used to detect M-CSF in a biological sample *in vitro* or *in vivo*. In one embodiment, the invention provides a method for diagnosing the presence or location of a M-CSF-expressing tumor in a subject in need thereof, comprising the steps of injecting the antibody into the subject, determining the expression of M-CSF in the

subject by localizing where the antibody has bound, comparing the expression in the subject with that of a normal reference subject or standard, and diagnosing the presence or location of the tumor.

[0267] The anti-M-CSF antibodies can be used in a conventional immunoassay, including, without limitation, an ELISA, an RIA, FACS, tissue immunohistochemistry, Western blot or immunoprecipitation. The anti-M-CSF antibodies of the invention can be used to detect M-CSF from humans. In another embodiment, the anti-M-CSF antibodies can be used to detect M-CSF from primates such as cynomologus monkey, rhesus monkeys, chimpanzees or apes. The invention provides a method for detecting M-CSF in a biological sample comprising contacting a biological sample with an anti-M-CSF antibody of the invention and detecting the bound antibody. In one embodiment, the anti-M-CSF antibody is directly labeled with a detectable label. In another embodiment, the anti-M-CSF antibody (the first antibody) is unlabeled and a second antibody or other molecule that can bind the anti-M-CSF antibody is labeled. As is well known to one of skill in the art, a second antibody is chosen that is able to specifically bind the particular species and class of the first antibody. For example, if the anti-M-CSF antibody is a human IgG, then the secondary antibody could be an anti-human-IgG. Other molecules that can bind to antibodies include, without limitation, Protein A and Protein G, both of which are available commercially, e.g., from Pierce Chemical Co.

[0268] Suitable labels for the antibody or secondary antibody have been disclosed *supra*, and include various enzymes, prosthetic groups, fluorescent materials, luminescent materials and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an

example of a luminescent material includes luminol; and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{35}S or ^{3}H.

[0269] In other embodiments, M-CSF can be assayed in a biological sample by a competition immunoassay utilizing M-CSF standards labeled with a detectable substance and an unlabeled anti-M-CSF antibody. In this assay, the biological sample, the labeled M-CSF standards and the anti-M-CSF antibody are combined and the amount of labeled M-CSF standard bound to the unlabeled antibody is determined. The amount of M-CSF in the biological sample is inversely proportional to the amount of labeled M-CSF standard bound to the anti-M-CSF antibody.

[0270] One can use the immunoassays disclosed above for a number of purposes. For example, the anti-M-CSF antibodies can be used to detect M-CSF in cells or on the surface of cells in cell culture, or secreted into the tissue culture medium. The anti-M-CSF antibodies can be used to determine the amount of M-CSF on the surface of cells or secreted into the tissue culture medium that have been treated with various compounds. This method can be used to identify compounds that are useful to inhibit or activate M-CSF expression or secretion. According to this method, one sample of cells is treated with a test compound for a period of time while another sample is left untreated. If the total level of M-CSF is to be measured, the cells are lysed and the total M-CSF level is measured using one of the immunoassays described above. The total level of M-CSF in the treated versus the untreated cells is compared to determine the effect of the test compound.

[0271] An immunoassay for measuring total M-CSF levels is an ELISA or Western blot. If the cell surface level of M-CSF is to be measured, the cells are not lysed, and the M-CSF cell surface levels can be measured using one of the immunoassays described above. An immunoassay for determining cell surface levels of M-CSF can include the steps of labeling the cell surface proteins with a detectable label, such as biotin or ^{125}I, immunoprecipitating the M-CSF with an anti-M-CSF antibody and then detecting the labeled M-CSF. Another immunoassay for determining the

localization of M-CSF, e.g., cell surface levels, can be immunohistochemistry. Methods such as ELISA, RIA, Western blot, immunohistochemistry, cell surface labeling of integral membrane proteins and immunoprecipitation are well known in the art. See, e.g., Harlow and Lane, *supra*. In addition, the immunoassays can be scaled up for high throughput screening in order to test a large number of compounds for inhibition or activation of M-CSF.

[0272] Another example of an immunoassay for measuring secreted M-CSF levels can be an antigen capture assay, ELISA, immunohistochemistry assay, Western blot and the like using antibodies of the invention. If secreted M-CSF is to be measured, cell culture media or body fluid, such as blood serum, urine, or synovial fluid, can be assayed for secreted M-CSF and/or cells can be lysed to release produced, but not yet secreted M-CSF. An immunoassay for determining secreted levels of M-CSF includes the steps of labeling the secreted proteins with a detectable label, such as biotin or ^{125}I, immunoprecipitating the M-CSF with an anti-M-CSF antibody and then detecting the labeled M-CSF. Another immunoassay for determining secreted levels of M-CSF can include the steps of (a) pre-binding anti-M-CSF antibodies to the surface of a microtiter plate; (b) adding tissue culture cell media or body fluid containing the secreted M-CSF to the wells of the microtiter plate to bind to the anti-M-CSF antibodies; (c) adding an antibody that will detect the anti-M-CSF antibody, e.g., anti-M-CSF labeled with digoxigenin that binds to an epitope of M-CSF different from the anti-M-CSF antibody of step (a); (d) adding an antibody to digoxigenin conjugated to peroxidase; and (e) adding a peroxidase substrate that will yield a colored reaction product that can be quantitated to determine the level of secreted M-CSF in tissue culture cell media or a body fluid sample. Methods such as ELISA, RIA, Western blot, immunohistochemistry, and antigen capture assay are well known in the art. See, e.g., Harlow and Lane, *supra*. In addition, the immunoassays can be scaled up for high throughput screening in order to test a large number of compounds for inhibition or activation of M-CSF.

[0273] The anti-M-CSF antibodies of the invention can also be used to determine the levels of cell surface M-CSF in a tissue or in cells derived from the tissue. In some embodiments, the tissue is from a diseased tissue. In some embodiments, the tissue can be a tumor or a biopsy thereof. In some embodiments of the method, a tissue or a biopsy thereof can be excised from a patient. The tissue or biopsy can then be used in an immunoassay to determine, e.g., total M-CSF levels, cell surface levels of M-CSF, or localization of M-CSF by the methods discussed above.

[0274] The method can comprise the steps of administering a detectably labeled anti-M-CSF antibody or a composition comprising them to a patient in need of such a diagnostic test and subjecting the patient to imaging analysis to determine the location of the M-CSF-expressing tissues. Imaging analysis is well known in the medical art, and includes, without limitation, x-ray analysis, magnetic resonance imaging (MRI) or computed tomography (CE). The antibody can be labeled with any agent suitable for *in vivo* imaging, for example a contrast agent, such as barium, which can be used for x-ray analysis, or a magnetic contrast agent, such as a gadolinium chelate, which can be used for MRI or CE. Other labeling agents include, without limitation, radioisotopes, such as ^{99}Tc. In another embodiment, the anti-M-CSF antibody will be unlabeled and will be imaged by administering a second antibody or other molecule that is detectable and that can bind the anti-M-CSF antibody. In an embodiment, a biopsy is obtained from the patient to determine whether the tissue of interest expresses M-CSF.

[0275] The anti-M-CSF antibodies of the invention can also be used to determine the secreted levels of M-CSF in a body fluid such as blood serum, urine, or synovial fluid derived from a tissue. In some embodiments, the body fluid is from a diseased tissue. In some embodiments, the body fluid is from a tumor or a biopsy thereof. In some embodiments of the method, body fluid is removed from a patient. The body fluid is then used in an immunoassay to determine secreted M-CSF levels by the methods discussed above. One embodiment of the invention

is a method of assaying for the activity of a M-CSF antagonist comprising: administering a M-CSF antagonist to a primate or human subject and measuring the number of CD14+CD16+ monocytes in a biological sample.

Therapeutic Methods of Use

[0276] In another embodiment, the invention provides a method for inhibiting M-CSF activity by administering an anti-M-CSF antibody to a patient in need thereof. Any of the types of antibodies described herein may be used therapeutically. In a preferred embodiment, the anti-M-CSF antibody is a human, chimeric or humanized antibody. In another preferred embodiment, the M-CSF is human and the patient is a human patient. Alternatively, the patient may be a mammal that expresses a M-CSF that the anti-M-CSF antibody cross-reacts with. The antibody may be administered to a non-human mammal expressing a M-CSF with which the antibody cross-reacts (i.e. a primate) for veterinary purposes or as an animal model of human disease. Such animal models may be useful for evaluating the therapeutic efficacy of antibodies of this invention.

[0277] As used herein, the term "a disorder in which M-CSF activity is detrimental" is intended to include diseases and other disorders in which the presence of high levels of M-CSF in a subject suffering from the disorder has been shown to be or is suspected of being either responsible for the pathophysiology of the disorder or a factor that contributes to a worsening of the disorder. Such disorders may be evidenced, for example, by an increase in the levels of M-CSF secreted and/or on the cell surface or increased tyrosine autophosphorylation of *c-fms* in the affected cells or tissues of a subject suffering from the disorder. The increase in M-CSF levels may be detected, for example, using an anti-M-CSF antibody as described above.

[0278] In one embodiment, an anti-M-CSF antibody may be administered to a patient who has a *c-fms*-expressing tumor or a tumor that secretes M-CSF and/or that expresses M-CSF on its cell surface. Preferably, the tumor expresses a level of *c-fms* or M-CSF that is higher than a normal

tissue. The tumor may be a solid tumor or may be a non-solid tumor, such as a lymphoma. In a more preferred embodiment, an anti-M-CSF antibody may be administered to a patient who has a *c-fms*-expressing tumor, a M-CSF-expressing tumor, or a tumor that secretes M-CSF that is cancerous. Further, the tumor may be cancerous. In an even more preferred embodiment, the tumor is a cancer of lung, breast, prostate or colon. In another preferred embodiment, the anti-M-CSF antibody administered to a patient results in M-CSF no longer bound to the *c-fms* receptor. In a highly preferred embodiment, the method causes the tumor not to increase in weight or volume or to decrease in weight or volume. In another embodiment, the method causes *c-fms* on tumor cells to not be bound by M-CSF. In another embodiment, the method causes M-CSF on tumor cells to not be bound to *c-fms*. In another embodiment, the method causes secreted M-CSF of the tumor cells to not be bound to *c-fms*. In a preferred embodiment, the antibody is selected from 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 or 9.14.4G1, or comprises a heavy chain, light chain or antigen binding region thereof. In another embodiment, the antibody is an anti-M-CSF antibody that has a heavy chain, a light chain, or both a heavy chain and light chain, that is or are 90%, 91%, 92,%, 93%, 94%, 95%, 96%, 96%, 97%, 98%, or 99% identical to the heavy chain, light chain, or both the heavy and light chain of antibody 8.10.3F respectively.

[0279] In another preferred embodiment, an anti-M-CSF antibody may be administered to a patient who expresses inappropriately high levels of M-CSF. It is known in the art that high-level expression of M-CSF can lead to a variety of common cancers. In one embodiment, said method relates to the treatment of cancer such as brain, squamous cell, bladder, gastric, pancreatic, breast, head, neck, esophageal, prostate, colorectal, lung, renal, kidney, ovarian, gynecological or thyroid cancer. Patients that can be treated with a compounds of the invention according to the methods of

this invention include, for example, patients that have been diagnosed as having lung cancer, bone cancer, pancreatic cancer, skin cancer, cancer of the head and neck, cutaneous or intraocular melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, colon cancer, breast cancer, gynecologic tumors (e.g., uterine sarcomas, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina or carcinoma of the vulva), Hodgkin's disease, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system (e.g., cancer of the thyroid, parathyroid or adrenal glands), sarcomas of soft tissues, cancer of the urethra, cancer of the penis, prostate cancer, chronic or acute leukemia, solid tumors (e.g., sarcomas, carcinomas or lymphomas that are cancers of body tissues other than blood, bone marrow or the lymphatic system), solid tumors of childhood, lymphocytic lymphomas, cancer of the bladder, cancer of the kidney or ureter (e.g., renal cell carcinoma, carcinoma of the renal pelvis), or neoplasms of the central nervous system (e.g., primary CNS lymphoma, spinal axis tumors, brain stem gliomas or pituitary adenomas). In a more preferred embodiment, the anti-M-CSF antibody is administered to a patient with breast cancer, prostate cancer, lung cancer or colon cancer. In an even more preferred embodiment, the method causes the cancer to stop proliferating abnormally, or not to increase in weight or volume or to decrease in weight or volume.

[0280] In another preferred embodiment, an anti-M-CSF antibody may be administered to a patient who expresses inappropriately high levels of M-CSF,which can lead to a variety of inflammatory or immune disorders. Such disorders include, but are not limited to, lupus, including SLE, lupus nephritis, and cutaneous lupus, arthritis, psoriatic arthritis, Reiter's syndrome, gout, traumatic arthritis, rubella arthritis and acute synovitis, rheumatoid arthritis, rheumatoid spondylitis, ankylosing spondylitis, osteoarthritis, gouty arthritis and other arthritic conditions, sepsis, septic shock, endotoxic shock, gram negative sepsis, toxic shock syndrome, Alzheimer's disease, stroke, neurotrauma, asthma, adult respiratory

distress syndrome, cerebral malaria, chronic pulmonary inflammatory disease, silicosis, pulmonary sarcoidosis, bone resorption disease, osteoporosis, restenosis, cardiac and renal reperfusion injury, thrombosis, glomerularonephritis, diabetes, graft vs. host reaction, allograft rejection, inflammatory bowel disease, Crohn's disease, ulcerative colitis, multiple sclerosis, muscle degeneration, eczema, contact dermatitis, psoriasis, sunburn, and conjunctivitis,

[0281] The antibody may be administered once, but more preferably is administered multiple times. For example, the antibody may be administered from three times daily to once every six months or longer. The administering may be on a schedule such as three times daily, twice daily, once daily, once every two days, once every three days, once weekly, once every two weeks, once every month, once every two months, once every three months and once every six months. The antibody may also be administered continuously via a minipump. The antibody may be administered via an oral, mucosal, buccal, intranasal, inhalable, intravenous, subcutaneous, intramuscular, parenteral, intratumor or topical route. The antibody may be administered at the site of the tumor or inflamed body part, into the tumor or inflamed body part, or at a site distant from the site of the tumor or inflamed body part. The antibody may be administered once, at least twice or for at least the period of time until the condition is treated, palliated or cured. The antibody generally will be administered for as long as the tumor is present provided that the antibody causes the tumor or cancer to stop growing or to decrease in weight or volume or until the inflamed body part is healed. The antibody will generally be administered as part of a pharmaceutical composition as described *supra*. The dosage of antibody will generally be in the range of 0.1-100 mg/kg, more preferably 0.5-50 mg/kg, more preferably 1-20 mg/kg, and even more preferably 1-10 mg/kg. The serum concentration of the antibody may be measured by any method known in the art.

[0282] In another aspect, efficacy of the anti-M-CSF antibody in treating or preventing various disorders and diseases may be determined by

examining patients for changes in symptoms, various biomarkers, tissue histology, and physiological conditions, that are associated with the various disorders and diseases. Such symptoms, biomarkers, tissue histology and conditions are within the skill of a person skilled in the art to determine. For example, efficacy of the anti-M-CSF antibody in treating or preventing lupus in patients may include analyzing or observing changes in lupus related symptoms such as skin lesions, proteinuria, lymphadenopathy, serum M-CSF levels, anti-dsDNA antibody levels, and changes in kidney pathology such as by examining macrophage infiltration, inflammatory infiltrates, proteinaceous casts, size of glomerular tufts, glomerular IgG deposits, and C3 deposits. Changes in monocyte populations, such as CD14+CD16+ monocytes, and changes in osteoclast markers, such as uNTX-1 can also be examined. Biomarkers for systemic lupus, cutaneous lupus, and lupus nephritis may include erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), complement (C3/C4), Ig levels (IgA, IgM, IgG), antinuclear antibodies (ANA), extractable nuclear antigen (ENA), and anti-dsDNA antibodies.

[0283] In addition to pharmacodynamic biomarker data, proof of principle biomarkers may include markers for the M-CSF pathway, proinflammatory cytokines/chemokines, immune cell subsets (B cell, T cell, and DC), and other disease-related markers from whole blood, serum, urine, and tissue samples taken throughout clinical studies.

[0284] For example, an exploratory serum biomarker panel can be used to examine changes in serum levels of cytokines, chemokines, and additional serum proteins related to M-CSF, monocyte, macrophage, and lupus (systemic, nephritis, and cutaneous) activity. Serum biomarkers may be examined by standard immunoassay techniques that are well known to persons skilled in the art. A panel of cytokine/chemokine serum biomarkers may include, for example: IFN-gamma, IFN-alpha, IL-12, TNF-alpha, IL-2, IL-4, IL-5, IL-13, IL-15, IL-6, IL-10, TGF-beta, IL-1-alpha, IL-1-beta, IL-21, IL-22, IL-23, IL-17A, IL-17F, CXCL10, CXCL9, CCL2, CCL19, RANTES, CXCL11, CCL7, CCL3, CXCL13, CCL8, CXCL8, CD40L, soluble

TNFR, and soluble IL-1 receptor antagonist. A panel related to M-CSF, monocyte, and macrophage activity include: M-CSF, GM-CSF, RANKL, soluble CD14, and neopterin. A subset of serum biomarkers related to cutaneous lupus, systemic lupus may include: E-selectin, BAFF, soluble CD27, soluble CD154, and CCL17.

[0285] Urine from treated patients can be examined for changes in a panel of urine biomarkers that may include, among others: M-CSF, MCP-1, IL-6, IL-10, CCL3, and RANTES.

[0286] To evaluate changes in the circulating immune cell repertoire with anti-M-CSF antibody exposure, fluorescence activated cell sorting (FACS) of whole blood from treated human subjects may also be examined. Immune cell subsets can be defined by established surface marker expression patterns. For example, a B cell subset panel may include evaluation of total B cells, naïve B cells, memory B cells (non-switched and class-switched), plasma cells, double negative B cells, and IgD naïve B cells. A transitional B cell panel may include transitional B cells, immature B cells, and germinal center B cells. A T cell subset panel may delineate total T cells, NK T cells, NK cells, naïve T cells, memory T cells, central memory T cells, and effector memory T cells. A dendritic cell panel may examine myeloid dendritic cells as well as plasmacytoid dendritic cells. Such cell subsets are within the skill of a person skilled in the art to determine and detect.

[0287] In addition, changes in RNA expression with anti-M-CSF antibody exposure may be examined. RNA may be isolated from whole blood and tissue biopsy samples from treated patients. Gene expression can be quantitated from isolated RNA, for example, via standard Taqman RT-qPCR TLDA panel assay techniques. For example, Table 1C lists a panel of target genes that are associated with M-CSF pathway engagement, cytokine/chemokineactivity, and disease-related gene expression. RNA expression of one or more of the genes listed in Table 1C may be examined to determine efficacy of treatment.

[0288] For example, lesional and nonlesional skin biopsies from cutaneous lupus patients may be evaluated for M-CSF-dependent changes by immunohistochemistry (IHC) and RNA profiling. Cell populations to be examined by IHC include macrophage, dendritic cell (pDC and mDC) and T cell populations. Additional IHC staining for biomarkers listed in the serum biomarker panel are under consideration. Expression analysis of RNA isolated from skin biopsies may examine one or more of the genes listed in Table 1C.

Table 1C

GENE, GeneBook	Entrez Gene ID
granulocyte colony stimulating factor	0
alpha 2M receptor expression	2
ACACA	31
ACACB	32
ACP5	54
AKT1	207
Akt2 expression	208
ALAS2	212
ALOX15	246
Amd1	262
ANPEP	290
BIRC5	332
APOC1	341
APOE	348
FAS	355
FASLG	356
ARAF	369
ARF6 WT	382
ARG1	383
ARHGAP5	394
BAX	581
cyclin D1	595
BCL2	596
BCL2L1	598
high level CSF-1	629
bone morphogenetic protein-2-induced colony stimulating factor-1	650
CA2	760
OPGL/CSF-1 treatment	796

GENE, GeneBook	Entrez Gene ID
CALCR	799
CASP3	836
CSF-1	841
caspase 9	842
Runx1	861
CBL	867
CCND2	894
CSF-1	920
CD14	929
CD34 positive hematopoietic progenitor cells	947
CD36	948
CD44	960
CD47	961
CD68	968
CD97	976
CDH5	1003
CDK4	1019
CDKN1A	1026
CDKN2D	1032
CCR5	1234
COL3A1	1281
CP	1356
CPE	1363
CREB1	1385
CREB-binding protein	1387
CRP	1401
MAPK14	1432
CSF-1	1435
macrophages CSF-1 anti-sense oligodeoxynucleotide	1435

GENE, GeneBook	Entrez Gene ID
Anti-CSF I antibody	1435
Anti-CSF-1 Fab	1435
c-fms gene product	1436
CSF-1 receptor expressing BT20 breast cancer cell line	1436
c-fms proto-oncogene	1436
CSF-1 receptor mRNA	1436
mouse c-fms	1436
CSF1R	1436
granulocyte-macrophage colony-stimulating factor (GM-CSF)-stimulated BAC1.2F5 macrophages	1437
GM-CSF	1437
CSF2RA	1438
granulocyte colony stimulating factor	1440
c-Src kinase activity	1445
CTSK	1513
CYP11A1	1583
CYP17A1	1586
DCK	1633
DNMT1	1786
DUSP1	1843
epidermal growth factor (EGF)	1950
EGR1	1958
EGR2	1959
EGR3	1960
CSF-1-induced TCF/SAP-1 modification	2002
SLC29A1	2030
erythropoietin (EPO)	2056
ErbB2	2064
endogenous ets-2	2114

GENE, GeneBook	Entrez Gene ID
colony-stimulating factor 1	2114
ETS2	2114
ETV3	2117
plasma prothrombin fragment F1	2147
F2R	2149
F2RL1	2150
F2RL2	2151
FABP7	2173
PTK2B	2185
FASN	2194
FCER1G	2207
FCGR2A	2212
FCGR2B	2213
FCGR3A	2214
FLT3	2322
FN1	2335
c-fos	2353
FYN	2534
GAPDH	2597
growth factor independent-1	2672
CXCR3	2833
several other GRB2-associated proteins	2885
HCK	3055
HDAC1	3065
HDAC2	3066
HLX1	3142
HNRNPH1	3187
HRAS	3265
HSD17B1	3292
HSPA5	3309

GENE, GeneBook	Entrez Gene ID
HTR5A	3361
IRF8	3394
IFNB1	3456
IFN-gamma	3458
purified IFN-gamma	3458
IFN-gamma	3458
IFNGR1	3459
IGF-1	3479
IGF1R	3480
IGFBP2	3485
IGF binding protein-3	3486
IKK beta	3551
IL-1 alpha	3552
IL-1 beta	3553
murine interleukin-1 receptor antagonist	3557
IL-2-receptor	3558
interleukin-2 (IL-2)	3558
IL-3	3562
IL-4	3565
IL-6	3569
IL6ST	3572
IL-7 enhanced macrophage colony-stimulating factor (CSF-1)-induced colony formation	3574
IL-8	3576
IL8	3576
IL10 level	3586
IL10RA	3587
Il11ra2	3590
IL12A	3592
IL12B	3593

GENE, GeneBook	Entrez Gene ID
IL18	3606
CSF-1	3630
INPP5D	3635
INPPL1	3636
IRF7	3665
ITGA5	3678
ITGAM	3684
ITGAV	3685
all CD11c(high) DC subsets	3687
ITGB3	3690
ITGB5	3693
jun	3725
JUNB	3726
KCNA3	3738
KCNJ2	3759
LHB	3972
TNF-beta	4049
LYN	4067
Smad 4 increased CSF-1 transcription	4089
MAP3K3	4215
MMP2	4313
MMP9	4318
MMP12	4321
MMP16	4325
MSR1	4481
chimeric c-Myc transactivators	4609
NFATC1	4772
NF-kappaB site	4790
NFKB2	4791
CSF-1 antibody	4843

GENE, GeneBook	Entrez Gene ID
NPY1R	4886
NT5E	4907
chimeric colony-stimulating factor-1/TrkB-receptor	4915
colony-stimulating factor-1	4982
OPRD1	4985
CSF-1-mediated activation	4988
SERPINE1	5054
PAI-2	5055
PAK2	5062
PDE6A	5145
PDGFA	5154
PDGFB receptor	5155
PENK	5179
PIK3CA	5290
PIK3CB	5291
PIK3CD	5293
PI3Kgamma(-/	5294
PIK3R2	5296
phospholipase A2 activity	5319
PLA2G4A	5321
uPA promoter activation	5328
uPA receptor	5328
PLCG2	5336
PLD2	5338
PPARG	5468
PRKACA	5566
PRKCA	5578
MAPK1	5594
MAPK3	5595
ERK5/BMK1	5598

GENE, GeneBook	Entrez Gene ID
MAPK8	5599
MAPK9	5601
MAP2K1	5604
MAP2K2	5605
augmented PTH-induced increases	5741
anti-CSF-1	5741
anti-CSF-1	5744
anti-CSF-1	5745
PTK2	5747
SHP-1	5777
PTPN6	5777
anti-CD148 monoclonal antibody	5795
PTPRO	5800
RAC1	5879
not dominant-negative c-Raf-1	5894
RBP1	5947
RPS6KB2	6199
CCL3	6348
CCL5	6352
CCL13	6357
CX3CL1	6376
CSF-1	6446
SHC1	6464
INI1/hSNF5/BAF47	6598
SPI1	6688
SRC-1	6714
STAT1	6772
STAT3	6774
STX3	6809
Syk Piceatannol	6850

GENE, GeneBook	Entrez Gene ID
Substance P augmentation	6863
mCSF-1	6868
TGF-beta1	7040
TGF-beta 2	7042
Tpo	7066
TLR1	7096
TLR2	7097
TLR5	7100
12-0-tetradecanoyl-phorbol-13-acetate (TPA)-induced down-regulation	7112
TNF- alpha	7124
TNFAIP3	7128
TNFRSF1A	7132
TNFRSF1B	7133
TOP2A	7153
TP53	7157
Tpo	7173
Tpt1	7178
HSP90B1	7184
TRAF2	7186
vascular cell adhesion molecule-1 (VCAM-1)	7412
vascular endothelial growth factor (VEGF)-A production	7422
WAS	7454
CNBP binding	7555
CXCR4	7852
GPR68	8111
SHP-1	8431
OPGL	8600
RANKL	8600

GENE, GeneBook	Entrez Gene ID
SRC-1	8648
SOCS1	8651
MARCO	8685
RIPK1	8737
TNFRSF11A	8792
SKAP2	8935
CH25H	9023
DOK2	9046
MAYP	9050
IL1RL1	9173
CD163	9332
GRAP2	9402
ITM2B	9445
GDF15	9518
CLOCK	9575
IKBKE	9641
HCK (p)	9846
MAFB	9935
Abi1	10006
IL18BP	10068
STX6	10228
RAMP2	10266
SIGMAR1	10280
TRAIP	10293
SIRPB1	10326
TLR6	10333
KHDRBS1	10657
SLC7A9	11136
TRIM35	23087
CSF-1-induced TCF/SAP-1 modification	23415

GENE, GeneBook	Entrez Gene ID
STX12	23673
phospholipase A2 activity	26279
NKIRAS1	28512
RHOD	29984
TLR9	54106
HPP-CFC-1	55997
CXCR7	57007
SLAP-2	84174
ADSSL1	122622
OSCAR	126014
GAB3	139716
192162	192162
MPEG1	219972
ANXA8	653145
CCR2	729230
Hsd3b	3283/3284
BCLXL	
Fcrls	

[0289] In another aspect, the anti-M-CSF antibody may be co-administered with other therapeutic agents, such as anti-inflammatory agents, anti-coagulant agents, agents that will lower or reduce blood pressure, anti-neoplastic drugs or molecules, to a patient who is in need of treatment. In one aspect, the invention relates to a method for the treatment of the hyperproliferative disorder in a mammal comprising administering to said mammal a therapeutically effective amount of a compound of the invention in combination with an anti-tumor agent selected from the group consisting of, but not limited to, mitotic inhibitors, alkylating agents, anti-metabolites, intercalating agents, growth factor inhibitors, cell cycle inhibitors, enzymes, topoisomerase inhibitors, biological response modifiers, anti-hormones, kinase inhibitors, matrix

metalloprotease inhibitors, genetic therapeutics and anti-androgens. In a more preferred embodiment, the antibody may be administered with an antineoplastic agent, such as adriamycin or taxol. In another preferred embodiment, the antibody or combination therapy is administered along with radiotherapy, chemotherapy, photodynamic therapy, surgery or other immunotherapy. In yet another preferred embodiment, the antibody will be administered with another antibody. For example, the anti-M-CSF antibody may be administered with an antibody or other agent that is known to inhibit tumor or cancer cell proliferation, e.g., an antibody or agent that inhibits erbB2 receptor, EGF-R, CD20 or VEGF.

[0290] Co-administration of the antibody with an additional therapeutic agent (combination therapy) encompasses administering a pharmaceutical composition comprising the anti-M-CSF antibody and the additional therapeutic agent and administering two or more separate pharmaceutical compositions, one comprising the anti-M-CSF antibody and the other(s) comprising the additional therapeutic agent(s). Further, although co-administration or combination therapy generally means that the antibody and additional therapeutic agents are administered at the same time as one another, it also encompasses instances in which the antibody and additional therapeutic agents are administered at different times. For instance, the antibody may be administered once every three days, while the additional therapeutic agent is administered once daily. Alternatively, the antibody may be administered prior to or subsequent to treatment of the disorder with the additional therapeutic agent. Similarly, administration of the anti-M-CSF antibody may be administered prior to or subsequent to other therapy, such as radiotherapy, chemotherapy, photodynamic therapy, surgery or other immunotherapy

[0291] The antibody and one or more additional therapeutic agents (the combination therapy) may be administered once, twice or at least the period of time until the condition is treated, palliated or cured. Preferably, the combination therapy is administered multiple times. The combination therapy may be administered from three times daily to once every six

months. The administering may be on a schedule such as three times daily, twice daily, once daily, once every two days, once every three days, once weekly, once every two weeks, once every month, once every two months, once every three months and once every six months, or may be administered continuously via a minipump. The combination therapy may be administered via an oral, mucosal, buccal, intranasal, inhalable, intravenous, subcutaneous, intramuscular, parenteral, intratumor or topical route. The combination therapy may be administered at a site distant from the site of the tumor. The combination therapy generally will be administered for as long as the tumor is present provided that the antibody causes the tumor or cancer to stop growing or to decrease in weight or volume.

[0292] In a still further embodiment, the anti-M-CSF antibody is labeled with a radiolabel, an immunotoxin or a toxin, or is a fusion protein comprising a toxic peptide. The anti-M-CSF antibody or anti-M-CSF antibody fusion protein directs the radiolabel, immunotoxin, toxin or toxic peptide to the M-CSF-expressing cell. In a preferred embodiment, the radiolabel, immunotoxin, toxin or toxic peptide is internalized after the anti-M-CSF antibody binds to the M-CSF on the surface of the target cell.

[0293] In another aspect, the anti-M-CSF antibody may be used to treat noncancerous states in which high levels of M-CSF and/or M-CSF have been associated with the noncancerous state or disease. In one embodiment, the method comprises the step of administering an anti-M-CSF antibody to a patient who has a noncancerous pathological state caused or exacerbated by high levels of M-CSF and/or M-CSF levels or activity. In a more preferred embodiment, the anti-M-CSF antibody slows the progress of the noncancerous pathological state. In a more preferred embodiment, the anti-M-CSF antibody stops or reverses, at least in part, the noncancerous pathological state.

Gene Therapy

[0294] The nucleic acid molecules of the instant invention can be administered to a patient in need thereof via gene therapy. The therapy

may be either *in vivo* or *ex vivo*. In a preferred embodiment, nucleic acid molecules encoding both a heavy chain and a light chain are administered to a patient. In a more preferred embodiment, the nucleic acid molecules are administered such that they are stably integrated into chromosomes of B cells because these cells are specialized for producing antibodies. In a preferred embodiment, precursor B cells are transfected or infected *ex vivo* and re-transplanted into a patient in need thereof. In another embodiment, precursor B cells or other cells are infected *in vivo* using a virus known to infect the cell type of interest. Typical vectors used for gene therapy include liposomes, plasmids and viral vectors. Exemplary viral vectors are retroviruses, adenoviruses and adeno-associated viruses. After infection either *in vivo* or *ex vivo*, levels of antibody expression can be monitored by taking a sample from the treated patient and using any immunoassay known in the art or discussed herein.

[0295] In a preferred embodiment, the gene therapy method comprises the steps of administering an isolated nucleic acid molecule encoding the heavy chain or an antigen-binding portion thereof of an anti-M-CSF antibody and expressing the nucleic acid molecule. In another embodiment, the gene therapy method comprises the steps of administering an isolated nucleic acid molecule encoding the light chain or an antigen-binding portion thereof of an anti-M-CSF antibody and expressing the nucleic acid molecule. In a more preferred method, the gene therapy method comprises the steps of administering of an isolated nucleic acid molecule encoding the heavy chain or an antigen-binding portion thereof and an isolated nucleic acid molecule encoding the light chain or the antigen-binding portion thereof of an anti-M-CSF antibody of the invention and expressing the nucleic acid molecules. The gene therapy method may also comprise the step of administering another anti-cancer agent, such as taxol or adriamycin.

[0296] In order that this invention may be better understood, the following examples are set forth. These examples are for purposes of illustration

only and are not to be construed as limiting the scope of the invention in any manner.

EXAMPLE I

Generation of Cell Lines Producing Anti-M-CSF Antibody

[0297] Antibodies of the invention were prepared, selected, and assayed as follows:

Immunization and hybridoma generation

Eight to ten week old XENOMOUSE™ mice were immunized intraperitoneally or in their hind footpads with human M-CSF (10 µg/dose/mouse). This dose was repeated five to seven times over a three to eight week period. Four days before fusion, the mice were given a final injection of human M-CSF in PBS. The spleen and lymph node lymphocytes from immunized mice were fused with the non-secretory myeloma P3-X63-Ag8.653 cell line, and the fused cells were subjected to HAT selection as previously described (Galfre and Milstein, *Methods Enzymol.* 73:3-46, 1981). A panel of hybridomas all secreting M-CSF specific human IgG2 and IgG4 antibodies was recovered. Antibodies also were generated using XENOMAX™ technology as described in Babcook, J.S. *et al.*, *Proc. Natl. Acad. Sci.* USA 93:7843-48, 1996. Nine cell lines engineered to produce antibodies of the invention were selected for further study and designated 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4, 8.10.3 and 9.7.2. The hybridomas were deposited under terms in accordance with the Budapest Treaty with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, VA 20110-2209 on August 8, 2003. The hybridomas have been assigned the following accession numbers:

Hybridoma 3.8.3 (LN 15891)	PTA-5390
Hybridoma 2.7.3 (LN 15892)	PTA-5391
Hybridoma 1.120.1 (LN 15893)	PTA-5392
Hybridoma 9.7.2 (LN 15894)	PTA-5393
Hybridoma 9.14.4 (LN 15895)	PTA-5394
Hybridoma 8.10.3 (LN 15896)	PTA-5395
Hybridoma 88-gamma (UC 25489)	PTA-5396
Hybridoma 88-kappa (UC 25490)	PTA-5397
Hybridoma 100-gamma (UC 25491)	PTA-5398
Hybridoma 100-kappa (UC 25492)	PTA-5399
Hybridoma 252-gamma (UC 25493)	PTA-5400
Hybridoma 252-kappa (UC 25494)	PTA-5401

EXAMPLE II

Gene Utilization Analysis

[0298] DNA encoding the heavy and light chains of monoclonal antibodies 252, 88, 100, 3.8.3, 2.7.3, 1,120.1, 9.14.4, 8.10.3 and 9.7.2 was cloned from the respective hybridoma cell lines and the DNA sequences were determined by methods known to one skilled in the art. Additionally, DNA from the hybridoma cell lines 9.14.4, 8.10.3 and 9.7.2 was mutated at specific framework regions in the variable domain and/or isotype-switched to obtain, for example, 9.14.4I, 8.10.3F, and 9.7.2IF, respectively. From nucleic acid sequence and predicted amino acid sequence of the antibodies, the identity of the gene usage for each antibody chain was determined ("VBASE"). Table 2 sets forth the gene utilization of selected antibodies in accordance with the invention:

Table 2

Heavy and Light Chain Gene Utilization

Clone	Heavy Chain				Kappa Light Chain		
	SEQ ID NO:	V_H	D_H	J_H	SEQ ID NO:	V_K	J_K
252	1, 2	3-11	7-27	6	3, 4	O12	3
88	5, 6	3-7	6-13	4	7, 8	O12	3
100	9, 10	3-23	1-26	4	11, 12	L2	3
3.8.3	14	3-11	7-27	4	16	L5	3
2.7.3	18	3-33	1-26	4	20	L5	4
1.120.1	22	1-18	4-23	4	24	B3	1
9.14.4I	25, 26	3-11	7-27	4b	27, 28	O12	3
8.10.3F	29, 30	3-48	1-26	4b	31, 32	A27	4
9.7.2IF	33, 34	3-11	6-13	6b	35, 36	O12	3
9.14.4	37, 38	3-11	7-27	4b	27, 28	O12	3
8.10.3	29, 30	3-48	1-26	4b	43, 44	A27	4
9.7.2	45, 46	3-11	6-13	6b	47, 48	O12	3
8.10.3FG1	97, 98	3-48	1-26	4b	31, 32	A27	4
9.14.4G1	101, 102	3-11	7-27	4b	27, 28	O12	3
9.14.4C-Ser	54	3-11	7-27	4b	56	O12	3
9.14.4-CG2	74	3-11	7-27	4b	56	O12	3
9.14.4-CG4	78	3-11	7-27	4b	56	O12	3
8.10.3C-Ser	58	3-48	1-26	4b	60	A27	4
8.10.3-CG2	62	3-48	1-26	4b	60	A27	4
8.10.3-CG4	94	3-48	1-26	4b	60	A27	4
8.10.3-Ser	90	3-48	1-26	4b	43, 44	A27	4
9.7.2C-Ser	50	3-11	6-13	6b	52	O12	3
9.7.2-CG2	66	3-11	6-13	6b	52	O12	3
9.7.2-CG4	70	3-11	6-13	6b	52	O12	3
9.7.2-Ser	86	3-11	6-13	6b	47, 48	O12	3
9.14.4-Ser	82	3-11	7-27	4b	27, 28	O12	3

[0299] Mutagenesis of specific residues of the heavy and light chains was carried out by designing primers and using the QuickChange Site Directed Mutagenesis Kit from Stratagene, according to the manufacturer's instructions. Mutations were confirmed by automated sequencing, and mutagenized inserts were subcloned into expression vectors. The expression vectors were transfected into HEK293 cells to produce enough of the antibodies for characterization.

EXAMPLE III

M-CSF Mouse Monocytic Cell Proliferation Assay

[0300] *In vitro* assays were conducted to measure M-CSF-dependent mouse monocytic cell proliferation in the presence of anti-M-CSF antibodies to determine the degree of inhibition by anti-M-CSF antibodies.

[0301] Mouse monocytic cells, M-NFS-60 cells, from American Type Culture Collection (ATCC) (Manassas, VA), were obtained and maintained in RPMI-1640 medium containing 2 mM L-glutamine (ATCC), 10% heat inactivated fetal bovine serum (FBS) (Invitrogen, Carlsbad, CA), 0.05 mM 2-mercaptoethanol (Sigma, St. Louis MO) (assay medium), with 15 ng/ml human M-CSF. M-NSF-60 cells were split to 5×10^4 for next day use or to 2.5×10^4 for use in 2 days. Prior to use in the assay, the cells were washed three times with RPMI-1640, counted and the volume adjusted with assay medium to yield 2×10^5 cells/ml. All conditions were conducted in triplicate in 96-well treated tissue culture plates (Corning, Corning, NY). To each well 50 µl of the washed cells, either 100 pM or 1000 pM M-CSF in a volume of 25 µl and test or control antibody at various concentrations in a volume of 25 µl in acetate buffer (140 mM sodium chloride, 20 mM sodium acetate, and 0.2 mg/ml polysorbate 80, pH 5.5) to a final volume of 100 µl was added. Antibodies of the invention were tested alone and with human M-CFS. The plates were incubated for 24 hours (hrs) at 37°C with 5% CO_2.

[0302] After 24 hrs, 10 µl/well of 0.5 µCi ^{3}H-thymidine (Amersham Biosciences, Piscataway, NJ) was added and pulsed with the cells for 3 hrs. To detect the amount of incorporated thymidine, the cells were harvested onto pre-wet unifilter GF/C filterplates (Packard, Meriden, CT) and washed 10 times with water. The plates were allowed to dry overnight. Bottom seals were added to the filterplates. Next, 45 µl Microscint 20 (Packard, Meriden, CT) per well was added. After a top seal was added, the plates were counted in a Trilux microbeta counter (Wallac, Norton, OH).

[0303] These experiments demonstrate that anti-M-CSF antibodies of the invention inhibit mouse monocytic cell proliferation in response to M-CSF. Further, by using various concentrations of antibodies, the IC_{50} for inhibition of mouse nonocytic cell proliferation was determined for antibodies 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, and 9.7.2 (Cell Proliferation Assay, Table 3a and Table 3b).

Table 3a

Antibody	**252**	**88**	**100**	**3.8.3**	**2.7.3**	**1.120.1**
M-CSF Mouse Monocytic Cell Proliferation Assay [IC_{50}, M]	1.86 x 10^{-10}	2.31 x 10^{-10}	7.44 x 10^{-10}	7.3 x 10^{-11}	1.96 x 10^{-10}	1.99 x 10^{-10}
Human Whole Blood Monocyte Activation Assay [IC_{50}, M]	8.67 x 10^{-10}	5.80 x 10^{-10}	1.53 x 10^{-10}	8.6 x 10^{-11}	7.15 x 10^{-10}	8.85 x 10^{-10}
Receptor Binding Inhibition Assay [IC_{50}, M]	7.47 x 10^{-10}	4.45 x 10^{-10}	1.252 x 10^{-9}	7.0 x 10^{-11}	3.08 x 10^{-10}	1.57 x 10^{-10}

Table 3b

Antibody	**9.14.4I**	**8.10.3F**	**9.7.2IF**	**9.14.4**	**8.10.3**	**9.7.2**
M-CSF Mouse Monocytic Cell Proliferation Assay [IC_{50}, M]	2.02 x 10^{-10}	4.13 x 10^{-10}	7.37 x 10^{-10}	2.02 x 10^{-10}	4.13 x 10^{-10}	7.37 x 10^{-10}
Human Whole Blood Monocyte Activation Assay [IC_{50}, M]	2.49 x 10^{-10}	4.46 x 10^{-10}	1.125 x 10^{-9}	6.48 x 10^{-10}	2.8 x 10^{-10}	1.98 x 10^{-10}
Receptor Binding Inhibition Assay [IC_{50}, M]	2.97 x 10^{-10}	9.8 x 10^{-11}	5.29 x 10^{-10}	4.1 x 10^{-11}	1.5 x 10^{-9}	6 x 10^{-12}

EXAMPLE IV

Human Whole Blood Monocyte Activation Assay

[0304] *In vitro* assays were conducted to measure M-CSF dependent monocyte shape changes in the presence of anti-M-CSF antibodies to determine if the anti-M-CSF antibodies were capable of inhibiting whole blood monocyte activation and their degree of inhibition of monocyte shape changes.

[0305] In individual wells of a 96-well tissue culture plate, 6 µl of 1.7 nM anti-M-CSF and 94 µl of whole human blood for a final concentration of 102 pM anti-M-CSF antibody were mixed. The plates were incubated at 37˚C in a CO_2 tissue culture incubator. Next, the plates were removed from the incubator. To each well, 100 µl of a fixative solution (0.5% formalin in phosphate buffered saline without $MgCl_2$ or $CaCl_2$) was added and the plates were incubated for 10 minutes at room temperature. For each sample, 180 µl from each well and 1 ml of Red Cell Lysis Buffer were mixed. The tubes were vortexed for 2 seconds. Next, the samples were incubated at 37˚C for 5 minutes in a shaking water bath to lyse the red blood cells, but to leave monocytes intact. Immediately following this incubation, the samples were read on a fluorescence-activated cell scanning (FACS) machine (BD Beckman FACS) and data was analyzed using FACS Station Software Version 3.4.

[0306] These experiments demonstrate that anti-M-CSF antibodies of the invention inhibit monocyte shape changes compared to control samples. Using the monocyte shape change assay, the IC_{50} was determined for antibodies 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, and 9.7.2 (Human Whole Blood Monocyte Activation, Table 3a and Table 3b).

EXAMPLE V

c-fms Receptor Binding Inhibition Assay

[0307] *In vitro* assays were conducted to measure M-CSF binding to *c-fms* receptor in the presence of anti-M-CSF antibodies to determine if the anti-M-CSF antibodies were capable of inhibiting M-CSF binding to *c-fms* receptor and their degree of inhibition.

[0308] NIH-3T3 cells transfected with human *c-fms* or M-NSF-60 cells maintained in Dulbecco's phosphate buffered saline without magnesium or calcium were washed. NIH-3T3 cells were removed from tissue culture plates with 5 mM ethylene-diamine-tetra-acetate (EDTA), pH 7.4. The NIH-

3T3 cells were returned to the tissue culture incubator for 1-2 minutes and the flask(s) were tapped to loosen the cells. The NIH-3T3 cells and the M-NSF-60 cells were transferred to 50 ml tubes and washed twice with reaction buffer (1x RPMI without sodium bicarbonate containing 50 mM N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), pH 7.4). Next, the NIH-3T3 cells were resuspended in reaction buffer for a final concentration of 1.5 x 10^5 cell/ml. The M-NSF-60 cells were resuspended in a reaction buffer for a final concentration of 2.5 x 10^6 cells/ml.

[0309] For the assay, 9 µl of a sterile 0.4 M sucrose solution, 100 µl of ^{125}I-M-CSF (Amersham, IMQ7228v) at a final concentration of 200 pM in RPMI-1640 containing 50 mM HEPES (pH 7.4), 0.2% bovine serum albumin, and 100 µl of unlabeled M-CSF at a final concentration of 200 nM were mixed in a binding tube. Next, 50 µl/tube of increasing concentrations of a test antibody was added. In order to determine non-specific binding of the antibodies, we included samples to which we also added 200 nM M-CSF. To control tubes, we did not add antibody. Next, 15,000 NIH-3T3 cells or 250,000 M-NSF-60 cells were added per tube. All tubes were incubated at room temperature for 3 hrs and subjected to centrifugation at 10,000 rpm for 2 min. The tips of the tubes containing the cell pellets were cut off and the amount of M-CSF bound to the cells was determined using a Packard Cobra II Gamma counter. The specific binding was determined by subtracting non-specific binding from total binding. All assays were performed in duplicate. The binding data was analyzed using the computer program, Graph Pad Prism 2.01.

[0310] These experiments demonstrate that anti-M-CSF antibodies of the invention inhibit the binding of M-CSF to *c-fms* receptor compared to control samples. Further, by using various concentrations of antibodies, the IC_{50} for inhibition of receptor binding was determined for antibodies 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, and 9.7.2 (Receptor Binding Inhibition Assay, Table 3a and Table 3b).

EXAMPLE VI

Determination of Affinity Constants (K_D) of Anti-M-CSF Monoclonal Antibodies by BIACORE™

[0311] Affinity measures of purified antibodies were performed by surface plasmon resonance using the BIACORE™ 3000 instrument, following the manufacturer's protocols.

[0312] For antibodies 3.8.3, 2.7.3 and 1.120.1, the experiments were performed in a BIACORE™ 3000 instrument at 25°C in Dulbecco's phosphate buffered saline containing 0.0005% Tween-20. Protein concentrations were obtained from sedimentation velocity experiments or by measuring the wavelength of the sample at 280 nm using theoretical extinction coefficients derived from amino acid sequences. For experiments measuring the binding of antibody to immobilized antigens, M-CSF was immobilized on a B1 chip by standard direct amine coupling procedures. Antibody samples were prepared at 0.69 µM for 3.8.3, 2.7.3 and 1.120.1. These samples were diluted 3-fold serially to 8.5 nM or 2.8 nM for roughly a 100-fold range in concentrations. For each concentration, the samples were injected in duplicate at 5 µl/min flow for 4 min. The dissociation was monitored for 2000 seconds. The data were fit globally to a simple 1:1 binding model using BIACORE™ Biaevaluation software. In all cases, this method was used to obtain k_{off} and it was found that this data set compared well to data obtained from global fit of association and dissociation data.

[0313] For antibodies 252, 88 and 100, the experiments were performed in a BIACORE™ 3000 instrument at 25°C in HBS-EP Buffer (0.01M HEPES, pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.005% Surfactant P20). For experiments measuring the binding of antibody to immobilized antigens, a M-CSF was immobilized on a CM5 Research Grade Sensor chip by standard direct amine coupling procedures. Antibody samples were prepared at 12.5 nM for antibodies 252 and 100 and at 25.0 nM for antibody 88. These samples were two-fold serially diluted to 0.78 nM for roughly a 15-30 fold range in concentrations. For each concentration, the

samples were injected in duplicate in random order at 30 µl/min flow for 3 min. The dissociation was monitored for 300 sec. The data were fit globally to a simple 1:1 binding model using BIACORE™ Biaevaluation software. In all cases, this method was used to obtain k_{off} and it was found that this data set compared well to data obtained from global fit of association and dissociation data.

[0314] Table 4 shows results for antibodies 252, 88, 100, 3.8.3, 2.7.3 and 1.120.1.

Table 4

	252	88	100	3.8.3	2.7.3	1.120.1
K_D (M)	1.33×10^{-11}	1.33×10^{-9}	2.0×10^{-11}	4.0×10^{-10}	4.7×10^{-9}	5.4×10^{-9}
k_{off} (1/s)	1.03×10^{-6}	7.3×10^{-5}	1.7×10^{-5}			

EXAMPLE VII

Production of 8.10.3 antibodies from 8.10.3 hybridoma cells

[0315] Antibody 8.10.3 was produced in 3L sparged spinners. The 3L sparged spinner flask is a glass vessel where cultures are mixed with an impeller controlled by a magnetic platform. The spinner is connected to gas lines to provide 5% CO_2 and air. 8.10.3 hybridoma cells were initially thawed into T-25 cell culture flasks. The cells were progressively expanded until there was a sufficient number of cells to seed the sparged spinners.

[0316] Two 3L sparged spinner flasks were seeded with 8.10.3 hybridoma cells in Hybridoma Serum-Free Medium with the additions noted on Table 5, for the two sparged flasks. The concentrations for Ultra low IgG serum (Gibco cat# 16250-078), L-glutamine (JRH Biosciences cat# 59202-500M), Non-Essential Amino Acids (Gibco cat# 11140-050), Peptone (Difco cat# 211693), glucose (In-house stock prepared from JT Baker cat# 1920-07), and Anti-foam C (Sigma cat.# A-8011) are given at

their final concentrations in the media. The balance of the volume in each reactor is Hybridoma Serum-Free Medium.

Table 5. Conditions for Growing Hybridoma 8.10.3 in two 3L sparged spinners.

Conditions	Spinner 1	Spinner 2
Seeding density ($1x10^6$ cells/ml)	0.16 ml	0.16 ml
Hybridoma Serum-Free Medium (Gibco cat# 12045-076)	Balance	Balance
Ultra low IgG serum (Gibco cat# 16250-078)	5%	5%
L-glutamine (JRH Biosciences cat# 59202-500M)	8 mmol/L	8mmol/L
Non-Essential Amino Acids (Gibco cat# 11140-050)	1%	1%
Peptone (Difco cat# 211693)	1g/L	1g/L
2M glucose (In-house stock prepared from JT Baker cat# 1920-07)	8g/L	8g/L
Anti-foam C (Sigma cat.# A-8011)	1ml/L	1ml/L

[0317] The cultures were grown for 15 days and were harvested when the viability was below 20%. Viability was determined by trypan blue exclusion method with an automated cell counter (Cedex, Innovatis). Harvesting was accomplished by centrifugation and subsequent filtration. Clarified supernatant was obtained after centrifugation for 15 minutes at 7000 rpm and subsequent filtration with a sterile 0.22 µm 4" Opticap Millipore filter (cat# KVSCO4HB3) into a 10L sterile TC-Tech bag (cat # P/N 12420 Bag Style CC-10-112420). The filtrate was then purified in the following example.

EXAMPLE VIII

Purification of an Anti-M-CSF Antibody

[0318] A Protein A column (Amersham Pharmacia) was prepped by washing with 3 column volumes of 8M Urea, followed by an equilibration

wash with 20 mM Tris (pH 8). The final filtrate from Example VII was spiked with 2% v/v of 1M Tris pH 8.3 and 0.02% NaN_3 before being loaded onto the Protein A column via gravity-drip mode. After load was complete, the resin was washed with 5 column volumes of 20 mM Tris (pH 8), followed by 5 column volumes of the elution buffer (0.1 M Glycine pH 3.0). Any precipitation was noted, and then a 10% v/v spike of 1M Tris pH 8.3 was added to the eluted antibody. The eluted protein was then dialyzed into 100 fold the volume amount of eluted material of dialysis buffer (140 mM NaCl/20mM Sodium Acetate pH 5.5). Following dialysis, the antibody was sterile filtered with a 0.22 μm filter and stored until further use.

EXAMPLE IX

Monkey Treatment and Monocyte Counts

[0319] One male and one female cynomolgus monkey per dosage group were intravenously administered vehicle or antibody 8.10.3 (produced as describe in Examples VII and VIII) at 0, 0.1, 1, or 5 mg/kg in a dose volume of 3.79 mL/kg over an approximately 5 minute period. Blood samples for clinical laboratory analysis were collected at 24 and 72 hours postdose and weekly for 3 weeks. The monocyte counts were determined by light scatter using an Abbott Diagnostics Inc. Cell Dyn system (Abbott Park, Illinois).

[0320] A dose-related decrease (~25% to 85%) in total monocytes at all doses (Figures 1A and 1B) was observed. Monocyte counts at the 0.1 and 1 mg/kg appeared to rebound to near control levels by week 2, while monocyte counts at 5 mg/kg were still decreased at 3 weeks.

CD14+CD16+ monocyte subset analysis

[0321] Primate whole blood was drawn into Vacutainer tubes containing sodium heparin. 0.2 ml of each blood sample was added to a 15 ml conical polypropylene centrifuge tube containing 10 ml of red blood cell lysis buffer (Sigma), and incubated in a 37°C water bath for 15 minutes. The tubes were then centrifuged in a Sorvall RT7 centrifuge for 5 minutes at 1,200 rpm. The supernatant was aspirated, the pellet resuspended in 10 ml of

4°C FACS buffer (Hanks' Balanced Salt Solution/2%FBS/0.02% sodium azide), and the tube centrifuged again for 5 minutes at 1,200 rpm. The supernatant was aspirated and the pellet resuspended in an antibody cocktail consisting of 80 µl 4°C FACS buffer, 10 µl FITC-conjugated anti-human CD14 monoclonal antibody (BD Biosciences, San Diego, CA), 0.5 µl Cy5-PE-conjugated anti-human CD16 monoclonal antibody (BD Biosciences, San Diego, CA), and 10 µl PE-conjugated anti-human CD89 monoclonal antibody (BD Biosciences, San Diego, CA). The cell suspension was incubated on ice for 20 minutes, after which 10 ml of 4°C FACS buffer was added and the cells centrifuged as before. The supernatant was aspirated, and the cell pellet resuspended in 400 µl FACS buffer and the cells analyzed on a FACSCaliber flow cytometer (BD Biosciences, San Jose, CA). Data for 30,000 cells were collected from each sample.

[0322] The monocyte population was identified by a combination of forward angle light scatter and orthogonal light scatter. Cells within the monocyte gate were further analyzed for expression of CD14 and CD16. Two distinct population of monocytes were observed, one expressing high levels of CD14 with little or no CD16 expression (CD14++CD16-) and the other expressing lower levels of CD14, but high levels of CD16 (CD14+CD16+), similar to the two monocyte subsets previously described in human peripheral blood (Ziegler-Heitbrock H.W., *Immunology Today* 17:424-428 (1996)). For each primate tested, the percentage of monocytes within the CD14+CD16+ subset was determined after each blood draw, on days 1, 3, 7, 14, and 21 after 8.10.3 injection.

[0323] In general, 8.10.3 treatment resulted in a reduction in the percentage of CD14+CD16+ monocytes (see Figures 2A and 2B). Monkeys not receiving 8.10.3 Antibody demonstrated relatively stable CD14+CD16+ monocyte levels. CD14+CD16+ monocytes have been termed "proinflammatory" because they produce higher levels of TNF-α and other inflammatory cytokines (Frankenberger, M.T., *et al.*, *Blood* 87:373-377 (1996)). It has also been reported that the differentiation of

monocytes from the conventional CD14++CD16- phenotype to the proinflammatory phenotype is dependent on M-CSF (Saleh M.N., *et al.*, *Blood* 85: 2910-2917 (1995)).

EXAMPLE X

Monkey Treatment and Monocyte Counts

[0324] Three male cynomolgus monkeys per dosage group were intravenously administered vehicle (20 mM Sodium acetate, pH 5.5, 140 mM NaCl), purified antibody 8.10.3F, or purified antibody 9.14.4I at 0, 1, or 5 mg/kg in a dose volume of 3.79 mL/kg over an approximately 5 minute period. The monkeys were 4 to 9 years of age and weighed 6 to 10 kg. Blood samples for clinical laboratory analysis were collected at 2, 4, 8, 15, 23, and 29 days. Monocyte counts were determined by light scatter using an Abbott Diagnostics Inc. Cell Dyn system (Abbott Park, Illinois).

[0325] A decrease in the percentage change in total monocytes at all doses of antibody 8.10.3F and antibody 9.14.4I as compared to pre-test levels of monocytes (Figures 3A and 3B) was observed (see e.g., day 4, 8, 15, and 23 in Figures 3A and 3B).

EXAMPLE XI

Effect of Anti-M-CSF Antibody in Murine Lupus Models

[0326] In this example, the effects of M-CSF neutralization by the rat anti-murine M-CSF antibody on the generation of lupus-like disease was tested in 2 separate murine models: the MRL-Fas^{lpr} and the NZBWF1/J. The effects of the anti-M-CSF antibody were also compared with murine CTLA-4Ig, which has been previously shown to reduce disease in MRL-Fas^{lpr} mice.

[0327] Several candidates for treatment of human disease have been evaluated in murine models of SLE which exhibit many of the same features as human disease (Perry *et al, J Biomed Biotechnol.* 2011:271694. Epub 2011 Feb 14).

[0328] MRL-Fas^{lpr} mice spontaneously develop symptoms resembling those observed in human lupus, including high titers of circulating anti-double-stranded DNA (dsDNA) serum autoantibodies, IgG deposits in the glomeruli, and, in severe disease, proteinuria, lymphadenopathy, and skin lesions. The development of lymphadenopathy is due to an accumulation of double negative (CD4- CD8-) and B220+ T-cells (Watson *et al, Journal of Experimental Medicine.* 176(6):1645-56 (1992)). Cytotoxic T-cell lymphocyte antigen-4 (CTLA-4) is involved in regulating T lymphocyte activation, and the presence of serum CTLA-4Ig is associated with decreased expansion of autoreactive B lymphocytes, decreased numbers of CD4+ T lymphocytes, and a beneficial effect in a mouse model of SLE (Mihara et al, *J Clin Invest.* 106(1):91-101(2002)). MRL-Fas^{lpr} mice deficient in M-CSF are protected from nephritis (Lenda *et al. J Immunol.* 173(7):4644-4754 (2004)).

[0329] The NZBWF1/J model is the oldest classical model of lupus generated by the F1 hybrid between the NZB and NZW strains which develops a severe lupus-like phenotype comparable to that of lupus patients including lymphadenopathy, splenomegaly, elevated serum antinuclear autoantibodies including anti-dsDNA IgG. Immune complex-mediated glomerulonephritis becomes apparent at 5 - 6 months of age, leading to kidney failure and death at 10 - 12 months (Mihara et al.). As in human SLE, disease in the NZBWF1 is strongly biased in favor of females (Theofilopoulos & Dixon, *Adv Immunol.* 37:269-390 (1985)).

Materials and Methods

[0330] The antibodies and control proteins used in this example are summarized in Table 6.

Table 6

Protein[a]	Activity
Rat anti-mouse M-CSF Ab 5A1	Neutralizing antibody for mouse M-CSF
CHOCK IgG1	Rat isotype control immunoglobulin
CTLA-4Ig	Positive control for reducing lupus disease

CHOCK = Chinese hamster ovary cells expressing CK-1; CTLA = cytotoxic T lymphocyte-associated antigen; Ig = immunoglobulin; M-CSF = macrophage colony stimulating factor.
[a] Proteins were manufactured at Pfizer.

[0331] Female MRL-Fas^{lpr} and NZBWF1/J (Jackson Laboratory, Bar Harbor, ME) were housed in the pathogen-free animal facility at Pfizer. Mice were used according to protocols approved by the Pfizer Animal Care and Use Committee.

SLE Studies

[0332] The effect of M-CSF neutralization on the development of lupus-like disease was examined using both the MRL-Fas^{lpr} and NZBWF1/J models of SLE. Ten week old female MRL-Fas^{lpr} or 26 week old female NZBWF1/J mice were treated 3 times per week intraperitoneally (IP) for 10 weeks with saline, 10 mg/kg of 5A1 anti-M-CSF, CHOCK IgG1 isotype control antibody or CTLA-4 Ig (MRL model only). MRL-Fas^{lpr} mice were examined bi-weekly for proteinuria, skin lesions and lymphadenopathy, and NZBWF1/J mice were examined bi-weekly for proteinuria. Proteinuria was measured using Albustix (Bayer, Tarrytown, NY) and scored on a scale of 0 - 5 where 0 = none; 1 = trace; 2 = 30 mg/dL; 3 = 100 mg/dL; 4 = 300 mg/dL; and 5 = ≥2000 mg/dL. Lymph nodes were palpated and lymphadenopathy was scored on a scale of 0 - 3 where 0 = none; 1 = small; 2 = moderate, at two different sites; 3 = large, at three or more different sites. Skin lesions were assessed by gross pathology and scored on a scale of 0 - 3 where 0 = none; 1 = small (face, ears); 2 = moderate (<2 cm face, ears and back); 3 = severe (>2 cm face, ears and back). Serum

in both models was also collected bi-weekly and examined for anti-dsDNA IgG serum antibodies by enzyme immunosorbent assay (ELISA). At the conclusion of the study, brain, lung and kidney were collected in either 10% non-buffered formalin for pathology or frozen in optimal cutting temperature (OCT) compound for immunohistochemistry.

Anti-dsDNA Serum Antibody and M-CSF ELISA

[0333] Anti-dsDNA IgG serum antibodies were measured by ELISA. Briefly, Immulon 1B plates (Thermolab Systems, Billerica, MA) were UV irradiated over night and then coated with 2 µg/mL calf thymus DNA (Sigma Aldrich, St. Louis, MO) for 1 hour at room temperature. Plates were blocked with phosphate buffered saline (PBS) plus 1% bovine serum albumin (BSA), diluted serum samples were added (starting at 1:100 dilution), and bound antibody was detected with horseradish peroxidase (HRP) -conjugated goat antibodies directed against mouse IgG antibody (Southern Biotech, Birmingham, AL). Plates were developed using 3,3',5,5'-tetramethylbenzidine (TMB; KPL, Gaithersburg, MD) and reactions were stopped with 2N sulfuric acid. Absorbancies were read at 450 nm using a SpectraMax Plus 384 microplate reader with SoftMax Pro software (Molecular Devices, Sunnyvale, CA). Arbitrary units of antibody were determined using standard positive control serum pooled from diseased MRL-Fas^{lpr} or NZBWF1/J mice. Serum levels of M-CSF were determined by an anti-murine M-CSF kit (R & D Systems, Minneapolis, MN; Cat.# MC00) as specified by the manufacturer.

Histology

[0334] In the MRL-lpr study, kidneys were examined for pathology and Ig and C3 deposits. Formalin-fixed specimens of right and left kidneys were submitted for hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS; with hematoxylin counterstain) -staining. Specimens of right and left kidneys were also frozen in liquid nitrogen and were stained immunohistochemically for C3, IgG, and IgM. All tissue sections were

examined by a board certified veterinary pathologist. F4/80 stained macrophages were examined by immunohistochemistry.

Results

[0335] A study was set up in 8 week old MRL-lpr mice to test the effect of blocking M-CSF with a neutralizing antibody, 5A1 (see, for example, Campbell I.K., *et al.*, *J. Leuk. Biol.* 68:144-150 (2000) and ATCC Number CRL-2702). Mice were treated with 400 μg antibody (10 mg/kg) intraperitoneally (IP), daily for 12 weeks. Control mice were treated with the same dose of an isotype control antibody, CHOCK IgG1 (see, for example, ATCC Number HB-9421) or saline. Mice were also treated with murine CTLA-4Ig (extracellular domain of murine CTLA4 fused to murine IgG2a containing effector function null mutation) as a positive control. Mice were examined for anti-dsDNA serum antibody, proteinuria, skin lesions and lymphadenopathy bi-weekly. The study design is outlined in Table7.

Table 7. Study Design for Testing the Efficacy of Anti-M-CSF Antibody in MRL^{lpr} Lupus Model

Treatment	Number	IP Dose	Animals examined microscopically[a]
Saline	10	100 μL 3x/week	1-10
CTLA-4Ig	10	10 mg/kg, 3x/week	11-20
Rat anti-M-CSF Ab 5A1	10	400 μg 3x/week	31-40
Rat CHOCK IgG1 control Ab	10	400 μg 3x/week	41-40

a. One H&E-stained, one PAS-stained, one C3-immunostained, one IgG-immunostained, one IgM immunostained, and one control antibody-immunostained slide were examined for each animal.

[0336] As shown in Figure 5, treatments with the positive control protein CTLA-4Ig and anti-M-CSF antibody significantly reduced the severity of lymphadenopathy in MRL-lpr mice. Figure 6 shows that treatment with anti-M-CSF antibody also significantly reduced the severity of the skin lesions that developed in this model. Interestingly, treatment with anti-M-CSF prevented the development of skin lesions in MRL-lpr mice, in contrast to treatment with CTLA-4Ig, which reduced the severity of skin lesions in MRL-lpr mice.

[0337] As shown in Figure 7, mice also developed less anti-dsDNA antibodies at the 12-week time point in this study when compared with the isotype control treated mice; whereas, treatment with CTLA-4Ig was very effective in reducing the development of anti-dsDNA antibodies at the 4 time-points tested.

[0338] In this study, mice did not develop significant levels of proteinuria; therefore, kidney function was not assessed. Microscopically, only administration of CTLA-4Ig was associated with beneficial effects on the severity of inflammatory infiltrates and proteinaceous casts, the size of glomerular tufts, glomerular cellularity, and the incidence and severity of glomerular IgG deposits. As shown in Figure 8, administration of rat anti-M-CSF antibody was associated with lower glomerular cellularity, and with a slightly lower group mean immunohistochemical staining score for C3 compared with administration of saline or isotype control. However, this was not associated with any other clear beneficial effects on any other parameters measured.

[0339] A subsequent study evaluated the efficacy of anti-M-CSF antibody 5A1 in ameliorating lupus nephritis disease in the NZBWF1/J model. Twenty-six week old mice were treated with 400 μg (10 mg/kg) of either control Ig or anti-M-CSF or saline, 3 times per week IP for 10 weeks. Mice were scored bi-weekly for proteinuria, weight gain/loss, and anti-dsDNA titers. Kidneys were harvested after 10 weeks of dosing and examined for pathology and immune complex deposition. The study design is described in Table 8.

Table 8. Study Design for Testing Efficacy of Anti-M-CSF Antibody in the NZBWF1/J Lupus Model

Treatment	Number	Dose (IP)	Animals Examined Microscopically: Kidneys[a]	Animals Examined Microscopically: Kidneys[b]
Saline	10	100 μL 3x/week	46, 47, 48, 49, 50, 51, 52, 53, 54, 55	46, 47, 48, 49, 50, 51, 52, 53, 54, 55
Rat anti-M-CSF Ab 5A1	10	400 μg 3x/week	57, 58, 59, 60, 61, 62, 63, 64, 65	57, 58, 60, 61, 62, 63, 64, 65
Rat CHOCK IgG1 control Ab	10	400 μg 3x/week	66, 67, 69, 70, 71, 72, 73, 74, 75	66, 67, 69, 70, 71, 72, 73, 74, 75

a. One H&E and one PAS stained tissue section and two F4/80 immunostained tissue sections were examined from each animal.
b. Four immunostained tissue sections for IgG, IgM and C3 were examined from each animal

[0340] As shown in Figure 9, anti-M-CSF treatment had a significant effect on the development of proteinuria when compared with the isotype control starting at 4 weeks after dosing until the end of the study in the NZBWF1/J lupus model. Treatment with antibody to M-CSF however did not affect the development of anti-dsDNA autoantibodies that contribute to immune complex deposition and kidney damage in this model. As shown in Figure 10, anti-dsDNA antibody levels were similar in saline, isotype control, and anti-M-CSF treated mice at the 6 and 10 week time points after dosing. Figure 11 shows that treatment with M-CSF antibody raises the serum level of M-CSF as determined by ELISA, indicating that the antibody is reacting with its target and sequesters M-CSF in the serum.

[0341] Microscopic examination of kidneys collected at the termination of the study and stained for immune deposits showed similar levels of IgG, IgM and C3 staining in all 3 treated groups, shown in Figure 12. Interestingly, kidneys obtained from mice treated with anti-M-CSF showed a slight reduction in staining for macrophages (F4/80 positive a

transmembrane protein expressed by mature macrophages.) when compared with the isotype control treated mice. Renal F4/80 staining for macrophages is present in interstitial cells in the outer medulla and the inner cortex in all mice. Staining is present in fewer cells in these locations in mice administered saline or rat anti-M-CSF compared with mice administered CHOCK IgG1 isotype control antibody suggesting that M-CSF blockade had an effect on macrophage infiltration in the kidney in this model. Anti-M-CSF treatment also appeared to reduce renal proteinaceous casts and tubular basophilia and degeneration in kidney of NZBWF1/J mice. The findings are less severe in animals administered saline or anti-M-CSF compared with mice administered CHOCK IgG1 isotype control.

EXAMPLE XII

Study Design

[0342] This was a randomized, double-blind (sponsor unblinded), placebo-controlled, dose-escalating, parallel-group study in 6 sequential cohorts investigating the safety and tolerability of 6 single dose levels of human monoclonal anti-M-CSF antibody 8.10.3F, administered as an intravenous (IV) formulation, in healthy adult volunteers.

Subject Disposition

[0343] A total of 48 subjects were enrolled in the study. Subjects in Cohorts 1 through 5 received doses of 3, 10, 30, 100, or 300 mg antibody 8.10.3F or placebo; these subjects were confined to the CRU (clinical research unit) for 3 days after dosing, were discharged, and returned to the CRU for scheduled assessments. Subjects in Cohort 6 were treated with 100 mg antibody 8.10.3F or placebo and were confined to the CRU for 21 days after dosing, were discharged, and returned to the CRU for scheduled assessments. At each dose level, 6 subjects were treated with single doses of antibody 8.10.3F and 2 with single doses of placebo. All enrolled subjects completed the study.

Pharmacokinetic Evaluations

A) Serum for Analysis of Antibody 8.10.3F

[0344] Samples for analysis of antibody 8.10.3F were collected pre-dose and at protocol-specified times post-dose through Day 28, as well as each additional outpatient visit after Day 28. Three mL of venous blood was collected into appropriately labeled tubes containing no additive, centrifuged within 40 minutes of collection, and the serum was stored frozen at approximately -70 degrees Celsius within 60 minutes of collection.

[0345] Serum samples were analyzed for antibody 8.10.3F at PPD Development (2244 Dabney Road, Richmond, VA 23230, USA) using a validated analytical assay. Antibody 8.10.3F samples were assayed using a validated, sensitive and specific Enzyme-Linked Immunosorbent Assay (ELISA). The serum specimens were stored at approximately -70°C until assay, and samples were assayed within 439 days of established matrix stability data. Sample concentrations were determined by interpolation from a calibration standard curve (over the range of 35.0 ng/mL to 1600 ng/mL) that had been fit using a 4-parameter logistic equation. Those samples with concentrations above the upper limit of quantification (1600 ng/mL) were adequately diluted into calibration range. The LLOQ (lower limit of quantification) for antibody 8.10.3F was 35.0 ng/mL. Clinical specimens with serum antibody 8.10.3F concentrations below the LLOQ are reported as <35.0 ng/mL.

[0346] The between-day assay accuracy, expressed as the ratio (%) of the estimated to the theoretical Quality Control (QC) concentrations, ranged from -7.81% to 2.22% for low, medium, high and diluted QC samples. Assay precision, expressed as the between-day coefficients of variation (CV%) of the estimated concentrations of QC samples was less than 10.0% for low (75.0 ng/mL), medium (320 ng/mL), high (1200 ng/mL) and diluted (1200 ng/mL, 16000 ng/mL and 160000 ng/mL) concentrations.

B) Calculation of Pharmacokinetic Parameters

[0347] Antibody 8.10.3F PK parameter values were calculated for each subject for each treatment using noncompartmental analysis of serum concentration-time data. The study design did not include PK sampling during the IV infusion. Given the anticipated long terminal t½ of antibody 8.10.3F, area under the concentration-time curve (AUC) during the infusion was expected to be minimal relative to the total AUC; therefore no attempt was made to estimate concentrations during or at the end of the infusion.

[0348] Samples below the LLOQ were included as zero. Actual sample collection times were used for the PK analysis. Pharmacokinetic parameter values were calculated using WinNonlin version 4.0.1.

Pharmacodynamic Evaluations

A) Whole Blood for Analysis of $CD14^{+}16^{+}$ Monocytes

[0349] Samples for analysis of $CD14^{+}16^{+}$ monocytes were collected pre-dose and on Days 2, 4, 7, 14, and 28, as well as at each additional outpatient visit after Day 28. Three mL of venous blood was collected into each of 2 tubes; one containing lithium heparin, and the other containing potassium EDTA. Samples were analyzed within 2 hours of collection. Whole blood samples were analyzed for the percent monocyte subpopulations (CD14 and CD16) in human peripheral blood at Pfizer Drug Safety Research & Development (DSRD), PGRD, Ann Arbor, Michigan using a validated, sensitive and specific flow cytometry assay (Becton-Dickinson FACSCalibur Flow Cytometry). Monocyte subpopulations were differentiated based on the ability of cells to scatter light and emit fluorescent signals.

[0350] For the assay performance characteristics for overall sample analysis, BD CaliBRITE beads were used to adjust instrument settings, to set fluorescence compensation and to evaluate instrument sensitivity before each run of the monocyte subsetting-CD14/16 assay.

B) Serum for Bone Specific Alkaline Phosphatase (BSAP)

[0351] Samples for analysis of BSAP were collected pre-dose and on Days 2, 4, 7, 14, and 28, as well as at each additional outpatient visit after Day 28. Five mL of venous blood was collected into a tube containing no additive, centrifuged within 40 minutes, and approximately equal volumes of serum were transferred into 2 tubes and stored frozen at approximately -70°C within 60 minutes of collection.

[0352] Samples were analyzed for BSAP concentration at Pacific Biometrics Inc. (PBI) (220 West Harrison Street, Seattle, Washington 98119, US) using a validated analytical assay. BSAP samples were assayed using a validated, sensitive and specific Enzyme Immunoassay (EIA). The performance of the method during validation was documented. The serum specimens were stored at approximately -70°C until assay, and samples were assayed within 365 days of established stability data generated during validation. Sample concentrations were determined by interpolation from a calibration standard curve that had been fit using a quadratic fitting equation. Those samples with concentrations above the ULOQ (140 U/L) were adequately diluted into calibration range. The assay sensitivity expressed as the observed limit of detection (LOD) for BSAP was 0.4 U/L. Clinical specimens with serum BSAP concentrations below the LOD are reported as below limit of detection.

[0353] The assay performance characteristics were evaluated by QC. Three levels of QC were placed on each run. The run acceptance criteria were: 2 out of 3 QC results must be within 2.0 standard deviation index (SDI), and the third must be within 2.5 SDI. For all four sample analysis runs, the mean run SDI ranged from -1.1 to 0.8 for QC samples.

C) Urine for Analysis of NTX-1

[0354] Samples for analysis of urinary NTX-1 were collected pre-dose and on Days 2, 4, 7, 14, and 28, as well as at each additional outpatient visit after Day 28. The second morning void urine was collected in a clean container. At each timepoint, a 5-mL aliquot was collected for NTX-1. Two

additional 5-mL aliquots were collected for exploratory biomarkers as scheduled. Samples were frozen at approximately -70°C.

[0355] Urine samples were analyzed for urinary N-telopeptide of cross-linked collagen I (uNTX-1) concentration at Pacific Biometrics Inc. (PBI) (220 West Harrison Street, Seattle, Washington 98119, USA) using validated analytical assays. uNTX-1 samples were assayed using a validated, sensitive and specific ELISA for NTX-1 and a validated kinetic Jaffe for urine creatinine.

[0356] The urine specimens were stored at approximately -70°C until assay, and samples were assayed within 365 days of established stability data generated during validation. Sample NTX-1 concentrations were determined by interpolation from a calibration standard curve that has been fit using a 4-parameter fitting equation. Urine creatinine values were determined from a linear calibration. Those samples with NTX-1 concentrations above the ULOQ (3000 nM equivalents per liter) were adequately diluted into calibration range. The NTX-1 assay sensitivity expressed as the LLOQ for NTX-1 was 44.0 nM equivalents per liter. Clinical specimens with NTX-1 concentrations below the LLOQ are reported as below limit of detection.

[0357] NTX-1 assay values were standardized to an equivalent amount of bone collagen, and are expressed in nanomoles bone collagen equivalents per liter (nmol BCE/L). uNTX-1 assay values are corrected for urinary dilution by urinary creatinine analysis and expressed in nanomole bone collagen equivalents per liter (nM BCE) per millimole creatinine per liter (mM creatinine).

[0358] The assay performance characteristics were evaluated by quality controls (QC). Three levels of QC were placed on each run. The run acceptance criteria were: 2 out of 3 QC results must be within 2.0 standard deviation index (SDI), and the third must be within 2.5 SDI. For all four sample analysis runs, the mean run SDI ranged from -0.2 to 0.8 for NTX-1 QC samples, and from -0.1 to 0.2 for urine creatinine QC samples.

D) K_2EDTA Plasma for Analysis of Total Human M-CSF

[0359] K_2EDTA plasma samples collected for exploratory biomarkers were used for analysis of total M-CSF. Samples were collected pre-dose and on Days 2, 7, and 28, as well as at each additional outpatient visit after Day 28. A 10 mL venous blood sample was obtained in a blood collection tube containing no potassium EDTA (K_2EDTA) as an anticoagulant. After centrifugation, the plasma was stored frozen at approximately -70°C. ELISA assay kit (DMC00) from R & D Systems, Inc. (614 McKinley Place NE, Minneapolis 55413). The quantitative sandwich enzyme immunoassay technique was employed in this assay and the assay procedures and critical reagents were followed the kit insert. The K_2EDTA plasma specimens were stored at approximately -70°C until assay. Sample concentrations are determined by interpolation from a calibration standard curve (over the range of 31.2pg/mL to 2000 pg/mL) that has been fit using a 4-parameter logistic equation. Those samples with concentrations above the upper limit of quantification (2000 pg/mL) were adequately diluted into calibration range. The lower limit of quantification (LLOQ) for M-CSF was 31.2 pg/mL. Clinical specimens with serum M-CSF concentrations below the LLOQ are reported as <31.2 pg/mL.

[0360] K_2EDTA plasma samples were analyzed for M-CSF concentration at Pfizer Discovery-Molecular Pharmacology, PGRD, Ann Arbor, Michigan using a commercially available

Results

A) Serum Antibody 8.10.3F Pharmacokinetics

[0361] Table 9 and Table 10 contain summaries of serum antibody 8.10.3F pharmacokinetic parameters following administration of a single IV dose in healthy patients. Mean serum concentration-time profiles following administration of each dose are depicted in Figure 13. Plots of Cmax and AUC values vs. Antibody 8.10.3F dose are shown in Figure 14.

[0362] Following a single infusion of antibody 8.10.3F administered over 1 hour to healthy adult volunteers, Cmax increased in a dose-proportional

manner. However, the extent of systemic exposure, AUC(0-∞), increased in a greater than dose-proportional manner over the dose range of 3-300 mg. Because of the nonlinearity of the system, the apparent terminal t½ (used to calculate AUC[0-∞]) was not felt to be a meaningful measure of the duration of exposure. Instead, the study day at which antibody 8.10.3F concentrations were below the LLOQ for all subjects was determined by inspection of the concentration-time tables. Serum antibody 8.10.3F concentrations were below the LLOQ following the 3, 10, 30, 100, and 300 mg doses by Days 7, 14, 28, 56, and 84, respectively.

Table 9. Summary of Serum Antibody 8.10.3F Pharmacokinetic Parameter Values Following Administration of Single Intravenous Solution Doses to Healthy Subjects

	Parameter Summary Statistics[a] by Antibody 8.10.3F Dose				
Parameter, units	3 mg	10 mg	30 mg	100 mg[b]	300 mg
N	6	6	6	12	6
Cmax, µg/mL	0.825 (24)	2.39 (21)	9.57 (23)	36.0 (19)	90.7 (18)
tmax, hr	0.25 (0.25-0.28)	0.76 (0.25-4.0)	2.0 (1.3-4.0)	1.3 (1.3-2.0)	3.1 (1.8-4.0)
AUC(0-tlqc), µg*hr/mL	13.6 (49)	97.7 (33)	601 (40)	3870 (18)	17800 (22)
AUC(0-∞), µg*hr/mL	16.9 (31)	105 (30)	640 (36)	3870 (18)	18100 (23)
T-BLQ[c]	7	14	21	56	84
CL, mL/min	3.18 (27)	1.72 (29)	0.842 (25)	0.442 (17)	0.290 (24)
Vd, mL/min	4.20 (23)	3.63 (26)	3.21 (24)	2.33 (22)	2.92 (15)
Duration of exposure (Days)[c]	7	14	21	56	84

[a] Median (range) for tmax; arithmetic mean (%CV) for all other parameters
[b] Outpatient and inpatient cohorts combined.
[c] Study Day on which serum concentrations were below the lower limit of quantitation (LLQ=0.035 µg/ml) in all subjects
N = Number of subjects

Table 10. Mean Serum Antibody 8.10.3F Concentrations (ng/mL) Following Administration of Single Intravenous Solution Doses to Healthy Subjects

Study Day	**1**	**2**	**7**	**28[a]**	**56[a]**	**84**
Dose	3 mg					
N	6	6	6			
Mean	0.00	235.83	0.00			
Median	0.00	224.50	0.00			
STD	0.00	53.16	0.00			
Dose	10 mg					
N	6	6	6	6		
Mean	0.00	1531.67	25.88[b]	0.00		
Median	0.00	1495.00	18.30[b]	0.00		
STD	0.00	386.13	32.03	0.00		
Dose	30 mg					
N	6	6	6	6		
Mean	0.00	5991.67	1057.83	0.00		
Median	0.00	5480.00	803.50	0.00		
STD	0.00	1597.09	717.84	0.00		
Dose	100 mg					
N	12	12	12	12	12	
Mean	0.00	24958.33	9751.67	22.93[b]	0.00	
Median	0.00	25400.00	9810.00	0.00	0.00	
STD	0.00	4170.78	2216.52	28.90	0.00	
Dose	300 mg					
N	6	6	6	6	6	6
Mean	0.00	72800.00	40333.33	2476.00	11.82[b]	0.00
Median	0.00	75850.00	42300.00	1614.50	0.00	0.00
STD	0.00	13733.17	8571.74	2266.54	28.94	0.00

[a] Data is summarized by nominal Study Day: Day 28 for the 300 mg dose group combines data collected on Day 28 for 5 of 6 Subjects with those collected on Day 29 for the other subject. Similarly, Day 56 for the 300 mg dose group combines data from 4 or 6 subjects collected on Day 56 with data from the other 2 of 6 subjects collected on Day 58..

[b]Mean and/or median values below the limit of quantitation reflect inclusion of samples reported as BLQ (<35 ng/mL) as 0 in calculations

B) Total Serum M-CSF

[0363] Total (free and antibody 8.10.3F bound) M-CSF concentrations are presented in Table 11 by dose and Study Day and illustrated for the 100-mg dose in Figure 15.

[0364] In the placebo treatment group, mean M-CSF concentration was 0.21 ng/mL at baseline and did not change substantially (range 0.21-0.28 ng/mL) following the administration of placebo to healthy adult volunteers, up to Day 84 (Table 11). Following administration of antibody 8.10.3F, the maximum mean M-CSF concentration increased with increasing dose. Peak concentrations of total M-CSF were attained on Day 2 or 7. Using the equilibrium dissociation constant for antibody 8.10.3F (K_D 2.8 x 10^{-10} M) and the concentrations of M-CSF and of antibody 8.10.3F to determine ratios of free and bound ligand, it was calculated that during the time that antibody 8.10.3F was detectable, 100% of the measured M-CSF ligand was bound to antibody 8.10.3F. Although the sampling frequency for M-CSF was not as frequent as it was for antibody 8.10.3F, M-CSF concentrations declined in parallel with antibody 8.10.3F, suggesting that the temporary increase in M-CSF was subsequently cleared as the antibody-antigen complex.

Table 11. Mean M-CSF Concentrations (ng/mL) Following Administration of Single Intravenous Solution Doses to Healthy Subjects

Study Day	**1**	**2**	**7**	**28**	**56a**	**84**
Dose	0 mg					
N	12	12	12	10	6	2
Mean	0.21	0.24	0.28	0.23	0.22	0.21
Median	0.21	0.20	0.19	0.19	0.22	0.21
STD	0.04	0.07	0.19	0.10	0.03	0.01
Dose	3 mg					
N	6	6	6			
Mean	0.20	4.26	0.24			
Median	0.20	4.43	0.24			
SDT	0.02	1.16	0.04			
Dose	10 mg					
N	6	6	6	6		
Mean	0.18	21.07	0.49	0.21		
Median	0.19	18.37	0.40	0.21		
STD	0.01	6.18	0.25	0.02		
Dose	30 mg					
N	6	6	6	6		
Mean	0.18	34.28	56.10	0.35		
Median	0.18	33.30	34.45	0.25		
STD	0.02	7.28	57.48	0.19		
Dose	100 mg					
N	12	12	12	12	12	
Mean	0.20	42.75	429.33	0.54	0.26	
Median	0.20	46.84	468.84	0.46	0.25	
STD	0.03	14.15	104.64	0.19	0.03	
Dose	300 mg					
N	6	6	6	6	6	6
Mean	0.21	22.89	482.74	247.85	0.35	0.24
Median	0.21	23.93	391.79	256.58	0.32	0.24
STD	0.03	5.37	176.28	181.54	0.08	0.03

[a] Day 56 for the 100 mg dose combines measurements taken on Day 56 in Cohort 4 and Day 52 in Cohort 6.
Primary data stored in ePharm as artifact number 1236578.

5

C) $CD14^+16^+$ Monocytes

[0365] Dose response for $CD14^+16^+$ monocyte count (the sum of $CD14^{bright}CD16^+$ and $CD14^{dim}CD16^+$) on Study Day 28 is presented in Figure 16. Mean $CD14^+16^+$ monocyte cell counts are presented in Table 12 by dose and Study Day and illustrated for the 100-mg dose in Figure 17 and Figure 18. The data in Figure 17 on Day 56 combines measurements made on Day 56 for Cohort 4 and Day 52 for Cohort 6. All figures presenting $CD14^+16^+$ cell count data exclude a single observation that was considered an outlier. Subject 1031 (100-mg) on Study Day 28 had a reported value of 179.0 cells/mcl.

[0366] Treatment with antibody 8.10.3F caused a rapid decline in absolute numbers of peripheral circulating $CD14^+CD16^+$ monocytes with a nadir ranging from approximately 60 to 80% suppression on Day 4 for all doses. Increasing doses maintained suppression of $CD14^+CD16^+$ monocytes for increasing duration. Absolute numbers of $CD14^+CD16^+$ monocytes returned to baseline within 14 days for the 3 and 10 mg doses, within 28 days for the 30 mg dose, and within 56 days for the 100 and 300 mg dose.

Table 12. CD14+16+ Monocyte Counts (cells/μL) Following Administration of Single Intravenous Solution Doses to Healthy Subjects

Study Day	**1**	**2**	**4**	**7**	**14**	**28**	**56**	**84**
Dose	0 mg							
N	11	12	12	12	12	10	5	2
Mean	32.72	34.19	37.53	40.62	37.42	35.91	36.66	39.95
Median	26.6	30.50	36.00	36.70	37.50	32.00	41.20	39.95
STD	16.55	18.16	16.03	18.14	14.99	17.46	13.29	15.20
Dose	3 mg							
N	6	6	6	6	6			
Mean	27.70	23.05	12.32	22.22	40.95			
Median	25.80	16.25	10.90	19.70	42.40			
STD	11.17	18.72	6.10	11.19	15.17			
Dose	10 mg							
N	6	6	6	6	6	6		
Mean	19.88	13.82	8.82	14.90	37.50	32.58		
Median	17.60	10.70	8.50	17.05	29.30	30.60		
STD	9.99	9.37	7.11	9.43	30.59	16.95		
Dose	30 mg							
N	6	6	6	6	6	6		
Mean	23.90	13.72	3.92	5.03	8.52	28.47		
Median	19.00	12.05	4.25	4.80	8.75	22.75		
STD	7.78	8.11	1.95	3.21	2.54	17.39		
Dose	100 mg							
N	12	12	12	12	11	12	12	
Mean	35.61	14.98	5.55	8.93	9.37	37.43	41.89	
Median	29.85	14.00	5.05	5.60	7.90	18.10	30.25	
STD	18.66	5.58	3.50	7.73	5.75	52.98	27.64	
Dose	300 mg							
N	6	6	6	6	6	6	6	6
Mean	36.35	10.92	6.42	11.00	12.05	15.73	38.52	35.77
Median	34.80	8.95	6.25	7.75	9.55	14.85	29.80	27.35
STD	17.41	5.82	3.18	10.54	8.34	9.48	23.51	20.84

D) BSAP and uNTX-1

[0367] Treatment with antibody 8.10.3F was associated with dose and time dependent decrease in uNTX-1, a marker of the bone resorptive activity of osteoclasts. The rate of decrease of uNTX-1 was slower than for $CD14^+CD16^+$ monocytes and recovery from suppression was more protracted. In the 30, 100, and 300 mg dose groups, uNTX-1 declined to a minimum of 64.4, 60.5, and 39.9% of baseline values, which occurred on Days 7, 14, and 28, respectively. On Day 28, mean uNTX-1 was 78.0, 69.4, and 39.3% for the same respective dose groups. uNTX-1 returned to baseline by approximately Day 56 for doses ≤100 mg.

[0368] Mean uNTX-1 is presented in Table 13 by dose and Study Day and illustrated for the 100-mg dose in Figure 19.

[0369] BSAP levels were not affected by treatment at doses up to and including 100 mg. At 300 mg, there was a trend toward increasing BSAP noted on Days 7 through 28 followed by a return to baseline by Day 56. However, the maximum absolute values observed in subjects receiving 300 mg were comparable to that observed in other treatment groups including placebo.

Table 13. Mean uNTX-1 (nM BCE/mM creatinine) Following Administration of Single Intravenous Solution Doses to Healthy Subjects

Study Day	**1**	**2**	**4**	**7**	**14**	**28**	**56**	**84**
Dose	0 mg							
N	12	12	12	12	12	10	5	2
Mean	51.73	54.12	46.48	51.46	51.73	40.58	38.08	0.21
Median	45.25	51.70	47.75	42.75	47.05	34.50	34.50	0.21
STD	24.89	24.37	22.12	23.44	22.89	18.18	12.21	0.01
Dose	3 mg							
N	6	6	6	6	6			
Mean	59.02	50.65	49.50	52.58	45.57			
Median	58.35	51.00	44.75	52.25	44.90			
STD	23.79	17.03	12.09	14.09	10.62			
Dose	10 mg							
N	6	6	6	6	6	6		
Mean	44.65	37.50	32.50	36.72	38.02	44.55		
Median	31.85	33.25	27.80	31.75	27.40	46.05		
STD	31.60	25.40	21.25	17.76	24.56	20.90		
Dose	30 mg							
N	6	6	6	6	6	6		
Mean	49.38	41.12	38.58	31.78	37.83	38.50		
Median	49.70	46.55	39.95	32.10	36.75	39.95		
STD	18.55	16.59	13.63	13.18	12.67	13.49		
Dose	100 mg							
N	12	12	12	12	12	12	12	
Mean	46.01	43.67	38.59	29.04	27.83	31.95	43.60	
Median	45.65	41.55	35.20	28.15	25.30	27.45	43.05	
STD	13.79	15.97	22.99	8.61	15.64	13.80	17.53	
Dose	300 mg							
N	6	6	6	6	6	6	5	6
Mean	43.00	32.98	31.13	24.08	17.42	17.17	30.26	0.24
Median	41.35	32.70	30.00	22.50	17.40	13.90	23.80	0.24
STD	17.11	10.16	11.82	7.49	2.76	5.91	20.20	0.03

Discussion

[0370] Antibody 8.10.3F was generally well tolerated following administration of a single intravenous dose of 3-300 mg to healthy adult volunteers. Treatment with antibody 8.10.3F caused rapid changes in pharmacodynamic markers that were reversible following clearance of the drug. The primary biomarker for mechanism was the number of circulating $CD14^+CD16^+$ monocytes, which had been linked to efficacy in preclinical arthritis models. All doses of antibody 8.10.3F caused a rapid decline in $CD14^+CD16^+$ monocytes with a nadir ranging from approximately 60 to 80% suppression on Day 4. Increasing doses maintained suppression of $CD14^+CD16^+$ monocytes for increasing duration. Absolute numbers of $CD14^+CD16^+$ monocytes returned to baseline within 14 days for the 3 and 10 mg doses, within 28 days for the 30 mg dose, and within 56 days for the 100 and 300 mg dose in this study.

[0371] Based upon preclinical models using a surrogate antibody, doses predicted to provide therapeutic benefit are those that maintain suppression of $CD14^+CD16^+$ monocytes at levels ≤50% of baseline values. In this Study, doses ≥30 mg were able to suppress circulating $CD14^+CD16^+$ monocytes below 50% of baseline for at least 14 days. The 30 mg dose had median $CD14^+CD16^+$ monocyte values of 25%, 46%, and 120% of baseline on Study Days 7, 14, and 28, respectively. The 100 mg dose had median $CD14^+CD16^+$ monocyte values of 19%, 26%, and 61% of baseline on Study Days 7, 14, and 28, respectively. $CD14^+CD16^+$ monocytes may decline further with repeated dosing.

[0372] Inhibition of M-CSF was predicted to inhibit osteoclast differentiation leading to inhibition of bone resorption. Treatment with antibody 8.10.3F was associated with dose and time dependent decrease in uNTX-1. The rate of decrease of uNTX-1 was slower than for $CD14^+CD16^+$ monocytes and recovery from suppression was more protracted. In the 30, 100, and 300 mg dose groups, uNTX-1 declined to a minimum of 64.4, 60.5, and 39.9% of baseline values, which occurred on Days 7, 14, and 28, respectively. On Day 28, mean uNTX-1 was 78.0,

69.4, and 39.3% for the same respective dose groups. uNTX-1 returned to baseline by approximately Day 56 for doses ≤100 mg. BSAP levels were not affected by treatment at doses up to and including 100 mg. At 300 mg, there was a trend toward increasing BSAP noted on Days 7 through 28 followed by a return to baseline by Day 56. However, the maximum absolute values observed in subjects receiving 300 mg were comparable to that observed in other treatment groups including placebo.

