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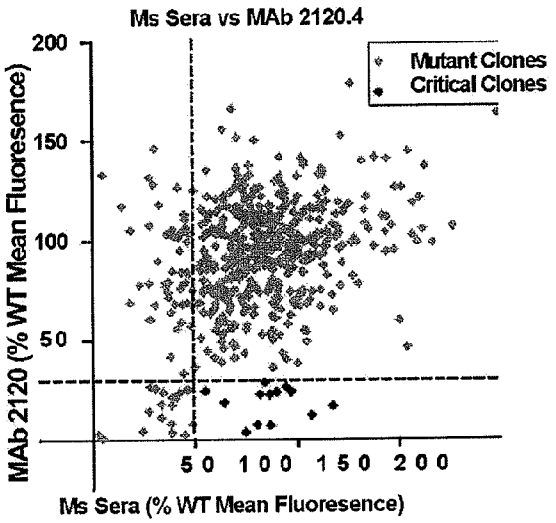
[Continued on next page]

(54) Title: ANTI-MCAM ANTIBODIES AND ASSOCIATED METHODS OF USE

FIG.1



(57) Abstract: The invention provides anti-MCAM antibodies that inhibit the ability of human MCAM to bind a laminin alpha-4 chain, dosage regimes, monitoring regimes, and treatment regimes with agent inhibiting MCAM expression.

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Declarations under Rule 4.17:

— *as to the identity of the inventor (Rule 4.17(i))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

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— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

ANTI-MCAM ANTIBODIES AND ASSOCIATED METHODS OF USE

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims the benefit of US Application Nos. 62/303,360 and 62/303,369 both filed March 3, 2016, each incorporated by reference in its entirety for all purposes.

REFERENCE TO A SEQUENCE LISTING, A TABLE, OR A COMPUTER PROGRAM LISTING

[0002] The Sequence Listing written in file 489314_SEQLST.txt, created on March 1, 2017, for "ANTI-MCAM ANTIBODIES AND ASSOCIATED METHODS OF USE" is 158,072 bytes. The information contained in this file is hereby incorporated by reference.

BACKGROUND

[0003] A subset of CD4+ T cells, termed TH17 cells (T helper 17 cells), has been implicated in the pathogenesis of a number of immune disorders including autoimmune diseases, particularly those neuroinflammatory conditions involving CNS infiltration of T cells, such as multiple sclerosis and the animal model, experimental autoimmune encephalomyelitis (EAE). TH17 cells have been reported to secrete a number of select cytokines including IL-17 and IL-22. TH17 cells have been reported to undergo specific recruitment and infiltration of tissue. MCAM has been reported to be expressed on TH17 cells and to bind laminin alpha-4 as a ligand.

SUMMARY OF THE CLAIMED INVENTION

[0004] The invention provides a method of treating or effecting prophylaxis of a patient having or at risk of a neuroinflammatory, autoimmune disease, immune disease mediated at least in part by TH17 cells, or cancer, comprising administering to the patient an effective regime of an antibody comprising a mature heavy chain variable region at least 90% identical to SEQ ID NO: 161, and a mature light chain variable region at least 90% identical to SEQ ID NO: 123, wherein the effective regime comprises a dose of 0.3 to 50 mg/kg administered on multiple occasions at intervals of 2-5 weeks.

[0005] In some methods, the antibody is a human IgG1 kappa antibody comprising a mature heavy chain variable region of SEQ ID NO: 161 and a mature light chain variable region of SEQ ID NO: 123.

[0006] In some methods, the antibody is administered as a component of a formulation comprising

(a) the antibody at a concentration within a range from about 10 mM to about 50 mM;

(b) one or more sugars and polyols ("sugar/polyol") selected from:

(i) sucrose present at a concentration within the range from about 200 mM to about 260 mM; and

(ii) trehalose present at a concentration within the range from about 200 mM to about 260 mM;

(c) histidine at 10-30 mM; and

(d) polysorbate 20 present at a concentration within the range from about 0.005% to about 0.05% by weight;

wherein the pharmaceutical formulation is characterized by a pH within the range from about 5.5 to about 7.

[0007] In some methods, the formulation comprises the antibody at 40 mg/ml, histidine at 20 mM, sucrose at 220 mM, polysorbate at 0.2 g/L and pH 6.

[0008] In some methods, the antibody is administered intravenously. In some methods, the antibody is administered in an interval of four weeks. In some methods, the dose is administered at least three times. In some methods, the dose is administered at least ten times. In some methods, the dose is administered at least until a steady state intravenous concentration is obtained. In some methods, the dose is administered for the rest of the patient's life. In some methods, the same dose is administered at the same interval on the multiple occasions.