[0373] All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

SEQUENCES

Key:

Signal peptide: underlined lower case
CDRs 1,2,3: underlined UPPERCASE
Variable domain: UPPER CASE
Constant domain: lower case
Mutations from germline in bold

SEQ ID NO: 1
252 Heavy Chain [Gamma chain] nucleotide sequence
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SEQ ID NO: 9
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SEQ ID NO: 11
100 Light Chain [Kappa chain] nucleotide sequence
atggaagccccagctcagcttctcttcctcctgctactctggctcccagataccactggaGAAATAGT
GATGACGCAGTCTCCAGCCACCCTGTCTGTGTCTCCAGGGGAAAGAG
CCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAGCAACTTAGCC
TGGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTATGG
TGCATCCACCAGGGCCAGTGGTATCCCAGACAGGATCAGTGGCAGTG
GGTCTGGAACAGAGTTCACTCTCATCATCAGCAGCCTGCAGTCTGAA
GATTTTGCAGTTTATTACTGTCAGCAGTCTAATAACTGGCCATTCACTT
TCGGCCCTGGGACCAAAGTGGATATCAAACGAactgtggctgcaccatctgtcttc
atcttcccgccatctgatgagcagttgaaatctggaactgctagcgttgtgtgcctgctgaataacttctatc
ccagagaggccaaagtacagtggaaggtggataacgccctccaatcgggtaactcccaggagagtg
tcacagagcaggacagcaaggacagcacctacagcctcagcagcaccctgacgctgagcaaagc
agactacgagaaacacaaagtctacgcctgcgaagtcacccatcagggcctgagctcgcccgtcac
aaagagcttcaacaggggagagtgt

SEQ ID NO: 12
100 Light Chain [Kappa chain] protein sequence
meapaqllfllllwlpdttgEIVMTQSPATLSVSPGERATLSCRASQSVSSNLAWYQ
QKPGQAPRLLIYGASTRASGIPDRISGSGSGTEFTLIISSLQSEDFAVYYC
QQSNNWPFTFGPGTKVDIKRtvaapsvfifppsdeqlksgtasvvcllnnfypreakvqwkv
dnalqsgnsqesvteqdskdstyslsstltlskadyekhkvyacevthqglsspvtksfnrgec

SEQ ID NO: 14
3.8.3 Heavy Chain [Gamma chain] protein sequence
mefglswvflvaiikgvqcQVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMS
WIRQAPGKGLEWFSYISSSGSTIYYADSVKGRFTISRDNAKNSLSLQMNS
LRAEDTAVYYCARGLTGDYWGQGTLVTVSSAstkgpsvfplapcsrstsestaalgcl
vkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssnfgtqtytcnvdhkpsntkvdktve
rkccvecppcpappvagpsvflfppkpkdtlmisrtpevtcvvvdvshedpevqfnwyvdgvevhna
ktkpreeqfnstfrvvsvltvvhqdwlngkeykckvsnkglpapiektisktkgqprepqvytlppsree
mtknqvsltclvkgfypsdiavewesngqpennykttppmldsdgsfflyskltvdksrwqqgnvfscs
vmhealhnhytqkslslspgk

SEQ ID NO: 16
3.8.3 Light Chain [Kappa chain] protein sequence
mdmrvpaqllgllllwfpgsrcDIQMTQSPSSVSASVGDRVTISCRASQDISGWLA
WYQQKPGKAPKLLISATSSLHSGVPSRFSGSGSGTDFTLTISSLQPEDFA
TYYCQQTNSFPFTFGPGTKVDIKRtvaapsvfifppsdeqlksgtasvvcllnnfypreakv
qwkvdnalqsgnsqesvteqdskdstyslsstltlskadyekhkvyacevthqglsspvtksfnrgec

SEQ ID NO: 18
2.7.3 Heavy Chain [Gamma chain] protein sequence
mefglswvflvallrgcqcQVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMH
WVRQAPGKGLEWVA**F**IWYDGSNKYYADSVKGRFTISRDNSKNTLYLQM
NSLRAEDTAVYYCARGY**RV**YFDYWGQGTLVTVSSAstkgpsvfplapcsrstses
taalgclvkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssslgtktytcnvdhkpsntk
vdkrveskygppcpscpapeflggpsvflfppkpkdtlmisrtpevtcvvvdvsqedpevqfnwyvdg
vevhnaktkpreeqfnstyrvvsvltvlhqdwlngkeykckvsnkglpssiektiskakgqprepqvytl
ppsqeemtknqvsltclvkgfypsdiavewesngqpennykttppvldsdgsfflysrltvdksrwqeg
nvfscsvmhealhnhytqkslslspgk

SEQ ID NO: 20
2.7.3 Light Chain [Kappa chain] protein sequence
mdmrvpaqllgllllwfpgsrcDIQMTQSPSSVSASVGDRVTITCRASQ**D**ISSWLA
WYQ**R**KPGKAPKL**Q**IYAASSL**E**SGVPSRF**N**GSGSGTDFTL**S**ISSLQPEDFA
TYYCQQTNSFPLTFGGGTKVEIKRtvaapsvfifppsdeqlksgtasvvcllnnfypreakv
qwkvdnalqsgnsqesvteqdskdstyslsstltlskadyekhkvyacevthqglsspvtksfnrgec

SEQ ID NO: 22
1.120.1 Heavy Chain [Gamma chain] protein sequence
mewtwsflflvaaatgahsQVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGIS
WVRQAPGQGLEWMGWISAYNGNTNYAQKLQ**D**RVTMTTDTST**T**TAYME
LRSLRSDDTAVYYCA**RRA**YG**AN**F**F**DYWGQGTLVTVSSAstkgpsvfplapcsr
stsestaalgclvkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssnfgtqtytcnvdhk
psntkvdktverkccvecppcpappvagpsvflfppkpkdtlmisrtpevtcvvvdvshedpevqfnw
yvdgvevhnaktkpreeqfnstfrvvsvltvvhqdwlngkeykckvsnkglpapiektisktkgqprepq
vytlppsreemtknqvsltclvkgfypsdiavewesngqpennykttppmldsdgsfflyskltvdksrw
qqgnvfscsvmhealhnhytqkslslspgk

SEQ ID NO: 24
1.120.1 Light Chain [Kappa chain] protein sequence
mvlqtqvfisllwisgaygDIVMTQSPDSLAVSLGERATINCKSSQS**I**L**FF**SNNKNY
LAWY**R**QKPGQPP**N**LLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAE
DVAVYYCQQYYS**S**PWTFGQGTKVEIKRtvaapsvfifppsdeqlksgtasvvcllnnfy
preakvqwkvdnalqsgnsqesvteqdskdstyslsstltlskadyekhkvyacevthqglsspvtksf
nrgec

SEQ ID NO: 25
9.14.4I Heavy Chain [Gamma Chain] nucleotide sequence
atggagtttgggctgagctgggttttccttgttgctattataaaaggtgtCCAGTGTCAGGTGCAG
CTGGTGGAGTCTGGGGGAGGCTTGGTCAAGCCTGGAGGGTCCCTGA
GACTCTCCTGTGCAGCCTCTGGATTCACCTTCAGTGACTACTATATGA
GCTGGATCCGCCAGGCTCCAGGGAAGGGACTGGAGTGGGTTTCATA
CATTAGTAGTAGTGGTAGTACCATATACTACGCAGACTCTGTGAAGGG
CCGATTCACCATCTCCAGGGACAACGCCAAGAACTCACTGTATCTGCA
AATGAACAGCCTGAGAGCCGAGGACACGGCCGTGTATTACTGTGCGA
GAGGCCTAACTGGGGACTACTGGGGCCAGGGAACCCTGGTCACCGT
CTCCTCAGCTtccaccaagggcccatccgtcttccccctggcgccctgctctagaagcacctccg
agagcacagcggccctgggctgcctggtcaaggactacttccccgaaccggtgacggtgtcgtggaa
ctcaggcgctctgaccagcggcgtgcacaccttcccagctgtcctacagtcctcaggactctactccctc
agcagcgtggtgaccgtgccctccagcaacttcggcacccagacctacacctgcaacgtagatcaca
agcccagcaacaccaaggtggacaagacagttgagcgcaaatgttgtgtcgagtgcccaccgtgccc
agcaccacctgtggcaggaccgtcagtcttcctcttcccccaaaacccaaggacaccctcatgatctc
ccggaccctgaggtcacgtgcgtggtggtggacgtgagccacgaagaccccgaggtccagttcaac
tggtacgtggacggcgtggaggtgcataatgccaagacaaagccacgggaggagcagttcaacagc
acgttccgtgtggtcagcgtcctcaccgttgtgcaccaggactggctgaacggcaaggagtacaagtgc
aaggtctccaacaaaggcctcccagcccccatcgagaaaaccatctccaaaaccaaagggcagcc
ccgagaaccacaggtgtacaccctgcccccatcccgggaggagatgaccaagaaccaggtcagcct
gacctgcctggtcaaaggcttctaccccagcgacatcgccgtggagtgggagagcaatgggcagccg
gagaacaactacaagaccacacctcccatgctggactccgacggctccttcttcctctacagcaagctc
accgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgca
caaccactacacgcagaagagcctctccctgtctccgggtaaa

SEQ ID NO: 26
9.14.4I Heavy Chain [Gamma Chain] protein sequence
mefglswvflvaiikgvgcQVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMS
WIRQAPGKGLEWVSYISSSGSTIYYADSVKGRFTISRDNAKNSLYLQMNS
LRAEDTAVYYCARGLTGDYWGQGTLVTVSSAstkgpsvfplapcsrstsestaalgcl
vkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssnfgtqtytcnvdhkpsntkvdktve
rkccvecppcpappvagpsvflfppkpkdtlmisrtpevtcvvvdvshedpevqfnwyvdgvevhna
ktkpreeqfnstfrvvsvltvvhqdwlngkeykckvsnkglpapiektisktkgqprepqvytlppsree
mtknqvsltclvkgfypsdiavewesngqpennykttppmldsdgsfflyskltvdksrwqqgnvfscs
vmhealhnhytqkslslspgk

SEQ ID NO: 27
9.14.4, 9.14.4I, 9.14.4-Ser and 9.14.4-G1 Light Chain [Kappa Chain]
nucleotide sequence
atggacatgagggtccccgctcagctcctggggctcctgctactctggctccgaggtgccagatgTGA
CATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTCGGAGA
CAGAGTCACCATCACTTGCCGGCCAAGTCAGATCATTAGCAGTTTATT
AAATTGGTATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCCA
TGCTGCATCCAGTTTGCAAAGTGGGGTCCCATCAAGGTTCAGTGGCA
GTGGATCTGGGACAGATTTCACTCTCACCATCAGTAGTCTGCAACCTG
AAGATTTTGCAACTTACTACTGTCAACAGAGTTACAGTACCCCATTCAC
TTTCGGCCCTGGGACCAAAGTGGATATCAAACGAactgtggctgcaccatctgtc
ttcatcttcccgccatctgatgagcagttgaaatctggaactgcctctgttgtgtgcctgctgaataacttctat
cccagagaggccaaagtacagtggaaggtggataacgccctccaatcgggtaactcccaggagagt
gtcacagagcaggacagcaaggacagcacctacagcctcagcagcaccctgacgctgagcaaag
cagactacgagaaacacaaagtctacgcctgcgaagtcacccatcagggcctgagctcgcccgtcac
aaagagcttcaacaggggagagtgt

SEQ ID NO: 28
9.14.4, 9.14.4I, 9.14.4-Ser and 9.14.4-G1 Light Chain [Kappa Chain]
protein sequence
mdmrvpaqllgllllwlrgarcDIQMTQSPSSLSASVGDRVTITCRPSQIISSLLNWY
QQKPGKAPKLLIHAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATY
YCQQSYSTPFTFGPGTKVDIKRtvaapsvfifppsdeqlksgtasvvcllnnfypreakvqw
kvdnalqsgnsqesvteqdskdstyslsstltlskadyekhkvyacevthqglsspvtksfnrgec

SEQ ID NO: 37
9.14.4 Heavy Chain [Gamma Chain] nucleotide sequence
atggagtttgggctgagctgggttttccttgttgctattataaaaggtgtCCAGTGTCAGGTGCAG
CTGGTGGAGTCTGGGGGAGGCTTGGTCAAGCCTGGAGGGTCCCTGA
GACTCTCCTGTGCAGCCTCTGGATTCACCTTCAGTGACTACTATATGA
GCTGGATCCGCCAGGCTCCAGGGAAGGGACTGGAGTGGGTTTCATA
CATTAGTAGTAGTGGTAGTACCATATACTACGCAGACTCTGTGAAGGG
CCGATTCACCATCTCCAGGGACAACGCCAAGAACTCACTGTATCTGCA
AATGAACAGCCTGAGAGCCGAGGACACGGCCGTGTATTACTGTGCGA
GAGGCCTAACTGGGGACTACTGGGGCCAGGGAACCCTGGTCACCGT
CTCCTCAGCTtccaccaagggcccatccgtcttccccctggcgccctgctctagaagcacctccg
agagcacagcggccctgggctgcctggtcaaggactacttccccgaaccggtgacggtgtcgtggaa
ctcaggcgctctgaccagcggcgtgcacaccttcccagctgtcctacagtcctcaggactctactccctc
agcagcgtggtgaccgtgccctccagcagcttgggcacgaagacctacacctgcaacgtagatcaca
agcccagcaacaccaaggtggacaagagagttgagtccaaatatggtcccccatgcccatcatgccc
agcacctgagttcctgggggggaccatcagtcttcctgttcccccaaaacccaaggacactctcatgatc
tcccggaccctgaggtcacgtgcgtggtggtggacgtgagccaggaagaccccgaggtccagttca
actggtacgtggatggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagttcaaca
gcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaacggcaaggagtacaa
gtgcaaggtctccaacaaaggcctcccgtcctccatcgagaaaaccatctccaaagccaaagggca
gccccgagagccacaggtgtacaccctgcccccatcccaggaggagatgaccaagaaccaggtca
gcctgacctgcctggtcaaaggcttctaccccagcgacatcgccgtggagtgggagagcaatgggca
gccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcctctacagca
ggctaaccgtggacaagagcaggtggcaggaggggaatgtcttctcatgctccgtgatgcatgaggct
ctgcacaaccactacacacagaagagcctctccctgtctccgggtaaa

SEQ ID NO: 38
9.14.4 Heavy Chain [Gamma Chain] protein sequence
mefglswvflvaiikgvgcQVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMS
WIRQAPGKGLEWVSYISSSGSTIYYADSVKGRFTISRDNAKNSLYLQMNS
LRAEDTAVYYCARGLTGDYWGQGTLVTVSSAstkgpsvfplapcsrstsestaalgcl
vkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssslgtktytcnvdhkpsntkvdkrve
skygppcpscpapeflggpsvflfppkpkdtlmisrtpevtcvvvdvsqedpevqfnwyvdgvevhn
aktkpreeqfnstyrvvsvltvlhqdwlngkeykckvsnkglpssiektiskakgqprepqvytlppsqe
emtknqvsltclvkgfypsdiavewesngqpennykttppvldsdgsfflysrltvdksrwqegnvfscs
vmhealhnhytqkslslspgk

SEQ ID NO: 54
9.14.4C-Ser Heavy Chain [Gamma chain] protein sequence
mefglswvflvaiikgvgcQVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMS
WIRQAPGKGLEWVSYISSSGSTIYYADSVKGRFTISRDNAKNSLYLQMNS
LRAEDTAVYYCARGLTGDYWGQGTLVTVSSAstkgpsvfplapcsrstsestaalgcl
vkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssslgtktytcnvdhkpsntkvdkrve
skygppcp**p**cpapeflggpsvflfppkpkdtlmisrtpevtcvvvdvsqedpevqfnwyvdgvevhn
aktkpreeqfnstyrvvsvltvlhqdwlngkeykckvsnkglpssiektiskakgqprepqvytlppsqe
emtknqvsltclvkgfypsdiavewesngqpennykttppvldsdgsfflysrltvdksrwqegnvfscs
vmhealhnhytqkslslspgk

SEQ ID NO: 56
9.14.4C-Ser, 9.14.4-CG2 and 9.14.4-CG4 Light Chain [Kappa chain] protein sequence
mdmrvpaqllglllwlrgarcDIQMTQSPSSLSASVGDRVTITCRPSQIISSLLNWY
QQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATY
YCQQSYSTPFTFGPGTKVDIKRtvaapsvfifppsdeqlksgtasvvcllnnfypreakvqw
kvdnalqsgnsqesvteqdskdstyslsstltlskadyekhkvyacevthqglsspvtksfnrgec

SEQ ID NO: 74
9.14.4-CG2 Heavy Chain [Gamma chain] protein sequence
mefglswvflvaiikgvqcQVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMS
WIRQAPGKGLEWVSYISSSGSTIYYADSVKGRFTISRDNAKNSLYLQMNS
LRAEDTAVYYCARGLTGDYWGQGTLVTVSSAstkgpsvfplapcsrstsestaalgcl
vkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssnfgtqtytcnvdhkpsntkvdktve
rkccvecppcpappvagpsvflfppkpkdtlmisrtpevtcvvvdvshedpevqfnwyvdgvevhna
ktkpreeqfnstfrvvsvltvvhqdwlngkeykckvsnkglpapiektisktkgqprepqvytlppsree
mtknqvsltclvkgfypsdiavewesngqpennykttppmldsdgsfflyskltvdksrwqqgnvfscs
vmhealhnhytqkslslspgk

SEQ ID NO: 78
9.14.4-CG4 Heavy Chain [Gamma chain] protein sequence
mefglswvflvaiikgvqcQVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMS
WIRQAPGKGLEWVSYISSSGSTIYYADSVKGRFTISRDNAKNSLYLQMNS
LRAEDTAVYYCARGLTGDYWGQGTLVTVSSAstkgpsvfplapcsrstsestaalgcl
vkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpsssigtktytcnvdhkpsntkvdkrve
skygppcpscpapeflggpsvflfppkpkdtlmisrtpevtcvvvdvsqedpevqfnwyvdgvevhn
aktkpreeqfnstyrvvsvltvlhqdwlngkeykckvsnkglpssiektiskakgqprepqvytlppsqe
emtknqvsltclvkgfypsdiavewesngqpennykttppvldsdgsfflysrltvdksrwqegnvfscs
vmhealhnhytqkslslspgk

SEQ ID NO: 82
9.14.4-Ser Heavy Chain [Gamma chain] protein sequence
mefglswvflvaiikgvqcQVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMS
WIRQAPGKGLEWVSYISSSGSTIYYADSVKGRFTISRDNAKNSLYLQMNS
LRAEDTAVYYCARGLTGDYWGQGTLVTVSSAstkgpsvfplapcsrstsestaalgcl
vkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpsssigtktytcnvdhkpsntkvdkrve
skygppcppcpapeflggpsvflfppkpkdtlmisrtpevtcvvvdvsqedpevqfnwyvdgvevhn
aktkpreeqfnstyrvvsvltvlhqdwlngkeykckvsnkglpssiektiskakgqprepqvytlppsqe
emtknqvsltclvkgfypsdiavewesngqpennykttppvldsdgsfflysrltvdksrwqegnvfscs
vmhealhnhytqkslslspgk

SEQ ID NO. 101
9.14.4G1 Heavy chain (gamma chain) nucleotide sequence
atggagtttgggctgagctgggttttccttgttgctattataaaaggtgtccagtgtCAGGTGCAGCT
GGTGGAGTCTGGGGGAGGCTTGGTCAAGCCTGGAGGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCACCTTCAGTGACTACTATATGAGC
TGGATCCGCCAGGCTCCAGGGAAGGGACTGGAGTGGGTTTCATACAT
TAGTAGTAGTGGTAGTACCATATACTACGCAGACTCTGTGAAGGGCCG
ATTCACCATCTCCAGGGACAACGCCAAGAACTCACTGTATCTGCAAAT
GAACAGCCTGAGAGCCGAGGACACGGCCGTGTATTACTGTGCGAGA
GGCCTAACTGGGGACTACTGGGGCCAGGGAACCCTGGTCACCGTCT
CCTCAGCTtccaccaagggcccatcggtcttcccctggcaccctcctccaagagcacctctggg
ggcacagcggccctgggctgcctggtcaaggactacttccccgaaccggtgacggtgtcgtggaactc
aggcgccctgaccagcggcgtgcacaccttcccggctgtcctacagtcctcaggactctactccctcag
cagcgtggtgaccgtgccctccagcagcttgggcacccagacctacatctgcaacgtgaatcacaagc
ccagcaacaccaaggtggacaagaaagttgagcccaaatcttgtgacaaaactcacacatgcccac
cgtgcccagcacctgaactcctgggggggaccgtcagtcttcctcttcccccaaaacccaaggacacc
ctcatgatctcccggacccctgaggtcacatgcgtggtggtggacgtgagccacgaagaccctgaggt
caagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagca
gtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaagg
agtacaagtgcaaggtctccaacaaagccctcccagccccatcgagaaaaccatctccaaagcca
aagggcagccccgagaaccacaggtgtacaccctgcccccatcccgggatgagctgaccaagaac
caggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaa
tgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcctcta
cagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcat
gaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatag

SEQ ID NO 102
9.14.4G1 Heavy chain (gamma chain) protein sequence
mefglswvflvaiikgvgcQVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMS
WIRQAPGKGLEWVSYISSSGSTIYYADSVKGRFTISRDNAKNSLYLQMNS
LRAEDTAVYYCARGLTGDYWGQGTLVTVSSAstkgpsvfplapsskstsggtaalgc
lvkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssslgtqtyicnvnhkpsntkvdkkv
epkscdkthtcppcpapellggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnwyvdgvev
hnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepqvytlpps
rdeltknqvsltclvkgfypsdiavewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfsc
svmhealhnhytqkslslspgk

SEQ ID NO: 29
8.10.3 and 8.10.3F Heavy Chain [Gamma chain] nucleotide sequence
atggagttgggctgtgctgggttttccttgttgctattttagaaggtgtccagtgtGAGGTGCAGCT
GGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCACCTTCAGTAGTTTTAGTATGACC
TGGGTCCGCCAGGCTCCAGGAAAGGGGCTGGAGTGGGTTTCATACAT
TAGTAGTAGAAGTAGTACCATATCCTACGCAGACTCTGTGAAGGGCCG
ATTCACCATCTCCAGAGACAATGCCAAGAACTCACTGTATCTGCAAAT
GAACAGCCTGAGAGACGAGGACACGGCTGTGTATTACTGTGCGAGAG
ATCCTCTTCTAGCGGGAGCTACCTTCTTTGACTACTGGGGCCAGGGA
ACCCTGGTCACCGTCTCCTCAGCCtccaccaagggcccatcggtcttcccctggcgc
cctgctccaggagcacctccgagagcacagcggccctgggctgcctggtcaaggactacttccccga
accggtgacggtgtcgtggaactcaggcgctctgaccagcggcgtgcacaccttcccagctgtcctaca
gtcctcaggactctactccctcagcagcgtggtgaccgtgccctccagcaacttcggcacccagaccta
cacctgcaacgtagatcacaagcccagcaacaccaaggtggacaagacagttgagcgcaaatgttg
tgtcgagtgcccaccgtgcccagcaccacctgtggcaggaccgtcagtcttcctcttcccccaaaacc
caaggacaccctcatgatctcccggacccctgaggtcacgtgcgtggtggtggacgtgagccacgaa
gaccccgaggtccagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagcca
cgggaggagcagttcaacagcacgttccgtgtggtcagcgtcctcaccgttgtgcaccaggactggctg
aacggcaaggagtacaagtgcaaggtctccaacaaaggcctcccagcccccatcgagaaaaccat
ctccaaaaccaaagggcagccccgagaaccacaggtgtacaccctgcccccatcccgggaggaga
tgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctaccccagcgacatcgccgtgga
gtgggagagcaatgggcagccggagaacaactacaagaccacacctcccatgctggactccgacg
gctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcat
gctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaa

SEQ ID NO: 30
8.10.3 and 8.10.3F Heavy Chain [Gamma chain] protein sequence
melglcwvflvailegvqcEVQLVESGGGLVQPGGSLRLSCAASGFTFSS**F**SM**T**
WVRQAPGKGLEWVSYISS**R**SST**I**SYADSVKGRFTISRDNAKNSLYLQMN
SLRDEDTAVYYCAR**DPLLA**G**AT**FFDYWGQGTLVTVSSAstkgpsvfplapcsrs
tsestaalgclvkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssnfgtqtytcnvdhkp
sntkvdktverkccvecppcpappvagpsvflfppkpkdtlmisrtpevtcvvvdvshedpevqfnwy
vdgvevhnaktkpreeqfnstfrvvsvltvvhqdwlngkeykckvsnkglpapiektisktkgqprepqv
ytlppsreemtknqvsltclvkgfypsdiavewesngqpennykttppmldsdgsfflyskltvdksrwq
qgnvfscsvmhealhnhytqkslslspgk

SEQ ID NO: 31
8.10.3FG1 and 8.10.3F Light Chain [Kappa chain] nucleotide sequence
atggaaaccccagcgcagcttctcttcctcctgctactctggctcccagataccaccggaGAATTTG
TGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGA
GCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAGCAGTTACTT
AGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCT
ATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACAGGTTCAGTGGC
AGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGCC
TGAAGATTTTGCAGTGTATTACTGTCAGCAGTATGGTAGCTCACCTCT
CACTTTCGGCGGAGGGACCAAGGTGGAGATCAAACGAactgtggctgcacc
atctgtcttcatcttcccgccatctgatgagcagttgaaatctggaactgcctctgttgtgtgcctgctgaata
acttctatcccagagaggccaaagtacagtggaaggtggataacgccctccaatcgggtaactcccag
gagagtgtcacagagcaggacagcaaggacagcacctacagcctcagcagcaccctgacgctgag
caaagcagactacgagaaacacaaagtctacgcctgcgaagtcacccatcagggcctgagctcgcc
cgtcacaaagagcttcaacaggggagagtgt

SEQ ID NO: 32
8.10.3FG1 and 8.10.3F Light Chain [Kappa chain] protein sequence
metpaqllfllllwlpdttgEFVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWY
QQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYY
CQQYGSSPLTFGGGTKVEIKRtvaapsvfifppsdeqlksgtasvvcllnnfypreakvqwk
vdnalqsgnsqesvteqdskdstyslsstltlskadyekhkvyacevthqglsspvtksfnrgec

SEQ ID NO: 43
8.10.3 and 8.10.3-Ser Light Chain [Kappa chain] nucleotide sequence
atggaaaccccagcgcagcttctcttcctcctgctactctggctcccagataccaccggaGAATTTG
TGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGA
GCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAGCAGTTACTT
AGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCT
ATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACAGGTTCAGTGGC
AGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGCC
TGAAGATTTTGTAGTGTATTACTGTCAGCAGTATGGTAGCTCACCTCTC
ACTTTCGGCGGAGGGACCAAGGTGGAGATCAAACGAactgtggctgcaccat
ctgtcttcatcttcccgccatctgatgagcagttgaaatctggaactgcctctgttgtgtgcctgctgaataac
ttctatcccagagaggccaaagtacagtggaaggtggataacgccctccaatcgggtaactcccagg
agagtgtcacagagcaggacagcaaggacagcacctacagcctcagcagcaccctgacgctgagc
aaagcagactacgagaaacacaaagtctacgcctgcgaagtcacccatcagggcctgagctcgccc
gtcacaaagagcttcaacaggggagagtgt

SEQ ID NO: 44
8.10.3 and 8.10.3-Ser Light Chain [Kappa chain] protein sequence
metpaqllfllllwlpdttgEFVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWY
QQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDF**V**VYY
CQQYGSSPLTFGGGTKVEIKRtvaapsvfifppsdeqlksgtasvvcllnnfypreakvqwk
vdnalqsgnsqesvteqdskdstyslsstltlskadyekhkvyacevthqglsspvtksfnrgec

SEQ ID NO: 58
8.10.3C-Ser Heavy Chain [Gamma chain] protein sequence
melglcwvflvailegvqcEVQLVESGGGLVQPGGSLRLSCAASGFTFSSFSMT
WVRQAPGKGLEWVSYISSRSSTISYADSVKGRFTISRDNAKNSLYLQMN
SLRDEDTAVYYCARDPLLAGATFFDYWGQGTLVTVSSAstkgpsvfplapcsrs
tsestaalgclvkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssslgtktytcnvdhkp
sntkvdkrveskygppcppcpapeflggpsvflfppkpkdtlmisrtpevtcvvvdvsqedpevqfnw
yvdgvevhnaktkpreeqfnstyrvvsvltvlhqdwlngkeykckvsnkglpssiektiskakgqprep
qvytlppsqeemtknqvsltclvkgfypsdiavewesngqpennykttppvldsdgsfflysrltvdksr
wqegnvfscsvmhealhnhytqkslslspgk

SEQ ID NO: 60
8.10.3-CG2, 8.10.3-CG4 and 8.10.3C-Ser Light Chain [kappa chain] protein sequence
metpaqllfllllwlpdttgEIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQ
QKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC
QQYGSSPLTFGGGTKVEIKRtvaapsvfifppsdeqlksgtasvvcllnnfypreakvqwkv
dnalqsgnsqesvteqdskdstyslsstltlskadyekhkvyacevthqglsspvtksfnrgec

SEQ ID NO: 62
8.10.3-CG2 Heavy Chain [Gamma chain] protein sequence
melglcwvflvailegvqcEVQLVESGGGLVQPGGSLRLSCAASGFTFSSFSMT
WVRQAPGKGLEWVSYISSRSSTISYADSVKGRFTISRDNAKNSLYLQMN
SLRDEDTAVYYCARDPLLAGATFFDYWGQGTLVTVSSAstkgpsvfplapcsrs
tsestaalgclvkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssnfgtqtytcnvdhkp
sntkvdktverkccvecppcpappvagpsvflfppkpkdtlmisrtpevtcvvvdvshedpevqfnwy
vdgvevhnaktkpreeqfnstfrvvsvltvvhqdwlngkeykckvsnkglpapiektisktkgqprepqv
ytlppsreemtknqvsltclvkgfypsdiavewesngqpennykttppmldsdgsfflyskltvdksrwq
qgnvfscsvmhealhnhytqkslslspgk

SEQ ID NO: 90
8.10.3-Ser Heavy Chain [Gamma chain] protein sequence
melglcwvflvailegvqcEVQLVESGGGLVQPGGSLRLSCAASGFTFSSFSMT
WVRQAPGKGLEWVSYISSRSSTISYADSVKGRFTISRDNAKNSLYLQMN
SLRDEDTAVYYCARDPLLAGATFFDYWGQGTLVTVSSAstkgpsvfplapcsrs
tsestaalgclvkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssslgtktytcnvdhkp
sntkvdkrveskygppcppcpapeflggpsvflfppkpkdtlmisrtpevtcvvvdvsqedpevqfnw
yvdgvevhnaktkpreeqfnstyrvvsvltvlhqdwlngkeykckvsnkglpssiektiskakgqprep
qvytlppsqeemtknqvsltclvkgfypsdiavewesngqpennykttppvldsdgsfflysrltvdksr
wqegnvfscsvmhealhnhytqkslslspgk

SEQ ID NO: 94
8.10.3-CG4 Heavy Chain [Gamma chain] protein sequence
melglcwvflvailegvqcEVQLVESGGGLVQPGGSLRLSCAASGFTFSSFSMT
WVRQAPGKGLEWVSYISSRSSTISYADSVKGRFTISRDNAKNSLYLQMN
SLRDEDTAVYYCARDPLLAGATFFDYWGQGTLVTVSSAstkgpsvfplapcsrs
tsestaalgclvkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssslgtktytcnvdhkp
sntkvdkrveskygppcpscpapeflggpsvflfppkpkdtlmisrtpevtcvvvdvsqedpevqfnwy
vdgvevhnaktkpreeqfnstyrvvsvltvlhqdwlngkeykckvsnkglpssiektiskakgqprepq
vytlppsqeemtknqvsltclvkgfypsdiavewesngqpennykttppvldsdgsfflysrltvdksrw
qegnvfscsvmhealhnhytqkslslspgk

SEQ ID NO: 97
8.10.3FG1 Heavy Chain nucleotide sequence
atggagttggggctgagctgggttttccttgttgctattataaaaggtgtccagtgtGAGGTGCAGCT
GGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCACCTTCAGTAGTTTTAGTATGACC
TGGGTCCGCCAGGCTCCAGGAAAGGGGCTGGAGTGGGTTTCATACAT
TAGTAGTAGAAGTAGTACCATATCCTACGCAGACTCTGTGAAGGGCCG
ATTCACCATCTCCAGAGACAATGCCAAGAACTCACTGTATCTGCAAAT
GAACAGCCTGAGAGACGAGGACACGGCTGTGTATTACTGTGCGAGAG
ATCCTCTTCTAGCGGGAGCTACCTTCTTTGACTACTGGGGCCAGGGA
ACCCTGGTCACCGTCTCCTCAGCCtccaccaagggcccatcggtcttcccctggcac
cctcctccaagagcacctctgggggcacagcggccctgggctgcctggtcaaggactacttccccgaa
ccggtgacggtgtcgtggaactcaggcgccctgaccagcggcgtgcacaccttcccggctgtcctaca
gtcctcaggactctactccctcagcagcgtggtgaccgtgccctccagcagcttgggcacccagaccta
catctgcaacgtgaatcacaagcccagcaacaccaaggtggacaagaaagttgagcccaaatcttgt
gacaaaactcacacatgcccaccgtgcccagcacctgaactcctgggggaccgtcagtcttcctcttc
cccccaaaacccaaggacaccctcatgatctcccggaccctgaggtcacatgcgtggtggtggacgt
gagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaag
acaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcac
caggactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccctcccagcccccatc
gagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgcccccatc
ccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgac
atcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctg
gactccgacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcagggga
acgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtct
ccgggtaaatag

SEQ ID NO: 98
8.10.3FG1 Heavy chain (gamma chain) protein sequence
melglcwvflvailegvqcEVQLVESGGGLVQPGGSLRLSCAASGFTFSSFSMT
WVRQAPGKGLEWVSYISSRSSTISYADSVKGRFTISRDNAKNSLYLQMN
SLRDEDTAVYYCARDPLLAGATFFDYWGQGTLVTVSSAstkgpsvfplapssk
stsggtaalgclvkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssslgtqtyicnvnhk
psntkvdkkvepkscdkthtcppcpapellggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkf
nwyvdgvevhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqp
repqvytlppsrdeltknqvsltclvkgfypsdiavewesngqpennykttppvldsdgsfflyskltvdks
rwqqgnvfscsvmhealhnhytqkslslspgk

SEQ ID NO: 33
9.7.2IF Heavy Chain [Gamma chain] nucleotide sequence
atggagtttgggctgagctgggttttccttgttgctattataaaaggtgtccagtgtcAGGTGCAGCT
GGTGGAGTCTGGGGGAGGCTTGGTCAAGCCTGGAGGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCACCTTCAGTGACTACTACATGAGC
TGGATCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTTTCATACAT
TAGTAGTAGTGGTAGTACCATATACTACGCAGACTCTGTGAAGGGCCG
ATTCACCATCTCCAGGGACAACGCCAAGAATTCACTGTATCTGCAAAT
GAACAGCCTGAGAGCCGAGGACACGGCCGTGTATTACTGTGCGAGG
CGTATAGGAGGTATGGACGTCTGGGGCCAAGGGACCACGGTCACCG
TCTCCTCAGCTtccaccaagggcccatccgtcttccccctggcgccctgctctagaagcacctcc
gagagcacagcggccctgggctgcctggtcaaggactacttccccgaaccggtgacggtgtcgtgga
actcaggcgctctgaccagcggcgtgcacaccttcccagctgtcctacagtcctcaggactctactccct
cagcagcgtggtgaccgtgccctccagcaacttcggcacccagacctacacctgcaacgtagatcac
aagcccagcaacaccaaggtggacaagacagttgagcgcaaatgttgtgtcgagtgcccaccgtgcc
cagcaccacctgtggcaggaccgtcagtcttcctcttcccccaaaacccaaggacaccctcatgatct
cccggacccctgaggtcacgtgcgtggtggtggacgtgagccacgaagaccccgaggtccagttcaa
ctggtacgtggacggcgtggaggtgcataatgccaagacaaagccacgggaggagcagttcaacag
cacgttccgtgtggtcagcgtcctcaccgttgtgcaccaggactggctgaacggcaaggagtacaagtg
caaggtctccaacaaaggcctcccagcccccatcgagaaaaccatctccaaaaccaaagggcagc
cccgagaaccacaggtgtacaccctgcccccatcccgggaggagatgaccaagaaccaggtcagc
ctgacctgcctggtcaaaggcttctaccccagcgacatcgccgtggagtgggagagcaatgggcagc
cggagaacaactacaagaccacacctcccatgctggactccgacggctccttcttcctctacagcaag
ctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctct
gcacaaccactacacgcagaagagcctctccctgtctccgggtaaa

SEQ ID NO: 34
9.7.2IF Heavy Chain [Gamma Chain] protein sequence
mefglswvflvaiikgvqcQVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMS
WIRQAPGKGLEWVSYISSSGSTIYYADSVKGRFTISRDNAKNSLYLQMNS
LRAEDTAVYYCARRIGGMDVWGQGTTVTVSSAstkgpsvfplapcsrstsestaalg
clvkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssnfgtqtytcnvdhkpsntkvdkt
verkccvecppcpappvagpsvflfppkpkdtlmisrtpevtcvvvdvshedpevqfnwyvdgvevh
naktkpreeqfnstfrvvsvltvvhqdwlngkeykckvsnkglpapiektisktkgqprepqvytlppsre
emtknqvsltclvkgfypsdiavewesngqpennykttppmldsdgsfflyskltvdksrwqqgnvfsc
svmhealhnhytqkslslspgk
SEQ ID NO: 35

9.7.2IF Light Chain [Kappa chain] nucleotide sequence
atggacatgagggtccccgctcagctcctggggctcctgctactctggctccgaggtgccagatgtGA
CATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGA
CAGAGTCACCATCACTTGCCGGGCAAGTCAGAGCATTAGCGGCTTTTT
AATTTGGTATCAGCAGAGACCAGGGAAAGCCCCTAAGCTCCTGATCTA
TGCTACATCCAGTTTACAAAGTGGGGTCCCATCAAGGTTCAGTGGCAG
TGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTCTGCAACCTGA
AGATTTTGCAACTTACTACTGTCAACAGAGTTACAGTACCCCATTCACT
TTCGGCCCTGGGACCAAAGTGGATATCAAACGAactgtggctgcaccatctgtctt
catcttcccgccatctgatgagcagttgaaatctggaactgcctctgttgtgtgcctgctgaataacttctatc
ccagagaggccaaagtacagtggaaggtggataacgccctccaatcgggtaactcccaggagagtg
tcacagagcaggacagcaaggacagcacctacagcctcagcagcaccctgacgctgagcaaagc
agactacgagaaacacaaagtctacgcctgcgaagtcacccatcagggcctgagctcgcccgtcac
aaagagcttcaacaggggagagtgt

SEQ ID NO: 36
9.7.2IF Light Chain [Kappa chain] protein sequence
mdmrvpaqllgllllwlrgarcDIQMTQSPSSLSASVGDRVTITCRASQSIS**GFLI**WY
QQ**R**PGKAPKLLIYATSSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATY
YCQQSYSTPFTFGPGTKVDIKRtvaapsvfifppsdeqlksgtasvvcllnnfypreakvqw
kvdnalqsgnsqesvteqdskdstyslsstltlskadyekhkvyacevthqglsspvtksfnrgec

SEQ ID NO: 45
9.7.2 Heavy Chain [Gamma chain] nucleotide sequence
atggagtttgggctgagctgggttttccttgttgctattataaaaggtgtccagtgtcAGGTGCAGCT
GGTGGAGTCTGGGGGAGGCTTGGTCAAGCCTGGAGGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCACCTTCAGTGACTACTACATGAGC
TGGATCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTTTCATACAT
TAGTAGTAGTGGTAGTACCATATACTACGCAGACTCTGTGAAGGGCCG
ATTCACCATCTCCAGGGACAACGCCAAGAATTCACTGTATCTGCAAAT
GAACAGCCTGAGAGCCGAGGACACGGCCGTGTATTACTGTGCGAGG
CGTATAGGAGGTATGGACGTCTGGGGCCAAGGGACCACGGTCACCG
TCTCCTCAGCTtccaccaagggcccatccgtcttccccctggcgccctgctctagaagcacctcc
gagagcacagcggccctgggctgcctggtcaaggactacttccccgaaccggtgacggtgtcgtgga
actcaggcgctctgaccagcggcgtgcacaccttcccagctgtcctacagtcctcaggactctactccct
cagcagcgtggtgaccgtgccctccagcagcttgggcacgaagacctacacctgcaacgtagatcac
aagcccagcaacaccaaggtggacaagagagttgagtccaaatatggtcccccatgcccatcatgcc
cagcacctgagttcctgggggaccatcagtcttcctgttcccccaaaacccaaggacactctcatgat
ctcccggacccctgaggtcacgtgcgtggtggtggacgtgagccaggaagaccccgaggtccagttc
aactggtacgtggatggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagttcaac
agcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaacggcaaggagtaca
agtgcaaggtctccaacaaaggcctcccgtcctccatcgagaaaaccatctccaaagccaaagggc
agccccgagagccacaggtgtacaccctgcccccatcccaggaggagatgaccaagaaccaggtc
agcctgacctgcctggtcaaaggcttctaccccagcgacatcgccgtggagtgggagagcaatgggc
agccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcctctacagc
aggctaaccgtggacaagagcaggtggcaggaggggaatgtcttctcatgctccgtgatgcatgaggc
tctgcacaaccactacacacagaagagcctctccctgtctccgggtaaa

SEQ ID NO: 46
9.7.2 Heavy Chain [Gamma Chain] protein sequence
mefglswvflvaiikgvqcQVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMS
WIRQAPGKGLEWVSYISSSGSTIYYADSVKGRFTISRDNAKNSLYLQMNS
LRAEDTAVYYCA**RRIG**GMDVWGQGTTVTVSSAstkgpsvfplapcsrstsestaalg
clvkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssslgtktytcnvdhkpsntkvdkrv
eskygppcpscpapeflggpsvflfppkpkdtlmisrtpevtcvvvdvsqedpevqfnwyvdgvevh
naktkpreeqfnstyrvvsvltvlhqdwlngkeykckvsnkglpssiektiskakgqprepqvytlppsq
eemtknqvsltclvkgfypsdiavewesngqpennykttppvldsdgsfflysrltvdksrwqegnvfsc
svmhealhnhytqkslslspgk

SEQ ID NO: 47
9.7.2 and 9.7.2-Ser Light Chain [Kappa chain] nucleotide sequence
atggacatgagggtccccgctcagctcctggggctcctgctactctggctccgaggtgccagatgtGA
CATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGA
CAGAGTCACCATCACTTGCCGGGCAAGTCAGAGCATTAGCGGCTTTTT
AATTTGGTATCAGCAGAGACCAGGGAAAGCCCCTAAGCTCCTGATCTA
TGCTACATCCAGTTTACAAAGTGGGGTCCCATTAAGGTTCAGTGGCAG
TGAATCTGGGACAGATTTCACTCTCACCATCAGCAGTCTGCAACCTGA
AGATTTTGCAACTTACTACTGTCAACAGAGTTACAGTACCCCATTCACT
TTCGGCCCTGGGACCAAAGTGGATATCAAACGAactgtggctgcaccatctgtctt
catcttcccgccatctgatgagcagttgaaatctggaactgcctctgttgtgtgcctgctgaataacttctatc
ccagagaggccaaagtacagtggaaggtggataacgccctccaatcgggtaactcccaggagagtg
tcacagagcaggacagcaaggacagcacctacagcctcagcagcaccctgacgctgagcaaagc
agactacgagaaacacaaagtctacgcctgcgaagtcacccatcagggcctgagctcgcccgtcac
aaagagcttcaacaggggagagtgt

SEQ ID NO: 48
9.7.2 and 9.7.2-Ser Light Chain [Kappa chain] protein sequence
mdmrvpaqllgllllwlrgarcDIQMTQSPSSLSASVGDRVTITCRASQSIS**GFLI**WY
QQ**R**PGKAPKLLIYATSSLQSGVP**L**RFSGS**E**SGTDFTLTISSLQPEDFATYY
CQQSYSTPFTFGPGTKVDI**K**Rtvaapsvfifppsdeqlksgtasvvcllnnfypreakvqwk
vdnalqsgnsqesvteqdskdstyslsstltlskadyekhkvyacevthqglsspvtksfnrgec

SEQ ID NO: 50
9.7.2C-Ser Heavy Chain [Gamma chain] protein sequence
mefglswvflvaiikgvqcQVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMS
WIRQAPGKGLEWVSYISSSGSTIYYADSVKGRFTISRDNAKNSLYLQMNS
LRAEDTAVYYCAI**RIG**GMDVWGQGTTVTVSSAstkgpsvfplapcsrstsestaalgc
lvkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssslgtktytcnvdhkpsntkvdkrve
skygppcp**p**cpapeflggpsvflfppkpkdtlmisrtpevtcvvvdvsqedpevqfnwyvdgvevhn
aktkpreeqfnstyrvvsvltvlhqdwlngkeykckvsnkglpssiektiskakgqprepqvytlppsqe
emtknqvsltclvkgfypsdiavewesngqpennykttppvldsdgsfflysrltvdksrwqegnvfscs
vmhealhnhytqkslslspgk

SEQ ID NO: 52

9.7.2C-Ser, 9.7.2-CG2 and 9.7.2-CG4 Light Chain [Kappa chain] protein sequence
mdmrvpaqllgllllwlrgarcDIQMTQSPSSLSASVGDRVTITCRASQSISGFLIWY
QQKPGKAPKLLIYATSSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYY
CQQSYSTPFTFGPGTKVDIKRtvaapsvfifppsdeqlksgtasvvcllnnfypreakvqwk
vdnalqsgnsqesvteqdskdstyslsstltlskadyekhkvyacevthqglsspvtksfnrgec

SEQ ID NO: 66
9.7.2-CG2 Heavy Chain [Gamma chain] protein sequence
mefglswvflvaiikgvqcQVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMS
WIRQAPGKGLEWVSYISSSGSTIYYADSVKGRFTISRDNAKNSLYLQMNS
LRAEDTAVYYCAIRIGGMDVWGQGTTVTVSSAstkgpsvfplapcsrstsestaalgc
lvkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssnfgtqtytcnvdhkpsntkvdktv
erkccvecppcpappvagpsvflfppkpkdtlmisrtpevtcvvvdvshedpevqfnwyvdgvevhn
aktkpreeqfnstfrvvsvltvvhqdwlngkeykckvsnkglpapiektisktkgqprepqvytlppsree
mtknqvsltclvkgfypsdiavewesngqpennykttppmldsdgsfflyskltvdksrwqqgnvfscs
vmhealhnhytqkslslspgk

SEQ ID NO: 70
9.7.2-CG4 Heavy Chain [Gamma chain] protein sequence
mefglswvflvaiikgvqcQVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMS
WIRQAPGKGLEWVSYISSSGSTIYYADSVKGRFTISRDNAKNSLYLQMNS
LRAEDTAVYYCARIGGMDVWGQGTTVTVSSAstkgpsvfplapcsrstsestaalgcl
vkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssslgtktytcnvdhkpsntkvdkrve
skygppcpscpapeflggpsvflfppkpkdtlmisrtpevtcvvvdvsqedpevqfnwyvdgvevhn
aktkpreeqfnstyrvvsvltvlhqdwlngkeykckvsnkglpssiektiskakgqprepqvytlppsqe
emtknqvsltclvkgfypsdiavewesngqpennykttppvldsdgsfflysrltvdksrwqegnvfscs
vmhealhnhytqkslslspgk

SEQ ID NO: 86
9.7.2-Ser Heavy Chain [Gamma chain] protein sequence
mefglswvflvaiikgvqcQVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMS
WIRQAPGKGLEWVSYISSSGSTIYYADSVKGRFTISRDNAKNSLYLQMNS
LRAEDTAVYYCARRIGGMDVWGQGTTVTVSSAstkgpsvfplapcsrstsestaalg
clvkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssslgtktytcnvdhkpsntkvdkrv
eskygppcppcpapeflggpsvflfppkpkdtlmisrtpevtcvvvdvsqedpevqfnwyvdgvevh
naktkpreeqfnstyrvvsvltvlhqdwlngkeykckvsnkglpssiektiskakgqprepqvytlppsq
eemtknqvsltclvkgfypsdiavewesngqpennykttppvldsdgsfflysrltvdksrwqegnvfsc
svmhealhnhytqkslslspgk

What is claimed:

1. A method for treating lupus comprising administering to a subject in need thereof a therapeutically effective amount of a human monoclonal antibody or an antigen-binding portion thereof that specifically binds to M-CSF.

2. The method according to claim 1, wherein said antibody or antigen-binding portion possesses at least one of the following properties:

a) binds to human secreted isoforms of M-CSF and membrane bound isoforms of M-CSF;

b) has a selectivity for M-CSF that is at least 100 times greater than its selectivity for GM-CSF or G-CSF;

c) binds to M-CSF with a K_D of 1.0×10^{-7} M or less;

d) has an off rate (k_{off}) for M-CSF of $2.0 \times 10^{-4} s^{-1}$ or smaller; or

e) binds human M-CSF in the presence of human *c-fms*.

3. The method according to claim 2, wherein said antibody or antigen-binding portion blocks binding to *c-fms* and binds M-CSF with a K_D of 1.0×10^{-7} M or less.

4. The method according to claim 1, wherein the antibody or antigen-binding portion has at least one property selected from the group consisting of:

a) cross-competes for binding to M-CSF with an antibody selected from the group consisting of: antibody 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 and 9.14.4G1;

b) competes for binding to M-CSF with an antibody selected from the group consisting of: 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 and 9.14.4G1;

c) binds to the same epitope of M-CSF as an antibody selected from the group consisting of: antibody 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 and 9.14.4G1;

d) binds to M-CSF with substantially the same K_D as an antibody selected from the group consisting of: antibody 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 and 9.14.4G1; and

e) binds to M-CSF with substantially the same off rate as an antibody selected from the group consisting of: antibody 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 and 9.14.4G1.

5. The method according to claim 1, wherein the antibody or antigen-binding portion is selected from the group consisting of:

a) an antibody comprising the heavy chain amino acid sequence set forth in SEQ ID NO: 2 and the light chain amino acid sequence set forth in SEQ ID NO: 4, without the signal sequences;

b) an antibody comprising the heavy chain amino acid sequence set forth in SEQ ID NO: 6 and the light chain amino acid sequence set forth in SEQ ID NO: 8, without the signal sequences;

c) an antibody comprising the heavy chain amino acid sequence set forth in SEQ ID NO: 10 and the light chain amino acid sequence set forth in SEQ ID NO: 12, without the signal sequences;

d) an antibody comprising the heavy chain amino acid sequence set forth in SEQ ID NO: 14 and the light chain amino acid sequence set forth in SEQ ID NO: 16, without the signal sequences;

e) an antibody comprising the heavy chain amino acid sequence set forth in SEQ ID NO: 18 and the light chain amino acid sequence set forth in SEQ ID NO: 20, without the signal sequences;

f) an antibody comprising the heavy chain amino acid sequence set forth in SEQ ID NO: 22 and the light chain amino acid sequence set forth in SEQ ID NO: 24, without the signal sequences;

g) an antibody comprising the heavy chain amino acid sequence set forth in SEQ ID NO: 26 and the light chain amino acid sequence set forth in SEQ ID NO: 28, without the signal sequences;

h) an antibody comprising the heavy chain amino acid sequence set forth in SEQ ID NO: 38and the light chain amino acid sequence set forth in SEQ ID NO: 28, without the signal sequences;

i) an antibody comprising the heavy chain amino acid sequence set forth in SEQ ID NO: 54 and the light chain amino acid sequence set forth in SEQ ID NO: 56, without the signal sequences;

j) an antibody comprising the heavy chain amino acid sequence set forth in SEQ ID NO: 74 and the light chain amino acid sequence set forth in SEQ ID NO: 56, without the signal sequences;

k) an antibody comprising the heavy chain amino acid sequence set forth in SEQ ID NO: 78 and the light chain amino acid sequence set forth in SEQ ID NO: 56, without the signal sequences;

l) an antibody comprising the heavy chain amino acid sequence set forth in SEQ ID NO: 82 and the light chain amino acid sequence set forth in SEQ ID NO: 28, without the signal sequences;

m) an antibody comprising the heavy chain amino acid sequence set forth in SEQ ID NO: 102 and the light chain amino acid sequence set forth in SEQ ID NO: 28, without the signal sequences;

n) an antibody comprising the heavy chain amino acid sequence set forth in SEQ ID NO: 30 and the light chain amino acid sequence set forth in SEQ ID NO: 32, without the signal sequences;

o) an antibody comprising the heavy chain amino acid sequence set forth in SEQ ID NO: 30 and the light chain amino acid sequence set forth in SEQ ID NO: 44, without the signal sequences;

p) an antibody comprising the heavy chain amino acid sequence set forth in SEQ ID NO: 58 and the light chain amino acid sequence set forth in SEQ ID NO: 60, without the signal sequences;

q) an antibody comprising the heavy chain amino acid sequence set forth in SEQ ID NO: 62 and the light chain amino acid sequence set forth in SEQ ID NO: 60, without the signal sequences;

r) an antibody comprising the heavy chain amino acid sequence set forth in SEQ ID NO: 90 and the light chain amino acid sequence set forth in SEQ ID NO: 44, without the signal sequences;

s) an antibody comprising the heavy chain amino acid sequence set forth in SEQ ID NO: 94 and the light chain amino acid sequence set forth in SEQ ID NO: 60, without the signal sequences;

t) an antibody comprising the heavy chain amino acid sequence set forth in SEQ ID NO: 98 and the light chain amino acid sequence set forth in SEQ ID NO: 32, without the signal sequences;

u) an antibody comprising the heavy chain amino acid sequence set forth in SEQ ID NO: 34 and the light chain amino acid sequence set forth in SEQ ID NO: 36, without the signal sequences;

v) an antibody comprising the heavy chain amino acid sequence set forth in SEQ ID NO: 46 and the light chain amino acid sequence set forth in SEQ ID NO: 48, without the signal sequences;

w) an antibody comprising the heavy chain amino acid sequence set forth in SEQ ID NO: 50 and the light chain amino acid sequence set forth in SEQ ID NO: 52,without the signal sequences;

x) an antibody comprising the heavy chain amino acid sequence set forth in SEQ ID NO: 66 and the light chain amino acid sequence set forth in SEQ ID NO: 52, without the signal sequences;

y) an antibody comprising the heavy chain amino acid sequence set forth in SEQ ID NO: 70 and the light chain amino acid sequence set forth in SEQ ID NO: 52, without the signal sequences; and

z) an antibody comprising the heavy chain amino acid sequence set forth in SEQ ID NO: 86 and the light chain amino acid sequence set forth in SEQ ID NO: 48, without the signal sequences.

6. The method according to claim 1, wherein said antibody or antigen-binding portion comprises:

a) a heavy chain CDR1, CDR2 and CDR3 independently selected from the heavy chain of an antibody selected from the group consisting of: monoclonal antibody 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 and 9.14.4G1; or

b) a light chain CDR1, CDR2 and CDR3 independently selected from the light chain of an antibody selected from the group consisting of: monoclonal antibody 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 and 9.14.4G1.

7. The method according to claim 1, wherein:

a) the antibody or antigen binding portion comprises the heavy chain CDR1, CDR2 and CDR3 of an antibody selected from the group consisting of: antibody 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 and 9.14.4G1;

b) the antibody or antigen binding portion comprises the heavy chain CDR1, CDR2 and CDR3 of an antibody selected from the group consisting of: antibody 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 and 9.14.4G1;

c) the antibody comprises a heavy chain of (a) and a light chain of (b); or

d) the antibody or antigen binding portion comprises a heavy chain of (a) and a light chain of (b), wherein the heavy chain and light chain CDR amino acid sequences are selected from the same antibody.

8. The method according to claim 1, wherein the antibody or antigen-binding portion comprises:

a) a heavy chain comprising the amino acid sequence from the beginning of the CDR1 through the end of the CDR3 of the heavy chain of an antibody selected from the group consisting of: antibody 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 and 9.14.4G1;

b) a light chain comprising the amino acid sequence from the beginning of the CDR1through the end of the CDR3 of an antibody selected from the group consisting of: antibody 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2,

9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 and 9.14.4G1;

c) the heavy chain of (a) and the light chain of (b); or

d) the heavy chain of (a) and the light chain of (b)

wherein the heavy chain and light chain sequences are selected from the same antibody.

9. The method according to claim 1, wherein said monoclonal antibody or antigen-binding portion comprises:

a) the heavy chain variable domain (VH) amino acid sequence, without a signal sequence, of an antibody selected from the group consisting of: antibody 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 and 9.14.4G1;

b) the light chain variable domain (VL) amino acid sequence, without a signal sequence, of an antibody selected from the group consisting of: antibody 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 and 9.14.4G1;

c) the VH amino acid sequence of (a) and the VL amino acid sequence of (b); or

d) the VH amino acid sequence of (a) and the VL amino acid sequence of (b), wherein the VH and VL are from the same antibody.

10. The method according to claim 1, wherein said antibody or antigen-binding portion comprises one or more of an FR1, FR2, FR3 or FR4 amino acid sequence of an antibody selected from the group consisting of: antibody 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser,

8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 and 9.14.4G1.

11. The method according to any one of claims 1-10, wherein the C-terminal lysine of the heavy chain of said antibody or antigen-binding portion is not present

12. The method according to claim 1, wherein the antibody comprises:

a) a heavy chain amino acid sequence that is at least 90% identical to the heavy chain amino acid sequence of monoclonal antibody 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 or 9.14.4G1, without the signal sequence;

b) a light chain amino acid sequence that is at least 90% identical to the light chain amino acid sequence of monoclonal antibody 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 or 9.14.4G1, without the signal sequence; or

c) the heavy chain amino acid sequence of (a) and the light chain amino acid sequence of (b).

13. The method according to claim 1, wherein the antibody or antigen binding portion comprises:

a) a heavy chain amino acid sequence that is at least 90% identical to the heavy chain amino acid sequence of monoclonal antibody 8.10.3F without the signal sequence;

b) a light chain amino acid sequence that is at least 90% identical to the light chain amino acid sequence of monoclonal antibody 8.10.3F;

c) the heavy chain amino acid sequence of (a) and the light chain amino acid sequence of (b); or

d) a heavy chain amino acid sequence and a light chain amino acid sequence that together are at least 90% identical to the heavy chain amino acid sequence and the light chain amino acid sequence of antibody 8.10.3F.

14. The method according to claim 13, wherein the heavy chain amino acid sequence of the antibody or antigen-binding portion is at least 95% identical to the heavy chain amino acid sequence of monoclonal antibody 8.10.3F without the signal sequence.

15. The method according to claim 13, wherein the light chain amino acid sequence of the antibody or antigen-binding portion is at least 95% identical to the light chain amino acid sequence of monoclonal antibody 8.10.3F without the signal sequence.

16. The method according to claim 13, wherein both the heavy chain amino acid sequence and light chain amino acid sequence of the antibody or antigen-binding portion together are at least 95% identical to the heavy chain amino acid sequence and light chain amino acid sequence of monoclonal antibody 8.10.3F without the signal sequence.

17. The method according to claim 13, wherein the heavy chain amino acid sequence of the antibody or antigen-binding portion is at least 97% identical to the heavy chain amino acid sequence of monoclonal antibody 8.10.3F without the signal sequence.

18. The method according to claim 13, wherein the light chain amino acid sequence of the antibody or antigen-binding portion is at least 97% identical to the light chain amino acid sequence of monoclonal antibody 8.10.3F without the signal sequence.

19. The method according to claim 13, wherein both the heavy chain amino acid sequence and light chain amino acid sequence of the antibody or antigen-binding portion together are at least 97% identical to the heavy chain amino acid sequence and light chain amino acid sequence of monoclonal antibody 8.10.3F without the signal sequence.

20. The method according to claim 1 wherein the antibody is monoclonal anti-M-CSF antibody 8.10.3F.

21. The method according to claim 1, wherein the antibody or antigen-binding portion comprises the heavy chain CDR1, CDR2, CDR3 and the light chain CDR1, CDR2, CDR3 of antibody 8.10.3F.

22. The method according to claim 1, wherein the antibody or antigen-binding portion comprises the variable heavy chain and variable light chain portion of antibody 8.10.3F..

23. The method according to claim 1, wherein the lupus is systemic lupus erythematosus (SLE).

24. The method according to claim 1, wherein the lupus is lupus nephritis.

25. The method according to claim 1, wherein the lupus is cutaneous lupus

26. The method according to claim 1, wherein efficacy of the anti-M-CSF antibody in treating lupus is determined by examining patients for changes in conditions selected from symptoms, biomarkers, histological samples, and physiological conditions.

27. The method according to claim 26, wherein the patients are examined for changes in conditions selected from skin lesions, proteinuria, lymphadenopathy, serum M-CSF levels, anti-dsDNA antibody levels, CD14+CD16+ monocyte population, osteocyte markers, and kidney pathology.

28. The method according to claim 27, wherein kidney pathology is determined by examining conditions selected from macrophage infiltration, inflammatory infiltrates, proteinaceous casts, size of glomerular tufts, glomerular IgG deposits, and C3 deposits.

29. The method according to claim 26, wherein patients may be examined for changes in one or more biomarkers selected from the genes in Table 1C, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), complement (C3/C4), Ig levels (IgA, IgM, IgG), antinuclear antibodies (ANA), extractable nuclear antigen (ENA), and anti-dsDNA antibodies.

30. Use of an anti-M-CSF antibody according to any one of claims 1-23 for the treatment of lupus in a patient in need thereof.

31. The use according to claim 30, wherein the lupus is SLE.

32. The use according to claim 30, wherein the lupus is lupus nephritis.

33. The use according to claim 30, wherein the lupus is cutaneous lupus.

34. Use of anti-M-CSF antibody according to any one of claims 1-23 in the manufacture of a medicament for the treatment of lupus.

35. The use according to claim 34, wherein the lupus is SLE.

36. The use according to claim 34, wherein the lupus is lupus nephritis.

37. The use according to claim 34, wherein the lupus is cutaneous lupus.

FIGURE 1A

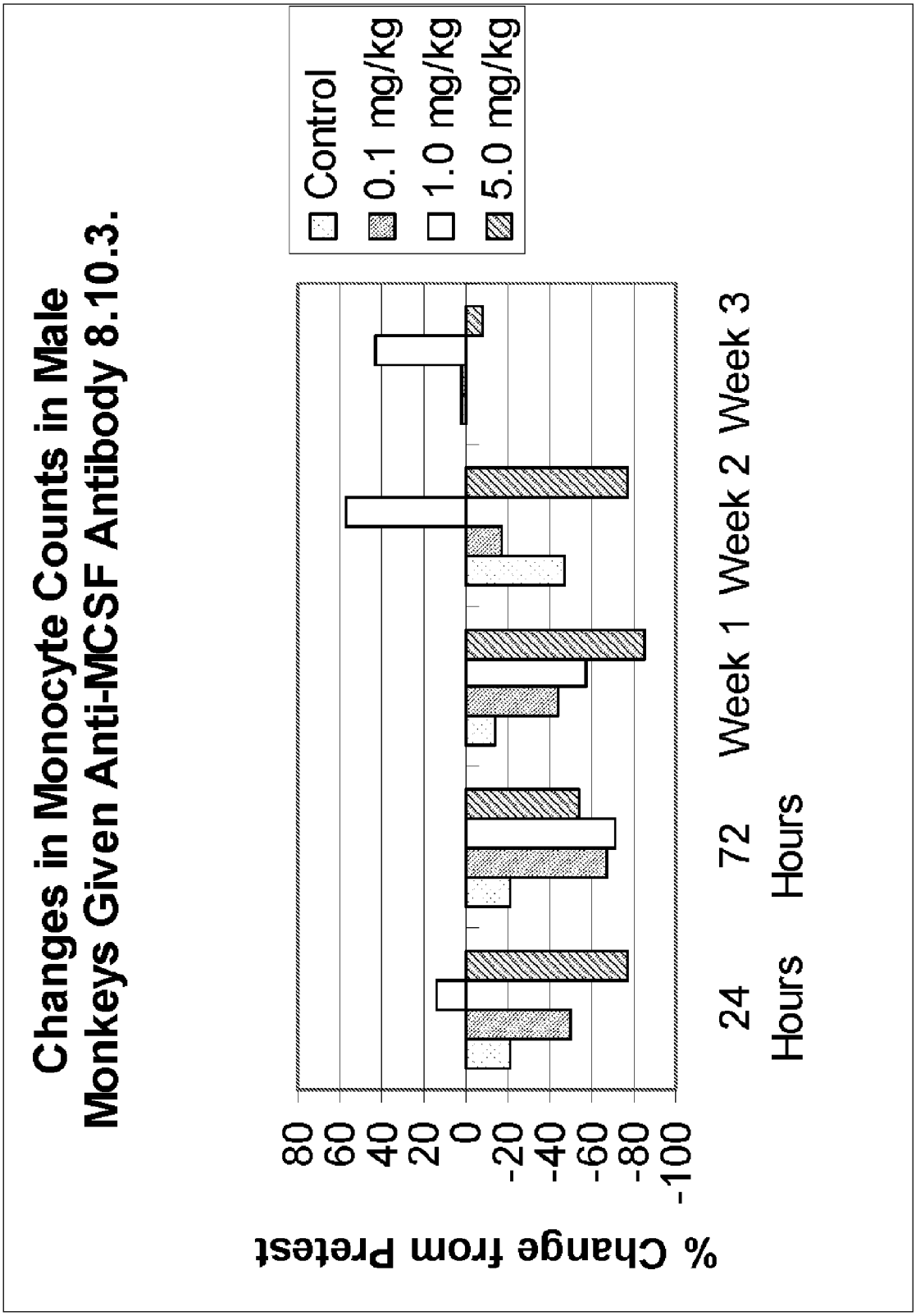

Changes in Monocyte Counts in Male Monkeys Given Anti-MCSF Antibody 8.10.3.
Control
0.1 mg/kg
1.0 mg/kg
5.0 mg/kg
% Change from Pretest
80
60
40
20
0
-20
-40
-60
-80
-100
24 Hours
72 Hours
Week 1
Week 2
Week 3

FIGURE 1B

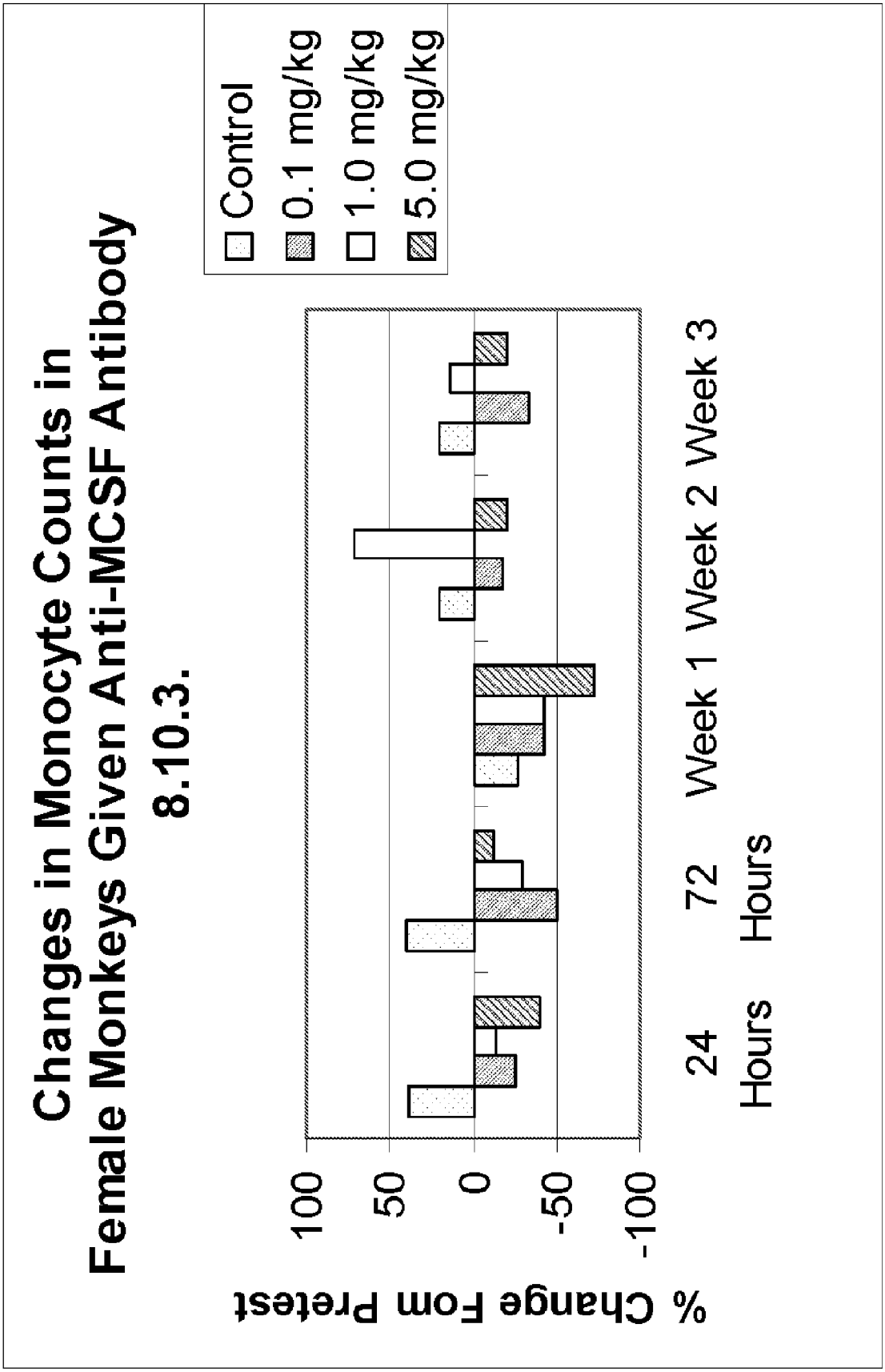

Changes in Monocyte Counts in Female Monkeys Given Anti-MCSF Antibody 8.10.3.
Control
0.1 mg/kg
1.0 mg/kg
5.0 mg/kg
% Change Fom Pretest
100
50
0
-50
-100
24 Hours
72 Hours
Week 1
Week 2
Week 3

FIGURE 2A

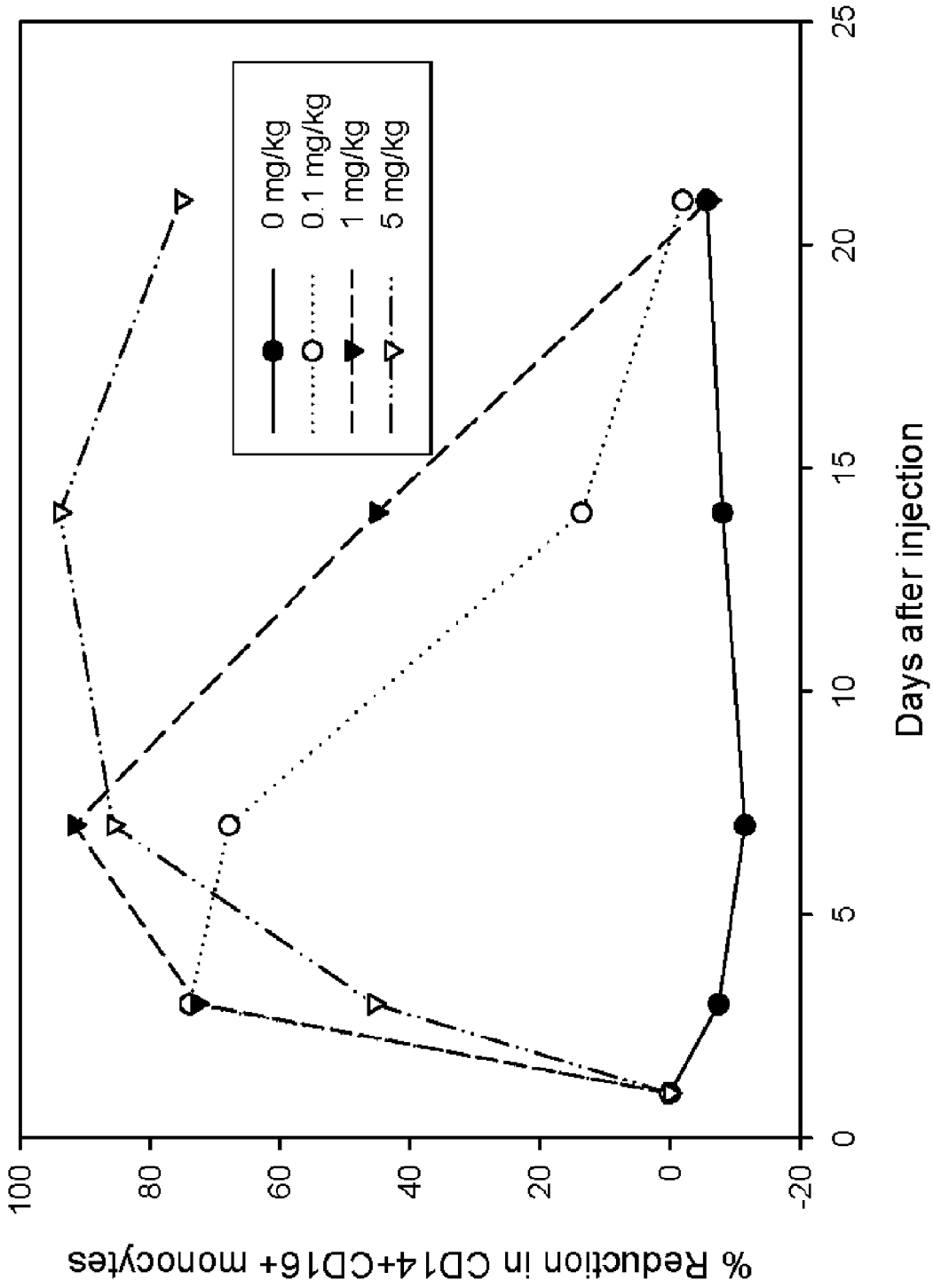

% Reduction in CD14+CD16+ monocytes
100
80
60
40
20
0
-20
0
5
10
15
20
25
Days after injection
0 mg/kg
0.1 mg/kg
1 mg/kg
5 mg/kg

FIGURE 2B

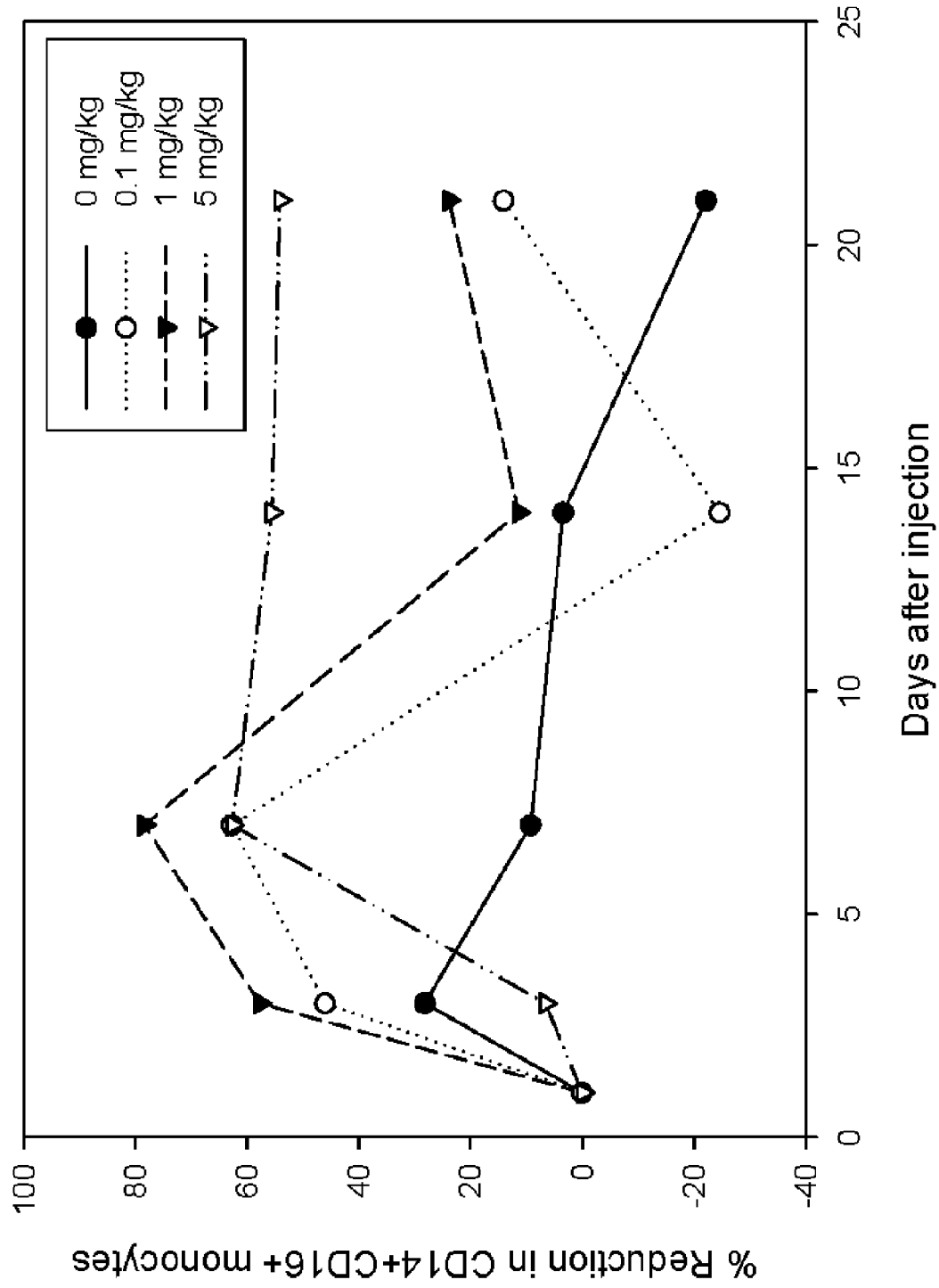

0 mg/kg
0.1 mg/kg
1 mg/kg
5 mg/kg
% Reduction in CD14+CD16+ monocytes
100
80
60
40
20
0
-20
-40
0
5
10
15
20
25
Days after injection

FIGURE 3A

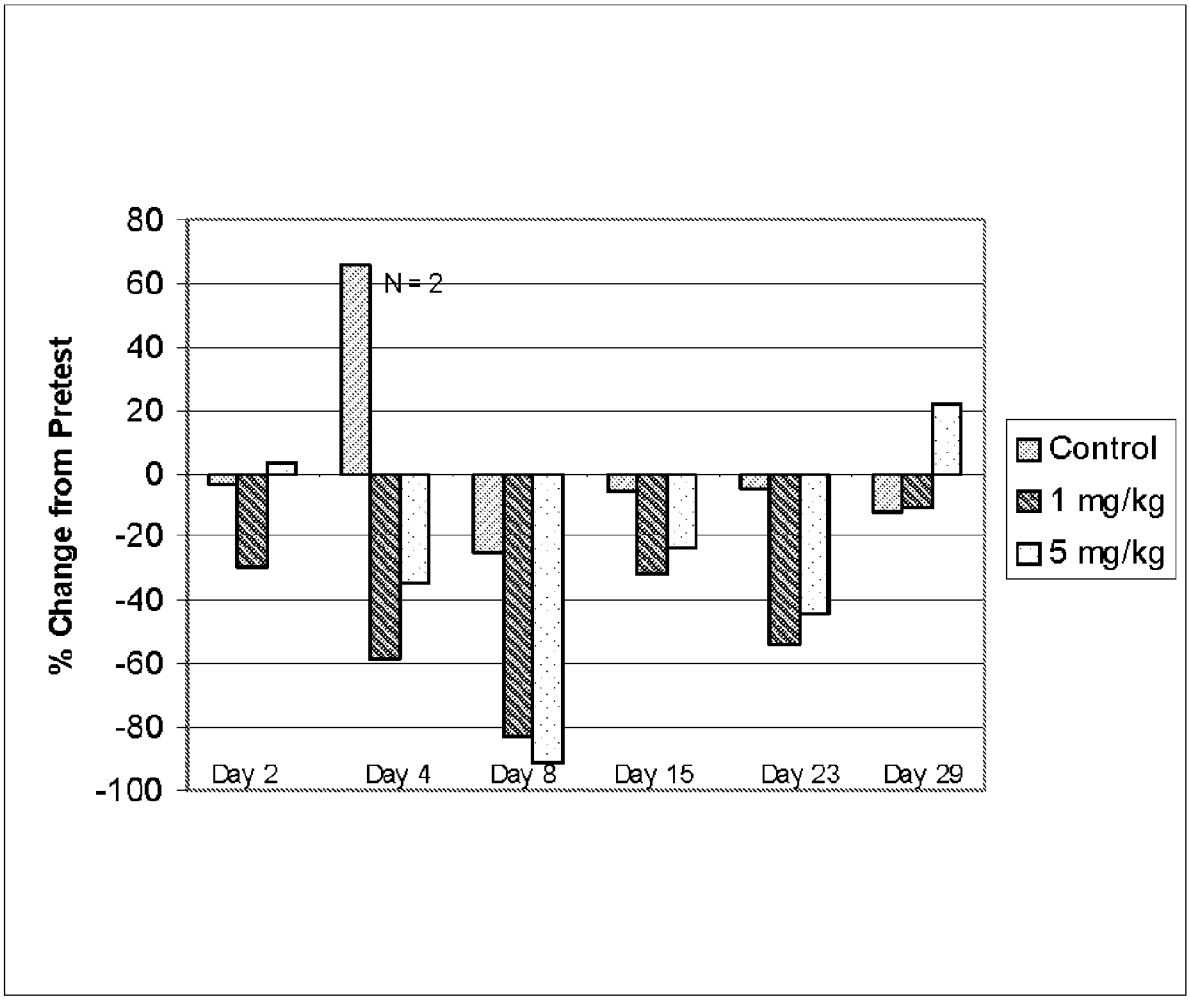

% Change from Pretest
80
60
40
20
0
-20
-40
-60
-80
-100
N = 2
Day 2
Day 4
Day 8
Day 15
Day 23
Day 29
Control
1 mg/kg
5 mg/kg

FIGURE 3B

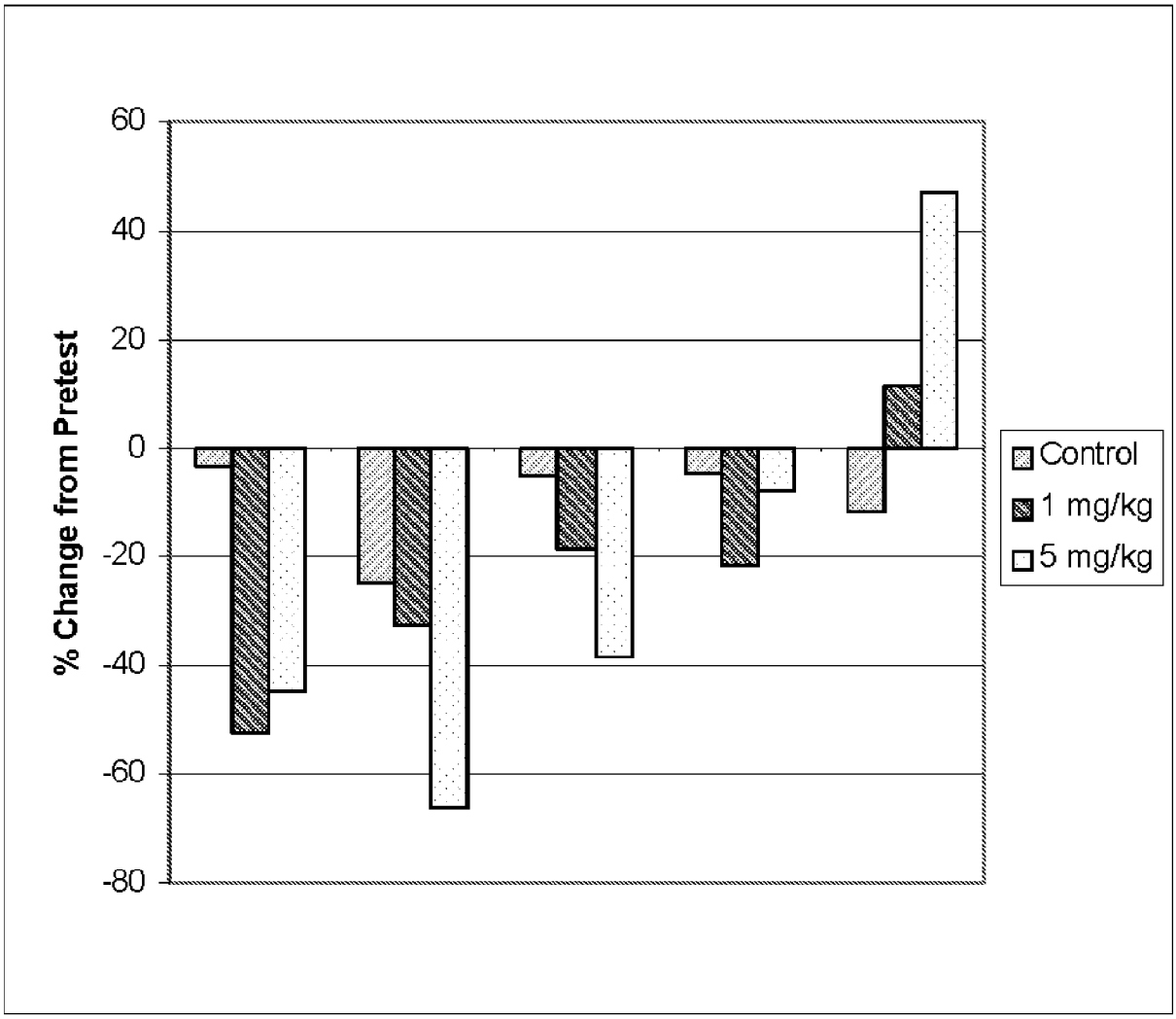


FIGURE 4A

```
Germline V=O12, J=JK3
252          ---------------------- -------GF-- --------------- -T----- ---F---------------------------- -----V---

Germ         DIQMTQSPSSLSASVGDRVTITC RASQSISSYLN WYQQKPGKAPKLLIY AASSLQS GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC QQSYSTPFT
                       FR1               CDR1         FR2          CDR2              FR3                  CDR3

252          ---------- (residues 21-127 of SEQ ID NO: 4)

Germ         FGPGTKVDIK (SEQ ID NO: 103)
                  J
```

FIGURE 4B

```
Germline V=O12, J=JK3
88           ---------------------- -P--D------ --------------- ------- ---L---------------------------- ---------

Germ         DIQMTQSPSSLSASVGDRVTITC RASQSISSYLN WYQQKPGKAPKLLIY AASSLQS GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC QQSYSTPFT
                       FR1               CDR1         FR2          CDR2              FR3                  CDR3

88           ---------- (residues 21-127 of SEQ ID NO: 8)

Germ         FGPGTKVDIK (SEQ ID NO: 103)
                  J
```

FIGURE 4C

```
Germline V=L2, J=JK3
100          ---------------------- ---------- --------------- ------S ---D-I--------------------------- ---------
Germ         EIVMTQSPATLSVSPGERATLSC RASQSVSSNLA WYQQKPGQAPRLLIY GASTRAT GIPARFSGSGSGTEFTLTISSLQSEDFAVYYC QQYNNWPFT
                       FR1               CDR1         FR2          CDR2              FR3                  CDR3

100          ---------- (residues 21-127 of SEQ ID NO: 12)

Germ         FGPGTKVDIK (SEQ ID NO: 107)
                  J
```

FIGURE 4D

Germline V=L5, J=JK3

3.8.3 ----------S- ----D--G--- ----------S -T---H- ---------- --T------ ----------

(residues 23-130 of SEQ ID NO: 16)

Germ	DIQMTQSPSSVSASVGDRVTITC	RASQGISSWLA	WYQQKPGKAPKLLIY	AASSLQS	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQANSFPFT	FGPGTKVDIKR
(SEQ ID NO: 109)	FR1	CDR1	FR2	CDR2	FR3	CDR3	J

FIGURE 4E

Germline V=L5, J=JK4

2.7.3 ---------- ----D------ ---R--------Q-- -----E- ------N---------S---------- --T------ ----------

(residues 23-130 of SEQ ID NO: 20)

Germ	DIQMTQSPSSVSASVGDRVTITC	RASQGISSWLA	WYQQKPGKAPKLLIY	AASSLQS	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQANSFPLT	FGGGTKVEIKR
(SEQ ID NO: 117)	FR1	CDR1	FR2	CDR2	FR3	CDR3	J

FIGURE 4F

Germline V=B3, J=JK1

1.120.1 ---------- -----I-FF-------- --R-------N---- ------- ---------- -----S---

Germ	DIVMTQSPDSLAVSLGERATINC	KSSQSVLYSSNNKNYLA	WYQQKPGQPPKLLIY	WASTRES	GVPDRFSGSGSGTDFTLTISSLQAEDVAVYYC	QQYYSTPWT	
	FR1	CDR1	FR2	CDR2	FR3	CDR3	

1.120.1 ---------- (residues 21-134 of SEQ ID NO: 24)

Germ	FGQGTKVEIKR	(SEQ ID NO: 112)
	J	

FIGURE 4G

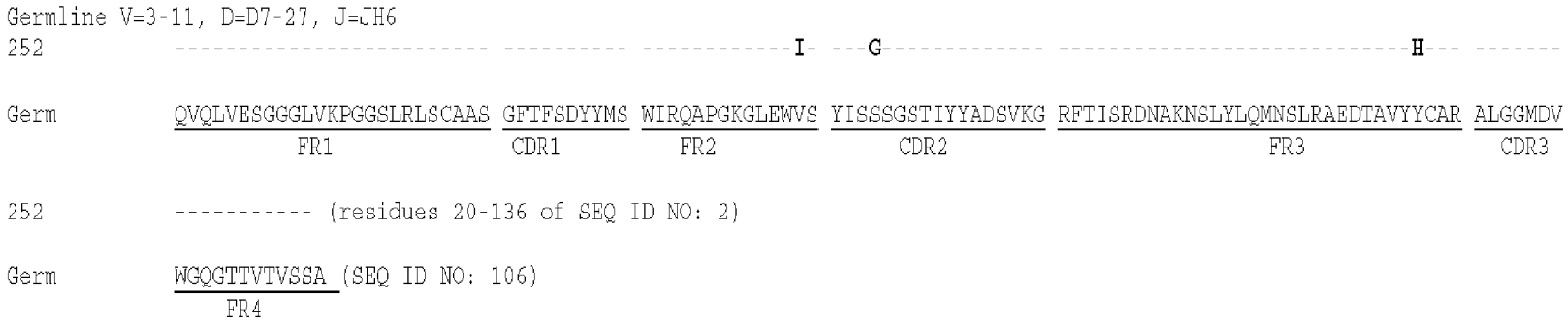


FIGURE 4H

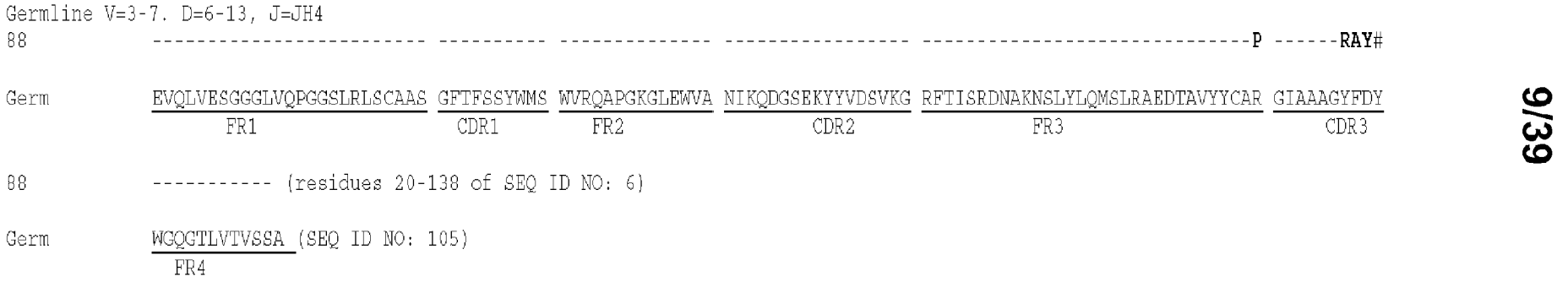


FIGURE 4I

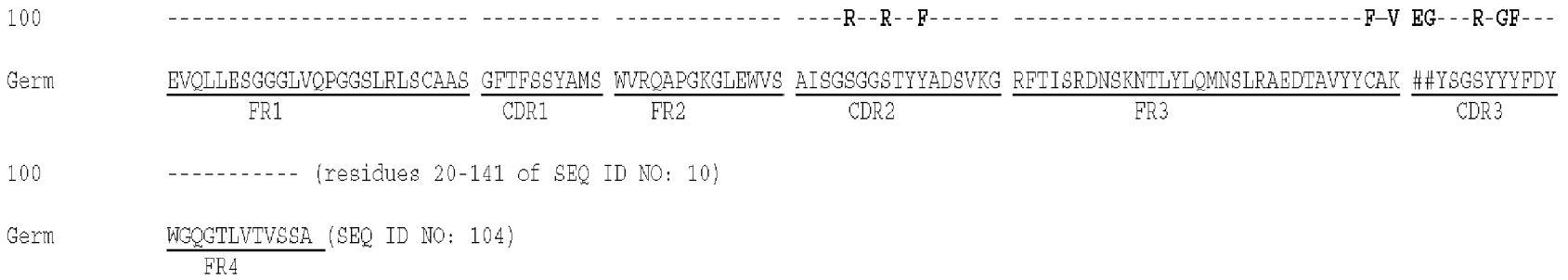


FIGURE 4J

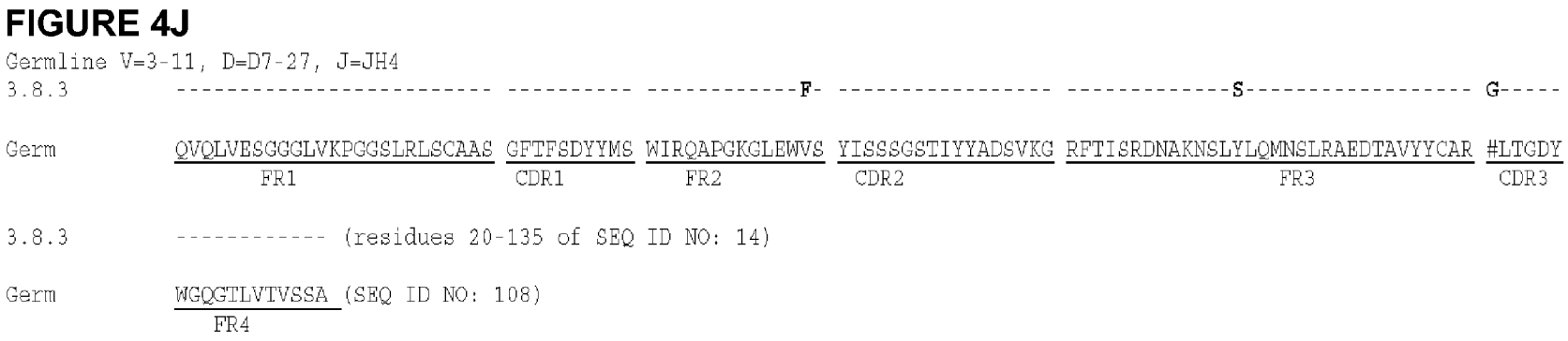


FIGURE 4K

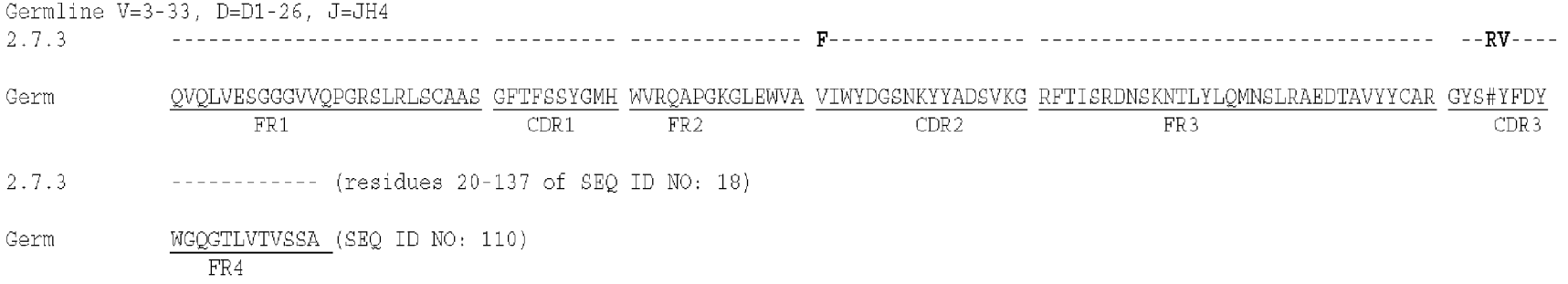


FIGURE 4L

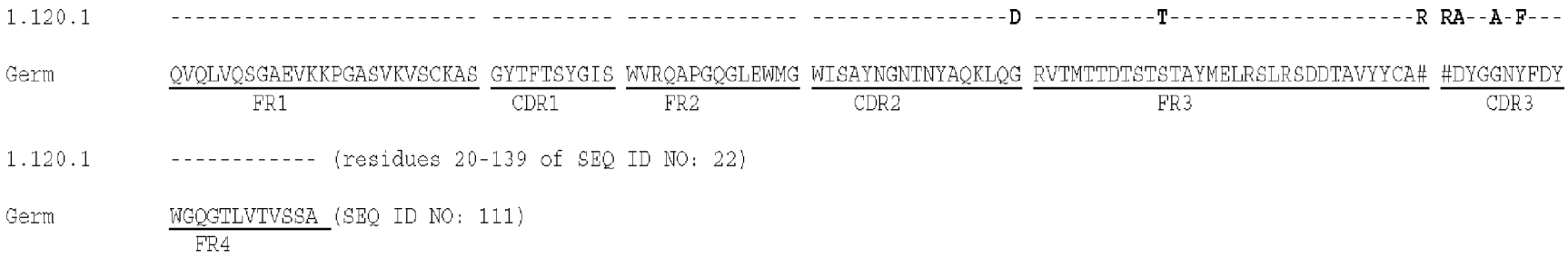


FIGURE 4M

Germline V=A27, J=JK4

8.10.3 -**F**---------- ---------- ---------- ------- ----------**V**----

Germ EIVLTQSPGTLSLSPGERATLSC RASQSVSSSYLA WYQQKPGQAPRLLIY GASSRAT GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC
FR1 CDR1 FR2 CDR2 FR3

8.10.3 --------- ---------- (residues 21-129 of SEQ ID NO: 44)

Germ QQYGSSPLT FGGGTKVEIKR (SEQ ID NO: 114)
CDR3 J

FIGURE 4N

Germline V=VH3-48, D=D1-26, J=JH4b

8.10.3 ---------- ------**F**--**T** ---------- ----**R**----**S**------- ---------- **DPLLA-ATF**---

Germ EVQLVESGGGLVQPGGSLRLSCAAS GFTFSSYSMN WVRQAPGKGLEWVS YISSSSSTIYYADSVKG RFTISRDNAKNSLYLQMNSLRDEDTAVYYCAR ###IVG###FDY
FR1 CDR1 FR2 CDR2 FR3 CDR3

8.10.3 ---------- (residues 20-141 of SEQ ID NO: 30)

Germ WGQGTLVTVSSA (SEQ ID NO: 113)
J

FIGURE 4O

Germline V=012, J=JK3

9.14.4 ---------- -**P**--**I**---**L**-- ----------**H** ------- ---------- --------- ----------
(residues 23-130 of SEQ ID NO: 28)

Germ DIQMTQSPSSLSASVGDRVTITC RASQSISSYLN WYQQKPGKAPKLLIY AASSLQS GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC QQSYSTPFT FGPGTKVDIKR
(SEQ ID NO: 103) FR1 CDR1 FR2 CDR2 FR3 CDR3 J

FIGURE 4P

```
Germline V=VH3-11, D=D7-27, J=JH4b
9.14.4      ------------------------ ---------- -------------- ---------------- ------------------------------- G-----

Germ        QVQLVESGGGLVKPGGSLRLSCAAS GFTFSDYYMS WIRQAPGKGLEWVS YISSSGSTIYYADSVKG RFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR #LTGDY
                      FR1               CDR1        FR2              CDR2                    FR3                  CDR3

9.14.4      ------------ (residues 20-135 of SEQ ID NO: 38)

Germ        WGQGTLVTVSSA (SEQ ID NO: 116)
                 J
```