[0009] In some methods the antibody is administered in a dosage of 0.3, 1.0, 3.0, 10, 30 or 50 mg/kg. In some methods the antibody is administered in a dosage of 0.3-1 mg/kg. In some methods the antibody is administered in a dosage of 1-3 mg/kg. In some methods the antibody is administered in a dosage of 3-10 mg/kg. In some methods the antibody is administered in a dosage of 10-30 mg/kg. In some methods the antibody is administered in a dosage of 30-50 mg/kg. In some methods the antibody is administered in a dosage of 3-10 mg/kg every two weeks administered intravenously. In some methods the antibody is administered in a dosage of 20-30 mg/kg every four weeks administered intravenously.

[0010] Such diseases include multiple sclerosis, psoriasis, psoriatic arthritis, Behcet's disease, Giant Cell Arteritis, Polymyalgia Rheumatica, Takayasu's Arteritis, Crohn's disease, rheumatoid arthritis, and granulomatous lung disease, such as chronic beryllium disease) and glioblastoma.

[0011] In some methods the antibody is administered in combination with methotrexate, a steroid, a nonsteroidal anti-inflammatory drug, or a combination thereof. In some methods the antibody is administered as a monotherapy.

[0012] The invention further provides a method of treating or effecting prophylaxis of a patient having or at risk of a neuroinflammatory, autoimmune disease, or immune disorder mediated at least in part by TH17 cells, comprising administering an inhibitor of expression of MCAM. In some methods the inhibitor is a zinc finger protein, a transcriptional activator like effector (TALE), an siRNA, an anti-sense RNA, a ribozyme, or Cas9 enzyme and a guide RNA that directs the Cas9 enzyme to binding human MCAM DNA.

[0013] The invention further provides a method of monitoring patients having or at risk of a neuroinflammatory, autoimmune disease, immune disease mediated at least in part by TH17 cells, or cancer, undergoing treatment with an antibody that specifically binds human MCAM and inhibits its interaction with lamin-alpha4, comprising measuring a level of MCAM expressed by CD4 T-cells of the patients or a level of MCAM extracellular domain in a blood sample of the patients, wherein a reduction of a level of MCAM expressed by CD4 T cells or an increase in MCAM extracellular in the blood responsive to treatment indicates a positive response to treatment.

[0014] In some methods, some patients show a positive response to treatment and some patients do not show a positive response to treatment, and a higher percentage of patients showing a positive response to treatment continue to undergo treatment with the antibody than patients not showing a positive response to treatment.

[0015] The invention further provides a method of treating or effecting prophylaxis of a patient having or at risk of psoriasis, psoriatic arthritis, graft versus host disease, giant cell arteritis, granulomatous lung disease, such as chronic beryllium disease, Crohn's disease, rheumatoid arthritis or glioblastoma, comprising administering to the patient an effective regime of pharmaceutical composition, wherein:

a. the pharmaceutical composition has a pH of about 6 and comprises about

i. 40 mg/ml of an antibody comprising a mature heavy chain variable region of SEQ ID NO: 161 and a mature light chain variable region of SEQ ID NO: 123,

ii. 20 mM histidine,

iii. 220 mM sucrose, and

iv. 0.02% polysorbate 20;

b. the effective regime comprises a dose of 1.0, 3.0 or 10-30 mg/kg administered intravenously every 2-5 weeks.

[0016] In some methods the antibody is administered in a dosage of 3 mg/kg and is administered about every four weeks. In some methods the antibody is administered in a dosage of 10 mg/kg and is administered about every four weeks. In some methods the antibody is administered in a dosage of 30 mg/kg and is administered about every four weeks.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] FIG. 1 depicts the identification of critical clones. The mean 2120.4.19 binding value plotted as a function of its mean surface expression value (gray diamonds). Thresholds of <30%

monoclonal antibody reactivity and >50% mouse sera binding were applied to identify clones (black diamonds) that were negative for antibody binding but positive for surface expression

[0018] FIGS. 2A-C. FIG. 2A a homology model of human MCAM, represented by a ribbon diagram. FIG. 2B depicts a partial alignment of human BCAM, human MCAM, and mouse MCAM sequences indicating residues of interest at position 141 (I141) and position 145 (P145) of human MCAM. FIG. 2C depicts a ribbon diagram depicting the location and exposure of the I141 and P145 residues of human MCAM.

[0019] FIG. 3A shows the alignment of sequences of the variable heavy chains for the following: rat 2120.4.19 anti-MCAM antibody (2120.4.19.6_VH_topo_pro; SEQ ID NO: 114); 2120 VH1 humanized anti-MCAM antibody (h2120VH1; SEQ ID NO: 115); 2120 VH2 humanized anti-MCAM antibody (h2120VH2; SEQ ID NO: 116); 2120 VH3 humanized anti-MCAM antibody (h2120VH3; SEQ ID NO: 117); 2120 VH4 humanized anti-MCAM antibody (h2120VH4; SEQ ID NO: 118); 2120 VH5 humanized anti-MCAM antibody (h2120VH5; SEQ ID NO: 119); and heavy chain human variable AF062133 IGHV2-26*01 sequence used as the framework donor (AF062133_VH; SEQ ID NO: 108). Kabat numbering is used and hypervariable regions (HVRs) grafted from the rat 2120.4.19.6 antibody to the variable heavy chain variable AF062133 IGHV2-26*01 framework are boxed. The S30T, I37V, L48I and K71R mutations combined with (i) mutations of the boxed N/D residues in CDR-H1, *e.g.*, N32S (VH3); N32Q (VH4); or G33A (VH5)), provides an N deamidation mutant. The bolded amino acid residues in the humanized antibody sequences differ from the corresponding residues in the rat antibody sequence. The position of canonical and interface amino acid residues that may affect CDR contact or CDR structure are indicated by an asterisk. Residues where mutations were focused due to the presence of *N*-deamination sites or *N*-glycosylation sites are shown in the bracketed box.

[0020] FIG. 3B shows the alignment of sequences of the variable light chains for the following: rat 2120.4.19.6 anti-MCAM antibody (2120.4.19.6_VL_topo_pro; SEQ ID NO: 120); 2120 VL1 humanized anti-MCAM antibody (h2120VL1 SEQ ID NO: 121); 2120 VL2 humanized anti-MCAM antibody (h2120VL2 SEQ ID NO: 122); 2120 VL3 humanized anti-MCAM antibody (h2120VL3 SEQ ID NO: 123); and light chain human variable X84343 IGKV2-26*01 sequence

used as the framework donor (X84343_VL SEQ ID NO: 124). Kabat numbering is used and hypervariable regions (HVRs) grafted from the rat 2120.4.19.6 antibody to the variable light chain variable X84343 IGKV2-26*01 framework are boxed. The bolded amino acid residues in the humanized antibody sequences differ from the corresponding residues in the rat antibody sequence. The position of canonical and interface amino acid residues that may affect CDR contact or CDR structure are indicated by an asterisk.

[0021] FIG. 4A shows the alignment of sequences of the mature heavy chain variable regions for the following: rat 2120.4.19 anti-MCAM antibody (2120.4.19.6_VH_topo_pro; SEQ ID NO: 114); 2120 VH1.Q1E humanized anti-MCAM antibody (h2120VH1.Q1E; SEQ ID NO: 157); 2120 VH2.Q1E humanized anti-MCAM antibody (h2120VH2.Q1E; SEQ ID NO: 158); 2120 VH3.Q1E humanized anti-MCAM antibody (h2120VH3.Q1E; SEQ ID NO: 159); 2120 VH4.Q1E humanized anti-MCAM antibody (h2120VH4.Q1E; SEQ ID NO: 160); 2120 VH5.Q1E humanized anti-MCAM antibody (h2120VH5.Q1E; SEQ ID NO: 161); and heavy chain human variable AF062133 IGHV2-26*01 sequence used as the framework donor (AF062133_VH; SEQ ID NO: 108). Kabat numbering is used and hypervariable regions (HVRs) grafted from the rat 2120.4.19.6 antibody to the variable heavy chain variable AF062133 IGHV2-26*01 framework are boxed. The position Q1E substitution is outlined by a box.

[0022] FIG. 4B shows the alignment of sequences of the variable light chains for the following: rat 2120.4.19.6 anti-MCAM antibody (2120.4.19.6_VL_topo_pro; SEQ ID NO: 120); 2120 VL1 humanized anti-MCAM antibody (h2120VL1; SEQ ID NO: 121); 2120 VL2 humanized anti-MCAM antibody (h2120VL2; SEQ ID NO: 122); 2120 VL3 humanized anti-MCAM antibody (h2120VL3; SEQ ID NO: 123); and light chain human variable X84343 IGKV2-26*01 sequence used as the framework donor (X84343_VL SEQ ID NO: 124). Kabat numbering is used and hypervariable regions (HVRs) grafted from the rat 2120.4.19.6 antibody to the variable light chain variable X84343 IGKV2-26*01 framework are boxed.

[0023] FIGS. 5A-C show MCAM expression is dose- and time-dependently down regulated by PRX003 treatment in hMCAM transgenic mice as shown in a single mouse after single injections. FIG. 5A shows MCAM expression at predose. FIGS. 5B & C show MCAM expression levels at six hours and 96 hours.

[0024] FIGS. 6A & B show expression and subsequent reduction of MCAM on NK cells after a single PRX-003 dose (FIG. 6A) and time-dependently down regulated by PRX003 treatment in hMCAM transgenic mice as shown in multiple mice after repeated injections (FIG 6B).

[0025] FIGS. 7A & B. FIG. 7A shows PRX003 selectively down regulates hMCAM and does not affect the viability of $MCAM^+$ cells. FIG. 7B shows PRX003 selectively down regulates mMCAM and does not affect the viability of $MCAM^+$ cells.