FIGURE 4Q

```
Germline V=012, J=JK3
9.7.2       ---------------------- -------GF-I ----R---------- -T----- ---L-----E---------------------- --------- -----------FR1
                    CDR1          FR2        CDR2                  FR3                  CDR3                  J
(residues 23-130 of SEQ ID NO: 48)
Germ        DIQMTQSPSSLSASVGDRVTITC RASQSISSYLN WYQQKPGKAPKLLIY AASSLQS GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC QQSYSTPFT FGPGTKVDIKR
(SEQ ID NO: 103)   FR1                   CDR1          FR2        CDR2                  FR3                  CDR3       J
```

FIGURE 4R

```
Germline V=VH3-11, D=D6-13, J=JH6b
9.7.2       ------------------------ ---------- -------------- ---------------- -------------------------------R R-G----

Germ        QVQLVESGGGLVKPGGSLRLSCAAS GFTFSDYYMS WIRQAPGKGLEWVS YISSSGSTIYYADSVKG RFTISRDNAKNSLYLQMNSLRAEDTAVYYCA# #I#GMDV
                      FR1               CDR1        FR2              CDR2                    FR3                  CDR3


9.7.2       ------------ (residues 20-136 of SEQ ID NO: 46)

Germ        WGQGTTVTVSSA (SEQ ID NO: 115)
                 J
```

FIGURE 4S

```
Germline V=012, J=JK3
9.14.4I      ---------------------- -P--I---L-- --------------H ------- ------------------------------ --------- -----------
(residues 23-130 of SEQ ID NO: 28)

Germ         DIQMTQSPSSLSASVGDRVTITC RASQSISSYLN WYQQKPGKAPKLLIY AASSLQS GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC QQSYSTPFT FGPGTKVDIKR
(SEQ ID NO: 103)  FR1                  CDR1         FR2         CDR2      FR3                  CDR3       J
```

FIGURE 4T

```
Germline V=VH3-11, D=D7-27, J=JH4b
9.14.4I      ------------------------ ---------- ------------- ----------------- ------------------------------- G-----

Germ         QVQLVESGGGLVKPGGSLRLSCAAS GFTFSDYYMS WIRQAPGKGLEWVS YISSSGSTIYYADSVKG RFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR #LTGDY
                       FR1               CDR1       FR2          CDR2                FR3                  CDR3

9.14.4I      ------------ (residues 20-135 of SEQ ID NO: 26)

Germ         WGQGTLVTVSSA (SEQ ID NO: 116)
                  J
```

FIGURE 4U

```
Germline V=A27, J=JK4
8.10.3F      -F--------------------- ------------ --------------- ------- -------------------------------

Germ         EIVLTQSPGTLSLSPGERATLSC RASQSVSSSYLA WYQQKPGQAPRLLIY GASSRAT GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC
                      FR1               CDR1         FR2          CDR2      FR3

8.10.3F      --------- ----------- (residues 21-129 of SEQ ID NO: 32)

Germ         QQYGSSPLT FGGGTKVEIKR (SEQ ID NO: 114)
               CDR3         J
```

FIGURE 4V

Germline V=VH3-48, D=D1-26, J=JH4b

8.10.3F ------------------------- ------F--T ------------- ----R----S------- ------------------------------ DPLLA-ATF---

Germ EVQLVESGGGLVQPGGSLRLSCAAS GFTFSSYSMN WVRQAPGKGLEWVS YISSSSSTIYYADSVKG RFTISRDNAKNSLYLQMNSLRDEDTAVYYCAR ###IVG###FDY
FR1 CDR1 FR2 CDR2 FR3 CDR3

8.10.3F ------------ (residues 20-141 of SEQ ID NO: 30)

Germ WGQGTLVTVSSA (SEQ ID NO: 113)
J

FIGURE 4W

Germline V=012, J=JK3

9.7.2IF ---------------------- -------GF-I ----R---------- -T----- ------------------------------ --------- -----------
FR1 CDR1 FR2 CDR2 FR3 CDR3 J
(residues 23-130 of SEQ ID NO: 36)

Germ DIQMTQSPSSLSASVGDRVTITC RASQSISSYLN WYQQKPGKAPKLLIY AASSLQS GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC QQSYSTPFT FGPGTKVDIKR
(SEQ ID NO: 103) FR1 CDR1 FR2 CDR2 FR3 CDR3 J

FIGURE 4X

Germline V=VH3-11, D=D6-13, J=JH6b

9.7.2IF ------------------------- ---------- ------------- ----------------- ------------------------------R R-G----

Germ QVQLVESGGGLVKPGGSLRLSCAAS GFTFSDYYMS WIRQAPGKGLEWVS YISSSGSTIYYADSVKG RFTISRDNAKNSLYLQMNSLRAEDTAVYYCA# #I#GMDV
FR1 CDR1 FR2 CDR2 FR3 CDR3

9.7.2IF ------------ (residues 20-136 of SEQ ID NO: 34)

Germ WGQGTIVTVSSA (SEQ ID NO: 115)
J

FIGURE 4Y

Germline V=012, J=JK3

```
9.7.2C-Ser   ---------------------- -------GF-I -------------- -T----- ------------------------------- --------- -----------
                       FR1             CDR1          FR2        CDR2                FR3                CDR3         J
(residues 23-130 of SEQ ID NO: 52)

Germ         DIQMTQSPSSLSASVGDRVTITC RASQSISSYLN WYQQKPGKAPKLLIY AASSLQS GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC QQSYSTPFT FGPGTKVDIKR
(SEQ ID NO: 103)      FR1               CDR1          FR2        CDR2                FR3                CDR3         J
```

FIGURE 4Z

Germline V=VH3-11, D=D6-13, J=JH6b

```
9.7.2C-Ser   ------------------------ ---------- ------------- ----------------- ------------------------------ R-G----

Germ         QVQLVESGGGLVKPGGSLRLSCAAS GFTFSDYYMS WIRQAPGKGLEWVS YISSSGSTIYYADSVKG RFTISRDNAKNSLYLQMNSLRAEDTAVYYCA #I#GMDV
                       FR1               CDR1          FR2            CDR2                  FR3                 CDR3

9.7.2C-Ser   ------------ (residues 20-136 of SEQ ID NO: 50)

Germ         WGQGTTVTVSSA (SEQ ID NO: 115)
                    J
```

FIGURE 4AA

Germline V=012, J=JK3

```
9.14.4C-Ser  ---------------------- -P--I---L-- -------------- ------- ------------------------------- --------- -----------
(residues 23-130 of SEQ ID NO: 56)

Germ         DIQMTQSPSSLSASVGDRVTITC RASQSISSYLN WYQQKPGKAPKLLIY AASSLQS GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC QQSYSTPFT FGPGTKVDIKR
(SEQ ID NO: 103)               FR1         CDR1          FR2        CDR2                FR3                CDR3         J
```

FIGURE 4BB

Germline V=VH3-11, D=D7-27, J=JH4b

9.14.4C-Ser ---------- ---------- ---------- ---------- ---------- G-----

Germ QVQLVESGGGLVKPGGSLRLSCAAS GFTFSDYYMS WIRQAPGKGLEWVS YISSSGSTIYYADSVKG RFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR #LTGDY
FR1 CDR1 FR2 CDR2 FR3 CDR3

9.14.4C-Ser ---------- (residues 20-135 of SEQ ID NO: 54)

Germ WGQGTLVTVSSA (SEQ ID NO: 116)
J

FIGURE 4CC

Germline V=A27, J=JK4

8.10.3C-Ser ---------- ---------- ---------- ------- ----------

Germ EIVLTQSPGTLSLSPGERATLSC RASQSVSSSYLA WYQQKPGQAPRLLIY GASSRAT GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC
FR1 CDR1 FR2 CDR2 FR3

Germ QQYGSSPLT FGGGTKVEIKR (residues 21-129 of SEQ ID NO: 60)
CDR3 J

8.10.3 --------- ---------- (SEQ ID NO: 114)

FIGURE 4DD

Germline V=VH3-48, D=D1-26, J=JH4b

8.10.3C-Ser ---------- ------**F--T** ---------- ----**R----S**------- ---------- **DPLLA-ATF**---

Germ EVQLVESGGGLVQPGGSLRLSCAAS GFTFSSYSMN WVRQAPGKGLEWVS YISSSSSTIYYADSVKG RFTISRDNAKNSLYLQMNSLRDEDTAVYYCAR ###IVG###FDY
FR1 CDR1 FR2 CDR2 FR3 CDR3

8.10.3C-Ser ---------- (residues 20-141 of SEQ ID NO: 58)

Germ WGQGTLVTVSSA (SEQ ID NO: 113)
J

FIGURE 4EE

Germline V=A27, J=JK4

8.10.3-CG2 ---------- ---------- ---------- ------- ----------

Germ EIVLTQSPGTLSLSPGERATLSC RASQSVSSSYLA WYQQKPGQAPRLLIY GASSRAT GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC
FR1 CDR1 FR2 CDR2 FR3

8.10.3-CG2 --------- ---------- (residues 21-129 of SEQ ID NO: 60)

Germ QQYGSSPLT FGGGTKVEIKR (SEQ ID NO: 114)
CDR3 J

FIGURE 4FF

Germline V=VH3-48, D=D1-26, J=JH4b

8.10.3-CG2 ---------- ------**F**--**T** ---------- ----**R**----**S**------- ---------- **DPLLA**-**ATF**---

Germ EVQLVESGGGLVQPGGSLRLSCAAS GFTFSSYSMN WVRQAPGKGLEWVS YISSSSSTIYYADSVKG RFTISRDNAKNSLYLQMNSLRDEDTAVYYCAR ###IVG###FDY
FR1 CDR1 FR2 CDR2 FR3 CDR3

8.10.3-CG2 ---------- (residues 20-141 of SEQ ID NO: 62)

Germ WGQGTLVTVSSA (SEQ ID NO: 113)
J

FIGURE 4GG

Germline V=012, J=JK3

9.7.2-CG2 ---------- -------**GF**-**I** ---------- -**T**----- ---------- --------- ----------
FR1 CDR1 FR2 CDR2 FR3 CDR3 J
(residues 23-130 of SEQ ID NO: 52)

Germ DIQMTQSPSSLSASVGDRVTITC RASQSISSYLN WYQQKPGKAPKLLIY AASSLQS GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC QQSYSTPFT FGPGTKVDIKR
(SEQ ID NO: 103) FR1 CDR1 FR2 CDR2 FR3 CDR3 J

FIGURE 4HH

Germline V=VH3-11, D=D6-13, J=JH6b

9.7.2-CG2 ---------- ---------- ---------- ---------- ---------- R-G----

(residues 20-136 of SEQ ID NO: 66)

Germ	QVQLVESGGGLVKPGGSLRLSCAAS	GFTFSDYYMS	WIRQAPGKGLEWVS	YISSSGSTIYYADSVKG	RFTISRDNAKNSLYLQMNSLRAEDTAVYYCA	#I#GMDV	
(SEQ ID NO: 115)	FR1	CDR1	FR2	CDR2	FR3	CDR3	

9.7.2-CG2 ----------

Germ	WGQGTTVTVSSA	
	J	

FIGURE 4II

Germline V=012, J=JK3

9.7.2-CG4 ---------- -------GF-I ---------- -T----- ---------- --------- ----------

(residues 23-130 of SEQ ID NO: 52)

Germ	DIQMTQSPSSLSASVGDRVTITC	RASQSISSYLN	WYQQKPGKAPKLLIY	AASSLQS	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQSYSTPFT	FGPGTKVDIKR	
(SEQ ID NO: 103)	FR1	CDR1	FR2	CDR2	FR3	CDR3	J	

FIGURE 4JJ

Germline V=VH3-11, D=D6-13, J=JH6b

9.7.2-CG4 ---------- ---------- ---------- ---------- ---------- R-G----

Germ	QVQLVESGGGLVKPGGSLRLSCAAS	GFTFSDYYMS	WIRQAPGKGLEWVS	YISSSGSTIYYADSVKG	RFTISRDNAKNSLYLQMNSLRAEDTAVYYCA	#I#GMDV	
	FR1	CDR1	FR2	CDR2	FR3	CDR3	

9.7.2-CG4 ---------- (residues 20-135 of SEQ ID NO: 70)

Germ	WGQGTTVTVSSA (SEQ ID NO: 115)	
	J	

FIGURE 4KK

Germline V=012, J=JK3

9.14.4-CG2 ---------- -P--I---L-- ---------- ------- ---------- --------- ----------

(residues 23-130 of SEQ ID NO: 56)

Germ	DIQMTQSPSSLSASVGDRVTITC	RASQSISSYLN	WYQQKPGKAPKLLIY	AASSLQS	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQSYSTPFT	FGPGTKVDIKR
(SEQ ID NO: 103)	FR1	CDR1	FR2	CDR2	FR3	CDR3	J

FIGURE 4LL

Germline V=VH3-11, D=D7-27, J=JH4b

9.14.4-CG2 ---------- ---------- ---------- ---------- ---------- G-----

Germ	QVQLVESGGGLVKPGGSLRLSCAAS	GFTFSDYYMS	WIRQAPGKGLEWVS	YISSSGSTIYYADSVKG	RFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR	#LTGDY	
	FR1	CDR1	FR2	CDR2	FR3	CDR3	

9.14.4-CG2 ---------- (residues 20-135 of SEQ ID NO: 74)

Germ	WGQGTLVTVSSA	(SEQ ID NO: 116)
	J	

FIGURE 4MM

Germline V=012, J=JK3

9.14.4-CG4 ---------- -P--I---L-- ---------- ------- ---------- --------- ----------

(residues 23-130 of SEQ ID NO: 56)

Germ	DIQMTQSPSSLSASVGDRVTITC	RASQSISSYLN	WYQQKPGKAPKLLIY	AASSLQS	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQSYSTPFT	FGPGTKVDIKR
(SEQ ID NO: 103)	FR1	CDR1	FR2	CDR2	FR3	CDR3	J

FIGURE 4NN

Germline V=VH3-11, D=D7-27, J=JH4b

9.14.4-CG4 ---------- ---------- ---------- ---------- ---------- G-----

Germ QVQLVESGGGLVKPGGSLRLSCAAS GFTFSDYYMS WIRQAPGKGLEWVS YISSSGSTIYYADSVKG RFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR #LTGDY
FR1 CDR1 FR2 CDR2 FR3 CDR3

9.14.4-CG4 ---------- (residues 20-135 of SEQ ID NO: 78)

Germ WGQGTLVTVSSA (SEQ ID NO: 116)
J

FIGURE 4OO

Germline V=012, J=JK3

9.14.4-Ser ---------- -P--I---L-- ----------H ------- ---------- --------- ----------
(residues 23-130 of SEQ ID NO: 28)

Germ DIQMTQSPSSLSASVGDRVTITC RASQSISSYLN WYQQKPGKAPKLLIY AASSLQS GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC QQSYSTPFT FGPGTKVDIKR
(SEQ ID NO: 103) FR1 CDR1 FR2 CDR2 FR3 CDR3 J

FIGURE 4PP

Germline V=VH3-11, D=D7-27, J=JH4b

9.14.4-Ser ---------- ---------- ---------- ---------- ---------- G-----

Germ QVQLVESGGGLVKPGGSLRLSCAAS GFTFSDYYMS WIRQAPGKGLEWVS YISSSGSTIYYADSVKG RFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR #LTGDY
FR1 CDR1 FR2 CDR2 FR3 CDR3

9.14.4-Ser ---------- (residues 20-135 of SEQ ID NO: 82)

Germ WGQGTLVTVSSA (SEQ ID NO: 116)
J

FIGURE 4QQ

Germline V=012, J=JK3

9.7.2-Ser ---------- -------**GF-I** ----**R**---------- -**T**----- ---**L**-----**E**---------- --------- ----------

FR1 CDR1 FR2 CDR2 FR3 CDR3 J

(residues 23-130 of SEQ ID NO: 48)

Germ DIQMTQSPSSLSASVGDRVTITC RASQSISSYLN WYQQKPGKAPKLLIY AASSLQS GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC QQSYSTPFT FGPGTKVDIKR

(SEQ ID NO: 103) FR1 CDR1 FR2 CDR2 FR3 CDR3 J

FIGURE 4RR

Germline V=VH3-11, D=D6-13, J=JH6b

9.7.2-Ser ---------- ---------- ---------- ---------- ---------- **R-G**----

Germ QVQLVESGGGLVKPGGSLRLSCAAS GFTFSDYYMS WIRQAPGKGLEWVS YISSSGSTIYYADSVKG RFTISRDNAKNSLYLQMNSLRAEDTAVYYCA #I#GMDV

FR1 CDR1 FR2 CDR2 FR3 CDR3

9.7.2-Ser ---------- (residues 20-136 of SEQ ID NO: 50)

Germ WGQGTTVTVSSA (SEQ ID NO: 115)

J

FIGURE 4SS

Germline V=A27, J=JK4

8.10.3-Ser -**F**---------- ---------- ---------- ------- ----------**V**----

Germ EIVLTQSPGTLSLSPGERATLSC RASQSVSSSYLA WYQQKPGQAPRLLIY GASSRAT GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC

FR1 CDR1 FR2 CDR2 FR3

8.10.3-Ser --------- ---------- (residues 21-129 of SEQ ID NO: 44)

Germ QQYGSSPLT FGGGTKVEIKR (SEQ ID NO: 114)

CDR3 J

FIGURE 4TT

Germline V=VH3-48, D=D1-26, J=JH4b

8.10.3-Ser ------------------------- ------**F**--**T** ------------- ----**R**----**S**------- ------------------------------ **DPLLA-ATF**---

Germ EVQLVESGGGLVQPGGSLRLSCAAS GFTFSSYSMN WVRQAPGKGLEWVS YISSSSSTIYYADSVKG RFTISRDNAKNSLYLQMNSLRDEDTAVYYCAR ###IVG###FDY
FR1 CDR1 FR2 CDR2 FR3 CDR3

8.10.3-Ser ------------ (residues 20-141 of SEQ ID NO: 90)

Germ WGQGTLVTVSSA (SEQ ID NO: 113)
J

FIGURE 4UU

Germline V=A27, J=JK4

8.10.3-CG4 ---------------------- ----------- -------------- ------- ------------------------------

Germ EIVLTQSPGTLSLSPGERATLSC RASQSVSSSYLA WYQQKPGQAPRLLIY GASSRAT GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC
FR1 CDR1 FR2 CDR2 FR3

8.10.3-CG4 --------- ---------- (residues 21-129 of SEQ ID NO: 60)

Germ QQYGSSPLT FGGGTKVEIKR (SEQ ID NO: 114)
CDR3 J

FIGURE 4VV

Germline V=VH3-48, D=D1-26, J=JH4b

8.10.3-CG4 ------------------------- ------**F**--**T** ------------- ----**R**----**S**------- ------------------------------ **DPLLA-ATF**---

Germ EVQLVESGGGLVQPGGSLRLSCAAS GFTFSSYSMN WVRQAPGKGLEWVS YISSSSSTIYYADSVKG RFTISRDNAKNSLYLQMNSLRDEDTAVYYCAR ###IVG###FDY
FR1 CDR1 FR2 CDR2 FR3 CDR3

8.10.3-CG4 ------------ (residues 20-141 of SEQ ID NO: 94)

Germ WGQGTLVTVSSA (SEQ ID NO: 113)
J

FIGURE 4WW

Germline V=012, J=JK3

9.14.4G1 ---------- -P--I---L-- ----------H ------- ---------- --------- ----------

(residues 23-130 of SEQ ID NO: 28)

Germ	DIQMTQSPSSLSASVGDRVTITC	RASQSISSYLN	WYQQKPGKAPKLLIY	AASSLQS	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQSYSTPFT	FGPGTKVDIKR
(SEQ ID NO: 103)	FR1	CDR1	FR2	CDR2	FR3	CDR3	J

FIGURE 4XX

Germline V=VH3-11, D=D7-27, J=JH4b

9.14.4G1 ---------- ---------- ---------- ---------- ---------- G-----

Germ	QVQLVESGGGLVKPGGSLRLSCAAS	GFTFSDYYMS	WIRQAPGKGLEWVS	YISSSGSTIYYADSVKG	RFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR	#LTGDY
	FR1	CDR1	FR2	CDR2	FR3	CDR3

9.14.4G1 ---------- (residues 20-135 of SEQ ID NO: 102)

Germ	WGQGTLVTVSSA	(SEQ ID NO: 116)
	J	

FIGURE 4YY

Germline V=A27, J=JK4

8.10.3FG1 -F---------- ---------- ---------- ------- ----------

Germ	EIVLTQSPGTLSLSPGERATLSC	RASQSVSSSYLA	WYQQKPGQAPRLLIY	GASSRAT	GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC	
	FR1	CDR1	FR2	CDR2	FR3	

8.10.3FG1 --------- ---------- (residues 21-129 of SEQ ID NO: 32)

Germ	QQYGSSPLT	FGGGTKVEIKR	(SEQ ID NO: 114)
	CDR3	J	

FIGURE 4ZZ

Germline V=VH3-48, D=D1-26, J=JH4b

8.10.3FG1 ----------F-T----------R---S---------- DPLLA-ATF--

Germ EVQLVESGGGLVQPGGSLRLSCAAS GFTFSSYSMN WVRQAPGKGLEWVS YISSSSSTIYYADSVKG RFTISRDNAKNSLYLQMNSLRDEDTAVYYCAR ###IVG##FDY

FR1 CDR1 FR2 CDR2 FR3 CDR3

8.10.3FG1 ---------- (residues 20-141 of SEQ ID NO: 98)

Germ WGQGTLVTVSSA (SEQ ID NO: 113)

J

FIGURE 5

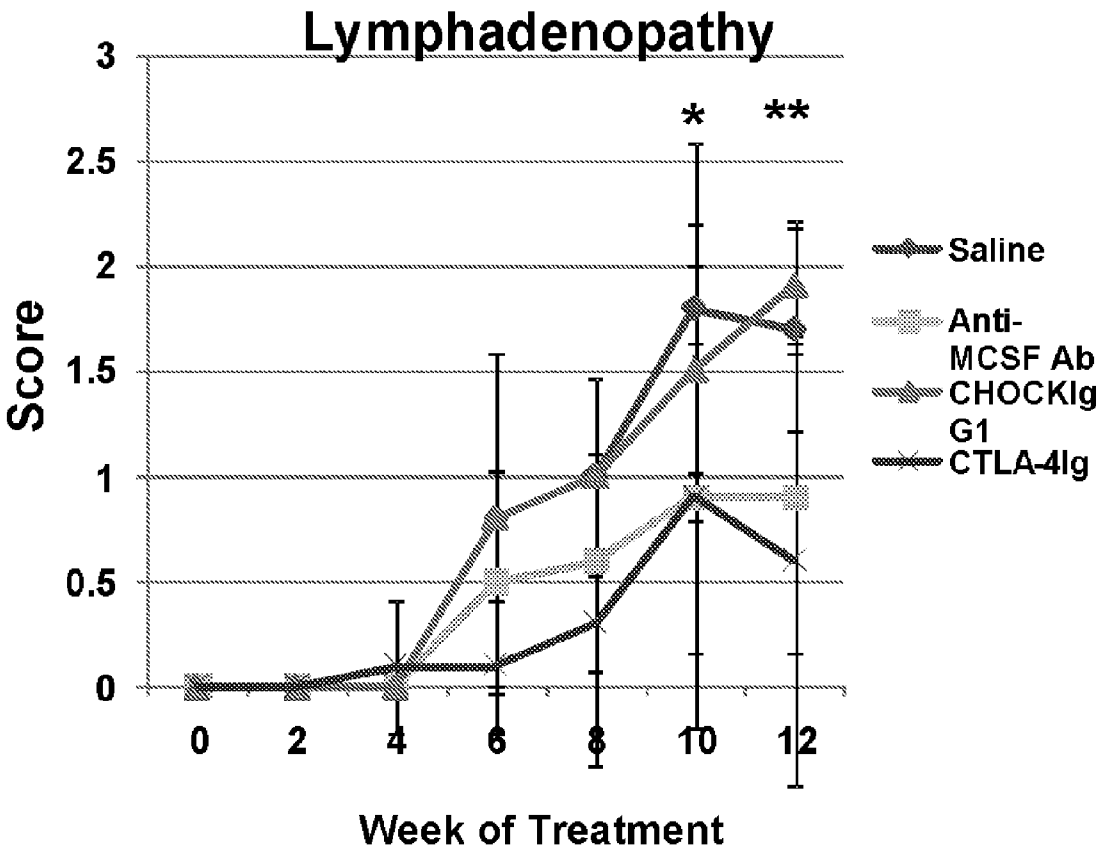

Lymphadenopathy
Score
3
2.5
2
1.5
1
0.5
0
0
2
4
6
8
10
12
*
**
Week of Treatment
Saline
Anti-MCSF Ab
CHOCKIg G1
CTLA-4Ig

FIGURE 6

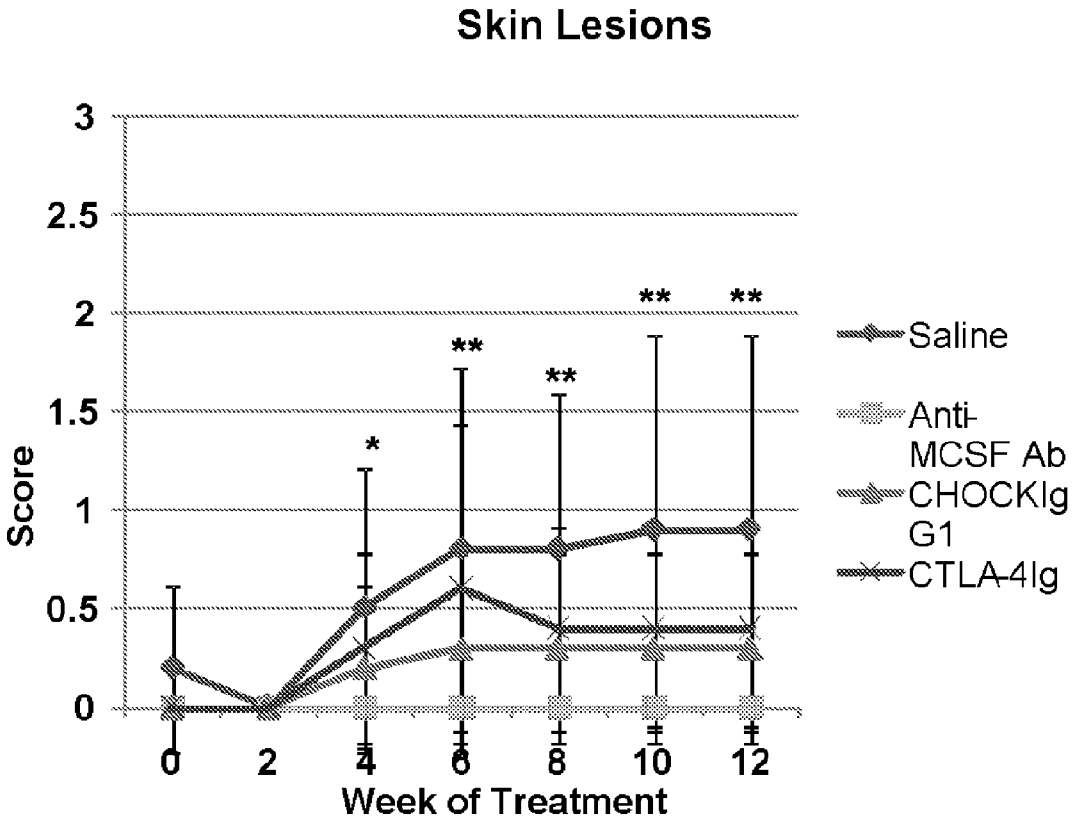

Skin Lesions
Score
3
2.5
2
1.5
1
0.5
0
0
2
4
6
8
10
12
Week of Treatment
*
**
**
**
**
Saline
Anti-MCSF Ab
CHOCKIg G1
CTLA-4Ig

FIGURE 7

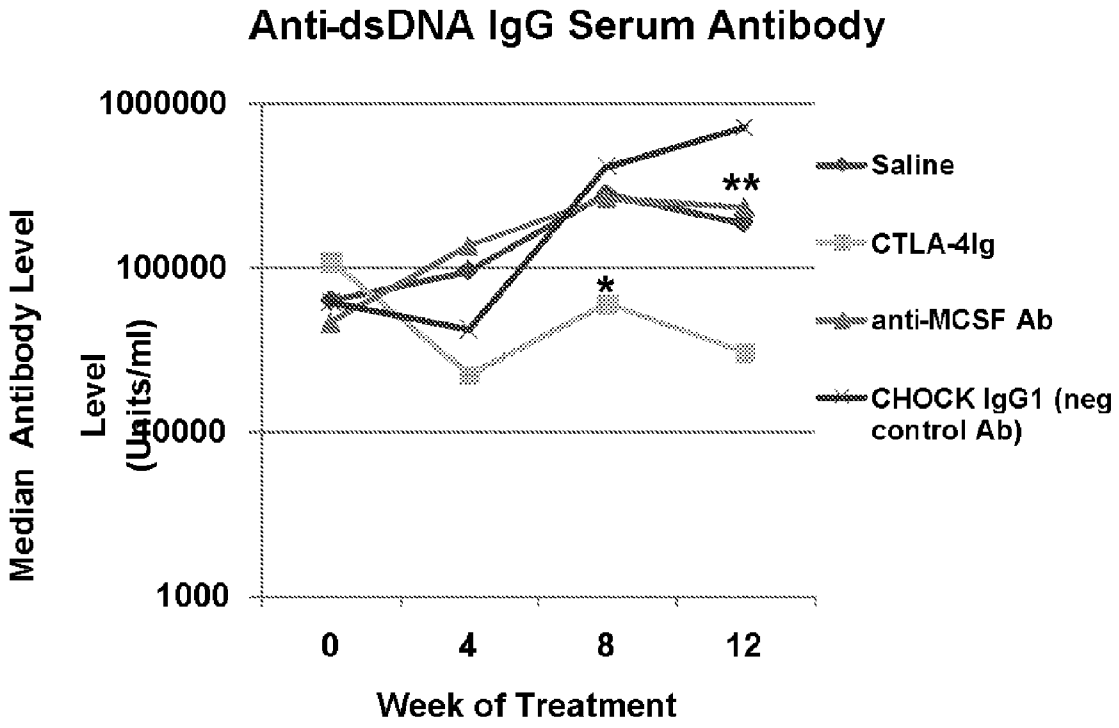

Anti-dsDNA IgG Serum Antibody
Median Antibody Level
Level (Units/ml)
1000000
100000
10000
1000
0
4
8
12
Week of Treatment
**
*
Saline
CTLA-4Ig
anti-MCSF Ab
CHOCK IgG1 (neg control Ab)

FIGURE 8

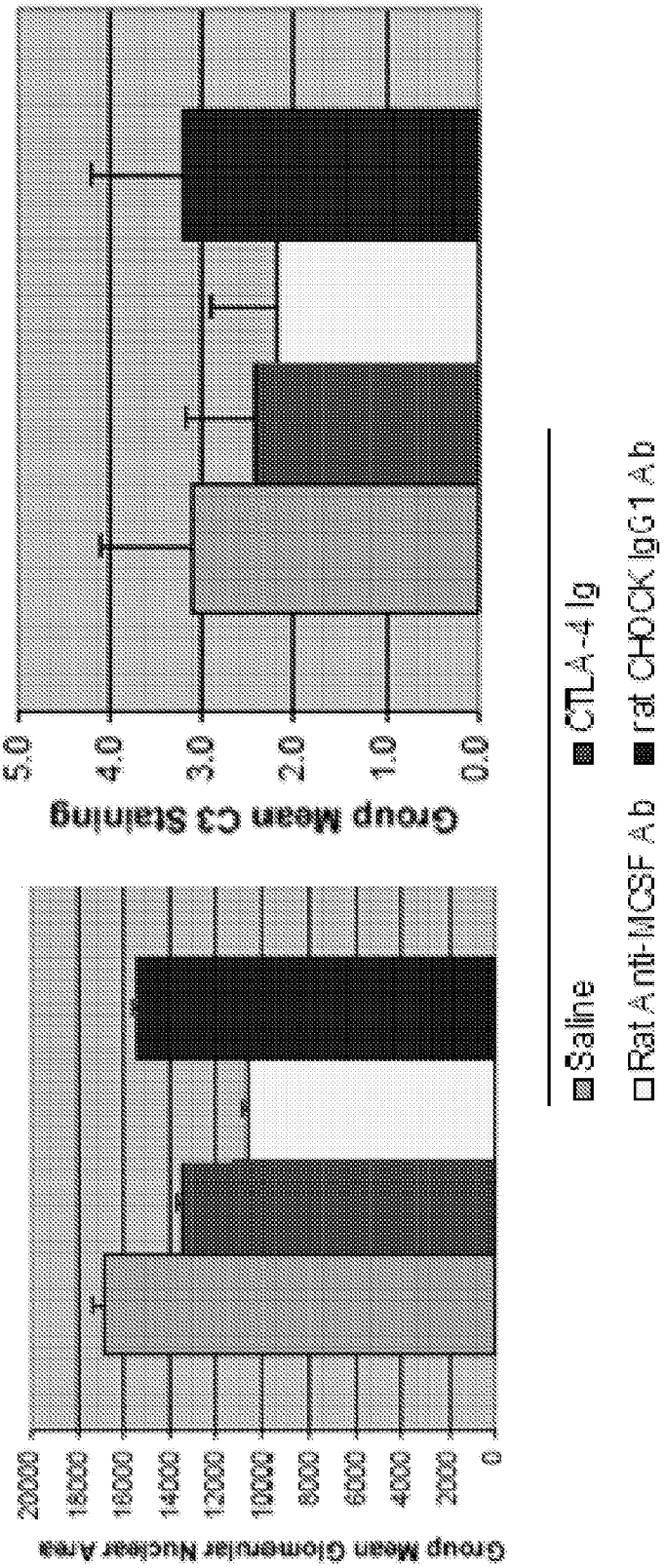

Group Mean C3 Staining
5.0
4.0
3.0
2.0
1.0
0.0
Group Mean Glomerular Nuclear Area
200000
180000
160000
140000
120000
100000
80000
60000
40000
20000
0
Saline
CTLA-4 Ig
Rat Anti-MCSF Ab
rat CHOCK IgG1 Ab

FIGURE 9A

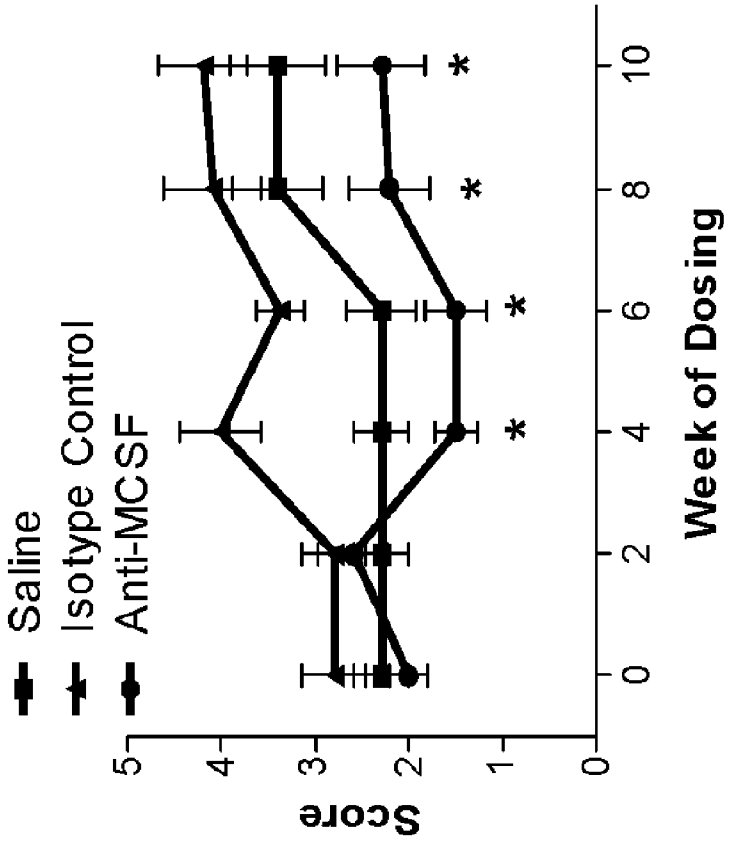


FIGURE 9B

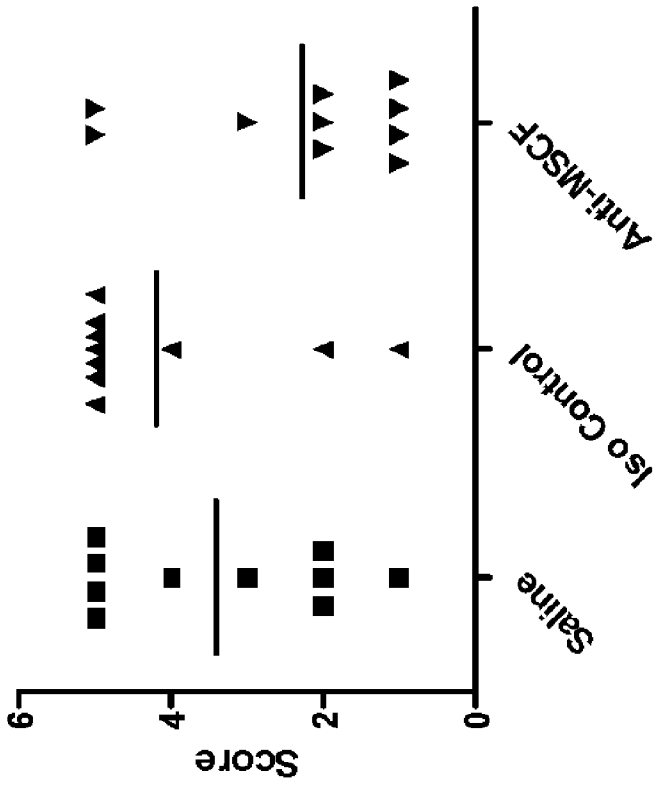


FIGURE 10A

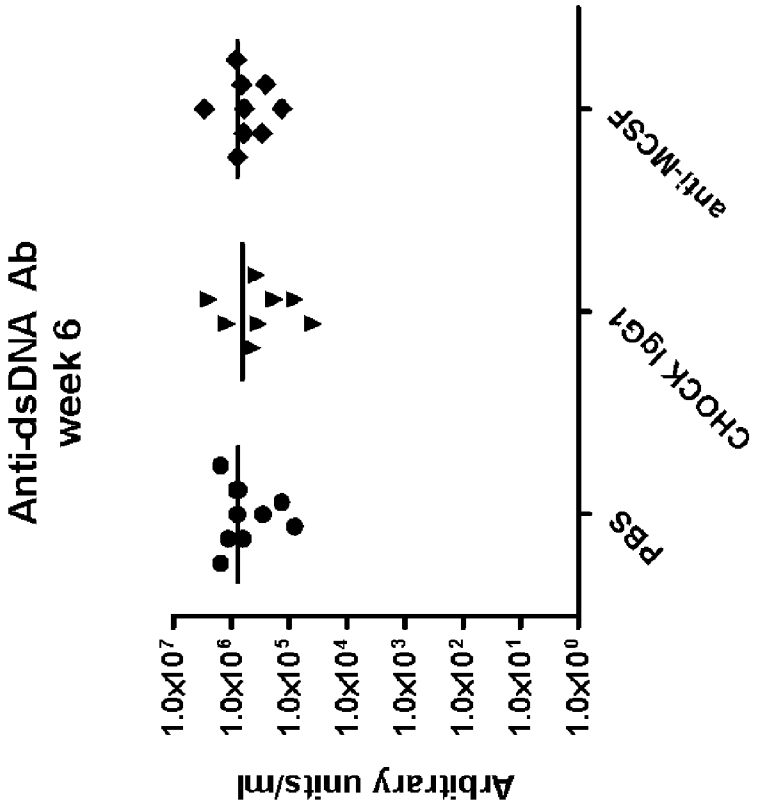


FIGURE 10B

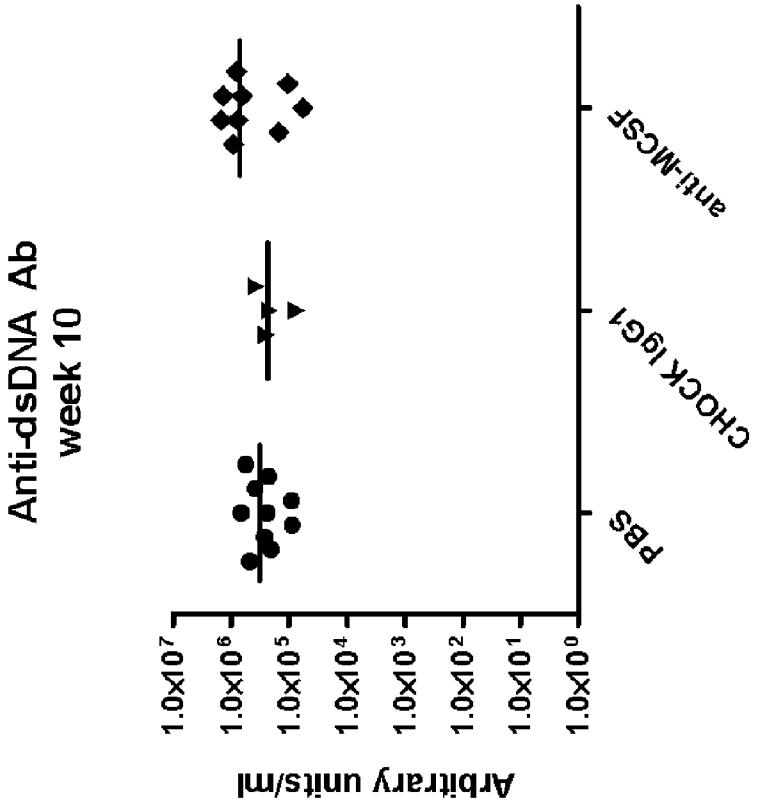


FIGURE 11

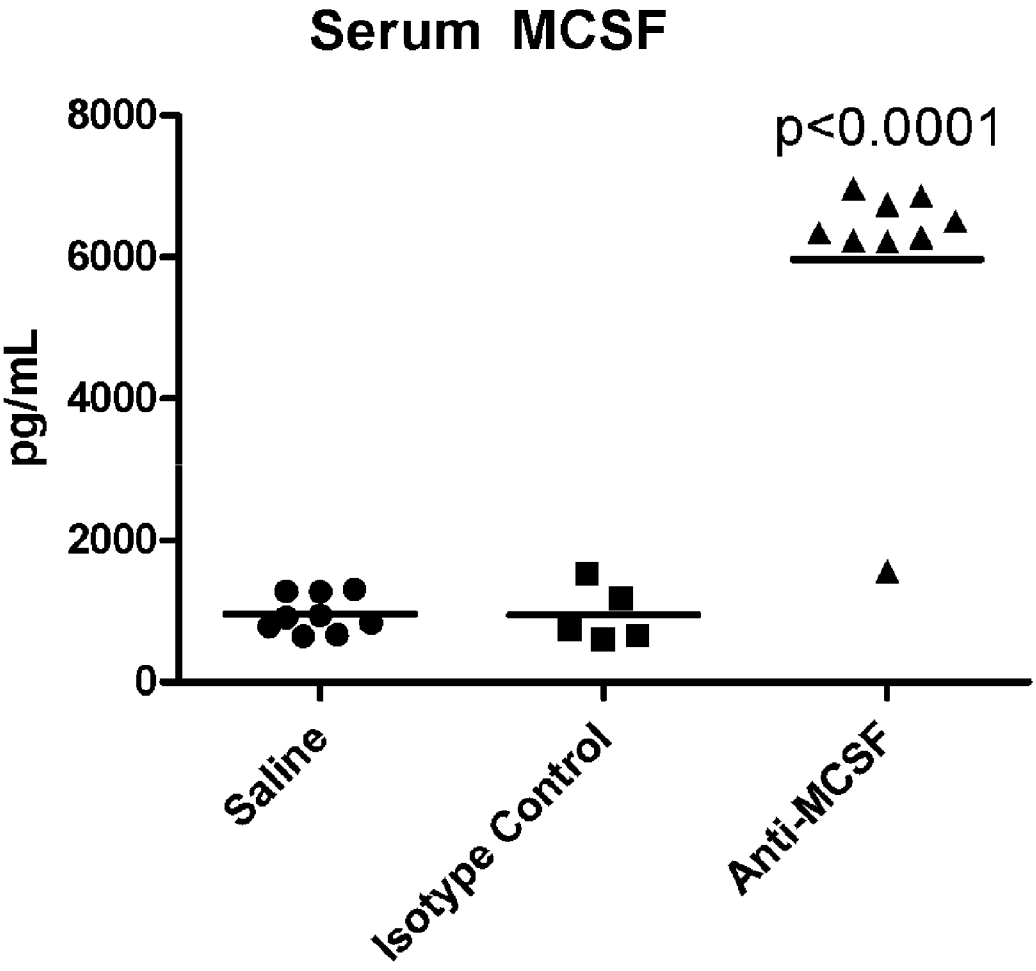

Serum MCSF
p<0.0001
8000
6000
4000
2000
0
pg/mL
Saline
Isotype Control
Anti-MCSF

FIGURE 12

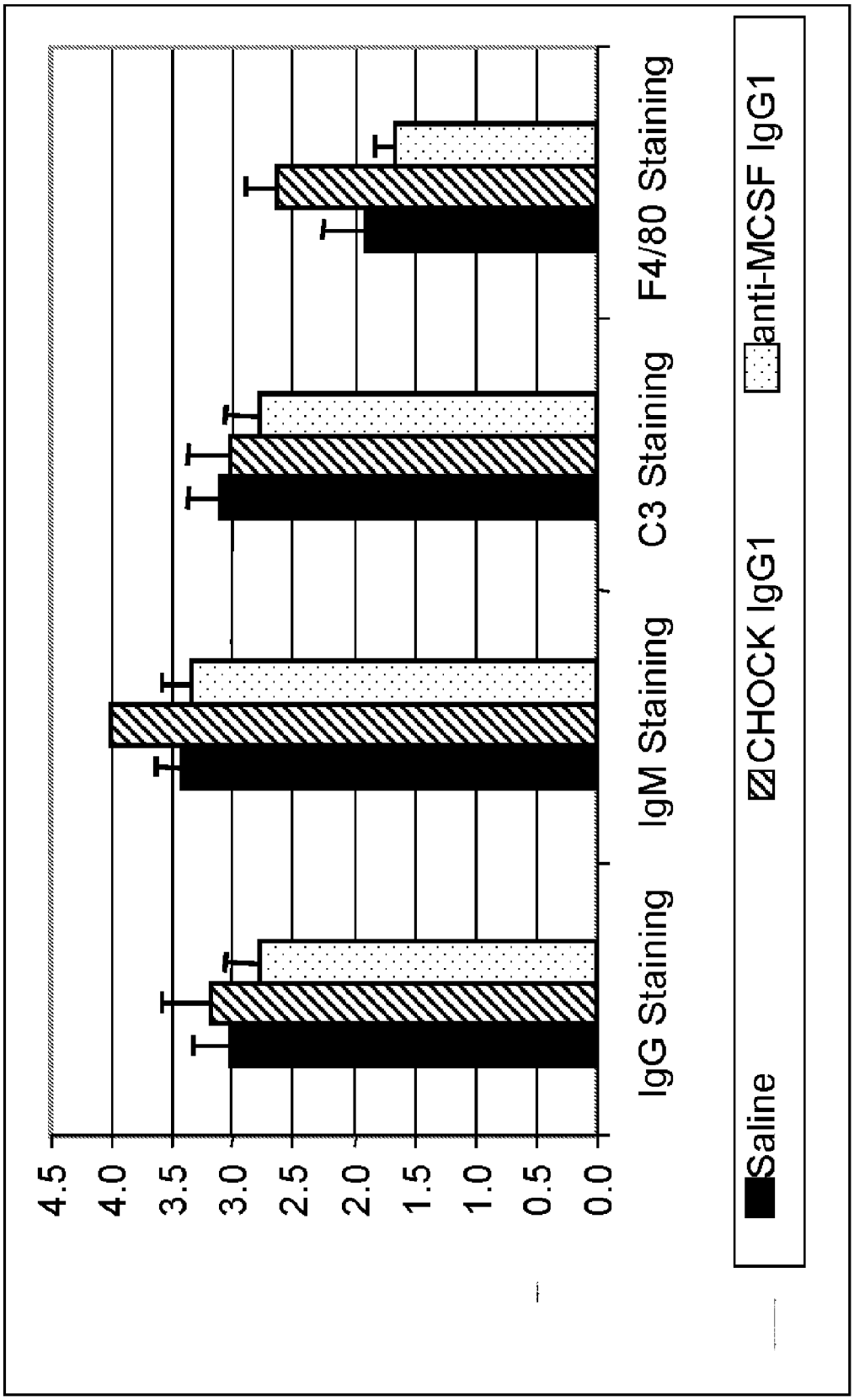

4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
IgG Staining
IgM Staining
C3 Staining
F4/80 Staining
Saline
CHOCK IgG1
anti-MCSF IgG1

FIGURE 13

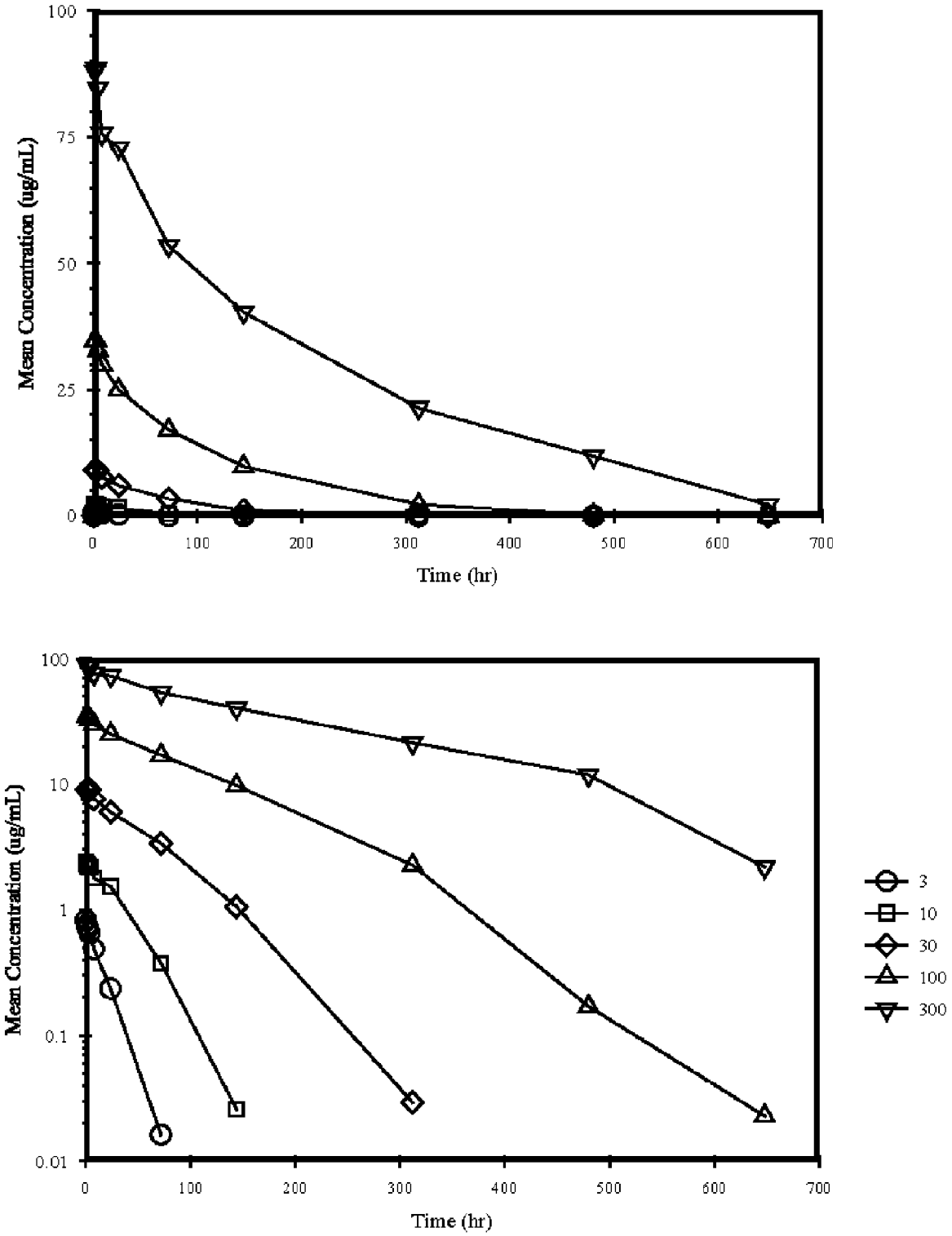

Mean Concentration (ug/mL)
100
75
50
25
0
0
100
200
300
400
500
600
700
Time (hr)
Mean Concentration (ug/mL)
100
10
1
0.1
0.01
0
100
200
300
400
500
600
700
Time (hr)
3
10
30
100
300

FIGURE 14

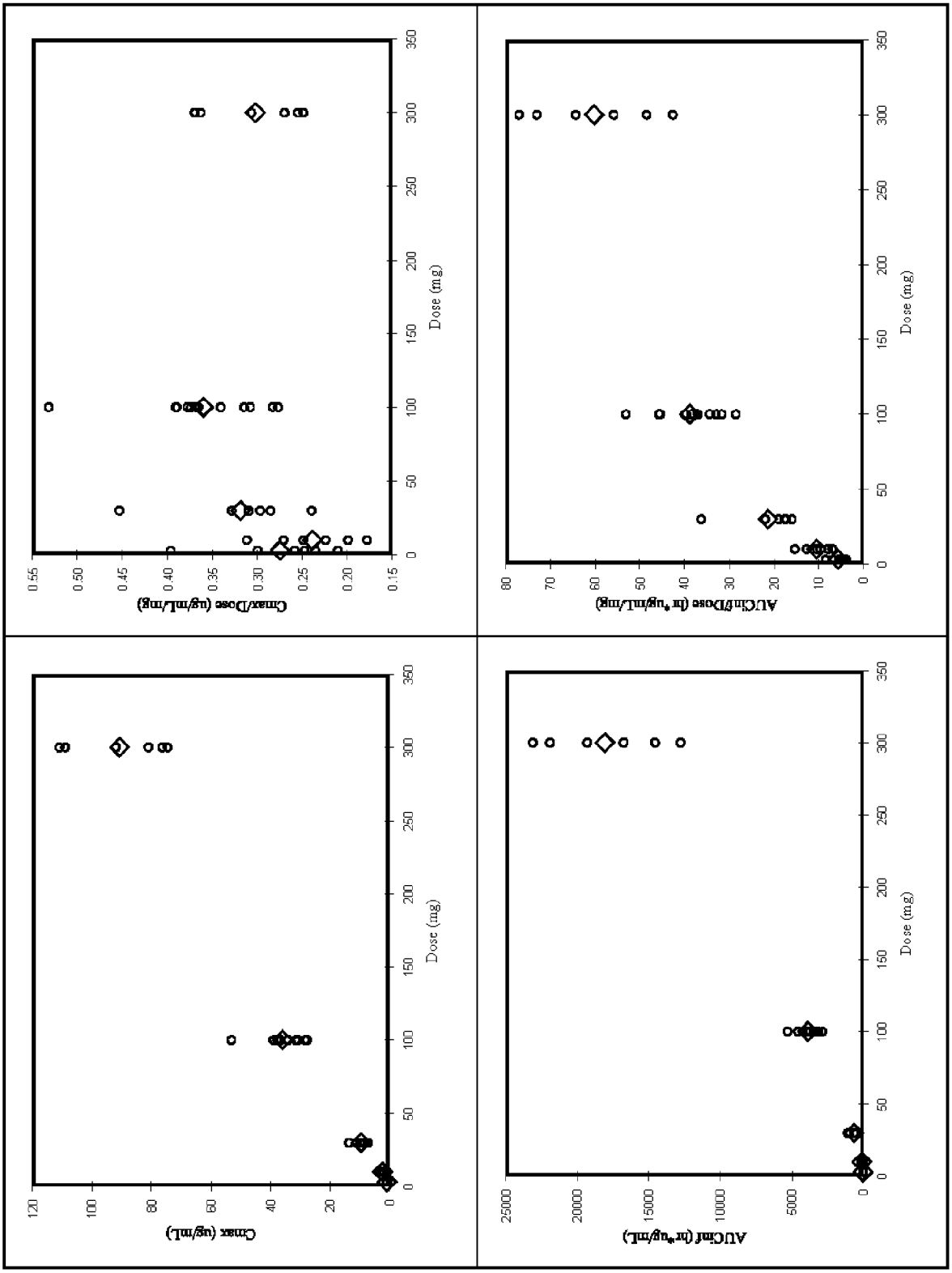

Cmax (ug/mL)
Dose (mg)
Cmax/Dose (ug/mL/mg)
Dose (mg)
AUCinf (hr*ug/mL)
Dose (mg)
AUCinf/Dose (hr*ug/mL/mg)
Dose (mg)

FIGURE 15

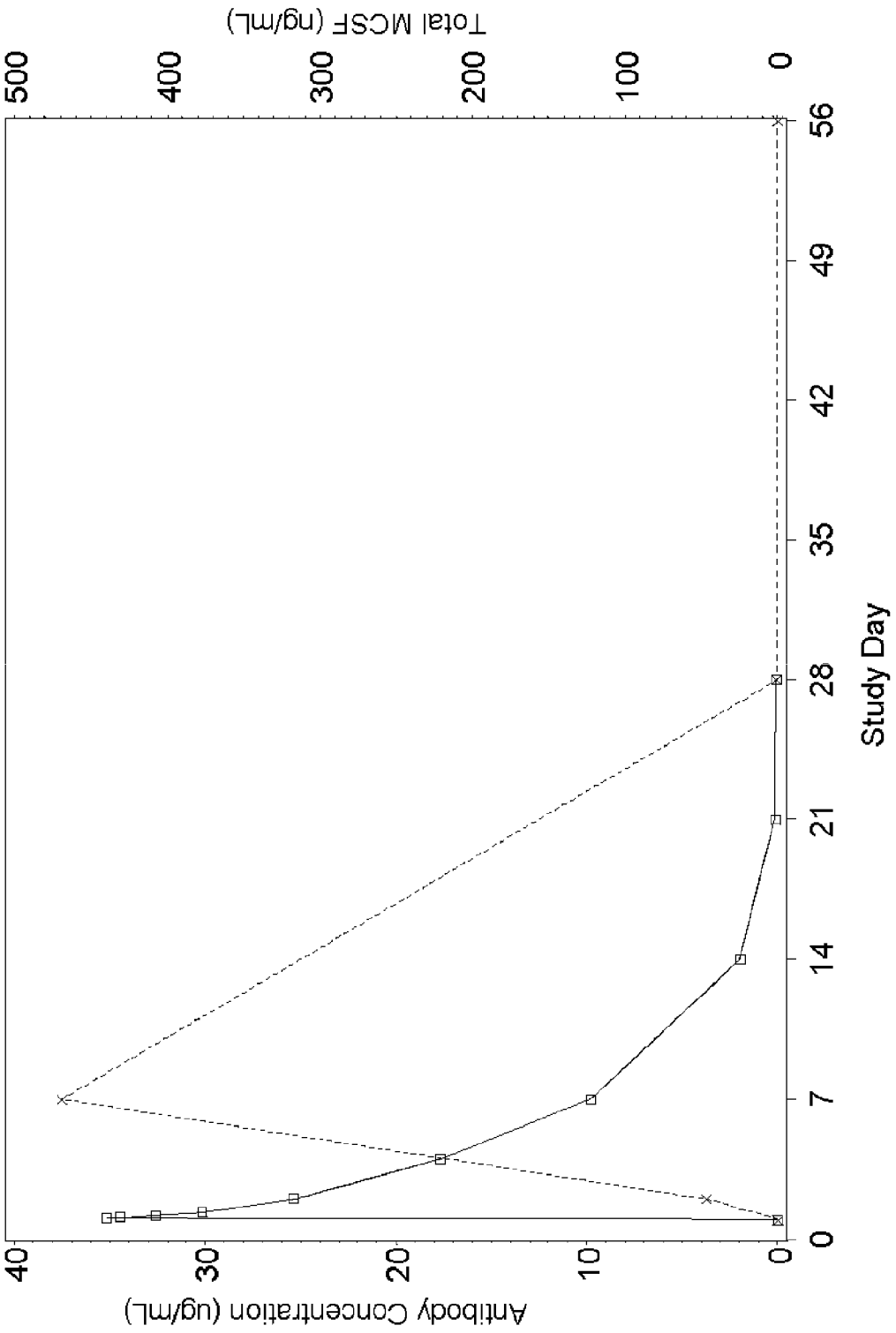

Total MCSF (ng/mL)
500
400
300
200
100
0
Antibody Concentration (ug/mL)
40
30
20
10
0
0
7
14
21
28
35
42
49
56
Study Day

FIGURE 16

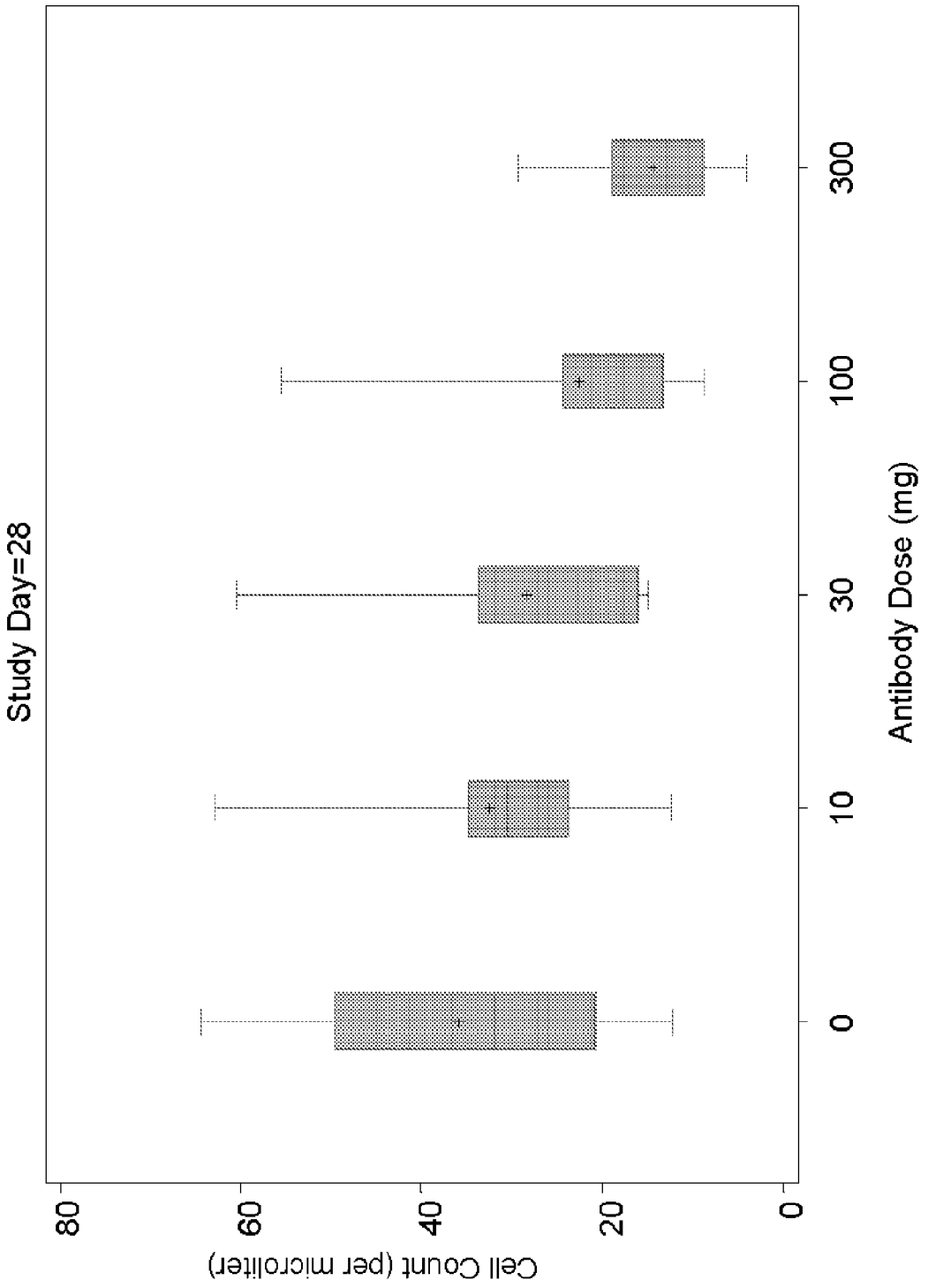

Study Day=28
Cell Count (per microliter)
0
20
40
60
80
0
10
30
100
300
Antibody Dose (mg)

FIGURE 17

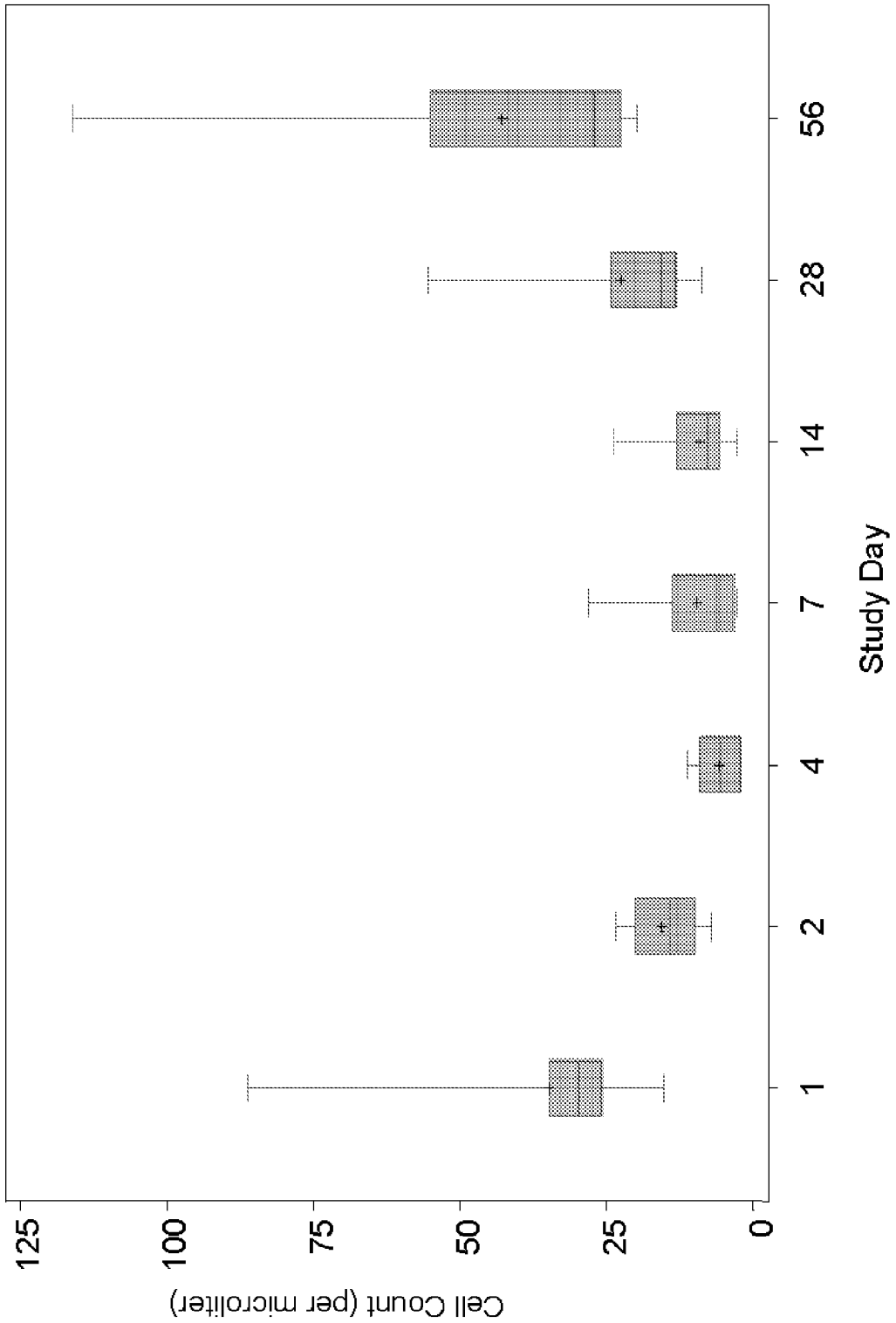

Cell Count (per microliter)
0
25
50
75
100
125
Study Day
1
2
4
7
14
28
56

FIGURE 18

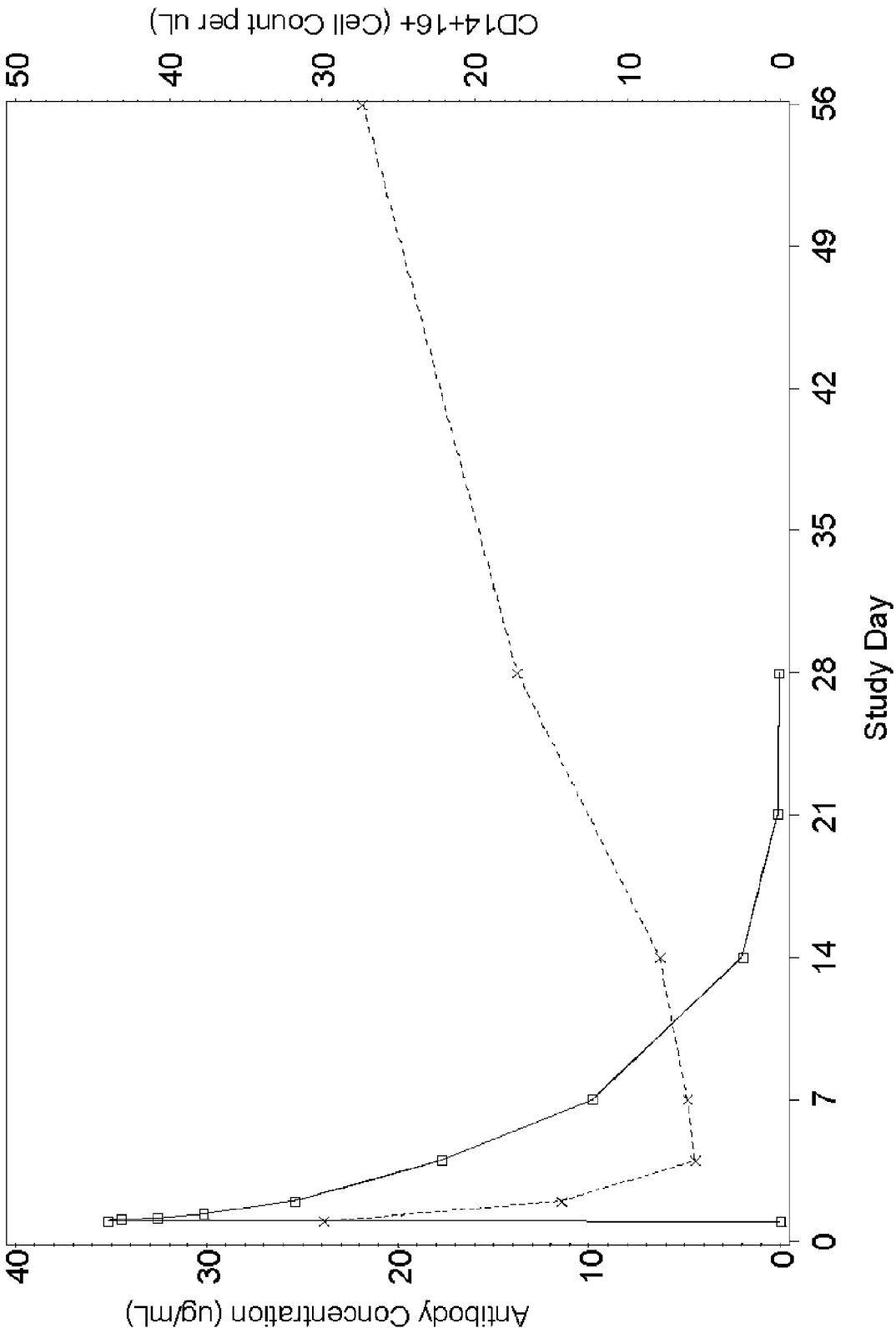
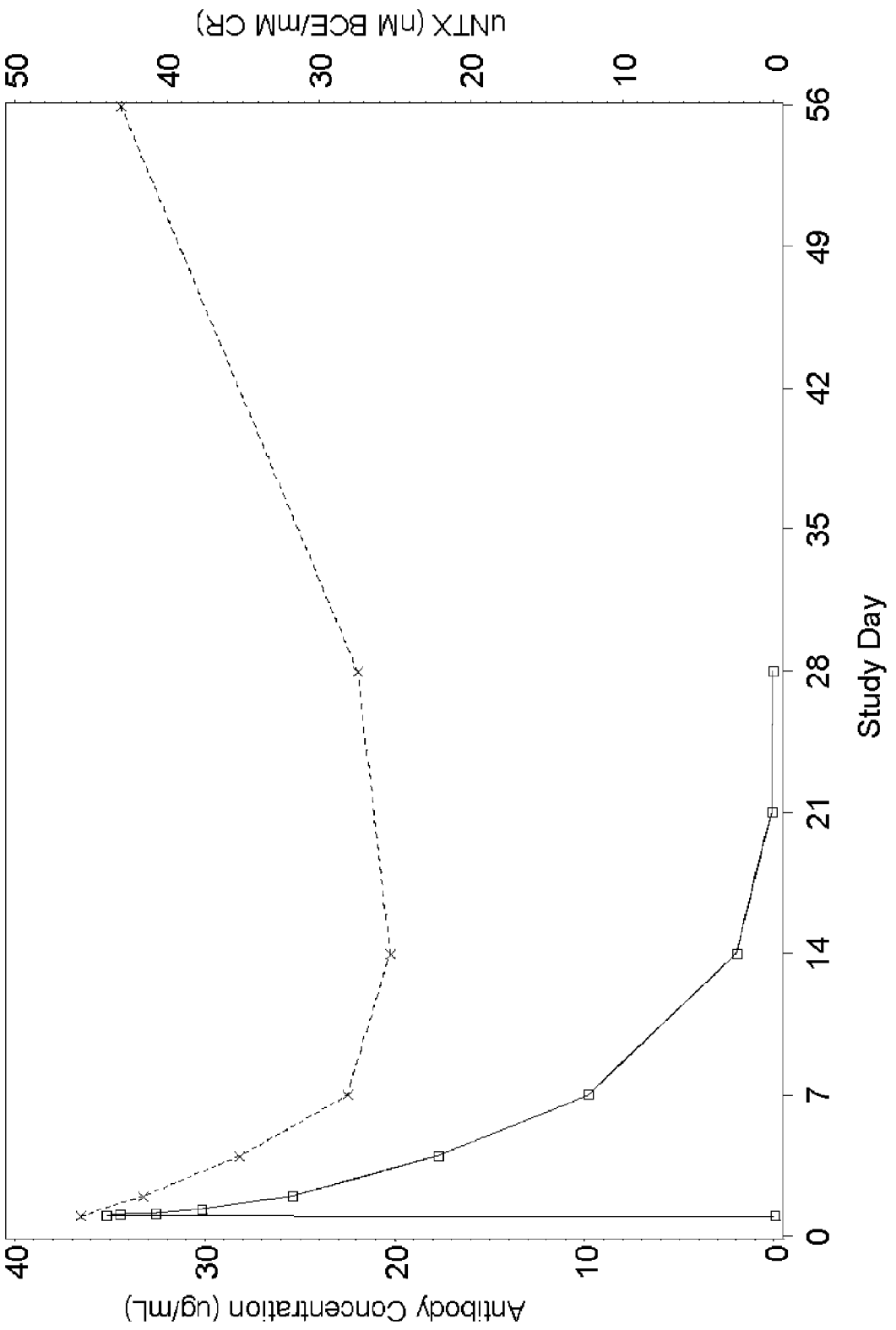

Antibody Concentration (ug/mL)
CD14+16+ (Cell Count per uL)
Study Day
0
10
20
30
40
50
0
7
14
21
28
35
42
49
56

FIGURE 19


Antibody Concentration (ug/mL)
uNTX (nM BCE/mM CR)
Study Day
0
10
20
30
40
50
0
7
14
21
28
35
42
49
56

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2012/056125

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/24 A61K39/395 A61P29/00
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)
C07K A61K

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, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	US 2002/141994 A1 (DEVALARAJA MADHAV N [US] ET AL) 3 October 2002 (2002-10-03) the whole document in particular, pages 2-6 -----	1,2,23, 30,31, 34,35 3-22, 24-29, 32,33, 36,37
Y	WO 2005/030124 A2 (WARNER LAMBERT CO [US]; ABGENIX INC [US]; BEDIAN VAHE [US]; DEVALARAJA) 7 April 2005 (2005-04-07) cited in the application the whole document ----- -/--	3-22, 24-29, 32,33, 36,37

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

[X] See patent family annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 January 2013	01/03/2013

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	Pérez-Mato, Isabel

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2012/056125

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	J. MENKE ET AL: "Distinct Roles of CSF-1 Isoforms in Lupus Nephritis", JOURNAL OF THE AMERICAN SOCIETY OF NEPHROLOGY, vol. 22, no. 10, 1 October 2011 (2011-10-01), pages 1821-1833, XP055049338, ISSN: 1046-6673, DOI: 10.1681/ASN.2011010038 the whole document	24,32,36
Y	J. MENKE ET AL: "Circulating CSF-1 Promotes Monocyte and Macrophage Phenotypes that Enhance Lupus Nephritis", JOURNAL OF THE AMERICAN SOCIETY OF NEPHROLOGY, vol. 20, no. 12, 1 December 2009 (2009-12-01), pages 2581-2592, XP055049341, ISSN: 1046-6673, DOI: 10.1681/ASN.2009050499 the whole document	24,32,36
Y	RUBIN KELLEY V ET AL: "Pivotal role of colony stimulating factor-1 in lupus nephritis.", KIDNEY INTERNATIONAL. SUPPLEMENT FEB 1994, vol. 45, February 1994 (1994-02), pages S83-S85, XP009166135, ISSN: 0098-6577 the whole document	24,32,36
Y	JULIA MENKE ET AL: "Sunlight triggers cutaneous lupus through a CSF-1-dependent mechanism in MRL-Fas(lpr) mice.", THE JOURNAL OF IMMUNOLOGY, vol. 181, no. 10, 1 November 2008 (2008-11-01), pages 7367-7379, XP055049414, ISSN: 0022-1767 the whole document	25,33,37
Y	FURUKAWA F ET AL: "Animal models of spontaneous and drug-induced cutaneous lupus erythematosus", AUTOIMMUNITY REVIEWS, ELSEVIER, AMSTERDAM, NL, vol. 4, no. 6, 1 July 2005 (2005-07-01), pages 345-350, XP027686749, ISSN: 1568-9972 [retrieved on 2005-07-01] the whole document	25,33,37

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INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2012/056125

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	J FONT ET AL: "Clusters of clinical and immunologic features in systemic lupus erythematosus: analysis of 600 patients from a single center", SEMINARS IN ARTHRITIS AND RHEUMATISM, vol. 33, no. 4, 1 February 2004 (2004-02-01), pages 217-230, XP55049410, ISSN: 0049-0172, DOI: 10.1053/S0049-0172(03)00133-1 the whole document	26-29
A	DEBORAH LENDA ET AL: "Negative role of colony-stimulating factor-1 in macrophage, T cell, and B cell mediated autoimmune disease in MRL-Fas(lpr) mice.", THE JOURNAL OF IMMUNOLOGY, vol. 173, no. 7, 1 October 2004 (2004-10-01), pages 4744-4754, XP055049345, ISSN: 0022-1767 the whole document	1-37
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A	BLOOM R D ET AL: "Colony stimulating factor-1 in the induction of lupus nephritis.", KIDNEY INTERNATIONAL MAY 1993, vol. 43, no. 5, May 1993 (1993-05), pages 1000-1009, XP002690161, ISSN: 0085-2538 the whole document	1-37

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2012/056125

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		NZ 546369 A	28-05-2010
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		ZA 200601969 A	30-05-2007

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WO2013/068902 EN 2013.05.16
(83)生物保藏信息
ATCC PTA-5401 2003.08.08
ATCC PTA-5390 2003.08.08
ATCC PTA-5391 2003.08.08
ATCC PTA-5392 2003.08.08
ATCC PTA-5393 2003.08.08
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ATCC PTA-5397 2003.08.08
ATCC PTA-5398 2003.08.08
ATCC PTA-5399 2003.08.08
ATCC PTA-5400 2003.08.08

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J·E·毕比 R·福格尔
M·宏科扎兰克 D·贝德勒
P·S·雷迪 D·J·沃沙克
(74)专利代理机构 永新专利商标代理有限公司 72002
代理人 左路 林晓红

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A61K 39/395(2006.01)
A61P 29/00(2006.01)

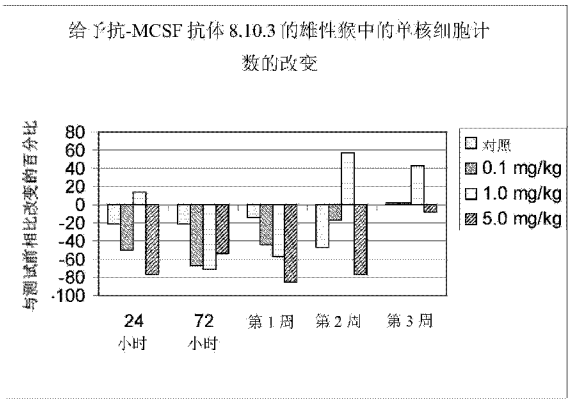
权利要求书6页 说明书88页
序列表80页 附图36页

(54)发明名称

使用抗M-CSF抗体治疗炎性疾病的方法

(57)摘要

本发明涉及抗体及其抗原结合部分，其特异性结合M-CSF，优选地人M-CSF，并抑制M-CSF。本发明还涉及人抗M-CSF抗体及其抗原结合部分。本发明的抗体是嵌合抗体、双特异性抗体、衍生化抗体、单链抗体或是融合蛋白的一部分。本发明还涉及衍生自人抗M-CSF抗体的分离的重链和轻链免疫球蛋白和编码此类免疫球蛋白的核酸分子。本发明还涉及制备人抗M-CSF抗体、包含这些抗体的组合物的方法，以及使用所述抗体和组合物进行诊断和治疗的方法。本发明还提供使用编码包含人抗M-CSF抗体的重链和/或轻链免疫球蛋白分子的核酸分子的基因治疗方法。本发明还涉及包含本发明的核酸分子的转基因动物和转基因植物。



CN 104271599 A

1. 一种治疗狼疮的方法，其包括给有需要的个体施用治疗有效量的特异性结合 M-CSF 的人单克隆抗体或其抗原结合部分。

2. 权利要求 1 的方法，其中所述抗体或抗原结合部分具有以下特性中的至少一种：

a) 结合人 M-CSF 的分泌亚型和 M-CSF 的膜结合亚型；

b) 对 M-CSF 的选择性是其对 GM-CSF 或 G-CSF 的选择性的至少 100 倍；

c) 以 $1.0x10^{-7}$M 或更低的 K_D 结合 M-CSF；

d) 对 M-CSF 的解离速率 (k_{off}) 为 $2.0x10^{-4}s^{-1}$ 或更小；或

e) 在存在人 c-fms 的情况下结合人 M-CSF。

3. 权利要求 2 的方法，其中所述抗体或抗原结合部分阻断与 c-fms 的结合并以 $1.0x10^{-7}$M 或更低的 K_D 结合 M-CSF。

4. 权利要求 1 的方法，其中所述抗体或抗原结合部分具有以下特性中的至少一种：

a) 与选自如下一组的抗体交叉竞争结合 M-CSF：抗体 252、88、100、3.8.3、2.7.3、1.120.1、9.14.4I、8.10.3F、9.7.2IF、9.14.4、8.10.3、9.7.2、9.7.2C-Ser、9.14.4C-Ser、8.10.3C-Ser、8.10.3-CG2、9.7.2-CG2、9.7.2-CG4、9.14.4-CG2、9.14.4-CG4、9.14.4-Ser、9.7.2-Ser、8.10.3-Ser、8.10.3-CG4、8.10.3FG1 和 9.14.4G1；

b) 与选自如下一组的抗体竞争结合 M-CSF：252、88、100、3.8.3、2.7.3、1.120.1、9.14.4I、8.10.3F、9.7.2IF、9.14.4、8.10.3、9.7.2、9.7.2C-Ser、9.14.4C-Ser、8.10.3C-Ser、8.10.3-CG2、9.7.2-CG2、9.7.2-CG4、9.14.4-CG2、9.14.4-CG4、9.14.4-Ser、9.7.2-Ser、8.10.3-Ser、8.10.3-CG4、8.10.3FG1 和 9.14.4G1；

c) 与选自如下一组的抗体结合 M-CSF 的相同的表位：抗体 252、88、100、3.8.3、2.7.3、1.120.1、9.14.4I、8.10.3F、9.7.2IF、9.14.4、8.10.3、9.7.2、9.7.2C-Ser、9.14.4C-Ser、8.10.3C-Ser、8.10.3-CG2、9.7.2-CG2、9.7.2-CG4、9.14.4-CG2、9.14.4-CG4、9.14.4-Ser、9.7.2-Ser、8.10.3-Ser、8.10.3-CG4、8.10.3FG1 和 9.14.4G1；

d) 以与选自如下一组的抗体基本上相同的 K_D 结合 M-CSF：抗体 252、88、100、3.8.3、2.7.3、1.120.1、9.14.4I、8.10.3F、9.7.2IF、9.14.4、8.10.3、9.7.2、9.7.2C-Ser、9.14.4C-Ser、8.10.3C-Ser、8.10.3-CG2、9.7.2-CG2、9.7.2-CG4、9.14.4-CG2、9.14.4-CG4、9.14.4-Ser、9.7.2-Ser、8.10.3-Ser、8.10.3-CG4、8.10.3FG1 和 9.14.4G1；和

e) 以与选自如下一组的抗体基本上相同的解离速率结合 M-CSF：抗体 252、88、100、3.8.3、2.7.3、1.120.1、9.14.4I、8.10.3F、9.7.2IF、9.14.4、8.10.3、9.7.2、9.7.2C-Ser、9.14.4C-Ser、8.10.3C-Ser、8.10.3-CG2、9.7.2-CG2、9.7.2-CG4、9.14.4-CG2、9.14.4-CG4、9.14.4-Ser、9.7.2-Ser、8.10.3-Ser、8.10.3-CG4、8.10.3FG1 和 9.14.4G1。

5. 权利要求 1 的方法，其中所述抗体或抗原结合部分选自以下一组：

a) 抗体，其包含 SEQ ID NO:2 所示的重链氨基酸序列和 SEQ ID NO:4 所示的轻链氨基酸序列，但不具有信号序列；

b) 抗体，其包含 SEQ ID NO:6 所示的重链氨基酸序列和 SEQ ID NO:8 所示的轻链氨基酸序列，但不具有信号序列；

c) 抗体，其包含 SEQ ID NO:10 所示的重链氨基酸序列和 SEQ ID NO:12 所示的轻链氨基酸序列，但不具有信号序列；

d) 抗体，其包含 SEQ ID NO:14 所示的重链氨基酸序列和 SEQ ID NO:16 所示的轻链氨

基酸序列，但不具有信号序列；

e) 抗体，其包含 SEQ ID NO:18 所示的重链氨基酸序列和 SEQ ID NO:20 所示的轻链氨基酸序列，但不具有信号序列；

f) 抗体，其包含 SEQ ID NO:22 所示的重链氨基酸序列和 SEQ ID NO:24 所示的轻链氨基酸序列，但不具有信号序列；

g) 抗体，其包含 SEQ ID NO:26 所示的重链氨基酸序列和 SEQ ID NO:28 所示的轻链氨基酸序列，但不具有信号序列；

h) 抗体，其包含 SEQ ID NO:38 所示的重链氨基酸序列和 SEQ ID NO:28 所示的轻链氨基酸序列，但不具有信号序列；

i) 抗体，其包含 SEQ ID NO:54 所示的重链氨基酸序列和 SEQ ID NO:56 所示的轻链氨基酸序列，但不具有信号序列；

j) 抗体，其包含 SEQ ID NO:74 所示的重链氨基酸序列和 SEQ ID NO:56 所示的轻链氨基酸序列，但不具有信号序列；

k) 抗体，其包含 SEQ ID NO:78 所示的重链氨基酸序列和 SEQ ID NO:56 所示的轻链氨基酸序列，但不具有信号序列；

l) 抗体，其包含 SEQ ID NO:82 所示的重链氨基酸序列和 SEQ ID NO:28 所示的轻链氨基酸序列，但不具有信号序列；

m) 抗体，其包含 SEQ ID NO:102 所示的重链氨基酸序列和 SEQ ID NO:28 所示的轻链氨基酸序列，但不具有信号序列；

n) 抗体，其包含 SEQ ID NO:30 所示的重链氨基酸序列和 SEQ ID NO:32 所示的轻链氨基酸序列，但不具有信号序列；

o) 抗体，其包含 SEQ ID NO:30 所示的重链氨基酸序列和 SEQ ID NO:44 所示的轻链氨基酸序列，但不具有信号序列；

p) 抗体，其包含 SEQ ID NO:58 所示的重链氨基酸序列和 SEQ ID NO:60 所示的轻链氨基酸序列，但不具有信号序列；

q) 抗体，其包含 SEQ ID NO:62 所示的重链氨基酸序列和 SEQ ID NO:60 所示的轻链氨基酸序列，但不具有信号序列；

r) 抗体，其包含 SEQ ID NO:90 所示的重链氨基酸序列和 SEQ ID NO:44 所示的轻链氨基酸序列，但不具有信号序列；

s) 抗体，其包含 SEQ ID NO:94 所示的重链氨基酸序列和 SEQ ID NO:60 所示的轻链氨基酸序列，但不具有信号序列；

t) 抗体，其包含 SEQ ID NO:98 所示的重链氨基酸序列和 SEQ ID NO:32 所示的轻链氨基酸序列，但不具有信号序列；

u) 抗体，其包含 SEQ ID NO:34 所示的重链氨基酸序列和 SEQ ID NO:36 所示的轻链氨基酸序列，但不具有信号序列；

v) 抗体，其包含 SEQ ID NO:46 所示的重链氨基酸序列和 SEQ ID NO:48 所示的轻链氨基酸序列，但不具有信号序列；

w) 抗体，其包含 SEQ ID NO:50 所示的重链氨基酸序列和 SEQ ID NO:52 所示的轻链氨基酸序列，但不具有信号序列；

x) 抗体，其包含 SEQ ID NO:66 所示的重链氨基酸序列和 SEQ ID NO:52 所示的轻链氨基酸序列，但不具有信号序列；

y) 抗体，其包含 SEQ ID NO:70 所示的重链氨基酸序列和 SEQ ID NO:52 所示的轻链氨基酸序列，但不具有信号序列；和

z) 抗体，其包含 SEQ ID NO:86 所示的重链氨基酸序列和 SEQ ID NO:48 所示的轻链氨基酸序列，但不具有信号序列。

6. 权利要求 1 的方法，其中所述抗体或抗原结合部分包含：

a) 重链 CDR1、CDR2 和 CDR3，其独立地选自如下一组的抗体的重链：单克隆抗体 252、88、100、3.8.3、2.7.3、1.120.1、9.14.4I、8.10.3F、9.7.2IF、9.14.4、8.10.3、9.7.2、9.7.2C-Ser、9.14.4C-Ser、8.10.3C-Ser、8.10.3-CG2、9.7.2-CG2、9.7.2-CG4、9.14.4-CG2、9.14.4-CG4、9.14.4-Ser、9.7.2-Ser、8.10.3-Ser、8.10.3-CG4、8.10.3FG1 和 9.14.4G1；或

b) 轻链 CDR1、CDR2 和 CDR3，其独立地选自如下一组的抗体的轻链：单克隆抗体 252、88、100、3.8.3、2.7.3、1.120.1、9.14.4I、8.10.3F、9.7.2IF、9.14.4、8.10.3、9.7.2、9.7.2C-Ser、9.14.4C-Ser、8.10.3C-Ser、8.10.3-CG2、9.7.2-CG2、9.7.2-CG4、9.14.4-CG2、9.14.4-CG4、9.14.4-Ser、9.7.2-Ser、8.10.3-Ser、8.10.3-CG4、8.10.3FG1 和 9.14.4G1。

7. 权利要求 1 的方法，其中：

a) 所述抗体或抗原结合部分包含选自如下一组的抗体的重链 CDR1、CDR2 和 CDR3：抗体 252、88、100、3.8.3、2.7.3、1.120.1、9.14.4I、8.10.3F、9.7.2IF、9.14.4、8.10.3、9.7.2、9.7.2C-Ser、9.14.4C-Ser、8.10.3C-Ser、8.10.3-CG2、9.7.2-CG2、9.7.2-CG4、9.14.4-CG2、9.14.4-CG4、9.14.4-Ser、9.7.2-Ser、8.10.3-Ser、8.10.3-CG4、8.10.3FG1 和 9.14.4G1；

b) 所述抗体或抗原结合部分包含选自如下一组的抗体的重链 CDR1、CDR2 和 CDR3：抗体 252、88、100、3.8.3、2.7.3、1.120.1、9.14.4I、8.10.3F、9.7.2IF、9.14.4、8.10.3、9.7.2、9.7.2C-Ser、9.14.4C-Ser、8.10.3C-Ser、8.10.3-CG2、9.7.2-CG2、9.7.2-CG4、9.14.4-CG2、9.14.4-CG4、9.14.4-Ser、9.7.2-Ser、8.10.3-Ser、8.10.3-CG4、8.10.3FG1 和 9.14.4G1；

c) 所述抗体包含 (a) 的重链和 (b) 的轻链；或

d) 所述抗体或抗原结合部分包含 (a) 的重链和 (b) 的轻链，其中所述重链和轻链 CDR 氨基酸序列选自同一抗体。

8. 权利要求 1 的方法，其中所述抗体或抗原结合部分包含：

a) 重链，其包含选自如下一组的抗体的重链中从 CDR1 起始处至 CDR3 结束处的氨基酸序列：抗体 252、88、100、3.8.3、2.7.3、1.120.1、9.14.4I、8.10.3F、9.7.2IF、9.14.4、8.10.3、9.7.2、9.7.2C-Ser、9.14.4C-Ser、8.10.3C-Ser、8.10.3-CG2、9.7.2-CG2、9.7.2-CG4、9.14.4-CG2、9.14.4-CG4、9.14.4-Ser、9.7.2-Ser、8.10.3-Ser、8.10.3-CG4、8.10.3FG1 和 9.14.4G1；

b) 轻链，其包含选自如下一组的抗体的从 CDR1 起始处至 CDR3 结束处的氨基酸序列：抗体 252、88、100、3.8.3、2.7.3、1.120.1、9.14.4I、8.10.3F、9.7.2IF、9.14.4、8.10.3、9.7.2、9.7.2C-Ser、9.14.4C-Ser、8.10.3C-Ser、8.10.3-CG2、9.7.2-CG2、9.7.2-CG4、9.14.4-CG2、9.14.4-CG4、9.14.4-Ser、9.7.2-Ser、8.10.3-Ser、8.10.3-CG4、8.10.3FG1 和 9.14.4G1；

c) (a) 的重链和 (b) 的轻链；或

d)(a) 的重链和 (b) 的轻链，其中所述重链和轻链序列选自同一抗体。

9. 权利要求 1 的方法，其中所述单克隆抗体或抗原结合部分包含：

a) 选自如下一组的抗体的重链可变区 (VH) 氨基酸序列：抗体 252、88、100、3.8.3、2.7.3、1.120.1、9.14.4I、8.10.3F、9.7.2IF、9.14.4、8.10.3、9.7.2、9.7.2C-Ser、9.14.4C-Ser、8.10.3C-Ser、8.10.3-CG2、9.7.2-CG2、9.7.2-CG4、9.14.4-CG2、9.14.4-CG4、9.14.4-Ser、9.7.2-Ser、8.10.3-Ser、8.10.3-CG4、8.10.3FG1 和 9.14.4G1，但不具有信号序列；

b) 选自如下一组的抗体的轻链可变区 (VL) 氨基酸序列：抗体 252、88、100、3.8.3、2.7.3、1.120.1、9.14.4I、8.10.3F、9.7.2IF、9.14.4、8.10.3、9.7.2、9.7.2C-Ser、9.14.4C-Ser、8.10.3C-Ser、8.10.3-CG2、9.7.2-CG2、9.7.2-CG4、9.14.4-CG2、9.14.4-CG4、9.14.4-Ser、9.7.2-Ser、8.10.3-Ser、8.10.3-CG4、8.10.3FG1 和 9.14.4G1，但不具有信号序列；

c)(a) 的 VH 氨基酸序列和 (b) 的 VL 氨基酸序列；或

d)(a) 的 VH 氨基酸序列和 (b) 的 VL 氨基酸序列，其中所述 VH 和 VL 来自同一抗体。

10. 权利要求 1 的方法，其中所述抗体或抗原结合部分包含选自如下一组的抗体的一或多个 FR1、FR2、FR3 或 FR4 氨基酸序列：抗体 252、88、100、3.8.3、2.7.3、1.120.1、9.14.4I、8.10.3F、9.7.2IF、9.14.4、8.10.3、9.7.2、9.7.2C-Ser、9.14.4C-Ser、8.10.3C-Ser、8.10.3-CG2、9.7.2-CG2、9.7.2-CG4、9.14.4-CG2、9.14.4-CG4、9.14.4-Ser、9.7.2-Ser、8.10.3-Ser、8.10.3-CG4、8.10.3FG1 和 9.14.4G1。