[0026] FIGS. 8A-H shows dynamic changes in soluble MCAM induced by PRX003 are tightly coupled with cellular downregulation of MCAM.

[0027] FIGS. 9A-C show anti-MCAM lowers inflammation and clinical score in a mouse model of EAE, known to involve TH17 cells: treatment period (FIG. 9A); $MCAM^+$ cells (FIG. 9B); and, $CD4^+/MCAM^+$ T cells (FIG 9C).

BRIEF DESCRIPTION OF THE SEQUENCES

[0028] SEQ ID NO: 1 is the nucleic acid sequence encoding the mature light chain variable region of antibody clone 17.

[0029] SEQ ID NO: 2 is the amino acid sequence of the mature light chain variable region of antibody clone 17.

[0030] SEQ ID NO: 3 is the amino acid sequence of CDRL1 of the antibody clone 17.

[0031] SEQ ID NO: 4 is the amino acid sequence of CDRL2 of the antibody clone 17.

[0032] SEQ ID NO: 5 is the amino acid sequence of CDRL3 of the antibody clone 17.

[0033] SEQ ID NO: 6 is the nucleic acid sequence encoding the mature heavy chain variable region of antibody clone 17.

[0034] SEQ ID NO: 7 is the amino acid sequence of the mature heavy chain variable region of antibody clone 17.

[0035] SEQ ID NO: 8 is the amino acid sequence of CDRH1 of the antibody clone 17.

[0036] SEQ ID NO: 9 is the amino acid sequence of CDRH2 of the antibody clone 17.

[0037] SEQ ID NO: 10 is the amino acid sequence of CDRH3 of the antibody clone 17.

[0038] SEQ ID NO: 11 is the amino acid sequence of human MCAM Accession No. CAA48332.

[0039] SEQ ID NO: 12 is the nucleic acid sequence encoding the mature light chain variable region of antibody clone 15.

[0040] SEQ ID NO: 13 is the amino acid sequence of the mature light chain variable region of antibody clone 15.

[0041] SEQ ID NO: 14 is the amino acid sequence of CDRL1 of the antibody clone 15.

[0042] SEQ ID NO: 15 is the amino acid sequence of CDRL2 of the antibody clone 15.

[0043] SEQ ID NO: 16 is the amino acid sequence of CDRL3 of the antibody clone 15.

[0044] SEQ ID NO: 17 is the nucleic acid sequence encoding the mature heavy chain variable region of antibody clone 15.

[0045] SEQ ID NO: 18 is the amino acid sequence of the mature heavy chain variable region of antibody clone 15.

[0046] SEQ ID NO: 19 is the amino acid sequence of CDRH1 of the antibody clone 15.

[0047] SEQ ID NO: 20 is the amino acid sequence of CDRH2 of the antibody clone 15.

[0048] SEQ ID NO: 21 is the amino acid sequence of CDRH3 of the antibody clone 15.

[0049] SEQ ID NO: 22 is the amino acid sequence of human MCAM domain 1 (residues 19-129).

[0050] SEQ ID NO: 23 is the amino acid sequence of human MCAM domain 2 (residues 139-242).

[0051] SEQ ID NO: 24 is the amino acid sequence of human MCAM domain 3 (residues 244-321).

[0052] SEQ ID NO: 25 is the amino acid sequence of human MCAM domain 4 (residues 355-424).

[0053] SEQ ID NO: 26 is the amino acid sequence of human MCAM domain 5 (residues 430-510).

[0054] SEQ ID NO: 27 is the amino acid sequence of an α4-chain isoform of human laminin 411 (Accession No. NP001098676).

[0055] SEQ ID NO: 28 is the amino acid sequence of an α4-chain isoform of human laminin 411 (Accession No. CAA48332).

[0056] SEQ ID NO: 29 is the nucleic acid sequence encoding the mature light chain variable region of antibody 1174.1.3.

[0057] SEQ ID NO: 30 is the amino acid sequence of the mature light chain variable region of antibody 1174.1.3.

[0058] SEQ ID NO: 31 is the amino acid sequence of CDRL1 of antibody 1174.1.3.

[0059] SEQ ID NO: 32 is the amino acid sequence of CDRL2 of antibody 1174.1.3.

[0060] SEQ ID NO: 33 is the amino acid sequence of CDRL3 of antibody 1174.1.3.

[0061] SEQ ID NO: 34 is the nucleic acid sequence encoding the mature heavy chain variable region of antibody 1174.1.3.

[0062] SEQ ID NO: 35 is the amino acid sequence of the mature heavy chain variable region of antibody 1174.1.3.

[0063] SEQ ID NO: 36 is the amino acid sequence of CDRH1 of antibody 1174.1.3.