11. 权利要求 1-10 中任一项的方法，其中所述抗体或抗原结合部分的重链无 C 末端赖氨酸。

12. 权利要求 1 的方法，其中所述抗体包含：

a) 重链氨基酸序列，其与单克隆抗体 252、88、100、3.8.3、2.7.3、1.120.1、9.14.4I、8.10.3F、9.7.2IF、9.14.4、8.10.3、9.7.2、9.7.2C-Ser、9.14.4C-Ser、8.10.3C-Ser、8.10.3-CG2、9.7.2-CG2、9.7.2-CG4、9.14.4-CG2、9.14.4-CG4、9.14.4-Ser、9.7.2-Ser、8.10.3-Ser、8.10.3-CG4、8.10.3FG1 或 9.14.4G1 的重链氨基酸序列具有至少 90％的相同性，但不具有信号序列；

b) 轻链氨基酸序列，其与单克隆抗体 252、88、100、3.8.3、2.7.3、1.120.1、9.14.4I、8.10.3F、9.7.2IF、9.14.4、8.10.3、9.7.2、9.7.2C-Ser、9.14.4C-Ser、8.10.3C-Ser、8.10.3-CG2、9.7.2-CG2、9.7.2-CG4、9.14.4-CG2、9.14.4-CG4、9.14.4-Ser、9.7.2-Ser、8.10.3-Ser、8.10.3-CG4、8.10.3FG1 或 9.14.4G1 的轻链氨基酸序列具有至少 90％的相同性，但不具有信号序列；或

c)(a) 的重链氨基酸序列和 (b) 的轻链氨基酸序列。

13. 权利要求 1 的方法，其中所述抗体或抗原结合部分包含：

a) 重链氨基酸序列，其与单克隆抗体 8.10.3F 的重链氨基酸序列具有至少 90％的相同性，但不具有信号序列；

b) 轻链氨基酸序列，其与单克隆抗体 8.10.3F 的轻链氨基酸序列具有至少 90％的相同性；

c)(a) 的重链氨基酸序列和 (b) 的轻链氨基酸序列；或

d) 重链氨基酸序列和轻链氨基酸序列，它们一起与抗体 8.10.3F 的重链氨基酸序列和轻链氨基酸序列具有至少 90％的相同性。

14. 权利要求 13 的方法，其中所述抗体或抗原结合部分的重链氨基酸序列与单克隆抗体 8.10.3F 的重链氨基酸序列具有至少 95％的相同性，但不具有信号序列。

15. 权利要求 13 的方法，其中所述抗体或抗原结合部分的轻链氨基酸序列与单克隆抗体 8.10.3F 的轻链氨基酸序列具有至少 95％的相同性，但不具有信号序列。

16. 权利要求 13 的方法，其中所述抗体或抗原结合部分的重链氨基酸序列和轻链氨基酸序列两者一起与单克隆抗体 8.10.3F 的重链氨基酸序列和轻链氨基酸序列具有至少 95％的相同性，但不具有信号序列。

17. 权利要求 13 的方法，其中所述抗体或抗原结合部分的重链氨基酸序列与单克隆抗体 8.10.3F 的重链氨基酸序列具有至少 97％的相同性，但不具有信号序列。

18. 权利要求 13 的方法，其中所述抗体或抗原结合部分的轻链氨基酸序列与单克隆抗体 8.10.3F 的轻链氨基酸序列具有至少 97％的相同性，但不具有信号序列。

19. 权利要求 13 的方法，其中所述抗体或抗原结合部分的重链氨基酸序列和轻链氨基酸序列两者一起与单克隆抗体 8.10.3F 的重链氨基酸序列和轻链氨基酸序列具有至少 97％的相同性，但不具有信号序列。

20. 权利要求 1 的方法，其中所述抗体是单克隆抗 M-CSF 抗体 8.10.3F。

21. 权利要求 1 的方法，其中所述抗体或抗原结合部分包含抗体 8.10.3F 的重链 CDR1、CDR2、CDR3 和轻链 CDR1、CDR2、CDR3。

22. 权利要求 1 的方法，其中所述抗体或抗原结合部分包含抗体 8.10.3F 的可变重链和可变轻链部分。

23. 权利要求 1 的方法，其中所述狼疮是系统性红斑狼疮 (SLE)。

24. 权利要求 1 的方法，其中所述狼疮是狼疮性肾炎。

25. 权利要求 1 的方法，其中所述狼疮是皮肤狼疮。

26. 权利要求 1 的方法，其中通过检查患者情况的改变来确定抗 M-CSF 抗体治疗狼疮的功效，所述情况选自症状、生物学标记物、组织学样品和生理条件。

27. 权利要求 26 的方法，其中检查患者选自以下一组的情况改变：皮肤损害、蛋白尿、淋巴结病、血清 M-CSF 水平、抗 dsDNA 抗体水平、CD14+CD16+ 单核细胞群、骨细胞标记物和肾脏病理学。

28. 权利要求 27 的方法，其中通过检查选自以下一组的情况来确定肾脏病理学：巨噬细胞浸润、炎性浸润、蛋白管型、肾小球簇大小、肾小球 IgG 沉积和 C3 沉积。

29. 权利要求 26 的方法，其中可检查患者选自以下一组的一或多种生物学标记物的改变：表 1C 中的基因、红细胞沉降率 (ESR)、C- 反应蛋白 (CRP)、补体 (C3/C4)、Ig 水平 (IgA、IgM、IgG)、抗核抗体 (ANA)、可提取性核抗原 (ENA) 和抗 dsDNA 抗体。

30. 权利要求 1-23 任一项的抗 M-CSF 抗体在治疗有需要的患者的狼疮中的用途。

31. 权利要求 30 的用途，其中所述狼疮是 SLE。

32. 权利要求 30 的用途，其中所述狼疮是狼疮性肾炎。

33. 权利要求 30 的用途，其中所述狼疮是皮肤狼疮。

34. 权利要求 1-23 任一项的抗 M-CSF 抗体在制备治疗狼疮的药物中的用途。

35. 权利要求 34 的用途，其中所述狼疮是 SLE。

36. 权利要求 34 的用途，其中所述狼疮是狼疮性肾炎。

37. 权利要求 34 的用途，其中所述狼疮是皮肤狼疮。

使用抗 M-CSF 抗体治疗炎性疾病的方法

[0001] 相关申请

[0002] 本申请要求 2011 年 11 月 8 日提交的美国临时申请 No.61/557,175 的权益，其全部内容通过引用并入本申请。

[0003] 发明背景

[0004] 巨噬细胞集落刺激因子 (M-CSF) 是称为集落刺激因子 (CSF) 的蛋白质家族的成员。M-CSF 是分泌型的或细胞表面的糖蛋白，其由通过二硫键连接的两个亚基组成，总分子量为 40-90kD(Stanley E.R.,et al.,Mol.Reprod.Dev.,46:4-10(1997))。类似于其他 CSF，M-CSF 由巨噬细胞、单核细胞和人关节组织细胞例如软骨细胞和滑膜成纤维细胞等应答于例如白介素 -1 或肿瘤坏死因子 α 等蛋白质而产生。M-CSF 刺激多能造血前体干细胞形成巨噬细胞集落 (Stanley E.R.,et al.,Mol.Reprod.Dev.,46:4-10(1997))。

[0005] M-CSF 典型地结合其受体 c-fms 而发挥其生物学效应。c-fms 含有五个细胞外 Ig 结构域，一个跨膜结构域和具有两个激酶结构域的细胞内结构域。M-CSF 结合 c-fms 之后，受体发生同二聚化并启动一连串信号转导途径，包括 JAK/STAT、PI3K 和 ERK 途径。

[0006] M-CSF 是单核细胞 / 巨噬细胞功能、活化和存活的重要调节物。多种动物模型已经证实了 M-CSF 参与多种疾病，包括类风湿性关节炎 (RA) 和癌症。巨噬细胞是 RA 中的关键效应细胞。已经发现 RA 中滑膜的巨噬细胞浸润程度与潜在的关节破坏程度密切相关。由单核细胞 / 巨噬细胞、成纤维细胞和内皮细胞在类风湿关节中内源性产生的 M-CSF 作用于单核细胞 / 巨噬细胞系列的细胞，促进它们的存活并分化为具有骨破坏性的破骨细胞，并增强促炎性细胞功能，例如细胞毒性、过氧化物产生、吞噬作用、趋化作用和产生次级细胞因子。例如，以 M-CSF 处理无乳链球菌超声处理物诱导的实验性关节炎大鼠模型导致加重的病理学表现 (Abd,A.H.,et al.,Lymphokine Cytokine Res.10:43-50(1991))。类似地，给胶原诱导的关节炎 (CIA) 小鼠模型 (其是一种 RA 模型) 皮下注射 M-CSF，导致 RA 疾病症状明显加重 (Campbell I.K.,et al.,J.Leuk.Biol.68:144-150(2000))。不仅如此，对 RA 和其他自身免疫性疾病高度易感的 MRL/lpr 小鼠具有升高的基线 M-CSF 血清浓度 (Yui M.A.,et al.,Am.J.Pathol.139:255-261(1991))。通过使用 M-CSF 中和性小鼠单克隆抗体显著降低了已建立的疾病的严重程度，证实了内源性 M-CSF 对于维持 CIA 是必需的这一事实 (Campbell I.K.,et al.,J.Leuk.Biol.68:144-150(2000))。

[0007] 对于癌症，在小鼠中用反义寡核苷酸抑制集落刺激因子，通过减慢巨噬细胞介导的 ECM 破坏抑制了胚胎和结肠肿瘤异种移植物的肿瘤生长 (Seyedhossein,A.,et al.,Cancer Research,62:5317-5324(2002))。

[0008] M-CSF 结合于 c-fms 并随后活化单核细胞 / 巨噬细胞，这在多种疾病状态中具有重要性。除了 RA 和癌症，M-CSF 相关疾病状态的其他实例包括骨质疏松、破坏性关节炎、动脉粥样化形成、肾小球肾炎、川崎病、HIV-1 感染，在这些疾病中，单核细胞 / 巨噬细胞和有关细胞类型发挥了作用。例如，破骨细胞类似于巨噬细胞，并在一定程度上受到 M-CSF 的调控。在破骨细胞成熟的初始阶段，M-CSF 所诱导的生长和分化信号对于其随后在骨骼中发挥破骨活性是必需的。

[0009] 破骨细胞介导的骨丢失，既可以是局灶性骨侵蚀，也可以是更加弥漫性的近关节处骨质疏松的形式，其是 RA 的难以解决的主要问题。这种骨丢失的后果包括关节变形、功能丧失、骨折风险增加和死亡率升高。M-CSF 对于破骨细胞形成具有独特的必要性，在关节炎动物模型中实验性阻断该细胞因子成功地消除了关节破坏。已知类似的破坏途径也在其他形式的破坏性关节炎例如牛皮癣关节炎中发挥作用，并可能代表了类似的干预措施的靶点。

[0010] 绝经后骨丢失继发于因雌激素缺乏所导致的破骨细胞介导的过度骨再吸收且骨形成不足的缺陷型骨重塑。在小鼠中已经发现，使用阻断性抗体体内中和 M-CSF 可完全防止卵巢切除术所诱导的破骨细胞数量升高、骨吸收升高和随后的骨丢失。

[0011] 一些线索表明，M-CSF 在动脉粥样化形成以及动脉壁机械创伤后的增殖性内膜增生中发挥核心作用。已经发现，粥样硬化病变中的所有主要细胞类型均表达 M-CSF，并且暴露于氧化的脂蛋白可使之进一步上调。使用中和性 c-fms 抗体阻断 M-CSF 信号途径，降低了巨噬细胞衍生的泡沫细胞在以高脂饮食饲养的载脂蛋白 E 缺陷小鼠的主动脉根部的聚集。

[0012] 在实验性肾小球肾炎和人肾小球肾炎中均发现，肾小球 M-CSF 表达与局部巨噬细胞聚集、活化和增殖共定位，并与肾小球损伤和蛋白尿的程度相关。通过使用针对其受体 c-fms 的抗体阻断 M-CSF 信号途径，显著下调了小鼠中实验性单侧输尿管结扎所诱导的肾脏炎症反应过程中的局部巨噬细胞聚集。

[0013] 川崎病 (KD) 是一种原因不明的急性发热性小儿血管炎。其最常见和严重的并发症累及冠状血管，表现为动脉瘤扩张。川崎病的急性期，血清 M-CSF 水平显著升高，在静脉输注免疫球蛋白治疗后正常。巨细胞动脉炎 (GCA) 是一种炎性血管病变，主要出现在老年患者，其中 T 细胞和巨噬细胞浸润中等和大动脉壁，引起包括继发于动脉阻塞的失明和中风等临床后果。在血管病变处存在升高水平的来自巨噬细胞的炎性介质是巨噬细胞活跃参与 GCA 的证据。

[0014] 已经报道了 M-CSF 导致人单核细胞来源的巨噬细胞在体外更加易感于 HIV-1 感染。近期研究发现，M-CSF 增加了单核细胞来源的巨噬细胞被感染的频率、每个感染细胞所表达的 HIV mRNA 的量以及每个感染培养物所表达的原病毒 DNA 水平。

[0015] 鉴于 M-CSF 在各种疾病中的作用，亟需抑制 M-CSF 活性的方法。

[0016] 迫切需要治疗性抗 M-CSF 抗体。

发明内容

[0017] 本发明提供分离的特异性结合人 M-CSF 并作为 M-CSF 拮抗剂的人抗体或其抗原结合部分以及包含所述抗体或部分的组合物。

[0018] 本发明还提供组合物，其包含有效用于此种治疗的抗 M-CSF 抗体的重链和 / 或轻链、其可变区、或其抗原结合部分，或编码本发明的抗体、抗体链或其可变区的核酸分子，以及药用可接受载体。在某些实施方式中，所述组合物可进一步包含其他组分，例如治疗剂或诊断剂。本发明还提供诊断和治疗方法。在某些实施方式中，所述组合物以治疗或预防特定疾病或病症所需的治疗有效量使用。

[0019] 本发明还提供以有效量的本发明的抗 M-CSF 抗体或其抗原结合部分、编码所述抗体或其重链和 / 或轻链、可变区或抗原结合部分的核酸治疗或预防多种疾病和病症的方

法，例如但不限于，狼疮、炎症、癌症、动脉粥样化形成、神经系统疾病和心脏病。

[0020] 本发明提供产生抗 M-CSF 抗体或其抗原结合部分的分离的细胞系，例如杂交瘤。

[0021] 本发明还提供编码抗 M-CSF 抗体的重链和 / 或轻链、其可变区、或其抗原结合部分的核酸分子。

[0022] 本发明提供包含所述核酸分子的载体和宿主细胞、以及重组产生由所述核酸分子编码的多肽的方法。

[0023] 本发明还提供表达所述抗 M-CSF 抗体的重链和 / 或轻链或其抗原结合部分的非人转基因动物或植物。

[0024] 本申请以及公开号为 WO2005/030124 的 PCT 申请 PCT/US2004/029390 详细描述了所述抗 M-CSF 抗体或其抗原结合部分、组合物、细胞系、核酸分子、载体、宿主细胞和是用所述抗 M-CSF 抗体治疗或预防疾病的方法，通过引用将该 PCT 申请的全部内容并入本申请。

附图说明

[0025] 图 1A 和 1B 显示了抗 M-CSF 抗体随时间以剂量相关的方式降低雄猴和雌猴的总单核细胞计数。使用 Abbott Diagnostics Inc. Cell Dyn system 通过光散射方法确定单核细胞计数。以 3.79mL/kg 的剂量体积在大约 5 分钟时间内施用空载体或抗体 8.10.3(0、0.1、1 或 5mg/kg)，监测施用后 24 小时至 3 周的单核细胞计数。

[0026] 图 1A 雄猴。

[0027] 图 1B 雌猴。

[0028] 图 2A 和 2B 显示了抗 M-CSF 抗体处理降低雄猴和雌猴的 CD14+CD16+ 单核细胞百分比。以 3.79mL/kg 的剂量体积在大约 5 分钟时间内施用空载体或抗体 8.10.3(0、0.1、1 或 5mg/kg)，施用后 0-21 天。对每只测试的猴子，在注射 8.10.3 后的 1、3、7、14 和 21 天测定 CD14+CD16+ 亚群内的单核细胞百分比。

[0029] 图 2A 雄猴。

[0030] 图 2B 雌猴。

[0031] 图 3A 和 3B 显示了与单核细胞的测试前水平相比，使用抗体 8.10.3F 和抗体 9.14.4I 的抗 M-CSF 处理在所有剂量上均降低了总单核细胞的百分比改变。

[0032] 图 3A 显示了使用抗体 8.10.3F 得到的实验数据。

[0033] 图 3B 显示了使用抗体 9.14.4I 得到的实验数据。

[0034] 图 4 是来自 26 种抗 M-CSF 抗体的轻链和重链可变区的推定的氨基酸序列与相应的可变区基因的种系氨基酸序列相比的序列比对。抗体序列与种系基因序列之间的差异以粗体字显示。破折号表示与种系相比无改变。每一比对中以下划线标出的序列从左到右代表 FR1、CDR1、FR2、CDR2、FR3、CDR3 和 FR4 的序列。

[0035] 图 4A 显示了抗体 252 的轻链可变区的推定的氨基酸序列 (SEQ ID NO:4 的残基 21-127) 与种系 V_{κ}O12, J_{κ}3 序列 (SEQ ID NO:103) 之间的比对。

[0036] 图 4B 显示了抗体 88 的轻链可变区的推定的氨基酸序列 (SEQ ID NO:8 的残基 21-127) 与种系 V_{κ}O12, J_{κ}3 序列 (SEQ ID NO:103) 之间的比对。

[0037] 图 4C 显示了抗体 100 的轻链可变区的推定的氨基酸序列 (SEQ ID NO:12 的残基 21-127) 与种系 V_{κ}L2, J_{κ}3 序列 (SEQ ID NO:107) 之间的比对。

[0038] 图 4D 显示了抗体 3.8.3 的轻链可变区的推定的氨基酸序列 (SEQ ID NO:16 的残基 23-130) 与种系 V_{κ}L5, J_{κ}3 序列 (SEQ ID NO:109) 之间的比对。

[0039] 图 4E 显示了抗体 2.7.3 的轻链可变区的推定的氨基酸序列 (SEQ ID NO:20 的残基 23-130) 与种系 V_{κ}L5, J_{κ}4 序列 (SEQ ID NO:117) 之间的比对。

[0040] 图 4F 显示了抗体 1.120.1 的轻链可变区的推定的氨基酸序列 (SEQ ID NO:24 的残基 21-134) 与种系 V_{κ}B3, J_{κ}1 序列 (SEQ ID NO:112) 之间的比对。

[0041] 图 4G 显示了抗体 252 的重链可变区的推定的氨基酸序列 (SEQ ID NO:2 的残基 20-136) 与种系 V_H3-11, D_H7-27 J_H6 序列 (SEQ ID NO:106) 之间的比对。

[0042] 图 4H 显示了抗体 88 的重链可变区的推定的氨基酸序列 (SEQ ID NO:6 的残基 20-138) 与种系 V_H3-7, D_H6-13, J_H4 序列 (SEQ ID NO:105) 之间的比对。

[0043] 图 4I 显示了抗体 100 的重链可变区的推定的氨基酸序列 (SEQ ID NO:10 的残基 20-141) 与种系 V_H3-23, D_H1-26, J_H4 序列 (SEQ ID NO:104) 之间的比对。

[0044] 图 4J 显示了抗体 3.8.3 的重链可变区的推定的氨基酸序列 (SEQ ID NO:14 的残基 20-135) 与种系 V_H3-11, D_H7-27, J_H4 序列 (SEQ ID NO:108) 之间的比对。

[0045] 图 4K 显示了抗体 2.7.3 的重链可变区的推定的氨基酸序列 (SEQ ID NO:18 的残基 20-137) 与种系 V_H3-33, D_H1-26, J_H4 序列 (SEQ ID NO:110) 之间的比对。

[0046] 图 4L 显示了抗体 1.120.1 的重链可变区的推定的氨基酸序列 (SEQ ID NO:22 的残基 20-139) 与种系 V_H1-18, D_H4-23, J_H4 序列 (SEQ ID NO:111) 之间的比对。

[0047] 图 4M 显示了抗体 8.10.3 的轻链可变区的推定的氨基酸序列 (SEQ ID NO:44 的残基 21-129) 与种系 V_{κ}A27, J_{κ}4 序列 (SEQ ID NO:114) 之间的比对。

[0048] 图 4N 显示了抗体 8.10.3 的重链可变区的推定的氨基酸序列 (SEQ ID NO:30 的残基 20-141) 与种系 V_H3-48, D_H1-26, J_H4b 序列 (SEQ ID NO:113) 之间的比对。

[0049] 图 4O 显示了抗体 9.14.4 的轻链可变区的推定的氨基酸序列 (SEQ ID NO:28 的残基 23-130) 与种系 V_{κ}O12, J_{κ}3 序列 (SEQ ID NO:103) 之间的比对。

[0050] 图 4P 显示了抗体 9.14.4 的重链可变区的推定的氨基酸序列 (SEQ ID NO:38 的残基 20-135) 与种系 V_H3-11, D_H7-27, J_H4b 序列 (SEQ ID NO:116) 之间的比对。

[0051] 图 4Q 显示了抗体 9.7.2 的轻链可变区的推定的氨基酸序列 (SEQ ID NO:48 的残基 23-130) 与种系 V_{κ}O12, J_{κ}3 序列 (SEQ ID NO:103) 之间的比对。

[0052] 图 4R 显示了抗体 9.7.2 的重链可变区的推定的氨基酸序列 (SEQ ID NO:46 的残基 20-136) 与种系 V_H3-11, D_H6-13, J_H6b 序列 (SEQ ID NO:115) 之间的比对。

[0053] 图 4S 显示了抗体 9.14.4I 的轻链可变区的推定的氨基酸序列 (SEQ ID NO:28 的残基 23-130) 与种系 V_{κ}O12J_{κ}3 序列 (SEQ ID NO:103) 之间的比对。

[0054] 图 4T 显示了抗体 9.14.4I 的重链可变区的推定的氨基酸序列 (SEQ ID NO:26 的残基 20-135) 与种系 V_H3-11, D_H7-27, J_H4b 序列 (SEQ ID NO:116) 之间的比对。

[0055] 图 4U 显示了抗体 8.10.3F 的轻链可变区的推定的氨基酸序列 (SEQ ID NO:32 的残基 21-129) 与种系 V_{κ}A27, J_{κ}4 序列 (SEQ ID NO:114) 之间的比对。

[0056] 图 4V 显示了抗体 8.10.3F 的重链可变区的推定的氨基酸序列 (SEQ ID NO:30 的残基 20-141) 与种系 V_H3-48, D_H1-26, J_H4b 序列 (SEQ ID NO:113) 之间的比对。

[0057] 图 4W 显示了抗体 9.7.2IF 的轻链可变区的推定的氨基酸序列 (SEQ ID NO:36 的

残基 23-130）与种系 $V_{\kappa}012$, $J_{\kappa}3$ 序列（SEQ ID NO:103）之间的比对。

[0058] 图 4X 显示了抗体 9.7.2IF 的重链可变区的推定的氨基酸序列（SEQ ID NO:34 的残基 20-136）与种系 V_H3-11, D_H6-13, J_H6b 序列（SEQ ID NO:115）之间的比对。

[0059] 图 4Y 显示了抗体 9.7.2C-Ser 的轻链可变区的推定的氨基酸序列（SEQ ID NO:52 的残基 23-130）与种系 $V_{\kappa}012$, $J_{\kappa}3$ 序列（SEQ ID NO:103）之间的比对。

[0060] 图 4Z 显示了抗体 9.7.2C-Ser 的重链可变区的推定的氨基酸序列（SEQ ID NO:50 的残基 20-136）与种系 V_H3-11, D_H6-13, J_H6b 序列（SEQ ID NO:115）之间的比对。

[0061] 图 4AA 显示了抗体 9.14.4C-Ser 的轻链可变区的推定的氨基酸序列（SEQ ID NO:56 的残基 23-130）与种系 $V_{\kappa}012$, $J_{\kappa}3$ 序列（SEQ ID NO:103）。

[0062] 图 4BB 显示了抗体 9.14.4C-Ser 的重链可变区的推定的氨基酸序列（SEQ ID NO:54 的残基 20-135）与种系 V_H3-11, D_H7-27, J_H4b 序列（SEQ ID NO:116）之间的比对。

[0063] 图 4CC 显示了抗体 8.10.3C-Ser 的轻链可变区的推定的氨基酸序列（SEQID NO:60 的残基 21-129）与种系 $V_{\kappa}A27$, $J_{\kappa}4$ 序列（SEQ ID NO:114）之间的比对。

[0064] 图 4DD 显示了抗体 8.10.3C-Ser 的重链可变区的推定的氨基酸序列（SEQ ID NO:58 的残基 20-141）与种系 V_H3-48, D_H1-26, J_H4b 序列（SEQ ID NO:113）之间的比对。

[0065] 图 4EE 显示了抗体 8.10.3-CG2 的轻链可变区的推定的氨基酸序列（SEQ ID NO:60 的残基 21-129）与种系 $V_{\kappa}A27$, $J_{\kappa}4$ 序列（SEQ ID NO:114）之间的比对。

[0066] 图 4FF 显示了抗体 8.10.3-CG2 的重链可变区的推定的氨基酸序列（SEQ ID NO:62 的残基 20-141）与种系 V_H3-48, D_H1-26, J_H4b 序列（SEQ ID NO:113）之间的比对。

[0067] 图 4GG 显示了抗体 9.7.2-CG2 的轻链可变区的推定的氨基酸序列（SEQ ID NO:52 的残基 23-130）与种系 $V_{\kappa}012$, $J_{\kappa}3$ 序列（SEQ ID NO:103）之间的比对。

[0068] 图 4HH 显示了抗体 9.7.2-CG2 的重链可变区的推定的氨基酸序列（SEQ ID NO:66 的残基 20-136）与种系 V_H3-11, D_H6-13, J_H6b 序列（SEQ ID NO:115）之间的比对。

[0069] 图 4II 显示了抗体 9.7.2-CG4 的轻链可变区的推定的氨基酸序列（SEQ ID NO:52 的残基 23-130）与种系 $V_{\kappa}012$, $J_{\kappa}3$ 序列（SEQ ID NO:103）之间的比对。

[0070] 图 4JJ 显示了抗体 9.7.2-CG4 的重链可变区的推定的氨基酸序列（SEQ ID NO:70 的残基 20-135）与种系 V_H3-11, D_H6-13, J_H6b 序列（SEQ ID NO:115）之间的比对。

[0071] 图 4KK 显示了抗体 9.14.4-CG2 的轻链可变区的推定的氨基酸序列（SEQ ID NO:56 的残基 23-130）与种系 $V_{\kappa}012$, $J_{\kappa}3$ 序列（SEQ ID NO:103）之间的比对。

[0072] 图 4LL 显示了抗体 9.14.4-CG2 的重链可变区的推定的氨基酸序列（SEQ ID NO:74 的残基 20-135）与种系 V_H3-11, D_H7-27, J_H4b 序列（SEQ ID NO:116）之间的比对。

[0073] 图 4MM 显示了抗体 9.14.4-CG4 的轻链可变区的推定的氨基酸序列（SEQ ID NO:56 的残基 23-130）与种系 $V_{\kappa}012$, $J_{\kappa}3$ 序列（SEQ ID NO:103）之间的比对。

[0074] 图 4NN 显示了抗体 9.14.4-CG4 的重链可变区的推定的氨基酸序列（SEQ ID NO:78 的残基 20-135）与种系 V_H3-11, D_H7-27, J_H4b 序列（SEQ ID NO:116）之间的比对。

[0075] 图 4OO 显示了抗体 9.14.4-Ser 的轻链可变区的推定的氨基酸序列（SEQ ID NO:28 的残基 23-130）与种系 $V_{\kappa}012$, $J_{\kappa}3$ 序列（SEQ ID NO:103）之间的比对。

[0076] 图 4PP 显示了抗体 9.14.4-Ser 的重链可变区的推定的氨基酸序列（SEQ ID NO:82 的残基 20-135）与种系 V_H3-11, D_H7-27, J_H4b 序列（SEQ ID NO:116）之间的比对。

[0077] 图 4QQ 显示了抗体 9.7.2-Ser 的轻链可变区的推定的氨基酸序列 (SEQ ID NO:48 的残基 23-130) 与种系 V_{κ}012,J_{κ}3 序列 (SEQ ID NO:103) 之间的比对。

[0078] 图 4RR 显示了抗体 9.7.2-Ser 的重链可变区的推定的氨基酸序列 (SEQ ID NO:86 的残基 20-136) 与种系 V_H3-11,D_H6-13,J_H6b 序列 (SEQ ID NO:115) 之间的比对。

[0079] 图 4SS 显示了抗体 8.10.3-Ser 的轻链可变区的推定的氨基酸序列 (SEQ ID NO:44 的残基 21-129) 与种系 V_{κ}A27,J_{κ}4 序列 (SEQ ID NO:114) 之间的比对。

[0080] 图 4TT 显示了抗体 8.10.3-Ser 的重链可变区的推定的氨基酸序列 (SEQ ID NO:90 的残基 20-141) 与种系 V_H3-48,D_H1-26,J_H4b 序列 (SEQ ID NO:113) 之间的比对。

[0081] 图 4UU 显示了抗体 8.10.3-CG4 的轻链可变区的推定的氨基酸序列 (SEQ ID NO:60 的残基 21-129) 与种系 V_{κ}A27,J_{κ}4 序列 (SEQ ID NO:114) 之间的比对。

[0082] 图 4VV 显示了抗体 8.10.3-CG4 的重链可变区的推定的氨基酸序列 (SEQ ID NO:94 的残基 20-141) 与种系 V_H3-48,D_H1-26,J_H4b 序列 (SEQ ID NO:113) 之间的比对。

[0083] 图 4WW 显示了抗体 9.14.4G1 的轻链可变区的推定的氨基酸序列 (SEQ ID NO:28 的残基 23-130) 与种系 V_{κ}012J_{κ}3 序列 (SEQ ID NO:103) 之间的比对。

[0084] 图 4XX 显示了抗体 9.14.4G1 的重链可变区的推定的氨基酸序列 (SEQ ID NO:102 的残基 20-135) 与种系 V_H3-11,D_H7-27,J_H4b 序列 (SEQ ID NO:116) 之间的比对。

[0085] 图 4YY 显示了抗体 8.10.3FG1 的轻链可变区的推定的氨基酸序列 (SEQ ID NO:32 的残基 21-129) 与种系 V_{κ}A27,J_{κ}4 序列 (SEQ ID NO:114) 之间的比对。

[0086] 图 4ZZ 显示了抗体 8.10.3FG1 的重链可变区的推定的氨基酸序列 (SEQ ID NO:98 的残基 20-141) 与种系 V_H3-48,D_H1-26,J_H4b 序列 (SEQ ID NO:113) 之间的比对。

[0087] 图 5 显示了抗 M-CSF 抗体对小鼠 MRL-lpr 狼疮模型淋巴结病发展的影响。按照实施例所述,给小鼠 (n = 10/ 组) 施用盐水 (菱形)、抗 M-CSF Ab5A1 (方形)、CHOCK IgG1 (三角) 或 CTLA-4Ig(X),3x/ 周,共 12 周,并对淋巴结病评分。*抗 M-CSF 处理组显著不同于盐水 ($p<0.05$)。**抗 M-CSF 处理组显著不同于盐水和 CTLA-4Ig($p<0.05$)。抗 M-CSF 组与 CHOCK IgG1 处理组无显著差异 ($p = 0.065$,6 周至 12 周)。

[0088] 图 6 显示了抗 M-CSF 抗体对小鼠 MRL-lpr 狼疮模型皮肤病变发展的影响。按照实施例所述,给小鼠 (n = 10/ 组) 施用盐水 (菱形)、抗 M-CSF Ab5A1 (方形)、CHOCK IgG1 (三角) 或 CTLA-4Ig(X),3x/ 周,共 12 周,并对皮肤病变评分。*抗 M-CSF 处理组显著不同于盐水 ($p<0.05$)。**抗 M-CSF 处理组显著不同于盐水和 CTLA-4Ig($p<0.05$)。抗 M-CSF 组与 CHOCK IgG1 处理组无显著差异 ($p = 0.065$,6 周至 12 周)。

[0089] 图 7 显示了抗 M-CSF 抗体对小鼠 MRL-lpr 狼疮模型抗 dsDNA 自身抗体发展的影响。以盐水 (菱形)、CTLA-4Ig (方形)、抗 M-CSF Ab5A1 (三角) 或 CHOCK IgG1 同种型对照 (X) 处理小鼠,在 4 个时间点测定抗 dsDNA 抗体滴度。*CTLA-4Ig 显著不同于盐水和抗 M-CSF($p<0.05$)。**抗 M-CSF 处理组显著不同于 CHOCK IgG1($p<0.05$)。

[0090] 图 8 显示了抗 M-CSF 抗体对小鼠 MRL-lpr 狼疮模型肾小球核面积和 C3 沉积的影响。在研究结束时收集以盐水、CTLA-4Ig、抗 M-CSF Ab5A1 或 CHOCK IgG1 同种型对照处理的小鼠的肾脏,显微镜下检查肾小球核面积 (左) 和 C3 沉积免疫组织化学染色 (右)。数据条形代表平均积分,短线是标准误。

[0091] 图 9 显示了抗 M-CSF 抗体对小鼠 NZBWF1/J 狼疮模型蛋白尿发展的影响。以盐水

（方形）、CHOCK IgG1 同种型对照（三角）或抗 M-CSF 抗体（圆形）处理小鼠，使用以下量表对蛋白尿进行评分：0 ＝阴性；1 ＝微量；2 ＝ >30mg/dL；3 ＝ >100mg/dL；4 ＝ >300mg/dL；5 ＝ >2000mg/dL。图 9A 显示了各组的蛋白尿平均积分，每两周测定一次。图 9B 显示了个体小鼠第 10 周时的蛋白尿积分。

[0092] 图 10 显示了抗 M-CSF 抗体对小鼠 NZBWF1/J 狼疮模型抗 dsDNA 抗体滴度的影响。如实施例所述，以盐水（圆形）、CHOCK IgG1 同种型对照（三角）或抗 M-CSF 抗体（菱形）处理小鼠，通过 ELISA 测定抗 dsDNA 抗体水平。图 10A 显示了第 6 周时的抗体滴度。图 10B 显示了第 10 周时的抗体滴度。

[0093] 图 11 显示了抗 M-CSF 抗体对小鼠 NZBWF1/J 狼疮模型血清 M-CSF 水平的影响。以盐水（圆形）、同种型对照（方形）或抗 M-CSF（三角）处理小鼠，在研究结束时收集血清，通过特异性 ELISA 测定 M-CSF 的血清水平。

[0094] 图 12 显示了抗 M-CSF 抗体对 NZBWF1/J 小鼠肾脏中免疫复合物沉积和巨噬细胞浸润的影响。以盐水、CHOCK IgG1 对照或抗 M-CSF 抗体处理小鼠，条形代表每组评价肾脏免疫组织化学染色积分，短线表示标准误。

[0095] 图 13 显示了给健康对象施用单剂量静脉注射液后平均血清抗体 8.10.3F 浓度随时间的变化。上图和下图分别是线性和半对数刻度。图例是以 mg 表示的抗体剂量。700 小时后的浓度为 0（低于定量低限）或代表 <3subjects。

[0096] 图 14 显示了给健康对象施用单剂量静脉注射液后抗体 8.10.3F Cmax（上图）和 AUC(0- ∞)（下图）的数值。左侧显示了观测值；右侧显示了剂量 - 标准化值。圆形是个体对象，菱形是算数平均值。

[0097] 图 15 显示了给健康对象施用单剂量 100-mg 静脉注射液后抗体 8.10.3F（空白方形）和 M-CSF(X) 的平均浓度。

[0098] 图 16 显示了给健康对象施用单剂量静脉注射液后，在研究的第 28 天时 $CD14^{+}16^{+}$ 单核细胞的剂量应答。

[0099] 图 17 显示了给健康对象施用单剂量 100-mg 静脉注射液后 $CD14^{+}16^{+}$ 的时间应答。

[0100] 图 18 显示了给健康对象施用单剂量 100-mg 静脉注射液后的平均抗体 8.10.3F（空白方形）浓度和 $CD14^{+}16^{+}$ 单核细胞计数 (X)。

[0101] 图 19 显示了给健康对象施用单剂量 100-mg 静脉注射液后的平均抗体 8.10.3F 浓度（空白方形）和 uNTX-1(X)。

[0102] 发明详述

[0103] 定义和通用技术

[0104] 除非特别指明，否则本发明中使用的科技术语应具有本领域人员通常理解的含义。此外，除非上下文有要求，否则单数术语应包括多个，且复数术语应包括单个。通常，涉及本申请所述的细胞和组织培养、分子生物学、免疫学、微生物学、遗传学以及蛋白质和核酸化学和杂交的命名法和技术均是本领域熟知和常用的。

[0105] 除非另有说明，本发明的方法和技术通常根据本领域熟知的常规方法实施，这些常规方法描述于本说明书中所引用和讨论的各种通用的以及更加具体的参考文献中。参见例如 Sambrook et al.,Molecular Cloning:A Laboratory Manual,2d ed.,Cold Spring Harbor Laboratory Press,Cold Spring Harbor,N.Y.(1989) 和 Ausubel et al.,Current

Protocols in Molecular Biology,Greene Publishing Associates(1992)，以及 Harlow and Lane Antibodies:A Laboratory Manual,Cold Spring Harbor Laboratory Press,Cold Spring Harbor,N.Y.(1990)，通过引用将这些文献并入本文。酶反应和纯化技术依照生产商的说明书，以本领域通常采用或本申请所描述的那样进行。涉及本申请所描述的分析化学、有机合成化学以及医学和药物化学的命名法和实验室流程和技术均是本领域所熟知和常用的。采用标准技术进行化合物合成、化合物分析、药物制备、制剂和递送、以及患者治疗。

[0106] 除非另有说明，否则以下术语应理解为具有如下含义：

[0107] 术语“多肽”涵盖天然的或人工合成的蛋白质、蛋白质片段和蛋白质序列的多肽类似物。多肽可以是单体或多聚体。

[0108] 术语“分离的蛋白质”、“分离的多肽”或“分离的抗体”是指其来源或者衍生来源具有如下 1-4 种特点的蛋白质、多肽或抗体：(1) 与在其天然状态中伴随的天然关联成分不相关，(2) 没有来自相同物种的其它蛋白质，(3) 由不同物种的细胞表达，或者 (4) 不是天然产生的。因此，化学合成的多肽或者是在与其从中天然产生的细胞不同的细胞系统中合成的多肽是与其天然关联的成分相“分离的”。也可以通过本领域熟知的分离和蛋白质纯化技术使得蛋白质与其天然关联的成分基本上分开。

[0109] 分离的抗体的实例包括使用 M-CSF 经亲和纯化的抗 M-CSF 抗体、由杂交瘤或其他细胞系体外合成的抗 M-CSF 抗体以及来自转基因小鼠的人抗 M-CSF 抗体。

[0110] 当样品的至少大约 60-75％呈现出单一多肽时，该蛋白质或多肽是“基本上纯的”、“基本上均质的”或者“基本上纯化的”。蛋白质或多肽可以是单体或者是多聚体。基本上纯的蛋白质或多肽可典型包含大约 50％、60％、70％、80％或者 90％ w/w 的蛋白质样品，更通常为大约 95％，优选是 99％以上纯的。蛋白质纯度或者均质性可以通过本领域已知的许多手段检测，如蛋白质样品的聚丙烯酰胺凝胶电泳，继以本领域熟知的染料对凝胶进行染色观察到单一多肽条带。对于特定的目的，使用 HPLC 或其他本领域熟知的纯化技术可提供更高的分辨率。

[0111] 术语“多肽片段”在本文中指的是这样的多肽，其具有氨基端和 / 或羧基端的缺失，但剩下的氨基酸序列与天然存在的序列中的相应位置相同。在一些实施方式中，片段的长度为至少 5、6、8 或 10 个氨基酸。在其他一些实施方式中，片段的长度为至少 14、至少 20、至少 50、或至少 70、80、90、100、150 或 200 个氨基酸。

[0112] 术语“多肽类似物”在本文中指的是这样的多肽，其包含与氨基酸序列的一部分基本上相同的节段并具有至少一种以下特性：(1) 在合适的结合条件下特异性结合 M-CSF，(2) 能够抑制 M-CSF。

[0113] 典型地，相对于正常存在的序列，多肽类似物包含保守性氨基酸取代（或插入或缺失）。类似物的长度典型地为至少 20 或 25 个氨基酸，优选地至少 50、60、70、80、90、100、150 或 200 个氨基酸或更长，且通常可与全长多肽一样长。

[0114] 在某些实施方式中，所述抗体或其抗原结合部分的氨基酸取代是这样的取代：(1) 其降低对蛋白水解作用的易感性，(2) 其降低对氧化作用的易感性，(3) 其改变形成蛋白复合物的结合亲和力，或者 (4) 其赋予或改变此类类似物的其他生理化学或功能特性。类似物可包括各种突变蛋白质，其序列不同于正常存在的肽序列。例如，可在正常存在的序列中

造成单个或多个氨基酸取代（优选地保守性氨基酸取代），优选地在多肽的形成分子间接触的结构域之外的部分。

[0115] 保守性氨基酸取代不应明显改变亲本序列的结构特征。例如，置换的氨基酸不应改变形成亲本序列中存在的免疫球蛋白结合结构域的反向平行 β 折叠，或破坏亲本序列特征性的其他类型的二级结构。通常，甘氨酸和脯氨酸类似物不会被用于反向平行 β 折叠中。本领域已知的多肽二级结构和三级结构的实例可参见 Proteins, Structures and Molecular Principles (Creighton, Ed., W. H. Freeman and Company, New York (1984)); Introduction to Protein Structure (C. Branden and J. Tooze, eds., Garland Publishing, New York, N. Y. (1991)); 和 Thornton et al., Nature 354:105 (1991)，通过引用将上述各文献并入本申请。

[0116] 制药业中经常将非肽类似物用作具有与模板肽相类似的特性的药物。这些类型的非肽化合物称为“肽模拟物”或“模拟肽”。Fauchere, J. Adv. Drug Res. 15:29 (1986); Veber and Freidinger, TINS p. 392 (1985); 和 Evans et al., J. Med. Chem. 30:1229 (1987)，通过引用将上述各文献并入本申请。通常借助于计算机化的分子建模而开发此类化合物。可使用结构上类似于具有治疗用途的肽的肽模拟物来产生等同的治疗或预防效果。通常，模拟肽在结构上类似范例多肽 (paradigm polypeptide)（即具有所需的生物化学特性或药理学活性的多肽），例如人抗体，但其一个或多个肽键任选地通过本领域熟知的方法由选自以下一组的键替代：$-CH_2NH-$、$-CH_2S-$、$-CH_2-CH_2-$、-CH ＝ CH-（顺式和反式）、$-COCH_2-$、-CH(OH)CH_2- 和 $-CH_2SO-$。也可用相同类型的 D- 氨基酸系统地取代共有序列的一个或多个氨基酸（如 D- 赖氨酸代替 L 赖氨酸）来生产更稳定的肽。此外，可通过本领域已知的方法产生包含共有序列或基本上相同的共有序列变异的限制性肽 (constrained peptide) (Rizo and Gierasch, Ann. Rev. Biochem. 61:387 (1992)，通过引用并入本申请）；例如通过加入能形成分子内二硫键使肽环化的内部半胱氨酸残基。

[0117] “抗体”指完整的抗体或抗原结合部分，该抗原结合部分与完整的抗体竞争特异性结合。通常参见，Fundamental Immunology, Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N. Y. (1989))（为所有目的，通过引用将其全部内容并入本申请）。可通过重组 DNA 技术或通过对完整抗体的酶或化学裂解产生抗原结合部分。在一些实施方式中，抗原结合部分包括 Fab、Fab'、$F(ab')_2$、Fd、Fv、dAb 和互补性决定区 (CDR) 片段、单链抗体 (scFv)、嵌合抗体、双特异抗体和包含抗体的至少一部分的多肽，该部分足以使多肽特异性结合抗原。

[0118] 从 N- 端至 C- 端，成熟的轻链和重链可变结构域都包含 FR1、CDR1、FR2、CDR2、FR3、CDR3 和 FR4 区。依照 Kabat, Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, Md. (1987and 1991)), Chothia&Lesk, J. Mol. Biol. 196:901-917 (1987) 或 Chothia et al., Nature 342:878-883 (1989) 的定义给各结构域分配氨基酸。

[0119] 在本文中，以编号命名的抗体是与从具有相同编号的杂交瘤获得的单克隆抗体相同的抗体。例如，单克隆抗体 3.8.3 是与从杂交瘤 3.8.3 获得的抗体相同的抗体。

[0120] 在本文中，Fd 片段指的是由 V_H 和 C_H1 结构域组成的抗体片段；F_v 片段由抗体单臂的 V_L 和 V_H 结构域组成；而 dAb 片段 (Ward et al., Nature 341:544-546 (1989)) 由 V_H 结构域组成。

[0121] 在一些实施方式中，抗体是单链抗体（scFv），其中通过合成的连接序列使 V_L 和 V_H 结构域成对以形成单价分子的抗体，所述连接序列能使 V_L 和 V_H 区作为单条蛋白质链而制备（Bird et al.，Science242:423-426(1988) 和 Huston et al.，Proc.Natl.Acad.Sci.USA85:5879-5883(1988)）。在一些实施方式中，抗体是双特异抗体，即二价抗体，其中 V_H 和 V_L 结构域在单一的多肽链上表达，但使用太短而使得同一链上的两个结构域之间不能形成配对的连接序列，从而迫使结构域与另一条链的互补结构域成对并产生两个抗原结合位点（参见例如，Holliger P.et al.，Proc.Natl.Acad.Sci.USA90:6444-6448(1993) 和 Poljak R.J.et al.，Structure2:1121-1123(1994)）。在一些实施方式中，来自本发明的抗体的一个或多个 CDR 可共价或非共价地整合至分子中以使其成为特异性结合至 M-CSF 的免疫粘合素。在此类实施方式中，CDR 可作为更大的多肽链的部分而整合，可共价地连接至另一个多肽链，或可非共价地整合。

[0122] 在具有一个或多个结合位点的实施方式中，结合位点可彼此相同或不同。

[0123] 在本文中，术语“人抗体”指的是其中可变区和恒定区序列是人序列的任何抗体。该术语涵盖具有源于人基因但经改变（如用以降低可能的免疫原性、增强亲和力、消除可能引起不希望的折叠的半胱氨酸等）的序列的抗体。该术语涵盖在非人细胞中重组产生的这种抗体，这可以造成抗体发生人细胞中不常见的糖基化。这些抗体可通过下文所述的多种方法制备。

[0124] 术语“嵌合抗体”在本文中指的是包含来自两种或更多种不同抗体的结构域的抗体。在一个实施方式中，一个或多个 CDR 源自人抗 M-CSF 抗体。在另一实施方式中，所有的 CDR 源自人抗 M-CSF 抗体。在另一实施方式中，嵌合抗体中组合的 CDR 源自多于一种的人抗 M-CSF 抗体。例如，嵌合抗体可包含来自第一人抗 M-CSF 抗体的轻链的 CDR1、来自第二人抗 M-CSF 抗体的轻链的 CDR2 和来自第三人抗 M-CSF 抗体的轻链的 CDR3，而来自重链的 CDR 可源自一或多种其他抗 M-CSF 抗体。此外，框架区可以源自从中获得一或多个 CDR 的抗 M-CSF 抗体之一或源自一或多种不同人抗体。

[0125] 依照本说明书的教导，本领域人员可容易地制备抗体或免疫球蛋白分子的片段或类似物。片段或类似物的优选的氨基端和羧基端位于功能结构域的边界附近。通过将核苷酸序列和 / 或氨基酸序列数据与公共或私有序列数据库相比较，可鉴定到结构和功能结构域。优选地，使用计算机化的比较法来鉴定存在于已知结构和 / 或功能的其它蛋白质中的基序或推定的蛋白质构象结构域。用于鉴定折叠成已知三维结构的蛋白质序列的方法是已知的，见 Bowie et al.，Science253:164(1991)。

[0126] 术语“表面等离子共振”在本文中指的是一种光学现象，其使得能通过检测生物传感器基质内的蛋白质浓度的改变来分析实时生物特异性相互作用，例如使用 BIACORE™ 系统 (Pharmacia Biosensor AB,Uppsala,Sweden and Piscataway,N.J.)。进一步的描述可参见 Jonsson U.et al.，Ann.Biol.Clin.51:19-26(1993)；Jonsson U.et al.，Biotechniques11:620-627(1991)；Jonsson B.et al.，J.Mol.Recognit.8:125-131(1995)；和 Johnsson B.et al.，Anal.Biochem.198:268-277(1991)。

[0127] 术语“K_D”指特定的抗体 - 抗原相互作用的平衡解离常数。

[0128] 术语“表位”包括能特异性结合至免疫球蛋白或 T 细胞受体或以其它方式与分子相互作用的任何蛋白质决定簇。表位决定簇通常由分子的化学活性表面簇例如氨基酸或糖

侧链组成，并通常具有特定的三维结构特征及特定电荷特征。表位可以是“线性的”或“构象的”。在线性表位中，蛋白质和相互作用分子（如抗体）之间相互作用的所有点均线性地沿着该蛋白质的一级氨基酸序列存在。在构象表位中，相互作用点跨越蛋白质上彼此分开的氨基酸残基。当解离常数≤ 1mM，优选地≤ 100nM，且最优选地≤ 10nM 时，称抗体特异性结合抗原。在某些实施方式中，K_D 为 1pM 至 500pM。在其他一些实施方式中，K_D 在 500pM 至 1μM 之间。在其他一些实施方式中，K_D 在 1μM 至 100nM 之间。在其他一些实施方式中，K_D 在 100mM 至 10nM 之间。确定抗原上的所需表位之后，便可以产生针对该表位的抗体，例如采用本发明中所描述的技术。或者，在发现过程中，抗体的产生和表征可阐明关于合意表位的信息。根据这些信息，便可以竞争性地筛选结合同一表位的抗体。实现这一目的的方法是进行交叉竞争研究，以便找到彼此竞争结合的抗体，例如竞争结合该抗体的抗体。国际申请 WO03/48731 描述了基于抗体的交叉竞争而将抗体“装箱 (binning)”的高通量方法。

[0129] 在本文中，20 个常规氨基酸及其缩写遵循常规用法。见 Immunology-A Synthesis(2nd Edition, E. S. Golub and D. R. Gren, Eds., Sinauer Associates, Sunderland, Mass. (1991))，通过引用将其并入本申请。

[0130] 术语“多核苷酸”在本文中是指长度为至少 10 个碱基的核苷酸的聚合形式，所述核苷酸可以是核糖核酸或脱氧核糖核酸或任一类型核苷酸的修饰形式。该术语包括单链和双链形式。

[0131] 术语“分离的多核苷酸”在本文中指基因组、cDNA 或合成来源的多核苷酸或它们的一些组合，由于其来源，该“分离的多核苷酸”具有以下 1-3 的特征：(1) 与其中该“分离的多核苷酸”在自然界中得以发现处的全部或部分多核苷酸不关联，(2) 可操纵地连接至在自然界中不与其连接的多核苷酸，或 (3) 在自然中不作为更大序列的一部分存在。

[0132] 术语“寡核苷酸”在本文中包括通过天然存在和非天然存在的寡核苷酸键连接在一起的天然存在的和经修饰的核苷酸。寡核苷酸是通常包含 200 碱基或更少碱基长度的多核苷酸子集。优选地，寡核苷酸的长度是 10-60 个碱基，最优选地，其长度是 12、13、14、15、16、17、18、19 或 20-40 个碱基。虽然寡核苷酸可以是双链，如用于基因突变体的构建，但寡核苷酸通常为单链，如用于引物和探针。本发明的寡核苷酸可以是有义或反义寡核苷酸。

[0133] 术语“天然存在的核苷酸”在本文中包括脱氧核糖核苷酸和核糖核甘酸。术语“经修饰的核苷酸”在本文中包括具有经修饰或取代的糖基等的核苷酸。术语“寡核苷酸键”在本文中包括寡核苷酸连键如硫代磷酸酯、二硫代磷酸酯、硒代磷酸酯、二硒代磷酸酯、苯胺基硫代磷酸酯 (phosphoroanilothioate)、苯胺基磷酸酯 (phoshoraniladate)、phosphoroamidate 等。见例如，LaPlanche et al., Nucl. Acids Res. 14:9081(1986)；Stec et al., J. Am. Chem. Soc. 106:6077(1984)；Stein et al., Nucl. Acids Res. 16:3209(1988)；Zon et al., Anti-Cancer Drug Design6:539(1991)；Zon et al., Oligonucleotides and Analogues:A Practical Approach, pp. 87-108(F. Eckstein, Ed., Oxford University Press, Oxford England(1991))；美国专利 No. 5,151,510；Uhlmann and Peyman, Chemical Reviews90:543(1990)，通过引用将这些公开内容并入本申请。如果需要，寡核苷酸可包括用于检测的标记物。

[0134] “可操纵地连接的”序列包括邻接目的基因的表达调控序列和通过反式作用控制目的基因或相隔一段距离控制目的基因的表达调控序列。术语“表达调控序列”在本文中

指实现其连接的编码序列的表达和加工所需的多核苷酸序列。表达调控序列包括适当的转录起始、终止、启动子和增强子序列；有效的 RNA 加工信号如剪接和多腺苷酸化信号；稳定细胞质 mRNA 的序列；增强翻译效率的序列（即 Kozak 共有序列）；增强蛋白质稳定性的序列；以及当需要时，包括增强蛋白分泌的序列。在不同的宿主生物体，这种调控序列的性质不同；在原核生物中，这种调控序列一般包括启动子、核糖体结合位点和转录终止序列；在真核生物中，通常，这种调控序列包括启动子和转录终止序列。术语“调控序列”意在包括（最少）其存在对表达和加工处理是必需的所有成分，并还可包括其存在是有利的额外成分，例如引导序列和融合伴侣序列。

[0135] 术语“载体”在本文中意指能运输已与之连接的另外的核酸的核酸分子。在一些实施方式中，载体是“质粒”，即可将额外的 DNA 片段连接至其中的环状双链 DNA 环。在一些实施方式中，载体是病毒载体，其中额外的 DNA 节段可连接至该病毒基因组中。在一些实施方式中，载体能在其被导入的宿主细胞中自主复制（如，具有细菌复制起点的细菌载体和附加型哺乳动物载体）。在其他一些实施方式中，载体（如非附加型哺乳动物载体）在将其导入宿主细胞后可整合至宿主细胞的基因组中，并因而可随宿主基因组一起复制。而且，某些载体能指导与它们可操纵地连接的基因的表达。此类载体在此称为“重组表达载体”（或简称“表达载体”）。

[0136] 术语“重组宿主细胞”（或简称“宿主细胞”）在本文中意指已经导入重组表达载体的细胞。应该理解，“重组宿主细胞”和“宿主细胞”不仅指特定的对象细胞而且指这种细胞的后代。因为某些修饰由于突变或环境的影响可能发生在随后的子代，实际上，这种子代可能与亲本细胞不完全相同，但仍然包括在本文中术语“宿主细胞”的范围内。

[0137] 在此涉及的术语“选择性杂交”指的是以可检测且特异性的方式结合。本发明的多核苷酸、寡核苷酸及其片段在杂交和洗涤条件下可选择性地杂交至核酸链，所述的杂交和洗涤条件使可检测的与非特异性核酸的结合的可感知量最小化。正如本领域已知的并在此讨论的那样，可采用“高严格性”或“高度严格的”条件获得选择性杂交条件。“高严格性”或“高度严格的”条件的实例是于 42℃杂交温度、在 6X SSPE 或 SSC，50％甲酰胺，5X Denhardt 试剂，0.5％ SDS，100μg/ml 变性的片段化鲑精 DNA 的杂交缓冲液中将多核苷酸与另一种多核苷酸温育 12-16 小时，其中可将一种多核苷酸固定至固体表面如膜上，随后，于 55℃用 1X SSC，0.5％ SDS 的洗涤缓冲液洗涤两次。也见 Sambrook et al.，见上，pp.9.50-9.55.

[0138] 在提及核苷酸序列的上下文中，术语“百分比序列相同性”指当第一连续序列与第二连续序列相比较达到最大对应的比对时的残基百分比。序列相同性比较的长度可超过至少约 9 个核苷酸、通常至少约 18 个核苷酸、更通常为至少约 24 个核苷酸、典型地为至少约 28 个核苷酸、更典型的为至少约 32 个核苷酸，并且优选地为至少约 36、48 或更多核苷酸的一段序列。本领域已知的可用于测量核苷酸序列相同性的不同算法有许多。例如，可使用 FASTA、Gap 或 Bestfit（均为 Wisconsin Package Version10.0 的程序，Genetics Computer Group(GCG)，Madison，Wisconsin）比较多核苷酸序列。FASTA，其包括如 FASTA2 和 FASTA3 程序，提供了查询序列和检索序列之间最佳重叠区域的比对和百分比序列相同性 (Pearson，Methods Enzymol.183:63-98(1990)；Pearson，Methods Mol.Biol.132:185-219(2000)；Pearson，Methods Enzymol.266:227-258(1996)；Pearson，J.Mol.Biol.276:71-84(1998)，通过引用并入本申请）。除非另外指定，使用特定程序或算法

的默认参数。例如，核苷酸序列之间的百分比序列相同性可使用采用默认参数（字段大小为 6 和 NOPAM 因子用于评分矩阵）的 FASTA 或使用采用 GCG Version6.1 所提供的默认参数的 Gap 来测定，在此通过引用将其并入本申请。

[0139] 除非另外说明，对核苷酸序列的指代涵盖其互补链。因此，对具有特定序列的核酸分子的指代应该理解为涵盖具有其互补序列的其互补链。

[0140] 术语“百分比序列相同性”指的是一个比例，以相同残基的数量占所比较的残基数量的百分数表示。

[0141] 当涉及核酸或其片段时，术语“显著相似性”或“显著序列相似性”表示当以合适的核苷酸插入或删除达到与另外的核苷酸（或其互补链）最优地比对时，在至少约 85％，优选地至少约 90％、91％、92％、93％、94％、95％、96％、97％、98％或 99％的核苷酸碱基中存在核苷酸序列相同性，如通过任何熟知的序列相同性算法测量，如上面讨论的 FASTA、BLAST 或 Gap。

[0142] 当应用于多肽时，术语“显著相同性”表示两个肽序列，当例如通过采用默认缺口权重 (gap weight) 的程序 GAP 或 BESTFIT 最优比对时，具有至少 70％、75％、80％或 85％的序列相同性，优选地至少 90％、91％、92％、93％、94％ 95％、96％、97％、98％或 99％的序列相同性。在某些实施方式中，不一致的残基位置的区别在于保守性氨基酸取代。“保守性氨基酸取代”是指其中氨基酸残基由带有具有相似化学性质（如电荷或疏水性）的侧链 R 基团的另外的氨基酸残基取代。一般而言，保守性氨基酸取代基本不会改变蛋白质的功能特性。在两个或多个氨基酸序列由于保守取代而彼此不同的情况下，可上调百分比序列相同性以校正取代的保守性质。进行这种调节的方法是本领域人员熟知的。参见例如 Pearson,Methods Mol.Biol.243:307-31(1994)。带有具有相似化学性质的侧链的氨基酸组的实例包括 :1) 脂族侧链 :甘氨酸、丙氨酸、缬氨酸、亮氨酸和异亮氨酸 ;2) 脂族 - 羟基侧链 :丝氨酸和苏氨酸 ;3) 含酰胺的侧链 :天冬酰胺和谷氨酰胺 ;4) 芳香侧链 :苯丙氨酸、酪氨酸和色氨酸 ;5) 碱性侧链 :赖氨酸、精氨酸和组氨酸 ;6) 酸性侧链 :天冬氨酸和谷氨酸 ;和 7) 包含硫的侧链 :半胱氨酸和蛋氨酸。保守氨基酸取代组是 :缬氨酸 - 亮氨酸 - 异亮氨酸、苯丙氨酸 - 酪氨酸、赖氨酸 - 精氨酸、丙氨酸 - 缬氨酸、谷氨酸 - 天冬氨酸和天冬酰胺 - 谷氨酰胺。

[0143] 或者，保守性取代是在 PAM250log- 可能性矩阵 (Gonnet et al.,Science 256:1443-45(1992)，这里将其通过引用并入本申请）中具有正值的任何改变。“适度保守性”取代是在 PAM250log- 可能性矩阵中具有非负值的任何改变。

[0144] 多肽的序列相同性通常使用序列分析软件测量。蛋白质分析软件通过赋予不同取代、缺失和其它修饰（包括保守性氨基酸取代）的相似性度量而匹配序列。例如，GCG 包括程序如“Gap”和“Bestfit”，可采用其程序所述的默认参数测定紧密相关的多肽如来自不同生物体物种的同源性多肽之间或野生型蛋白质和其突变型蛋白之间的序列同源性或序列相同性。见，例如 GCG Version6.1。也可以使用 FASTA，采用默认或推荐的参数来比较多肽序列，见 GCG Version6.1.(University of Wisconsin WI)。FASTA（例如，FASTA2 和 FASTA3）提供了查询序列和搜索序列之间最佳重叠的区域的比对和百分比序列相同性 (Pearson,Methods Enzymol.183:63-98(1990) ;Pearson,Methods Mol.Biol.132:185-219(2000))。当将本发明的序列和含有大量来自不同生物体的序列的数据

库相比较时，另外优选的算法是计算机程序 BLAST，特别是 blastp 或 tblastn，采用程序随带的默认参数。参见，例如，Altschul et al.，J.Mol.Biol.215:403-410(1990)；Altschul et al.，Nucleic Acids Res.25:3389-402(1997)。

[0145] 比较同源性的多肽序列的长度一般为至少约 16 个氨基酸残基，通常至少约 20 个残基，更通常为至少约 24 个残基，典型的为至少约 28 个残基，并且优选多于约 35 个残基。当搜索含有来自大量不同生物体的序列的数据库时，优选比较氨基酸序列。

[0146] 在本文中，术语“标记”或“经标记的”指将另一种分子掺入抗体中。在一个实施方式中，该标记是可检测的标记，如掺入放射性标记的氨基酸或给多肽附着可通过标记的抗生物素蛋白（如，含有可通过光学或比色法检测的荧光标记或酶活性的抗生物素蛋白链菌素）检测的生物素基部分。在另一实施方式中，标记物或标记可以是治疗性的，如药物缀合物或毒素。标记多肽和糖蛋白的多种方法是本领域已知的并可使用。多肽标记的实例包括但不限于：放射性同位素或放射性核素（例如，^{3}H, ^{14}C, ^{15}N, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I）、荧光标记（如 FITC、若丹明、稀土元素荧光体）、酶标记（如辣根过氧化物酶、β－半乳糖苷酶、荧光素酶、碱性磷酸酶）、化学发光标记、生物素基、由第二报道分子识别的预定的多肽表位（如亮氨酸拉链对序列、二抗的结合位点、金属结合结构域、表位标签）、磁性试剂如钆螯合物、毒素如百日咳毒素、紫杉醇、细胞松弛素 B、短杆菌肽 D、溴化乙锭、吐根碱、丝裂霉素、依托泊苷、鬼臼噻吩甙、长春新碱、长春花碱、秋水仙碱、阿霉素、柔红霉素、二羟基蒽二酮(dihydroxy anthracin dione)、米托蒽醌、光辉霉素、放线菌素D、1-去氢睾酮、糖皮质激素、普鲁卡因、丁卡因、利多卡因、普萘洛尔以及嘌呤霉素和其类似物或同系物。在一些实施方式中，标记由不同长度的间隔臂连接以减少可能的位阻。

[0147] 在说明书和权利要求书中，“包含”应理解为意味着包括所述的整数或整数组，但不排除任何其他整数或整数组。

[0148] 人抗 M-CSF 抗体及其表征

[0149] 在一个实施方式中，本发明提供人源化抗 M-CSF 抗体。在另一实施方式中，本发明提供人抗 M-CSF 抗体。在一些实施方式中，人抗 M-CSF 抗体通过免疫非人转基因动物而产生，所述动物例如为啮齿动物，其基因组包含人免疫球蛋白基因以使得所述啮齿动物产生人抗体。

[0150] 本发明的抗 M-CSF 抗体可包含人 κ 或人 λ 轻链或源自它们的氨基酸序列。在包含 κ 轻链的一些实施方式中，轻链可变区 (VL) 部分地由人 $V_{\kappa}O12$、$V_{\kappa}L2$、$V_{\kappa}L5$、$V_{\kappa}A27$ 或 $V_{\kappa}B3$ 基因和 $J_{\kappa}1$、$J_{\kappa}2$、$J_{\kappa}3$ 或 $J_{\kappa}4$ 基因编码。在本发明的特定实施方式中，轻链可变区由 $V_{\kappa}O12/J\kappa 3$、$V_{\kappa}L2/J\kappa 3$、$V_{\kappa}L5/J\kappa 3$、$V_{\kappa}L5/J\kappa 4$、$V_{\kappa}A27/J\kappa 4$ 或 $V_{\kappa}B3/J\kappa 1$ 基因编码。

[0151] 在一些实施方式中，M-CSF 抗体的 VL 相对于种系氨基酸序列包含一或多个氨基酸取代。在一些实施方式中，抗 M-CSF 抗体的 V_L 相对于种系氨基酸序列包含 1、2、3、4、5、6、7、8、9 或 10 个氨基酸取代。在一些实施方式中，对种系的这些取代中的一或多个位于轻链的 CDR 区。在一些实施方式中，所述相对于种系的氨基酸取代与抗体 252、88、100、3.8.3、2.7.3、1.120.1、9.14.4I、8.10.3F、9.7.2IF、9.14.4、8.10.3、9.7.2、9.7.2C-Ser、9.14.4C-Ser、8.10.3C-Ser、8.10.3-CG2、9.7.2-CG2、9.7.2-CG4、9.14.4-CG2、9.14.4-CG4、9.14.4-Ser、9.7.2-Ser、8.10.3-Ser、8.10.3-CG4、8.10.3FG1 或 9.14.4G1 的任何一或多个 V_L 中相对于种系的那些取代位于一或多个相同的位置。例如，抗 M-CSF 抗体的 V_L 具有一或

多个与种系相比在抗体88的V_L中出现的氨基酸取代以及与种系相比在抗体252的V_L中出现的其他氨基酸取代，抗体252与抗体88使用相同的V_K基因。在一些实施方式中，所述氨基酸改变位于一或多个相同的位置，但涉及与参照抗体不同的突变。

[0152] 在一些实施方式中，相对于种系的氨基酸改变出现于一或多个与抗体252、88、100、3.8.3、2.7.3、1.120.1、9.14.4I、8.10.3F、9.7.2IF、9.14.4、8.10.3、9.7.2、9.7.2C-Ser、9.14.4C-Ser、8.10.3C-Ser、8.10.3-CG2、9.7.2-CG2、9.7.2-CG4、9.14.4-CG2、9.14.4-CG4、9.14.4-Ser、9.7.2-Ser、8.10.3-Ser、8.10.3-CG4、8.10.3FG1或9.14.4G1的任一V_L的氨基酸改变相同的位置，但这些改变可代表在这些位置上相对于参照抗体中的氨基酸的保守性氨基酸取代。例如，如果这些抗体之一在特定位置相对于种系发生改变且该位置是谷氨酸，那么可以在该位置以天冬氨酸取代。类似地，如果相对于种系的氨基酸取代是丝氨酸，可以用苏氨酸取代该位置的丝氨酸。保守性氨基酸取代已在上文中讨论。

[0153] 在一些实施方式中，人抗M-CSF抗体的轻链包含与抗体252(SEQ ID NO:4)、88(SEQ ID NO:8)、100(SEQ ID NO:12)、3.8.3(SEQ ID NO:16)、2.7.3(SEQ ID NO:20)、1.120.1(SEQ ID NO:24)、9.14.4I(SEQ ID NO:28)、8.10.3F(SEQ ID NO:32)、9.7.2IF(SEQ ID NO:36)、9.14.4(SEQ ID NO:28)、8.10.3(SEQ ID NO:44)、9.7.2(SEQ ID NO:48)、9.7.2C-Ser(SEQ ID NO:52)、9.14.4C-Ser(SEQ ID NO:56)、8.10.3C-Ser(SEQ ID NO:60)、8.10.3-CG2(SEQ ID NO:60)、9.7.2-CG2(SEQ ID NO:52)、9.7.2-CG4(SEQ ID NO:52)、9.14.4-CG2(SEQ ID NO:56)、9.14.4-CG4(SEQ ID NO:56)、9.14.4-Ser(SEQ ID NO:28)、9.7.2-Ser(SEQ ID NO:48)、8.10.3-Ser(SEQ ID NO:44)、8.10.3-CG4(SEQ ID NO:60)8.10.3FG1(SEQ ID NO:32)或9.14.4G1(SEQ ID NO:28)的V_L的氨基酸序列相同的氨基酸序列，或者具有多达1、2、3、4、5、6、7、8、9或10个保守性氨基酸取代和/或总共多达3个非保守性氨基酸取代的所述氨基酸序列。在一些实施方式中，轻链包含任一前述抗体的从CDR1起始处至CDR3结束处的氨基酸序列。

[0154] 在一些实施方式中，抗M-CSF抗体的轻链至少包含种系或在此所述的抗体序列的轻链CDR1、CDR2或CDR3。在另一实施方式中，轻链可包含独立地选自252、88、100、3.8.3、2.7.3、1.120.1、9.14.4I、8.10.3F、9.7.2IF、9.14.4、8.10.3、9.7.2、9.7.2C-Ser、9.14.4C-Ser、8.10.3C-Ser、8.10.3-CG2、9.7.2-CG2、9.7.2-CG4、9.14.4-CG2、9.14.4-CG4、9.14.4-Ser、9.7.2-Ser、8.10.3-Ser、8.10.3-CG4、8.10.3FG1或9.14.4G1的抗体的CDR1、CDR2或CDR3区，或者各自具有少于4个或少于3个保守性氨基酸取代和/或总共3个或更少的非保守性氨基酸取代的所述CDR区。在其他一些实施方式中，抗M-CSF抗体的轻链包含轻链CDR1、CDR2或CDR3，其中每一CDR独立地选自如下抗体的CDR1、CDR2和CDR3区，所述抗体具有的轻链可变区包含选自SEQ ID NO:4、8、12、16、20、24、28、32、36、44、48、52、56或60的V_L区氨基酸序列，或由选自SEQ ID NO:3、7、11、27、31、35、43或47的编码V_L区的核酸分子所编码的氨基酸序列。抗M-CSF抗体的轻链可包含抗体的CDR1、CDR2和CDR3区，所述抗体包含选自252、88、100、3.8.3、2.7.3、1.120.1、9.14.4I、8.10.3F、9.7.2IF、9.14.4、8.10.3、9.7.2、9.7.2C-Ser、9.14.4C-Ser、8.10.3C-Ser、8.10.3-CG2、9.7.2-CG2、9.7.2-CG4、9.14.4-CG2、9.14.4-CG4、9.14.4-Ser、9.7.2-Ser、8.10.3-Ser、8.10.3-CG4、8.10.3FG1或9.14.4G1的V_L区的氨基酸序列或SEQ ID NO:4、8、12、16、20、24、28、32、36、44、48、52、56或60的V_L区氨基酸序列。

[0155] 在一些实施方式中，轻链包含抗体 252、88、100、3.8.3、2.7.3、1.120.1、9.14.4I、8.10.3F、9.7.2IF、9.14.4、8.10.3、9.7.2、9.7.2C-Ser、9.14.4C-Ser、8.10.3C-Ser、8.10.3-CG2、9.7.2-CG2、9.7.2-CG4、9.14.4-CG2、9.14.4-CG4、9.14.4-Ser、9.7.2-Ser、8.10.3-Ser、8.10.3-CG4、8.10.3FG1 或 9.14.4G1 的 CDR1、CDR2 和 CDR3 区，或者各自具有少于 4 个或少于 3 个保守性氨基酸取代和 / 或总共 3 个或更少的非保守性氨基酸取代的所述 CDR 区。

[0156] 对于重链，在一些实施方式中，重链氨基酸序列的可变区部分地由人 V_H3-11、V_H3-23、V_H3-7、V_H1-18、V_H3-33、V_H3-48 基因和 J_H4、J_H6、J_H4b 或 J_H6b 基因编码。在本发明的特定实施方式中，重链可变区由 V_H3-11/D_H7-27/J_H6、V_H3-7/D_H6-13/J_H4、V_H3-23/D_H1-26/J_H4、V_H3-11/D_H7-27/J_H4、V_H3-33/D_H1-26/J_H4、V_H1-18/D_H4-23/J_H4、V_H3-11/D_H7-27/J_H4b、V_H3-48/D_H1-26/J_H4b、V_H3-11/D_H6-13/J_H6b、V_H3-11/D_H7-27/J_H4b、V_H3-48/D_H1-6/J_H4b 或 V_H3-11/D_H6-13/J_H6b 基因编码。在一些实施方式中，抗 M-CSF 抗体的 V_H 相对于种系氨基酸序列含有一或多个氨基酸取代、缺失或插入（添加）。在一些实施方式中，重链可变区与种系氨基酸序列相比包含 1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17 或 18 个突变。在一些实施方式中，与种系氨基酸序列相比所述突变是非保守性取代。在一些实施方式中，所述突变位于重链的 CDR 区。在一些实施方式中，所述氨基酸改变与抗体 252、88、100、3.8.3、2.7.3、1.120.1、9.14.4I、8.10.3F、9.7.2IF、9.14.4、8.10.3、9.7.2、9.7.2C-Ser、9.14.4C-Ser、8.10.3C-Ser、8.10.3-CG2、9.7.2-CG2、9.7.2-CG4、9.14.4-CG2、9.14.4-CG4、9.14.4-Ser、9.7.2-Ser、8.10.3-Ser、8.10.3-CG4、8.10.3FG1 或 9.14.4G1 的任何一或多个 V_H 中与种系相比的那些突变位于一或多个相同的位置。在其他一些实施方式中，所述氨基酸改变位于一或多个相同的位置，但涉及与参照抗体不同的突变。

[0157] 在一些实施方式中，重链包含抗体 252(SEQ ID NO:2)、88(SEQ ID NO:6)、100(SEQ ID NO:10)、3.8.3(SEQ ID NO:14)、2.7.3(SEQ.ID NO:18)、1.120.1(SEQ.ID NO:22)、9.14.4I(SEQ ID NO:26)、8.10.3F(SEQ ID NO:30)、9.7.2IF(SEQ ID NO:34)、9.14.4(SEQ ID NO:38)、8.10.3(SEQ ID NO:30)、9.7.2(SEQ ID NO:46)、9.7.2C-Ser(SEQ ID NO:50)、9.14.4C-Ser(SEQ ID NO:54)、8.10.3C-Ser(SEQ ID NO:58)、8.10.3-CG2(SEQ ID NO:62)、9.7.2-CG2(SEQ ID NO:66)、9.7.2-CG4(SEQ ID NO:70)、9.14.4-CG2(SEQ ID NO:74)、9.14.4-CG4(SEQ ID NO:78)、9.14.4-Ser(SEQ ID NO:82)、9.7.2-Ser(SEQ ID NO:86)、8.10.3-Ser(SEQ ID NO:90)8.10.3-CG4(SEQ ID NO:94)、8.10.3FG1(SEQ ID NO:98) 或 9.14.4G1(SEQ ID NO:102) 的可变结构域 (V_H) 的氨基酸序列，或者具有多达 1、2、3、4、5、6、7、8、9 或 10 个保守性氨基酸取代和 / 或总共多达 3 个的非保守性氨基酸取代的所述氨基酸序列。在一些实施方式中，重链包含任一前述抗体的从 CDR1 起始处至 CDR3 结束处的氨基酸序列。

[0158] 在一些实施方式中，重链包含抗体 252、88、100、3.8.3、2.7.3、1.120.1、9.14.4I、8.10.3F、9.7.2IF、9.14.4、8.10.3、9.7.2、9.7.2C-Ser、9.14.4C-Ser、8.10.3C-Ser、8.10.3-CG2、9.7.2-CG2、9.7.2-CG4、9.14.4-CG2、9.14.4-CG4、9.14.4-Ser、9.7.2-Ser、8.10.3-Ser、8.10.3-CG4、8.10.3FG1 或 9.14.4G1 的重链 CDR1、CDR2 和 CDR3 区，或者各自具有少于 8 个、少于 6 个、少于 4 个或少于 3 个的保守性氨基酸取代和 / 或总共 3 个或更少的非保守性氨基酸取代的所述 CDR 区。

[0159] 在一些实施方式中，重链包含种系或如上所述的具有在此所述的抗体序列的抗体CDR3，并还可包含种系序列的CDR1和CDR2区，或者可包含抗体序列的CDR1和CDR2，它们各自独立地选自包含选自252、88、100、3.8.3、2.7.3、1.120.1、9.14.4I、8.10.3F、9.7.2IF、9.14.4、8.10.3、9.7.2、9.7.2C-Ser、9.14.4C-Ser、8.10.3C-Ser、8.10.3-CG2、9.7.2-CG2、9.7.2-CG4、9.14.4-CG2、9.14.4-CG4、9.14.4-Ser、9.7.2-Ser、8.10.3-Ser、8.10.3-CG4、8.10.3FG1或9.14.4G1的抗体的重链的抗体。在另一实施方式中，重链包含在此所述的抗体序列的CDR3，并还可包含CDR1和CDR2区，它们各自独立地选自如下所述的重链可变区的CDR1和CDR2区，所述重链可变区包含选自SEQ ID NO:2、6、10、14、18、22、26、30、34、38、46、50、54、58、62、66、70、74、78、82、86、90、94、98或102的V_H区氨基酸序列，或由选自SEQ ID NO:1、5、9、25、29、33、37、45、97或101的编码V_H区的核酸序列所编码的氨基酸序列。在另一实施方式中，所述抗体包含如上所述的轻链和如上所述的重链。

[0160] 可以进行的一种类型的氨基酸取代是改变抗体中的一或多个半胱氨酸，半胱氨酸可与另一残基具有化学反应性，例如但不限于，丙氨酸或丝氨酸。在一个实施方式中，其是非规范半胱氨酸(non-canonical cysteine)的取代。取代可以出现在抗体可变结构域的框架区或恒定结构域。在另一实施方式中，半胱氨酸位于抗体的非规范区域(non-canonical region)。

[0161] 可以进行的另一类型的氨基酸取代是去除抗体中的任何潜在的蛋白水解位点，特别是那些位于抗体可变结构域的CDR或框架区或者恒定结构域内的潜在的蛋白水解位点。取代半胱氨酸残基和去除蛋白水解位点可降低抗体产物的异质性并增加其均质性。另一类型的氨基酸取代是消除天冬酰胺-甘氨酸对，它们形成潜在的脱酰胺位点，这可通过改变这两个残基之一或两者而实现。

[0162] 在一些实施方式中，本发明的抗M-CSF抗体的重链没有C端赖氨酸(Lewis D.A.,et al.,Anal.Chem,66(5):585-95(1994))。在本发明的各种实施方式中，抗M-CSF抗体的重链和轻链看任选地包括单一序列。

[0163] 在一个方面，本发明涉及人抗M-CSF单克隆抗体和经工程化以产生所述抗体的细胞系。表1A列出了以下单克隆抗体的编码重链和轻链可变区的核酸的序列号(SEQ ID NO)以及相应的推定的氨基酸序列：单克隆抗体252、88、100、3.8.3、2.7.3、1.120.1、9.14.4I、8.10.3F、9.7.2IF、9.14.4、8.10.3和9.7.2。可通过本领域人员已知的方法制备额外的变体抗体9.7.2C-Ser、9.14.4C-Ser、8.10.3C-Ser、8.10.3-CG2、9.7.2-CG2、9.7.2-CG4、9.14.4-CG2、9.14.4-CG4、9.14.4-Ser、9.7.2-Ser、8.10.3-Ser、8.10.3-CG48.10.3FG1或9.14.4G1。表1B列出了抗体252、88、100、3.8.3、2.7.3、1.120.1、9.7.2、9.14.4、8.10.3、8.10.3C-Ser、8.10.3-CG2、8.10.3-Ser、8.10.3-CG4和8.10.3FG1的重链、轻链以及重链和轻链分别与抗体8.10.3F的重链、轻链以及重链和轻链相比的氨基酸百分比相同性。

[0164] 在另一方面，本发明涉及与单克隆抗体8,10.3F基本上类似的抗M-CSF单克隆抗体，其中所述抗体的重链或轻链或者重链和轻链的氨基酸序列与抗体8.10.3F的重链或轻链或者重链和轻链分别享有至少大约70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％或99％的相同性。

[0165] 表1A

[0166]

人抗 M-CSF 抗体				
MAb	序列号 (SEQ ID NO:)			
	全长			
	重链		轻链	
	DNA	蛋白质	DNA	蛋白质
252	1	2	3	4
88	5	6	7	8
100	9	10	11	12
3.8.3		14		16
2.7.3		18		20
1.120.1		22		24
9.14.4I	25	26	27	28
9.14.4	37	38	27	28
9.14.4C-Ser		54		56
9.14.4-CG2		74		56
9.14.4-CG4		78		56
9.14.4-Ser		82	27	28
9.14.4-G1	101	102	27	28
8.10.3F	29	30	31	32
8.10.3	29	30	43	44
8.10.3C-Ser		58		60
8.10.3-CG2		62		60
8.10.3-Ser		90	43	44
8.10.3-CG4		94		60
8.10.3FG1	97	98	31	32
9.7.2IF	33	34	35	36
9.7.2	45	46	47	48
9.7.2C-Ser		50		52
9.7.2-CG2		66		52
9.7.2-CG4		70		52
9.7.2-Ser		86	47	48

[0167] 表 1B

[0168]

MAb	与抗体 8.10.3F 的重链、轻链或者重链和轻链的百分比相同性		
	重链	轻链	重链和轻链组合
8.10.3-CG2	100	99	99
8.10.3-CG4	94	99	96
8.10.3-Ser	95	99	96
8.10.3C-Ser	95	99	96
8.10.3FG1	94	100	96
8.10.3	100	99	99
1.120.1	87	85	86
100	94	90	93
2.7.3	89	84	87
252	95	84	91
3.8.3	96	83	92
88	95	84	91
9.14.4	91	83	88
9.7.2	90	83	88

[0169] 抗 M-CSF 抗体的类别和亚类

[0170] 可通过本领域已知的任何方法确定抗 M-CSF 抗体的类别和亚类。通常，抗体的类别和亚类可使用对特定类别和亚类的抗体具有特异性的抗体来测定。这些抗体是可从商业获得的。通过 ELISA、蛋白质印迹法及其它技术能测定类别和亚类。或者，可通过测序抗体重链和 / 或轻链的全部或部分恒定区，将它们的氨基酸序列与各种不同类别和亚类的免疫球蛋白的已知氨基酸序列相比较，而确定抗体的类别和亚类。

[0171] 在一些实施方式中，抗 M-CSF 抗体是单克隆抗体。抗 M-CSF 抗体可以是 IgG、IgM、IgE、IgA 或 IgD 分子。在优选的实施方式中，抗 M-CSF 抗体是 IgG，且是 IgG1、IgG2、IgG3 或 IgG4 亚类。在其他优选的实施方式中，抗体是 IgG2 或 IgG4 亚类。在另一优选的实施方式中，抗体是 IgG1 亚类。

[0172] 物种和分子选择性

[0173] 在本发明的另一方面，该抗 M-CSF 抗体显示出物种和分子选择性。在一些实施方式中，抗 M-CSF 抗体结合人、食蟹猴和鼠的 M-CSF。遵循本说明书的教导，可以使用本领域熟知的方法确定抗 M-CSF 抗体的物种选择性。例如，可使用蛋白质印迹法、FACS、ELISA、RIA、细胞增殖测定法或 M-CSF 受体结合测定法来确定物种选择性。在优选的实施方式中，可使用细胞增殖测定法或 ELISA 确定物种选择性。

[0174] 在另一实施方式中，抗 M-CSF 抗体对 M-CSF 的选择性是其对 GM-/G-CSF 的选择性的至少 100 倍。在一些实施方式中，抗 M-CSF 抗体对 M-CSF 以外的其他蛋白质不显示任何可检测到的特异性结合。根据本说明书的教导，采用本领域熟知的方法，本领域人员可确定抗 M-CSF 抗体对 M-CSF 的选择性。例如可采用蛋白质印迹法、FACS、ELISA 或 RIA 来确定所

述选择性。

[0175] 鉴定由抗 M-CSF 抗体识别的 M-CSF 表位

[0176] 本发明提供结合 M-CSF 的人抗 M-CSF 单克隆抗体，其与以下抗体竞争结合 M-CSF、交叉竞争结合 M-CSF 和 / 或结合 M-CSF 的相同表位和 / 或以相同的 K_D 结合 M-CSF ：(a) 抗体，其选自 252、88、100、3.8.3、2.7.3、1.120.1、9.14.4I、8.10.3F、9.7.2IF、9.14.4、8.10.3、9.7.2、9.7.2C-Ser、9.14.4C-Ser、8.10.3C-Ser、8.10.3-CG2、9.7.2-CG2、9.7.2-CG4、9.14.4-CG2、9.14.4-CG4、9.14.4-Ser、9.7.2-Ser、8.10.3-Ser、8.10.3-CG4、8.10.3FG1 或 9.14.4G1 ；(b) 抗体，其包含具有氨基酸序列 SEQ ID NO:2、6、10、14、18、22、26、30、34、38、46、50、54、58、62、66、70、74、78、82、86、90、94、98 或 102 的重链可变区 ；(c) 抗体，其包含具有氨基酸序列 SEQ ID NO:4、8、12、16、20、24、28、32、36、44、48、52、56 或 60 的轻链可变区 ；(d) 抗体，其包含 (b) 中所定义的重链可变区和 (c) 中所定义的轻链可变区。

[0177] 可使用本领域已知的方法确定一种抗体是否与抗 M-CSF 抗体结合相同的表位、竞争结合、交叉竞争结合或以相同的 K_D 结合 M-CSF。在一个实施方式中，可在饱和条件下将本发明的抗 M-CSF 抗体结合至 M-CSF，并随后测量测试抗体结合至 M-CSF 的能力。如果测试抗体能与抗 M-CSF 抗体同时结合至 M-CSF，那么该测试抗体与抗 M-CSF 抗体结合至不同的表位。然而，如果该测试抗体不能同时结合至 M-CSF，那么该测试抗体结合的表位与该人抗 M-CSF 抗体结合的表位是相同的表位、重叠的表位或是非常接近的表位。可采用 ELISA、RIA 或 FACS 来进行这一实验。在优选的实施方式中，采用 BIACORE™ 进行该实验。

[0178] 抗 M-CSF 抗体对 M-CSF 的结合亲和力

[0179] 在本发明的一些实施方式中，抗 M-CSF 抗体以高亲和力结合 M-CSF。在一些实施方式中，抗 M-CSF 抗体以 $1x10^{-7}M$ 或更低的 K_D 结合 M-CSF。在其他优选的实施方式中，该抗体以 $1x10^{-8}M$、$1x10^{-9}M$、$1x10^{-10}M$、$1x10^{-11}M$、$1x10^{-12}M$ 或更低的 K_D 结合 M-CSF。在某些实施方式中，K_D 为 1pM 至 500pM。在其他一些实施方式中，K_D 在 500pM 至 1μM 之间。在其他一些实施方式中，K_D 在 1μM 至 100nM 之间。在其他一些实施方式中，K_D 在 100mM 至 10nM 之间。在更加优选的实施方式中，抗体以与选自如下一组的抗体基本上相同的 K_D 结合 M-CSF ：抗体 252、88、100、3.8.3、2.7.3、1.120.1、9.14.4I、8.10.3F、9.7.2IF、9.14.4、8.10.3、9.7.2、9.7.2C-Ser、9.14.4C-Ser、8.10.3C-Ser、8.10.3-CG2、9.7.2-CG2、9.7.2-CG4、9.14.4-CG2、9.14.4-CG4、9.14.4-Ser、9.7.2-Ser、8.10.3-Ser、8.10.3-CG4、8.10.3FG1 或 9.14.4G1。在另一优选的实施方式中，抗体以与包含来自选自如下一组的抗体的轻链 CDR2 和 / 或重链 CDR3 的抗体基本上相同的 K_D 结合 M-CSF ：抗体 252、88、100、3.8.3、2.7.3、1.120.1、9.14.4I、8.10.3F、9.7.2IF、9.14.4、8.10.3、9.7.2、9.7.2C-Ser、9.14.4C-Ser、8.10.3C-Ser、8.10.3-CG2、9.7.2-CG2、9.7.2-CG4、9.14.4-CG2、9.14.4-CG4、9.14.4-Ser、9.7.2-Ser、8.10.3-Ser、8.10.3-CG4、8.10.3FG1 或 9.14.4G1。在另一优选的实施方式中，抗体以与包含氨基酸序列 SEQ ID NO:2、6、10、14、18、22、26、30、34、38、46、50、54、58、62、66、70、74、78、82、86、90、94、98 或 102 的重链可变区或包含氨基酸序列 SEQ ID NO:4、8、12、16、20、24、28、32、36、44、48、52、56 或 60 的轻链可变区的抗体基本上相同的 K_D 结合 M-CSF。在另一优选的实施方式中，抗体以与包含具有 V_L 区氨基酸序列 SEQ ID NO:4、8、12、16、20、24、28、32、36、44、48、52、56 或 60 的轻链可变区的 CDR2 且可任选地包含其 CDR1 和 / 或 CDR3 的抗体、或与包含具有 V_H 区氨基酸序列 SEQ ID NO:2、6、10、14、18、22、26、30、34、38、46、50、54、58、62、66、70、74、78、

82、86、90、94、98 或 102 的重链可变区 CDR3 且可任选地包含其 CDR1 和 / 或 CDR2 的抗体基本上相同的 K_D 结合 M-CSF。

[0180] 在一些实施方式中，抗 M-CSF 抗体具有低解离速率。在一些实施方式中，抗 M-CSF 抗体的 k_{off} 为 $2.0x10^{-4}s^{-1}$ 或更低。在其他优选的实施方式中，抗体结合 M-CSF 的 k_{off} 为 $2.0x10^{-5}$ 或 $k_{off}2.0x10^{-6}s^{-1}$ 或更低。在一些实施方式中，K_{off} 与在此所述的抗体基本上相同，这样的抗体选自 252、88、100、3.8.3、2.7.3、1.120.1、9.14.4I、8.10.3F、9.7.2IF、9.14.4、8.10.3、9.7.2、9.7.2C-Ser、9.14.4C-Ser、8.10.3C-Ser、8.10.3-CG2、9.7.2-CG2、9.7.2-CG4、9.14.4-CG2、9.14.4-CG4、9.14.4-Ser、9.7.2-Ser、8.10.3-Ser、8.10.3-CG4、8.10.3FG1 或 9.14.4G1。在一些实施方式中，抗体以与如下所述的抗体基本上相同的 k_{off} 结合 M-CSF：(a) 抗体，其包含选自 252、88、100、3.8.3、2.7.3、1.120.1、9.14.4I、8.10.3F、9.7.2IF、9.14.4、8.10.3、9.7.2、9.7.2C-Ser、9.14.4C-Ser、8.10.3C-Ser、8.10.3-CG2、9.7.2-CG2、9.7.2-CG4、9.14.4-CG2、9.14.4-CG4、9.14.4-Ser、9.7.2-Ser、8.10.3-Ser、8.10.3-CG4、8.10.3FG1 或 9.14.4G1 的抗体的重链的 CDR3 且可任选地包含其 CDR1 和 / 或 CDR2；或 (b) 抗体，其包含选自 252、88、100、3.8.3、2.7.3、1.120.1、9.14.4I、8.10.3F、9.7.2IF、9.14.4、8.10.3、9.7.2、9.7.2C-Ser、9.14.4C-Ser、8.10.3C-Ser、8.10.3-CG2、9.7.2-CG2、9.7.2-CG4、9.14.4-CG2、9.14.4-CG4、9.14.4-Ser、9.7.2-Ser、8.10.3-Ser、8.10.3-CG4、8.10.3FG1 或 9.14.4G1 的抗体的轻链的 CDR2 且可任选地包含其 CDR1 和 / 或 CDR3。在一些实施方式中，抗体以与包含具有氨基酸序列 SEQ ID NO:2、6、10、14、18、22、26、30、34、38、46、50、54、58、62、66、70、74、78、82、86、90、94、98 或 102 的重链可变区的抗体、或包含具有氨基酸序列 SEQ ID NO:4、8、12、16、20、24、28、32、36、44、48、52、56 或 60 的轻链可变区抗体基本上相同的 k_{off} 结合 M-CSF。在另一优选的实施方式中，抗体以与包含具有氨基酸序列 SEQ ID NO:4、8、12、16、20、24、28、32、36、44、48、52、56 或 60 的轻链可变区的 CDR2 且可任选地包含其 CDR1 和 / 或 CDR3 的抗体、或包含具有氨基酸序列 SEQ ID NO:2、6、10、14、18、22、26、30、34、38、46、50、54、58、62、66、70、74、78、82、86、90、94、98 或 102 的重链可变区的 CDR3 且可任选地包含其 CDR1 和 / 或 CDR2 的抗体基本上相同的 k_{off} 结合 M-CSF。

[0181] 可通过本领域已知的方法确定抗 M-CSF 抗体与 M-CSF 的解离速率。可通过竞争性 ELISA、RIA 或表面等离子共振（例如，通过采用 BIACORE™ 技术）来测定结合亲和力。可通过表面等离子共振测定解离速率。优选地，通过表面等离子共振测定结合亲和力和解离速率。更优选地，采用 BIACORE™ 技术测定结合亲和力和解离速率。实施例 VI 举例阐述了通过 BIACORE™ 技术确定抗 M-CSF 单克隆抗体的亲和力常数的方法。

[0182] 抗 M-CSF 抗体抑制 M-CSF 的活性

[0183] 抑制 M-CSF 与 c-fms 结合

[0184] 在另一实施方式中，本发明提供抗 M-CSF 抗体，其抑制 M-CSF 与 c-fms 受体的结合并阻断或预防 c-fms 的活化。在优选的实施方式中，M-CSF 是人的 M-CSF。在另一优选的实施方式中，抗 M-CSF 抗体人抗体。可通过如下方法测定 IC_{50}：ELISA、RIA 和基于细胞的测定法例如细胞增殖测定法、全血单核细胞形态改变测定法或受体结合抑制测定法。在一个实施方式中，通过细胞增殖测定法测定，抗体或其部分抑制细胞增殖的 IC_{50} 为不超过 $8.0x10^{-7}M$、优选地不超过 $3x10^{-7}M$ 或更优选地不超过 $8x10^{-8}M$。在另一实施方式中，通过单核细胞形态改变测定法测定的 IC_{50} 为不超过 $2x10^{-6}M$、优选地不超过 $9.0x10^{-7}M$ 或更优选地不超过 $9x10^{-8}M$。

在另一优选的实施方式中，通过受体结合测定法测定的 IC_{50} 为不超过 $2x10^{-6}M$、优选地不超过 $8.0x10^{-7}M$ 或更优选地不超过 $7.0x10^{-8}M$。实施例 III、IV 和 V 例证了各种类型的测定法。

[0185] 在另一方面，本发明的抗 M-CSF 抗体抑制单核细胞 / 巨噬细胞应答于 M-CSF 的细胞增殖，与不存在抗体时的细胞增殖相比，抑制至少 20％、更优选地 40％、45％、50％、55％、60％、65％、70％、80％、85％、90％、95％或 100％。

[0186] 抗体和抗体产生细胞系的产生方法

[0187] 免疫

[0188] 在一些实施方式中，用 M-CSF 抗原免疫其基因组中包含部分或全部人免疫球蛋白重链和轻链基因座的非人动物而产生人抗体。在优选的实施方式中，非人动物是 XENOMOUSE™ 动物 (Abgenix Inc.,Fremont,CA)。可使用的另一种非人动物是由 Medarex(Medarex,Inc.,Princeton,NJ) 产生的转基因动物。

[0189] XENOMOUSE™ 小鼠是遗传工程化的小鼠品系，其包含人免疫球蛋白重链和轻链基因座的大片段，且在产生小鼠抗体方面具有缺陷。参见，例如，Green et al.,Nature Genetics7:13-21(1994) 和美国专利 5,916,771、5,939,598、5,985,615、5,998,209、6,075,181、6,091,001、6,114,598、6,130,364、6,162,963 和 6,150,584。另见 WO91/10741、WO94/02602、WO96/34096、WO96/33735、WO98/16654、WO98/24893、WO98/50433、WO99/45031、WO99/53049、WO00/09560 和 WO00/037504。

[0190] 在另一方面，本发明提供用 M-CSF 抗原免疫包含人免疫球蛋白基因座的非人转基因动物而从非人、非小鼠动物制备抗 M-CSF 抗体的方法。可使用上述文献描述的方法产生这些动物。可如美国专利 5,994,619 中所述对这些文献中公开的方法进行改良。美国专利 5,994,619 描述了用于产生新的培养的内细胞团 (CICM) 细胞和细胞系 (其源于猪和奶牛)，以及异源 DNA 已经插入其中的转基因 CICM 细胞的方法。CICM 转基因细胞可用于产生克隆的转基因胚胎、胎儿和后代。该‘619 专利也描述了用于产生能将异源 DNA 传递至其后代的转基因动物的方法。在优选的实施方式中，非人动物是大鼠、绵羊、猪、山羊、牛或马。

[0191] 该 XENOMOUSE™ 小鼠产生成人样完全人抗体库 (repertoire) 并产生抗原特异性人抗体。在一些实施方式中，XENOMOUSE™ 小鼠通过引入兆碱基大小的人重链基因座和 κ 轻链基因座的种系构型酵母人工染色体 (YAC) 片段而含有约 80％人抗体 V 基因库 (repertoire)。在其他一些实施方式中，XENOMOUSE™ 小鼠进一步含有大约全部的 λ 轻链基因座。见 Mendez et al.,Nature Genetics15:146-156(1997),Green and Jakobovits,J.Exp.Med.188:483-495(1998) 和 WO98/24893，将其公开内容在此通过引用并入本申请。

[0192] 在一些实施方式中，含有人免疫球蛋白基因的非人动物是具有人免疫球蛋白的“小基因座 (minilocus)”的动物。在小基因座方法中，通过引入来自 Ig 基因座的单个基因来模拟外源 Ig 基因座。从而，将一个或多个 V_H 基因、一个或多个 D_H 基因、一个或多个 J_H 基因、μ 恒定区和第二恒定区 (优选地 γ 恒定区) 构成一种构建体用于插入至动物中。该方法描述于，例如，美国专利 5,545,807、5,545,806、5,569,825、5,625,126、5,633,425、5,661,016、5,770,429、5,789,650、5,814,318、5,591,669、5,612,205、5,721,367、5,789,215 和 5,643,763，在此通过引用将其并入本申请。

[0193] 在另一方面，本发明提供制备人源化抗 M-CSF 抗体的方法。在一些实施方式中，如下所述在允许抗体产生的条件下用 M-CSF 抗原免疫非人动物。从动物分离抗体产生细胞，

与骨髓瘤融合以产生杂交瘤，并分离编码所需抗 M-CSF 抗体的重链和轻链的核酸。随后采用本领域已知的技术并如下文所述对这些核酸进行工程化处理，以降低非人序列的量，即，对抗体进行人源化以降低在人体中的免疫应答。

[0194] 在一些实施方式中，M-CSF 抗原是分离的和 / 或纯化的 M-CSF。在优选的实施方式中，M-CSF 抗原是人 M-CSF。在一些实施方式中，M-CSF 抗原是 M-CSF 的片段。在一些实施方式中，M-CSF 片段是 M-CSF 的细胞外结构域。在一些实施方式中，M-CSF 片段包含 M-CSF 的至少一种表位。在其他一些实施方式中，M-CSF 抗原是细胞，所述细胞在其表面表达或过表达 M-CSF 或其免疫原性片段。在一些实施方式中，M-CSF 抗原是 M-CSF 融合蛋白。M-CSF 可通过已知的技术纯化自天然来源。重组 M-CSF 已经商品化。

[0195] 可通过本领域已知的任何方法免疫动物。参见，例如，Harlow and Lane, Antibodies:A Laboratory Manual, New York:Cold Spring Harbor Press, 1990。用于免疫非人动物如小鼠、大鼠、绵羊、山羊、猪、牛和马的方法是本领域熟知的。参见，例如，Harlow and Lane，见上，和美国专利 5,994,619。在优选的实施方式中，M-CSF 抗原与一种佐剂一起施用以刺激免疫应答。这种佐剂包括完全或不完全弗氏佐剂、RIBI（胞壁酰二肽）或 ISCOM（免疫刺激复合物）。这种佐剂可通过将多肽隔离于局部沉积物中而保护多肽免于迅速分散，或它们可含有刺激宿主分泌对巨噬细胞和免疫系统的其它成分具有趋化性的因子的物质。优选地，如果施用多肽，该免疫接种程序表将包含两次或更多次施用多肽，在数周内展开。实施例 I 例证了在 XENOMOUSE™ 小鼠中产生抗 M-CSF 单克隆抗体的方法。

[0196] 抗体和抗体产生细胞系的产生

[0197] 用 M-CSF 抗原免疫动物后，可从该动物中获得抗体和 / 或产生抗体的细胞。在一些实施方式中，含有抗 M-CSF 抗体的血清可通过放血或处死动物从该动物中获得。血清可如其从动物中获得那样使用，可从该血清获得免疫球蛋白部分，或可从血清中纯化抗 M-CSF 抗体。

[0198] 在一些实施方式中，可从分离自经免疫的动物的细胞制备产生抗体的永生化细胞系。免疫后，处死动物并使淋巴结和 / 或脾脏的 B 细胞永生化。使细胞永生化的方法包括但不限于用癌基因转染它们、用致癌病毒感染它们、将其在选择永生化细胞的条件下培养、使其接受致癌或突变化合物影响、将其与永生化细胞如骨髓瘤细胞融合、以及使肿瘤抑制基因失活。参见，例如，Harlow and Lane，见上。如果采用与骨髓瘤细胞融合的方式，骨髓瘤细胞优选地不分泌免疫球蛋白多肽（非分泌细胞系）。使用 M-CSF、它的一部分或表达 M-CSF 的细胞筛选永生化细胞。在优选的实施方式中，初次筛选使用酶联免疫测定 (ELISA) 或放射免疫测定法进行。WO00/37504 给出了 ELISA 筛选的实例，在此通过引用将其并入本申请。

[0199] 如下文中进一步讨论的那样，对产生抗 M-CSF 抗体的细胞如杂交瘤进行选择、克隆并进一步筛选所需的特征，包括健康的细胞生长、高抗体产量及所需的抗体特征。杂交瘤可在体内在同系动物、在缺乏免疫系统的动物如裸鼠中，或在体外细胞培养物中扩增。选择、克隆和扩增杂交瘤的方法是本领域人员熟知的。

[0200] 在优选的实施方式中，免疫的动物是表达人免疫球蛋白基因的非人动物，将脾脏的 B 细胞融合至源于与该非人动物相同物种的骨髓瘤。在更加优选的实施方式中，免疫的动物是 XENOMOUSE™ 动物并且骨髓瘤细胞系是非分泌型小鼠骨髓瘤。在更加优选的实施方

式中，骨髓瘤细胞系是 P3-X63-AG8-653。参见，例如，实施例 I。

[0201] 因此，在一个实施方式中，本发明提供用于产生能产生针对 M-CSF 的人单克隆抗体或其片段的细胞系的方法，所述方法包括 (a) 用 M-CSF、M-CSF 的一部分或表达 M-CSF 的细胞或组织免疫在此描述的非人转基因动物；(b) 让该转基因动物产生对 M-CSF 的免疫应答；(c) 从转基因动物中分离 B 淋巴细胞；(d) 使 B 淋巴细胞永生化；(e) 建立永生化的 B 淋巴细胞的独立单克隆群；并 (f) 筛选该永生化的 B 淋巴细胞以鉴定针对 M-CSF 的抗体。

[0202] 在另一方面，本发明提供产生人抗 M-CSF 抗体的杂交瘤。在优选的实施方式中，杂交瘤是小鼠杂交瘤，如上描述的。在其他一些实施方式中，杂交瘤在非人、非小鼠物种如大鼠、绵羊、猪、山羊、牛或马中产生。在另一实施方式中，杂交瘤是人杂交瘤。

[0203] 在另一优选的实施方式中，用 M-CSF 免疫转基因动物，从经免疫的转基因动物分离原代细胞，例如脾脏细胞或者外周血细胞，并鉴定产生对所需抗原具有特异性的抗体的独立细胞。分离来自各独立细胞的多腺苷酸化 mRNA 并进行逆转录多聚酶链反应 (RT-PCR)，其中使用退火至至可变区序列的有义引物，例如识别大部分或全部人重链和轻链可变区基因 FR1 区的简并引物，以及退火至恒定区或链接区序列的反义引物。然后克隆重链和轻链可变区的 cDNA，并在任何合适的宿主细胞例如骨髓瘤细胞中，作为与免疫球蛋白恒定区例如重链和 κ 或 λ 恒定区的嵌合抗体的形式表达。见 Babcook, J. S. et al., Proc. Natl. Acad. Sci. USA93:7843-48, 1996，通过引用将其并入本申请。然后可如在此所述的方式鉴定并分离抗 M-CSF 抗体。

[0204] 在另一实施方式中，可使用噬菌体展示技术来提供含有对 M-CSF 具有不同亲和力的抗体库 (repertoire) 的文库。产生此类库 (repertoire) 不需要使得来自被免疫动物的 B 细胞永生化。反之，原代 B 细胞可直接作为 DNA 的来源。使用获得自例如源自脾脏的 B 细胞的 cDNA 混合物制备表达文库，例如，转染至大肠杆菌的噬菌体展示文库。测试所得细胞对 M-CSF 的免疫反应性。从此类文库中鉴定高亲和力人抗体的技术可参见 Griffiths et al., EMBO J., 13:3245-3260(1994)；Nissim et al., ibid, pp. 692-698 和 Griffiths et al., ibid, 12:725-734。最终从文库中鉴定到产生对抗原具有所需结合亲和力水平的克隆，回收编码负责此结合的产物的 DNA 并用于标准重组表达操作。也可使用以前操作的核苷酸序列构建噬菌体展示文库并以类似的方式筛选。通常，编码重链和轻链的 cDNA 独立地提供或链接形成 Fv 类似物用于产生噬菌体文库。

[0205] 随后筛选噬菌体文库找到对 M-CSF 具有最高亲和力的抗体并从合适的克隆回收遗传物质。多轮次的筛选可提高所分离的初始抗体的亲和力。

[0206] 在另一方面，本发明提供产生人抗 M-CSF 抗体的杂交瘤。在优选的实施方式中，杂交瘤是如上所述的小鼠杂交瘤。在其他一些实施方式中，杂交瘤在非人、非小鼠物种如大鼠、绵羊、猪、山羊、牛或马中产生。在另一实施方式中，杂交瘤是人杂交瘤。

[0207] 制备抗体的核酸、载体、宿主细胞和重组方法

[0208] 核酸

[0209] 本发明还涵盖编码抗 M-CSF 抗体的核酸分子。在一些实施方式中，不同的核酸分子编码抗 M-CSF 免疫球蛋白的重链和轻链。在其他一些实施方式中，同一核酸分子编码抗 M-CSF 免疫球蛋白的重链和轻链。在一个实施方式中，核酸编码本发明的 M-CSF 抗体。

[0210] 在一些实施方式中，所述编码轻链可变结构域的核酸分子包含人的 V_{κ}L5、012、L2、

B3、A27 基因和 Jκ1、Jκ2、Jκ3 或 Jκ4 基因。

[0211] 在一些实施方式中，所述编码轻链的核酸分子编码与种系氨基酸序列相比包含 1、2、3、4、5、6、7、8、9 或 10 个突变的氨基酸序列。在一些实施方式中，所述核酸分子包含编码与种系序列相比包含 1、2、3、4、5、6、7、8、9 或 10 个非保守性氨基酸取代和 / 或 1、2 或 3 个非保守性取代的 V_L 氨基酸序列的核苷酸序列。取代可以在 CDR 区、框架区或在恒定区内。

[0212] 在一些实施方式中，所述核酸分子编码与种系序列相比包含一或多个变异的 V_L 氨基酸序列，所述一或多个变异与抗体 252、88、100、3.8.3、2.7.3、1.120.1、9.14.4I、8.10.3F、9.7.2IF、9.14.4、8.10.3、9.7.2、9.7.2C-Ser、9.14.4C-Ser、8.10.3C-Ser、8.10.3-CG2、9.7.2-CG2、9.7.2-CG4、9.14.4-CG2、9.14.4-CG4、9.14.4-Ser、9.7.2-Ser、8.10.3-Ser、8.10.3-CG4、8.10.3FG1 或 9.14.4G1 中任一抗体的 V_L 中的那些变异相同。

[0213] 在一些实施方式中，与种系序列相比，所述核酸分子编码抗体 252、88、100、3.8.3、2.7.3、1.120.1、9.14.4、8.10.3 或 9.7.2 中任一抗体的 V_L 中的至少 3 个氨基酸突变。

[0214] 在一些实施方式中，所述核酸分子包含编码单克隆抗体 252(SEQ ID NO:4)、88(SEQ ID NO:8)、100(SEQ ID NO:12)、3.8.3(SEQ ID NO:16)、2.7.3(SEQ ID NO:20)、1.120.1(SEQ ID NO:24)、9.14.4I(SEQ ID NO:28)、8.10.3F(SEQ ID NO:32)、9.7.2IF(SEQ ID NO:36)、9.14.4(SEQ ID NO:28)、8.10.3(SEQ ID NO:44)、9.7.2(SEQ ID NO:48)、9.7.2C-Ser(SEQ ID NO:52)、9.14.4C-Ser(SEQ ID NO:56)、8.10.3C-Ser(SEQ ID NO:60)、8.10.3-CG2(SEQ ID NO:60)、9.7.2-CG2(SEQ ID NO:52)、9.7.2-CG4(SEQ ID NO:52)、9.14.4-CG2(SEQ ID NO:56)、9.14.4-CG4(SEQ ID NO:56)、9.14.4-Ser(SEQ ID NO:28)、9.7.2-Ser(SEQ ID NO:48)、8.10.3-Ser(SEQ ID NO:44)、8.10.3-CG4(SEQ ID NO:60)8.10.3FG1(SEQ ID NO:32) 或 9.14.4G1(SEQ ID NO:28) 的 V_L 氨基酸序列或其部分的核苷酸序列。在一些实施方式中，所述部分至少包含 CDR2 区。在一些实施方式中，核酸编码所述抗体的轻链 CDR 的氨基酸序列。在一些实施方式中，所述部分是包含 CDR1-CDR3 的连续部分。

[0215] 在一些实施方式中，所述核酸分子包含编码 SEQ ID NO:4、8、12、16、20、24、28、32、36、44、48、52、56 或 60 之一的轻链氨基酸序列的核苷酸序列。在一些优选的实施方式中，核酸分子包含 SEQ ID NO:3、7、11、27、31、35、43 或 47 的轻链核苷酸序列或其部分。

[0216] 在一些实施方式中，所述核酸分子编码与图 1 所示的 VL 氨基酸序列或者与抗体 252、88、100、3.8.3、2.7.3、1.120.1、9.14.4I、8.10.3F、9.7.2IF、9.14.4、8.10.3、9.7.2、9.7.2C-Ser、9.14.4C-Ser、8.10.3C-Ser、8.10.3-CG2、9.7.2-CG2、9.7.2-CG4、9.14.4-CG2、9.14.4-CG4、9.14.4-Ser、9.7.2-Ser、8.10.3-Ser、8.10.3-CG4、8.10.3FG1 或 9.14.4G1 中任一抗体的 VL 氨基酸序列、或与 SEQ ID NO:4、8、12、16、20、24、28、32、36、44、48、52、56 或 60 中任一氨基酸序列具有至少 70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98% 或 99% 的相同性的 V_L 氨基酸序列。本发明的核酸分子包括在高严格性条件（例如如上所述的条件）下与编码 SEQ ID NO:4、8、12、16、20、24、28、32、36、44、48、52、56 或 60 的轻链氨基酸序列的核酸序列、或与具有 SEQ ID NO:3、7、11、27、31、35、43 或 47 的轻链核酸序列的核酸序列杂交的核酸。

[0217] 在另一实施方式中，核酸编码选自 252、88、100、3.8.3、2.7.3、1.120.1、9.14.4I、8.10.3F、9.7.2IF、9.14.4、8.10.3、9.7.2、9.7.2C-Ser、9.14.4C-Ser、8.10.3C-Ser、

8.10.3-CG2、9.7.2-CG2、9.7.2-CG4、9.14.4-CG2、9.14.4-CG4、9.14.4-Ser、9.7.2-Ser、8.10.3-Ser、8.10.3-CG4、8.10.3FG1 或 9.14.4G1 的抗体的全长轻链、或包含 SEQ ID NO:4、8、12、16、20、24、28、32、36、44、48、52、56 或 60 的氨基酸序列和轻链恒定区的轻链、或包含突变的轻链。此外，上所述核酸可包含 SEQ ID NO:3、7、11、27、31、35、43 或 47 的轻链核苷酸序列和编码轻链恒定区的核苷酸序列、或编码包含突变的轻链的核酸分子。

[0218] 在另一优选的实施方式中，所述核酸分子编码重链可变区 (V_H)，其包含人 VH1-18、3-33、3-11、3-23、3-48 或 3-7 基因序列或从其衍生的序列。在各种实施方式中，核酸分子包含人 V_H1-18 基因、D_H4-23 基因和人 J_H4 基因；人 V_H3-33 基因、人 D_H1-26 基因和人 J_H4 基因；人 V_H3-11 基因、人 D_H7-27 基因和人 J_H4 基因；人 V_H3-11 基因、人 D_H7-27 基因和人 J_H6 基因；人 V_H3-23 基因、人 D_H1-26 基因和人 J_H4 基因；人 V_H3-7 基因、人 D_H6-13 基因和人 J_H4 基因；人 V_H3-11 基因、人 D_H7-27 基因和人 J_H4b 基因；人 V_H3-48 基因、人 D_H1-26 基因和人 J_H4b 基因；人 V_H3-11 基因、人 D_H6-13 基因和人 J_H6b 基因、或衍生自所述人基因的序列。

[0219] 在一些实施方式中，所述核酸分子编码与人 V、D 或 J 基因的种系氨基酸序列相比包含 1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17 或 18 个突变的氨基酸序列。在一些实施方式中，所述突变位于 V_H 区。在一些实施方式中，所述突变位于 CDR 区。

[0220] 在一些实施方式中，与种系序列相比，所述核酸分子编码与单克隆抗体 252、88、100、3.8.3、2.7.3、1.120.1、9.14.4I、8.10.3F、9.7.2IF、9.14.4、8.10.3、9.7.2、9.7.2C-Ser、9.14.4C-Ser、8.10.3C-Ser、8.10.3-CG2、9.7.2-CG2、9.7.2-CG4、9.14.4-CG2、9.14.4-CG4、9.14.4-Ser、9.7.2-Ser、8.10.3-Ser、8.10.3-CG4、8.10.3FG1 或 9.14.4G1 的 V_H 中的那些氨基酸突变相同的一或多个氨基酸突变。在一些实施方式中，与种系序列相比，所述核酸编码与上述单克隆抗体之一中的至少 3 个氨基酸突变相同的至少 3 个氨基酸突变。

[0221] 在一些实施方式中，所述核酸分子包含核苷酸序列，其编码抗体 252(SEQ ID NO:4)、88(SEQ ID NO:8)、100(SEQ ID NO:12)、3.8.3(SEQ ID NO:16)、2.7.3(SEQ ID NO:20)、1.120.1(SEQ ID NO:24)、9.14.4I(SEQ ID NO:28)、8.10.3F(SEQ ID NO:32)、9.7.2IF(SEQ ID NO:36)、9.14.4(SEQ ID NO:28)、8.10.3(SEQ ID NO:44)、9.7.2(SEQ ID NO:48)、9.7.2C-Ser(SEQ ID NO:52)、9.14.4C-Ser(SEQ ID NO:56)、8.10.3C-Ser(SEQ ID NO:60)、8.10.3-CG2(SEQ ID NO:60)、9.7.2-CG2(SEQ ID NO:52)、9.7.2-CG4(SEQ ID NO:52)、9.14.4-CG2(SEQ ID NO:56)、9.14.4-CG4(SEQ ID NO:56)、9.14.4-Ser(SEQ ID NO:28)、9.7.2-Ser(SEQ ID NO:48)、8.10.3-Ser(SEQ ID NO:44)、8.10.3-CG4(SEQ ID NO:60)8.10.3FG1(SEQ ID NO:32) 或 9.14.4G1(SEQ ID NO:28) 的 V_H 氨基酸序列的至少一部分、或具有保守性氨基酸突变和 / 或总共 3 个或更少的非保守性氨基酸取代的所述序列的至少一部分。在各种实施方式中，所述序列编码一或多个 CDR 区、优选地 CDR3 区、所有 3 个 CDR 区、包括 CDR1-CDR3 的连续部分或完整 V_H 区。

[0222] 在一些实施方式中，所述核酸分子包含编码 SEQ ID NO:2、6、10、14、18、22、26、30、34、38、46、50、54、58、62、66、70、74、78、82、86、90、94、98 或 102 之一的氨基酸序列的重链核苷酸序列。在一些优选的实施方式中，核酸分子包含 SEQ ID NO:1、5、9、25、29、33、37、45、97 或 101 的重链核苷酸序列的至少一部分。在一些实施方式中，所述部分编码 V_H 区、CDR3 区、所有 3 个 CDR 区、或包括 CDR1-CDR3 的连续区域。

[0223] 在一些实施方式中，所述核酸分子编码 VH 氨基酸序列，其与图 4 所示的 V_H 氨基酸序列或与 SEQ ID NO:2、6、10、14、18、22、26、30、34、38、46、50、54、58、62、66、70、74、78、82、86、90、94、98 或 102 中任一 V_H 氨基酸序列具有至少 70％、75％、80％、85％、90％、91％、92％、93％、94％，95％、96％、97％、98％或 99％的相同性。本发明的核酸分子包括在高严格性条件（例如如上所述的条件）下与编码 SEQ ID NO:2、6、10、14、18、22、26、30、34、38、46、50、54、58、62、66、70、74、78、82、86、90、94、98 或 102 的重链氨基酸序列的核酸序列、或与具有 SEQ ID NO:1、5、9、25、29、33、37、45、97 或 101 的核苷酸序列的核酸序列杂交的核酸。

[0224] 在另一实施方式中，还是编码选自 252、88、100、3.8.3、2.7.3、1.120.1、9.14.4I、8.10.3F、9.7.2IF、9.14.4、8.10.3、9.7.2、9.7.2C-Ser、9.14.4C-Ser、8.10.3C-Ser、8.10.3-CG2、9.7.2-CG2、9.7.2-CG4、9.14.4-CG2、9.14.4-CG4、9.14.4-Ser、9.7.2-Ser、8.10.3-Ser、8.10.3-CG4、8.10.3FG1 或 9.14.4G1 的抗体的全长重链、或具有 SEQ ID NO:2、6、10、14、18、22、26、30、34、38、46、50、54、58、62、66、70、74、78、82、86、90、94、98 或 102 的氨基酸序列和重链恒定区的重链、或包含突变的重链。此外，上所述核酸可包含 SEQ ID NO:1、5、9、25、29、33、37、45、97 或 101 的重链核苷酸序列和编码轻链恒定区的核苷酸序列、或编码包含突变的重链的核酸分子。

[0225] 编码抗 M-CSF 抗体的完整重链或轻链或其部分的核酸分子可分离自产生该抗体的任何来源。在各种实施方式中，核酸分子可分离自来自以 M-CSF 免疫的动物的 B 细胞，或分离自源自这样的 B 细胞且表达抗 M-CSF 抗体的永生化细胞。分离编码抗体的 mRNA 的方法是本领域熟知的。参见，例如，Sambrook et al。mRNA 可用于产生 cDNA 以便用于聚合酶链反应 (PCR) 或 cDNA 克隆抗体基因。在优选的实施方式中，所述核酸分子分离自杂交瘤，杂交瘤具有作为其融合伴侣之一的来自非人转基因动物的产生人免疫球蛋白的细胞。在更加优选的实施方式中，产生人免疫球蛋白的细胞分离自 XENOMOUSE™ 动物。在另一实施方式中，产生人免疫球蛋白的细胞来自如上所述的非人、非小鼠转基因动物。在另一实施方式中，核酸分离自非人、非转基因的动物。分离自非人、非转基因的动物的核酸分子可用于，例如，人源化抗体。

[0226] 在一些实施方式中，编码本发明的抗 M-CSF 抗体的重链的核酸可包含与编码任意来源的重链恒定结构域的核苷酸序列框内连接的编码本发明的 V_H 结构域的核苷酸序列。类似地，编码本发明的抗 M-CSF 抗体的轻链的核酸分子可包含与编码任意来源的轻链恒定结构域的核苷酸序列框内连接的编码本发明的 V_L 结构域的核苷酸序列。

[0227] 在本发明的另一方面，编码重链 (V_H) 轻链 (V_L) 可变结构域的核酸分子被“转换”成全长抗体基因。在一个实施方式中，编码 V_H 或 V_L 结构域的核酸分子可通过将其插入至分别已经编码重链恒定区 (C_H) 或轻链恒定区 (C_L) 的表达载体，使得 V_H 节段可操纵地连接至载体内的 C_H 节段而 V_L 节段可操纵地连接至载体内的 C_L 节段，从而转换为全长抗体基因。在另一实施方式中，编码 V_H 和 / 或 V_L 结构域的核酸分子通过使用标准的分子生物学技术通过连接，例如连接编码 V_H 和 / 或 V_L 结构域的核酸分子至编码 C_H 和 / 或 C_L 结构域的核酸分子而使其转换成全长抗体基因。使用编码 VL 和 CL 链的核酸分子可达到一样的结果。人重链和轻链免疫球蛋白恒定结构域基因的核酸序列是本领域已知的。参见，例如，Kabat et al.,Sequences of Proteins of Immunological Interest,5th Ed.,NIH Publ.

No.91-3242,1991。编码全长重链和/或轻链的核酸分子可随后由已经导入所述核酸分子的细胞表达,并可随后分离抗 M-CSF 抗体。

[0228] 可使用核酸分子来重组表达大量抗 M-CSF 抗体。核酸分子还可用于产生嵌合抗体、双特异性抗体、单链抗体、免疫粘合素、二价抗体、突变抗体和抗体衍生物,如下文所述。如果核酸分子源自非人、非转基因的动物,核酸分子可用于抗体的人源化,如下文所述。

[0229] 在另一实施方式中,本发明的核酸分子用作特定抗体序列的探针或 PCR 引物。例如,所述核酸可作为探针用于诊断方法或作为 PCR 引物用于扩增可能使用的 DNA 区域,例如,用于分离编码抗 M-CSF 抗体可变结构域的其他核酸分子。在一些实施方式中,所述核酸分子是寡核苷酸。在一些实施方式中,寡核苷酸来自的目标抗体的重链和轻链的高可变区。在一些实施方式中,寡核苷酸编码抗体 252、88、100、3.8.3、2.7.3 或 1.120.1 的一或多个 CDR 的全部或部分、或在此所述的它们的变体。

[0230] 载体

[0231] 本发明提供包含编码本发明的抗 M-CSF 抗体或其抗原结合部分的重链的核酸分子的载体。本发明也提供包含编码此类抗体或其抗原结合部分的轻链的核酸分子的载体。本发明还提供包含编码融合蛋白、修饰抗体、抗体片段和其探针的核酸分子的载体。

[0232] 在一些实施方式中,通过将如上所述获得的编码部分或全长轻链和重链的 DNA 插入至表达载体,使得基因可操纵地连接至必需的表达调控序列例如转录和翻译调控序列,从而表达本发明的抗 M-CSF 抗体或抗原结合部分。表达载体包括质粒、逆转录病毒、腺病毒、腺伴随病毒 (AAV)、植物病毒如花椰菜花叶病毒、烟草花叶病毒、粘粒、YAC、EBV 衍生的附加体等。将抗体基因连接进载体中,使得载体中的转录和翻译调控序列发挥它们预期的调节该抗体基因转录和翻译的功能。选择表达载体和表达调控序列使其与使用的表达宿主细胞相容。抗体轻链的基因和抗体重链的基因可插入分开的载体中。在优选的实施方式中,两种基因都插入同一表达载体。抗体基因可通过标准方法(例如连接抗体基因片段和载体上的互补性限制位点,或如果无限制位点则进行平端连接)插入表达载体。

[0233] 方便的载体是编码功能完全的人 C_H 或 C_L 免疫球蛋白序列的载体,通过工程化造成合适的限制位点使得任何 V_H 或 V_L 序列能如上所述容易地插入和表达。在这种载体中,剪接通常发生在插入的 J 区的剪接供体位点和在人 C 区之前的剪接受体位点之间,也发生在人 C_H 外显子中存在的剪接区域。多腺苷酸化和转录终止发生在编码区下游的天然染色体位点。重组表达载体也能编码促进抗体链从宿主细胞分泌的信号肽。抗体链基因可克隆进载体中使得信号肽框内连接至免疫球蛋白链的氨基末端。信号肽可以是免疫球蛋白信号肽或异源信号肽(即来自非免疫球蛋白的信号肽)。

[0234] 除了抗体链基因,本发明的重组表达载体还携带有控制抗体链基因在宿主细胞中表达的调节序列。本领域技术人员将理解表达载体的设计,包括对调节序列的选择可依赖于待转化的宿主细胞的选择、期望的蛋白质表达水平等因素。用于哺乳动物宿主细胞表达的优选的调节序列包括在哺乳动物细胞中指导高水平蛋白质表达的病毒元件,例如源于逆转录病毒 LTR、巨细胞病毒 (CMV)(如 CMV 启动子/增强子)、猿猴病毒 40(SV40)(例如 SV40 启动子/增强子)、腺病毒(如腺病毒主要晚期启动子 (AdMLP))的启动子和/或增强子,多瘤和强的哺乳动物启动子如天然免疫球蛋白和肌动蛋白启动子。对于进一步的病毒调控元件和其序列的描述,见例如,美国专利 No.5,168,062、美国专利 No.4,510,245 和美国专利

No.4,968,615。用于在植物中表达抗体的方法，包括对启动子和载体的描述以及植物的转化在本领域是已知的。参见，例如，美国专利6,517,529，通过引用将其并入本申请。在细菌细胞或真菌细胞如酵母细胞中表达多肽的方法也是本领域熟知的。

[0235] 除了抗体链基因和调节序列外，本发明的重组表达载体可携带另外的序列，如调节该载体在宿主细胞中的复制的序列（如复制起点）和选择标记基因。选择标记基因便于对载体已经导入其中的宿主细胞进行选择（参见例如，美国专利No.4,399,216、4,634,665和5,179,017）。例如，通常选择标记基因赋予载体已经导入其中的宿主细胞以药物抗性，例如对G418、潮霉素或甲氨蝶呤的抗性。优选的选择标记基因包括二氢叶酸还原酶(DHFR)基因（用于以甲氨蝶呤选择/扩增dhfr-宿主细胞）、新霉素耐药基因（用于G418选择）以及谷氨酸合成酶基因。

[0236] 用于重组产生蛋白质的非杂交瘤宿主细胞和方法

[0237] 编码抗M-CSF抗体的核酸分子和包含这些核酸分子的载体可用于转化合适的哺乳动物、植物、细菌或酵母宿主细胞。转化可通过用于将多核苷酸导入宿主细胞的任何已知方法完成。用于将异源多核苷酸导入哺乳动物细胞的方法是本领域熟知的，包括葡聚糖介导的转染、磷酸钙沉淀、聚凝胺-介导的转染、原生质体融合、电穿孔术、脂质体包囊化的多核苷酸和直接显微注射DNA至胞核。另外，核酸分子可通过病毒载体引入至哺乳动物细胞。转化细胞的方法是本领域熟知的。参见，例如，美国专利No.4,399,216、4,912,040、4,740,461和4,959,455（通过引用将这些专利并入本申请）。转化植物细胞的方法是本领域熟知的，包括如农杆菌属介导的转化、基因枪转化、直接注射、电穿孔和病毒转化。转化细菌和酵母细胞的方法也是本领域熟知的。

[0238] 可用作表达用宿主的哺乳动物细胞系是本领域熟知的，包括许多可从美国典型培养物保藏中心(ATCC)获得的永生化细胞系。这些例如包括中国仓鼠卵巢(CHO)细胞、NSO、SP2细胞、HeLa细胞、幼仓鼠肾(BHK)细胞、猴肾细胞(COS)、人肝细胞癌细胞（如Hep G2）、A549细胞和许多其它的细胞系。通过确定哪种细胞系具有高表达水平来选择特别优选的细胞系。其它可使用的细胞系是昆虫细胞系如Sf9细胞。当将编码抗体基因的重组表达载体引入至哺乳动物宿主细胞时，可通过培养宿主细胞一段时间来产生抗体，所述的时间足以使该抗体在宿主细胞中表达，或更优选地使该抗体分泌至宿主细胞生长的培养基中。可使用标准蛋白质纯化方法从培养基回收抗体。植物宿主细胞包括如烟草、拟南芥、浮萍、玉米、小麦、马铃薯等。细菌宿主细胞包括大肠杆菌和链霉菌属种类。酵母宿主细胞包括粟酒裂殖酵母、酿酒酵母和巴斯得毕赤酵母。

[0239] 此外，可使用许多已知的技术增强本发明的抗体（或其其它部分）从生产细胞系中的表达。例如，谷氨酰胺合成酶基因表达系统(GS系统）是用于在一定条件下改善表达的普通方法。该GS系统在欧洲专利No.0 216 846、0 256 055和0 323 997以及欧洲专利申请No.89303964.4中有完整或部分讨论。

[0240] 通过不同细胞系表达或在转基因动物中表达的抗体可能彼此具有不同的糖基化。然而，由在此提供的核酸分子编码或包含在此提供的氨基酸序列的所有抗体是本发明的一部分，无需考虑该抗体的糖基化状态或形式或修饰情况。

[0241] 转基因动物和植物

[0242] 也可通过产生以目标免疫球蛋白重链和轻链序列转基因的哺乳动物或植物而以

转基因的方式产生本发明的抗 M-CSF 抗体，且所产生的抗体是可自其回收的形式。就在哺乳动物中转基因产生而言，抗 M-CSF 抗体可产生于山羊、牛或其他哺乳动物的乳中，并可从中回收。参见，例如，美国专利 No.5,827,690、5,756,687、5,750,172 和 5,741,957。在一些实施方式中，如上所述，以 M-CSF 或其免疫原性部分免疫包含人免疫球蛋白基因座的非人转基因动物。在植物、酵母或真菌 / 藻类中制备抗体的方法描述于，例如，美国专利 6,046,037 和 US5,959,177。

[0243] 在一些实施方式中，非人转基因动物或转基因植物可通过标准转基因技术将一或多种编码本发明的抗 M-CSF 抗体的核酸分子导入动物或植物中而产生。参见 Hogan 和美国专利 6,417,429，见上。用于生产转基因动物的转基因细胞可以是胚胎干细胞或体细胞。转基因非人生物体可以是嵌合、非嵌合的杂合子及非嵌合的纯合子。参见，例如，Hogan et al.,Manipulating the Mouse Embryo:A Laboratory Manual2ed.,Cold Spring Harbor Press (1999);Jackson et al.,Mouse Genetics and Transgenics:A Practical Approach,Oxford University Press(2000);和 Pinkert,Transgenic Animal Technology:A Laboratory Handbook,Academic Press(1999)。在一些实施方式中，转基因非人动物具有通过编码目标重链和 / 或轻链的靶向构建体所造成的靶向断裂和替换。在优选的实施方式中，转基因动物包含并表达编码特异性结合至 M-CSF，优选地结合至人 M-CSF 的重链和轻链的核酸分子。在一些实施方式中，转基因动物包含编码修饰的抗体如单链抗体、嵌合抗体或人源化抗体的核酸分子。抗 M-CSF 抗体可在任何转基因动物中生产。在优选的实施方式中，非人动物是小鼠、大鼠、绵羊、猪、山羊、牛或马。非人转基因动物将所述编码的多肽表达于血、乳、尿、唾液、泪液、粘液和其它体液中。

[0244] 噬菌体展示文库

[0245] 本发明提供制备抗 M-CSF 抗体或其抗原结合部分的方法，其包括如下步骤：在噬菌体上合成人抗体文库，筛选含有 M-CSF 或其部分的文库，分离与 M-CSF 结合的噬菌体，并从该噬菌体获得抗体。例如，制备抗体文库用于噬菌体展示技术的方法包括如下步骤：以 M-CSF 或其抗原部分免疫包括人免疫球蛋白基因座的非 - 人动物以便引起免疫应答，由经免疫的动物提取产生抗体的细胞；由该提取的细胞分离 RNA，将 RNA 反转录以产生 cDNA，使用引物扩增 cDNA，并将 cDNA 插入噬菌体展示载体以便抗体在噬菌体上表达。可以此方式获得本发明的重组抗 M-CSF 抗体。

[0246] 本发明的重组抗 M-CSF 人抗体可通过筛选重组的组合抗体文库来分离。优选地，该抗体文库为 scFv 噬菌体展示文库，其通过使用分离自 B 细胞的 mRNA 所制备的人 V_L 和 V_H cDNA 产生。制备及筛选此类文库的方法是本领域已知的。已有用于产生噬菌体展示文库的商品化试剂盒（例如，Pharmacia Recombinant Phage Antibody System, 目录号 27-9400-01；和 Stratagene SurfZAP™phage display kit, 目录号 240612)。也有其它可用于产生及筛选抗体展示文库的方法及试剂（参见，例如，美国专利 No.5,223,409；PCT 出版物 WO92/18619、WO91/17271、WO92/20791、WO92/15679、WO93/01288、WO92/01047、WO92/09690；Fuchs et al.,Bio/Technology 9:1370-1372(1991);Hay et al.,Hum. Antibod.Hybridomas3:81-85(1992);Huse et al.,Science246:1275-1281(1989); McCafferty et al.,Nature 348:552-554(1990);Griffiths et al.,EMBO J.12:725-734(1993);Hawkins et al.,J.Mol.Biol.226:889-896(1992);

Clackson et al.,Nature352:624-628(1991);Gram et al.,Proc.Natl.Acad.Sci.USA89:3576-3580(1992);Garrad et al.,Bio/Technology9:1373-1377(1991);Hoogenboom et al.,Nuc.Acid Res.19:4133-4137(1991);和 Barbas et al.,Proc.Natl.Acad.Sci.USA88:7978-7982(1991))。

[0247] 在一个实施方式中,为分离具有所需特性的人抗 M-CSF 抗体,首先采用在此所述的人抗 M-CSF 抗体来选择对 M-CSF 具有类似结合活性的人重链及轻链序列,其使用 PCT 出版物 WO93/06213 中描述的表位印记法 (epitope imprinting)。用于此方法的抗体文库优选为 scFv 文库,其制备及筛选描述于 PCT 出版物 WO92/01047,McCafferty et al.,Nature348:552-554(1990);和 Griffiths et al.,EMBO J.12:725-734(1993)。scFv 抗体文库优选使用人 M-CSF 作为抗原来筛选。

[0248] 一旦选定最初的人 V_L 和 V_H 结构域,即可进行"混合及匹配"试验,其中筛选不同对的最初选择的 V_L 和 V_H 节段的 M-CSF 结合情况,以选出优选的 V_L/V_H 配对组合。此外,为进一步改善抗体品质,可对优选的 V_L/V_H 对的 V_L 和 V_H 节段进行随机突变,优选在 V_H 和 / 或 V_L 的 CDR3 区内,其方法类似于体内导致天然免疫应答过程中抗体的亲和力成熟的体细胞突变过程。这种体外亲和力成熟可通过使用分别互补于 V_H CDR3 或 V_L CDR3 的 PCR 引物扩增 V_H 和 V_L 结构域而实现,其中该引物的某些位置已经掺入四种核苷酸碱基的随机混合物,以使得所得 PCR 产物所编码的 V_H 和 V_L 节段中的 V_H 和 / 或 V_L CDR3 区中导入了随机突变。可再次就其 M-CSF 结合性对这些随机突变的 V_H 和 V_L 节段进行筛选。

[0249] 从重组免疫球蛋白展示文库筛选并分离本发明的抗 M-CSF 抗体之后,可回收来自展示包装(如来自噬菌体基因组)的编码选定抗体的核酸,并通过标准重组 DNA 技术亚克隆入其它表达载体。若需要时,该核酸可进一步如下所述加以操作以产生其它本发明的抗体形式。为表达通过筛选组合文库所分离的重组人抗体,如上所述将编码抗体的 DNA 克隆入重组表达载体并导入哺乳动物宿主细胞中。

[0250] 类别转换

[0251] 本发明的另一方面提供一种方法,用于将抗 M-CSF 抗体的类别或亚类转换为另一种类别或亚类。在一些实施方式中,使用本领域所熟知的方法来分离编码 V_L 或 V_H 但不包含任何编码 C_L 或 C_H 的核酸序列的核酸分子。接着将该核酸分子可操作地连接至编码来自所需免疫球蛋白类别或亚类的 C_L 或 C_H 的核酸序列。其可通过使用包括 C_L 或 C_H 链的载体或核酸分子而实现,如前所述。例如,原本为 IgM 的抗 M-CSF 抗体可转换类别成为 IgG。此外,类别转换可用于将 IgG 亚类转换为另一种,如由 IgG1 转换为 IgG2。另一制备包括所需同种型的本发明的抗体的方法包括以下步骤:分离编码抗 M-CSF 抗体的重链的核酸及编码抗 M-CSF 抗体的轻链的核酸,分离编码 V_H 区的序列,连接 V_H 序列至编码所需同种型的重链恒定结构域的序列,在细胞中表达轻链基因及重链构建体,并收集具有所需同种型的抗 M-CSF 抗体。

[0252] 在一些实施方式中,本发明的抗 M-CSF 抗体的重链第 228 位(根据欧洲编号惯例)的丝氨酸变为脯氨酸。因此,IgG4 的 F_C 区中的 CPSC 亚序列变为 IgG1 中的亚序列 CPPC(Aalberse,R.C.and Schuurman,J.,Immunology,105:9-19(2002))。例如,位于 SEQ ID NO:46 的残基 243(其相当于欧洲编号惯例中的残基 228)的丝氨酸可变为脯氨酸。类似地,位于 SEQ ID NO:38 的残基 242(其相当于欧洲编号惯例中的残基 228)的丝氨酸可变为脯氨

酸。在一些实施方式中，IgG4 抗体的框架区可回复突变成为种系构架序列。某些具体实施方式同时包括构架区的回复突变及 FC 区的丝氨酸变为脯氨酸。参见，例如，表 1A 中的 SEQ ID NO:54（抗体 9.14.4C-Ser）和 SEQ ID NO:58（抗体 8.10.3C-Ser）。

[0253] 去免疫化的抗体

[0254] 另一产生具有减低免疫原性的抗体的方法为抗体去免疫化。在本发明的另一方面，抗体可使用描述于如 PCT 出版物 WO98/52976 和 WO00/34317（其全文以引用的方式并入本申请）的技术来进行去免疫化。

[0255] 突变的抗体

[0256] 在另一实施方式中，核酸分子、载体及宿主细胞可用于制备突变的抗 M-CSF 抗体。该抗体可于重链和 / 或轻链的可变结构域内产生突变，如改变抗体的结合特性。例如，可于一个或多个 CDR 区中产生突变以增加或降低抗体对 M-CSF 的 K_D，以增加或降低 k_{off}，或者改变抗体的结合特异性。定点突变技术是本领域中所熟知的。参见，例如，Sambrook et al. 和 Ausubel et al.，见上。在优选的实施方式中，与种系相比，已知突变发生在抗 M-CSF 抗体的可变结构域内要改变的氨基酸残基位置。在另一实施方式中，与种系相比，已知一或多个突变发生在单克隆抗体 252、88、100、3.8.3、2.7.3、1.120.1、9.14.4I、8.10.3F、9.7.2IF、9.14.4、8.10.3、9.7.2、9.7.2C-Ser、9.14.4C-Ser、8.10.3C-Ser、8.10.3-CG2、9.7.2-CG2、9.7.2-CG4、9.14.4-CG2、9.14.4-CG4、9.14.4-Ser、9.7.2-Ser、8.10.3-Ser、8.10.3-CG4、8.10.3FG1 或 9.14.4G1 的可变结构域的 CDR 区或框架区内或恒定结构域内要改变的氨基酸残基位置。在另一实施方式中，与种系相比，已知一或多个突变发生在选自 SEQ ID NO:2、6、10、14、18、22、26、30、34、38、46、50、54、58、62、66、70、74、78、82、86、90、94、98 或 102 的重链氨基酸序列或其重链核苷酸序列为 SEQ ID NO:1、5、9、25、29、33、37、45、97 或 101 的重链氨基酸序列的可变结构域的 CDR 区或框架区内要改变的氨基酸残基位置。在另一实施方式中，与种系相比，已知一或多个突变发生在选自 SEQ ID NO:4、8、12、16、20、24、28、32、36、44、48、52、56 或 60 的轻链氨基酸序列或其轻链核苷酸序列为 SEQ ID NO:3、7、11、27、31、35、43 或 47 的轻链氨基酸序列的可变结构域的 CDR 区或框架区内要改变的氨基酸残基位置。

[0257] 在一个实施方式中，构架区已经突变以便所产生的构架区具有对应的种系基因的氨基酸序列。突变发生于构架区或恒定区以增加抗 M-CSF 抗体的半衰期。请见如 PCT 出版物 WO00/09560，通过引用将其并入本申请。也可在构架区或恒定区中产生突变以改变抗体的免疫原性，以提供共价或非共价结合另一分子的位点，或改变如补体结合的特性、FcR 结合及抗体依赖性细胞介导的细胞毒性 (ADCC)。根据本发明，单一抗体可在可变结构域的一或多个 CDR 或框架区内或者是恒定结构域内具有突变。

[0258] 在一些实施方式中，与突变前的抗 M-CSF 抗体相比，在突变的抗 M-CSF 抗体的 V_H 或 V_L 结构域中有由 1 至 8 个（包含其间任何数字）氨基酸突变。上述任一情况中，突变可发生于一个或多个 CDR 区。而且，任一突变都可为保守性氨基酸取代。在一些实施方式中，恒定区含有不超过 5、4、3、2 或 1 个氨基酸变化。

[0259] 修饰的抗体

[0260] 在另一实施方式中，可产生融合抗体或免疫粘附素，其包括连接至另一多肽的本发明的抗 M-CSF 抗体的全部或部分。在优选的实施方式中，仅抗 M-CSF 抗体的可变结构域连

接至多肽。在另一优选的实施方式中，抗 M-CSF 抗体的 V_H 结构域连接第一多肽，而抗 M-CSF 抗体的 V_L 结构域连接第二多肽，第二多肽与第一多肽的关联方式使得 V_H 和 V_L 结构域能彼此相互作用从而形成抗体结合位点。在另一优选的实施方式中，V_H 结构域通过接头与 V_L 结构域分离，以便 V_H 和 V_L 结构域可彼此互相作用（请见下文有关单链抗体的部分）。V_H- 接头 -V_L 抗体再连接至目的多肽。融合抗体可用以将多肽指引向表达 M-CSF 的细胞或组织。该多肽可为一种治疗剂，如毒素、生长因子或其它调节蛋白质，或者其可为诊断剂，例如易于观察的酶，如辣根过氧化物酶。此外，产生的融合抗体中两个（或多个）单链抗体可互相连接。这对于希望在单一多肽链上产生二价或多价抗体或者希望产生双特异性抗体而言是有用的。

[0261] 为产生单链抗体 (scFv)，将编码 V_H 和 V_L 的 DNA 片段可操作地连接至另一编码柔性接头的片段（如编码氨基酸序列 $(Gly_4-Ser)_3$），使得 V_H 和 V_L 序列可作为连续的单链蛋白质表达，且该 V_L 和 V_H 结构域以柔性接头连结。参见，例如，Bird et al.,Science242:423-426(1988) ;Huston et al.,Proc.Natl.Acad.Sci.USA85:5879-5883(1988) ;McCafferty et al.,Nature348:552-554(1990)。单链抗体可为单价（若仅使用单一 V_H 和 V_L）、二价（若使用两种 V_H 和 V_L）或多价（若使用多于两种 V_H 和 V_L）。可产生能与 M-CSF 及另一分子特异性结合的双特异性或多价抗体。

[0262] 在其他一些实施方式中，可使用编码抗 M-CSF 抗体的核酸分子制备其它经修饰的抗体。例如，可依照说明书教导采用标准的分子生物技术来制备“κ 抗体”(Ill et al.,Protein Eng.10:949-57(1997))、“微型抗体”(Martin et al.,EMBO J.13:5303-9(1994))、“二价抗体”(Holliger et al.,Proc.Natl.Acad.Sci.USA90:6444-6448(1993)) 或“Janusins”(Traunecker et al.,EMBO J.10:3655-3659(1991) 和 Traunecker et al.,Int.J.Cancer(Suppl.)7:51-52(1992))。

[0263] 可通过多种方法来制备双特异性抗体或抗原结合片段，包括杂交瘤的融合或 Fab' 片段的连接等。参见，例如，Songsivilai&Lachmann,Clin.Exp.Immunol.79:315-321(1990),Kostelny et al.,J.Immunol.148:1547-1553(1992)。另外，可形成“二价抗体”或“Janusins”形式的双特异性抗体。在一些实施方式中，双特异性抗体可与两种不同的 M-CSF 表位结合。在一些实施方式中，双特异性抗体具有来自单克隆抗体 252、88、100、3.8.3、2.7.3、1.120.1、9.14.4I、8.10.3F、9.7.2IF、9.14.4、8.10.3 或 9.7.2 的第一重链及第一轻链，以及另外的抗体重链及轻链。在一些实施方式中，另外的轻链及重链也来自上述单克隆抗体之一，但不同于所述第一重链及轻链。

[0264] 在一些实施方式中，可使用来自在此所提供的人抗 M-CSF 单克隆抗体、该单克隆抗体的氨基酸序列、或由编码该单克隆抗体的核酸序列所编码的重链或轻链的一或多个可变区或 CDR 区制备上述经修饰抗体。

[0265] 衍生及标记的抗体

[0266] 本发明的抗 M-CSF 抗体或抗原结合部分可经另一分子（如另一肽或蛋白质）衍生化或与之连接。通常，抗体或其部分的衍生方式使得该衍生或标记不会不利影响其与 M-CSF 结合。因此，本发明的抗体与抗体部分意欲包括在此所述的完整及经修饰形式的人抗 M-CSF 抗体。例如，本发明的抗体或抗体部分可功能性地连接（通过化学结合、基因融合、非共价键结合等）至一或多个其它分子实体，如另一抗体（如双特异性抗体或二价抗体）、检测剂、

细胞毒性剂、药剂和 / 或可介导所述抗体或抗体部分与另一分子（如链霉抗生物素蛋白核心区或聚组氨酸标签）结合的蛋白质或肽。

[0267] 一种衍生的抗体是通过使两个或多个抗体（各为同类或不同类，如产生双特异性抗体）交联而制备的。适当的交联接头是异双官能团，具有两个以适当间隔子（如间 - 马来酰亚胺苯甲酰基 -N- 羟基琥珀酰亚胺酯）分隔的不同反应基团，或是同种双官能团（如琥珀酰亚胺基辛二酸酯）的。此种接头可得自 Pierce Chemical Company, Rockford, Ill。

[0268] 另一种衍生抗体为标记抗体。可用于衍生本发明的抗体或抗原结合部分的检测剂包含荧光化合物，包含荧光素、异硫氰酸荧光素、罗丹明、5- 二甲胺 -1- 萘磺酰氯、藻红素、镧系元素磷光体等。抗体也可标记上用于检测的酶，如辣根过氧化物酶、β - 半乳糖苷酶、萤光素酶、碱性磷酸酶、葡萄糖氧化酶等。当抗体以可检测的酶标记时，其通过添加额外的试剂来检测，所述的试剂被酶利用产生可识别的反应产物。例如，存在辣根过氧化物酶试剂时，添加过氧化氢及二氨基联苯胺能产生可检测的有色反应产物。抗体亦可以生物素标记，并通过间接测量抗生物素蛋白或链霉抗生物素蛋白结合进行检测。抗体还可由能以二级报告子（如亮氨酸拉链对序列、二抗的结合部位、金属结合区、表位标签）识别的预先测定的多肽表位标记。在一些实施方式中，标记可通过不同长度的间隔臂附着以减少可能的空间障碍。

[0269] 抗 M-CSF 抗体也可以放射标记氨基酸标记。放射标记的抗 M-CSF 抗体可用于诊断及治疗目的。例如放射标记的抗 M-CSF 抗体可通过 x- 射线或其它诊断技术来检测能表达 M-CSF 的肿瘤。另外，放射标记的抗 M-CSF 抗体可治疗性地用作癌细胞或肿瘤的毒素。标记多肽实例包含（不限于）下列放射性同位素或放射核素 -^{3}H、^{14}C、^{15}N、^{35}S、^{90}Y、^{99}Tc、^{111}In、^{125}I 和 ^{131}I。

[0270] 抗 M-CSF 抗体也可用化学基团例如聚乙二醇 (PEG)、甲基或乙基或碳水化合物基团来衍生。这些基团可用于改善抗体的生物学特性，如增加血清半衰期或增强组织结合。

[0271] 药物组合物及试剂盒

[0272] 本发明还涉及包含人抗 M-CSF 拮抗剂抗体的组合物，其用于有需要治疗的对象来治疗类风湿性关节炎、骨质疏松症或动脉硬化。在一些实施方式中，治疗的对象为人。在其他一些实施方式中，对象为兽医对象。可以本发明拮抗剂抗 M-CSF 抗体治疗的单核细胞在其中发挥重要作用的过度增殖性病症可涉及任何组织或器官并包含（但不限于）脑癌、肺癌、鳞状细胞癌、膀胱癌、胃癌、胰脏癌、乳腺癌、头癌、颈癌、肝癌、肾癌、卵巢癌、前列腺癌、结肠直肠、食道癌、妇科癌症、鼻咽癌或甲状腺癌、黑色素瘤、淋巴瘤、白血病或多发性骨髓瘤。特别地，本发明的人拮抗剂抗 M-CSF 抗体可用于治疗或预防乳癌、前列腺癌、结肠癌及肺癌。

[0273] 本发明还涵盖用于治疗选自下列病症的组合物 :哺乳动物（包含人）的包括系统性红斑狼疮、狼疮性肾炎和皮肤狼疮的狼疮、关节炎、牛皮癣性关节炎、Reiter's 综合征、痛风、创伤性关节炎、风疹关节炎与急性滑膜炎、类风湿性关节炎、类风湿性脊椎炎、强直性脊柱炎、骨关节炎、痛风性关节炎及其它关节炎病症、脓毒症、脓毒性休克、内毒素休克、革兰氏阴性菌脓毒症、中毒性休克综合征、阿尔茨海默氏症、中风、神经创伤、哮喘、成人呼吸道窘迫综合征、脑型疟、慢性肺炎性疾病、硅肺、肺部类肉瘤病、骨质再吸收疾病、骨质疏松症、再狭窄、心脏及肾脏再输注伤害、血栓形成、肾小球肾炎、糖尿病、移植物抗宿主反应、同种

异体移植物排斥、炎性肠病、局限性回肠炎、溃疡性结肠炎、多发性硬化症、肌肉变性、湿疹、接触性皮炎、牛皮癣、晒伤或结膜炎性，该组合物包含治疗有效量的本发明人抗 M-CSF 单克隆抗体及药用可接受载体。

[0274] 治疗可包含施用一或多种本发明的拮抗剂抗 M-CSF 单克隆抗体或其抗原结合片段，其可单独或与药用可接受载体共同给药。在本文中，"药用可接受载体"意指任何及所有生理相容的溶剂、分散介质、包衣、抗菌剂及抗真菌剂、等渗剂及吸收延缓剂等等。药用可接受载体的一些实例为水、盐水、磷酸缓冲盐水、葡萄糖、甘油、乙醇等以及其混合物。在许多情况下，优选为组合物中包含等渗剂，如糖类、多元醇类如甘露醇、山梨糖醇或氯化钠。额外的药用可接受物质实例为可增加抗体的保存期或效力的湿润剂或少量助剂，如湿润剂或乳化剂、防腐剂或缓冲剂。

[0275] 本发明的抗 M-CSF 抗体及包括这些抗体的组合物可与一种或多种其它治疗剂、诊断剂或预防剂组合给药。额外的治疗剂包含其它抗新生物、抗肿瘤、抗血管形成或化学治疗剂。此种额外的药剂可包含于相同组合物中或分别给药。在一些实施方式中，一种或多种的本发明抑制性抗 M-CSF 抗体可做为疫苗或疫苗的佐剂。

[0276] 本发明的组合物可为多种形式，例如液体、半固体及固体剂型，如液体溶液（如可注射及输注溶液）、分散液或悬浮液、片剂、丸剂、粉剂、脂质体及栓剂。优选形式取决于预期的给药方式及治疗用途。典型的优选组合物为可注射或输注溶液形式，如类似运用于人被动免疫的组合物。优选给药方式为胃肠外给药（如静脉、皮下、腹腔、肌肉）。在优选的实施方式中，抗体是以静脉输注或注射给药。在另一优选的实施方式中，该抗体可肌肉注射或皮下注射给药。在另一实施方式中，本发明包括以可特异性结合 M-CSF 的抗体或其抗原结合部分治疗需治疗的对象的方法，其步骤包括：(a) 施用有效量的编码重链或其抗原结合部分的分离核酸分子、编码轻链或其抗原结合部分的分离核酸分子，或同时施用编码轻链及重链或其抗原结合部分的核酸分子；及 (b) 表达该核酸分子。

[0277] 治疗性组合物典型地必须是无菌且于制造及贮存条件下稳定。组合物可调配为溶液、微乳化溶液、分散液、脂质体或其它适于高药物浓度的规定结构。无菌可注射溶液的制备可通过将含需要量抗 M-CSF 抗体的合适溶剂与视需要的一种上列成分或上列成分的组合相混合，然后无菌过滤。通常，分散剂的制备是通过将活性化合物掺入包含基础分散介质及必需的上列其它成分的无菌载体中。至于可供制备无菌可注射溶液的无菌粉剂，优选制备方法为真空干燥及冷冻干燥处理预先无菌过滤的溶液以产生包括活性成分及任何附加所需成分的粉末。可通过如使用包衣（如卵磷脂）、于分散液中维持所需颗粒大小及使用表面活性剂维持溶液的适当流动性。可通过在组合物中纳入可延缓吸收的药剂（如单硬脂酸盐类及明胶）延长可注射组合物的吸收。

[0278] 本发明的抗体可通过多种本领域中已知的方法给药，不过对许多治疗用途而言，优选给药途径 / 方法为皮下注射、肌肉注射或静脉输注。本领域人员应了解给药途径和 / 或方式取决于所需的结果。

[0279] 在某些实施方式中，可用能防止抗体快速释放的载体处理该抗体组合物的活性化合物，制备成例如控制释放制剂，包含植入物、经皮贴片及微胶囊递送系统。可使用生物可分解、生物兼容性聚合物，如乙烯乙酸乙烯酯、聚酐类、聚乙醇酸、胶原蛋白、聚原酸酯及聚乳酸。许多可制备此种制剂的方法都已申请专利或普遍为本领域人员所熟知。参见，例如，

Sustained and Controlled Release Drug Delivery Systems(J.R.Robinson,ed.,Marcel Dekker,Inc.,New York,1978)。

[0280] 在某些实施方式中，本发明的抗 M-CSF 抗体可口服给药，例如与惰性稀释剂或可吸收的食用载体合用。该化合物（及视需要的其它成分）亦可封入硬胶囊或软明胶胶囊中，压缩为片剂或直接掺入对象饮食中。对治疗性的口服给药，抗 M-CSF 抗体可与赋形剂并用，并以可食片剂、口腔片、含片、胶囊、酏剂、悬浮液、糖浆、薄片等形式运用。除了胃肠外施用本发明化合物外，该化合物可能需要以能预防其失活的物质包衣或与可预防其失活的物质共同施用。

[0281] 额外的活性化合物亦可掺入所述组合物中。在某些实施方式中，本发明的抗 M-CSF 抗体可与一种或多种额外的治疗剂共同调配和 / 或共同给药。这些药剂包含可与其它靶结合的抗体、抗癌剂、抗肿瘤剂、化学治疗剂、抑制 M-CSF 的肽类似物、能与 M-CSF 结合的可溶性 c-fms、一种或多种抑制 M-CSF 的化学剂、抗炎剂、抗凝血剂、降血压药剂（即血管紧张素 - 转化酶 (ACE) 抑制剂）。此种组合疗法可需要较低剂量的抗 M-CSF 抗体以及共同施用的药剂，从而避免与各种单一药物疗法相关的可能毒性或并发症。

[0282] 本发明的抑制性抗 M-CSF 抗体及包括该抗体的组合物亦可与其它治疗方案组合施用，特别是与放射疗法并用治疗癌症。本发明化合物亦可与抗癌剂（如血管内皮抑制素及血管抑制素 (angiostatin)）或细胞毒性药物（如阿霉素）、柔红霉素、顺铂、鬼臼噻吩甙、紫杉醇、泰素帝及生物碱（如长春花新碱、法呢基转移酶抑制剂、VEGF 抑制剂）及抗代谢剂（如氨甲喋呤）并用。

[0283] 本发明的化合物亦可与抗病毒剂（如 Viracept、AZT、阿昔洛韦及泛昔洛韦、以及抗脓毒症化合物（如 Valant）并用。

[0284] 本发明的组合物可包含“治疗有效量”或“预防有效量”的本发明的抗体或其抗原结合部分。“治疗有效量″意指，以剂量及所需时间而言，可有效达到所需的治疗效果的量。抗体或抗体部分的治疗有效量可依各种因素而不同，如个体的疾病状态、年龄、性别及体重，以及抗体或抗体部分诱发个体所需反应的能力。治疗有效量也是指该剂量时抗体或抗体部分的有益治疗作用胜过其任何毒性或有害作用。“预防有效量″意指，以剂量及所需时间而言，可有效达到所需的预防作用的量。典型地，由于预防性剂量是用于染病前或患病早期的对象，预防有效量将小于治疗有效量。

[0285] 可调整剂量方案以提供最佳所需反应（如治疗或预防反应）。例如可施用单一冲击剂量、可于一段时间内施用数次分割剂量或该剂量可视治疗状态的迫切需要而成比例地减少或增加。将胃肠外组合物调配成为单位剂型特别有利，其可易于给药且剂量统一。在本文中，单位剂型意指物理上离散的单位，其适合用作欲治疗的哺乳动物对象的单位剂型；各单位包含预先定量的活性化合物，其加上所需的药用载体、经计算可产生所需疗效。本发明的剂量形式的规格直接取决于 (a) 抗 M-CSF 抗体或部分的独特特性以及欲实现的特定治疗或预防作用，及 (b) 配制此抗体用于治疗敏感个体的技术中的内在限制因素。

[0286] 本发明的抗体或抗体部分的治疗或预防有效量的示范性非限制性范围为 0.025 至 50mg/kg，更优选地为 0.1 至 50mg/kg，更优选地为 0.1 至 25、0.1 至 10 或 0.1 至 3mg/kg。应注意的是剂量值可随所需减轻的病症类型及严重性而变化。应进一步了解对任何特定对象而言，特定的剂量方案应于一段时间后依个体需要及给药者或监督施用组合物者的专业

判断来加以调整，且在此所示的剂量范围仅为示范性的且并非意欲限制本发明的组合物的范围或运用。

[0287] 本发明的另一方面提供试剂盒，其包括本发明的抗 M-CSF 抗体或抗原结合部分或包括此种抗体或部分的组合物。除抗体或组合物外，试剂盒可包含诊断剂或治疗剂。试剂盒亦可包含用于诊断或治疗法的说明。在优选的实施方式中，该试剂盒包含了抗体或包括该抗体的组合物及可用于下述方法中的诊断剂。在另一优选的实施方式中，该试剂盒包含抗体或包含其的组合物及一种或多种可用于下述方法中的治疗剂。本发明的一个实施方式为试剂盒，其包括容器、对患有炎性疾病的人施用抗 M-CSF 抗体的说明、或测量生物学样品中的 CD14+CD16+ 单核细胞数量的说明、以及抗 M-CSF 抗体。

[0288] 本发明还涉及抑制哺乳动物中异常细胞生长的组合物，其包括一定量的本发明的抗体以及一定量的化学治疗剂，其中的化合物、盐类、溶剂化物或前药及化学治疗剂的量可一起有效抑制异常细胞的生长。许多化学治疗剂为本领域中已知。在一些实施方式中，该化学治疗剂选自：有丝分裂抑制剂、烷化剂、抗代谢剂、嵌入抗生素、生长因子抑制剂、细胞周期抑制剂、酶、拓扑异构酶抑制剂、生物反应调节剂、抗激素、如抗雄激素及抗血管形成剂。

[0289] 抗血管形成剂，如 MMP-2（基质金属蛋白酶 2）抑制剂、MMP-9（基质金属蛋白酶 -9）抑制剂及 COX-II（环氧化酶 II）抑制剂，可与本发明的抗 M-CSF 抗体组合。可用的 COX-II 抑制剂实例包含 CELEBREX™（塞来考昔 (celecoxib)）、伐地考昔 (valdecoxib) 及罗非考昔 (rofecoxib)。可用的基质金属蛋白酶抑制剂实例描述于 WO96/33172（1996 年 10 月 24 日公开）、WO96/27583（1996 年 3 月 7 日公开）、欧洲专利申请号 97304971.1（1997 年 7 月 8 日申请）、欧洲专利申请号 99308617.2（1999 年 10 月 29 申请）、WO98/07697（1998 年 2 月 26 日公开）、WO98/03516（1998 年 1 月 29 日公开）、WO98/34918（1998 年 8 月 13 日公开）、WO98/34915（1998 年 8 月 13 日公开）、W98/33768（1998 年 8 月 6 日公开）、WO98/30566（1998 年 7 月 16 日公开）、欧洲专利申请 606,046（1994 年 7 月 13 日公开）、欧洲专利公开 931,788（1999 年 7 月 28 日公开）、WO90/05719（1990 年 5 月 31 日公开）、WO99/52910（1999 年 10 月 21 日公开）、WO99/52889（1999 年 10 月 21 日公开）、WO99/29667（1999 年 6 月 17 日公开）、PCT 国际申请 PCT/IB98/01113（1998 年 7 月 21 日申请）、欧洲专利申请 99302232.1（1999 年 3 月 25 日申请）、英国专利申请 9912961.1（1999 年 6 月 3 日申请）、美国临时申请 60/148,464（1999 年 8 月 12 日申请）、美国专利 5,863,949（1999 年 1 月 26 日颁予）、美国专利 5,861,510（1999 年 1 月 19 日颁予）及欧洲专利公开 780,386（1997 年 6 月 25 日公开），其全部内容皆以引用的方式并入本申请。优选的 MMP 抑制剂为不会导致关节疼痛。更优选的为相对于其它基质金属蛋白酶（即 MMP-1、MMP-3、MMP-4、MMP-5、MMP-6、MMP-7、MMP-8、MMP-10、MMP-11、MMP-12 及 MMP-13）可选择性抑制 MMP-2 和 / 或 MMP-9 的 MMP 抑制剂。用于本发明中的 MMP 抑制剂的一些具体实例为 AG-3340、RO32-3555、RS13-0830、以及下列化合物：3-[[4-(4- 氟 - 苯氧基)- 苯磺酰]-(1- 羟基氨甲酰基 - 环戊基)- 氨基]- 丙酸；3- 外 -3-[4-(4- 氟 - 苯氧基)- 苯磺酰氨基]-8- 氧杂 - 二环 [3.2.1] 辛烷 -3- 羧酸羟基酰胺；(2R,3R)1-[4-(2- 氯 -4- 氟 - 苄氧基)- 苯磺酰]-3- 羟基 -3- 甲基 - 哌啶 -2- 羧酸羟基酰胺；4-[4-(4- 氟 - 苯氧基)- 苯磺酰氨基] 四氧 - 噻 -4- 羧酸羟基酰胺；3-[[4-(4- 氟 - 苯氧基)- 苯磺酰]-(1- 羟基氨甲酰基 - 环丁基)- 氨基]- 丙酸；4-[4-(4- 氯 - 苯氧基)- 苯磺酰氨基]- 四氢 - 吡喃 -4- 羧酸羟基酰胺；(R)3-[4-(4- 氯 - 苯氧基)- 苯磺酰氨基]- 四氢 - 吡

喃 -3- 羧酸羟基酰胺 ;(2R,3R)1-[4-(4- 氟 -2- 甲基 - 苄氧基)- 苯磺酰]-3- 羟基 -3- 甲基 - 哌啶 -2- 羧酸羟基酰胺 ;3-[[4-(4- 氟 - 苯氧基)- 苯磺酰]-(1- 羟基氨甲酰基 -1- 甲基 - 乙基)- 氨基]- 丙酸 ;3-[[4-(4- 氟 - 苯氧基)- 苯磺酰]-(4- 羟基氨甲酰基 - 四氢 - 吡喃 -4- 基)- 氨基]- 丙酸 ;3- 外 -3-[4-(4- 氯 - 苯氧基)- 苯磺酰氨基]-8- 氧杂 - 二环 [3.2.1] 辛烷 -3- 羧酸羟基酰胺 ;3- 内 -3-[4-(4- 氟 - 苯氧基)- 苯磺酰胺基]-8- 氧杂 - 二环 [3.2.1] 辛烷 -3- 羧酸羟基酰胺 ;和 (R)3-[4-(4- 氟 - 苯氧基)- 苯磺酰氨基]- 四氢 - 呋喃 -3- 羧酸羟基酰胺 ;及上述化合物药学上可接受盐类及溶剂化物。

[0290] 包括本发明的人抗 M-CSF 单克隆抗体的化合物可与信号转导抑制剂一起使用,如可抑制 EGF-R(表皮生长因子受体) 反应的药剂,如 EGF-R 抗体、EGF 抗体及 EGF-R 抑制剂分子 ;VEGF(血管内皮生长因子) 抑制剂,如可抑制 VEGF 的 VEGF 受体及分子 ;及 erbB2 受体抑制剂,如有机分子或可与 erbB2 受体结合的抗体,如 HERCEPTIN™(Genentech, Inc.)。EGF-R 抑制剂描述于如 WO95/19970 中 (1995 年 7 月 27 日公开)、WO98/14451(1998 年 4 月 9 日公开)、WO98/02434(1998 年 1 月 22 日公开) 及美国专利 5,747,498(1998 年 5 月 5 日颁予),且此种物质可如在此所述运用于本发明。EGFR- 抑制剂包含 (但不限于) 单克隆抗体 C225 及抗 EGFR22Mab(ImClone systems Incorporated)、ABX-EGF(Abgenix/Cell Genesys)、EMD-7200(Merck KgaA)、EMD-5590(Merck KgaA)、MDX-447/H-477(Medarex Inc.and Merck KgaA) 和化合物 ZD-1834,ZD-1838 和 ZD-1839(AstraZeneca)、PKI-166(Novartis)、PKI-166/CGP-75166(Novartis)、PTK787(Novartis)、CP701(Cephalon)、leflunomide(Pharmacia/Sugen)、CI-1033(Warner Lambert Parke Davis)、CI-1033/PD183,805(Warner Lambert Parke Davis)、CL-387,785(Wyeth-Ayerst)、BBR-1611(Boehringer Mannheim GmbH/Roche)、Naamidine A(Bristol Myers Squibb)、RC-3940-II(Pharmacia)、BIBX-1382(Boehringer Ingelheim)、OLX-103(Merck&Co.)、VRCTC-310(Ventech Research)、EGF 融合毒素 (Seragen Inc.)、DAB-389(Seragen/Lilgand)、ZM-252808(Imperial Cancer Research Fund)、RG-50864(INSERM)、LFM-A12(Parker Hughes Cancer Center)、WHI-P97(ParkerHughes Cancer Center)、GW-282974(Glaxo)、KT-8391(Kyowa Hakko) 和 EGF-R 疫苗 (York Medical/Centro de Immunologia Molecular(CIM))。这些和其他 EGF-R 抑制剂和用于本发明。

[0291] VEGF 抑制剂,例如 SU-5416 及 SU-6668(Sugen Inc.)、AVASTIN™(Genentech)、SH-268(Schering) 及 NX-1838(NeXstar) 亦可与本发明化合物组合。VEGF 抑制剂描述于,例如,WO99/24440(1999 年 5 月 20 日公开)、PCT 国际申请 PCT/IB99/00797(1999 年 5 月 3 日申请)、WO95/21613(1995 年 8 月 17 日公开)、WO99/61422(1999 年 12 月 2 日公开)、美国专利 5,834,504(1998 年 11 月 10 日颁予)、WO98/50356(1998 年 11 月 12 日公开)、美国专利 5,883,113(1999 年 3 月 16 日颁予)、美国专利 5,886,020(1999 年 3 月 23 日颁予)、美国专利 5,792,783(1998 年 8 月 11 日颁予)、WO99/10349(1999 年 3 月 4 日公开)、W97/32856(1997 年 9 月 12 日公开)、W97/22596(1997 年 6 月 26 日公开)、WO98/54093(1998 年 12 月 3 日公开)、WO98/02438(1998 年 1 月 22 日公开)、WO99/16755(1999 年 4 月 8 日公开) 及 WO98/02437(1998 年 1 月 22 日公开),其全文以引用的方式并入本文中。其它一些可用于本发明的特异性 VEGF 抑制剂的实例为 IM862(Cytran Inc.) ;Genentech, Inc. 的抗 VEGF 单克隆抗体 ;以及 angiozyme,一种来自 Ribozyme and Chiron 公司的合成核酶。这些及其它

VEGF 抑制剂可如在此所述运用于本发明。ErbB2 受体抑制剂，如 GW-282974(Glaxo Wellcome plc) 及单克隆抗体 AR-209(Aronex Pharmaceuticals Inc.) 及 2B-1(Chiron)，可进一步与本发明化合物并用，如那些描述于 WO98/02434(1998 年 1 月 22 日公开)、WO99/35146(1999 年 7 月 15 日公开)、WO99/35132(1999 年 7 月 15 日公开)、WO98/02437(1998 年 1 月 22 日公开)、WO97/13760(1997 年 4 月 17 日公开)、WO95/19970(1995 年 7 月 27 日公开)、美国专利 5,587,458(1996 年 12 月 M 日颁予) 及美国专利号 5,877,305(1999 年 3 月 2 日颁予)，其全部内容以引用的方式并入本文中。可用于本发明的 ErbB2 受体抑制剂也描述于美国专利 6,465,449(2002 年 10 月 15 日颁予) 及美国专利 6,284,764(2001 年 9 月 4 日颁予) 中，两者全文内容皆以引用的方式并入本文中。ErbB2 受体抑制剂化合物及物质 (描述于上述 PCT 申请案、美国专利与美国临时申请案中)，以及其它可抑制 erbB2 受体的化合物与物质，可依本发明所述与本发明化合物并用。

[0292] 抗生存 (anti-survival) 剂包含抗 IGF-IR 抗体及抗整合素剂，如抗整合素抗体。

[0293] 抗炎剂可与本发明的抗 M-CSF 抗体并用。对治疗类风湿性关节炎而言，本发明的人抗 M-CSF 抗体可与下列药剂并用 :如 TNF-α 抑制剂，如 TNF 药物 (如 REMICADE™、CDP-870 及 HUMIRA™) 及 TNF 受体免疫球蛋白分子 (如 ENBREL™)、IL-1 抑制剂、受体拮抗剂或可溶性 IL-1ra(如 Kineret 或 ICE 抑制剂)、COX-2 抑制剂 (如塞来考昔、罗非考昔、伐地考昔及依托考昔)、金属蛋白酶抑制剂 (优选为 MMP-13 选择性抑制剂)、p2X7 抑制剂、α2δ 配体 (如 NEUROTIN™ 及 PREGABALIN™)、低剂量氨甲蝶呤、Ieflunomide、hydroxychloroquine、d- 青霉胺、auranofin 或胃肠外或口服金制剂。本发明的化合物亦可与现存可治疗骨关节炎的治疗剂组合使用。可组合使用的适当药剂包含标准非类固醇抗炎剂 (以下称为 NSAID)，如炎痛喜康、双氯酚酸钠、丙酸类如萘普生、氟比洛芬 (flurbiprofen)、fenoprofen、ketoprofen 和布洛芬、芬那美特 (fenamates)(如甲灭酸)、吲哚美辛、苏林大 (sulindac)、apazone、pyrazolones(如苯基保泰松)、水杨酸类 (如阿司匹林)、COX-2 抑制剂如塞来考昔、伐地考昔、罗非考昔及依托考昔，止痛剂及关节内治疗剂，如类固醇及透明质酸如 hyalgan 及欣维可 (synvisc)。

[0294] 抗凝剂可与本发明的抗 M-CSF 抗体并用。抗凝剂实例包含 (但不限于) 华法令 (COUMADIN™)、肝素及 enoxaparin(LOVENOX™)。

[0295] 本发明的人抗 M-CSF 抗体亦可与心血管药剂，如钙通道阻断剂、降血脂制剂，如抑制素 (statins)、fibrates、β- 阻断剂、Ace 抑制剂、血管紧张素 -2 受体拮抗剂及血小板聚集抑制剂组合使用。本发明的化合物亦可与 CNS 药物并用，如抗抑郁剂 (如舍曲林)、抗帕金森氏症药 (如司立吉林 (deprenyl)、L- 多巴、REQUIP™、MIRAPEX™、MAOB 抑制剂，如司来吉兰 (selegine) 及雷沙吉兰 (rasagiline)、comP 抑制剂 (如答是美 (Tasmar))、A-2 抑制剂、多巴胺再摄取抑制剂、NMDA 拮抗剂、尼古丁激动剂、多巴胺激动剂及神经元氧化氮合酶抑制剂) 及抗阿尔茨海默症药物如 donepezil、tacrine、α2δ 配体 (如 NEUROTIN™ 和 PREGABALIN™) 抑制剂、COX-2 抑制剂、丙戊茶碱或 metryfonate。

[0296] 本发明的人抗 M-CSF 抗体亦可与骨质疏松症药物并用，如 roloxifene、屈洛昔芬 (droloxifene)、拉索昔芬 (Iasofoxifene) 或 fosomax 及免疫抑制剂，如 FK-506 和雷帕霉素。

[0297] 诊断用途的方法

[0298] 另一方面，本发明提供诊断方法。抗 M-CSF 抗体可用于检测体外或体内生物学样品的 M-CSF。在一个实施方式中，本发明提供一种可诊断有需要的对象体内表达 M-CSF 的肿瘤的存在或其位置的方法，其步骤包括将抗体注射至对象，通过定位抗体结合处而确定对象中的 M-CSF 表达，比较对象与正常参考对象或标准的表达并诊断肿瘤的存在或位置。

[0299] 抗 M-CSF 抗体可用于常规的免疫分析法，包含（但不限于）ELISA、RIA、FACS、组织免疫组化、蛋白质印迹法或免疫沉淀法。本发明的抗 M-CSF 抗体可用于检测来自人的 M-CSF。在另一实施方式中，抗 M-CSF 抗体可用于检测源自灵长类（如食蟹猴、恒河猴、黑猩猩或猿）的 M-CSF。本发明提供可检测生物学样品中 M-CSF 的方法，其包括将生物学样品与本发明的抗 M-CSF 抗体接触并检测已结合的抗体。在一个实施方式中，抗 M-CSF 抗体可直接以可检测的标记来标记。在另一实施方式中，抗 M-CSF 抗体（第一抗体）并未标记而第二抗体或可与抗 M-CSF 抗体结合的其它分子是标记的。例如，为本领域人员所熟知，所选的第二抗体可与第一抗体的特定物种及类别特异性结合。例如，若抗 M-CSF 抗体为人 IgG，那么二抗可为抗人 IgG。其它可结合抗体的分子包含（但不限于）蛋白质 A 及蛋白质 G，二者皆已商品化，如源自 Pierce Chemical Co。

[0300] 前文已公开了抗体或二抗的适当标记，包括各种酶、辅基、荧光物质、发光物质及放射性物质。适当的酶的实例包含辣根过氧化酶、碱性磷酸酶、β－半乳糖苷酶或乙酰胆碱酯酶；适当辅基复合物实例包含链霉抗生物素蛋白／生物素及抗生物素蛋白／生物素；适当荧光物质实例包含伞形酮、荧光素、异硫氰酸荧光素、罗丹明、二氯三嗪胺荧光素、丹磺酰氯或藻红素；发光物实例包含鲁米诺；适当放射性物实例包含 ^{125}I、^{131}I、^{35}S 或 ^{3}H。

[0301] 在其他一些实施方式中，M-CSF 可于生物学样品中通过竞争性免疫分析法、利用以可检测物质标记的 M-CSF 标准及未标记的抗 M-CSF 抗体进行分析。在此测定法析中，将生物学样品、已标记的 M-CSF 标准与抗 M-CSF 抗体组合，并确定标记的 M-CSF 标准与未标记抗体结合的量。生物学样品中的 M-CSF 的量为标记的 M-CSF 标准与抗 M-CSF 抗体结合的量成反比。

[0302] 上述免疫分析法可用于多种目的。如抗 M-CSF 抗体可用于检测细胞培养物中的细胞内或细胞表面上或分泌至组织培养基的 M-CSF。抗 M-CSF 抗体可用于测定细胞表面上或分泌至组织培养基中的 M-CSF 含量，其中该培养基已使用不同化合物处理。本方法可用以鉴别可用来抑制或活化 M-CSF 表达或分泌的化合物。依照该方法，将一种细胞样本以测试化合物处理一段时间而另一样本则未经处理。若欲测定总 M-CSF 水平，将细胞裂解并使用上述一种免疫测定法来测量 M-CSF 总水平。比较经处理与未处理细胞中 M-CSF 总水平以确定受试化合物的效果。

[0303] 一种可测量 M-CSF 总水平的免疫分析法是 ELISA 或 Wfestern 印迹法。若欲测量细胞表面的 M-CSF 水平，细胞不裂解，并可使用上述免疫分析法之一来测量细胞表面的 M-CSF 水平。可测定细胞表面 M-CSF 水平的免疫分析法包含步骤：以可检测标记（如生物素或 ^{125}I）来标记细胞表面蛋白质，以抗 M-CSF 抗体免疫沉淀 M-CSF，随后再检测标记的 M-CSF。另一种测定 M-CSF 定位的免疫分析法（如细胞表面水平）可为免疫组织化学法。如 ELISA、RIA、蛋白质印迹法、免疫组织化学、整合膜蛋白质的细胞表面标记及免疫沉淀等方法为本领域中所熟知。请见如 Harlow 和 Lane，见上。此外，免疫分析法可将规模放大以用于高通量筛选，以便测试大量抑制或活化 M-CSF 的化合物。

[0304] 另一可测量分泌的 M-CSF 水平的免疫分析法实例为抗原捕捉分析、ELISA、免疫组织染色分析、蛋白质印迹法及使用本发明的抗体的类似方法。若欲测量所分泌的 M-CSF，可分析细胞培养基或体液（如血清、尿液或滑液）中分泌的 M-CSF 和 / 或将细胞裂解以释出已产生但尚未分泌的 M-CSF。可测定分泌的 M-CSF 水平的免疫分析法包含步骤：以可检测的标记（如生物素或 ^{125}I）将所分泌的蛋白质加以标记，以抗 M-CSF 抗体免疫沉淀 M-CSF 并接着检测标记的 M-CSF。另一可测定分泌的 M-CSF 水平的免疫分析法包含步骤：(a) 预先将抗 M-CSF 抗体与微量滴定板表面结合；(b) 添加包含分泌 M-CSF 的组织培养细胞培养基或体液至微量滴定板的孔中与抗 M-CSF 抗体结合；(c) 添加可检测抗 M-CSF 抗体的抗体，如以地高辛配基标记的抗 M-CSF，其可与 M-CSF 的表位结合，但此表位与步骤 (a) 的抗 M-CSF 抗体所结合的不同；(d) 添加过氧化物酶缀合的地高辛配基抗体；及 (e) 添加过氧化物酶底物，其可产生一种有色反应产物，其可被定量以确定组织培养细胞培养基或体液样本中分泌的 M-CSF 水平。如 ELISA、RIA、蛋白质印迹法、免疫组织化学及抗原捕捉分析等方法是于本领域中所熟知。参见，例如，Harlow 和 Lane，见上。此外，为了测试大量抑制或活化 M-CSF 的化合物，免疫分析法可扩大规模以供高通量筛选。

[0305] 本发明的抗 M-CSF 抗体亦可用于测定组织或起源于组织的细胞中的细胞表面 M-CSF 水平。在一些实施方式中，组织是源自患病组织。在一些实施方式中，该组织可为肿瘤或其活检物。在本方法的一些实施方式中，可自患者切除组织或活检物。然后该组织或活检物可用于免疫分析法，以通过上述方法测定如总 M-CSF 总水平、细胞表面 M-CSF 水平或 M-CSF 的定位。

[0306] 该方法可包括步骤：对需进行该诊断试验的病患施用可检测的标记的抗 M-CSF 抗体或包括该抗体的组合物并对病患进行成像分析以测定 M-CSF 表达组织的位置。成像分析是医学领域中所熟知的方法，且包含（但不限于）x- 射线分析、核磁共振成像 (MRI) 或计算机断层 (CE)。抗体可以任何适用于体内成像的物质标记，如造影剂，如钡（其可用于 x- 射线分析）或磁性造影剂，如钆螯合物，其可用于 MRI 或 CE。其它标记物质包含（但不限于）放射性同位素，如 ^{99}Tc。在另一实施方式中，抗 M-CSF 抗体将不标记，其通过施用第二抗体或其它可与抗 M-CSF 抗体结合的可检测分子来成像。在一个实施方式中，活检物自患者获得以测定目的组织是否表达 M-CSF。

[0307] 本发明的抗 M-CSF 抗体亦可用于测定体液（如血清、尿液或源自组织的滑液）中 M-CSF 所分泌的水平。在一些实施方式中，体液源自患病组织。在一些实施方式中，体液源自肿瘤或其活检物。在本方法的一些实施方式中，体液取自病患，随后将体液运用于免疫分析法中以通过前述方法测定分泌的 M-CSF 水平。本发明的一个实施方式为一种分析 M-CSF 拮抗剂活性的方法，其包括：对灵长类或人对象施用 M-CSF 拮抗剂并测量生物学样品中 CD14+CD16+ 单核细胞数量。

[0308] 治疗用途的方法

[0309] 在另一实施方式中，本发明提供一种方法，其可通过对需要的患者施用抗 M-CSF 抗体而抑制 M-CSF 活性。在此所述的任何类型抗体皆可做治疗用。在优选的实施方式中，抗 M-CSF 抗体为人嵌合抗体或人源化抗体。在另一优选的实施方式中，M-CSF 属于人且患者为人患者。或者，该患者可为表达可与抗 M-CSF 抗体交叉反应的 M-CSF 的哺乳动物。该抗体可对表达与抗体交叉反应的 M-CSF 的非人哺乳动物（即灵长类）给药作为兽医用途或

一种人疾病的动物模型。此种动物模型可用于评估本发明的抗体的疗效。

[0310] 在本文中，术语"M-CSF 活性在其中有害的病症"意欲包含疾病及其它病症，其中罹患该病症的对象中高水平 M-CSF 的存在被发现或怀疑似为该病症的病理生理学的原因或为导致病症恶化的因素。此类病症可通过如分泌的和/或于细胞表面的 M-CSF 水平增加或患有该疾病的对象受影响细胞或组织中 c-fms 的酪氨酸自身磷酸化反应增加而证实。可使用如上述的抗 M-CSF 抗体检测 M-CSF 的水平增加。

[0311] 在一个实施方式中，抗 M-CSF 抗体可给药患有表达 c-fms 的肿瘤或分泌 M-CSF 和/或于细胞表面表达 M-CSF 的肿瘤的患者。优选地，该肿瘤所表达的 c-fms 或 M-CSF 的水平高于正常组织。肿瘤可为实体肿瘤或可为非实体肿瘤，如淋巴瘤。在更加优选的实施方式中，可对患有表达 c-fms 的肿瘤、表达 M-CSF 的肿瘤或分泌 M-CSF 的癌性肿瘤的病患施用抗 M-CSF 抗体。另外，肿瘤可为癌性。在更加优选的实施方式中，肿瘤为肺癌、乳腺癌、前列腺癌或结肠癌。在另一优选的实施方式中，对患者施用的抗 M-CSF 抗体造成 M-CSF 不再与 c-fms 受体结合。在一非常优选具体实施方式中，该方法导致肿瘤重量或体积不增加或减少。在另一实施方式中，该方法可导致肿瘤细胞上的 c-fms 不被 M-CSF 结合。在另一实施方式中，该方法可导致肿瘤细胞上的 M-CSF 不被 c-fms 结合。在另一具体实施方式中，该方法可导致肿瘤细胞分泌的 M-CSF 不被 c-fms 结合。在优选的实施方式中，选自 252、88、100、3.8.3、2.7.3、1.120.1、9.14.4I、8.10.3F、9.7.2IF、9.14.4、8.10.3、9.7.2、9.7.2C-Ser、9.14.4C-Ser、8.10.3C-Ser、8.10.3-CG2、9.7.2-CG2、9.7.2-CG4、9.14.4-CG2、9.14.4-CG4、9.14.4-Ser、9.7.2-Ser、8.10.3-Ser、8.10.3-CG4、8.10.3FG1 或 9.14.4G1，或抗体包含其重链、轻链或抗原结合区。在另一实施方式中，抗体是具有与抗体 8.10.3F 的重链、轻链、或者重链和轻链分别具有 90％、91％、92,％、93％、94％、95％、96％、96％、97％、98％或 99％的相同性的重链、轻链、或者重链和轻链的抗 M-CSF 抗体。

[0312] 在另一优选的实施方式中，给 M-CSF 表达水平不当升高的患者施用抗 M-CSF 抗体。本领域中已知高水平 M-CSF 表达可导致多种常见癌症。在一个实施方式中，该方法涉及治疗癌症，如脑癌、鳞状细胞癌、膀胱癌、胃癌、胰腺癌、乳腺癌、头部癌症、颈部癌症、食道癌、前列腺癌、结肠直肠癌、肺癌、肾癌、肾脏癌、卵巢癌、妇科癌或甲状腺癌。可以用本发明的化合物按照本发明的方法进行治疗的患者包括，例如，已诊断为患有肺癌、骨癌、胰腺癌、皮肤癌、头及颈部癌症、皮肤或眼内黑色素瘤、子宫癌、卵巢癌、直肠癌、肛门区癌症、胃癌、结肠癌、乳腺癌、妇科肿瘤（如子宫肉瘤、输卵管癌、子宫内膜癌、宫颈癌、阴道癌或外阴癌）、霍奇金氏病、食道癌、小肠癌、内分泌系统癌症（如甲状腺癌、甲状旁腺癌或肾上腺癌）、软组织的肉瘤、尿道癌、阴茎癌、前列腺癌、慢性或急性白血病、实体瘤（除血液、骨髓或淋巴系统外的身体组织癌症，如肉瘤、癌或淋巴瘤）、儿童期实体瘤、淋巴细胞性淋巴瘤、膀胱癌、肾脏或输尿管癌（如肾细胞癌、肾盂癌）或中枢神经系统肿瘤（如原发性 CNS 淋巴瘤、脊柱肿瘤、脑干神经胶质瘤或脑垂体腺瘤）的患者。在更加优选的实施方式中，给患有乳腺癌、前列腺癌、肺癌或结肠癌的患者施用抗 M-CSF 抗体。在更加优选的实施方式中，该方法可导致癌症停止异常地增殖，或者不增加或减少其重量或体积。

[0313] 在另一优选的实施方式中，给 M-CSF 表达水平不当升高可导致各种炎症或免疫疾病的患者施用抗 M-CSF 抗体。此类疾病包括但不限于，狼疮包括系统性红斑狼疮、狼疮性肾炎和皮肤狼疮、关节炎、牛皮癣性关节炎、Reiter's 综合征、痛风、创伤性关节炎、风疹关节

炎与急性滑膜炎、类风湿性关节炎、类风湿性脊椎炎、强直性脊柱炎、骨关节炎、痛风性关节炎及其它关节炎病症、脓毒症、脓毒性休克、内毒素休克、革兰氏阴性菌脓毒症、中毒性休克综合征、阿尔茨海默氏症、中风、神经创伤、哮喘、成人呼吸道窘迫综合征、脑型疟、慢性肺炎性疾病、硅肺、肺部类肉瘤病、骨质再吸收疾病、骨质疏松症、再狭窄、心脏及肾脏再输注伤害、血栓形成、肾小球肾炎、糖尿病、移植物抗宿主反应、同种异体移植物排斥、炎性肠病、局限性回肠炎、溃疡性结肠炎、多发性硬化症、肌肉变性、湿疹、接触性皮炎、牛皮癣、晒伤或结膜炎性。

[0314] 抗体可给药一次，但更优选为多次给药。如该抗体可自每日给药三次至每六个月或更久给药一次。给药时间表可为如每日三次、每日二次、每日一次、每二日一次、每三日一次、每周一次、每二周一次、每月一次、每二月一次、每三月一次及每六个月一次。抗体亦可经由微型泵持续给药。该抗体可口服、粘膜、颊内、鼻内、吸入、静脉、皮下、肌内、肠胃外、肿瘤内或局部给药途径进行给药。抗体可于肿瘤的部位或发炎的身体部分给药，投入至肿瘤或发炎的身体部分内或于远离肿瘤的位置或发炎的身体部分处给药。该抗体可给药一次、至少二次或至少施用一段时间直到病症经治疗、减轻或治愈。只要肿瘤存在，该抗体通常可持续施用，前提为该抗体导致肿瘤或癌症停止生长或减少重量或体积或直到发炎的身体部分治愈。抗体通常可以前述药物组合物的一部分来施用。抗体剂量范围通常为 0.1-100mg/kg，更优选地 0.5-50mg/kg，更优选地 1-20mg/kg，且更优选为 1-10mg/kg。抗体的血清浓度可通过任何本领域中已知的方法测量。

[0315] 在另一方面，可通过检查患者与各种疾病有关的症状、各种生物学标记物、组织学和生理条件的改变来确定抗 M-CSF 抗体治疗或预防各种疾病的功效。本领域人员已知如何确定此类症状、生物学标记物、组织学和条件。例如，确定抗 M-CSF 抗体在治疗或预防患者的狼疮中的功效可包括分析或观察狼疮相关症状的改变，例如皮肤损害、蛋白尿、淋巴结病、血清 M-CSF 水平、抗 dsDNA 抗体水平和肾脏病理学改变，例如检查巨噬细胞浸润、炎性浸润、蛋白管型、肾小球簇大小、肾小球 IgG 沉积和 C3 沉积。也可检查单核细胞群例如 CD14+CD16+ 单核细胞的改变，以及破骨细胞标记物例如 uNTX-1 的改变。系统性红斑狼疮、皮肤狼疮、和狼疮性肾炎的生物学标记物可包括红细胞沉降率 (ESR)、C- 反应蛋白 (CRP)、补体 (C3/C4)、Ig 水平 (IgA、IgM、IgG)、抗核抗体 (ANA)、可提取性核抗原 (ENA) 和抗 dsDNA 抗体。

[0316] 除了药代动力学生物学标记物数据，原理验证 (proof of principle) 生物学标记物可包括临床研究过程中所取得的 M-CSF 途径标记物、促炎性细胞因子 / 趋化因子、免疫细胞亚组 (B 细胞、T 细胞和 DC) 以及来自全血、血清、尿液和组织样品的其他疾病相关性标记物。

[0317] 例如，可使用探究性血清标记物组来检查与 M-CSF、单核细胞、巨噬细胞和狼疮 (系统性、肾炎和皮肤) 活动有关的细胞因子、趋化因子和其他血清的蛋白质的血清水平改变。可通过本领域人员熟知的标准免疫测定技术检查血清生物学标记物。细胞因子 / 趋化因子血清生物学标记物组可包括，例如 :IFN-γ、IFN-α、IL-12、TNF-α、IL-2、IL-4、IL-5、IL-13、IL-15、IL-6、IL-10、TGF-β、IL-1-α、IL-1-β、IL-21、IL-22、IL-23、IL-17A、IL-17F、CXCL10、CXCL9、CCL2、CCL19、RANTES、CXCL11、CCL7、CCL3、CXCL13、CCL8、CXCL8、CD40L、可溶性 TNFR 和可溶性 IL-1 受体拮抗剂。与 M-CSF、单核细胞、和巨噬细胞活性相关

的组包括 :M-CSF、GM-CSF、RANKL、可溶性 CD14 和新蝶呤。与皮肤狼疮、系统性狼疮相关的血清生物学标记物亚组可包括 :E- 选择素、BAFF、可溶性 CD27、可溶性 CD154 和 CCL17。

[0318] 可检查来自经治疗的患者的尿液中尿液生物学标记物组的改变,其可包括例如 :M-CSF、MCP-1、IL-6、IL-10、CCL3 和 RANTES。

[0319] 为了评价循环免疫细胞库 (repertoire) 暴露于抗 M-CSF 抗体后的改变,也可对来自经治疗的人类对象的全血进行荧光激活细胞分选 (FACS) 检查。可通过已经建立的细胞表面标记物表达模式来确定免疫细胞亚群。例如,B 细胞亚群组可包括评价总 B 细胞、原初 B 细胞、记忆 B 细胞 (未转换的和已类别转换的)、浆细胞、双阴性 B 细胞和 IgD 原初 B 细胞。过渡 B 细胞 (transitional B cell) 组可包括过渡 B 细胞、未成熟 B 细胞和种系中心 B 细胞。T 细胞亚群组可分为总 T 细胞、NK T 细胞、NK 细胞、原初 T 细胞、记忆 T 细胞、中央记忆 T 细胞、和效应记忆 T 细胞。树突状细胞组可检查髓样树突细胞以及浆细胞样树突细胞。本领域人员知晓如何确定和检测此类细胞亚群。

[0320] 此外,可检查暴露于抗 M-CSF 抗体之后 RNA 表达的改变。RNA 可分离自来自经治疗的患者的全血和组织活检样品。可由分离的 RNA 定量基因表达,例如,通过标准的 Taqman RT-qPCR TLDA 组测定技术。例如,表 1C 列出了一组与 M-CSF 途径参与、细胞因子 / 趋化因子、和疾病相关基因表达有关的靶基因。可检查表 1C 中所列基因中一或多种 RNA 的表达以确定治疗的功效。

[0321] 例如,可通过免疫组化 (IHC) 和 RNA 表达谱评价来自皮肤狼疮患者的病损和非病损处皮肤活检样品的 M-CSF 依赖性改变。可通过 IHC 检查的细胞群包括巨噬细胞、树突状细胞 (pDC 和 mDC) 和 T 细胞群。可考虑针对血清生物学标记物组中所列的生物学标记物进行额外的 IHC 染色。对分离自皮肤活检样品的 RNA 表达分析可检查表 1C 中所列的一或多种基因。

[0322] 表 1C

[0323]

GENE, GeneBook	Entrez Gene ID
粒细胞集落刺激因子	0
α 2M 受体表达	2
ACACA	31
ACACB	32
ACP5	54
AKT1	207
Akt2 表达	208
ALAS2	212
ALOX15	246
Amd1	262
ANPEP	290
BIRC5	332
APOC1	341
APOE	348
FAS	355
FASLG	356
ARAF	369
ARF6 WT	382
ARG1	383
ARHGAP5	394
BAX	581
细胞周期蛋白 D1	595
BCL2	596
BCL2L1	598
高水平 CSF-1	629
骨形态发生蛋白-2-诱导的集落刺激因子-1	650
CA2	760
OPGL/CSF-1 处理	796
CALCR	799
CASP3	836
CSF-1	841
caspase 9	842
Runx1	861
CBL	867
CCND2	894
CSF-1	920
CD14	929
CD34 阳性造血前体细胞	947
CD36	948
CD44	960
CD47	961
CD68	968
CD97	976
CDH5	1003
CDK4	1019
CDKN1A	1026
CDKN2D	1032
CCR5	1234
COL3A1	1281
CP	1356
CPE	1363
CREB1	1385
CREB-结合蛋白	1387

[0324]

GENE, GeneBook	Entrez Gene ID
CRP	1401
MAPK14	1432
CSF-1	1435
巨噬细胞 CSF-1 抗-有义寡脱氧核苷酸	1435
抗 CSF I 抗体	1435
抗 CSF-1 Fab	1435
c-fms 基因产物	1436
表达 CSF-1 受体的 BT20 乳腺癌细胞系	1436
c-fms 原癌基因	1436
CSF-1 受体 mRNA	1436
mouse c-fms	1436
CSF1R	1436
粒细胞-巨噬细胞集落刺激因子(GM-CSF)-刺激的 BAC1.2F5 巨噬细胞	1437
GM-CSF	1437
CSF2RA	1438
粒细胞集落刺激因子	1440
c-Src 激酶活性	1445
CTSK	1513
CYP11A1	1583
CYP17A1	1586
DCK	1633
DNMT1	1786
DUSP1	1843
上皮生长因子(EGF)	1950
EGR1	1958
EGR2	1959
EGR3	1960
CSF-1-诱导的 TCF/SAP-1 改变	2002
SLC29A1	2030
促红细胞生成素(EPO)	2056
ErbB2	2064
endogenous ets-2	2114
集落刺激因子 1	2114
ETS2	2114
ETV3	2117
血浆凝血酶原片段 F1	2147
F2R	2149
F2RL1	2150
F2RL2	2151
FABP7	2173
PTK2B	2185
FASN	2194
FCER1G	2207
FCGR2A	2212
FCGR2B	2213
FCGR3A	2214
FLT3	2322
FN1	2335
c-fos	2353
FYN	2534
GAPDH	2597
生长因子依赖性-1	2672
CXCR3	2833