[0064] SEQ ID NO: 37 is the amino acid sequence of CDRH2 of antibody 1174.1.3.

[0065] SEQ ID NO: 38 is the amino acid sequence of CDRH3 of antibody 1174.1.3.

[0066] SEQ ID NO: 39 is the nucleic acid sequence encoding the mature light chain variable region of antibody 1414.1.2.

[0067] SEQ ID NO: 40 is the amino acid sequence of the mature light chain variable region of antibody 1414.1.2.

[0068] SEQ ID NO: 41 is the amino acid sequence of CDRL1 of antibody 1414.1.2.

[0069] SEQ ID NO: 42 is the amino acid sequence of CDRL2 of antibody 1414.1.2.

[0070] SEQ ID NO: 43 is the amino acid sequence of CDRL3 of antibody 1414.1.2.

[0071] SEQ ID NO: 44 is the nucleic acid sequence encoding the mature heavy chain variable region of antibody 1414.1.2.

[0072] SEQ ID NO: 45 is the amino acid sequence of the mature heavy chain variable region of antibody 1414.1.2.

[0073] SEQ ID NO: 46 is the amino acid sequence of CDRH1 of antibody 1414.1.2.

[0074] SEQ ID NO: 47 is the amino acid sequence of CDRH2 of antibody 1414.1.2.

[0075] SEQ ID NO: 48 is the amino acid sequence of CDRH3 of antibody 1414.1.2.

[0076] SEQ ID NO: 49 is the nucleic acid sequence encoding the mature light chain variable region of antibody 1415.1.1.

[0077] SEQ ID NO: 50 is the amino acid sequence of the mature light chain variable region of antibody 1415.1.1.

[0078] SEQ ID NO: 51 is the amino acid sequence of CDRL1 of antibody 1415.1.1.

[0079] SEQ ID NO: 52 is the amino acid sequence of CDRL2 of antibody 1415.1.1.

[0080] SEQ ID NO: 53 is the amino acid sequence of CDRL3 of antibody 1415.1.1.

[0081] SEQ ID NO: 54 is the nucleic acid sequence encoding the mature heavy chain variable region of antibody 1415.1.1.

[0082] SEQ ID NO: 55 is the amino acid sequence of the mature heavy chain variable region of antibody 1415.1.1.

[0083] SEQ ID NO: 56 is the amino acid sequence of CDRH1 of antibody 1415.1.1.

[0084] SEQ ID NO: 57 is the amino acid sequence of CDRH2 of antibody 1415.1.1.

[0085] SEQ ID NO: 58 is the amino acid sequence of CDRH3 of antibody 1415.1.1.

[0086] SEQ ID NO: 59 is the nucleic acid sequence encoding the mature light chain variable region of antibody 1749.1.3.

[0087] SEQ ID NO: 60 is the amino acid sequence of the mature light chain variable region of antibody 1749.1.3.

[0088] SEQ ID NO: 61 is the amino acid sequence of CDRL1 of antibody 1749.1.3.

[0089] SEQ ID NO: 62 is the amino acid sequence of CDRL2 of antibody 1749.1.3.

[0090] SEQ ID NO: 63 is the amino acid sequence of CDRL3 of antibody 1749.1.3.

[0091] SEQ ID NO: 64 is the nucleic acid sequence encoding the mature heavy chain variable region of antibody 1749.1.3.

[0092] SEQ ID NO: 65 is the amino acid sequence of the mature heavy chain variable region of antibody 1749.1.3.

[0093] SEQ ID NO: 66 is the amino acid sequence of CDRH1 of antibody 1749.1.3.

[0094] SEQ ID NO: 67 is the amino acid sequence of CDRH2 of antibody 1749.1.3.

[0095] SEQ ID NO: 68 is the amino acid sequence of CDRH3 of antibody 1749.1.3.

[0096] SEQ ID NO: 69 is the nucleic acid sequence encoding a mature light chain variable region of antibody 2120.4.19.

[0097] SEQ ID NO: 70 is the amino acid sequence of the mature light chain variable region of antibody 2120.4.19 set forth in SEQ ID NO: 69.

[0098] SEQ ID NO: 71 is the amino acid sequence of a mature light chain variable region of antibody 2120.4.19.

[0099] SEQ ID NO: 72 is the amino acid sequence of a mature light chain variable region of antibody 2120.4.19.

[0100] SEQ ID NO: 73 is the amino acid sequence of CDRL1 of antibody 2120.4.19.

[0101] SEQ ID NO: 74 is the amino acid sequence of CDRL2 of antibody 2120.4.19.

[0102] SEQ ID NO: 75 is the amino acid sequence of CDRL3 of antibody 2120.4.19.

[0103] SEQ ID NO: 76 is the nucleic acid sequence encoding the mature heavy chain variable region of antibody 2120.4.19.

[0104] SEQ ID NO: 77 is the amino acid sequence of the mature heavy chain variable region of antibody 2120.4.19.

[0105] SEQ ID NO: 78 is the amino acid sequence of CDRH1 of antibody 2120.4.19.

[0106] SEQ ID NO: 79 is the amino acid sequence of CDRH2 of antibody 2120.4.19.

[0107] SEQ ID NO: 80 is the amino acid sequence of CDRH3 of antibody 2120.4.19.

[0108] SEQ ID NO: 81 is a nucleic acid sequence encoding a mature light chain variable region of antibody 2107.4.10.

[0109] SEQ ID NO: 82 is the amino acid sequence of the mature light chain variable region of antibody 2107.4.10 set forth in SEQ ID NO: 81.

[0110] SEQ ID NO: 83 is a nucleic acid sequence encoding a mature light chain variable region of antibody 2107.4.10.

[0111] SEQ ID NO: 84 is the amino acid sequence of the mature light chain variable region of antibody 2107.4.10 set forth in SEQ ID NO: 83.

[0112] SEQ ID NO: 85 is the amino acid sequence of CDRL1 of antibody 2107.4.10.

[0113] SEQ ID NO: 86 is the amino acid sequence of CDRL2 of antibody 2107.4.10.

[0114] SEQ ID NO: 87 is the amino acid sequence of CDRL3 of antibody 2107.4.10.

[0115] SEQ ID NO: 88 is the nucleic acid sequence encoding the mature heavy chain variable region of antibody 2107.4.10.

[0116] SEQ ID NO: 89 is the amino acid sequence of the mature heavy chain variable region of antibody 2107.4.10.

[0117] SEQ ID NO: 90 is the amino acid sequence of CDRH1 of antibody 2107.4.10.

[0118] SEQ ID NO: 91 is the amino acid sequence of CDRH2 of antibody 2107.4.10.

[0119] SEQ ID NO: 92 is the amino acid sequence of CDRH3 of antibody 2107.4.10.

[0120] SEQ ID NO: 93 is the amino acid sequence of the mature heavy chain variable region of antibody 1749.1.3.

[0121] SEQ ID NO: 94 is the amino acid sequence of the mature heavy chain variable region of humanized antibody 1749 version 1 (VH1).

[0122] SEQ ID NO: 95 is the amino acid sequence of the mature heavy chain variable region of humanized antibody 1749 version 2 (VH2).

[0123] SEQ ID NO: 96 is the amino acid sequence of the heavy chain variable framework donor U96282_VH.

[0124] SEQ ID NO: 97 is the amino acid sequence of the mature light chain variable region of antibody 1749.1.3.

[0125] SEQ ID NO: 98 is the amino acid sequence of the mature light chain variable region of humanized antibody 1749 version 1 (VL1).

[0126] SEQ ID NO: 99 is the amino acid sequence of the mature light chain variable region of humanized antibody 1749 version 2 (VL2).

[0127] SEQ ID NO: 100 is the amino acid sequence of the light chain variable framework donor X02990_VL.

[0128] SEQ ID NO: 101 is the amino acid sequence of the mature heavy chain variable region of antibody 2107.4.10.18.

[0129] SEQ ID NO: 102 is the amino acid sequence of the mature heavy chain variable region of humanized antibody 2107 version 1 (VH1).

[0130] SEQ ID NO: 103 is the amino acid sequence of the mature heavy chain variable region of humanized antibody 2107 version 2 (VH2).

[0131] SEQ ID NO: 104 is the amino acid sequence of the mature heavy chain variable region of humanized antibody 2107 version 3 (VH3).

[0132] SEQ ID NO: 105 is the amino acid sequence of the mature heavy chain variable region of humanized antibody 2107 version 4A (VH4A).

[0133] SEQ ID NO: 106 is the amino acid sequence of the mature heavy chain variable region of humanized antibody 2107 version 5A (VH5A).

[0134] SEQ ID NO: 107 is the amino acid sequence of the mature heavy chain variable region of humanized antibody 2107 version 6 (VH6).

[0135] SEQ ID NO: 108 is the amino acid sequence of the heavy chain variable framework donor AF062133_VH.

[0136] SEQ ID NO: 109 is the amino acid sequence of the mature light chain variable region of antibody 2107.4.10.18.

[0137] SEQ ID NO: 110 is the amino acid sequence of the mature light chain variable region of humanized antibody 2107 version 1 (VL1).

[0138] SEQ ID NO: 111 is the amino acid sequence of the mature light chain variable region of humanized antibody 2107 version 2 (VL2).

[0139] SEQ ID NO: 112 is the amino acid sequence of the mature light chain variable region of humanized antibody 2107 version 3 (VL3).

[0140] SEQ ID NO: 113 is the amino acid sequence of the light chain variable framework donor U86803.

[0141] SEQ ID NO: 114 is the amino acid sequence of the mature heavy chain variable region of antibody 2120.4.19.6.