[0325]

GENE, GeneBook	Entrez Gene ID
若干其他 GRB2-相关蛋白质	2885
HCK	3055
HDAC1	3065
HDAC2	3066
HLX1	3142
HNRNPH1	3187
HRAS	3265
HSD17B1	3292
HSPA5	3309
HTR5A	3361
IRF8	3394
IFNB1	3456
IFN-γ	3458
纯化的 IFN-γ	3458
IFN-γ	3458
IFNGR1	3459
IGF-1	3479
IGF1R	3480
IGFBP2	3485
IGF 结合蛋白-3	3486
IKK β	3551
IL-1 α	3552
IL-1 β	3553
鼠白介素-1 受体拮抗剂	3557
IL-2-受体	3558
白介素-2 (IL-2)	3558
IL-3	3562
IL-4	3565
IL-6	3569
IL6ST	3572
IL-7 增强的巨噬细胞集落刺激因子(CSF-1)-诱导的集落形成	3574
IL-8	3576
IL8	3576
IL10 水平	3586
IL10RA	3587
Il11ra2	3590
IL12A	3592
IL12B	3593
IL18	3606
CSF-1	3630
INPP5D	3635
INPPL1	3636
IRF7	3665
ITGA5	3678
ITGAM	3684
ITGAV	3685
所有 CD11c(高) DC 亚群	3687
ITGB3	3690
ITGB5	3693
jun	3725
JUNB	3726
KCNA3	3738
KCNJ2	3759
LHB	3972

[0326]

GENE, GeneBook	Entrez Gene ID
TNF-β	4049
LYN	4067
Smad 4 增强的 CSF-1 转录	4089
MAP3K3	4215
MMP2	4313
MMP9	4318
MMP12	4321
MMP16	4325
MSR1	4481
嵌合 c-Myc 反式激活因子	4609
NFATC1	4772
NF-κB site	4790
NFKB2	4791
CSF-1 抗体	4843
NPY1R	4886
NT5E	4907
嵌合集落刺激因子-1/TrkB-受体	4915
集落刺激因子-1	4982
OPRD1	4985
CSF-1-介导的活化	4988
SERPINE1	5054
PAI-2	5055
PAK2	5062
PDE6A	5145
PDGFA	5154
PDGFB 受体	5155
PENK	5179
PIK3CA	5290
PIK3CB	5291
PIK3CD	5293
PI3Kγ(-/	5294
PIK3R2	5296
磷脂酶 A2 活性	5319
PLA2G4A	5321
uPA 启动子活化	5328
uPA 受体	5328
PLCG2	5336
PLD2	5338
PPARG	5468
PRKACA	5566
PRKCA	5578
MAPK1	5594
MAPK3	5595
ERK5/BMK1	5598
MAPK8	5599
MAPK9	5601
MAP2K1	5604
MAP2K2	5605
增强的 PTH-诱导的增加	5741
抗 CSF-1	5741
抗 CSF-1	5744
抗 CSF-1	5745
PTK2	5747
SHP-1	5777
PTPN6	5777

[0327]

GENE, GeneBook	Entrez Gene ID
抗 CD148 单克隆抗体	5795
PTPRO	5800
RAC1	5879
非显性负性 c-Raf-1	5894
RBP1	5947
RPS6KB2	6199
CCL3	6348
CCL5	6352
CCL13	6357
CX3CL1	6376
CSF-1	6446
SHC1	6464
INI1/hSNF5/BAF47	6598
SPI1	6688
SRC-1	6714
STAT1	6772
STAT3	6774
STX3	6809
Syk Piceatannol	6850
Substance P augmentation	6863
mCSF-1	6868
TGF-β1	7040
TGF-β 2	7042
Tpo	7066
TLR1	7096
TLR2	7097
TLR5	7100
12-0-十四酰-佛波酯-13-乙酸盐(TPA)-诱导的下调	7112
TNF-α	7124
TNFAIP3	7128
TNFRSF1A	7132
TNFRSF1B	7133
TOP2A	7153
TP53	7157
Tpo	7173
Tpt1	7178
HSP90B1	7184
TRAF2	7186
血管细胞粘附分子-1 (VCAM-1)	7412
血管内皮细胞生长因子(VEGF)-A 产生	7422
WAS	7454
CNBP 结合	7555
CXCR4	7852
GPR68	8111
SHP-1	8431
OPGL	8600
RANKL	8600
SRC-1	8648
SOCS1	8651
MARCO	8685
RIPK1	8737
TNFRSF11A	8792
SKAP2	8935
CH25H	9023
DOK2	9046
MAYP	9050

[0328]

GENE, GeneBook	Entrez Gene ID
IL1RL1	9173
CD163	9332
GRAP2	9402
ITM2B	9445
GDF15	9518
CLOCK	9575
IKBKE	9641
HCK (p)	9846
MAFB	9935
Abi1	10006
IL18BP	10068
STX6	10228
RAMP2	10266
SIGMAR1	10280
TRAIP	10293
SIRPB1	10326
TLR6	10333
KHDRBS1	10657
SLC7A9	11136
TRIM35	23087
CSF-1-诱导的 TCF/SAP-1 改变	23415
STX12	23673
磷脂酶 A2 活性	26279
NKIRAS1	28512
RHOD	29984
TLR9	54106
HPP-CFC-1	55997
CXCR7	57007
SLAP-2	84174
ADSSL1	122622
OSCAR	126014
GAB3	139716
192162	192162
MPEG1	219972
ANXA8	653145
CCR2	729230
Hsd3b	3283/3284
BCLXL	
Fcrls	

[0329] 另一方面，可给有治疗需要的患者共同施用抗 M-CSF 抗体与其它治疗剂（如抗炎剂、抗凝血剂、可降低血压的药物、抗癌剂或分子）。在一个方面，本发明涉及一种治疗哺乳动物过度增殖性病症的方法，其包括对该哺乳动物施用治疗有效量的本发明化合物并结合选自（但不限于）下列抗肿瘤剂：有丝分裂抑制剂、烷化剂、抗代谢剂、嵌入剂、生长因子抑制剂、细胞周期抑制剂、酶、拓扑异构酶抑制剂、生物反应调节剂、抗激素、激酶抑制剂、基质金属蛋白酶抑制剂、基因治疗剂及抗雄激素物质。在更加优选的实施方式中，抗体可与抗癌药共同给药，如阿霉素或紫杉醇。在另一优选的实施方式中，抗体或组合治疗是与放射疗法，化学疗法、光动力疗法、手术或其它免疫疗法一起施用。在另一优选的实施方式中，抗体可与另一抗体共同给药。例如，抗 M-CSF 抗体可与已知可抑制肿瘤或癌细胞增殖的抗体或其它药剂共同给药，如可抑制 erbB2 受体、EGF-R、CD20 或 VEGF 的抗体或药剂。

[0330] 共同施用抗体以及额外的治疗剂（组合疗法）包含施用一种包括抗 M-CSF 抗体及额外的治疗剂的药物组合物，以及施用两种或多种分开的药物组合物，一种包括抗 M-CSF 抗体而另一种包括额外的治疗剂。此外，虽然共同给药或组合疗法通常指抗体及额外的治疗剂于同一时间共同给药，但亦包含抗体及额外的治疗剂于不同时间给药的实例。例如，抗体可每三天给药一次，而额外的治疗剂则每日给药一次。或者，该抗体可于以额外的治疗剂治疗该病症前或治疗后施用。类似地，抗 M-CSF 抗体的可于进行其它疗法（如放射疗法、化学疗法、光动力疗法、手术或其它免疫疗法）之前或之后施用。

[0331] 抗体与一种或多种额外的治疗剂（组合疗法）可施用一次、二次或至少一段时间，直到病症已经治疗、减轻或治愈。优选地，组合疗法可多次施用。组合疗法可由每日给药三次至每六个月给药一次。给药时间表可为如每日三次、每日二次、每日一次、每二日一次、每三日一次、每周一次、每二周一次、每月一次、每两个月一次、每三个月一次及每六个月一次或者其可由微型泵持续给药。组合疗法可借口服、粘膜、颊内、鼻内、吸入、静脉、皮下、肌内、肠胃外、肿瘤内或局部途径施用。组合疗法可于距肿瘤部位较远的部位施用。只要该肿瘤存在，组合疗法通常可持续施用，前提为该抗体可导致肿瘤或癌症停止生长或重量或体积减少。

[0332] 在另一实施方式中，抗 M-CSF 抗体是以放射标记、免疫毒素或毒素标记的，或为一种包括毒性肽的融合蛋白。抗 M-CSF 抗体或抗 M-CSF 抗体融合蛋白将放射标记、免疫毒素、毒素或毒性肽导入可表达 M-CSF 的细胞中。在优选的实施方式中，放射标记、免疫毒素、毒素或毒性肽于抗 M-CSF 抗体与 M-CSF 在靶细胞表面结合后方才内在化。

[0333] 在另一方面，抗 M-CSF 抗体可用于治疗非癌性状态，其中的高水平 M-CSF 和 / 或 M-CSF 是与非癌性状态或疾病有关。在一个实施方式中，该方法包括步骤：给病患施用抗 M-CSF 抗体，该病患患有由高水平 M-CSF 和 / 或 M-CSF 水平或活性所引起或恶化的非癌性病理状态。在更加优选的实施方式中，抗 M-CSF 抗体可减缓非癌性病理状态的发展。在更加优选的实施方式中，抗 M-CSF 抗体可停止或逆转（至少部分）非癌性的病理状态。

[0334] 基因治疗

[0335] 本发明核酸分子可通过基因疗法给需要的患者施用。该疗法可用于体内或体外。在优选的实施方式中，给患者施用编码重链及轻链的核酸分子。在更加优选的实施方式中，可施用核酸分子以便其能稳定地整合至 B 细胞染色体中，因为这些细胞是专供产生抗体的。在优选的实施方式中，前体 B 细胞可经体外转染或感染并再移植至需要的患者。在另一实施方式中，使用已知可感染目的细胞类型的病毒体内感染前体 B 细胞或其它细胞。用于基因疗法的典型载体包含脂质体、质体及病毒性载体。示范性病毒载体为反转录病毒、腺病毒及腺相关病毒。在体内或体外感染后，抗体的表达水平可由接受治疗患者取得样本、使用任何本领域中已知或在此所述的免疫分析法监测。

[0336] 在优选的实施方式中，基因疗法包括步骤：施用经分离的编码抗 M-CSF 抗体的重链或其抗原结合部分的核酸分子，以及表达该核酸分子。在另一实施方式中，基因疗法包括步骤：施用经分离的编码抗 M-CSF 抗体轻链或其抗原结合部分的核酸分子，以及表达该核酸分子。在更优选方法中，基因疗法包括步骤：施用经分离的编码本发明的抗 M-CSF 抗体的重链或其抗原结合部分的核酸分子及分离的编码轻链或其抗原结合部分的核酸分子，以及表达该核酸分子。基因疗法亦可包括步骤：施用额外的抗癌剂，如紫杉醇或阿毒素。

[0337] 为使本发明被更好了解，提供下列实施例。这些实施例仅供说明的目的，不可理解为欲以任何方式限制本发明范围。

[0338] 实施例 I：制备产生抗 M-CSF 抗体的细胞系

[0339] 本发明的抗体如下制备，选择并分析：

[0340] 免疫和产生杂交瘤

[0341] 8-10 周龄 XENOMOUSE™ 小鼠经腹腔或在后足垫内用人 M-CSF(10μg/ 剂 / 小鼠)。3-8 周内重复该剂量 5-7 次。输注前四天，最后对小鼠注射人 M-CSF 的 PBS 溶液。经免疫小鼠的脾和淋巴结淋巴细胞与非分泌性骨髓瘤 P3-X63-Ag8.653 细胞系融合，然后如前所述对融合细胞进行 HAT 选择 (Galfre and Milstein, Methods Enzymol. 73:3-46, 1981)。收获了一组杂交瘤全部分泌 M-CSF 特异性人 IgG2 和 IgG4 抗体。还如 Babcook, J. S. et al., Proc. Natl. Acad. Sci. USA93:7843-48, 1996 所述，利用 XENOMAX™ 技术产生抗体。改造 9 个细胞系产生的本发明抗体经选择进行进一步研究，命名为 252、88、100、3.8.3、2.7.3、1.120.1、9.14.4、8.10.3 和 9.7.2。杂交瘤根据布达佩斯条约的规定于 2003 年 8 月 8 日保藏在美国典型培养物保藏中心 (ATCC), 10801University Blvd., Manassas, VA20110-2209。所述杂交瘤的保藏号如下：

[0342]

杂交瘤 3.8.3 (LN 15891)	PTA-5390
杂交瘤 2.7.3 (LN 15892)	PTA-5391
杂交瘤 1.120.1 (LN 15893)	PTA-5392
杂交瘤 9.7.2 (LN 15894)	PTA-5393
杂交瘤 9.14.4 (LN 15895)	PTA-5394
杂交瘤 8.10.3 (LN 15896)	PTA-5395
杂交瘤 88-γ (UC 25489)	PTA-5396
杂交瘤 88-κ (UC 25490)	PTA-5397
杂交瘤 100-γ (UC 25491)	PTA-5398
杂交瘤 100-κ (UC 25492)	PTA-5399
杂交瘤 252-γ (UC 25493)	PTA-5400
杂交瘤 252-κ (UC 25494)	PTA-5401

[0343] 实施例 II：基因利用分析

[0344] 分别从相应的杂交瘤细胞系克隆编码单克隆抗体 252、88、100、3.8.3、2.7.3、1,120.1、9.14.4、8.10.3 和 9.7.2 的重链和轻链的 DNA，并通过本领域已知的方法进行测定 DNA 序列。此外来自杂交瘤细胞系 9.14.4、8.10.3 和 9.7.2 的 DNA 在可变区特定读框区域突变和 / 或同种型转化以分别获得，例如，9.14.4I、8.10.3F、和 9.7.2IF。根据抗体的核酸序列以及推定的氨基酸序列，可测定各抗体链所使用基因的身份 (" VBASE")。表 2 给出了如本发明选定抗体的基因使用情况：

[0345] 表 2：重链和轻链基因使用情况

[0346]

克隆	重链				κ 轻链		
	SEQ ID NO:	V_H	D_H	J_H	SEQ ID NO:	$V_κ$	$J_κ$
252	1, 2	3-11	7-27	6	3, 4	O12	3
88	5, 6	3-7	6-13	4	7, 8	O12	3
100	9, 10	3-23	1-26	4	11, 12	L2	3
3.8.3	14	3-11	7-27	4	16	L5	3
2.7.3	18	3-33	1-26	4	20	L5	4
1.120.1	22	1-18	4-23	4	24	B3	1
9.14.4I	25, 26	3-11	7-27	4b	27, 28	O12	3
8.10.3F	29, 30	3-48	1-26	4b	31, 32	A27	4
9.7.2IF	33, 34	3-11	6-13	6b	35, 36	O12	3
9.14.4	37, 38	3-11	7-27	4b	27, 28	O12	3
8.10.3	29, 30	3-48	1-26	4b	43, 44	A27	4
9.7.2	45, 46	3-11	6-13	6b	47, 48	O12	3
8.10.3FG1	97, 98	3-48	1-26	4b	31, 32	A27	4
9.14.4G1	101, 102	3-11	7-27	4b	27, 28	O12	3
9.14.4C-Ser	54	3-11	7-27	4b	56	O12	3
9.14.4-CG2	74	3-11	7-27	4b	56	O12	3
9.14.4-CG4	78	3-11	7-27	4b	56	O12	3
8.10.3C-Ser	58	3-48	1-26	4b	60	A27	4
8.10.3-CG2	62	3-48	1-26	4b	60	A27	4
8.10.3-CG4	94	3-48	1-26	4b	60	A27	4
8.10.3-Ser	90	3-48	1-26	4b	43, 44	A27	4
9.7.2C-Ser	50	3-11	6-13	6b	52	O12	3
9.7.2-CG2	66	3-11	6-13	6b	52	O12	3
9.7.2-CG4	70	3-11	6-13	6b	52	O12	3
9.7.2-Ser	86	3-11	6-13	6b	47, 48	O12	3
9.14.4-Ser	82	3-11	7-27	4b	27, 28	O12	3

[0347] 通过设计引物并利用购自 Stratagene 的 QuickChange 位点特定诱变试剂盒根据厂商说明来进行重链和轻链的特定残基诱变。通过自动测序确定突变，并将诱变亚克隆到表达载体中。所述表达载体转染到 HEK293 细胞中产生足够的抗体用于定性。

[0348] 实施例 III :M-CSF 小鼠单核细胞增殖测定

[0349] 进行体外测定来测定在抗 M-CSF 抗体存在下 M-CSF- 依赖性小鼠单核细胞细胞增殖被抗 M-CSF 抗体抑制的程度。

[0350] 获得小鼠单核细胞，M-NFS-60 细胞（来自美国典型培养物保藏中心 (ATCC)

(Manassas,VA)) 并保存在含有以下成分的 RPMI-1640 培养基中:2mM L- 谷氨酸盐 (ATCC),10 % 热灭火的胚胎牛血清 (FBS)(Invitrogen,Carlsbad,CA),0.05mM2- 巯基乙醇 (Sigma,St.Louis MO)(测定介质),其中含有 15ng/ml 人 M-CSF。M-NSF-60 细胞分成 $5x10^4$ 第二天使用或分成 $2.5x10^4$ 两天内使用。在用于测定之前,所述细胞用 RPMI-1640 洗涤三次,计数并用测定培养基调整体积为 $2x10^5$ 细胞 /ml。所有条件一式三份重复用于 96- 孔处理的组织培养物板 (Corning,Corning,NY) 上。每个孔中加入 50μl 经洗涤的细胞,25μl100pM 或 1000pM M-CSF 或 25μl 各个浓度的对照抗体的醋酸缓冲液 (140mM 氯化钠,20mM 醋酸钠,以及 0.2mg/ml 聚山梨醇酯 80,pH5.5),使得终体积 100μl。本发明抗体单独测定并与人 M-CFS 共同测定。培养板在 37℃ 5% CO_2 下培养 24 小时。

[0351] 24 小时后,加入 10μl/ 孔的 0.5μCi3H- 胸苷 (Amersham Biosciences,Piscataway,NJ) 并脉冲细胞 3 小时。为了检测掺入胸苷的量,将细胞收获置于预先湿化的单滤 GF/C 滤板 (Packard,Meriden,CT) 并用水洗涤 10 次。允许该板干燥过夜。将封底剂加入滤板。然后,加入 45μl Microscint20(Packard,Meriden,CT) 每孔。加入封顶剂之后,在 Trilux microbeta 计数器 (Wallac,Norton,OH) 中计数该板。

[0352] 这些实验显示本发明抗 M-CSF 抗体抑制小鼠单核细胞对 M-CSF 的响应性增殖。此外,通过利用各种浓度的抗体,测定抗体 252、88、100、3.8.3、2.7.3、1.120.1、9.14.4I、8.10.3F、9.7.2IF、9.14.4、8.10.3 和 9.7.2 对小鼠单核细胞增殖抑制的 IC_{50}(细胞增殖测定,表 3a 和表 3b)。

[0353] 表 3a

[0354]

抗体	252	88	100	3.8.3	2.7.3	1.120.1
M-CSF 小鼠单核细胞增殖测定 [IC_{50}, M]	1.86×10^{-10}	2.31×10^{-10}	7.44×10^{-10}	7.3×10^{-11}	1.96×10^{-10}	1.99×10^{-10}
人全血单核细胞 活化测定 [IC_{50}, M]	8.67×10^{-10}	5.80×10^{-10}	1.53×10^{-10}	8.6×10^{-11}	7.15×10^{-10}	8.85×10^{-10}
受体结合抑制测定 [IC_{50}, M]	7.47×10^{-10}	4.45×10^{-10}	1.252×10^{-9}	7.0×10^{-11}	3.08×10^{-10}	1.57×10^{-10}

[0355] 表 3b

[0356]

抗体	9.14.4I	8.10.3F	9.7.2IF	9.14.4	8.10.3	9.7.2
M-CSF 小鼠单核细胞增殖测定 [IC_{50}, M]	2.02×10^{-10}	4.13×10^{-10}	7.37×10^{-10}	2.02×10^{-10}	4.13×10^{-10}	7.37×10^{-10}
人全血单核细胞 活化测定 [IC_{50}, M]	2.49×10^{-10}	4.46×10^{-10}	1.125×10^{-9}	6.48×10^{-10}	2.8×10^{-10}	1.98×10^{-10}
受体结合抑制测定 [IC_{50}, M]	2.97×10^{-10}	9.8×10^{-11}	5.29×10^{-10}	4.1×10^{-11}	1.5×10^{-9}	6×10^{-12}

[0357] 实施例 IV:人全血单核细胞活化测定

[0358] 体外测定 M-CSF 依赖性单核细胞在抗 M-CSF 抗体存在下的形状改变以确定所述抗 M-CSF 抗体能否抑制全血单核细胞活化以及其对单核细胞形状改变的抑制程度。

[0359] 在96孔组织培养板的各个孔中，混合6μl的1.7nM抗M-CSF和94μl人全血获得102pM终浓度的抗M-CSF抗体。将板在37℃ CO_2 组织培养保温箱中保温。然后，将板从保温箱中取出。在每个孔中，加入100μl固定液(0.5％福尔马林磷酸缓冲盐水，其中不含$MgCl_2$或$CaCl_2$)并将板在室温保温10分钟。对于每个样品，每孔取出180μl与1ml红细胞裂解缓冲液混合。涡旋试管2秒钟。然后，将样品在震摇水浴中在37℃保温5分钟以裂解红细胞但保留单核细胞完整。该次保温之后，立即将样品置于荧光即获得细胞淘选(FACS)仪(BD Beckman FACS)中读数，利用FACS Station Software Version3.4分析数据。

[0360] 这些试验证明，与对照样品相比本发明的抗M-CSF抗体与抑制单核细胞形状改变。利用单核细胞形状改变测定，测定抗体252、88、100、3.8.3、2.7.3、1.120.1、9.14.4I、8.10.3F、9.7.2IF、9.14.4、8.10.3和9.7.2的IC_{50}(人全血单核细胞活化，表3a和表3b)。

[0361] 实施例V：c-fms受体结合抑制实验

[0362] 进行体外实验以测定M-CSF与c-fms受体在抗M-CSF抗体存在下的结合从而确定抗M-CSF抗体是否抑制M-CSF与c-fms受体的结合以及其抑制程度。

[0363] 洗涤转染的人NIH-3T3细胞，所述细胞由保存在不含镁或钙的Dulbecco's磷酸缓冲盐水中的人c-fms或M-NSF-60细胞转染。用5mM乙二胺四乙酸(EDTA)，pH7.4，从组织培养板除去NIH-3T3细胞。将NIH-3T3细胞返回组织培养箱中1-2分钟并轻敲培养瓶以使细胞松散。NIH-3T3细胞和M-NSF-60细胞被转移到50ml试管中并用反应缓冲液洗涤两次(1xRPMI，不含碳酸氢钠的50mM N-2-羟乙基哌嗪-N'-2-乙磺酸(HEPES)，pH7.4)。然后，将NIH-3T3细胞重悬于反应缓冲液中使得终浓度为$1.5x10^5$细胞/ml。M-NSF-60细胞重悬于反应缓冲液中使得终浓度为$2.5x10^6$细胞/ml。

[0364] 为了进行测定，将9μl无菌0.4M蔗糖溶液，100μl终浓度为200pM的^{125}I-M-CSF(Amersham，IMQ7228v)在含有以下成分的RPMI-1640在结合试管中混合：50mM HEPES(pH7.4)，0.2％牛血清白蛋白，100μl终浓度为200nM的未标记M-CSF。然后，加入50μl/管的浓度递增的测定抗体。为了测定抗体的非特异性结合，我们纳入了其中还加入200nM M-CSF的样品。在对照试管中，我们没有加入抗体。然后，每个试管中加入15,000NIH-3T3细胞或250,000M-NSF-60细胞。所有试管在室温保温3小时然后在10,000rpm离心2min。含有细胞碎片的试管尖端被切下并利用Packard Cobra II Gamma计数器测定结合于细胞的M-CSF的量。特异性结合通过从总结合中减去非特异性结合来确定。所有测定一式两份进行。结合数据利用计算机程序Graph Pad Prism2.01分析。

[0365] 这些试验显示与对照样品相比本发明的抗M-CSF抑制M-CSF与c-fms受体的结合。此外，通过利用各种浓度的抗体，测定抗体252、88、100、3.8.3、2.7.3、1.120.1、9.14.4I、8.10.3F、9.7.2IF、9.14.4、8.10.3和9.7.2的受体结合抑制IC_{50}(受体结合抑制实验，表3a和表3b)。

[0366] 实施例VI：通过BIACORETM测定抗M-CSF单克隆抗体的亲和力常数(KD)

[0367] 纯化抗体的亲和力测定通过利用BIACORE™3000仪根据厂商说明利用表面等离子共振进行。

[0368] 对于抗体3.8.3、2.7.3和1.120.1，在BIACORE™3000仪中在25℃含有0.0005％Tween-20的Dulbecco's磷酸缓冲盐水中进行实验。蛋白浓度从沉淀速度试验获得或通过利用来自氨基酸序列的理论消光系数测定样品在280nm的波长。为了进行试验测定抗体与

固定化抗原的结合，通过标准直接胺偶联法将 M-CSF 固定在 B1 芯片上。对于 3.8.3, 2.7.3 和 1.120.1，制备 0.69μM 抗体样品。这些样品连续稀释 3 倍到 8.5nM 或 2.8nM，浓度范围大概 100 倍。对于每个浓度，所述样品一式两份以 5μl/min 的流速注射 4 分钟。监测解离 2000 秒。数据利用 BIACORE™ Biaevaluation 软件全面适应于简化 1:1 结合模型。在所有情况中，该方法用于获得 k_{off} 并发现该数据组与得自结合与解离的全面适应的数据相当。

[0369] 对于抗体 252、88 和 100，在 BIACORE™3000 仪在 25℃ HBS-EP 缓冲液 (0.01M HEPES, pH7.4, 0.15M NaCl, 3mM EDTA, 0.005% Surfactant P20) 中进行试验。对于测定抗体与固定抗原的结合的试验，通过标准直接胺偶联法将 M-CSF 固定在 CM5 研究级感应芯片上。制备抗体样品：12.5nM 的抗体 252 和 100 以及 25.0nM 的抗体 88。这些样品被两倍连续稀释到 0.78nM，大致浓度范围为 15-30 倍。对于每个浓度，所述样品一式两份随机顺序以 30μl/min 注射 3min。监测解离 300sec。数据利用 BIACORE™Biaevaluation 软件全面适应于简化 1:1 结合模型。在所有情况中，该方法用于获得 k_{off} 并发现该数据组与得自结合与解离的全面适应的数据相当。

[0370] 表 4 显示抗体 252, 88, 100, 3.8.3, 2.7.3 和 1.120.1 的结果。

[0371] 表 4

[0372]

	252	88	100	3.8.3	2.7.3	1.120.1
K_D(M)	$1.33x10^{-11}$	$1.33x10^{-9}$	$2.0x10^{-11}$	$4.0x10^{-10}$	$4.7x10^{-9}$	$5.4x10^{-9}$
k_{off}(1/s)	$1.03x10^{-6}$	$7.3x10^{-5}$	$1.7x10^{-5}$			

[0373] 实施例 VII：从 8.10.3 杂交瘤细胞制备 8.10.3 抗体

[0374] 于 3L 射流旋转器 (sparged spinners) 制备制备抗体 8.10.3。3L 射流旋转烧瓶为一种玻璃器皿，其使用以磁性平台所控制的推动器来混合培养物。将旋转器连接至气体管线以供给 5% CO2 及空气。首先将 8.10.3 杂交瘤细胞融解在 T-25 细胞培养瓶中。细胞逐渐扩增直到有足量细胞可供植入射流旋转器中。

[0375] 以 8.10.3 杂交瘤细胞接种于二个 3L 的射流旋转烧瓶内，培养基为无血清杂交瘤用培养基，添加物细节见表 5。所显示 UltraLow IgG 血清 (Gibco cat#16250-078)、L-谷氨酰胺 (JRH Biosciences cat#59202-500M)、非-必须氨基酸 (Gibco cat#11140-050)、蛋白胨 (Difco cat#211693)、葡萄糖 (由 JT Baker 自制储备物 cat#1920-07) 及消泡剂 C(Sigma cat.#A-8011) 的浓度为其于培养基的最终浓度。平衡各反应器体积使用无血清杂交瘤用培养基。

[0376] 表 5：杂交瘤 8.10.3 在两个 3L 射流旋转烧瓶中生长的条件

[0377]

条件	旋转烧瓶 1	旋转烧瓶 2
接种密度 ($1x10^6$ 细胞 /ml)	0.16ml	0.16ml
无血清杂交瘤用培养基 (Gibco cat#12045-076)	平衡	平衡

超低 IgG 血清 (Gibco cat#16250-078)	5%	5%
L- 谷氨酸盐 (JRH Biosciences cat#59202-500M)	8mmol/L	8mmol/L
非必需氨基酸 (Gibco cat#11140-050)	1%	1%
肽酮 (Difco cat#211693)	1g/L	1g/L
2M 葡萄糖 (制备自 JT Baker cat#1920-07 的内部批号)	8g/L	8g/L
消泡剂 C(Sigma cat.#A-8011)	1ml/L	1ml/L

[0378] 让培养物生长 15 天，当生存力低于 20％时进行收集。生存力可通过台盼蓝排除法以自动化细胞计数器 (Cedex，Innovatis) 测定。采集可由离心及随后的过滤完成。于 7000rpm 离心 15 分钟后可获得澄清的上清液并随后以无菌 0.22μm4”OpticapMillipore 滤 膜 (cat#KVSCO4HB3) 过 滤 至 10L 无 菌 TC-iTech 袋 (cat#P/N12420Bag Style CC-10-112420)。滤液随后于下列实施例中进行纯化。

[0379] 实施例 VIII :纯化抗 M-CSF 抗体

[0380] 首先如下预备蛋白质 A 柱 (Amersham Pharmacia)，以 3 柱体积的 8M 尿素洗涤，再以 20mM Tris(pH8) 平衡洗涤。来自实施例 VII 的最终滤出液与 2％ v/v 的 1M Tris pH8.3 及 0.02％的 NaN_3 混合，然后通过重力滴落方式装入蛋白质 A 柱。装填完成后，以 5 柱体积的 20mM Tris(pH8) 洗树脂，接着以 5 柱体积的洗脱缓冲液 (0.1M 甘氨酸 pH3.0) 冲洗。注意任何沉淀物，接着掺入 10％ v/v 的 1M Tris pH8.3 以洗脱抗体。被洗脱的蛋白质接着以 100 倍的洗脱物体积量的透析缓冲液 (140mM NaCl/20mM 醋酸钠 pH5.5) 进行透析洗脱物。于透析后，该抗体以 0.22μm 滤膜进行无菌过滤并贮存直到进一步应用。

[0381] 实施例 IX :猴子处理和单核细胞计数

[0382] 每剂量组采用一只雄性与一只雌性食蟹猴，于约 5 分钟的时间以静脉注射给予空载体或抗体 8.10.3(如实施例 VII 及 VIII 中所述制备)，剂量分别为 0、0.1、1 或 5mg/kg，体积为 3.79mL/kg。于给药后 24 及 72 小时及每周 (共 3 周) 采集供临床实验分析的血液样本。单核细胞计数是使用 Abbott Diagnostics Inc.Cell Dyn 系统 (Abbott Park,Illinois) 通过光散射测定。

[0383] 观察到所有剂量中单核细胞总数 (图 1A 及 1B) 的剂量相关性减少 (～ 25％至 85％)。于第 2 周时 0.1 及 1 mg/kg 的单核细胞计数似乎回升至接近对照组，而 5mg/kg 的单核细胞计数于第 3 周时仍减少。

[0384] CD14+CD16+ 单核细胞亚群分析

[0385] 将灵长类全血抽入含有肝素钠的 Vacutainer 管中。将 0.2ml 的各血液样本加入含 10ml 红细胞裂解缓冲液 (Sigma) 的 15ml 圆椎形聚丙烯离心试管中，并于 37℃水浴中温育 15 分钟。接着将该管于 Sorvall RT7 离心机以 1,200rpm 离心 5 分钟。吸出上清液，将沉淀再悬浮于 10ml 的 4℃ FACS 缓冲溶液中 (Hanks’ 平衡盐溶液 /2％ FBS/0.02％叠氮化钠)，并将管再次以 1,200rpm 离心 5 分钟。吸出上清液并将沉淀再悬浮于由下列各物所组成的抗体混合物中 :80μl 4℃ FACS 缓冲液、10μl FITC 缀合的抗人类 CD14 单

克隆抗体 (BD Biosciences, San Diego, CA)、0.5μl Cy5-PE- 缀合的抗人类 CD16 单克隆抗体 (BD Biosciences, San Diego, CA) 及 10μl PE 缀合的抗人类 CD89 单克隆抗体 (BD Biosciences,San Diego,CA)。细胞悬浮液于冰上温育 20 分钟,之后加入 4℃的 10ml FACS 缓冲液并将细胞如前述方式离心。吸出上清液,并将细胞沉淀再悬浮于 400μl FACS 缓冲溶液中并于 FACSCaliber 流式细胞分析仪 (BD Biosciences,SanJose,CA) 分析细胞。每样本收集 30,000 个细胞的数据。

[0386] 通过前角 (forward angle) 光散射及直角 (orthogonal) 光散射组合来鉴别单核细胞群。进一步分析单核细胞门控的细胞的 CD14 及 CD16 表达。可观察到二种明显不同的单核细胞群,一种可表达高水平的 CD14 及很少或无 CD16 表达 (CD14++CD16-),而另一种可表达较低水平的 CD14 及高水平的 CD16(CD14+CD16+),类似前述的人类外围血中的二种单核细胞亚群 (Ziegler-Heitbrock H.W.,Immunology Today17 :424-428(1996))。对受测的各灵长类而言,于以 8.10.3 注射后第 1、3、7、14 及 21 天各次抽血后进行测定 CD14+CD16+ 亚群中的单核细胞百分比。

[0387] 一般来说,以 8.10.3 处理可导致 CD14+CD16+ 单核细胞百分比下降 (请见图 2A 及 2B)。未接受 8.10.3 抗体的猴子显示相对稳定的 CD14+CD16+ 单核细胞水平。CD14+CD16+ 单核细胞被称为“促炎性”,因其可产生较高水平的 TNF-α 及其它炎症性细胞因子 (Frankenberger.,et al.,Blood87 :373-377(1996))。亦有报导显示单核细胞由常见的 CD14++CD16- 表型分化为促炎性表型是取决于 M-CSF(Saleh M.N.et al.,Blood85 : 2910-2917(1995))。实施例 X :猴子处理和单核细胞计数

[0388] 每剂量组三只雄食蟹猴于约 5 分钟的时间内以静脉内给予载体 (20mM 醋酸钠, pH5.5,140mM NaCl)、纯化抗体 8.10.3F 或纯化抗体 9.14.4I,其剂量为 0、1 或 5mg/kg,体积为 3.79mL/kg。猴子年龄为 4 至 9 岁而体重为 6 至 10kg。于 2、4、8、15、23 及 29 日采集供临床实验分析的血液样本。单核细胞计数使用 Abbott Diagnostics Inc.Cell Dyn 系统 (Abbott Park, Illinois) 通过光散射测定。

[0389] 观察到与测试前的单核细胞水平 (图 3A 及 3B) 相比,在抗体 8.10.3F 及抗体 9.14.4I 所有剂量 (请见如图 3A 及 3B 中的第 4、8、15 及 23 日) 的总单核细胞百分比改变皆减少。

[0390] 实施例 XI :抗 M-CSF 抗体在鼠狼疮模型中的效果

[0391] 在该实施例中,在两个独立的鼠模型 MRL-Fas^{lpr} 和 NZBWF1/J 中检测 M-CSF 被大鼠抗小鼠 M-CSF 抗体中和对狼疮样疾病的产生的效果。抗 M-CSF 抗体的效果也与鼠 CTLA-4Ig 进行比较,之前已经证明后者可降低 MRL-Fas^{lpr} 小鼠中的疾病。

[0392] 在显示与人疾病有多个相同特征的鼠 SLE 模型 (Perry et al,J Biomed Biotechnol。2011:271694。Epub2011Feb14) 中对用于治疗人疾病的多个候选物进行了评估。

[0393] MRL-Fas^{lpr} 小鼠自发症状模拟人狼疮的症状,包括高循环抗双链 DNA(dsDNA) 血清自身抗体、肾小球 IgG 沉积以及严重疾病中的蛋白尿、淋巴结病和皮肤损伤。淋巴结病的发生是由于聚集了双阴性 (CD4-CD8-) 和 B220+T- 细胞 (Watson et al,Journal of Experimental Medicine.176(6):1645-56(1992))。细胞毒 T 细胞淋巴细胞抗原 -4(CTLA-4) 参与调节 T 淋巴细胞活化,并且血清 CTLA-4Ig 的存在与自身反应性 B 淋巴

细胞扩增减少，CD4+T 淋巴细胞数量减少，以及小鼠 SLE 模型中的有利反应相关 (Mihara et al, J Clin Invest. 106(1):91-101(2002))。M-CSF 缺陷型 MRL-Fas^{lpr} 小鼠不发生肾炎 (Lenda et al. J Immunol. 173(7):4644-4754(2004))。

[0394] 通过 NZB 和 NZW 株之间的 F1 杂交产生的 NZBWF1/J 模型是狼疮的最早经典模型，其与狼疮患者发生类似的严重狼疮样表型，包括淋巴结病、脾肿大、血清抗核自身抗体升高，其中包括抗 dsDNA IgG。免疫复合物介导的肾小球肾炎在 5-6 月龄明显出现，导致肾衰并且在 10-12 月龄死亡 (Mihara et al.)。在人 SLE 中，NZBWF1 中的疾病在两性中明显不同并偏向于女性发病 (Theofilopoulos&Dixon, Adv Immunol. 37:269-390(1985))。

[0395] 材料和方法

[0396] 本实施例中使用的抗体和对照蛋白总结在表 6。

[0397] 表 6

[0398]

蛋白[a]	活性
大鼠抗小鼠 M-CSF Ab5A1	小鼠 M-CSF 的中和性抗体
CHOCK IgG1	大鼠同种型对照免疫球蛋白
CTLA-4Ig	减轻狼疮疾病的阳性对照

[0399] CHOCK ＝表达 CK-1 的中国仓鼠卵巢细胞；CTLA ＝细胞毒 T 淋巴细胞结合的抗原；Ig ＝免疫球蛋白；M-CSF ＝巨噬细胞集落刺激因子。

[0400] [a] 蛋白在 Pfizer 制备。

[0401] 雌性 MRL-Fas^{lpr} 和 NZBWF1/J(Jackson Laboratory, Bar Harbor, ME) 在 Pfizer 的无病原体动物饲养室中圈养。小鼠根据 Pfizer 动物关怀和利用委员会批准的方案使用。

[0402] SLE 研究

[0403] 利用 MRL-Fas^{lpr} 和 NZBWF1/J SLE 模型进行 M-CSF 中和对狼疮样疾病的出现的影响。10 周龄雌性 MRL-Fas^{lpr} 或 26 周龄雌性 NZBWF1/J 小鼠每周经腹腔内 (IP) 用以下物质处理 3 次：盐水、10mg/kg 的 5A1 抗 M-CSF、CHOCK IgG1 同种型对照抗体或 CTLA-4Ig(仅 MRL 模型)。MRL-Fas^{lpr} 小鼠每两周检查蛋白尿，皮肤损伤和淋巴结病，NZBWF1/J 小鼠每两周检查蛋白尿。蛋白尿利用 Albustix(Bayer, Tarrytown, NY) 测定并根据以下标准评分 0－5：0 ＝无；1 ＝痕量；2 ＝ 30mg/dL；3 ＝ 100mg/dL；4 ＝ 300mg/dL；和 5 ＝≥ 2000mg/dL。淋巴节可触及并对淋巴结病评分为 0－3：0 ＝无；1 ＝小；2 ＝中等，两个不同部位；3 ＝大，三个或更多不同部位。皮肤损伤利用宏观病理学评估并评分为 0－3：0 ＝无；1 ＝小 (面部，耳朵)；2 ＝中等 (<2cm 面部、耳朵和背部)；3 ＝严重 (>2cm 面部，耳朵和背部)。两个模型中的血清每两周收集一次用来通过酶联免疫测定 (ELISA) 检测抗 dsDNAIgG 血清抗体。在研究结束时，将脑、肺和肾收集在 10％非缓冲福尔马林中用于病理分析或冷冻在最佳切片温度 (OCT) 化合物中用于免疫组化分析。

[0404] 抗 dsDNA 血清抗体和 M-CSF ELISA

[0405] 抗 dsDNA IgG 血清抗体通过 ELISA 测定。简而言之，Immulon1B 板 (Thermolab Systems, Billerica, MA)UV 照射过夜然后用 2μg/mL 小牛胸腺 DNA(Sigma Aldrich, St.

Louis,MO) 在室温包被 1 小时。板用磷酸缓冲盐水 (PBS) 加 1％牛血清白蛋白 (BSA) 封闭，加入稀释血清样品（在 1:100 滴度开始），结合抗体利用抗小鼠 IgG 抗体的辣根过氧化物酶 (HRP) 缀合的山羊抗体 (Southern Biotech,Birmingham,AL) 检测。板用 3,3',5,5'- 四甲基联苯胺 (TMB ;KPL,Gaithersburg,MD) 显色并用 2N 硫磺酸终止。吸光度利用 SpectraMax Plus384 酶标仪和 SoftMax Pro 软件 (Molecular Devices,Sunnyvale,CA) 在 450nm 读数。通过利用收集自患病 MRL-Fas^{lpr} 或 NZBWF1/J 小鼠的标准阳性对照血清测定抗体（任意单位）。M-CSF 血清水平通过抗鼠 M-CSF 试剂盒 (R&D Systems,Minneapolis,MN ;Cat.#MC00) 如厂商说明测定。

[0406] 组织学

[0407] 在 MRL-lpr 研究中，检查肾脏的病理学和 Ig 和 C3 沉积。对福尔马林固定的左右肾脏标本进行苏木精和伊红 (H&E) 染色和过碘酸 - 希夫 (PAS ;以苏木精复染）染色。将左右肾脏冷冻在液氮中，然后针对 C3、IgG 和 IgM 进行免疫组化染色。所有组织切片均由具有全面资格的兽医病理学家检查。通过免疫组化检查 F4/80 染色的巨噬细胞。

[0408] 结果

[0409] 在 8 周龄的 MRL-lpr 小鼠上设计研究以测试以中和性抗体 5A1（参见，例如，Campbell I.K.,et al.,J.Leuk.Biol.68:144-150(2000) 和 ATCC Number CRL-2702) 阻断 M-CSF 的效应。每日给小鼠腹腔注射 (IP)400μg 抗体 (10mg/kg) 共 12 周。使用相同剂量的同种型对照抗体 CHOCK IgG1（参见，例如，ATCC Number HB-9421) 或盐水处理对照小鼠。小鼠还以 CTLA-4Ig（融合于含有效应物功能无效突变的小鼠 IgG2a 的小鼠 CTLA4 的胞外结构域）处理作为阳性对照。每两周检查小鼠的血清抗 dsDNA 抗体、蛋白尿、皮肤病变和淋巴结病。研究设计概述于表 7。

[0410] 表 7 :测试抗 M-CSF 抗体在 MRL^{lpr} 狼疮模型中的功效的研究设计

[0411]

处理	数量	IP 剂量	显微镜检查的动物[a]
盐水	10	100μL3x/ 周	1-10
CTLA-4Ig	10	10mg/kg,3x/ 周	11-20
大鼠抗 M-CSF Ab5A1	10	400μg3x/ 周	31-40
大鼠 CHOCK IgG1 对照 Ab	10	400μg3x/ 周	41-40

[0412] a. 每只动物检查一份 H&E 染色、一份 PAS 染色、一份 C3- 免疫染色、一份 IgG- 免疫染色、一份 IgM 免疫染色和一份对照抗体免疫染色的切片。

[0413] 如图 5 所示，阳性对照蛋白质 CTLA-4Ig 和抗 M-CSF 抗体处理显著降低了 MRL-lpr 小鼠淋巴结病的严重程度。图 6 显示，以抗 M-CSF 抗体处理还显著降低了该模型出现的皮肤病变的严重程度。有趣的是，抗 M-CSF 处理预防 MRL-lpr 小鼠出现皮肤病变，与此不同的是，CTLA-4Ig 处理降低了 MRL-lpr 小鼠皮肤病变的严重程度。

[0414] 如图 7 所示，与同种型对照处理的小鼠相比，这些小鼠在 12 周的时间点产生的抗 dsDNA 抗体较少 ;而以 CTLA-4Ig 处理在 4 个测试时间点均非常有效的降低了抗 dsDNA 抗体

产生。

[0415] 在这一研究中，小鼠没有出现显著的蛋白尿；因此也没有评价肾脏功能。显微镜检查发现，仅施用 CTLA-4Ig 与对炎性浸润和蛋白管型的严重程度、肾小球簇大小、肾小球构成和肾小球 IgG 沉积严重程度的有益影响相关。如图 8 所示，与施用盐水或同种型对照相比，施用大鼠抗 M-CSF 抗体与较低的肾小球构成和轻度降低的 C3 免疫组织化学染色积分的组平均值相关。然而，这并不与对所测量的任何其他参数的任何其他明显的有益影响相关。

[0416] 随后的研究评价了抗 M-CSF 抗体 5A1 在减轻 NZBWF1/J 模型的狼疮性肾炎中的功效。以 400μg(10mg/kg) 的对照 Ig 或抗 M-CSF 或盐水处理 26 周龄的小鼠，每周 3 次 IP 共 10 周。每两周对小鼠的蛋白尿、体重增加 / 减轻和抗 dsDNA 滴度进行评分。给药 10 周后手机肾脏并检查病理学和免疫复合物沉积。表 8 描述了该研究设计。

[0417] 表 8：测试抗 M-CSF 抗体在 NZBWF1/J 狼疮模型中的功效的研究设计

[0418]

处理	数量	剂量(IP)	显微镜检查的动物：肾脏 [a]	显微镜检查的动物：肾脏 [b]
盐水	10	100 μL 3x/周	46, 47, 48, 49, 50, 51, 52, 53, 54, 55	46, 47, 48, 49, 50, 51, 52, 53, 54, 55
大鼠抗 M-CSF Ab 5A1	10	400 μg 3x/周	57, 58, 59, 60, 61, 62, 63, 64, 65	57, 58, 60, 61, 62, 63, 64, 65
大鼠 CHOCK IgG1 对照 Ab	10	400 μg 3x/周	66, 67, 69, 70, 71, 72, 73, 74, 75	66, 67, 69, 70, 71, 72, 73, 74, 75

[0419] a. 每只动物检查一份 H&E 和一份 PAS 染色的切片和两份 F4/80 免疫染色的组织切片

[0420] b. 每只动物检查 4 份 IgG、IgM 和 C3 的免疫染色组织切片

[0421] 如图 9 所示，在 NZBWF1/J 狼疮模型中，与同种型对照相比，从给药后 4 周开始直至研究结束，抗 M-CSF 处理对蛋白尿的产生均具有显著的作用。然而，在这一模型中，以 M-CSF 抗体处理没有影响造成免疫复合物沉积和肾脏损害的抗 dsDNA 自身抗体的产生。如图 10 所示，在给药后的第 6 和 10 周时间点，抗 dsDNA 抗体水平与盐水、同种型对照和抗 M-CSF 处理的小鼠相似。图 11 显示，ELISA 测定发现，以 M-CSF 抗体升高了 M-CSF 的血清水平，表明该抗体与其靶反应并隔离了血清中的 M-CSF。

[0422] 如图 12 所示，对研究结束后收集的并对免疫沉积物染色的肾脏进行显微镜检查发现，在所有 3 个处理组，IgG、IgM 和 C3 染色的水平相似。有趣的是，与以同种型对照处理的小鼠相比，来自以抗 M-CSF 处理的小鼠的肾脏显示出轻度降低的巨噬细胞 (F4/80 阳性细胞，由成熟巨噬细胞表达的一种跨膜蛋白) 染色。肾脏的巨噬细胞 F4/80 染色出现于所有小鼠的外部髓质和内部皮质区的间质细胞。与施用 CHOCK IgG1 同种型对照抗体的小鼠相比，施用盐水或大鼠抗 M-CSF 的小鼠的这些部位较少的细胞出现染色，提示阻断 M-CSF 对该模型的肾脏中的巨噬细胞浸润具有影响。抗 M-CSF 处理似乎还减轻了 NZBWF1/J 小鼠肾脏内的肾脏蛋白管型以及嗜碱细胞增多和退化。与施用 CHOCK IgG1 同种型对照的小鼠相比，

施用盐水或抗 M-CSF 的动物中的这些表现严重程度较低。

[0423] 实施例 XII

[0424] 研究设计

[0425] 这是随机双盲（赞助商非盲）安慰剂对照剂量递增 6 组贯续平行研究，目的在于研究以静脉 (IV) 剂型施用于健康成年志愿者的 6 个单剂量水平的人单克隆抗 M-CSF 抗体 8.10.3F 的安全性和耐受性。

[0426] 对象处理

[0427] 总共 48 个对象入选本研究。第 1 至 5 组的对象接受剂量为 3、10、30、100 或 300mg 的抗体 8.10.3F 或者安慰剂，给药后这些对象在 CRU（重症监护室）中留观 3 天，然后出院，随后按照计划返回 CRU。第 6 组的对象以 100mg 抗体 8.10.3F 或安慰剂处理，给药后这些对象在 CRU 中留观 21 天，然后出院，随后按照计划返回 CRU。在各水平组中，6 个对象接受单剂量抗体 8.10.3F，2 个对象接受单剂量安慰剂。所有入选对象均完成了实验。

[0428] 药代动力学评估

[0429] A) 用于分析抗体 8.10.3F 的血清

[0430] 在给药前和方案设计的给药后时间点直至第 28 天以及第 28 天后每次门诊随诊时收集用于分析抗体 8.10.3F 的样品。将 3mL 静脉血收集到适当标记的含有添加物的管中，在收集后 40 分钟内离心，在收集后 60 分钟内将血清冻存在大约 -70℃。

[0431] 在 PPD Development(2244Dabney Road,Richmond,VA23230,USA) 使用有效的分析方法分析血清样品的抗体 8.10.3F。使用有效的敏感且特异性的酶联免疫吸附测定法 (ELISA) 测定抗体 8.10.3F 样品。血清标本储存在 -70℃直至使用，且样品在建立矩阵稳定性数据的 439 天之内测定。通过在校准的标准曲线（范围在 35.0ng/mL 至 1600ng/mL）上的插入值确定样品浓度，该曲线已经使用 4- 参数逻辑方程进行拟合。浓度超出定量上限值 (1600ng/mL) 的样品经充分稀释后插入至校准范围。抗体 8.10.3F 的 LLOQ（定量下限值）是 35.0ng/mL。血清抗体 8.10.3F 浓度低于 LLOQ 的临床标本报告为 <35.0ng/mL。

[0432] 日间测定准确度以估计值与理论质控 (QC) 浓度的比例（%）表示，低、中、高和稀释 QC 样品的范围在 -7.81% 至 2.22%。低 (75.0ng/mL)、中 (320ng/mL)、高 (1200ng/mL) 和稀释 (1200ng/mL,16000ng/mL 和 160000ng/mL) 浓度的测定精密度，表示为与 QC 样品的估计浓度的日间变异系数 (CV%)，均低于 10.0%。

[0433] B) 药代动力学参数的计算

[0434] 对血清浓度 - 时间数据进行不分区分析以计算经各种处理的各对象的抗体 8.10.3F PK 参数值。研究设计不包括在 IV 输注期间的 PK 采样。鉴于预期抗体 8.10.3F 具有长末段 t1/2，预期输注期间的浓度 - 时间曲线下面积 (AUC) 相对于 AUC 极小，因此未试图估计输注期间或结束时的浓度。

[0435] 低于 LLOQ 的样品被归于 0。样品的实际收集时间被用于 PK 分析。使用 WinNonlin version4.0.1 计算药代动力学参数值。

[0436] 药效动力学评估

[0437] A) 用于分析 $CD14^{+}16^{+}$ 单核细胞的全血

[0438] 在给药前和给药后第 2、4、7、14 和 28 天以及第 28 天后每次门诊随诊时收集用于分析 $CD14^{+}16^{+}$ 单核细胞的样品。将 3mL 静脉血收集到 2 个管中，其一含有肝素锂，另一

含有 EDTA 钾。在收集后 2 小时内分析样品。使用有效的敏感且特异性流式细胞术测定法 (Becton-Dickinson FACSCalibur Flow Cytometry) 以全血样品分析人外周血的单核细胞亚群 (CD14 和 CD16) 百分比，测定在 Pfizer Drug Safety Research&Development(DSRD),PGRD,Ann Arbor,Michigan 进行。基于细胞散射光和发射荧光信号的能力来区分单核细胞亚群。

[0439] 对于总体样品分析的测定性能特征，使用 BD CaliBRITE 珠来调整仪器的设置，在每一次单核细胞亚群 -CD14/16 测定之前设定荧光补偿并评估仪器的敏感性。

[0440] B) 用于骨骼特异性碱性磷酸酶 (BSAP) 的血清

[0441] 在给药前和给药后第 2、4、7、14 和 28 天以及第 28 天后每次门诊随诊时收集用于分析 BSAP 的样品。将 5mL 静脉血收集到不含添加物的管中，在收集后 40 分钟内离心，在收集后 60 分钟内将血清分为大约等体积转移至 2 个管中并冻存在大约 -70℃。

[0442] 在 Pacific Biometrics Inc.(PBI)(220West Harrison Street,Seattle,Washington98119,US) 使用有效的分析方法分析血清样品的 BSAP 浓度。使用有效的敏感且特异性的酶免疫测定法 (EIA) 测定 BSAP 样品。验证期间记录方法的性能。血清标本储存在 -70℃直至使用，且样品在验证期间产生的建立的稳定性数据的 365 天之内测定。通过在校准的标准曲线上的插入值确定样品浓度，该曲线已经使用 4- 参数逻辑方程进行拟合。浓度超出 ULOQ(140U/L) 的样品经充分稀释后插入至校准范围。以观测检测限 (LOD) 所表示的 BSAP 的测定敏感性为 0.4U/L。血清 BSAP 浓度低于 LOD 的临床标本报告为低于检测下限。

[0443] 通过 QC 评估测定性能特征。将 3 个水平的 QC 置于每次运行。运行接受度标准是 :3 个 QC 结果中的 2 个必须是在 2.0 标准差指数 (SDI) 之内，而第 3 个必须是在 2.5SDI 之内。对于所有 4 次样品分析运行，平均运行 SDI 的范围为 QC 样品的 -1.1 至 0.8。

[0444] C) 用于分析 NTX-1 的尿液

[0445] 在给药前和给药后第 2、4、7、14 和 28 天以及第 28 天后每次门诊随诊时收集用于分析 NTX-1 的尿液。将次日空腹晨尿收集到清洁容器中，在每一时间点收集 5ml 用于 NTX-1，收集 2 个额外的 5ml 用于计划中的探究性生物学标记物分析。样品冻存在大约 -70℃。

[0446] 在 Pacific Biometrics Inc.(PBI)(220West Harrison Street,Seattle,Washington98119,USA) 使用有效的分析方法分析用于分析尿液交联的胶原蛋白 I(uNTX-1) 的 N- 端肽浓度的尿液样品。使用有效的敏感且特异性的 NTX-1ELISA 和有效的尿液肌酐动力学 Jaffe 测定 uNTX-1 样品。

[0447] 尿液标本储存在 -70℃直至使用，且样品在验证期间产生的建立的稳定性数据的 365 天之内测定。通过在校准的标准曲线上的插入值确定样品 NTX-1 浓度，该曲线已经使用 4- 参数逻辑方程进行拟合。浓度超出 ULOQ(3000nM 当量 /L) 的样品经充分稀释后插入至校准范围。NTX-1 的以 LLOQ 所表示的 NTX-1 测定敏感性是 44.0nM 当量 /L。NTX-1 浓度低于 LLOQ 的临床标本报告为低于检测下限。

[0448] NTX-1 测定值被标准化为骨骼胶原蛋白的相当量，并以 nM 骨骼胶原蛋白当量 /L(nmol BCE/L) 表示。通过分析尿液肌酐针对尿液稀释来校正 uNTX-1 测定值，并以 nM 骨骼胶原蛋白当量 /L(nM BCE)/ 毫摩尔肌酐 /L(mM 肌酐) 表示。

[0449] 通过质控 (QC) 评估测定性能特征。将 3 个水平的 QC 置于每次运行。运行接受度标准是 :3 个 QC 结果中的 2 个必须是在 2.0 标准差指数 (SDI) 之内，而第 3 个必须是在

2.5SDI 之内。对于所有 4 次样品分析运行，平均运行 SDI 的范围为 NTX-1QC 样品的 -0.2 至 0.8，而对于尿液肌酐 QC 样品为 -0.1 至 0.2。

[0450] D) 用于分析总人 M-CSF 的 K_2EDTA 血浆

[0451] 使用针对探索性生物标记物收集的 K_2EDTA 血浆样品分析总 M-CSF。在给药前和第 2、7 和 28 天，以及第 28 天后的每次额外门诊患者访问时收集样品。在不含 EDTA 钾盐 (K2EDTA) 作为抗凝剂的血液收集管中获得 10mL 静脉血样品。离心后，血浆冷冻储存于大约 -70℃。ELISA 测定试剂盒 (DMC00) 来自 R&D Systems, Inc. (614McKinley Place NE, Minneapolis 55413)。在该测定中采用定量夹心酶免疫测定技术且测定操作和主要试剂遵循试剂盒说明。K_2EDTA 血浆样本储存于大约 -70℃直至测定。通过使用 4- 参数逻辑方程拟合的校准标准曲线 (跨越 31.2pg/mL 至 2000pg/mL 的范围) 的插值确定样品浓度。具有高于定量上限 (2000pg/mL) 的浓度的样品充分稀释至校准范围。M-CSF 的定量下限 (LLOQ) 为 31.2pg/mL。血清 M-CSF 浓度低于 LLOQ 的临床样品报道为 <31.2pg/mL。

[0452] 在 Pfizer Discovery-Molecular Pharmacology, PGRD, Ann Arbor, Michigan 使用商业可得的分析 K_2EDTA 血浆样品的 M-CSF 浓度。

[0453] 结果

[0454] A) 血清抗体 8.10.3F 药代动力学

[0455] 表 9 和表 10 含有健康患者中施用单一 IV 剂量后的血清抗体 8.10.3F 药代动力学参数的总结。施用每剂后的平均血清浓度 - 时间谱示于图 13。Cmax 和 AUC 值 vs. 抗体 8.10.3F 剂量的图示于图 14.

[0456] 在 1 小时给健康成人志愿者施用抗体 8.10.3F 的单一输注后，Cmax 以剂量比例方式增加。然而，全身性暴露的程度，AUC(0- ∞)，在 3-300mg 的剂量范围以比剂量比例方式更高的方式增加。因为系统的非线性，并不认为表观终点 t1/2(用于计算 AUC[0- ∞]) 是暴露持续时间的有意义的量度。相反，全部对象抗体 8.10.3F 浓度低于 LLOQ 的研究日通过调查浓度 - 时间表确定。血清抗体 8.10.3F 浓度在施用 3、10、30、100 和 300mg 剂量后分别在第 7、14、28、56 和 84 天低于 LLOQ。

[0457] 表 9 :给健康对象施用单一静脉内溶液剂量后血清抗体 8.10.3F 药代动力学参数值的总结

[0458]

	参数总结统计[a] 相对 抗体 8.10.3F 剂量				
参数, 单位	3 mg	10 mg	30 mg	100 mg[b]	300 mg
N	6	6	6	12	6
Cmax, μg/mL	0.825 (24)	2.39 (21)	9.57 (23)	36.0 (19)	90.7 (18)
tmax, hr	0.25 (0.25-0.28)	0.76 (0.25-4.0)	2.0 (1.3-4.0)	1.3 (1.3-2.0)	3.1 (1.8-4.0)
AUC(0-tlqc), μg*hr/mL	13.6 (49)	97.7 (33)	601 (40)	3870 (18)	17800 (22)
AUC(0-∞), μg*hr/mL	16.9 (31)	105 (30)	640 (36)	3870 (18)	18100 (23)
T-BLQ[c]	7	14	21	56	84
CL, mL/min	3.18 (27)	1.72 (29)	0.842 (25)	0.442 (17)	0.290 (24)
Vd, mL/min	4.20 (23)	3.63 (26)	3.21 (24)	2.33 (22)	2.92 (15)
暴露持续时间(天)[c]	7	14	21	56	84

[0459] [a] 对 tmax 为中位数（范围）;对其它参数为算数平均（% CV）

[0460] [b] 门诊患者和住院患者组合并。

[0461] [c] 所有对象中血清浓度低于定量下限（LLQ = 0.035 μg/ml）的研究日

[0462] N =对象数目

[0463] 表 10 :给健康对象施用单一静脉内溶液剂量后平均血清抗体 8.10.3F 浓度（ng/mL）

[0464]

研究日	**1**	**2**	**7**	**28[a]**	**56[a]**	**84**
剂量	3 mg					
N	6	6	6			
平均值	0.00	235.83	0.00			
中位数	0.00	224.50	0.00			
STD	0.00	53.16	0.00			
剂量	10 mg					
N	6	6	6	6		

[0465]

平均值	0.00	1531.67	25.88[b]	0.00		
中位数	0.00	1495.00	18.30[b]	0.00		
STD	0.00	386.13	32.03	0.00		
剂量	30 mg					
N	6	6	6	6		
平均值	0.00	5991.67	1057.83	0.00		
中位数	0.00	5480.00	803.50	0.00		
STD	0.00	1597.09	717.84	0.00		
剂量	100 mg					
N	12	12	12	12	12	
平均值	0.00	24958.33	9751.67	22.93[b]	0.00	
中位数	0.00	25400.00	9810.00	0.00	0.00	
STD	0.00	4170.78	2216.52	28.90	0.00	
剂量	300 mg					
N	6	6	6	6	6	6
平均值	0.00	72800.00	40333.33	2476.00	11.82[b]	0.00
中位数	0.00	75850.00	42300.00	1614.50	0.00	0.00
STD	0.00	13733.17	8571.74	2266.54	28.94	0.00

[0466] [a] 以标称研究日总结数据：针对 300mg 剂量组的第 28 天组合 6 个患者中的 5 个在第 28 天收集的数据和另一患者在第 29 天收集的数据。

[0467] 类似地，针对 300mg 剂量组的第 56 天组合 6 个患者中的 4 个在第 56 天收集的数据和 6 个患者中另外 2 个在第 58 天收集的数据。

[0468] [b] 低于定量限的平均值和 / 或中位数值反映包括在计算中报道为 BLQ(<35ng/mL) 为 0 的样品。

[0469] B) 总血清 M-CSF

[0470] 总（游离的和结合抗体 8.10.3F 的）M-CSF 浓度相对剂量和研究日示于表 11 且针对 100-mg 剂量示于图 15。

[0471] 在安慰剂处理组，基线处的平均 M-CSF 浓度为 0.21ng/mL，并且在给健康成年志愿者施用安慰剂后基本上不改变（范围 0.21-0.28ng/mL）直至第 84 天（表 11）。

[0472] 施用抗体 8.10.3F 后，最大平均 M-CSF 浓度随着剂量增加而增加。总 M-CSF 的峰浓度在第 2 或第 7 天获得。

[0473] 施用针对抗体 8.10.3F 的平衡解离常数 (K_D2.8x10^{-10}M) 以及 M-CSF 和抗体 8.10.3F 的浓度确定游离和结合配体的比率，计算出在抗体 8.10.3F 可检测的时间内，100％的测量

的 M-CSF 配体结合抗体 8.10.3F。尽管 M-CSF 的取样频率不如抗体 8.10.3F 的取样频繁，M-CSF 浓度与抗体 8.10.3F 平行地下降，提示 M-CSF 的暂时增加随后作为抗体 - 抗原复合物被清除。

[0474] 表 11 :给健康对象施用单一静脉内溶液剂量后的平均 M-CSF 浓度 (ng/mL)

研究日	**1**	**2**	**7**	**28**	**56a**	**84**
剂量	0 mg					
N	12	12	12	10	6	2
平均值	0.21	0.24	0.28	0.23	0.22	0.21
中位数	0.21	0.20	0.19	0.19	0.22	0.21
STD	0.04	0.07	0.19	0.10	0.03	0.01
剂量	3 mg					
N	6	6	6			
平均值	0.20	4.26	0.24			
中位数	0.20	4.43	0.24			
SDT	0.02	1.16	0.04			
剂量	10 mg					
N	6	6	6	6		
平均值	0.18	21.07	0.49	0.21		
中位数	0.19	18.37	0.40	0.21		
STD	0.01	6.18	0.25	0.02		
剂量	30 mg					
N	6	6	6	6		
平均值	0.18	34.28	56.10	0.35		
中位数	0.18	33.30	34.45	0.25		
STD	0.02	7.28	57.48	0.19		
剂量	100 mg					
N	12	12	12	12	12	

[0475]

	平均值	0.20	42.75	429.33	0.54	0.26	
	中位数	0.20	46.84	468.84	0.46	0.25	
	STD	0.03	14.15	104.64	0.19	0.03	
[0476]	剂量	300 mg					
	N	6	6	6	6	6	6
	平均值	0.21	22.89	482.74	247.85	0.35	0.24
	中位数	0.21	23.93	391.79	256.58	0.32	0.24
	STD	0.03	5.37	176.28	181.54	0.08	0.03

[0477] [a]100mg 剂量的第 56 天组合组 4 在第 56 天和组 6 在第 52 天进行的测量。

[0478] 原始数据储存于 ePharm，人工编号 1236578。

[0479] C)$CD14^{\pm}16^{\pm}$ 单核细胞

[0480] 图 16 显示研究 28 天时对 $CD14^{+}16^{+}$ 单核细胞计数的计量应答（$CD14^{bright}CD16^{+}$ 和 $CD14^{dim}CD16^{+}$ 的总数）。表 12 通过剂量和研究天数来显示平均 $CD14^{+}16^{+}$ 单核细胞计数，图 17 和图 18 对 100-mg 剂量进行了图示。图 17 中 56 天的数据与 56 天组 4 和 52 天组 6 的测量结果组合。显示 $CD14^{+}16^{+}$ 细胞计数数据的全部图不包括被认为是离群值的单个观察结果。研究 28 天的对象 1031(100-mg) 具有 179.0 细胞 /mcl 的报告值。

[0481] 对于全部剂量在 4 天时，用抗体 8.10.3F 治疗引起外周循环 $CD14^{+}CD16^{+}$ 单核细胞的绝对值的快速下降，最低值为约 60％至 80％的抑制率。增加剂量保持更长时期内 $CD14^{+}CD16^{+}$ 单核细胞的抑制率。对于 3 和 10mg 剂量，$CD14^{+}CD16^{+}$ 单核细胞的绝对值在 14 天内返回至基线；对于 30mg 剂量，$CD14^{+}CD16^{+}$ 单核细胞的绝对值在 28 天内返回至基线；对于 100 和 300mg 剂量，$CD14^{+}CD16^{+}$ 单核细胞的绝对值在 56 天内返回至基线。

[0482] 表 12：对健康对象施用单次静脉内溶液剂量后的 $CD14^{+}16^{+}$ 单核细胞计数（细胞 / μL）

	研究日	**1**	**2**	**4**	**7**	**14**	**28**	**56**	**84**
	剂量	0 mg							
[0483]	N	11	12	12	12	12	10	5	2
	平均值	32.72	34.19	37.53	40.62	37.42	35.91	36.66	39.95
	中位数	26.6	30.50	36.00	36.70	37.50	32.00	41.20	39.95

[0484]

STD	16.55	18.16	16.03	18.14	14.99	17.46	13.29	15.20
剂量	3 mg							
N	6	6	6	6	6			
平均值	27.70	23.05	12.32	22.22	40.95			
中位数	25.80	16.25	10.90	19.70	42.40			
STD	11.17	18.72	6.10	11.19	15.17			
剂量	10 mg							
N	6	6	6	6	6	6		
平均值	19.88	13.82	8.82	14.90	37.50	32.58		
中位数	17.60	10.70	8.50	17.05	29.30	30.60		
STD	9.99	9.37	7.11	9.43	30.59	16.95		
剂量	30 mg							
N	6	6	6	6	6	6		
平均值	23.90	13.72	3.92	5.03	8.52	28.47		
中位数	19.00	12.05	4.25	4.80	8.75	22.75		
STD	7.78	8.11	1.95	3.21	2.54	17.39		
剂量	100 mg							
N	12	12	12	12	11	12	12	
平均值	35.61	14.98	5.55	8.93	9.37	37.43	41.89	
中位数	29.85	14.00	5.05	5.60	7.90	18.10	30.25	
STD	18.66	5.58	3.50	7.73	5.75	52.98	27.64	
剂量	300 mg							
N	6	6	6	6	6	6	6	6
平均值	36.35	10.92	6.42	11.00	12.05	15.73	38.52	35.77
中位数	34.80	8.95	6.25	7.75	9.55	14.85	29.80	27.35
STD	17.41	5.82	3.18	10.54	8.34	9.48	23.51	20.84

[0485] D)BSAP 和 uNTX-1

[0486] 抗体 8.10.3F 的治疗与 uNTX-1（破骨细胞的骨再吸收活性的标志物）中剂量和

时间依赖性的降低相关。uNTX-1 的降低速率对于 $CD14^{+}CD16^{+}$ 单核细胞而言较慢，且抑制率的恢复被更加地延长。在 30、100 和 300mg 剂量组中，uNTX-1 降低至最低 64.4、60.5 和 39.9％的基线值，其分别出现在 7、14 和 28 天。对于相同的各剂量组，在 28 天，平均 uNTX-1 为 78.0、69.4 和 39.3％。对于≤ 100mg 的剂量，uNTX-1 在约 56 天返回至基线。

[0487] 表 13 通过剂量和研究天数来显示平均 uNTX-1，图 19 对 100-mg 剂量进行了图示。

[0488] BSAP 水平不受到剂量增加至 100mg（包括 100mg）的治疗的影响。在 300mg，在 7 至 28 天观察到 BSAP 增加的趋势，随后在 56 天返回至基线。然而，在接受 300mg 的对象中观察到的最大绝对值可与包括安慰剂在内的其它治疗组中观察到的值相匹配。

[0489] 表 13：对健康对象施用单次静脉内溶液剂量后的平均 uNTX-1（nM BCE/mM 肌酸酐）

[0490]

研究日	**1**	**2**	**4**	**7**	**14**	**28**	**56**	**84**
剂量	0 mg							
N	12	12	12	12	12	10	5	2
平均值	51.73	54.12	46.48	51.46	51.73	40.58	38.08	0.21
中位数	45.25	51.70	47.75	42.75	47.05	34.50	34.50	0.21
STD	24.89	24.37	22.12	23.44	22.89	18.18	12.21	0.01
剂量	3 mg							
N	6	6	6	6	6			
平均值	59.02	50.65	49.50	52.58	45.57			
中位数	58.35	51.00	44.75	52.25	44.90			
STD	23.79	17.03	12.09	14.09	10.62			
剂量	10 mg							
N	6	6	6	6	6	6		
平均值	44.65	37.50	32.50	36.72	38.02	44.55		
中位数	31.85	33.25	27.80	31.75	27.40	46.05		
STD	31.60	25.40	21.25	17.76	24.56	20.90		

[0491]

剂量	30 mg							
N	6	6	6	6	6	6		
平均值	49.38	41.12	38.58	31.78	37.83	38.50		
中位数	49.70	46.55	39.95	32.10	36.75	39.95		
STD	18.55	16.59	13.63	13.18	12.67	13.49		
剂量	100 mg							
N	12	12	12	12	12	12	12	
平均值	46.01	43.67	38.59	29.04	27.83	31.95	43.60	
中位数	45.65	41.55	35.20	28.15	25.30	27.45	43.05	
STD	13.79	15.97	22.99	8.61	15.64	13.80	17.53	
剂量	300 mg							
N	6	6	6	6	6	6	5	6
平均值	43.00	32.98	31.13	24.08	17.42	17.17	30.26	0.24
中位数	41.35	32.70	30.00	22.50	17.40	13.90	23.80	0.24
STD	17.11	10.16	11.82	7.49	2.76	5.91	20.20	0.03

[0492] 讨论

[0493] 在向健康成年志愿者施用 3-300mg 单次静脉内剂量后抗体 8.10.3F 通常很好地被耐受。抗体 8.10.3F 治疗引起药效标志物（其在药物清除后是可逆转的）的快速变化。此机理的主要生物标志物是大量循环 $CD14^+CD16^+$ 单核细胞，其已与临床前的关节炎模型的功效相结合。在 4 天时，全部剂量的抗体 8.10.3F 治疗引起 $CD14^+CD16^+$ 单核细胞的快速下降，最低值为约 60％至 80％的抑制率。增加剂量保持更长时期内 $CD14^+CD16^+$ 单核细胞的抑制率。对于 3 和 10mg 剂量，$CD14^+CD16^+$ 单核细胞的绝对值在 14 天内返回至基线；对于 30mg 剂量，$CD14^+CD16^+$ 单核细胞的绝对值在 28 天内返回至基线；对于 100 和 300mg 剂量，$CD14^+CD16^+$ 单核细胞的绝对值在 56 天内返回至基线。

[0494] 基于使用替代抗体的临床前模型，预测提供治疗益处的剂量是维持 $CD14^+CD16^+$ 单核细胞抑制率在基线值≤ 50％水平的剂量。在此研究中，≥ 30mg 的剂量能够持续至少 14 天抑制循环 $CD14^+CD16^+$ 单核细胞低于基线值的 50％。30mg 剂量在研究 7、14 和 28 天分别具有基线的 25％、46％和 120％的中位 $CD14^+CD16^+$ 单核细胞值。100mg 剂量在研究 7、14 和 28 天分别具有基线的 19％、26％和 61％的中位 $CD14^+CD16^+$ 单核细胞值。$CD14^+CD16^+$ 单核细胞可随着重复给药进一步降低。

[0495] 预测 M-CSF 的抑制会抑制导致骨再吸收的抑制的破骨细胞分化。抗体 8.10.3F 的治疗与 uNTX-1 中剂量和时间依赖性的降低相关。uNTX-1 的降低速率对于 $CD14^+CD16^+$ 单核细胞而言较慢，且抑制率的恢复被更加地延长。在 30、100 和 300mg 剂量组中，uNTX-1 降低

至最低 64.4、60.5 和 39.9％的基线值，其分别出现在 7、14 和 28 天。对于相同的各剂量组，在 28 天，平均 uNTX-1 为 78.0、69.4 和 39.3％。对于≤ 100mg 的剂量，uNTX-1 在约 56 天返回至基线。BSAP 水平不受到剂量增加至 100mg（包括 100mg）的治疗的影响。在 300mg，在 7 至 28 天观察到 BSAP 增加的趋势，随后在 56 天返回至基线。然而，在接受 300mg 的对象中观察到的最大绝对值可与包括安慰剂在内的其它治疗组中观察到的值相匹配。

[0496] 所有于本说明书所引用的公开及专利公开是以引用的方式并入本文中，其方式犹如各单独公开或专利公开是特别且单独指明以引用的方式并入本文中。为供清楚了解的目的，前述本发明通过说明及实施例的方式进行了一定程度的描述，但对熟谙本领域的一般技术人员而言，很明显的可按照本发明的指导而做出某些改变及修饰而不偏离所附申请权利要求的精神或范围。

[0497] 序列表

[0498] 关键词：

[0499] 信号肽：下划线小写字体

[0500] CDR1,2,3: 下划线大写字体

[0501] 可变区：大写字体

[0502] 恒定区：小写字体

[0503] 自种系的突变 :粗体

[0504] SEQ ID NO:1

[0505] 252 重链［γ 链］核苷酸序列

[0506] atggagttggggctgtgctggattttccttgttgctattataaaaggtgtccagtgtCAGGTGCAGCTG
GTGGAGTCTGGGGGAGGCTTGGTCAAGCCTGGAGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTCAG
TGACTACTACATGAGCTGGATCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGG**A**TTTCATACATTAGT**G**GTAGTGG
TAGTACCATATACTACGCAGACTCTGTGAAGGGCCGATTCACCATCTCCAGGGACAACGCCAAGAACTCACTGTATCT
GCAAATGAACAGCCTGAGAGCCGAGGACACGGCCGTGTAT**C**ACTGTGCGAGAGCCCTGGGTGGGATGGACGTCTGGG
GCCAAGGGACCACGGTCACCGTCTCCTCAGCTtccaccaagggcccatccgtcttccccctggcgccctgctctaga
agcacctccgagagcacagcggccctgggctgcctggtcaaggactacttccccgaaccggtgacggtgtcgtggaa
ctcaggcgctctgaccagcggcgtgcacaccttcccagctgtcctacagtcctcaggactctactccctcagcagcg
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gtggacaagacagttgagcgcaaatgttgtgtcgagtgcccaccgtgcccagcaccacctgtggcaggaccgtcagt
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tgagccacgaagaccccgaggtccagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagcca
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cccgagaaccacaggtgtacaccctgcccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcctg
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acctcccatgctggactccgacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcagg
ggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccg
ggtaaa

[0507] SEQ ID NO:2

[0508] 252 重链 [γ 链] 蛋白质序列

[0509] melglcwiflvaiikgvqcQVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMSWIRQAPGKGLEW I SYIS G SGSTIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVY H CARALGGMDVWGQGTTVTVSSAstkgp svfplapcsrstsestaalgclvkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssnfgtqtytc nvdhkpsntkvdktverkccvecppcpappvagpsvflfppkpkdtlmisrtpevtcvvvdvshedpevqfnwyvdg vevhnaktkpreeqfnstfrvvsvltvvhqdwlngkeykckvsnkglpapiektisktkgqprepqvytlppsreem tknqvsltclvkgfypsdiavewesngqpennykttppmldsdgsfflyskltvdksrwqqgnvfscsvmhealhnh ytqkslslspgk

[0510] SEQ ID NO:3

[0511] 252 轻链 [K 链] 核苷酸序列

[0512] atgagggtccctgctcagctcctggggctcctgctactctggctccgaggtgccagatgtGACATCC AGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTGCCGGGCAAGTCAG AGCATTAGCG G CT T TTTAAATTGGTATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATGCT A CATCCAGTTTGCAAAGTGGGGTCCCA TT CAGGTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAG CAGTCTGCAACCTGAAGATTTTGCAACTTATTACTGTCAACAGAGTTACAGT GT CCCATTCACTTTCGGCCCTGGG ACCAAAGTGGATATCAAACGAactgtggctgcaccatctgtcttcatcttcccgccatctgatgagcagttgaaatc tggaactgctagcgttgtgtgcctgctgaataacttctatcccagagaggccaaagtacagtggaaggtggataacg ccctccaatcgggtaactcccaggagagtgtcacagagcaggacagcaaggacagcacctacagcctcagcagcacc ctgacgctgagcaaagcagactacgagaaacacaaagtctacgcctgcgaagtcacccatcagggcctgagctcgcc cgtcacaaagagcttcaacaggggagagtgt

[0513] SEQ ID NO:4

[0514] 252 轻链 [K 链] 蛋白质序列

[0515] mrvpaqllgllllwlrgarcDIQMTQSPSSLSASVGDRVTITCRASQSIS GF LNWYQQKPGKAPKLLIYA T SSLQSGVP F RFSGSGSGTDFTLTISSLQPEDFATYYCQQSYS V PFTFGPGTKVD IKRtvaapsvfifppsdeqlksgtasvvcllnnfypreakvqwkvdnalqsgnsqesvteqdskdstyslsstltlsk adyekhkvyacevthqglsspvtksfnrgec

[0516] SEQ ID NO:5

[0517] 88 重链 [γ 链] 核苷酸序列

[0518] atggaatttgggctgtgctgggttttccttgttgctattttagaaggtgtccagtgtGAGGTGCAGCTG GTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTTAG TAGCTATTGGATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTGGCCAACATAAAGCAAGATGGAA GTGAGAAATACTATGTGGACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACTCACTGTATCTG CAAATGAACAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCTCCGGGTATAGCAGCAGCTGGTAGGGCCTA CTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAGCTtccaccaagggcccatccgtcttcccccctggcgccctgct ctagaagcacctccgagagcacagcggccctgggctgcctggtcaaggactacttccccgaaccggtgacggtgtcg tggaactcaggcgctctgaccagcggcgtgcacaccttcccagctgtcctacagtcctcaggactctactccctcag cagcgtggtgaccgtgccctccagcaacttcggcacccagacctacacctgcaacgtagatcacaagcccagcaaca ccaaggtggacaagacagttgagcgcaaatgttgtgtcgagtgcccaccgtgcccagcaccacctgtggcaggaccg tcagtcttcctcttcccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcacgtgcgtggtggt

ggacgtgagccacgaagaccccgaggtccagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaa
agccacgggaggagcagttcaacagcacgttccgtgtggtcagcgtcctcaccgttgtgcaccaggactggctgaac
ggcaaggagtacaagtgcaaggtctccaacaaaggcctcccagcccccatcgagaaaaccatctccaaaaccaaagg
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gcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgt
ctccgggtaaa

[0519] SEQ ID NO:6

[0520] 88 重链 [γ 链] 蛋白质序列

[0521] mefglcwvflvailegvqcEVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMSWVRQAPGKGLEWVAN
IKQDGSEKYYVDSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCA PGIAAAG RAY WGQGTLVTVSSAstkgp
svfplapcsrstsestaalgclvkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssnfgtqtytc
nvdhkpsntkvdktverkccvecppcpappvagpsvflfppkpkdtlmisrtpevtcvvvdvshedpevqfnwyvdg
vevhnaktkpreeqfnstfrvvsvltvvhqdwlngkeykckvsnkglpapiektisktkgqprepqvytlppsreem
tknqvsltclvkgfypsdiavewesngqpennykttppmldsdgsfflyskltvdksrwqqgnvfscsvmhealhnh
ytqkslslspgk

[0522] SEQ ID NO:7

[0523] 88 轻链 [K 链] 核苷酸序列

[0524] atgagggtccctgctcagctcctggggctcctgctactctggctccgaggtgccagatgtGACATCCAG
ATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTTGGAGACAGAGTCACCATCACTTGCCGG C CAAGTCAG GA C
ATTAGCAGTTATTTAAATTGGTATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATGCTGCATCCAGTTTG
CAAAGTGGGGTCCCAT T AAGGTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTCTGCAAC
CTGAAGATTTTGCAACTTACTACTGTCAACAGAGTTACAGTACCCCATTCACTTTCGGCCCTGGGACCAAAGTGGAT
ATCAAACGAactgtggctgcaccatctgtcttcatcttcccgccatctgatgagcagttgaaatctggaactgctag
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gtaactcccaggagagtgtcacagagcaggacagcaaggacagcacctacagcctcagcagcaccctgacgctgagc
aaagcagactacgagaaacacaaagtctacgcctgcgaagtcacccatcagggcctgagctcgcccgtcacaaagag
cttcaacaggggagagtgt

[0525] SEQ ID NO:8

[0526] 88 轻链 [K 链] 蛋白质序列

[0527] mrvpaqllgllllwlrgarcDIQMTQSPSSLSASVGDRVTITCR P SQ D
ISSYLNWYQQKPGKAPKLLIYAASSLQSGVP L RFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPFTFGPGTKV
DIKRtvaapsvfifppsdeqlksgtasvvcllnnfypreakvqwkvdnalqsgnsqesvteqdskdstyslsstltls
kadyekhkvyacevthqglsspvtksfnrgec

[0528] SEQ ID NO:9

[0529] 100 重链 [γ 链] 核苷酸序列

[0530] atggagtttgggctccgctggatttttcttgtggctatttaaaaggtgtccagtgtGAGGTGCAGCT
GTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTT

TAGCAGCTATGCCATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAATGGGTCTCAGCTATTAGTGGT C GTGGTGGTAG G ACATACT T CGCAGACTCCGTGAAGGGCCGGTTCACCATCTCCAGAGACAATTCCAAGAACACG CTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATT T CTGTGCG GT AGAAGGCTATAGTGGG CGCTACGGATTTTTTGACTACTGGGGCCAGGGAACCCTAGTCACCGTCTCCTCAGCCtccaccaagggcccatcggt cttccccctggcgccctgctctagaagcacctccgagagcacagcggccctgggctgcctggtcaaggactacttcc ccgaaccggtgacggtgtcgtggaactcaggcgctctgaccagcggcgtgcacaccttcccagctgtcctacagtcc tcaggactctactccctcagcagcgtggtgaccgtgccctccagcaacttcggcacccagacctacacctgcaacgt agatcacaagcccagcaacaccaaggtggacaagacagttgagcgcaaatgttgtgtcgagtgcccaccgtgcccag caccacctgtggcaggaccgtcagtcttcctcttcccccaaaacccaaggacaccctcatgatctcccggacccct gaggtcacgtgcgtggtggtggacgtgagccacgaagaccccgaggtccagttcaactggtacgtggacggcgtgga ggtgcataatgccaagacaaagccacgggaggagcagttcaacagcacgttccgtgtggtcagcgtcctcaccgttg tgcaccaggactggctgaacggcaaggagtacaagtgcaaggtctccaacaaaggcctcccagcccccatcgagaaa accatctccaaaaccaaagggcagccccgagaaccacaggtgtacaccctgcccccatcccgggaggagatgaccaa gaaccaggtcagcctgacctgcctggtcaaaggcttctaccccagcgacatcgccgtggagtgggagagcaatgggc agccggagaacaactacaagaccacacctcccatgctggactccgacggctccttcttcctctacagcaagctcacc gtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacac gcagaagagcctctccctgtctccgggtaaa

[0531] SEQ ID NO:10

[0532] 100 重链 [γ 链] 蛋白质序列

[0533] mefglrwiflvailkgvqcEVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSA ISG R GG R TY F ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVY F CA VEG YSG R Y GF FDYWGQGTLV TVSSAstkgpsvfplapcsrstsestaalgclvkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvps snfgtqtytcnvdhkpsntkvdktverkccvecppcpappvagpsvflfppkpkdtlmisrtpevtcvvvdvshedp evqfnwyvdgvevhnaktkpreeqfnstfrvvsvltvvhqdwlngkeykckvsnkglpapiektisktkgqprepqv ytlppsreemtknqvsltclvkgfypsdiavewesngqpennykttppmldsdgsfflyskltvdksrwqqgnvfsc svmhealhnhytqkslslspgk

[0534] SEQ ID NO:11

[0535] 100 轻链 [K 链] 核苷酸序列

[0536] atggaagccccagctcagcttctcttcctcctgctactctggctcccagataccactggaGAAATAGTG ATGACGCAGTCTCCAGCCACCCTGTCTGTGTCTCCAGGGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGT TAGCAGCAACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTATGGTGCATCCACCAGGGC CA G TGGTATCCCAG A CAGG A TCAGTGGCAGTGGGTCTGGAACAGAGTTCACTCTCATCATCAGCAGCCTGCAGT CTGAAGATTTTGCAGTTTATTACTGTCAGCAGTCTAATAACTGGCCATTCACTTTCGGCCCTGGGACCAAAGTGGAT ATCAAACGAactgtggctgcaccatctgtcttcatcttcccgccatctgatgagcagttgaaatctggaactgctag cgttgtgtgcctgctgaataacttctatcccagagaggccaaagtacagtggaaggtggataacgccctccaatcgg gtaactcccaggagagtgtcacagagcaggacagcaaggacagcacctacagcctcagcagcaccctgacgctgagc aaagcagactacgagaaacacaaagtctacgcctgcgaagtcacccatcagggcctgagctcgcccgtcacaaagag cttcaacaggggagagtgt

[0537] SEQ ID NO:12

[0538] 100 轻链 [K 链] 蛋白质序列

[0539] meapaqllfllllwlpdttgEIVMTQSPATLSVSPGERATLSCRASQSVSSNLAWYQQKPGQAPRLLIY GASTRASGIPDRISGSGSGTEFTLIISSLQSEDFAVYYCQQSNNWPFTFGPGTKVDIKRtvaapsvfifppsdeql ksgtasvvcllnnfypreakvqwkvdnalqsgnsqesvteqdskdstyslsstltlskadyekhkvyacevthqgls spvtksfnrgec

[0540] SEQ ID NO:14

[0541] 3.8.3 重链 [γ 链] 蛋白质序列

[0542] mefglswvflvaiikgvqcQVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMSWIRQAPGKGLEW FSYISSSGSTIYYADSVKGRFTISRDNAKNSLSLQMNSLRAEDTAVYYCARGLTGDYWGQGTLVTVSSAstkgpsv fplapcsrstsestaalgclvkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssnfgtqtytcnv dhkpsntkvdktverkccvecppcpappvagpsvflfppkpkdtlmisrtpevtcvvvdvshedpevqfnwyvdgve vhnaktkpreeqfnstfrvvsvltvvhqdwlngkeykckvsnkglpapiektisktkgqprepqvytlppsreemtk nqvsltclvkgfypsdiavewesngqpennykttppmldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhyt qkslslspgk

[0543] SEQ ID NO:16

[0544] 3.8.3 轻链 [K 链] 蛋白质序列

[0545] mdmrvpaqllgllllwfpgsrcDIQMTQSPSSVSASVGDRVTISCRASQDISGWLAWYQQKPGKAPKLLISATSSLHSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQTNSFPFTFGPGTKV DIKRtvaapsvfifppsdeqlksgtasvvcllnnfypreakvqwkvdnalqsgnsqesvteqdskdstyslsstltls kadyekhkvyacevthqglsspvtksfnrgec

[0546] SEQ ID NO:18

[0547] 2.7.3 重链 [γ 链] 蛋白质序列

[0548] mefglswvflvallrgcqcQVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWV AFIWYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGYRVYFDYWGQGTLVTVSSAstkgp svfplapcsrstsestaalgclvkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssslgtktytc nvdhkpsntkvdkrveskygppcpscpapeflggpsvflfppkpkdtlmisrtpevtcvvvdvsqedpevqfnwyvd gvevhnaktkpreeqfnstyrvvsvltvlhqdwlngkeykckvsnkglpssiektiskakgqprepqvytlppsqee mtknqvsltclvkgfypsdiavewesngqpennykttppvldsdgsfflysrltvdksrwqegnvfscsvmhealhn hytqkslslspgk

[0549] SEQ ID NO:20

[0550] 2.7.3 轻链 [K 链] 蛋白质序列

[0551] mdmrvpaqllgllllwfpgsrcDIQMTQSPSSVSASVGDRVTITCRASQDISSWLAWYQRKPGKAPKLQIYAASSLESGVPSRFNGSGSGTDFTLSISSLQPEDFATYYCQQTNSFPLTFGGGTKVEIKRt vaapsvfifppsdeqlksgtasvvcllnnfypreakvqwkvdnalqsgnsqesvteqdskdstyslsstltlskadye khkvyacevthqglsspvtksfnrgec

[0552] SEQ ID NO:22

[0553] 1.120.1 重链 [γ 链] 蛋白质序列

[0554] mewtwsflflvaaatgahsQVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGQGLEWMGW ISAYNGNTNYAQKLQDRVTMTTDTSTTAYMELRSLRSDDTAVYYCARRAYGANFFDYWGQGTLVTVSSA

stkgpsvfplapcsrstsestaalgclvkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssnfgt
qtytcnvdhkpsntkvdktverkccvecppcpappvagpsvflfppkpkdtlmisrtpevtcvvvdvshedpevqfn
wyvdgvevhnaktkpreeqfnstfrvvsvltvvhqdwlngkeykckvsnkglpapiektisktkgqprepqvytlpp
sreemtknqvsltclvkgfypsdiavewesngqpennykttppmldsdgsfflyskltvdksrwqqgnvfscsvmhe
alhnhytqkslslspgk

[0555] SEQ ID NO:24

[0556] 1.120.1 轻链 [K 链] 蛋白质序列

[0557] mvlqtqvfislllwisgaygDIVMTQSPDSLAVSLGERATINCKSSQS I L FF SNNKNYLAWY R
QKPGQPP N LLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYS S PWTFGQGTKVEIKRtvaa
psvfifppsdeqlksgtasvvcllnnfypreakvqwkvdnalqsgnsqesvteqdskdstyslsstltlskadyekhk
vyacevthqglsspvtksfnrgec

[0558] SEQ ID NO:25

[0559] 9.14.4I 重链 [γ 链] 核苷酸序列

[0560] atggagtttgggctgagctgggtttccttgttgctattataaaaggtgtCCAGTGTCAGGTGCAGCTG
GTGGAGTCTGGGGGAGGCTTGGTCAAGCCTGGAGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTCAG
TGACTACTATATGAGCTGGATCCGCCAGGCTCCAGGGAAGGGACTGGAGTGGGTTTCATACATTAGTAGTAGTGGTA
GTACCATATACTACGCAGACTCTGTGAAGGGCCGATTCACCATCTCCAGGGACAACGCCAAGAACTCACTGTATCTG
CAAATGAACAGCCTGAGAGCCGAGGACACGGCCGTGTATTACTGTGCGAGAGGCCTAACTGGGGACTACTGGGGCCA
GGGAACCCTGGTCACCGTCTCCTCAGCTtccaccaagggcccatccgtcttcccccctggcgccctgctctagaagca
cctccgagagcacagcggccctgggctgcctggtcaaggactacttccccgaaccggtgacggtgtcgtggaactca
ggcgctctgaccagcggcgtgcacaccttcccagctgtcctacagtcctcaggactctactccctcagcagcgtggt
gaccgtgccctccagcaacttcggcacccagacctacacctgcaacgtagatcacaagcccagcaacaccaaggtgg
acaagacagttgagcgcaaatgttgtgtcgagtgcccaccgtgcccagcaccacctgtggcaggaccgtcagtcttc
ctcttcccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcacgtgcgtggtggtggacgtgag
ccacgaagaccccgaggtccagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccacggg
aggagcagttcaacagcacgttccgtgtggtcagcgtcctcaccgttgtgcaccaggactggctgaacggcaaggag
tacaagtgcaaggtctccaacaaaggcctcccagccccatcgagaaaaccatctccaaaaccaaagggcagccccg
agaaccacaggtgtacaccctgcccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcctggtca
aaggcttctaccccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacacct
cccatgctggactccgacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaa
cgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggta
aa

[0561] SEQ ID NO:26

[0562] 9.14.4I 重链 [γ 链] 蛋白质序列

[0563] mefglswvflvaiikgvqcQVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMSWIRQAPGKGLEWVSY
ISSSGSTIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARG LTGDYWGQGTLVTVSSAstkgpsvfpla
pcsrstsestaalgclvkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssnfgtqtytcnvdhkp
sntkvdktverkccvecppcpappvagpsvflfppkpkdtlmisrtpevtcvvvdvshedpevqfnwyvdgvevhna
ktkpreeqfnstfrvvsvltvvhqdwlngkeykckvsnkglpapiektisktkgqprepqvytlppsreemtknqvs

ltclvkgfypsdiavewesngqpennykttppmldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqksl slspgk

[0564] SEQ ID NO:27

[0565] 9.14.4,9.14.4I,9.14.4-Ser 和 9.14.4-G1 轻链 [K 链] 核苷酸序列

[0566] atggacatgagggtccccgctcagctcctggggctcctgctactctggctccgaggtgccagatgTGAC ATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTCGGAGACAGAGTCACCATCACTTGCCGGCCAAGTCA GATCATTAGCAGTTTATTAAATTGGTATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCCATGCTGCATCCA GTTTGCAAAGTGGGGTCCCATCAAGGTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGTAGTCTG CAACCTGAAGATTTTGCAACTTACTACTGTCAACAGAGTTACAGTACCCCATTCACTTTCGGCCCTGGGACCAAAGT GGATATCAAACGAactgtggctgcaccatctgtcttcatcttcccgccatctgatgagcagttgaaatctggaactg cctctgttgtgtgcctgctgaataacttctatcccagagaggccaaagtacagtggaaggtggataacgccctccaa tcgggtaactcccaggagagtgtcacagagcaggacagcaaggacagcacctacagcctcagcagcaccctgacgct gagcaaagcagactacgagaaacacaaagtctacgcctgcgaagtcacccatcagggcctgagctcgcccgtcacaa agagcttcaacaggggagagtgt

[0567] SEQ ID NO:28

[0568] 9.14.4,9.14.4I,9.14.4-Ser 和 9.14.4-G1 轻链 [K 链] 蛋白质序列

[0569] mdmrvpaqllgllllwlrgarcDIQMTQSPSSLSASVGDRVTITCR **P** SQ **I** ISS **L** LNWYQQKPGKAPKLLI **H** AASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPFTFGPGTKVDIKR tvaapsv**fi**fppsdeqlksgtasvvcllnnfypreakvqwkvdnalqsgnsqesvteqdskdstyslsstltlskady ekhkvyacevthqglsspvtksfnrgec

[0570] SEQ ID NO:37

[0571] 9.14.4 重链 [γ 链] 核苷酸序列

[0572] atggagtttgggctgagctgggttttccttgttgctattataaaaggtgtCCAGTGTCAGGTGCAGCTG GTGGAGTCTGGGGGAGGCTTGGTCAAGCCTGGAGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTCAG TGACTACTATATGAGCTGGATCCGCCAGGCTCCAGGGAAGGGACTGGAGTGGGTTTCATACATTAGTAGTAGTGGTA GTACCATATACTACGCAGACTCTGTGAAGGGCCGATTCACCATCTCCAGGGACAACGCCAAGAACTCACTGTATCTG CAAATGAACAGCCTGAGAGCCGAGGACACGGCCGTGTATTACTGTGCGAGAGGCCTAACTGGGGACTACTGGGGCCA GGGAACCCTGGTCACCGTCTCCTCAGCTtccaccaagggcccatccgtcttcccccctggcgccctgctctagaagca cctccgagagcacagcggccctgggctgcctggtcaaggactacttccccgaaccggtgacggtgtcgtggaactca ggcgctctgaccagcggcgtgcacaccttcccagctgtcctacagtcctcaggactctactccctcagcagcgtggt gaccgtgccctccagcagcttgggcacgaagacctacacctgcaacgtagatcacaagcccagcaacaccaaggtgg acaagagagttgagtccaaatatggtcccccatgcccatcatgcccagcacctgagttcctgggggggaccatcagtc ttcctgttcccccccaaaacccaaggacactctcatgatctcccggacccctgaggtcacgtgcgtggtggtggacgt gagccaggaagaccccgaggtccagttcaactggtacgtggatggcgtggaggtgcataatgccaagacaaagccgc gggaggagcagttcaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaacggcaag gagtacaagtgcaaggtctccaacaaaggcctcccgtcctccatcgagaaaaccatctccaaagccaaagggcagcc ccgagagccacaggtgtacaccctgcccccatcccaggaggagatgaccaagaaccaggtcagcctgacctgcctgg tcaaaggcttctaccccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacg cctcccgtgctggactccgacggctccttcttcctctacagcaggctaaccgtggacaagagcaggtggcaggaggg

gaatgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacacagaagagcctctccctgtctccgg gtaaa