[0142] SEQ ID NO: 115 is the amino acid sequence of the mature heavy chain variable region of humanized antibody 2120 version 1 (VH1).

[0143] SEQ ID NO: 116 is the amino acid sequence of the mature heavy chain variable region of humanized antibody 2120 version 2 (VH2).

[0144] SEQ ID NO: 117 is the amino acid sequence of the mature heavy chain variable region of humanized antibody 2120 version 3 (VH3).

[0145] SEQ ID NO: 118 is the amino acid sequence of the mature heavy chain variable region of humanized antibody 2120 version 4 (VH4).

[0146] SEQ ID NO: 119 is the amino acid sequence of the mature heavy chain variable region of humanized antibody 2120 version 5 (VH5).

[0147] SEQ ID NO: 120 is the amino acid sequence of the mature light chain variable region of antibody 2120.4.19.6.

[0148] SEQ ID NO: 121 is the amino acid sequence of the mature light chain variable region of humanized antibody 2120 version 1 (VL1).

[0149] SEQ ID NO: 122 is the amino acid sequence of the mature light chain variable region of humanized antibody 2120 version 2 (VL2).

[0150] SEQ ID NO: 123 is the amino acid sequence of the mature light chain variable region of humanized antibody 2120 version 3 (VL3).

[0151] SEQ ID NO: 124 is the amino acid sequence of the light chain variable framework donor X84343_VL.

[0152] SEQ ID NO: 125 is the amino acid sequence of a humanized heavy chain framework region.

[0153] SEQ ID NO: 126 is the amino acid sequence of a humanized heavy chain framework region.

[0154] SEQ ID NO: 127 is the amino acid sequence of a humanized heavy chain framework region.

[0155] SEQ ID NO: 128 is the amino acid sequence of a humanized heavy chain/light chain framework region.

[0156] SEQ ID NO: 129 is the amino acid sequence of a humanized light chain framework region.

[0157] SEQ ID NO: 130 is the amino acid sequence of a humanized light chain framework region.

[0158] SEQ ID NO: 131 is the amino acid sequence of a humanized light chain framework region.

[0159] SEQ ID NO: 132 is the amino acid sequence of a humanized light chain framework region.

[0160] SEQ ID NO: 133 is the amino acid sequence of a humanized heavy chain framework region.

[0161] SEQ ID NO: 134 is the amino acid sequence of a humanized heavy chain framework region.

[0162] SEQ ID NO: 135 is the amino acid sequence of a humanized heavy chain framework region.

[0163] SEQ ID NO: 136 is the amino acid sequence of a humanized heavy chain framework region.

[0164] SEQ ID NO: 137 is the amino acid sequence of a humanized heavy chain framework region.

[0165] SEQ ID NO: 138 is the amino acid sequence of a humanized heavy chain framework region.

[0166] SEQ ID NO: 139 is the amino acid sequence of CDRH1 of humanized antibody 2120 version 3 (VH3).

[0167] SEQ ID NO: 140 is the amino acid sequence of CDRH1 of humanized antibody 2120 version 4 (VH4).

[0168] SEQ ID NO: 141 is the amino acid sequence of CDRH1 of humanized antibody 2120 version 5 (VH5).

[0169] SEQ ID NO: 142 is the amino acid sequence of a humanized light chain framework region.

[0170] SEQ ID NO: 143 is the amino acid sequence of a humanized light chain framework region.

[0171] SEQ ID NO: 144 is the amino acid sequence of a humanized light chain framework region.

[0172] SEQ ID NO: 145 is the amino acid sequence of a humanized light chain framework region.

[0173] SEQ ID NO: 146 is the amino acid sequence of a humanized light chain framework region.

[0174] SEQ ID NO: 147 is the amino acid sequence of a humanized light chain framework region.

[0175] SEQ ID NO: 148 is the amino acid sequence of a humanized light chain framework region.

[0176] SEQ ID NO: 149 is the amino acid sequence of a humanized light chain framework region.

[0177] SEQ ID NO: 150 is the amino acid sequence of a humanized light chain framework region.

[0178] SEQ ID NO: 151 is the amino acid sequence of CDRH1 of humanized antibody 2107 version 1 (VH1).

[0179] SEQ ID NO: 152 is the amino acid sequence of CDRH1 of humanized antibody 2107 version 4 (VH4).

[0180] SEQ ID NO: 153 is the amino acid sequence of CDRH3 of humanized antibody 2120 version 1-5 (VH1-VH5).

[0181] SEQ ID NO: 154 is the amino acid sequence of a humanized light chain framework region.

[0182] SEQ ID NO: 155 is the amino acid sequence of a humanized heavy chain framework region.

[0183] SEQ ID NO: 156 is the amino acid sequence of the mature heavy chain variable region of antibody 2120.4.19.Q1E, wherein position 1 (Kabat numbering) is occupied by E.