[0573] SEQ ID NO:38

[0574] 9.14.4 重链 [γ 链] 蛋白质序列

[0575] mefglswvflvaiikgvqcQVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMSWIRQAPGKGLEWVSY ISSSGSTIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARGLTGDYWGQGTLVTVSSAstkgpsvfpla pcsrstsestaalgclvkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssslgtktytcnvdhkp sntkvdkrveskygppcpscpapeflggpsvflfppkpkdtlmisrtpevtcvvvdvsqedpevqfnwyvdgvevhn aktkpreeqfnstyrvvsvltvlhqdwlngkeykckvsnkglpssiektiskakgqprepqvytlppsqeemtknqv sltclvkgfypsdiavewesngqpennykttppvldsdgsfflysrltvdksrwqegnvfscsvmhealhnhytqks lslspgk

[0576] SEQ ID NO:54

[0577] 9.14.4C-Ser 重链 [γ 链] 蛋白质序列

[0578] mefglswvflvaiikgvqcQVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMSWIRQAPGKGLEWVSY ISSSGSTIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARGLTGDYWGQGTLVTVSSAstkgpsvfpla pcsrstsestaalgclvkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssslgtktytcnvdhkps ntkvdkrveskygppcpPcpapeflggpsvflfppkpkdtlmisrtpevtcvvvdvsqedpevqfnwyvdgvevhnak tkpreeqfnstyrvvsvltvlhqdwlngkeykckvsnkglpssiektiskakgqprepqvytlppsqeemtknqvsl tclvkgfypsdiavewesngqpennykttppvldsdgsfflysrltvdksrwqegnvfscsvmhealhnhytqksls lspgk

[0579] SEQ ID NO:56

[0580] 9.14.4C-Ser,9.14.4-CG2 和 9.14.4-CG4 轻链 [K 链] 蛋白质序列

[0581] mdmrvpaqllgllllwlrgarcDIQMTQSPSSLSASVGDRVTITCRPSQISSLLNWYQQKPGKAP KLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPFTFGPGTKVDIKRtvaapsvfifppsd eqlksgtasvvcllnnfypreakvqwkvdnalqsgnsqesvteqdskdstyslsstltlskadyekhkvyacevthq glsspvtksfnrgec

[0582] SEQ ID NO:74

[0583] 9.14.4-CG2 重链 [γ 链] 蛋白质序列

[0584] mefglswvflvaiikgvqcQVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMSWIRQAPGKGLEWVSY ISSSGSTIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARGLTGDYWGQGTLVTVSSAstkgpsvfpla pcsrstsestaalgclvkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssnfgtqtytcnvdhkp sntkvdktverkccvecppcpappvagpsvflfppkpkdtlmisrtpevtcvvvdvshedpevqfnwyvdgvevhna ktkpreeqfnstfrvvsvltvvhqdwlngkeykckvsnkglpapiektisktkgqprepqvytlppsreemtknqvs ltclvkgfypsdiavewesngqpennykttppmldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqksl slspgk

[0585] SEQ ID NO:78

[0586] 9.14.4-CG4 重链 [γ 链] 蛋白质序列

[0587] mefglswvflvaiikgvqcQVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMSWIRQAPGKGLEWVSY ISSSGSTIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARGLTGDYWGQGTLVTVSSAstkgpsvfpla

pcsrstsestaalgclvkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssslgtktytcnvdhkp
sntkvdkrveskygppcpscpapeflggpsvflfppkpkdtlmisrtpevtcvvvdvsqedpevqfnwyvdgvevhn
aktkpreeqfnstyrvvsvltvlhqdwlngkeykckvsnkglpssiektiskakgqprepqvytlppsqeemtknqv
sltclvkgfypsdiavewesngqpennykttppvldsdgsfflysrltvdksrwqegnvfscsvmhealhnhytqks
lslspgk

[0588] SEQ ID NO:82

[0589] 9.14.4-Ser 重链 [γ 链] 蛋白质序列

[0590] mefglswvflvaiikgvqcQVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMSWIRQAPGKGLEWVSY
ISSSGSTIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARGLTGDYWGQGTLVTVSSAstkgpsvfpla
pcsrstsestaalgclvkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssslgtktytcnvdhkps
ntkvdkrveskygppcpPcpapeflggpsvflfppkpkdtlmisrtpevtcvvvdvsqedpevqfnwyvdgvevhnak
tkpreeqfnstyrvvsvltvlhqdwlngkeykckvsnkglpssiektiskakgqprepqvytlppsqeemtknqvsl
tclvkgfypsdiavewesngqpennykttppvldsdgsfflysrltvdksrwqegnvfscsvmhealhnhytqksls
lspgk

[0591] SEQ ID NO.101

[0592] 9.14.4G1 重链（γ 链）核苷酸序列

[0593] atggagtttgggctgagctgggtttccttgttgctattataaaaggtgtccagtgtCAGGTGCAGCTG
GTGGAGTCTGGGGGAGGCTTGGTCAAGCCTGGAGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTCAG
TGACTACTATATGAGCTGGATCCGCCAGGCTCCAGGGAAGGGACTGGAGTGGGTTTCATACATTAGTAGTAGTGGTA
GTACCATATACTACGCAGACTCTGTGAAGGGCCGATTCACCATCTCCAGGGACAACGCCAAGAACTCACTGTATCTG
CAAATGAACAGCCTGAGAGCCGAGGACACGGCCGTGTATTACTGTGCGAGAGGCCTAACTGGGGACTACTGGGGCCA
GGGAACCCTGGTCACCGTCTCCTCAGCTtccaccaagggcccatcggtcttcccctggcaccctcctccaagagca
cctctgggggcacagcggccctgggctgcctggtcaaggactacttccccgaaccggtgacggtgtcgtggaactca
ggcgccctgaccagcggcgtgcacaccttcccggctgtcctacagtcctcaggactctactccctcagcagcgtggt
gaccgtgccctccagcagcttgggcacccagacctacatctgcaacgtgaatcacaagcccagcaacaccaaggtgg
acaagaaagttgagcccaaatcttgtgacaaaactcacacatgcccaccgtgcccagcacctgaactcctgggggga
ccgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggaccccctgaggtcacatgcgtggt
ggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaaga
caaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctg
aatggcaaggagtacaagtgcaaggtctccaacaaagccctcccagcccccatcgagaaaaccatctccaaagccaa
agggcagccccgagaaccacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctga
cctgcctggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactac
aagaccacgcctcccgtgctggactccgacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtg
gcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccc
tgtctccgggtaaatag

[0594] SEQ ID NO102

[0595] 9.14.4G1 重链（γ 链）蛋白质序列

[0596] mefglswvflvaiikgvqcQVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMSWIRQAPGKGLEWVSY
ISSSGSTIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARGLTGDYWGQGTLVTVSSAstkgpsvfpla

psskstsggtaalgclvkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssslgtqtyicnvnhkp
sntkvdkkvepkscdkthtcppcpapellggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnwyvdgve
vhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepqvytlppsrdeltk
nqvsltclvkgfypsdiavewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhyt
qkslslspgk

[0597] SEQ ID NO:29

[0598] 8.10.3 和 8.10.3F 重链 [γ 链] 核苷酸序列

[0599] atggagttggggctgtgctgggttttccttgttgctattttagaaggtgtccagtgtGAGGTGCAGCTG
GTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTCAG
TAGTTTTAGTATGACCTGGGTCCGCCAGGCTCCAGGAAAGGGGCTGGAGTGGGTTTCATACATTAGTAGTAGAAGTA
GTACCATATCCTACGCAGACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAATGCCAAGAACTCACTGTATCTG
CAAATGAACAGCCTGAGAGACGAGGACACGGCTGTGTATTACTGTGCGAGAGATCCTCTTCTAGCGGGAGCTACCTT
CTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAGCCtccaccaagggcccatcggtcttcccctgg
cgccctgctccaggagcacctccgagagcacagcggccctgggctgcctggtcaaggactacttccccgaaccggtg
acggtgtcgtggaactcaggcgctctgaccagcggcgtgcacaccttcccagctgtcctacagtcctcaggactcta
ctccctcagcagcgtggtgaccgtgccctccagcaacttcggcacccagacctacacctgcaacgtagatcacaagc
ccagcaacaccaaggtggacaagacagttgagcgcaaatgttgtgtcgagtgcccaccgtgcccagcaccacctgtg
gcaggaccgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcacgtg
cgtggtggtggacgtgagccacgaagaccccgaggtccagttcaactggtacgtggacggcgtggaggtgcataatg
ccaagacaaagccacgggaggagcagttcaacagcacgttccgtgtggtcagcgtcctcaccgttgtgcaccaggac
tggctgaacggcaaggagtacaagtgcaaggtctccaacaaaggcctcccagcccccatcgagaaaaccatctccaa
aaccaaagggcagccccgagaaccacaggtgtacaccctgcccccatcccgggaggagatgaccaagaaccaggtca
gcctgacctgcctggtcaaaggcttctaccccagcgacatcgccgtggagtgggagagcaatgggcagccggagaac
aactacaagaccacacctcccatgctggactccgacggctccttcttcctctacagcaagctcaccgtggacaagag
caggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcc
tctccctgtctccgggtaaa

[0600] SEQ ID NO:30

[0601] 8.10.3 和 8.10.3F 重链 [γ 链] 蛋白质序列

[0602] melglcwvflvailegvqcEVQLVESGGGLVQPGGSLRLSCAASGFTFSS **F** SM **T**
WVRQAPGKGLEWVSYISS **R** SSTI **S** YADSVKGRFTISRDNAKNSLYLQMNSLRDEDTAVYYCAR **DPLLA** G **ATF**
FDYWGQGTLVTVSSAstkgpsvfplapcsrstsestaalgclvkdyfpepvtvswnsgaltsgvhtfpavlqssgly
slssvvtvpssnfgtqtytcnvdhkpsntkvdktverkccvecppcpappvagpsvflfppkpkdtlmisrtpevtc
vvvdvshedpevqfnwyvdgvevhnaktkpreeqfnstfrvvsvltvvhqdwlngkeykckvsnkglpapiektisk
tkgqprepqvytlppsreemtknqvsltclvkgfypsdiavewesngqpennykttppmldsdgsfflyskltvdks
rwqqgnvfscsvmhealhnhytqkslslspgk

[0603] SEQ ID NO:31

[0604] 8.10.3FG1 和 8.10.3F 轻链 [K 链] 核苷酸序列

[0605] atggaaaccccagcgcagcttctcttcctcctgctactctggctcccagataccaccggaGAATTTGTG
TTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGT

TAGCAGCAGTTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTATGGTGCATCCAGCA
GGGCCACTGGCATCCCAGACAGGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAG
CCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTATGGTAGCTCACCTCTCACTTTCGGCGGAGGGACCAAGGTGGA
GATCAAACGAactgtggctgcaccatctgtcttcatcttcccgccatctgatgagcagttgaaatctggaactgcct
ctgttgtgtgcctgctgaataacttctatcccagagaggccaaagtacagtggaaggtggataacgccctccaatcg
ggtaactcccaggagagtgtcacagagcaggacagcaaggacagcacctacagcctcagcagcaccctgacgctgag
caaagcagactacgagaaacacaaagtctacgcctgcgaagtcacccatcagggcctgagctcgcccgtcacaaaga
gcttcaacaggggagagtgt

[0606] SEQ ID NO:32

[0607] 8.10.3FG1 和 8.10.3F 轻链 [K 链] 蛋白质序列

[0608] metpaqllfllllwlpdttgE **F** VLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLL
IYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPLTFGGGTKVEIKRtvaapsv**fi**fppsdeql
ksgtasvvcllnnfypreakvqwkvdnalqsgnsqesvteqdskdstyslsstltlskadyekhkvyacevthqgls
spvtksfnrgec

[0609] SEQ ID NO:43

[0610] 8.10.3 和 8.10.3-Ser 轻链 [K 链] 核苷酸序列

[0611] atggaaaccccagcgcagcttctcttcctcctgctactctggctcccagataccaccggaGAATTTGTG
TTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGT
TAGCAGCAGTTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTATGGTGCATCCAGCA
GGGCCACTGGCATCCCAGACAGGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAG
CCTGAAGATTTTGTAGTGTATTACTGTCAGCAGTATGGTAGCTCACCTCTCACTTTCGGCGGAGGGACCAAGGTGGA
GATCAAACGAactgtggctgcaccatctgtcttcatcttcccgccatctgatgagcagttgaaatctggaactgcct
ctgttgtgtgcctgctgaataacttctatcccagagaggccaaagtacagtggaaggtggataacgccctccaatcg
ggtaactcccaggagagtgtcacagagcaggacagcaaggacagcacctacagcctcagcagcaccctgacgctgag
caaagcagactacgagaaacacaaagtctacgcctgcgaagtcacccatcagggcctgagctcgcccgtcacaaaga
gcttcaacaggggagagtgt

[0612] SEQ ID NO:44

[0613] 8.10.3 和 8.10.3-Ser 轻链 [K 链] 蛋白质序列

[0614] metpaqllfllllwlpdttgE **F** VLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLL
IYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDF**V**VYYCQQYGSSPLTFGGGTKVEIKRtvaapsv**fi**fppsdeql
ksgtasvvcllnnfypreakvqwkvdnalqsgnsqesvteqdskdstyslsstltlskadyekhkvyacevthqgls
spvtksfnrgec

[0615] SEQ ID NO:58

[0616] 8.10.3C-Ser 重链 [γ 链] 蛋白质序列

[0617] melglcwvflvailegvqcEVQLVESGGGLVQPGGSLRLSCAASGFTFSS **F** SM **T**
WVRQAPGKGLEWVSYISS**R**SSTI**S**YADSVKGRFTISRDNAKNSLYLQMNSLRDEDTAVYYCAR**DPLLA**GA**TF**
FDYWGQGTLVTVSSAstkgpsvfplapcsrstsestaalgclvkdyfpepvtvswnsgaltsgvhtfpavlqssgly
slssvvtvpssslgtktytcnvdhkpsntkvdkrveskygppcp**P**cpapeflggpsvflfppkpkdtlmisrtpevt
cvvvdvsqedpevqfnwyvdgvevhnaktkpreeqfnstyrvvsvltvlhqdwlngkeykckvsnkglpssiektis

kakgqprepqvytlppsqeemtknqvsltclvkgfypsdiavewesngqpennykttppvldsdgsfflysrltvdk srwqegnvfscsvmhealhnhytqkslslspgk

[0618] SEQ ID NO:60

[0619] 8.10.3-CG2,8.10.3-CG4 和 8.10.3C-Ser 轻链 [k 链] 蛋白质序列

[0620] metpaqllfllllwlpdttgEIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLI YGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPLTFGGGTKVEIKRtvaapsvfifppsdeqlk sgtasvvcllnnfypreakvqwkvdnalqsgnsqesvteqdskdstyslsstltlskadyekhkvyacevthqglss pvtksfnrgec

[0621] SEQ ID NO:62

[0622] 8.10.3-CG2 重链 [γ 链] 蛋白质序列

[0623] melglcwvflvailegvqcEVQLVESGGGLVQPGGSLRLSCAASGFTFSS F SM T WVRQAPGKGLEWVSYISS R SSTI S YADSVKGRFTISRDNAKNSLYLQMNSLRDEDTAVYYCAR DPLL A G ATF FDYWGQGTLVTVSSAstkgpsvfplapcsrstsestaalgclvkdyfpepvtvswnsgaltsgvhtfpavlqssgly slssvvtvpssnfgtqtytcnvdhkpsntkvdktverkccvecppcpappvagpsvflfppkpkdtlmisrtpevtc vvvdvshedpevqfnwyvdgvevhnaktkpreeqfnstfrvvsvltvvhqdwlngkeykckvsnkglpapiektisk tkgqprepqvytlppsreemtknqvsltclvkgfypsdiavewesngqpennykttppmldsdgsfflyskltvdks rwqqgnvfscsvmhealhnhytqkslslspgk

[0624] SEQ ID NO:90

[0625] 8.10.3-Ser 重链 [γ 链] 蛋白质序列

[0626] melglcwvflvailegvqcEVQLVESGGGLVQPGGSLRLSCAASGFTFSS F SM T WVRQAPGKGLEWVSYISS R SSTI S YADSVKGRFTISRDNAKNSLYLQMNSLRDEDTAVYYCAR DPLL A G ATF FDYWGQGTLVTVSSAstkgpsvfplapcsrstsestaalgclvkdyfpepvtvswnsgaltsgvhtfpavlqssgly slssvvtvpssslgtktytcnvdhkpsntkvdkrveskygppcp p cpapeflggpsvflfppkpkdtlmisrtpevtc vvvdvsqedpevqfnwyvdgvevhnaktkpreeqfnstyrvvsvltvlhqdwlngkeykckvsnkglpssiektisk akgqprepqvytlppsqeemtknqvsltclvkgfypsdiavewesngqpennykttppvldsdgsfflysrltvdks rwqegnvfscsvmhealhnhytqkslslspgk

[0627] SEQ ID NO:94

[0628] 8.10.3-CG4 重链 [γ 链] 蛋白质序列

[0629] melglcwvflvailegvqcEVQLVESGGGLVQPGGSLRLSCAASGFTFSS F SM T WVRQAPGKGLEWVSYISS R SSTI S YADSVKGRFTISRDNAKNSLYLQMNSLRDEDTAVYYCAR DPLL A G ATF FDYWGQGTLVTVSSAstkgpsvfplapcsrstsestaalgclvkdyfpepvtvswnsgaltsgvhtfpavl qssglyslssvvtvpssslgtktytcnvdhkpsntkvdkrveskygppcpscpapeflggpsvflfppkpkdtlmis rtpevtcvvvdvsqedpevqfnwyvdgvevhnaktkpreeqfnstyrvvsvltvlhqdwlngkeykckvsnkglpss iektiskakgqprepqvytlppsqeemtknqvsltclvkgfypsdiavewesngqpennykttppvldsdgsfflys rltvdksrwqegnvfscsvmhealhnhytqkslslspgk

[0630] SEQ ID NO:97

[0631] 8.10.3FG1 重链核苷酸序列

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[0633] SEQ ID NO:98

[0634] 8.10.3FG1 重链（γ 链）蛋白质序列

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[0636] SEQ ID NO:33

[0637] 9.7.2IF 重链［γ 链］核苷酸序列

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[0643] 9.7.2IF 轻链 [K 链] 核苷酸序列

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[0652] 9.7.2 重链[γ 链]蛋白质序列

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[0655] 9.7.2 和 9.7.2-Ser 轻链[K 链]核苷酸序列

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[0657] SEQ ID NO:48

[0658] 9.7.2 和 9.7.2-Ser 轻链 [K 链] 蛋白质序列

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[0660] SEQ ID NO:50

[0661] 9.7.2C-Ser 重链 [γ 链] 蛋白质序列

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[0663] SEQ ID NO:52

[0664] 9.7.2C-Ser,9.7.2-CG2 和 9.7.2-CG4 轻链 [K 链] 蛋白质序列

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[0666] SEQ ID NO:66

[0667] 9.7.2-CG2 重链 [γ 链] 蛋白质序列

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[0670] 9.7.2-CG4 重链 [γ 链] 蛋白质序列

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Glu Trp Ile Ser Tyr Ile Ser Gly Ser Gly Ser Thr Ile Tyr Tyr Ala
65 70 75 80

Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn
85 90 95

Ser Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val
100 105 110

Tyr His Cys Ala Arg Ala Leu Gly Gly Met Asp Val Trp Gly Gln Gly
115 120 125

Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
130 135 140

Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu
145 150 155 160

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
165 170 175

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
180 185 190

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser

[0003]

195 200 205

Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys Pro
210 215 220

Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Cys Cys Val Glu
225 230 235 240

Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu
245 250 255

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
260 265 270

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln
275 280 285

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
290 295 300

Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu
305 310 315 320

Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
325 330 335

Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
340 345 350

Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
355 360 365

Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
370 375 380

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
385 390 395 400

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser Asp Gly
405 410 415

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
420 425 430

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
435 440 445

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
450 455 460

<210> 3
<211> 702
<212> DNA
<213> Homo sapiens

[0004]

<400> 3
atgagggtcc ctgctcagct cctggggctc ctgctactct ggctccgagg tgccagatgt 60

gacatccaga tgacccagtc tccatcctcc ctgtctgcat ctgtaggaga cagagtcacc 120

atcacttgcc gggcaagtca gagcattagc ggcttttaa attggtatca gcagaaacca 180

gggaaagccc ctaagctcct gatctatgct acatccagtt tgcaaagtgg ggtcccattc 240

aggttcagtg gcagtggatc tgggacagat ttcactctca ccatcagcag tctgcaacct 300

gaagattttg caacttatta ctgtcaacag agttacagtg tcccattcac tttcggccct 360

gggaccaaag tggatatcaa acgaactgtg gctgcaccat ctgtcttcat cttcccgcca 420

tctgatgagc agttgaaatc tggaactgct agcgttgtgt gcctgctgaa taacttctat 480

cccagagagg ccaaagtaca gtggaaggtg gataacgccc tccaatcggg taactcccag 540

gagagtgtca cagagcagga cagcaaggac agcacctaca gcctcagcag caccctgacg 600

ctgagcaaag cagactacga gaaacacaaa gtctacgcct gcgaagtcac ccatcagggc 660

ctgagctcgc ccgtcacaaa gagcttcaac aggggagagt gt 702

<210> 4
<211> 234
<212> PRT
<213> Homo sapiens

<400> 4

Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp Leu Arg
1 5 10 15

Gly Ala Arg Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser
20 25 30

Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser
35 40 45

Ile Ser Gly Phe Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro
50 55 60

Lys Leu Leu Ile Tyr Ala Thr Ser Ser Leu Gln Ser Gly Val Pro Phe
65 70 75 80

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
85 90 95

Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr
100 105 110

Ser Val Pro Phe Thr Phe Gly Pro Gly Thr Lys Val Asp Ile Lys Arg
115 120 125

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
130 135 140

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr

[0005]

145 150 155 160

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
165 170 175

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
180 185 190

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
195 200 205

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
210 215 220

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225 230

<210> 5
<211> 1389
<212> DNA
<213> Homo sapiens

<400> 5
atggaatttg ggctgtgctg ggttttcctt gttgctattt tagaaggtgt ccagtgtgag 60

gtgcagctgg tggagtctgg gggaggcttg gtccagcctg gggggtccct gagactctcc 120

tgtgcagcct ctggattcac ctttagtagc tattggatga gctgggtccg ccaggctcca 180

gggaaggggc tggagtgggt ggccaacata aagcaagatg gaagtgagaa atactatgtg 240

gactctgtga agggccgatt caccatctcc agagacaacg ccaagaactc actgtatctg 300

caaatgaaca gcctgagagc cgaggacacg gctgtgtatt actgtgctcc gggtatagca 360

gcagctggta gggcctactg gggccaggga accctggtca ccgtctcctc agcttccacc 420

aagggcccat ccgtcttccc cctggcgccc tgctctagaa gcacctccga gagcacagcg 480

gccctgggct gcctggtcaa ggactacttc cccgaaccgg tgacggtgtc gtggaactca 540

ggcgctctga ccagcggcgt gcacaccttc ccagctgtcc tacagtcctc aggactctac 600

tccctcagca gcgtggtgac cgtgccctcc agcaacttcg gcacccagac ctacacctgc 660

aacgtagatc acaagcccag caacaccaag gtggacaaga cagttgagcg caaatgttgt 720

gtcgagtgcc caccgtgccc agcaccacct gtggcaggac cgtcagtctt cctcttcccc 780

ccaaaaccca aggacaccct catgatctcc cggacccctg aggtcacgtg cgtggtggtg 840

gacgtgagcc acgaagaccc cgaggtccag ttcaactggt acgtggacgg cgtggaggtg 900

cataatgcca agacaaagcc acgggaggag cagttcaaca gcacgttccg tgtggtcagc 960

gtcctcaccg ttgtgcacca ggactggctg aacggcaagg agtacaagtg caaggtctcc 1020

aacaaaggcc tcccagcccc catcgagaaa accatctcca aaaccaaagg gcagccccga 1080

gaaccacagg tgtacaccct gcccccatcc cgggaggaga tgaccaagaa ccaggtcagc 1140

ctgacctgcc tggtcaaagg cttctacccc agcgacatcg ccgtggagtg ggagagcaat 1200

gggcagccgg agaacaacta caagaccaca cctcccatgc tggactccga cggctccttc 1260

[0006]

ttcctctaca gcaagctcac cgtggacaag agcaggtggc agcaggggaa cgtcttctca 1320

tgctccgtga tgcatgaggc tctgcacaac cactacacgc agaagagcct ctccctgtct 1380

ccgggtaaa 1389

<210> 6
<211> 463
<212> PRT
<213> Homo sapiens

<400> 6

Met Glu Phe Gly Leu Cys Trp Val Phe Leu Val Ala Ile Leu Glu Gly
1 5 10 15

Val Gln Cys Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln
20 25 30

Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
35 40 45

Ser Ser Tyr Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
50 55 60

Glu Trp Val Ala Asn Ile Lys Gln Asp Gly Ser Glu Lys Tyr Tyr Val
65 70 75 80

Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn
85 90 95

Ser Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val
100 105 110

Tyr Tyr Cys Ala Pro Gly Ile Ala Ala Ala Gly Arg Ala Tyr Trp Gly
115 120 125

Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
130 135 140

Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala
145 150 155 160

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
165 170 175

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
180 185 190

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
195 200 205

Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His
210 215 220

Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Cys Cys

[0007]

225 230 235 240

Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val
245 250 255

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
260 265 270

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
275 280 285

Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
290 295 300

Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser
305 310 315 320

Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
325 330 335

Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile
340 345 350

Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
355 360 365

Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
370 375 380

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
385 390 395 400

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser
405 410 415

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
420 425 430

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
435 440 445

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
450 455 460

<210> 7
<211> 702
<212> DNA
<213> Homo sapiens

<400> 7
atgagggtcc ctgctcagct cctggggctc ctgctactct ggctccgagg tgccagatgt 60

gacatccaga tgacccagtc tccatcctcc ctgtctgcat ctgttggaga cagagtcacc 120

atcacttgcc ggccaagtca ggacattagc agttatttaa attggtatca gcagaaacca 180

[0008]

gggaaagccc ctaagctcct gatctatgct gcatccagtt tgcaaagtgg ggtcccatta 240

aggttcagtg gcagtggatc tgggacagat ttcactctca ccatcagcag tctgcaacct 300

gaagattttg caacttacta ctgtcaacag agttacagta ccccattcac tttcggccct 360

gggaccaaag tggatatcaa acgaactgtg gctgcaccat ctgtcttcat cttcccgcca 420

tctgatgagc agttgaaatc tggaactgct agcgttgtgt gcctgctgaa taacttctat 480

cccagagagg ccaaagtaca gtggaaggtg gataacgccc tccaatcggg taactcccag 540

gagagtgtca cagagcagga cagcaaggac agcacctaca gcctcagcag caccctgacg 600

ctgagcaaag cagactacga gaaacacaaa gtctacgcct gcgaagtcac ccatcagggc 660

ctgagctcgc ccgtcacaaa gagcttcaac aggggagagt gt 702

<210> 8
<211> 234
<212> PRT
<213> Homo sapiens

<400> 8

Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp Leu Arg
1 5 10 15

Gly Ala Arg Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser
20 25 30

Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Pro Ser Gln Asp
35 40 45

Ile Ser Ser Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro
50 55 60

Lys Leu Leu Ile Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Leu
65 70 75 80

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
85 90 95

Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr
100 105 110

Ser Thr Pro Phe Thr Phe Gly Pro Gly Thr Lys Val Asp Ile Lys Arg
115 120 125

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
130 135 140

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
145 150 155 160

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
165 170 175

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr

[0009]

180 185 190

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
195 200 205

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
210 215 220

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225 230

<210> 9
<211> 1398
<212> DNA
<213> Homo sapiens

<400> 9
atggagtttg ggctccgctg gatttttctt gtggctattt taaaaggtgt ccagtgtgag 60

gtgcagctgt tggagtctgg gggaggcttg gtacagcctg gggggtccct gagactctcc 120

tgtgcagcct ctggattcac ctttagcagc tatgccatga gctgggtccg ccaggctcca 180

gggaaggggc tggaatgggt ctcagctatt agtggtcgtg gtggtaggac atacttcgca 240

gactccgtga agggccggtt caccatctcc agagacaatt ccaagaacac gctgtatctg 300

caaatgaaca gcctgagagc cgaggacacg gccgtatatt tctgtgcggt agaaggctat 360

agtgggcgct acggattttt tgactactgg ggccagggaa ccctagtcac cgtctcctca 420

gcctccacca agggcccatc ggtcttcccc ctggcgccct gctctagaag cacctccgag 480

agcacagcgg ccctgggctg cctggtcaag gactacttcc ccgaaccggt gacggtgtcg 540

tggaactcag gcgctctgac cagcggcgtg cacaccttcc cagctgtcct acagtcctca 600

ggactctact ccctcagcag cgtggtgacc gtgccctcca gcaacttcgg cacccagacc 660

tacacctgca acgtagatca caagcccagc aacaccaagg tggacaagac agttgagcgc 720

aaatgttgtg tcgagtgccc accgtgccca gcaccacctg tggcaggacc gtcagtcttc 780

ctcttccccc caaaacccaa ggacaccctc atgatctccc ggacccctga ggtcacgtgc 840

gtggtggtgg acgtgagcca cgaagacccc gaggtccagt tcaactggta cgtggacggc 900

gtggaggtgc ataatgccaa gacaaagcca cgggaggagc agttcaacag cacgttccgt 960

gtggtcagcg tcctcaccgt tgtgcaccag gactggctga acggcaagga gtacaagtgc 1020

aaggtctcca acaaaggcct cccagccccc atcgagaaaa ccatctccaa aaccaaaggg 1080

cagccccgag aaccacaggt gtacaccctg cccccatccc gggaggagat gaccaagaac 1140

caggtcagcc tgacctgcct ggtcaaaggc ttctacccca gcgacatcgc cgtggagtgg 1200

gagagcaatg ggcagccgga gaacaactac aagaccacac ctcccatgct ggactccgac 1260

ggctccttct tcctctacag caagctcacc gtggacaaga gcaggtggca gcaggggaac 1320

gtcttctcat gctccgtgat gcatgaggct ctgcacaacc actacacgca gaagagcctc 1380

tccctgtctc cgggtaaa 1398

<210> 10

[0010]

<211> 466
<212> PRT
<213> Homo sapiens

<400> 10

Met Glu Phe Gly Leu Arg Trp Ile Phe Leu Val Ala Ile Leu Lys Gly
1 5 10 15

Val Gln Cys Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln
20 25 30

Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
35 40 45

Ser Ser Tyr Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
50 55 60

Glu Trp Val Ser Ala Ile Ser Gly Arg Gly Gly Arg Thr Tyr Phe Ala
65 70 75 80

Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn
85 90 95

Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val
100 105 110

Tyr Phe Cys Ala Val Glu Gly Tyr Ser Gly Arg Tyr Gly Phe Phe Asp
115 120 125

Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys
130 135 140

Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu
145 150 155 160

Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro
165 170 175

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr
180 185 190

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val
195 200 205

Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn
210 215 220

Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg
225 230 235 240

Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly
245 250 255

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile

[0011]

260 265 270

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
275 280 285

Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
290 295 300

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg
305 310 315 320

Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys
325 330 335

Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu
340 345 350

Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
355 360 365

Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
370 375 380

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
385 390 395 400

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met
405 410 415

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
420 425 430

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
435 440 445

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
450 455 460

Gly Lys
465

<210> 11
<211> 702
<212> DNA
<213> Homo sapiens

<400> 11
atggaagccc cagctcagct tctcttcctc ctgctactct ggctcccaga taccactgga 60

gaaatagtga tgacgcagtc tccagccacc ctgtctgtgt ctccagggga aagagccacc 120

ctctcctgca gggccagtca gagtgttagc agcaacttag cctggtacca gcagaaacct 180

ggccaggctc ccaggctcct catctatggt gcatccacca gggccagtgg tatcccagac 240

aggatcagtg gcagtgggtc tggaacagag ttcactctca tcatcagcag cctgcagtct 300

[0012]

gaagattttg cagtttatta ctgtcagcag tctaataact ggccattcac tttcggccct 360

gggaccaaag tggatatcaa acgaactgtg gctgcaccat ctgtcttcat cttcccgcca 420

tctgatgagc agttgaaatc tggaactgct agcgttgtgt gcctgctgaa taacttctat 480

cccagagagg ccaaagtaca gtggaaggtg gataacgccc tccaatcggg taactcccag 540

gagagtgtca cagagcagga cagcaaggac agcacctaca gcctcagcag caccctgacg 600

ctgagcaaag cagactacga gaaacacaaa gtctacgcct gcgaagtcac ccatcagggc 660

ctgagctcgc ccgtcacaaa gagcttcaac aggggagagt gt 702

<210> 12
<211> 234
<212> PRT
<213> Homo sapiens

<400> 12

Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
1 5 10 15

Asp Thr Thr Gly Glu Ile Val Met Thr Gln Ser Pro Ala Thr Leu Ser
20 25 30

Val Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser
35 40 45

Val Ser Ser Asn Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro
50 55 60

Arg Leu Leu Ile Tyr Gly Ala Ser Thr Arg Ala Ser Gly Ile Pro Asp
65 70 75 80

Arg Ile Ser Gly Ser Gly Ser Gly Thr Glu Phe Thr Leu Ile Ile Ser
85 90 95

Ser Leu Gln Ser Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Ser Asn
100 105 110

Asn Trp Pro Phe Thr Phe Gly Pro Gly Thr Lys Val Asp Ile Lys Arg
115 120 125

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
130 135 140

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
145 150 155 160

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
165 170 175

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
180 185 190

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys

[0013]

195 200 205

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
210 215 220

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225 230

<210> 13

<400> 13
000

<210> 14
<211> 460
<212> PRT
<213> Homo sapiens

<400> 14

Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Ile Ile Lys Gly
1 5 10 15

Val Gln Cys Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys
20 25 30

Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
35 40 45

Ser Asp Tyr Tyr Met Ser Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu
50 55 60

Glu Trp Phe Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala
65 70 75 80

Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn
85 90 95

Ser Leu Ser Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val
100 105 110

Tyr Tyr Cys Ala Arg Gly Leu Thr Gly Asp Tyr Trp Gly Gln Gly Thr
115 120 125

Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
130 135 140

Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly
145 150 155 160

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
165 170 175

Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
180 185 190

[0014]

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
195 200 205

Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser
210 215 220

Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Cys Cys Val Glu Cys
225 230 235 240

Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe
245 250 255

Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
260 265 270

Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln Phe
275 280 285

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
290 295 300

Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu Thr
305 310 315 320

Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
325 330 335

Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr
340 345 350

Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
355 360 365

Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
370 375 380

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
385 390 395 400

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser Asp Gly Ser
405 410 415

Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
420 425 430

Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
435 440 445

Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
450 455 460

<210> 15

<400> 15

[0015]

000

<210> 16
<211> 236
<212> PRT
<213> Homo sapiens

<400> 16

Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp
1 5 10 15

Phe Pro Gly Ser Arg Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Ser
20 25 30

Val Ser Ala Ser Val Gly Asp Arg Val Thr Ile Ser Cys Arg Ala Ser
35 40 45

Gln Asp Ile Ser Gly Trp Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys
50 55 60

Ala Pro Lys Leu Leu Ile Ser Ala Thr Ser Ser Leu His Ser Gly Val
65 70 75 80

Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
85 90 95

Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
100 105 110

Thr Asn Ser Phe Pro Phe Thr Phe Gly Pro Gly Thr Lys Val Asp Ile
115 120 125

Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp
130 135 140

Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn
145 150 155 160

Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu
165 170 175

Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp
180 185 190

Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr
195 200 205

Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser
210 215 220

Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225 230 235

<210> 17

[0016]

<400> 17
000

<210> 18
<211> 463
<212> PRT
<213> Homo sapiens

<400> 18

Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Leu Leu Arg Gly
1 5 10 15

Cys Gln Cys Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln
20 25 30

Pro Gly Arg Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
35 40 45

Ser Ser Tyr Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
50 55 60

Glu Trp Val Ala Phe Ile Trp Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala
65 70 75 80

Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn
85 90 95

Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val
100 105 110

Tyr Tyr Cys Ala Arg Gly Tyr Arg Val Tyr Phe Asp Tyr Trp Gly Gln
115 120 125

Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
130 135 140

Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala
145 150 155 160

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
165 170 175

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
180 185 190

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
195 200 205

Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys
210 215 220

Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro
225 230 235 240

Pro Cys Pro Ser Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val

[0017]

245 250 255

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
260 265 270

Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu
275 280 285

Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
290 295 300

Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser
305 310 315 320

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
325 330 335

Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile
340 345 350

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
355 360 365

Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
370 375 380

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
385 390 395 400

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
405 410 415

Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg
420 425 430

Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
435 440 445

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
450 455 460

<210> 19

<400> 19
000

<210> 20
<211> 236
<212> PRT
<213> Homo sapiens

<400> 20

Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp
1 5 10 15

[0018]

Phe Pro Gly Ser Arg Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Ser
20 25 30

Val Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser
35 40 45

Gln Asp Ile Ser Ser Trp Leu Ala Trp Tyr Gln Arg Lys Pro Gly Lys
50 55 60

Ala Pro Lys Leu Gln Ile Tyr Ala Ala Ser Ser Leu Glu Ser Gly Val
65 70 75 80

Pro Ser Arg Phe Asn Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Ser
85 90 95

Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
100 105 110

Thr Asn Ser Phe Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile
115 120 125

Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp
130 135 140

Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn
145 150 155 160

Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu
165 170 175

Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp
180 185 190

Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr
195 200 205

Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser
210 215 220

Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225 230 235

<210> 21

<400> 21
000

<210> 22
<211> 464
<212> PRT
<213> Homo sapiens

<400> 22

Met Glu Trp Thr Trp Ser Phe Leu Phe Leu Val Ala Ala Ala Thr Gly
1 5 10 15

[0019]

Ala His Ser Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys
20 25 30

Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe
35 40 45

Thr Ser Tyr Gly Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu
50 55 60

Glu Trp Met Gly Trp Ile Ser Ala Tyr Asn Gly Asn Thr Asn Tyr Ala
65 70 75 80

Gln Lys Leu Gln Asp Arg Val Thr Met Thr Thr Asp Thr Ser Thr Thr
85 90 95

Thr Ala Tyr Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val
100 105 110

Tyr Tyr Cys Ala Arg Arg Ala Tyr Gly Ala Asn Phe Phe Asp Tyr Trp
115 120 125

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
130 135 140

Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr
145 150 155 160

Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
165 170 175

Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
180 185 190

Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
195 200 205

Val Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp
210 215 220

His Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Cys
225 230 235 240

Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser
245 250 255

Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
260 265 270

Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
275 280 285

Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
290 295 300

[0020]

Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val
305 310 315 320

Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
325 330 335

Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr
340 345 350

Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
355 360 365

Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys
370 375 380

Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
385 390 395 400

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp
405 410 415

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
420 425 430

Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
435 440 445

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
450 455 460

<210> 23

<400> 23
000

<210> 24
<211> 240
<212> PRT
<213> Homo sapiens

<400> 24

Met Val Leu Gln Thr Gln Val Phe Ile Ser Leu Leu Leu Trp Ile Ser
1 5 10 15

Gly Ala Tyr Gly Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala
20 25 30

Val Ser Leu Gly Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser
35 40 45

Ile Leu Phe Phe Ser Asn Asn Lys Asn Tyr Leu Ala Trp Tyr Arg Gln
50 55 60

Lys Pro Gly Gln Pro Pro Asn Leu Leu Ile Tyr Trp Ala Ser Thr Arg

[0021]

65 70 75 80

Glu Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp
85 90 95

Phe Thr Leu Thr Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr
100 105 110

Tyr Cys Gln Gln Tyr Tyr Ser Ser Pro Trp Thr Phe Gly Gln Gly Thr
115 120 125

Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe
130 135 140

Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys
145 150 155 160

Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val
165 170 175

Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln
180 185 190

Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser
195 200 205

Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His
210 215 220

Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225 230 235 240

<210> 25
<211> 1380
<212> DNA
<213> Homo sapiens

<400> 25
atggagtttg ggctgagctg ggttttcctt gttgctatta taaaaggtgt ccagtgtcag 60

gtgcagctgg tggagtctgg gggaggcttg gtcaagcctg gagggtccct gagactctcc 120

tgtgcagcct ctggattcac cttcagtgac tactatatga gctggatccg ccaggctcca 180

gggaagggac tggagtgggt ttcatacatt agtagtagtg gtagtaccat atactacgca 240

gactctgtga agggccgatt caccatctcc agggacaacg ccaagaactc actgtatctg 300

caaatgaaca gcctgagagc cgaggacacg gccgtgtatt actgtgcgag aggcctaact 360

ggggactact ggggccaggg aaccctggtc accgtctcct cagcttccac caagggccca 420

tccgtcttcc ccctggcgcc ctgctctaga agcacctccg agagcacagc ggccctgggc 480

tgcctggtca aggactactt ccccgaaccg gtgacggtgt cgtggaactc aggcgctctg 540

accagcggcg tgcacacctt cccagctgtc ctacagtcct caggactcta ctccctcagc 600

agcgtggtga ccgtgccctc cagcaacttc ggcacccaga cctacacctg caacgtagat 660

[0022]

cacaagccca gcaacaccaa ggtggacaag acagttgagc gcaaatgttg tgtcgagtgc 720

ccaccgtgcc cagcaccacc tgtggcagga ccgtcagtct tcctcttccc cccaaaaccc 780

aaggacaccc tcatgatctc ccggaccccc gaggtcacgt gcgtggtggt ggacgtgagc 840

cacgaagacc ccgaggtcca gttcaactgg tacgtggacg gcgtggaggt gcataatgcc 900

aagacaaagc cacgggagga gcagttcaac agcacgttcc gtgtggtcag cgtcctcacc 960

gttgtgcacc aggactggct gaacggcaag gagtacaagt gcaaggtctc caacaaaggc 1020

ctcccagccc ccatcgagaa aaccatctcc aaaaccaaag ggcagccccg agaaccacag 1080

gtgtacaccc tgccccatc ccgggaggag atgaccaaga accaggtcag cctgacctgc 1140

ctggtcaaag gcttctaccc cagcgacatc gccgtggagt gggagagcaa tgggcagccg 1200

gagaacaact acaagaccac acctcccatg ctggactccg acggctcctt cttcctctac 1260

agcaagctca ccgtggacaa gagcaggtgg cagcagggga acgtcttctc atgctccgtg 1320

atgcatgagg ctctgcacaa ccactacacg cagaagagcc tctccctgtc tccgggtaaa 1380

<210> 26
<211> 460
<212> PRT
<213> Homo sapiens

<400> 26

Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Ile Ile Lys Gly
1 5 10 15

Val Gln Cys Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys
20 25 30

Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
35 40 45

Ser Asp Tyr Tyr Met Ser Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu
50 55 60

Glu Trp Val Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala
65 70 75 80

Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn
85 90 95

Ser Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val
100 105 110

Tyr Tyr Cys Ala Arg Gly Leu Thr Gly Asp Tyr Trp Gly Gln Gly Thr
115 120 125

Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
130 135 140

Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly
145 150 155 160

[0023]

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
165 170 175

Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
180 185 190

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
195 200 205

Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser
210 215 220

Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Cys Cys Val Glu Cys
225 230 235 240

Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe
245 250 255

Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
260 265 270

Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln Phe
275 280 285

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
290 295 300

Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu Thr
305 310 315 320

Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
325 330 335

Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr
340 345 350

Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
355 360 365

Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
370 375 380

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
385 390 395 400

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser Asp Gly Ser
405 410 415

Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
420 425 430

Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
435 440 445

[0024]

Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
450 455 460

<210> 27
<211> 708
<212> DNA
<213> Homo sapiens

<400> 27
atggacatga gggtccccgc tcagctcctg gggctcctgc tactctggct ccgaggtgcc 60

agatgtgaca tccagatgac ccagtctcca tcctccctgt ctgcatctgt cggagacaga 120

gtcaccatca cttgccggcc aagtcagatc attagcagtt tattaaattg gtatcagcag 180

aaaccaggga aagcccctaa gctcctgatc catgctgcat ccagtttgca aagtggggtc 240

ccatcaaggt tcagtggcag tggatctggg acagatttca ctctcaccat cagtagtctg 300

caacctgaag attttgcaac ttactactgt caacagagtt acagtacccc attcactttc 360

ggccctggga ccaaagtgga tatcaaacga actgtggctg caccatctgt cttcatcttc 420

ccgccatctg atgagcagtt gaaatctgga actgcctctg ttgtgtgcct gctgaataac 480

ttctatccca gagaggccaa agtacagtgg aaggtggata acgccctcca atcgggtaac 540

tcccaggaga gtgtcacaga gcaggacagc aaggacagca cctacagcct cagcagcacc 600

ctgacgctga gcaaagcaga ctacgagaaa cacaaagtct acgcctgcga agtcacccat 660

cagggcctga gctcgcccgt cacaaagagc ttcaacaggg gagagtgt 708

<210> 28
<211> 236
<212> PRT
<213> Homo sapiens

<400> 28

Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp
1 5 10 15

Leu Arg Gly Ala Arg Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Ser
20 25 30

Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Pro Ser
35 40 45

Gln Ile Ile Ser Ser Leu Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys
50 55 60

Ala Pro Lys Leu Leu Ile His Ala Ala Ser Ser Leu Gln Ser Gly Val
65 70 75 80

Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
85 90 95

Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
100 105 110

[0025]

Ser Tyr Ser Thr Pro Phe Thr Phe Gly Pro Gly Thr Lys Val Asp Ile
115 120 125

Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp
130 135 140

Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn
145 150 155 160

Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu
165 170 175

Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp
180 185 190

Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr
195 200 205

Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser
210 215 220

Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225 230 235

<210> 29
<211> 1398
<212> DNA
<213> Homo sapiens

<400> 29
atggagttgg ggctgtgctg ggttttcctt gttgctattt tagaaggtgt ccagtgtgag 60

gtgcagctgg tggagtctgg gggaggcttg gtacagcctg gggggtccct gagactctcc 120

tgtgcagcct ctggattcac cttcagtagt tttagtatga cctgggtccg ccaggctcca 180

ggaaaggggc tggagtgggt ttcatacatt agtagtagaa gtagtaccat atcctacgca 240

gactctgtga agggccgatt caccatctcc agagacaatg ccaagaactc actgtatctg 300

caaatgaaca gcctgagaga cgaggacacg gctgtgtatt actgtgcgag agatcctctt 360

ctagcgggag ctaccttctt tgactactgg ggccagggaa ccctggtcac cgtctcctca 420

gcctccacca agggcccatc ggtcttcccc ctggcgccct gctccaggag cacctccgag 480

agcacagcgg ccctgggctg cctggtcaag gactacttcc ccgaaccggt gacggtgtcg 540

tggaactcag gcgctctgac cagcggcgtg cacaccttcc cagctgtcct acagtcctca 600

ggactctact ccctcagcag cgtggtgacc gtgccctcca gcaacttcgg cacccagacc 660

tacacctgca acgtagatca caagcccagc aacaccaagg tggacaagac agttgagcgc 720

aaatgttgtg tcgagtgccc accgtgccca gcaccacctg tggcaggacc gtcagtcttc 780

ctcttccccc caaaacccaa ggacaccctc atgatctccc ggacccctga ggtcacgtgc 840

gtggtggtgg acgtgagcca cgaagacccc gaggtccagt tcaactggta cgtggacggc 900

gtggaggtgc ataatgccaa gacaaagcca cgggaggagc agttcaacag cacgttccgt 960

[0026]

gtggtcagcg tcctcaccgt tgtgcaccag gactggctga acggcaagga gtacaagtgc 1020

aaggtctcca acaaaggcct cccagccccc atcgagaaaa ccatctccaa aaccaaaggg 1080

cagccccgag aaccacaggt gtacaccctg cccccatccc gggaggagat gaccaagaac 1140

caggtcagcc tgacctgcct ggtcaaaggc ttctacccca gcgacatcgc cgtggagtgg 1200

gagagcaatg ggcagccgga gaacaactac aagaccacac ctcccatgct ggactccgac 1260

ggctccttct tcctctacag caagctcacc gtggacaaga gcaggtggca gcaggggaac 1320

gtcttctcat gctccgtgat gcatgaggct ctgcacaacc actacacgca gaagagcctc 1380

tccctgtctc cgggtaaa 1398

<210> 30
<211> 466
<212> PRT
<213> Homo sapiens

<400> 30

Met Glu Leu Gly Leu Cys Trp Val Phe Leu Val Ala Ile Leu Glu Gly
1 5 10 15

Val Gln Cys Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln
20 25 30

Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
35 40 45

Ser Ser Phe Ser Met Thr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
50 55 60

Glu Trp Val Ser Tyr Ile Ser Ser Arg Ser Ser Thr Ile Ser Tyr Ala
65 70 75 80

Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn
85 90 95

Ser Leu Tyr Leu Gln Met Asn Ser Leu Arg Asp Glu Asp Thr Ala Val
100 105 110

Tyr Tyr Cys Ala Arg Asp Pro Leu Leu Ala Gly Ala Thr Phe Phe Asp
115 120 125

Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys
130 135 140

Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu
145 150 155 160

Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro
165 170 175

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr
180 185 190

[0027]

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val
195 200 205

Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn
210 215 220

Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg
225 230 235 240

Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly
245 250 255

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
260 265 270

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
275 280 285

Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
290 295 300

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg
305 310 315 320

Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys
325 330 335

Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu
340 345 350

Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
355 360 365

Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
370 375 380

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
385 390 395 400

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met
405 410 415

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
420 425 430

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
435 440 445

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
450 455 460

Gly Lys
465

[0028]

<210> 31
<211> 705
<212> DNA
<213> Homo sapiens

<400> 31
atggaaaccc cagcgcagct tctcttcctc ctgctactct ggctcccaga taccaccgga 60

gaatttgtgt tgacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc 120

ctctcctgca gggccagtca gagtgttagc agcagttact tagcctggta ccagcagaaa 180

cctggccagg ctcccaggct cctcatctat ggtgcatcca gcagggccac tggcatccca 240

gacaggttca gtggcagtgg gtctgggaca gacttcactc tcaccatcag cagactggag 300

cctgaagatt ttgcagtgta ttactgtcag cagtatggta gctcacctct cactttcggc 360

ggagggacca aggtggagat caaacgaact gtggctgcac catctgtctt catcttcccg 420

ccatctgatg agcagttgaa atctggaact gcctctgttg tgtgcctgct gaataacttc 480

tatcccagag aggccaaagt acagtggaag gtggataacg ccctccaatc gggtaactcc 540

caggagagtg tcacagagca ggacagcaag gacagcacct acagcctcag cagcaccctg 600

acgctgagca aagcagacta cgagaaacac aaagtctacg cctgcgaagt cacccatcag 660

ggcctgagct cgcccgtcac aaagagcttc aacaggggag agtgt 705

<210> 32
<211> 235
<212> PRT
<213> Homo sapiens

<400> 32

Met Glu Thr Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
1 5 10 15

Asp Thr Thr Gly Glu Phe Val Leu Thr Gln Ser Pro Gly Thr Leu Ser
20 25 30

Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser
35 40 45

Val Ser Ser Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala
50 55 60

Pro Arg Leu Leu Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro
65 70 75 80

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
85 90 95

Ser Arg Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr
100 105 110

Gly Ser Ser Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
115 120 125

[0029]

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
130 135 140

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
145 150 155 160

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
165 170 175

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
180 185 190

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
195 200 205

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
210 215 220

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225 230 235

<210> 33
<211> 1383
<212> DNA
<213> Homo sapiens

<400> 33
atggagtttg ggctgagctg ggttttcctt gttgctatta taaaaggtgt ccagtgtcag 60

gtgcagctgg tggagtctgg gggaggcttg gtcaagcctg gagggtccct gagactctcc 120

tgtgcagcct ctggattcac cttcagtgac tactacatga gctggatccg ccaggctcca 180

gggaaggggc tggagtgggt ttcatacatt agtagtagtg gtagtaccat atactacgca 240

gactctgtga agggccgatt caccatctcc agggacaacg ccaagaattc actgtatctg 300

caaatgaaca gcctgagagc cgaggacacg gccgtgtatt actgtgcgag gcgtatagga 360

ggtatggacg tctggggcca agggaccacg gtcaccgtct cctcagcttc caccaagggc 420

ccatccgtct tccccctggc gccctgctct agaagcacct ccgagagcac agcggccctg 480

ggctgcctgg tcaaggacta cttccccgaa ccggtgacgg tgtcgtggaa ctcaggcgct 540

ctgaccagcg gcgtgcacac cttcccagct gtcctacagt cctcaggact ctactccctc 600

agcagcgtgg tgaccgtgcc ctccagcaac ttcggcaccc agacctacac ctgcaacgta 660

gatcacaagc ccagcaacac caaggtggac aagacagttg agcgcaaatg ttgtgtcgag 720

tgcccaccgt gcccagcacc acctgtggca ggaccgtcag tcttcctctt cccccccaaaa 780

cccaaggaca ccctcatgat ctcccggacc cctgaggtca cgtgcgtggt ggtggacgtg 840

agccacgaag accccgaggt ccagttcaac tggtacgtgg acggcgtgga ggtgcataat 900

gccaagacaa agccacggga ggagcagttc aacagcacgt tccgtgtggt cagcgtcctc 960

accgttgtgc accaggactg gctgaacggc aaggagtaca agtgcaaggt ctccaacaaa 1020

ggcctcccag ccccatcga gaaaaccatc tccaaaacca aagggcagcc ccgagaacca 1080

[0030]

caggtgtaca ccctgccccc atcccgggag gagatgacca agaaccaggt cagcctgacc 1140

tgcctggtca aaggcttcta ccccagcgac atcgccgtgg agtgggagag caatgggcag 1200

ccggagaaca actacaagac cacacctccc atgctggact ccgacggctc cttcttcctc 1260

tacagcaagc tcaccgtgga caagagcagg tggcagcagg ggaacgtctt ctcatgctcc 1320

gtgatgcatg aggctctgca caaccactac acgcagaaga gcctctccct gtctccgggt 1380

aaa 1383

<210> 34
<211> 461
<212> PRT
<213> Homo sapiens

<400> 34

Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Ile Ile Lys Gly
1 5 10 15

Val Gln Cys Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys
20 25 30

Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
35 40 45

Ser Asp Tyr Tyr Met Ser Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu
50 55 60

Glu Trp Val Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala
65 70 75 80

Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn
85 90 95

Ser Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val
100 105 110

Tyr Tyr Cys Ala Arg Arg Ile Gly Gly Met Asp Val Trp Gly Gln Gly
115 120 125

Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
130 135 140

Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu
145 150 155 160

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
165 170 175

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
180 185 190

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
195 200 205

[0031]

Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys Pro
210 215 220

Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Cys Cys Val Glu
225 230 235 240

Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu
245 250 255

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
260 265 270

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln
275 280 285

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
290 295 300

Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu
305 310 315 320

Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
325 330 335

Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
340 345 350

Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
355 360 365

Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
370 375 380

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
385 390 395 400

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser Asp Gly
405 410 415

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
420 425 430

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
435 440 445

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
450 455 460

<210> 35
<211> 708
<212> DNA
<213> Homo sapiens

<400> 35

[0032]

atggacatga gggtccccgc tcagctcctg gggctcctgc tactctggct ccgaggtgcc 60

agatgtgaca tccagatgac ccagtctcca tcctccctgt ctgcatctgt aggagacaga 120

gtcaccatca cttgccgggc aagtcagagc attagcggct ttttaatttg gtatcagcag 180

agaccaggga aagccctaa gctcctgatc tatgctacat ccagtttaca aagtggggtc 240

ccatcaaggt tcagtggcag tggatctggg acagatttca ctctcaccat cagcagtctg 300

caacctgaag attttgcaac ttactactgt caacagagtt acagtacccc attcactttc 360

ggccctggga ccaaagtgga tatcaaacga actgtggctg caccatctgt cttcatcttc 420

ccgccatctg atgagcagtt gaaatctgga actgcctctg ttgtgtgcct gctgaataac 480

ttctatccca gagaggccaa agtacagtgg aaggtggata acgccctcca atcgggtaac 540

tcccaggaga gtgtcacaga gcaggacagc aaggacagca cctacagcct cagcagcacc 600

ctgacgctga gcaaagcaga ctacgagaaa cacaaagtct acgcctgcga agtcacccat 660

cagggcctga gctcgcccgt cacaaagagc ttcaacaggg gagagtgt 708

<210> 36
<211> 236
<212> PRT
<213> Homo sapiens

<400> 36

Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp
1 5 10 15

Leu Arg Gly Ala Arg Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Ser
20 25 30

Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser
35 40 45

Gln Ser Ile Ser Gly Phe Leu Ile Trp Tyr Gln Gln Arg Pro Gly Lys
50 55 60

Ala Pro Lys Leu Leu Ile Tyr Ala Thr Ser Ser Leu Gln Ser Gly Val
65 70 75 80

Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
85 90 95

Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
100 105 110

Ser Tyr Ser Thr Pro Phe Thr Phe Gly Pro Gly Thr Lys Val Asp Ile
115 120 125

Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp
130 135 140

Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn
145 150 155 160

[0033]

Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu
165 170 175

Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp
180 185 190

Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr
195 200 205

Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser
210 215 220

Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225 230 235

<210> 37
<211> 1383
<212> DNA
<213> Homo sapiens

<400> 37
atggagtttg ggctgagctg ggttttcctt gttgctatta taaaaggtgt ccagtgtcag 60

gtgcagctgg tggagtctgg gggaggcttg gtcaagcctg gagggtccct gagactctcc 120

tgtgcagcct ctggattcac cttcagtgac tactatatga gctggatccg ccaggctcca 180

gggaagggac tggagtgggt ttcatacatt agtagtagtg gtagtaccat atactacgca 240

gactctgtga agggccgatt caccatctcc agggacaacg ccaagaactc actgtatctg 300

caaatgaaca gcctgagagc cgaggacacg gccgtgtatt actgtgcgag aggcctaact 360

ggggactact ggggccaggg aaccctggtc accgtctcct cagcttccac caagggccca 420

tccgtcttcc ccctggcgcc ctgctctaga agcacctccg agagcacagc ggccctgggc 480

tgcctggtca aggactactt ccccgaaccg gtgacggtgt cgtggaactc aggcgctctg 540

accagcggcg tgcacacctt cccagctgtc ctacagtcct caggactcta ctccctcagc 600

agcgtggtga ccgtgccctc cagcagcttg ggcacgaaga cctacacctg caacgtagat 660

cacaagccca gcaacaccaa ggtggacaag agagttgagt ccaaatatgg tcccccatgc 720

ccatcatgcc cagcacctga gttcctgggg ggaccatcag tcttcctgtt cccccccaaaa 780

cccaaggaca ctctcatgat ctcccggacc cctgaggtca cgtgcgtggt ggtggacgtg 840

agccaggaag accccgaggt ccagttcaac tggtacgtgg atggcgtgga ggtgcataat 900

gccaagacaa agccgcggga ggagcagttc aacagcacgt accgtgtggt cagcgtcctc 960

accgtcctgc accaggactg gctgaacggc aaggagtaca agtgcaaggt ctccaacaaa 1020

ggcctcccgt cctccatcga gaaaaccatc tccaaagcca aagggcagcc ccgagagcca 1080

caggtgtaca ccctgccccc atcccaggag gagatgacca agaaccaggt cagcctgacc 1140

tgcctggtca aaggcttcta ccccagcgac atcgccgtgg agtgggagag caatgggcag 1200

ccggagaaca actacaagac cacgcctccc gtgctggact ccgacggctc cttcttcctc 1260

tacagcaggc taaccgtgga caagagcagg tggcaggagg ggaatgtctt ctcatgctcc 1320

[0034]

gtgatgcatg aggctctgca caaccactac acacagaaga gcctctccct gtctccgggt 1380

aaa 1383

<210> 38
<211> 461
<212> PRT
<213> Homo sapiens

<400> 38

Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Ile Ile Lys Gly
1 5 10 15

Val Gln Cys Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys
20 25 30

Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
35 40 45

Ser Asp Tyr Tyr Met Ser Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu
50 55 60

Glu Trp Val Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala
65 70 75 80

Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn
85 90 95

Ser Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val
100 105 110

Tyr Tyr Cys Ala Arg Gly Leu Thr Gly Asp Tyr Trp Gly Gln Gly Thr
115 120 125

Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
130 135 140

Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly
145 150 155 160

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
165 170 175

Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
180 185 190

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
195 200 205

Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser
210 215 220

Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys
225 230 235 240

[0035]

Pro Ser Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu
245 250 255

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
260 265 270

Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln
275 280 285

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
290 295 300

Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu
305 310 315 320

Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
325 330 335

Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys
340 345 350

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
355 360 365

Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
370 375 380

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
385 390 395 400

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
405 410 415

Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln
420 425 430

Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
435 440 445

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
450 455 460

<210> 39

<400> 39
000

<210> 40

<400> 40
000

<210> 41

<400> 41
000

[0036]

<210> 42

<400> 42
000

<210> 43
<211> 705
<212> DNA
<213> Homo sapiens

<400> 43
atggaaaccc cagcgcagct tctcttcctc ctgctactct ggctcccaga taccaccgga 60

gaatttgtgt tgacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc 120

ctctcctgca gggccagtca gagtgttagc agcagttact tagcctggta ccagcagaaa 180

cctggccagg ctcccaggct cctcatctat ggtgcatcca gcagggccac tggcatccca 240

gacaggttca gtggcagtgg gtctgggaca gacttcactc tcaccatcag cagactggag 300

cctgaagatt ttgtagtgta ttactgtcag cagtatggta gctcacctct cactttcggc 360

ggagggacca aggtggagat caaacgaact gtggctgcac catctgtctt catcttcccg 420

ccatctgatg agcagttgaa atctggaact gcctctgttg tgtgcctgct gaataacttc 480

tatcccagag aggccaaagt acagtggaag gtggataacg ccctccaatc gggtaactcc 540

caggagagtg tcacagagca ggacagcaag gacagcacct acagcctcag cagcaccctg 600

acgctgagca aagcagacta cgagaaacac aaagtctacg cctgcgaagt cacccatcag 660

ggcctgagct cgcccgtcac aaagagcttc aacaggggag agtgt 705

<210> 44
<211> 235
<212> PRT
<213> Homo sapiens

<400> 44

Met Glu Thr Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
1 5 10 15

Asp Thr Thr Gly Glu Phe Val Leu Thr Gln Ser Pro Gly Thr Leu Ser
20 25 30

Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser
35 40 45

Val Ser Ser Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala
50 55 60

Pro Arg Leu Leu Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro
65 70 75 80

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
85 90 95

Ser Arg Leu Glu Pro Glu Asp Phe Val Val Tyr Tyr Cys Gln Gln Tyr
100 105 110

[0037]

Gly Ser Ser Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
115 120 125

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
130 135 140

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
145 150 155 160

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
165 170 175

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
180 185 190

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
195 200 205

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
210 215 220

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225 230 235

<210> 45
<211> 1386
<212> DNA
<213> Homo sapiens

<400> 45
atggagtttg ggctgagctg ggttttcctt gttgctatta taaaaggtgt ccagtgtcag 60

gtgcagctgg tggagtctgg gggaggcttg gtcaagcctg gagggtccct gagactctcc 120

tgtgcagcct ctggattcac cttcagtgac tactacatga gctggatccg ccaggctcca 180

gggaaggggc tggagtgggt ttcatacatt agtagtagtg gtagtaccat atactacgca 240

gactctgtga agggccgatt caccatctcc agggacaacg ccaagaattc actgtatctg 300

caaatgaaca gcctgagagc cgaggacacg gccgtgtatt actgtgcgag gcgtatagga 360

ggtatggacg tctggggcca agggaccacg gtcaccgtct cctcagcttc caccaagggc 420

ccatccgtct tccccctggc gccctgctct agaagcacct ccgagagcac agcggccctg 480

ggctgcctgg tcaaggacta cttccccgaa ccggtgacgg tgtcgtggaa ctcaggcgct 540

ctgaccagcg gcgtgcacac cttcccagct gtcctacagt cctcaggact ctactccctc 600

agcagcgtgg tgaccgtgcc ctccagcagc ttgggcacga agacctacac ctgcaacgta 660

gatcacaagc ccagcaacac caaggtggac aagagagttg agtccaaata tggtccccca 720

tgcccatcat gcccagcacc tgagttcctg gggggaccat cagtcttcct gttccccccca 780

aaacccaagg acactctcat gatctcccgg acccctgagg tcacgtgcgt ggtggtggac 840

gtgagccagg aagaccccga ggtccagttc aactggtacg tggatggcgt ggaggtgcat 900

aatgccaaga caaagccgcg ggaggagcag ttcaacagca cgtaccgtgt ggtcagcgtc 960

[0038]

ctcaccgtcc tgcaccagga ctggctgaac ggcaaggagt acaagtgcaa ggtctccaac 1020

aaaggcctcc cgtcctccat cgagaaaacc atctccaaag ccaaagggca gccccgagag 1080

ccacaggtgt acaccctgcc cccatcccag gaggagatga ccaagaacca ggtcagcctg 1140

acctgcctgg tcaaaggctt ctaccccagc gacatcgccg tggagtggga gagcaatggg 1200

cagccggaga acaactacaa gaccacgcct cccgtgctgg actccgacgg ctccttcttc 1260

ctctacagca ggctaaccgt ggacaagagc aggtggcagg aggggaatgt cttctcatgc 1320

tccgtgatgc atgaggctct gcacaaccac tacacacaga agagcctctc cctgtctccg 1380

ggtaaa 1386

<210> 16
<211> 462
<212> PRT
<213> Homo sapiens

<400> 46

Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Ile Ile Lys Gly
1 5 10 15

Val Gln Cys Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys
20 25 30

Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
35 40 45

Ser Asp Tyr Tyr Met Ser Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu
50 55 60

Glu Trp Val Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala
65 70 75 80

Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn
85 90 95

Ser Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val
100 105 110

Tyr Tyr Cys Ala Arg Arg Ile Gly Gly Met Asp Val Trp Gly Gln Gly
115 120 125

Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
130 135 140

Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu
145 150 155 160

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
165 170 175

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
180 185 190

[0039]

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
195 200 205

Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro
210 215 220

Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro
225 230 235 240

Cys Pro Ser Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val Phe
245 250 255

Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro
260 265 270

Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val
275 280 285

Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
290 295 300

Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val
305 310 315 320

Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
325 330 335

Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser
340 345 350

Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
355 360 365

Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
370 375 380

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
385 390 395 400

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
405 410 415

Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp
420 425 430

Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
435 440 445

Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
450 455 460

<210> 47
<211> 708

[0040]

<212> DNA
<213> Homo sapiens

<400> 47
atggacatga gggtccccgc tcagctcctg gggctcctgc tactctggct ccgaggtgcc 60

agatgtgaca tccagatgac ccagtctcca tcctccctgt ctgcatctgt aggagacaga 120

gtcaccatca cttgccgggc aagtcagagc attagcggct ttttaatttg gtatcagcag 180

agaccaggga aagccctaa gctcctgatc tatgctacat ccagtttaca aagtggggtc 240

ccattaaggt tcagtggcag tgaatctggg acagatttca ctctcaccat cagcagtctg 300

caacctgaag attttgcaac ttactactgt caacagagtt acagtacccc attcactttc 360

ggccctggga ccaaagtgga tatcaaacga actgtggctg caccatctgt cttcatcttc 420

ccgccatctg atgagcagtt gaaatctgga actgcctctg ttgtgtgcct gctgaataac 480

ttctatccca gagaggccaa agtacagtgg aaggtggata acgccctcca atcgggtaac 540

tcccaggaga gtgtcacaga gcaggacagc aaggacagca cctacagcct cagcagcacc 600

ctgacgctga gcaaagcaga ctacgagaaa cacaaagtct acgcctgcga agtcacccat 660

cagggcctga gctcgcccgt cacaaagagc ttcaacaggg gagagtgt 708

<210> 48
<211> 236
<212> PRT
<213> Homo sapiens

<400> 48

Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp
1 5 10 15

Leu Arg Gly Ala Arg Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Ser
20 25 30

Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser
35 40 45

Gln Ser Ile Ser Gly Phe Leu Ile Trp Tyr Gln Gln Arg Pro Gly Lys
50 55 60

Ala Pro Lys Leu Leu Ile Tyr Ala Thr Ser Ser Leu Gln Ser Gly Val
65 70 75 80

Pro Leu Arg Phe Ser Gly Ser Glu Ser Gly Thr Asp Phe Thr Leu Thr
85 90 95

Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
100 105 110

Ser Tyr Ser Thr Pro Phe Thr Phe Gly Pro Gly Thr Lys Val Asp Ile
115 120 125

Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp
130 135 140

[0041]

```
Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn
145                 150                 155                 160

Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu
                165                 170                 175

Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp
            180                 185                 190

Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr
        195                 200                 205

Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser
    210                 215                 220

Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225                 230                 235
```

<210> 49

<400> 49
000

<210> 50
<211> 462
<212> PRT
<213> Homo sapiens

<400> 50

```
Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Ile Ile Lys Gly
1               5                   10                  15

Val Gln Cys Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys
            20                  25                  30

Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
        35                  40                  45

Ser Asp Tyr Tyr Met Ser Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu
    50                  55                  60

Glu Trp Val Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala
65                  70                  75                  80

Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn
                85                  90                  95

Ser Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val
            100                 105                 110

Tyr Tyr Cys Ala Ile Arg Ile Gly Gly Met Asp Val Trp Gly Gln Gly
        115                 120                 125

Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
    130                 135                 140
```

[0042]

Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu
145 150 155 160

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
165 170 175

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
180 185 190

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
195 200 205

Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro
210 215 220

Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro
225 230 235 240

Cys Pro Pro Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val Phe
245 250 255

Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro
260 265 270

Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val
275 280 285

Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
290 295 300

Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val
305 310 315 320

Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
325 330 335

Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser
340 345 350

Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
355 360 365

Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
370 375 380

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
385 390 395 400

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
405 410 415

Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp

[0043]

420 425 430

Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
435 440 445

Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
450 455 460

<210> 51

<400> 51
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<210> 52
<211> 236
<212> PRT
<213> Homo sapiens

<400> 52

Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp
1 5 10 15

Leu Arg Gly Ala Arg Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Ser
20 25 30

Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser
35 40 45

Gln Ser Ile Ser Gly Phe Leu Ile Trp Tyr Gln Gln Lys Pro Gly Lys
50 55 60

Ala Pro Lys Leu Leu Ile Tyr Ala Thr Ser Ser Leu Gln Ser Gly Val
65 70 75 80

Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
85 90 95

Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
100 105 110

Ser Tyr Ser Thr Pro Phe Thr Phe Gly Pro Gly Thr Lys Val Asp Ile
115 120 125

Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp
130 135 140

Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn
145 150 155 160

Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu
165 170 175

Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp
180 185 190

[0044]

Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr
195 200 205

Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser
210 215 220

Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225 230 235

<210> 53

<400> 53
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<210> 54
<211> 461
<212> PRT
<213> Homo sapiens

<400> 54

Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Ile Ile Lys Gly
1 5 10 15

Val Gln Cys Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys
20 25 30

Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
35 40 45

Ser Asp Tyr Tyr Met Ser Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu
50 55 60

Glu Trp Val Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala
65 70 75 80

Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn
85 90 95

Ser Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val
100 105 110

Tyr Tyr Cys Ala Arg Gly Leu Thr Gly Asp Tyr Trp Gly Gln Gly Thr
115 120 125

Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
130 135 140

Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly
145 150 155 160

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
165 170 175

Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
180 185 190

[0045]

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
195 200 205

Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser
210 215 220

Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys
225 230 235 240

Pro Pro Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu
245 250 255

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
260 265 270

Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln
275 280 285

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
290 295 300

Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu
305 310 315 320

Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
325 330 335

Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys
340 345 350

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
355 360 365

Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
370 375 380

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
385 390 395 400

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
405 410 415

Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln
420 425 430

Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
435 440 445

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
450 455 460

<210> 55

[0046]

<400> 55
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<210> 56
<211> 236
<212> PRT
<213> Homo sapiens

<400> 56

Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp
1 5 10 15

Leu Arg Gly Ala Arg Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Ser
20 25 30

Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Pro Ser
35 40 45

Gln Ile Ile Ser Ser Leu Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys
50 55 60

Ala Pro Lys Leu Leu Ile Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val
65 70 75 80

Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
85 90 95

Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
100 105 110

Ser Tyr Ser Thr Pro Phe Thr Phe Gly Pro Gly Thr Lys Val Asp Ile
115 120 125

Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp
130 135 140

Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn
145 150 155 160

Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu
165 170 175

Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp
180 185 190

Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr
195 200 205

Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser
210 215 220

Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225 230 235

<210> 57

[0047]

<400> 57
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<210> 58
<211> 467
<212> PRT
<213> Homo sapiens

<400> 58

Met Glu Leu Gly Leu Cys Trp Val Phe Leu Val Ala Ile Leu Glu Gly
1 5 10 15

Val Gln Cys Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln
20 25 30

Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
35 40 45

Ser Ser Phe Ser Met Thr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
50 55 60

Glu Trp Val Ser Tyr Ile Ser Ser Arg Ser Ser Thr Ile Ser Tyr Ala
65 70 75 80

Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn
85 90 95

Ser Leu Tyr Leu Gln Met Asn Ser Leu Arg Asp Glu Asp Thr Ala Val
100 105 110

Tyr Tyr Cys Ala Arg Asp Pro Leu Leu Ala Gly Ala Thr Phe Phe Asp
115 120 125

Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys
130 135 140

Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu
145 150 155 160

Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro
165 170 175

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr
180 185 190

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val
195 200 205

Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn
210 215 220

Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser
225 230 235 240

[0048]

Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe Leu Gly
245 250 255

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
260 265 270

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln
275 280 285

Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val
290 295 300

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr
305 310 315 320

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
325 330 335

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile
340 345 350

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
355 360 365

Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser
370 375 380

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
385 390 395 400

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
405 410 415

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val
420 425 430

Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met
435 440 445

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
450 455 460

Pro Gly Lys
465

<210> 59

<400> 59
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<210> 60
<211> 235
<212> PRT
<213> Homo sapiens

<400> 60

[0049]

Met Glu Thr Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
1 5 10 15

Asp Thr Thr Gly Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser
20 25 30

Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser
35 40 45

Val Ser Ser Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala
50 55 60

Pro Arg Leu Leu Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro
65 70 75 80

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
85 90 95

Ser Arg Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr
100 105 110

Gly Ser Ser Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
115 120 125

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
130 135 140

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
145 150 155 160

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
165 170 175

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
180 185 190

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
195 200 205

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
210 215 220

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225 230 235

<210> 61

<400> 61
000

<210> 62
<211> 466
<212> PRT
<213> Homo sapiens

[0050]

<400> 62

Met Glu Leu Gly Leu Cys Trp Val Phe Leu Val Ala Ile Leu Glu Gly
1 5 10 15

Val Gln Cys Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln
20 25 30

Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
35 40 45

Ser Ser Phe Ser Met Thr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
50 55 60

Glu Trp Val Ser Tyr Ile Ser Ser Arg Ser Ser Thr Ile Ser Tyr Ala
65 70 75 80

Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn
85 90 95

Ser Leu Tyr Leu Gln Met Asn Ser Leu Arg Asp Glu Asp Thr Ala Val
100 105 110

Tyr Tyr Cys Ala Arg Asp Pro Leu Leu Ala Gly Ala Thr Phe Phe Asp
115 120 125

Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys
130 135 140

Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu
145 150 155 160

Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro
165 170 175

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr
180 185 190

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val
195 200 205

Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn
210 215 220

Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg
225 230 235 240

Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly
245 250 255

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
260 265 270

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu

[0051]

275 280 285

Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
290 295 300

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg
305 310 315 320

Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys
325 330 335

Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu
340 345 350

Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
355 360 365

Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
370 375 380

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
385 390 395 400

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met
405 410 415

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
420 425 430

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
435 440 445

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
450 455 460

Gly Lys
465

<210> 63

<400> 63
000

<210> 64

<400> 64
000

<210> 65

<400> 65
000

<210> 66
<211> 461
<212> PRT
<213> Homo sapiens

[0052]

<400> 66

Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Ile Ile Lys Gly
1 5 10 15

Val Gln Cys Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys
20 25 30

Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
35 40 45

Ser Asp Tyr Tyr Met Ser Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu
50 55 60

Glu Trp Val Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala
65 70 75 80

Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn
85 90 95

Ser Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val
100 105 110

Tyr Tyr Cys Ala Ile Arg Ile Gly Gly Met Asp Val Trp Gly Gln Gly
115 120 125

Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
130 135 140

Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu
145 150 155 160

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
165 170 175

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
180 185 190

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
195 200 205

Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys Pro
210 215 220

Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Cys Cys Val Glu
225 230 235 240

Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu
245 250 255

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
260 265 270

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln

[0053]

275 280 285

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
290 295 300

Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu
305 310 315 320

Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
325 330 335

Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
340 345 350

Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
355 360 365

Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
370 375 380

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
385 390 395 400

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser Asp Gly
405 410 415

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
420 425 430

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
435 440 445

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
450 455 460

<210> 67

<400> 67
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<210> 68

<400> 68
000

<210> 69

<400> 69
000

<210> 70
<211> 461
<212> PRT
<213> Homo sapiens

<400> 70

Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Ile Ile Lys Gly
1 5 10 15

[0054]

Val Gln Cys Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys
20 25 30

Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
35 40 45

Ser Asp Tyr Tyr Met Ser Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu
50 55 60

Glu Trp Val Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala
65 70 75 80

Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn
85 90 95

Ser Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val
100 105 110

Tyr Tyr Cys Ala Arg Ile Gly Gly Met Asp Val Trp Gly Gln Gly Thr
115 120 125

Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
130 135 140

Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly
145 150 155 160

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
165 170 175

Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
180 185 190

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
195 200 205

Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser
210 215 220

Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys
225 230 235 240

Pro Ser Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu
245 250 255

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
260 265 270

Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln
275 280 285

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys

[0055]

290 295 300

Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu
305 310 315 320

Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
325 330 335

Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys
340 345 350

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
355 360 365

Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
370 375 380

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
385 390 395 400

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
405 410 415

Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln
420 425 430

Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
435 440 445

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
450 455 460

<210> 71

<400> 71
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<210> 72

<400> 72
000

<210> 73

<400> 73
000

<210> 74
<211> 460
<212> PRT
<213> Homo sapiens

<400> 74

Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Ile Ile Lys Gly
1 5 10 15

Val Gln Cys Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys
20 25 30

[0056]

Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
35 40 45

Ser Asp Tyr Tyr Met Ser Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu
50 55 60

Glu Trp Val Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala
65 70 75 80

Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn
85 90 95

Ser Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val
100 105 110

Tyr Tyr Cys Ala Arg Gly Leu Thr Gly Asp Tyr Trp Gly Gln Gly Thr
115 120 125

Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
130 135 140

Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly
145 150 155 160

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
165 170 175

Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
180 185 190

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
195 200 205

Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser
210 215 220

Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Cys Cys Val Glu Cys
225 230 235 240

Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe
245 250 255

Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
260 265 270

Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln Phe
275 280 285

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
290 295 300

Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu Thr

[0057]

305 310 315 320

Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
325 330 335

Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr
340 345 350

Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
355 360 365

Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
370 375 380

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
385 390 395 400

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser Asp Gly Ser
405 410 415

Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
420 425 430

Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
435 440 445

Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
450 455 460

<210> 75

<400> 75
000

<210> 76

<400> 76
000

<210> 77

<400> 77
000

<210> 78
<211> 461
<212> PRT
<213> Homo sapiens

<400> 78

Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Ile Ile Lys Gly
1 5 10 15

Val Gln Cys Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys
20 25 30

Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
35 40 45

[0058]

Ser Asp Tyr Tyr Met Ser Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu
50 55 60

Glu Trp Val Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala
65 70 75 80

Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn
85 90 95

Ser Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val
100 105 110

Tyr Tyr Cys Ala Arg Gly Leu Thr Gly Asp Tyr Trp Gly Gln Gly Thr
115 120 125

Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
130 135 140

Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly
145 150 155 160

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
165 170 175

Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
180 185 190

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
195 200 205

Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser
210 215 220

Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys
225 230 235 240

Pro Ser Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu
245 250 255

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
260 265 270

Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln
275 280 285

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
290 295 300

Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu
305 310 315 320

Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys

[0059]

325 330 335

Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys
340 345 350

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
355 360 365

Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
370 375 380

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
385 390 395 400

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
405 410 415

Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln
420 425 430

Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
435 440 445

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
450 455 460

<210> 79

<400> 79
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<210> 80

<400> 80
000

<210> 81

<400> 81
000

<210> 82
<211> 461
<212> PRT
<213> Homo sapiens

<400> 82

Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Ile Ile Lys Gly
1 5 10 15

Val Gln Cys Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys
20 25 30

Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
35 40 45

Ser Asp Tyr Tyr Met Ser Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu
50 55 60

[0060]

Glu Trp Val Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala
65 70 75 80

Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn
85 90 95

Ser Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val
100 105 110

Tyr Tyr Cys Ala Arg Gly Leu Thr Gly Asp Tyr Trp Gly Gln Gly Thr
115 120 125

Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
130 135 140

Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly
145 150 155 160

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
165 170 175

Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
180 185 190

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
195 200 205

Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser
210 215 220

Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys
225 230 235 240

Pro Pro Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu
245 250 255

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
260 265 270

Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln
275 280 285

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
290 295 300

Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu
305 310 315 320

Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
325 330 335

Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys

[0061]

340 345 350

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
355 360 365

Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
370 375 380

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
385 390 395 400

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
405 410 415

Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln
420 425 430

Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
435 440 445

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
450 455 460

<210> 83

<400> 83
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<210> 84

<400> 84
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<210> 85

<400> 85
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<210> 86
<211> 462
<212> PRT
<213> Homo sapiens

<400> 86

Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Ile Ile Lys Gly
1 5 10 15

Val Gln Cys Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys
20 25 30

Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
35 40 45

Ser Asp Tyr Tyr Met Ser Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu
50 55 60

Glu Trp Val Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala
65 70 75 80

[0062]

Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn
85 90 95

Ser Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val
100 105 110

Tyr Tyr Cys Ala Arg Arg Ile Gly Gly Met Asp Val Trp Gly Gln Gly
115 120 125

Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
130 135 140

Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu
145 150 155 160

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
165 170 175

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
180 185 190

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
195 200 205

Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro
210 215 220

Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro
225 230 235 240

Cys Pro Pro Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val Phe
245 250 255

Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro
260 265 270

Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val
275 280 285

Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
290 295 300

Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val
305 310 315 320

Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
325 330 335

Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser
340 345 350

Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro

[0063]

355 360 365

Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
370 375 380

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
385 390 395 400

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
405 410 415

Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp
420 425 430

Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
435 440 445

Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
450 455 460

<210> 87

<400> 87
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<210> 88

<400> 88
000

<210> 89

<400> 89
000

<210> 90
<211> 467
<212> PRT
<213> Homo sapiens

<400> 90

Met Glu Leu Gly Leu Cys Trp Val Phe Leu Val Ala Ile Leu Glu Gly
1 5 10 15

Val Gln Cys Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln
20 25 30

Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
35 40 45

Ser Ser Phe Ser Met Thr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
50 55 60

Glu Trp Val Ser Tyr Ile Ser Ser Arg Ser Ser Thr Ile Ser Tyr Ala
65 70 75 80

Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn
85 90 95

[0064]

Ser Leu Tyr Leu Gln Met Asn Ser Leu Arg Asp Glu Asp Thr Ala Val
100 105 110

Tyr Tyr Cys Ala Arg Asp Pro Leu Leu Ala Gly Ala Thr Phe Phe Asp
115 120 125

Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys
130 135 140

Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu
145 150 155 160

Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro
165 170 175

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr
180 185 190

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val
195 200 205

Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn
210 215 220

Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser
225 230 235 240

Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe Leu Gly
245 250 255

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
260 265 270

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln
275 280 285

Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val
290 295 300

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr
305 310 315 320

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
325 330 335

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile
340 345 350

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
355 360 365

Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser

[0065]

370 375 380

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
385 390 395 400

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
405 410 415

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val
420 425 430

Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met
435 440 445

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
450 455 460

Pro Gly Lys
465

<210> 91

<400> 91
000

<210> 92

<400> 92
000

<210> 93

<400> 93
000

<210> 94
<211> 467
<212> PRT
<213> Homo sapiens

<400> 94

Met Glu Leu Gly Leu Cys Trp Val Phe Leu Val Ala Ile Leu Glu Gly
1 5 10 15

Val Gln Cys Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln
20 25 30

Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
35 40 45

Ser Ser Phe Ser Met Thr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
50 55 60

Glu Trp Val Ser Tyr Ile Ser Ser Arg Ser Ser Thr Ile Ser Tyr Ala
65 70 75 80

Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn
85 90 95

[0066]

Ser Leu Tyr Leu Gln Met Asn Ser Leu Arg Asp Glu Asp Thr Ala Val
100 105 110

Tyr Tyr Cys Ala Arg Asp Pro Leu Leu Ala Gly Ala Thr Phe Phe Asp
115 120 125

Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys
130 135 140

Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu
145 150 155 160

Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro
165 170 175

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr
180 185 190

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val
195 200 205

Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn
210 215 220

Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser
225 230 235 240

Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro Ala Pro Glu Phe Leu Gly
245 250 255

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
260 265 270

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln
275 280 285

Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val
290 295 300

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr
305 310 315 320

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
325 330 335

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile
340 345 350

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
355 360 365

Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser

[0067]

370 375 380

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
385 390 395 400

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
405 410 415

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val
420 425 430

Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met
435 440 445

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
450 455 460

Pro Gly Lys
465

<210> 95

<400> 95
000

<210> 96

<400> 96
000

<210> 97
<211> 1413
<212> DNA
<213> Homo sapiens

<400> 97
atggagttgg ggctgagctg ggttttcctt gttgctatta taaaaggtgt ccagtgtgag 60

gtgcagctgg tggagtctgg gggaggcttg gtacagcctg gggggtccct gagactctcc 120

tgtgcagcct ctggattcac cttcagtagt tttagtatga cctgggtccg ccaggctcca 180

ggaaaggggc tggagtgggt ttcatacatt agtagtagaa gtagtaccat atcctacgca 240

gactctgtga agggccgatt caccatctcc agagacaatg ccaagaactc actgtatctg 300

caaatgaaca gcctgagaga cgaggacacg gctgtgtatt actgtgcgag agatcctctt 360

ctagcgggag ctaccttctt tgactactgg ggccagggaa ccctggtcac cgtctcctca 420

gcctccacca agggcccatc ggtcttcccc ctggcaccct cctccaagag cacctctggg 480

ggcacagcgg ccctgggctg cctggtcaag gactacttcc ccgaaccggt gacggtgtcg 540

tggaactcag gcgccctgac cagcggcgtg cacaccttcc cggctgtcct acagtcctca 600

ggactctact ccctcagcag cgtggtgacc gtgccctcca gcagcttggg cacccagacc 660

tacatctgca acgtgaatca caagcccagc aacaccaagg tggacaagaa agttgagccc 720

aaatcttgtg acaaaactca cacatgccca ccgtgcccag cacctgaact cctgggggga 780

ccgtcagtct tcctcttccc cccaaaaccc aaggacaccc tcatgatctc ccggaccccc 840

[0068]

gaggtcacat gcgtggtggt ggacgtgagc cacgaagacc ctgaggtcaa gttcaactgg 900

tacgtggacg gcgtggaggt gcataatgcc aagacaaagc cgcgggagga gcagtacaac 960

agcacgtacc gtgtggtcag cgtcctcacc gtcctgcacc aggactggct gaatggcaag 1020

gagtacaagt gcaaggtctc caacaaagcc ctcccagccc ccatcgagaa aaccatctcc 1080

aaagccaaag ggcagccccg agaaccacag gtgtacaccc tgccccccatc ccgggatgag 1140

ctgaccaaga accaggtcag cctgacctgc ctggtcaaag gcttctatcc cagcgacatc 1200

gccgtggagt gggagagcaa tgggcagccg gagaacaact acaagaccac gcctcccgtg 1260

ctggactccg acggctcctt cttcctctac agcaagctca ccgtggacaa gagcaggtgg 1320

cagcagggga acgtcttctc atgctccgtg atgcatgagg ctctgcacaa ccactacacg 1380

cagaagagcc tctccctgtc tccgggtaaa tag 1413

<210> 98
<211> 470
<212> PRT
<213> Homo sapiens

<400> 98

Met Glu Leu Gly Leu Cys Trp Val Phe Leu Val Ala Ile Leu Glu Gly
1 5 10 15

Val Gln Cys Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln
20 25 30

Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
35 40 45

Ser Ser Phe Ser Met Thr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
50 55 60

Glu Trp Val Ser Tyr Ile Ser Ser Arg Ser Ser Thr Ile Ser Tyr Ala
65 70 75 80

Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn
85 90 95

Ser Leu Tyr Leu Gln Met Asn Ser Leu Arg Asp Glu Asp Thr Ala Val
100 105 110

Tyr Tyr Cys Ala Arg Asp Pro Leu Leu Ala Gly Ala Thr Phe Phe Asp
115 120 125

Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys
130 135 140

Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly
145 150 155 160

Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro
165 170 175

[0069]

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr
180 185 190

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val
195 200 205

Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn
210 215 220

Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro
225 230 235 240

Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu
245 250 255

Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
260 265 270

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
275 280 285

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
290 295 300

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn
305 310 315 320

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp
325 330 335

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro
340 345 350

Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu
355 360 365

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn
370 375 380

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
385 390 395 400

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
405 410 415

Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
420 425 430

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
435 440 445

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
450 455 460

[0070]

Ser Leu Ser Pro Gly Lys
465 470

<210> 99

<400> 99
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<210> 100

<400> 100
000

<210> 101
<211> 1395
<212> DNA
<213> Homo sapiens

<400> 101
atggagtttg ggctgagctg ggttttcctt gttgctatta taaaaggtgt ccagtgtcag 60

gtgcagctgg tggagtctgg gggaggcttg gtcaagcctg gagggtccct gagactctcc 120

tgtgcagcct ctggattcac cttcagtgac tactatatga gctggatccg ccaggctcca 180

gggaagggac tggagtgggt ttcatacatt agtagtagtg gtagtaccat atactacgca 240

gactctgtga agggccgatt caccatctcc agggacaacg ccaagaactc actgtatctg 300

caaatgaaca gcctgagagc cgaggacacg gccgtgtatt actgtgcgag aggcctaact 360

ggggactact ggggccaggg aaccctggtc accgtctcct cagcttccac caagggccca 420

tcggtcttcc ccctggcacc ctcctccaag agcacctctg ggggcacagc ggccctgggc 480

tgcctggtca aggactactt ccccgaaccg gtgacggtgt cgtggaactc aggcgccctg 540

accagcggcg tgcacacctt cccggctgtc ctacagtcct caggactcta ctccctcagc 600

agcgtggtga ccgtgccctc cagcagcttg ggcacccaga cctacatctg caacgtgaat 660

cacaagccca gcaacaccaa ggtggacaag aaagttgagc ccaaatcttg tgacaaaact 720

cacacatgcc caccgtgccc agcacctgaa ctcctggggg gaccgtcagt cttcctcttc 780

cccccaaaac ccaaggacac cctcatgatc tcccggaccc ctgaggtcac atgcgtggtg 840

gtggacgtga gccacgaaga ccctgaggtc aagttcaact ggtacgtgga cggcgtggag 900

gtgcataatg ccaagacaaa gccgcgggag gagcagtaca acagcacgta ccgtgtggtc 960

agcgtcctca ccgtcctgca ccaggactgg ctgaatggca aggagtacaa gtgcaaggtc 1020

tccaacaaag ccctcccagc ccccatcgag aaaaccatct ccaaagccaa agggcagccc 1080

cgagaaccac aggtgtacac cctgccccca tcccgggatg agctgaccaa gaaccaggtc 1140

agcctgacct gcctggtcaa aggcttctat cccagcgaca tcgccgtgga gtgggagagc 1200

aatgggcagc cggagaacaa ctacaagacc acgcctcccg tgctggactc cgacggctcc 1260

ttcttcctct acagcaagct caccgtggac aagagcaggt ggcagcaggg gaacgtcttc 1320

tcatgctccg tgatgcatga ggctctgcac aaccactaca cgcagaagag cctctccctg 1380

tctccgggta aatag 1395

[0071]

<210> 102
<211> 464
<212> PRT
<213> Homo sapiens

<400> 102

Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Ile Ile Lys Gly
1 5 10 15

Val Gln Cys Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys
20 25 30

Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
35 40 45

Ser Asp Tyr Tyr Met Ser Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu
50 55 60

Glu Trp Val Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala
65 70 75 80

Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn
85 90 95

Ser Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val
100 105 110

Tyr Tyr Cys Ala Arg Gly Leu Thr Gly Asp Tyr Trp Gly Gln Gly Thr
115 120 125

Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
130 135 140

Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly
145 150 155 160

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
165 170 175

Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
180 185 190

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
195 200 205

Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser
210 215 220

Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr
225 230 235 240

His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser
245 250 255

[0072]

Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
260 265 270

Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
275 280 285

Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
290 295 300

Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val
305 310 315 320

Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
325 330 335

Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
340 345 350

Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
355 360 365

Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys
370 375 380

Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
385 390 395 400

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
405 410 415

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
420 425 430

Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
435 440 445

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
450 455 460

<210> 103
<211> 107
<212> PRT
<213> Homo sapiens

<400> 103

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Ser Tyr
20 25 30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

[0073]

Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Ser Thr Pro Phe
85 90 95

Thr Phe Gly Pro Gly Thr Lys Val Asp Ile Lys
100 105

<210> 104
<211> 120
<212> PRT
<213> Homo sapiens

<400> 104

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Tyr Ser Gly Ser Tyr Tyr Tyr Phe Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ser Ala
115 120

<210> 105
<211> 119
<212> PRT
<213> Homo sapiens

<400> 105

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val

[0074]

35 40 45

Ala Asn Ile Lys Gln Asp Gly Ser Glu Lys Tyr Tyr Val Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Arg Gly Ile Ala Ala Ala Gly Tyr Phe Asp Tyr Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ser Ala
115

<210> 106
<211> 117
<212> PRT
<213> Homo sapiens

<400> 106

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Tyr Met Ser Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ala Leu Gly Gly Met Asp Val Trp Gly Gln Gly Thr Thr Val
100 105 110

Thr Val Ser Ser Ala
115

<210> 107
<211> 107
<212> PRT
<213> Homo sapiens

<400> 107

Glu Ile Val Met Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly
1 5 10 15

[0075]

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Asn
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
35 40 45

Tyr Gly Ala Ser Thr Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Ser
65 70 75 80

Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Asn Asn Trp Pro Phe
85 90 95

Thr Phe Gly Pro Gly Thr Lys Val Asp Ile Lys
100 105

<210> 108
<211> 115
<212> PRT
<213> Homo sapiens

<400> 108

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Tyr Met Ser Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Leu Thr Gly Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val
100 105 110

Ser Ser Ala
115

<210> 109
<211> 108
<212> PRT
<213> Homo sapiens

<400> 109

[0076]

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Val Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Trp
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ala Asn Ser Phe Pro Phe
85 90 95

Thr Phe Gly Pro Gly Thr Lys Val Asp Ile Lys Arg
100 105

<210> 110
<211> 117
<212> PRT
<213> Homo sapiens

<400> 110

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Val Ile Trp Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Gly Tyr Ser Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val
100 105 110

Thr Val Ser Ser Ala
115

<210> 111
<211> 118
<212> PRT

[0077]

<213> Homo sapiens

<400> 111

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30

Gly Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Ser Ala Tyr Asn Gly Asn Thr Asn Tyr Ala Gln Lys Leu
50 55 60

Gln Gly Arg Val Thr Met Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Asp Tyr Gly Gly Asn Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser Ala
115

<210> 112
<211> 114
<212> PRT
<213> Homo sapiens

<400> 112

Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
1 5 10 15

Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Val Leu Tyr Ser
20 25 30

Ser Asn Asn Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
35 40 45

Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
50 55 60

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65 70 75 80

Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln
85 90 95

Tyr Tyr Ser Thr Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
100 105 110

[0078]

Lys Arg

<210> 113
<211> 116
<212> PRT
<213> Homo sapiens

<400> 113

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ser Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Tyr Ile Ser Ser Ser Ser Ser Thr Ile Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Asp Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ile Val Gly Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala
115

<210> 114
<211> 109
<212> PRT
<213> Homo sapiens

<400> 114

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35 40 45

Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Ser Ser Pro

[0079]

85 90 95

Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg
100 105

<210> 115
<211> 114
<212> PRT
<213> Homo sapiens

<400> 115

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Tyr Met Ser Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ile Gly Met Asp Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser
100 105 110

Ser Ala

<210> 116
<211> 115
<212> PRT
<213> Homo sapiens

<400> 116

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Tyr Met Ser Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

[0080]

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Leu Thr Gly Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val
100 105 110

Ser Ser Ala
115

<210> 117
<211> 108
<212> PRT
<213> Homo sapiens

<400> 117

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Val Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Trp
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ala Asn Ser Phe Pro Leu
85 90 95

Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg
100 105

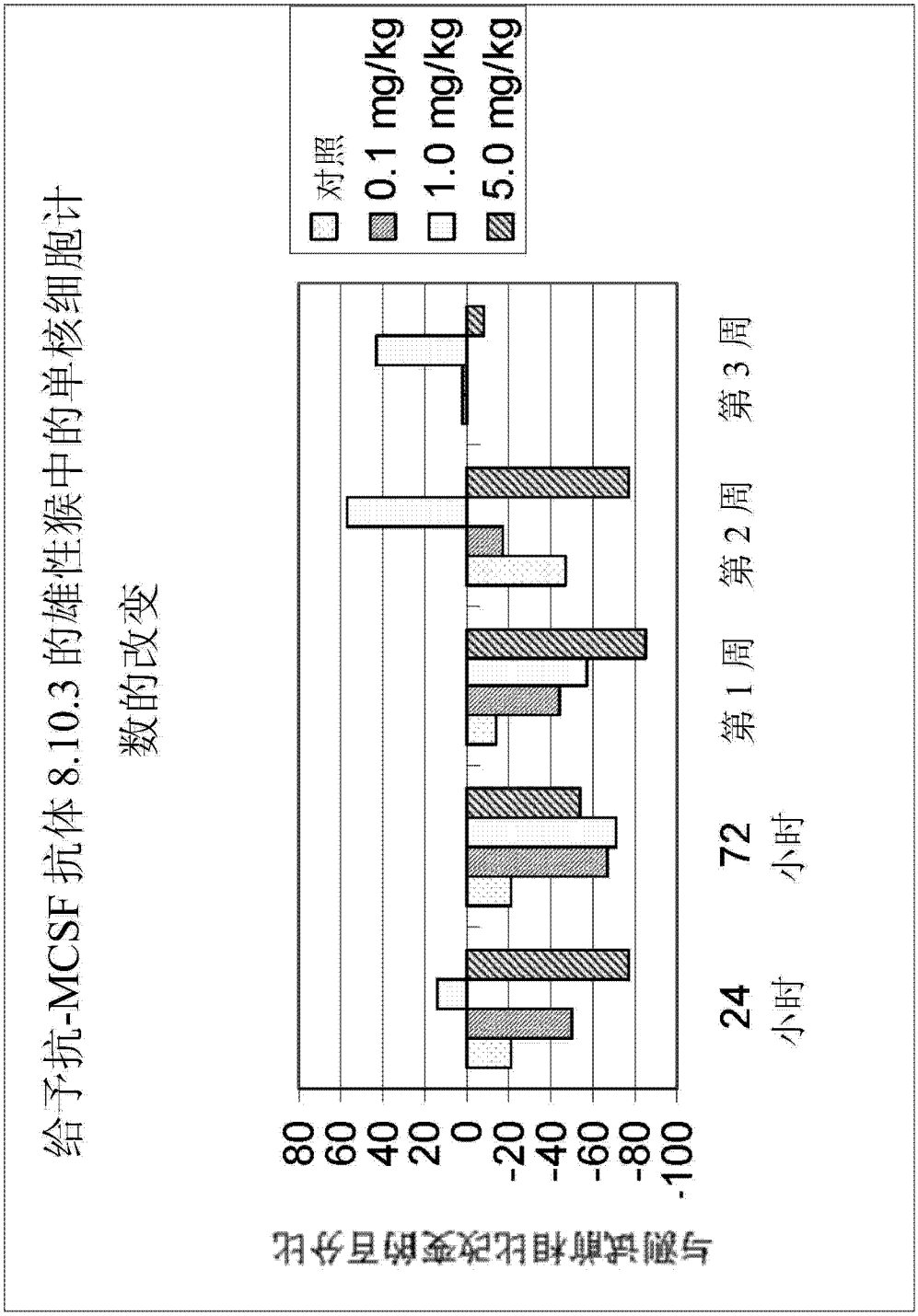

给予抗-MCSF 抗体 8.10.3 的雄性猴中的单核细胞计数的改变
对照
0.1 mg/kg
1.0 mg/kg
5.0 mg/kg
80
60
40
20
0
-20
-40
-60
-80
-100
与测试前相比改变的百分比
24 小时
72 小时
第 1 周
第 2 周
第 3 周

图 1A

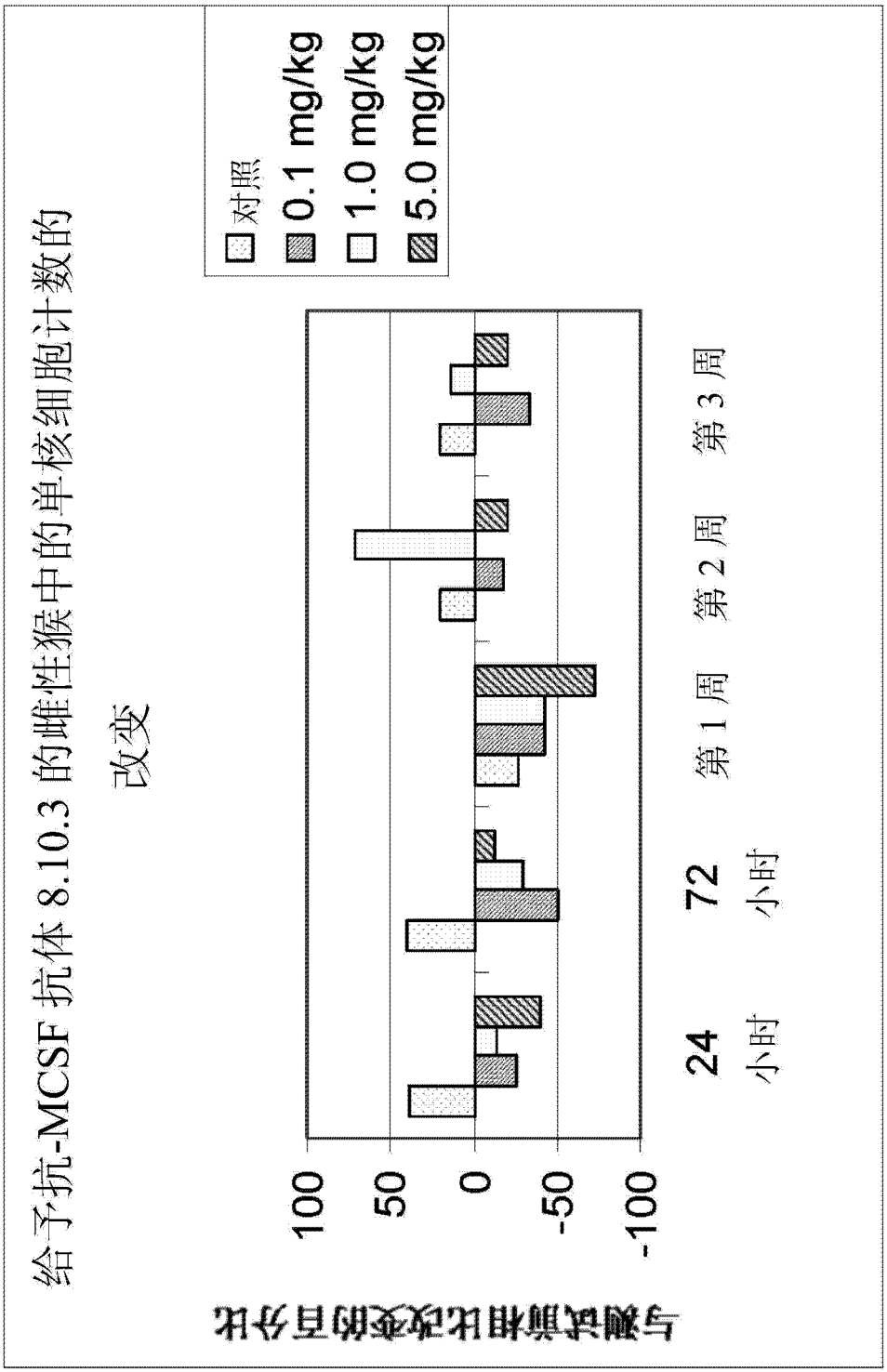

给予抗-MCSF 抗体 8.10.3 的雌性猴中的单核细胞计数的
改变
对照
0.1 mg/kg
1.0 mg/kg
5.0 mg/kg
与测试前相比改变的百分比
100
50
0
-50
-100
24
小时
72
小时
第 1 周
第 2 周
第 3 周

图 1B

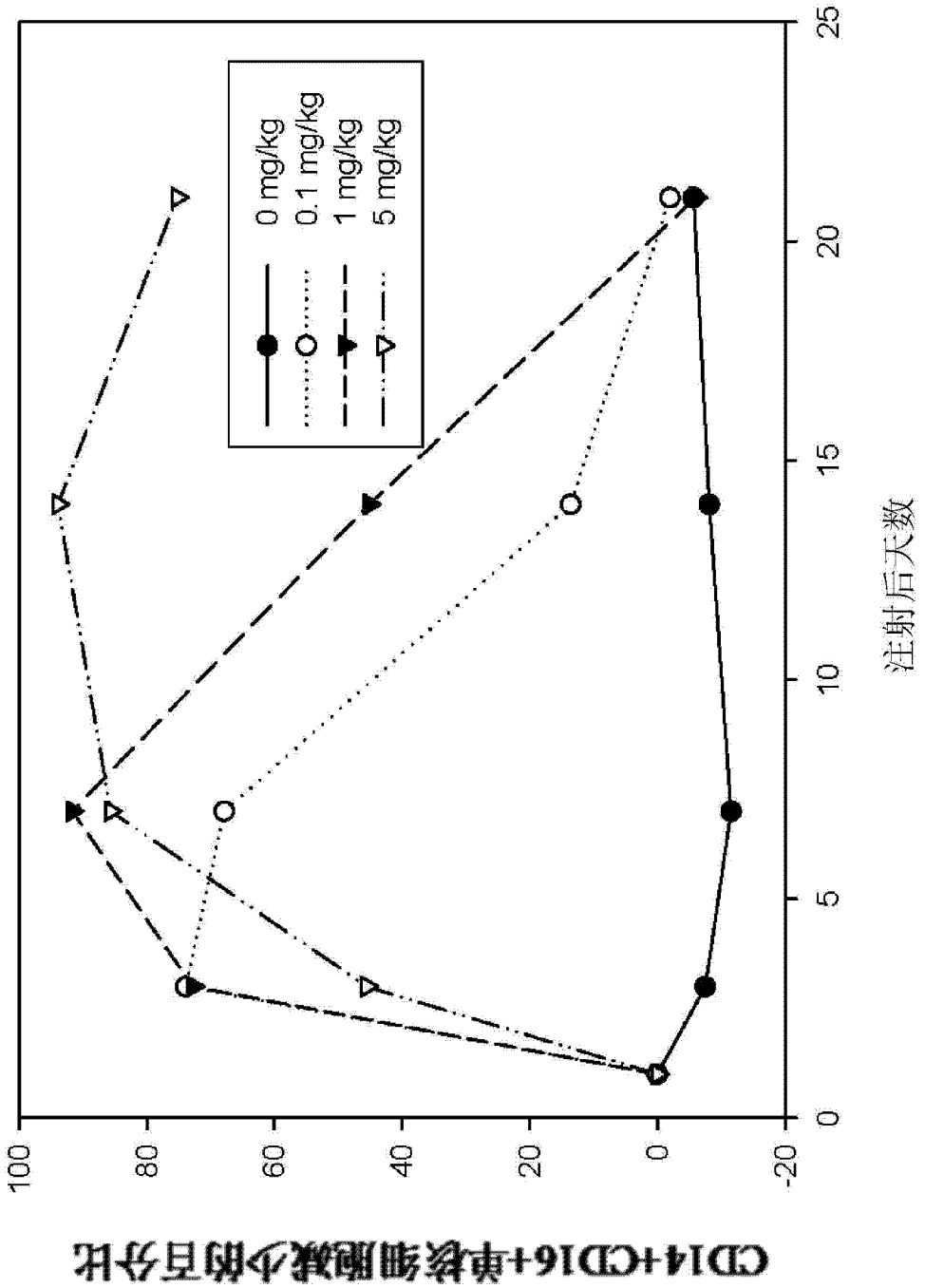

0 mg/kg
0.1 mg/kg
1 mg/kg
5 mg/kg
100
80
60
40
20
0
-20
0
5
10
15
20
25
注射后天数
CD14+CD16+单核细胞减少的百分比

图 2A

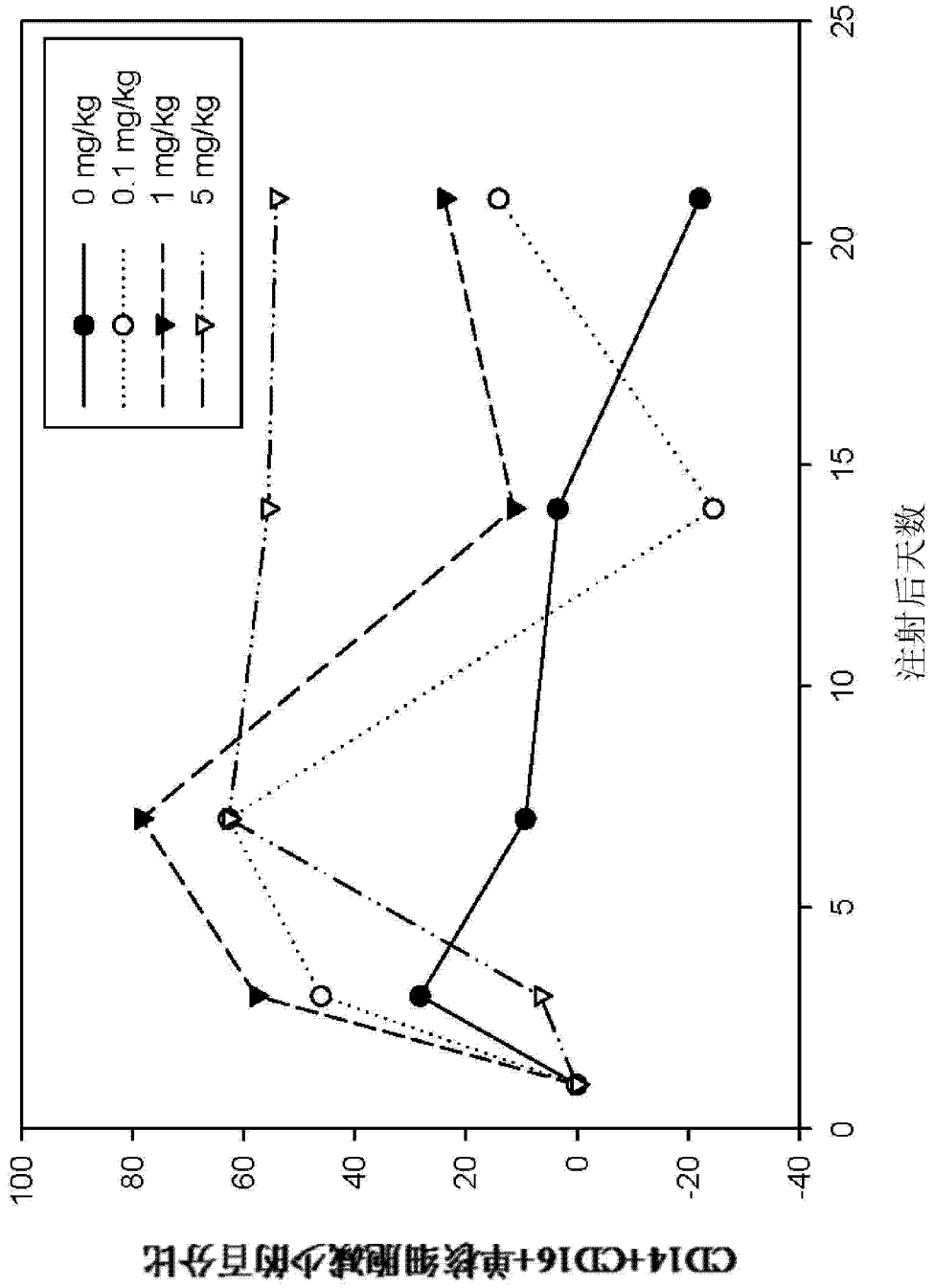

0 mg/kg
0.1 mg/kg
1 mg/kg
5 mg/kg
100
80
60
40
20
0
-20
-40
0
5
10
15
20
25
CD14+CD16+单核细胞减少的百分比
注射后天数

图 2B

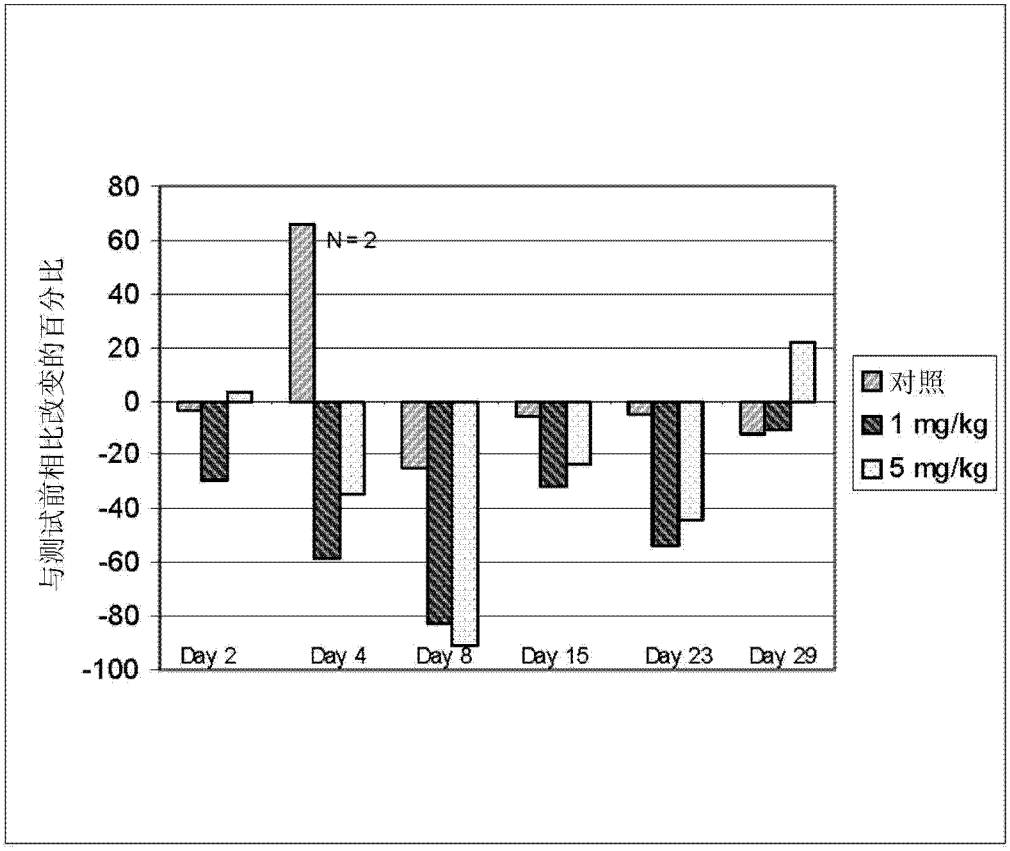

80
60
40
20
0
-20
-40
-60
-80
-100
与测试前相比改变的百分比
N=2
Day 2
Day 4
Day 8
Day 15
Day 23
Day 29
对照
1 mg/kg
5 mg/kg

图 3A

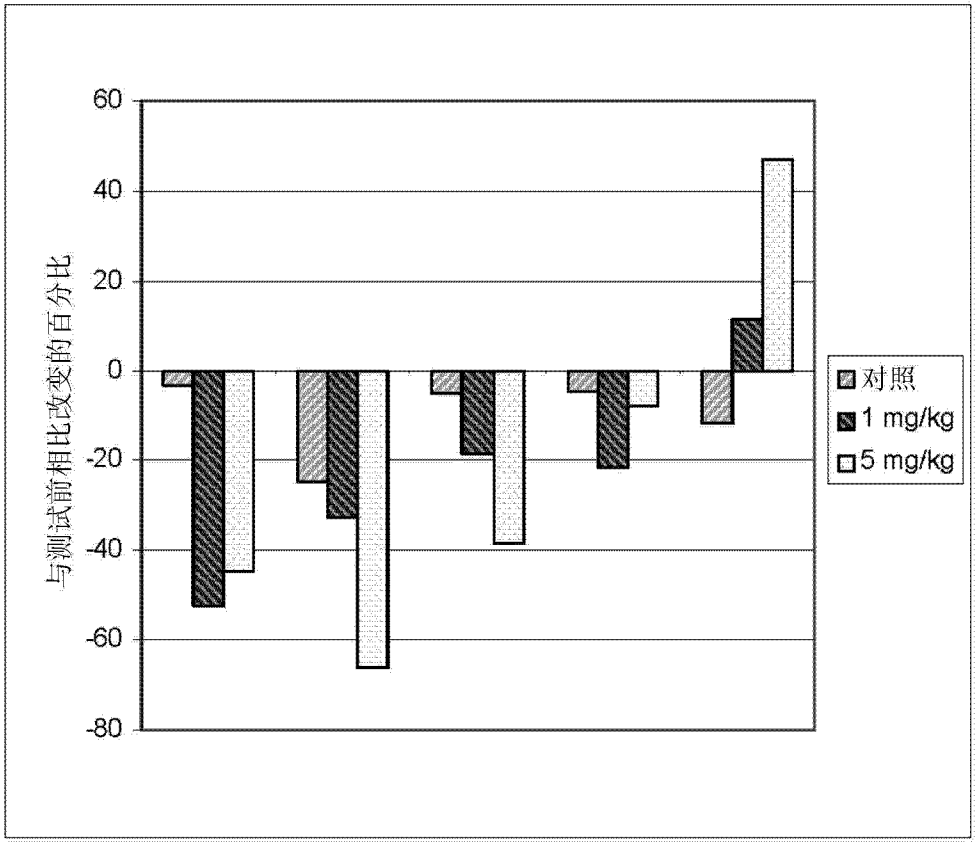


图 3B

```
种  系   V-012, J-JK3
252      ------------------------ --------GF-- ------------------ -T------ ---F---------------------- -----V---
种       DIQMTQSPSSLSASVGDRVTITC RASQSISSYLN WYQQKPGKAPKLLIY AASSLQS GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC QQSYSTPFT
                  FR1                CDR1          FR2          CDR2              FR3                CDR3
252      ---------- (残  基  21-127 of SEQ ID NO: 4)
种       FGPGTKVDIK (SEQ ID NO: 103)
                J
```

图 4A

```
种  系   V-012, J-JK3
88       ------------------------ -P--D------ ------------------ -------- ---L---------------------- ----------
种       DIQMTQSPSSLSASVGDRVTITC RASQSISSYLN WYQQKPGKAPKLLIY AASSLQS GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC QQSYSTPFT
                  FR1                CDR1          FR2          CDR2              FR3                CDR3
88       ---------- (残  基  21-127 of SEQ ID NO: 8)
种       FGPGTKVDIK (SEQ ID NO: 103)
                J
```

图 4B

```
种  系   V-L2, J-JK3
100      ------------------------ ------------------ ------S ---D-I---------------------- ----------
种       EIVMTQSPATLSVSPGERATLSC RASQSVSSNLA WYQQKPGQAPRLLIY GASTRAT GIPARFSGSGSGTEFTLTISSLQSEDFAVYYC QQYNNWPFT
                  FR1                CDR1          FR2          CDR2              FR3                CDR3
100      ---------- (残  基  21-127 of SEQ ID NO: 12)
种       FGPGTKVDIK (SEQ ID NO: 107)
                J
```

图 4C

```
种　系  V-L5, J-JK3
3.8.3   ------------------------S- ----D-G--- ----------------S -T--H- ------------------------------ --T------ ----------
(残　基  23-130 of SEQ ID NO: 16)

种          DIQMTQSPSSVSASVGDRVTITC RASQGISSWLA WYQQKPGKAPKLLIY AASSLQS GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC QQANSFPFT FGPGTKVDIKR
(SEQ ID NO: 109)    FR1             CDR1        FR2             CDR2        FR3                         CDR3      J
```

图 4D

```
种　系  V-L5, J-JK4
2.7.3   ------------------------ ----D------ ---R-------Q-- -----E- ------N---------S---------- --T------ ----------
(残　基  23-130 of SEQ ID NO: 20)

种          DIQMTQSPSSVSASVGDRVTITC RASQGISSWLA WYQQKPGKAPKLLIY AASSLQS GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC QQANSFPLT FGGGTKVEIKR
(SEQ ID NO: 117)    FR1             CDR1        FR2             CDR2        FR3                         CDR3      J
```

图 4E

```
种　系  V-B3, J-JK1
1.120.1 ------------------------ -----I-FF------- --R------N---- ------- ------------------------------ -----S--

种          DIVMTQSPDSLAVSLG残　基  ESQSVLYSSNNKNYLA WYQQKPGQPPKLLIY WASTRES GVPDRFSGSGSGTDFTLTISSLQAEDVAVYYC QQYYSTPWT
                FR1                 CDR1            FR2             CDR2        FR3                         CDR3

1.120.1 ---------- (残　基  21-134 of SEQ ID NO: 24)

种          FGQGTKVEIKR (SEQ ID NO: 112)
                J
```

图 4F

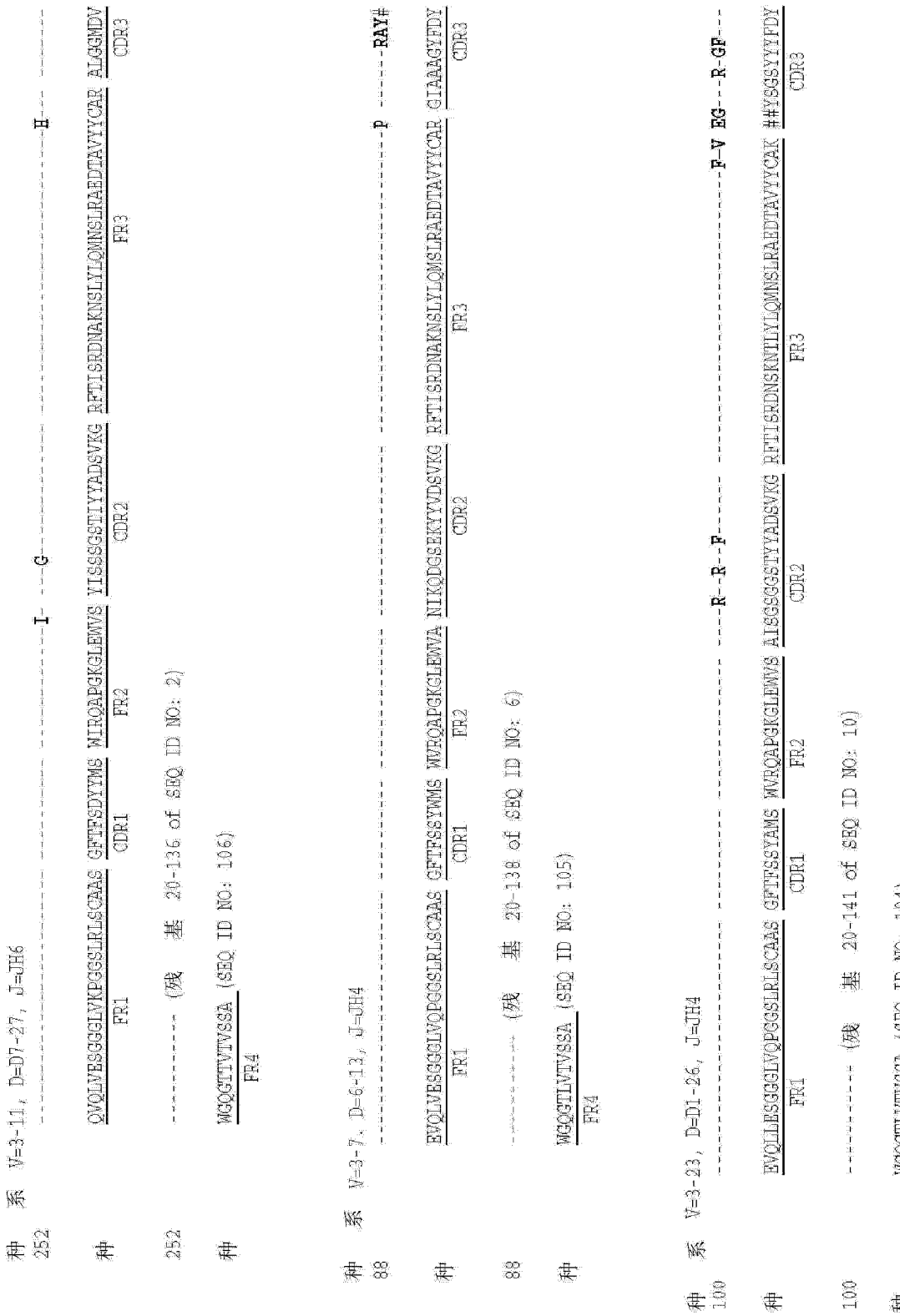

种 系 V=3-11, D=D7-27, J=JH6
252
种
252
种
FR1
CDR1
FR2
CDR2
FR3
CDR3
FR4
(残 基 20-136 of SEQ ID NO: 2)
(SEQ ID NO: 106)
种 系 V=3-7, D=6-13, J=JH4
88
种
88
种
(残 基 20-138 of SEQ ID NO: 6)
(SEQ ID NO: 105)
种 系 V=3-23, D=D1-26, J=JH4
100
种
100
种
(残 基 20-141 of SEQ ID NO: 10)
(SEQ ID NO: 104)

图 4G

图 4H

图 4I

```
种  系  V=3-11, D=D7-27, J=JH4
3.8.3   ----------  ----------  ----------F----------  ----------S----------  ----G-----

种      QVQLVESGGGLVKPGGSLRLSCAAS GFTFSDYYMS WIRQAPGKGLEWVS YISSSGSTIYYADSVKG RFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR #LTGDY
            FR1                   CDR1        FR2           CDR2                FR3                             CDR3
3.8.3       ----------（残  基  20-135 of SEQ ID NO: 14）
种      WGQGTLVTVSSA（SEQ ID NO: 108）
            FR4

种  系  V=3-33, D=D1-26, J=JH4
2.7.3   ----------  ----------  ----------F----------  ----------  ----------  --RV----

种      QVQLVESGGGVVQPGRSLRLSCAAS GFTFSSYGMH WVRQAPGKGLEWVA VIWYDGSNKYYADSVKG RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR GYSHYFDY
            FR1                   CDR1        FR2           CDR2                FR3                             CDR3
2.7.3       ----------（残  基  20-137 of SEQ ID NO: 18）
种      WGQGTLVTVSSA（SEQ ID NO: 110）
            FR4

种  系  V=1-18, D=D4-23, J=JH4
1.120.1 ----------  ----------  ----------  ----------D----------T----------  ----------R RA--A-F--

种      QVQLVQSGAEVKKPGASVKVSCKAS GYTFTSYGIS WVRQAPGQGLEWMG WISAYNGNTNYAQKLQG RVTMTTDTSTSTAYMELRSLRSDDTAVYYCA# #DYGGNYFDY
            FR1                   CDR1        FR2           CDR2                FR3                             CDR3
1.120.1     ----------（残  基  20-139 of SEQ ID NO: 22）
种      WGQGTLVTVSSA（SEQ ID NO: 111）
            FR4
```

图 4J

图 4K

图 4L

```
种  系  V=A27, J=JK4
8.10.3      -F-------------------- ---------- ---------- ------- ----------V----
种          EIVLTQSPGTLSLSPGERATLSC RASQSVSSSYLA WYQQKPGQAPRLLIY GASSRAT GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC
                    FR1             CDR1       FR2        CDR2        FR3
8.10.3      --------- ---------- (残  基  21-129 of SEQ ID NO: 44)
种          QQYGSSPLT FGGGTKVEIKR (SEQ ID NO: 114)
              CDR3      J
```

图 4M

```
种  系  V=VH3-48, D=D1-26, J=JH4b
8.10.3      ---------- ------P--T ---------- ----R---S------- ---------- ----------DPLLA-ATF---
种          EVQLVESGGGLVQPGGSLRLSCAAS GFTFSSYSMN WVRQAPGKGLEWVS YISSSSSTIYYADSVKG RFTISRDNAKNSLYLQMNSLRDEDTAVYYCAR ##IVG##FDY
                FR1                   CDR1       FR2        CDR2                  FR3                           CDR3
8.10.3      ---------- (残  基  20-141 of SEQ ID NO: 30)
种          WGQGTLVTVSSA (SEQ ID NO: 113)
              J
```

图 4N

```
种  系  V=012, J=JK3
9.14.4      ---------- -P--I---L-- ----------H ---------- ---------- ---------- ----------
(残  基  23-130 of SEQ ID NO: 28)
种          DIQMTQSPSSLSASVGDRVTITC RASQSISSYLN WYQQKPGKAPKLLIY AASSLQS GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC QQSYSTPFT FGPGTKVDIKR
(SEQ ID NO: 103)    FR1                CDR1       FR2        CDR2        FR3                           CDR3      J
```

图 4O

```
种  系  V=VH3-11, D=D7-27, J=JH4b
9.14.4
                                                
```

```
种  系  V=VH3-11, D=D7-27, J=JH4b
9.14.4I  ------------------------- ---------- ---------- ---------- ---------- ---------- ----------G-----

种                QVQLVESGGGLVKPGGSLRLSCAAS GFTFSDYYMS WIRQAPGKGLEWVS YISSSGSTIYYADSVKG RFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR #LTGDY
                        FR1                CDR1        FR2                CDR2                FR3                CDR3
9.14.4I   ------------- (残  基  20-135 of SEQ ID NO: 26)
种                WGQGTLVTVSSA (SEQ ID NO: 116)
                        J
```

图 4T

```
种  系  V=A27, J=JK4
8.10.3F  -P----------  ---------- ---------- ---------- ---------- ---------- ----------  ----------

种                EIVLTQSPGTLSLSPGERATLSC RASQSVSSSYLA WYQQKPGQAPRLLIY GASSRAT GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC
                        FR1                CDR1        FR2                CDR2        FR3
8.10.3F   ---------- ---------- (残  基  21-129 of SEQ ID NO: 32)
种                QQYGSSPLT FGGGTKVEIKR (SEQ ID NO: 114)
                        CDR3        J
```

图 4U

```
种  系  V=VH3-48, D=D1-26, J=JH4b
8.10.3F  ---------- ---------- ----------F--T ---------- ----------R---S---------- ---------- ----------DPLLA-ATF---

种                EVQLVESGGGLVQPGGSLRLSCAAS GFTFSSYSMN WVRQAPGKGLEWVS YISSSSSTIYYADSVKG RFTISRDNAKNSLYLQMNSLRDEDTAVYYCAR ###IVG##FDY
                        FR1                CDR1        FR2                CDR2                FR3                CDR3
8.10.3F   ---------- (残  基  20-141 of SEQ ID NO: 30)
种                WGQGTLVTVSSA (SEQ ID NO: 113)
                        J
```

图 4V

```
种  系  V=012, J=JK3
9.7.2IF  ----------  -------GF-I  ----R----------  -T-----  ----------  ----------  ----------
                  FR1          CDR1          FR2          CDR2          FR3          CDR3      J
(残  基  23-130 of SEQ ID NO: 36)

种          DIQMTQSPSSLSASVGDRVTITC  RASQSISSYLN  WYQQKPGKAPKLLIY  AASSLQS  GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC  QQSYSTPFT  FGPGTKVDIKR
(SEQ ID NO: 103)        FR1          CDR1          FR2          CDR2          FR3          CDR3      J
```

图 4W

```
种  系  V=VH3-11, D=D6-13, J=JH6b
9.7.2IF  ----------  ----------  ----------  ----------  ----------  ----------  ----------  ----R R-G----

种          QVQLVESGGGLVKPGGSLRLSCAAS  GFTFSDYYMS  WIRQAPGKGLEWVS  YISSSGSTIYYADSVKG  RFTISRDNAKNSLYLQMNSLRAEDTAVYYCA#  #I#GMDV
                  FR1          CDR1          FR2          CDR2          FR3          CDR3

9.7.2IF  ----------  (残  基  20-136 of SEQ ID NO: 34)

种  MGQGTTVTVSSA  (SEQ ID NO: 115)
                J
```

图 4X

```
种  系  V=012, J=JK3
9.7.2C-Ser  ----------  -------GF-I  ----------  -T-----  ----------  ----------  ----------
                  FR1          CDR1          FR2          CDR2          FR3          CDR3      J
(残  基  23-130 of SEQ ID NO: 52)

种          DIQMTQSPSSLSASVGDRVTITC  RASQSISSYLN  WYQQKPGKAPKLLIY  AASSLQS  GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC  QQSYSTPFT  FGPGTKVDIKR
(SEQ ID NO: 103)        FR1          CDR1          FR2          CDR2          FR3          CDR3      J
```

图 4Y

种 系 V=VH3-11, D=D6-13, J=JH6b
9.7.2C-Ser ---------- ---------- ---------- ---------- ---------- R-G----
种 QVQLVESGGGLVKPGGSLRLSCAAS GFTFSDYYMS WIRQAPGKGLEWVS YISSSGSTIYYADSVKG RFTISRDNAKNSLYLQMNSLRAEDTAVYYCA #I#GMDV
FR1 CDR1 FR2 CDR2 FR3 CDR3
9.7.2C-Ser ---------- (残 基 20-136 of SEQ ID NO: 50)
种 WGQGTTVTVSSA (SEQ ID NO: 115)
J

图 4Z

种 系 V=012, J=JK3
9.14.4C-Ser ---------- -P-I---L-- ---------- ---------- ---------- ----------
(残 基 23-130 of SEQ ID NO: 56)
种 DIQMTQSPSSLSASVGDRVTITC RASQSISSYLN WYQQKPGKAPKLLIY AASSLQS GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC QQSYSTPFT FGPGTKVDIKR
(SEQ ID NO: 103) FR1 CDR1 FR2 CDR2 FR3 CDR3 J

种 系 V=VH3-11, D=D7-27, J=JH4b
9.14.4C-Ser ---------- ---------- ---------- ---------- ---------- ----------
种 QVQLVESGGGLVKPGGSLRLSCAAS GFTFSDYYMS WIRQAPGKGLEWVS YISSSGSTIYYADSVKG RFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR #LTGDY
FR1 CDR1 FR2 CDR2 FR3 CDR3
9.14.4C-Ser ---------- (残 基 20-135 of SEQ ID NO: 54)
种 WGQGTLVTVSSA (SEQ ID NO: 116)
J

图 4BB

图 4AA

```
种  系  V-A27, J-JK4
8.10.3C-Ser  ---------- ---------- ---------- ---------- ----------

种                EIVLTQSPGTLSLSPGERATLSC RASQSVSSSYLA WYQQKPGQAPRLLIY GASSRAT GIPDRFSGSGSGSDFTLTISRLEPEDFAVYYC
                        FR1                 CDR1            FR2         CDR2        FR3
种          QQYGSSPLT FGGGTKVEIKR (残  基  21-129 of SEQ ID NO: 60)
              CDR3      J
8.10.3      --------- ---------- (SEQ ID NO: 114)
```

图 4CC

```
种  系  V-VH3-48, D=D1-26, J=JH4b
8.10.3C-Ser  ---------- ---------- ----F--T---------- ----R----S---------- ---------- ----------  DPLLA-ATF---

种                EVQLVESGGGLVQPGGSLRLSCAAS GFTFSSYSMN WVRQAPGKGLEWVS YISSSSSTIYYADSVKG RFTISRDNAKNSLYLQMNSLRDEDTAVYYCAR ###IVGH##FDY
                        FR1                 CDR1            FR2         CDR2        FR3                         CDR3
8.10.3C-Ser  ---------- (残  基  20-141 of SEQ ID NO: 58)
种          WGQGTLVTVSSA (SEQ ID NO: 113)
              J
```

图 4DD

```
种  系  V-A27, J-JK4
8.10.3-CG2  ---------- ---------- ---------- ---------- ----------

种                EIVLTQSPGTLSLSPGERATLSC RASQSVSSSYLA WYQQKPGQAPRLLIY GASSRAT GIPDRFSGSGSGSDFTLTISRLEPEDFAVYYC
                        FR1                 CDR1            FR2         CDR2        FR3
8.10.3-CG2  ---------- ---------- (残  基  21-129 of SEQ ID NO: 60)
种          QQYGSSPLT FGGGTKVEIKR (SEQ ID NO: 114)
              CDR3      J
```

图 4EE

```
种  系  V=VH3-48, D=D1-26, J=JH4b
8.10.3-CG2  ----------F--T----------R----S----------  DPLLA ATF--

种                  EVQLVESGGGLVQPGGSLRLSCAAS GFTFSSYSMN WVRQAPGKGLEWVS YISSSSSTIYYADSVKG RFTISRDNAKNSLYLQMNSLRDEDTAVYYCAR ##HIVG##H#FDY
                    FR1                   CDR1       FR2             CDR2                FR3                   CDR3
8.10.3-CG2  ----------  (残  基  20-141 of SEQ ID NO: 62)

种                  WGQGTLVTVSSA (SEQ ID NO: 113)
                    J

种  系  V=012, J=JK3
9.7.2-CG2   ----------  --------GF-I ----------  -T-----  ----------  ----------  ----------
                    FR1       CDR1       FR2       CDR2              FR3                   CDR3      J
(残  基  23-130 of SEQ ID NO: 52)

种                  DIQMTQSPSSLSASVGDRVTITC RASQSISSYLN WYQQKPGKAPKLLIY AASSLQS GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC QQSYSTPFT FGPGTKVDIKR
(SEQ ID NO: 103)    FR1                   CDR1       FR2             CDR2                FR3                   CDR3      J
```

图 4FF

```
种  系  V=VH3-11, D=D6-13, J=JH6b
9.7.2-CG2   ----------  ----------  ----------  ----------  ----------  ----------  ----------  R-G----
(残  基  20-136 of SEQ ID NO: 66)

种                  QVQLVESGGGLVKPGGSLRLSCAAS GFTFSDYYMS WIRQAPGKGLEWVS YISSSGSTIYYADSVKG RFTISRDNAKNSLYLQMNSLRAEDTAVYYCA #LH3MDV
(SEQ ID NO: 115)    FR1                   CDR1       FR2             CDR2                FR3                   CDR3
9.7.2-CG2   ----------

种                  NGQGTTVTVSSA
                    J
```

图 4GG

```
种  系  V=012, J=JK3
9.7.2-CG4   ----------  --------GF-I ----------  -T-----  ----------  ----------  ----------
(残  基  23-130 of SEQ ID NO: 52)

种                  DIQMTQSPSSLSASVGDRVTITC RASQSISSYLN WYQQKPGKAPKLLIY AASSLQS GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC QQSYSTPFT FGPGTKVDIKR
(SEQ ID NO: 103)    FR1                   CDR1       FR2             CDR2                FR3                   CDR3      J
```

图 4HH

图 4II

```
种  系  V=VH3-11, D=D6-13, J=JH6b
9.7.2-CG4  ------------------------------ ---------- ---------- ----------
```

```
种  系 V=VH3-11, D=D7-27, J=JH4b
9.14.4-CG4 ------------------------ ---------- ---------- ----------
```

```
种  系  V=VH3-11, D=D6-13, J=JH6b
9.7.2-Ser  ------------------------------------------------------------------------------------------------------------R-G----

种                 QVQLVESGGGLVKPGGSLRLSCAAS GFTFSDYYMS WIRQAPGKGLEWVS YISSSGSTIYYADSVKG RFTISRDNAKNSLYLQMNSLRAEDTAVYYCA #1#GMDV
                          FR1                  CDR1          FR2          CDR2                FR3                      CDR3
9.7.2-Ser  ---------- (残  基  20-136 of SEQ ID NO: 50)

种                 WGQGTTVTVSSA (SEQ ID NO: 115)
                         J
```

图 4RR

```
种  系  V=427, J=JK4
8.10.3-Ser  -F-------------------------- ---------- ---------- ---------- ---------- ----------V----

种                 EIVLTQSPGTLSLSPGERATLSC RASQSVSSSYLA WYQQKPGQAPRLLIY GASSRAT GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC
                          FR1                  CDR1          FR2          CDR2          FR3
8.10.3-Ser  ---------- ---------- (残  基  21-129 of SEQ ID NO: 44)

种                 QQYGSSPLT FGGGTKVEIKR (SEQ ID NO: 114)
                         CDR3      J
```

图 4SS

```
种  系  V=VH3-48, D=D1-26, J=JH4b
8.10.3-Ser  ---------- ---------- ----F-T ---------- ----R---S---------- ---------- ----------DPLLA-ATF---

种                 EVQLVESGGGLVQPGGSLRLSCAAS GFTFSSYSMN WVRQAPGKGLEWVS YISSSSSTIYYADSVKG RFTISRDNAKNSLYLQMNSLRDEDTAVYYCAR ###LVG##FDY
                          FR1                  CDR1          FR2          CDR2                FR3                      CDR3
8.10.3-Ser  ---------- (残  基  20-141 of SEQ ID NO: 90)

种                 WGQGTLVTVSSA (SEQ ID NO: 113)
                         J
```

图 4TT

```
种  系  V=A27, J=JK4
8.10.3-CG4  ----------  ----------  ----------  ----------  ----------  ----------  ----------

种                  EIVLTQSPGTLSLSPGERATLSC RASQSVSSSYLA WYQQKPGQAPRLLIY GASSRAT GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC
                                  FR1                CDR1          FR2          CDR2          FR3
8.10.3-CG4  ---------  ----------  (残  基  21-129 of SEQ ID NO: 60)
种                  QQYGSSPLT FGQGTKVEIKR (SEQ ID NO: 114)
                      CDR3      J
```

图 4UU

```
种  系  V=VH3-48, D=D1-26, J=UH4b
8.10.3-CG4  ----------  ----------  ----------F-T----------  ----------R---S----------  ----------  ----------  ----------  ----------  ----------  
```

```
种  系  V=A27, J=JK4
8.10.3FG1   -F----------  ----------  ----------  ----------  ----------  ----------  ----------

种          EIVLTQSPGTLSLSPGERATLSC RASQSVSSSYLA WYQQKPGQAPRLLIY GASSRAT GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC
                      FR1           CDR1          FR2           CDR2          FR3

8.10.3FG1   ---------  ----------  (残  基  21-129 of SEQ ID NO: 32)

种          QQYGSSPLT PGGGTKVEIKR (SEQ ID NO: 114)
            CDR3      J
```

图 4YY

```
种  系  V=VH3-48, D=D1-26, J=JH4b
8.10.3FG1   ----------  ----------  -----F-T----------  ----R---S----------  ----------  ----------  DPLLA-ATF--

种          EVQLVESGGGLVQPGGSLRLSCAAS GFTFSSYSMN WVRQAPGKGLEWVS YISSSSSTIYYADSVKG RFTISRDNAKNSLYLQMNSLRDEDTAVYYCAR ###IVG##FDY
                      FR1           CDR1          FR2           CDR2          FR3           CDR3

8.10.3FG1   ----------  (残  基  20-141 of SEQ ID NO: 98)

种          WGQGTLVTVSSA (SEQ ID NO: 113)
            J
```

图 4ZZ

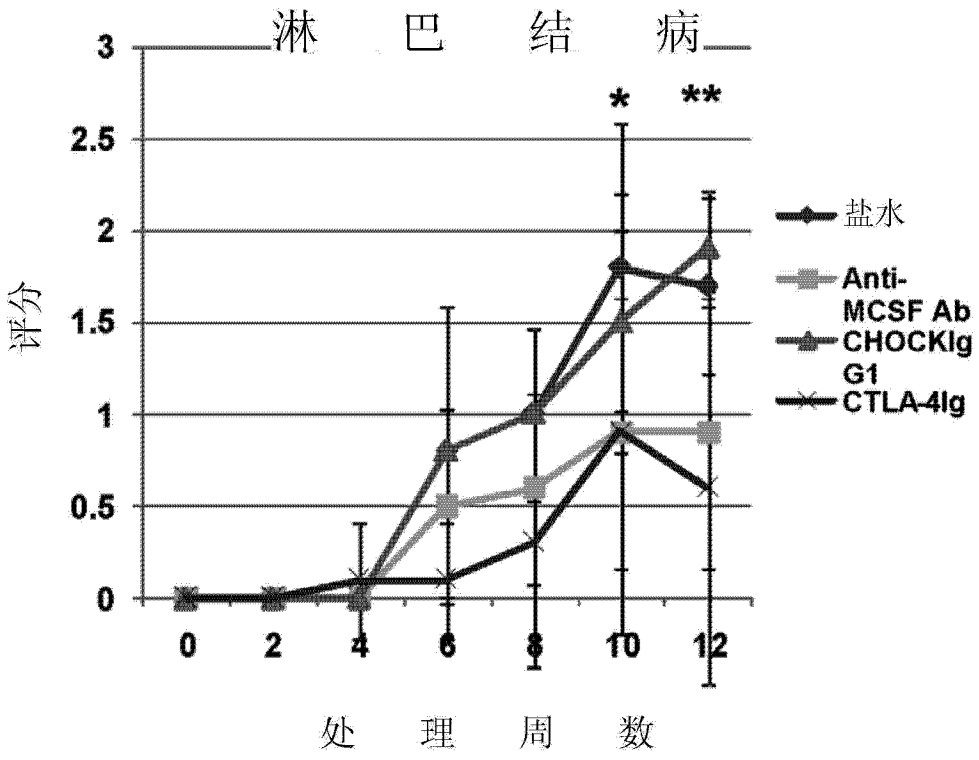

淋 巴 结 病
评分
处 理 周 数
盐水
Anti-MCSF Ab
CHOCKIg G1
CTLA-4Ig
*
**

图 5

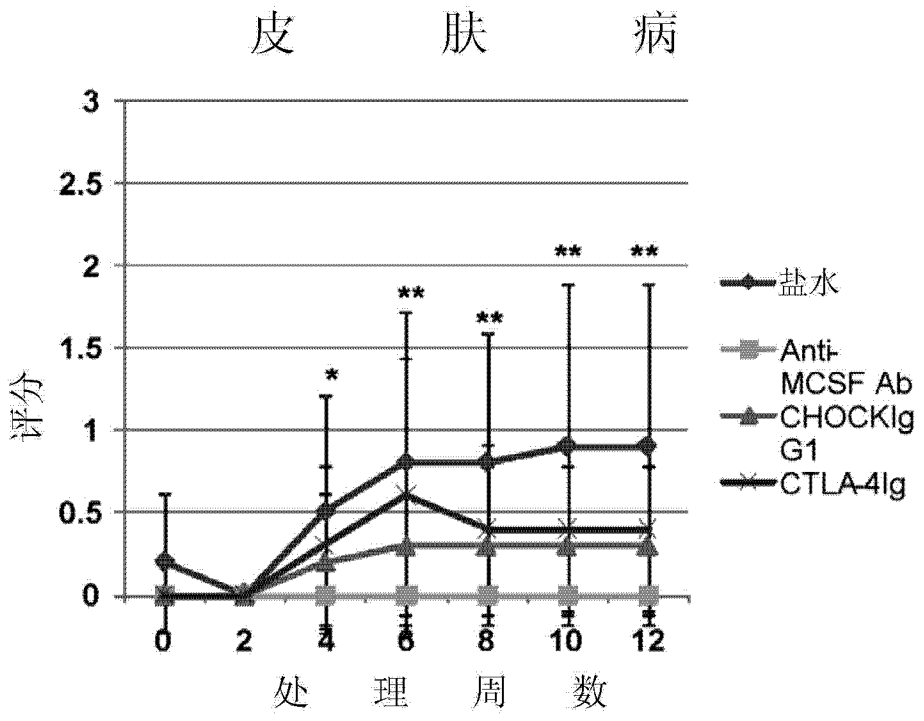

皮 肤 病
评分
处 理 周 数
盐水
Anti-MCSF Ab
CHOCKIg G1
CTLA-4Ig
*
**

图 6

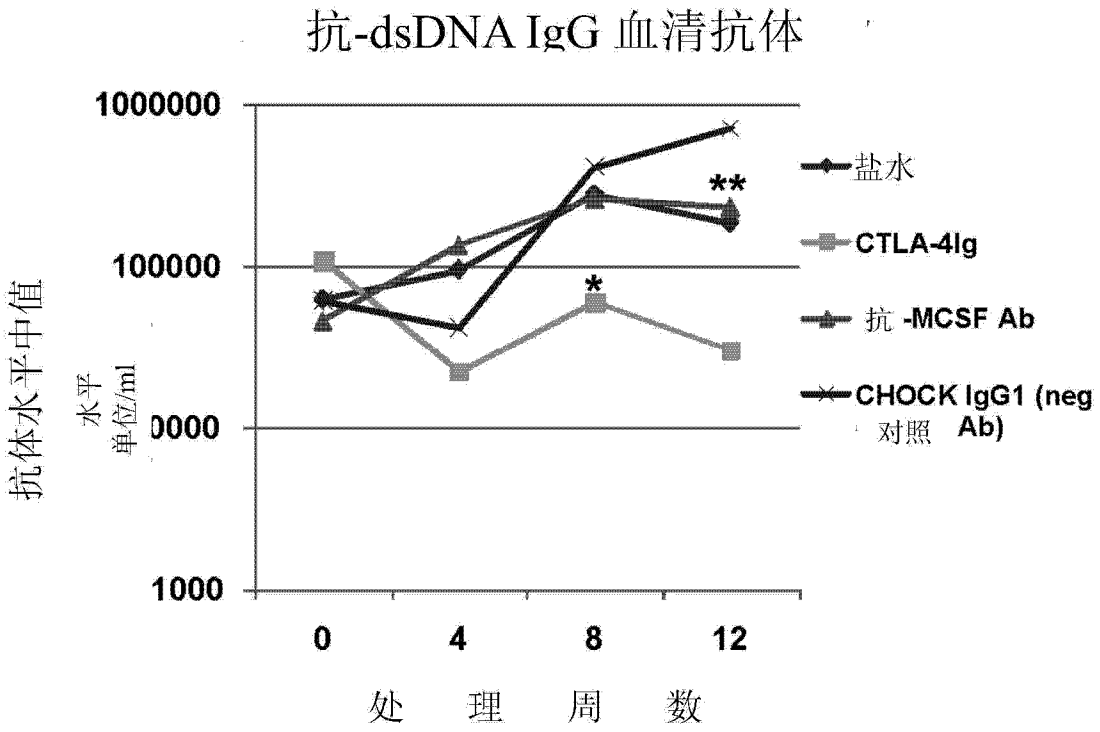

抗-dsDNA IgG 血清抗体
1000000
100000
0000
1000
抗体水平中值
水平
单位/ml
**
*
盐水
CTLA-4Ig
抗 -MCSF Ab
CHOCK IgG1 (neg
对照 Ab)
0
4
8
12
处 理 周 数

图 7

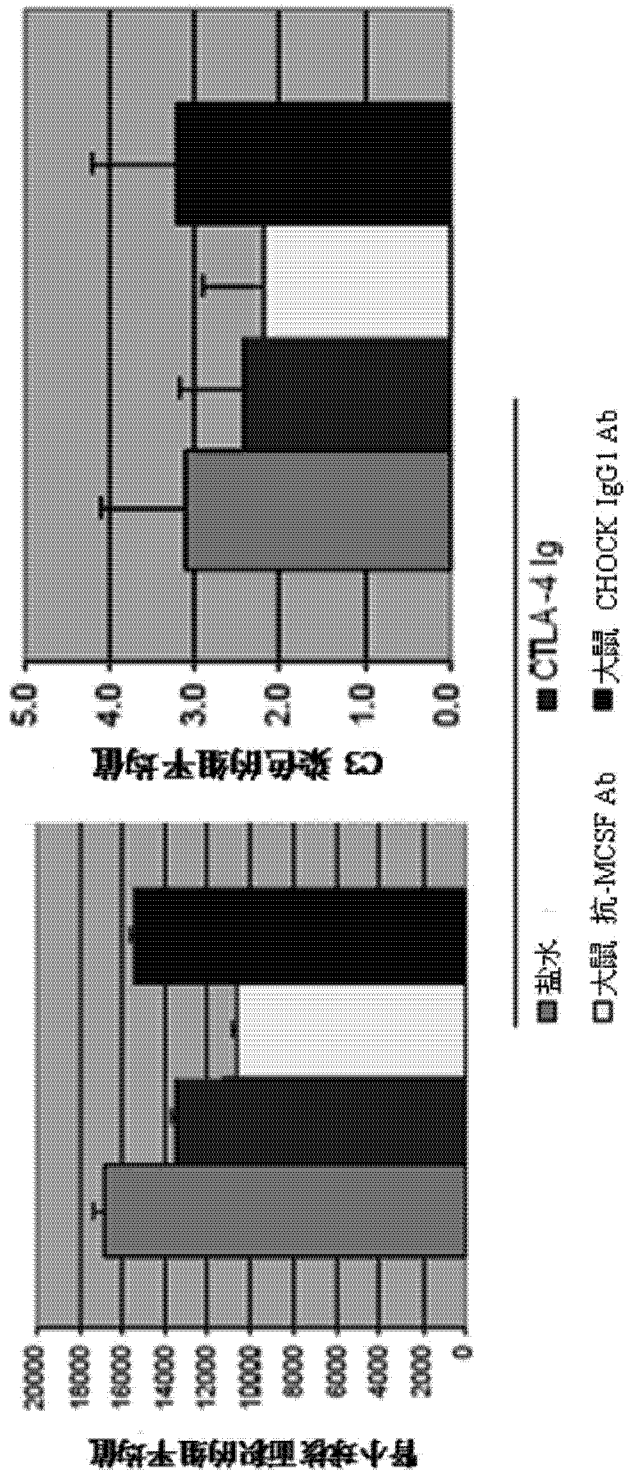

C3 染色的组平均值
5.0
4.0
3.0
2.0
1.0
0.0
骨小梁表面积的组平均值
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
盐水
CTLA-4 Ig
大鼠 抗-MCSF Ab
大鼠 CHOCK IgG1 Ab

图 8

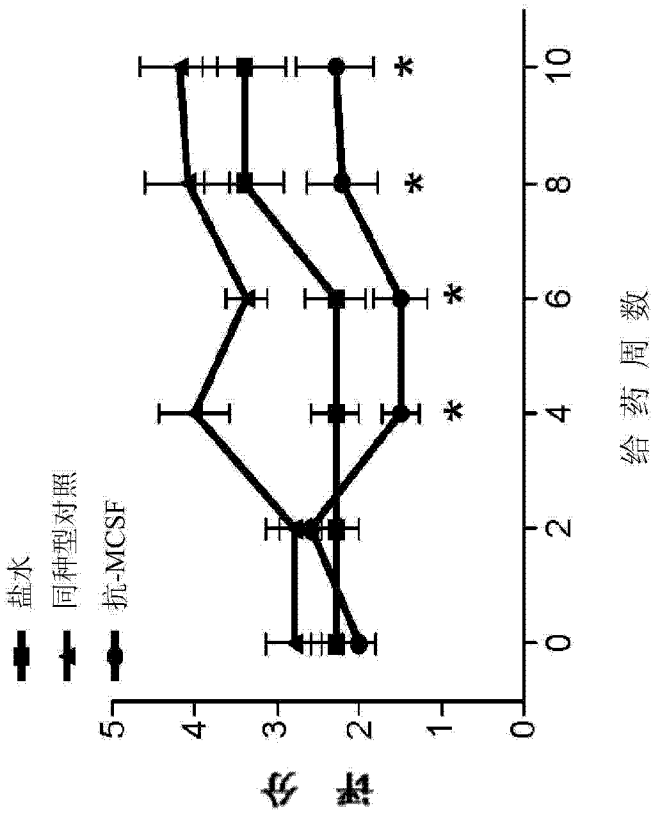

盐水
同种型对照
抗-MCSF
评分
给药周数
0
1
2
3
4
5
0
2
4
6
8
10
*
*
*
*

图 9A

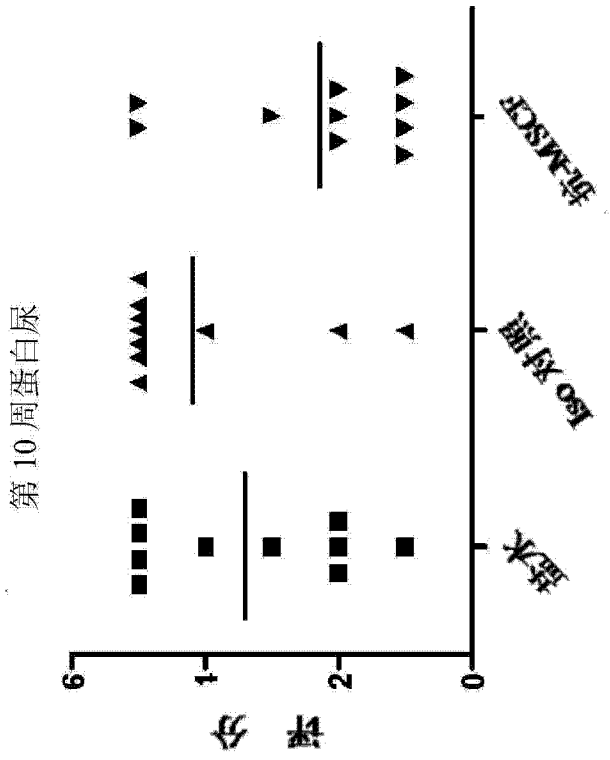

第 10 周蛋白尿
评分
0
2
4
6
载体
Iso 对照
抗-MCSF

图 9B

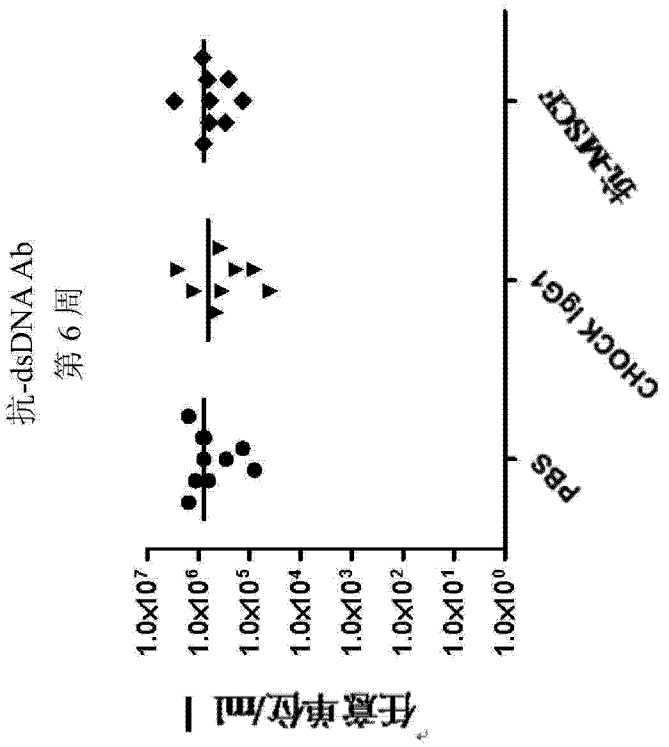

抗-dsDNA Ab
第 6 周
任意单位/ml
1.0x10^7
1.0x10^6
1.0x10^5
1.0x10^4
1.0x10^3
1.0x10^2
1.0x10^1
1.0x10^0
PBS
CHOCK IgG1
抗-MASCT

图 10A

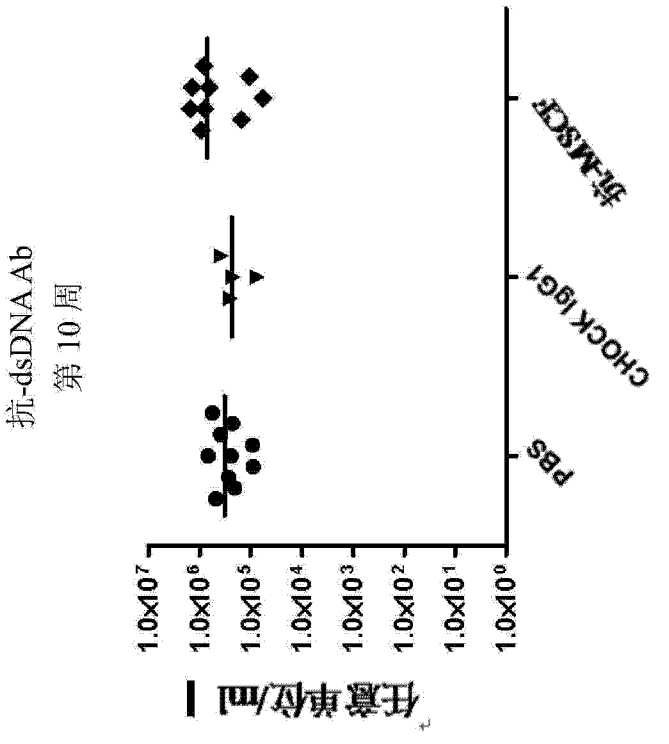

抗-dsDNA Ab
第 10 周
任意单位/ml
1.0x10^7
1.0x10^6
1.0x10^5
1.0x10^4
1.0x10^3
1.0x10^2
1.0x10^1
1.0x10^0
PBS
CHOCK IgG1
抗-MASCT

图 10B

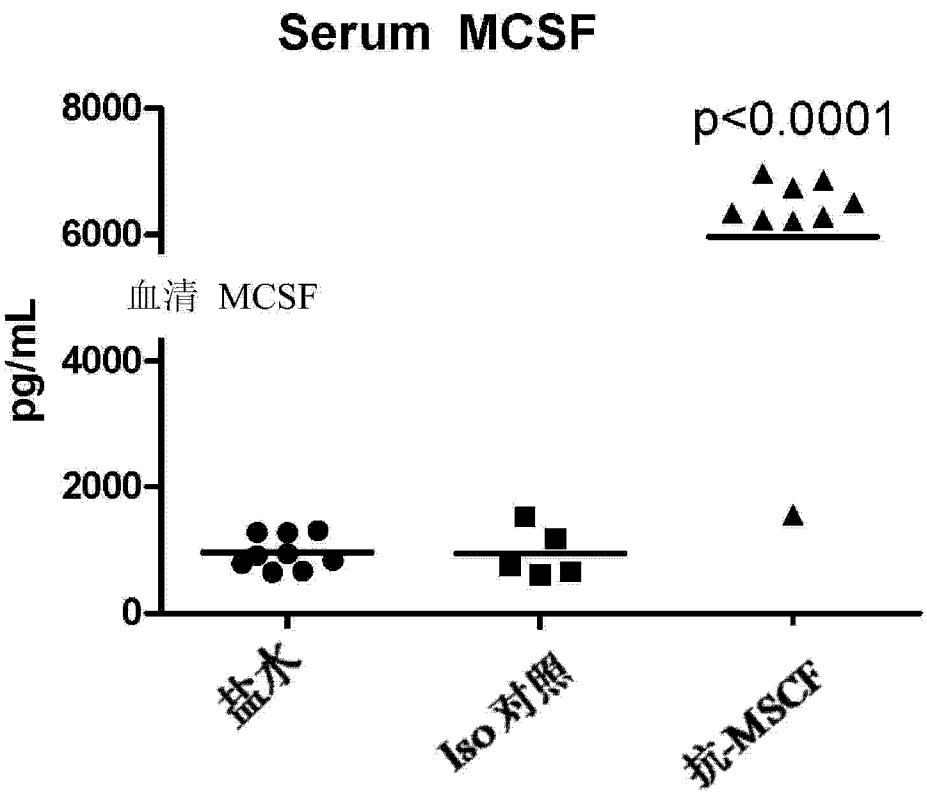

Serum MCSF
p<0.0001
8000
6000
血清 MCSF
pg/mL
4000
2000
0
盐水
Iso 对照
抗-MSCF

图 11

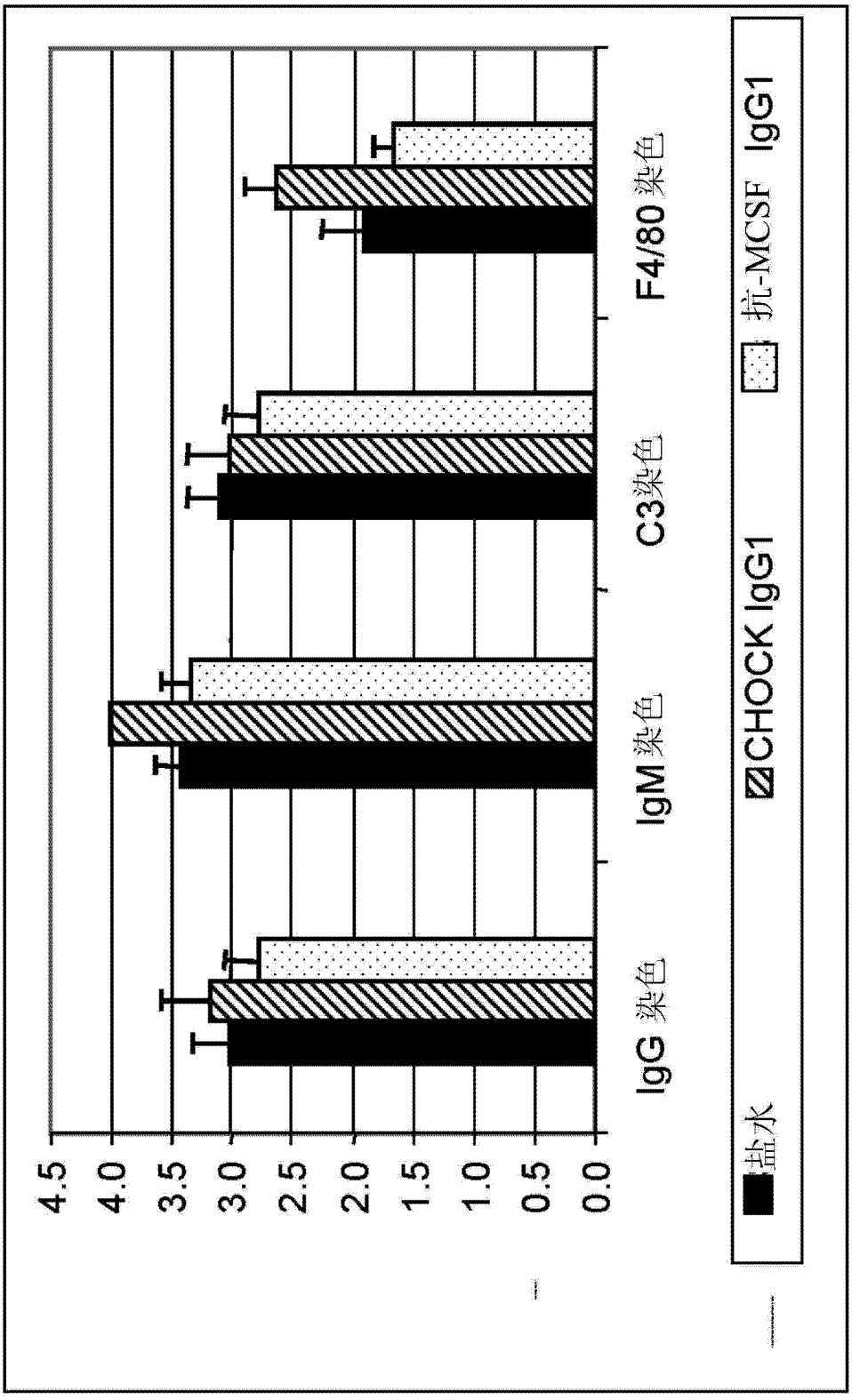

4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
IgG 染色
IgM 染色
C3染色
F4/80 染色
盐水
CHOCK IgG1
抗-MCSF IgG1

图 12

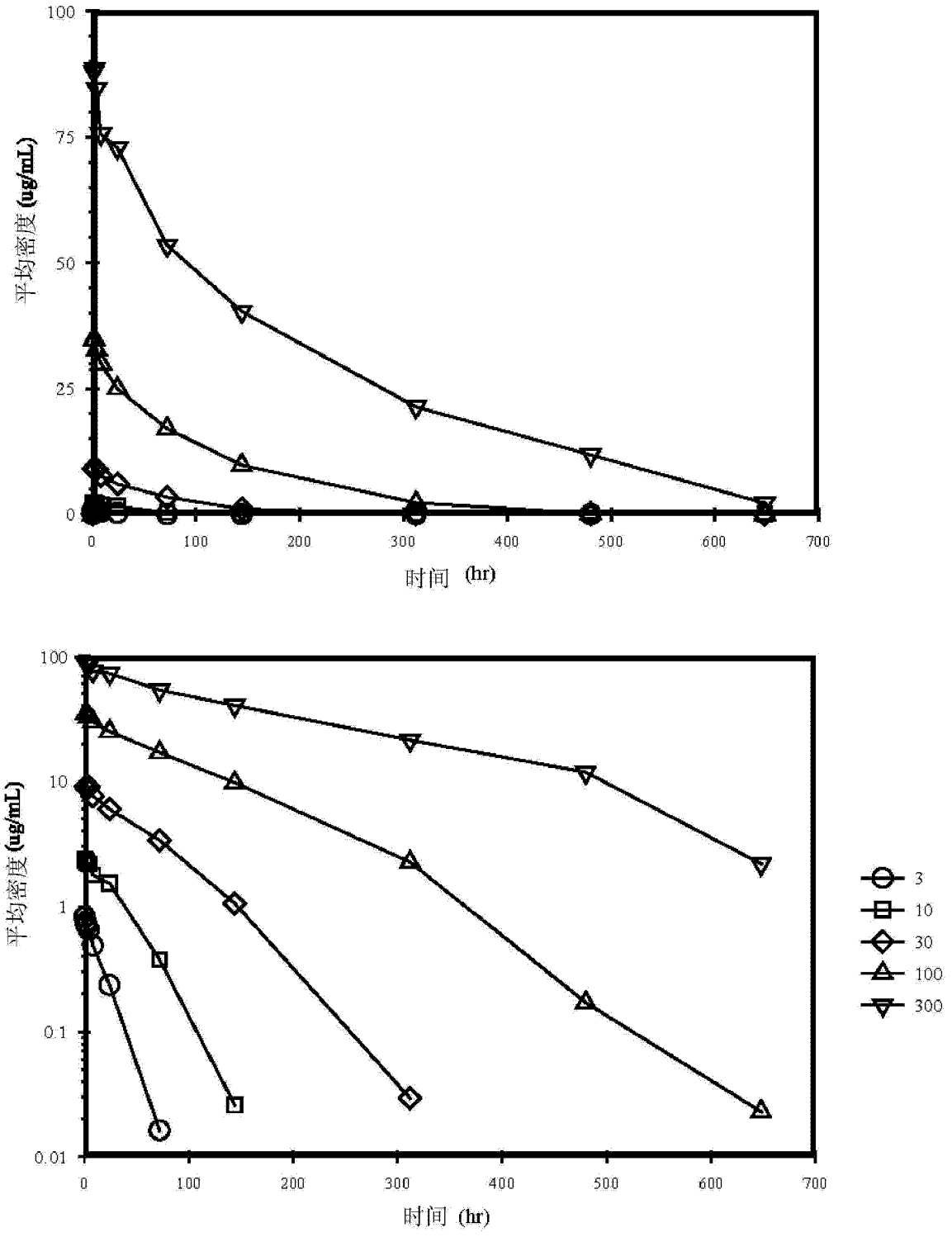

100
75
50
25
0
平均密度 (ug/mL)
0
100
200
300
400
500
600
700
时间 (hr)
100
10
1
0.1
0.01
平均密度 (ug/mL)
0
100
200
300
400
500
600
700
时间 (hr)
3
10
30
100
300

图 13

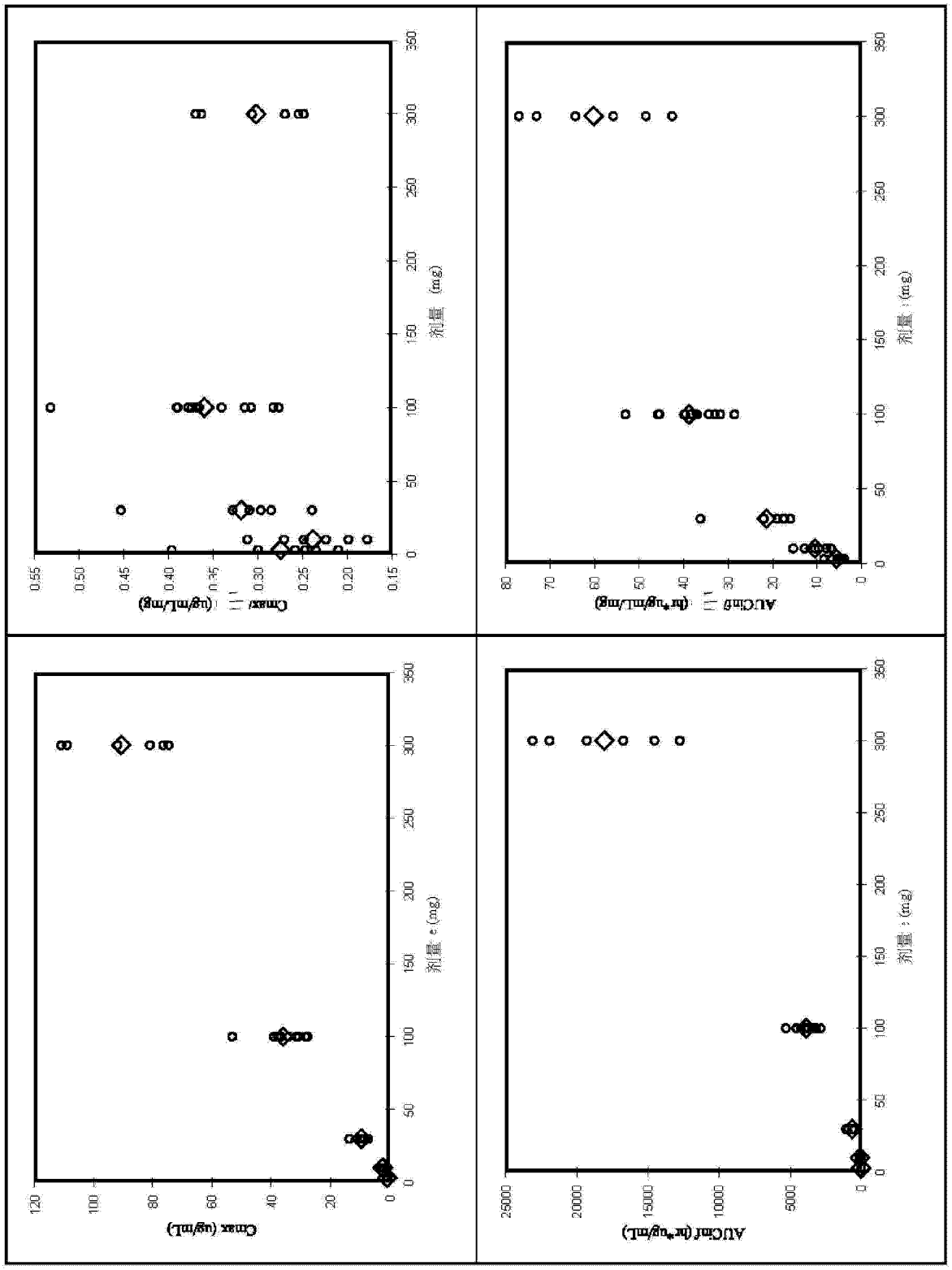

剂量 (mg)
AUCinf (hr*ug/mL)
AUCinf / 1 (hr*ug/mL/mg)
Cmax (ug/mL)
Cmax / 1 (ug/mL/mg)

图 14

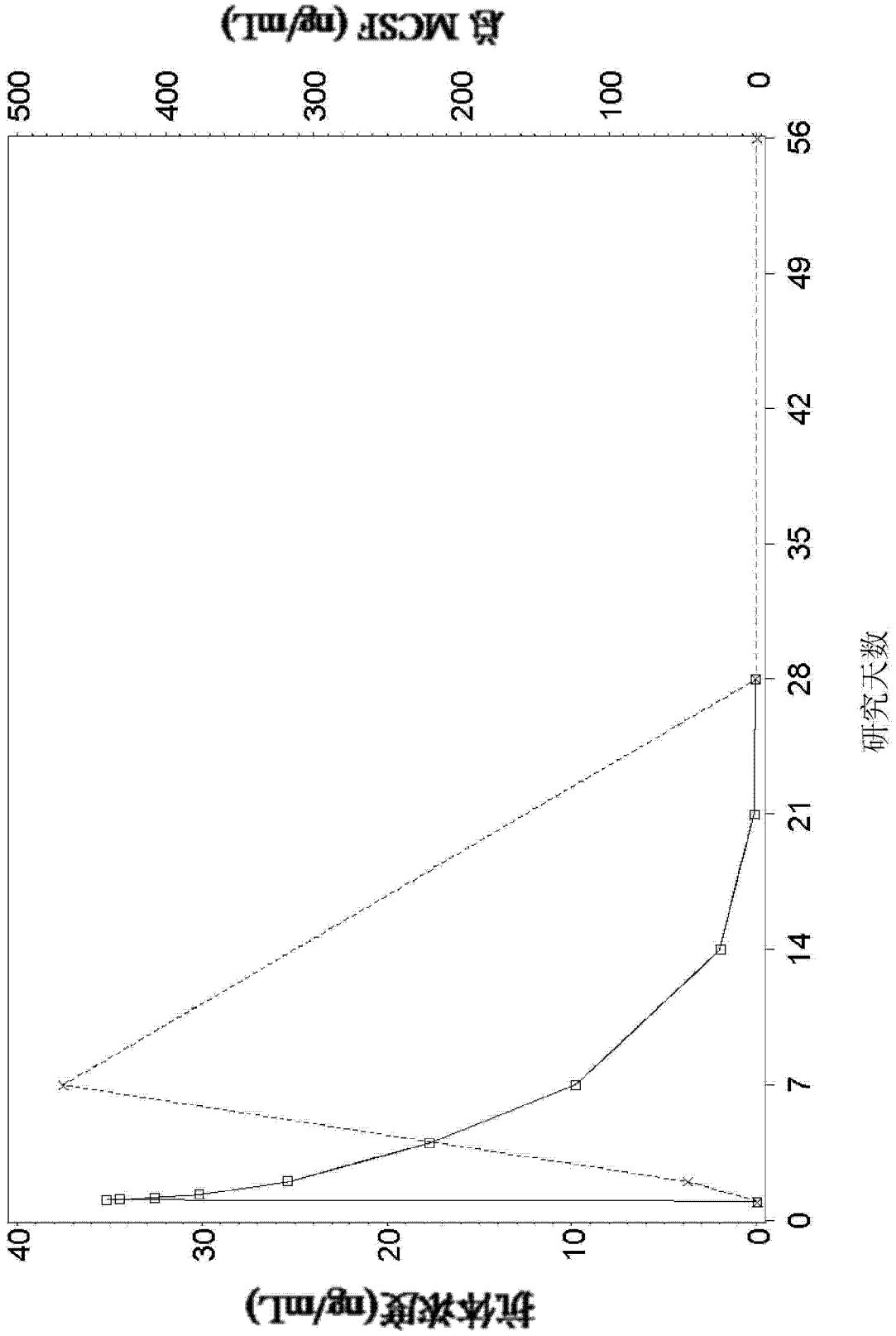

总 MCSF (ng/mL)
500
400
300
200
100
0
56
49
42
35
28
21
14
7
0
研究天数
40
30
20
10
0
抗体浓度(ng/mL)

图 15

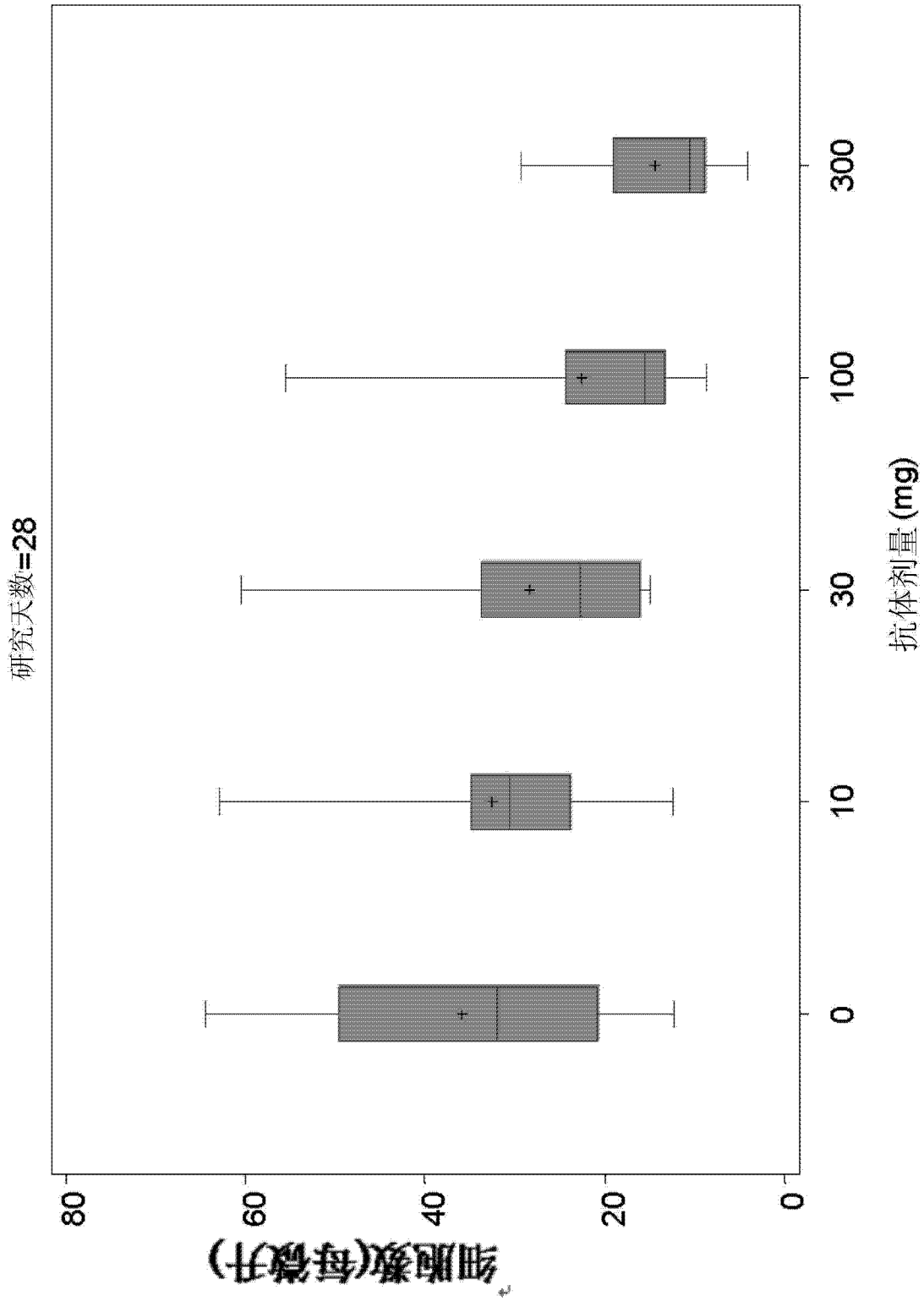


图 16

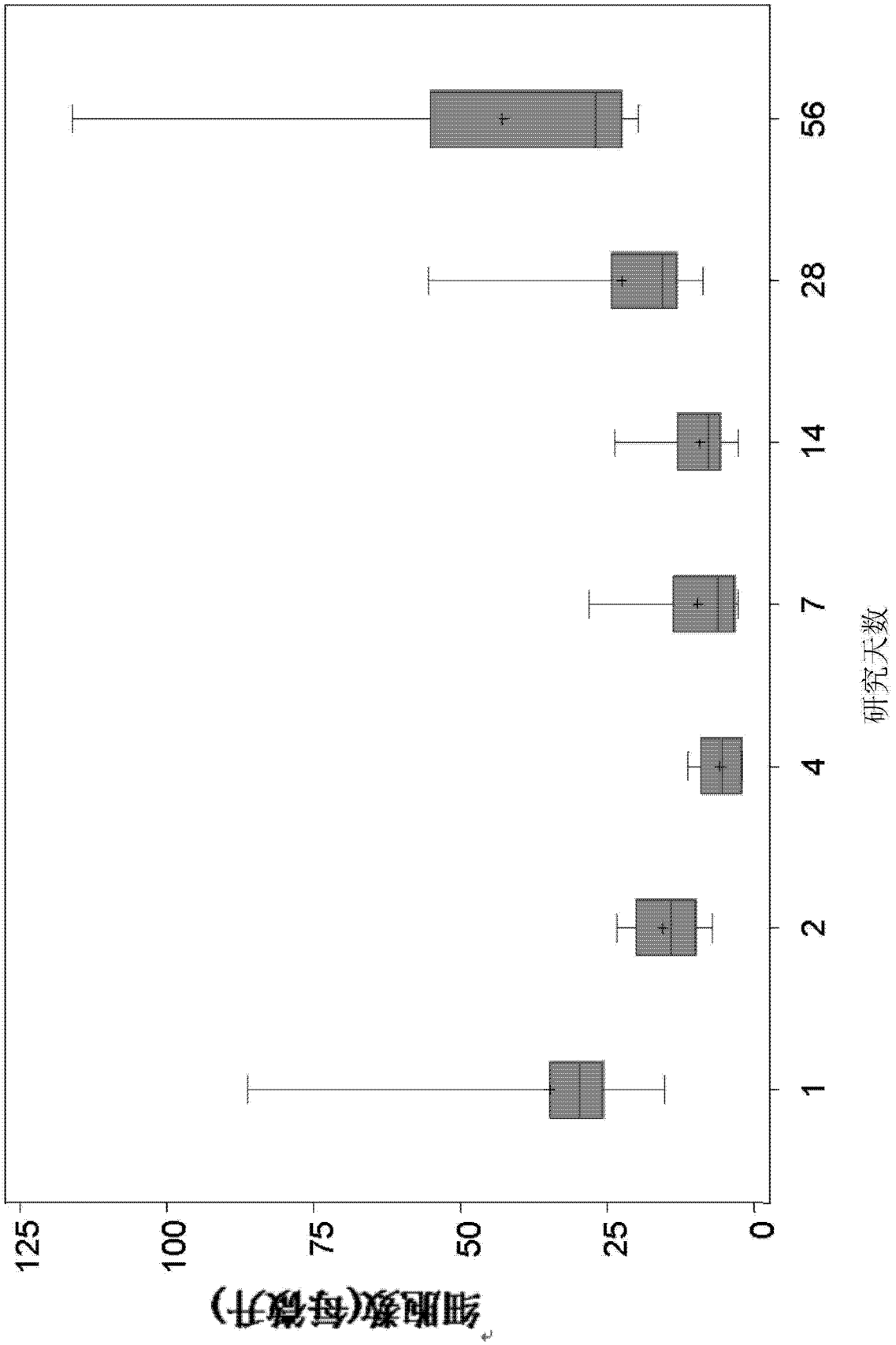


图 17

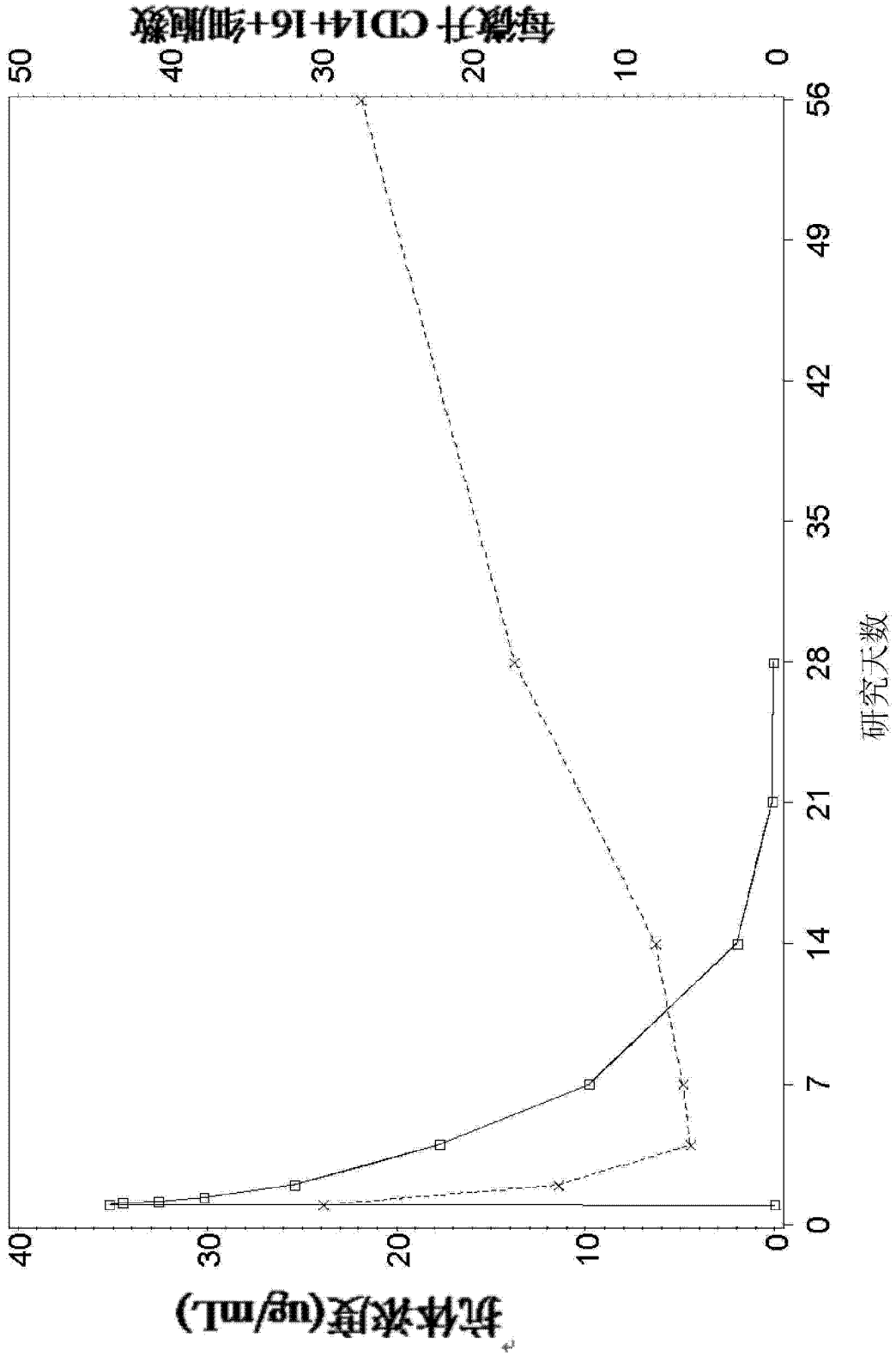

每微升 CD14+16+细胞数
50
40
30
20
10
0
56
49
42
35
28
21
14
7
0
研究天数
抗体浓度(ug/mL)
40
30
20
10
0

图 18

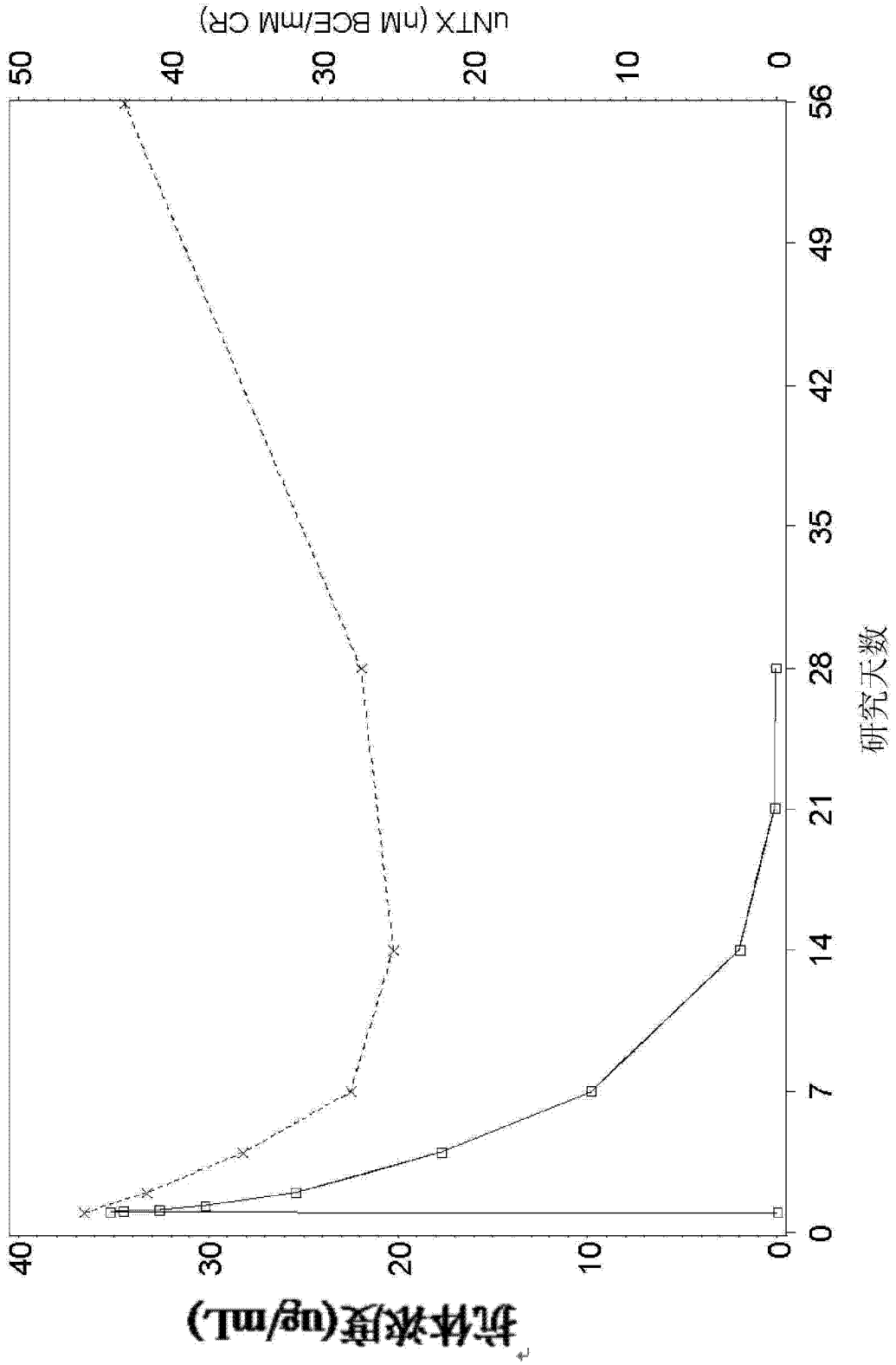

uNTX (nM BCE/mM CR)
0
10
20
30
40
50
抗体浓度(ug/mL)
0
10
20
30
40
0
7
14
21
28
35
42
49
56
研究天数

图 19

Abstract

The present invention relates to antibodies and antigen-binding portions thereof that specifically bind to a M-CSF, preferably human M-CSF, and that function to inhibit a M-CSF. The invention also relates to human anti-M-CSF antibodies and antigen-binding portions thereof. The invention also relates to antibodies that are chimeric, bispecific, derivatized, single chain antibodies or portions of fusion proteins. The invention also relates to isolated heavy and light chain immunoglobulins derived from human anti-M-CSF antibodies and nucleic acid molecules encoding such immunoglobulins. The present invention also relates to methods of making human anti-M-CSF antibodies, compositions comprising these antibodies and methods of using the antibodies and compositions for diagnosis and treatment. The invention also provides gene therapy methods using nucleic acid molecules encoding the heavy and/or light immunoglobulin molecules that comprise the human anti-M-CSF antibodies. The invention also relates to transgenic animals and transgenic plants comprising nucleic acid molecules of the present invention.