[0184] SEQ ID NO: 157 is the amino acid sequence of the mature heavy chain variable region of humanized antibody 2120 version 1 Q1E (VH1.Q1E), wherein position 1 (Kabat numbering) is occupied by E.

[0185] SEQ ID NO: 158 is the amino acid sequence of the mature heavy chain variable region of humanized antibody 2120 version 2 Q1E (VH2.Q1E), wherein position 1 (Kabat numbering) is occupied by E.

[0186] SEQ ID NO: 159 is the amino acid sequence of the mature heavy chain variable region of humanized antibody 2120 version 3 Q1E (VH3.Q1E), wherein position 1 (Kabat numbering) is occupied by E.

[0187] SEQ ID NO: 160 is the amino acid sequence of the mature heavy chain variable region of humanized antibody 2120 version 4 Q1E (VH4.Q1E), wherein position 1 (Kabat numbering) is occupied by E.

[0188] SEQ ID NO: 161 is the amino acid sequence of the mature heavy chain variable region of humanized antibody 2120 version 5 Q1E (VH5.Q1E), wherein position 1 (Kabat numbering) is occupied by E.

[0189] SEQ ID NO: 162 is the nucleic acid sequence encoding an exemplary signal peptide that can be fused to a mature heavy chain or mature light chain variable region.

[0190] SEQ ID NO: 163 is the amino acid sequence of the exemplary signal peptide encoded by the nucleic acid sequence of SEQ ID NO: 162.

[0191] SEQ ID NO: 164 is the nucleic acid sequence encoding an exemplary signal peptide that can be fused to a mature heavy chain or mature light chain variable region.

[0192] SEQ ID NO: 165 is the amino acid sequence of the exemplary signal peptide encoded by the nucleic acid sequence of SEQ ID NO: 164.

[0193] SEQ ID NO: 166 is the nucleic acid sequence encoding an exemplary signal peptide that can be fused to a mature heavy chain or mature light chain variable region.

[0194] SEQ ID NO: 167 is the amino acid sequence of the exemplary signal peptide encoded by the nucleic acid sequence of SEQ ID NO: 166.

[0195] SEQ ID NO: 168 is the amino acid sequence of a humanized 2120 light chain constant region, with Arginine at the N-terminus.

[0196] SEQ ID NO: 169 is the amino acid sequence of a humanized 2120 light chain constant region, without Arginine at the N-terminus.

[0197] SEQ ID NO: 170 is the amino acid sequence of a humanized 2120 heavy chain constant region.

[0198] SEQ ID NO: 171 is the amino acid sequence of a BIP version heavy chain G1m3 allotype constant region.

[0199] SEQ ID NO: 172 is the amino acid sequence of a BIP version heavy chain G1m3 allotype constant region.

[0200] SEQ ID NO: 173 is the amino acid sequence of a mature light chain region of humanized antibody 2120 version 3 (VL3 + light chain constant region).

[0201] SEQ ID NO: 174 is the amino acid sequence of a mature heavy chain region of humanized antibody 2120 version 5 (VH5 + BIP version heavy chain G1m3 allotype constant region).

[0202] SEQ ID NO: 175 is the amino acid sequence of a mature heavy chain region of humanized antibody 2120 version 5 (VH5 + BIP version heavy chain G1m3 allotype constant region).

[0203] SEQ ID NO: 176 is the amino acid sequence of a mature heavy chain region of humanized antibody 2120 version 5 Q1E (VH5.Q1E + BIP version heavy chain G1m3 allotype constant region).

[0204] SEQ ID NO: 177 is the amino acid sequence of a mature heavy chain region of humanized antibody 2120 version 5 Q1E (VH5.Q1E + BIP version heavy chain G1m3 allotype constant region).

[0205] SEQ ID NO: 178 is the amino acid sequence of the mature heavy chain variable region of humanized antibody 2107 version 4B (VH4B).

[0206] SEQ ID NO: 179 is the amino acid sequence of the mature heavy chain variable region of humanized antibody 2107 version 5B (VH5B).

DEFINITIONS

[0207] Monoclonal antibodies are typically provided in isolated form. This means that an antibody is typically at least 50% w/w pure of proteins and other macromolecules arising from its production or purification but does not exclude the possibility that the monoclonal antibody is combined with an excess of pharmaceutical acceptable carrier(s) or other vehicle intended to facilitate its use. Sometimes monoclonal antibodies are at least 60%, 70%, 80%, 90%, 95 or 99% w/w pure of proteins and other macromolecules from production or purification.

[0208] Specific binding of a monoclonal antibody to its target antigen means an affinity of at least 10^6, 10^7, 10^8, 10^9, or 10^{10} M^{-1}. Specific binding is detectably higher in magnitude and distinguishable from non-specific binding occurring to at least one unrelated target. Specific binding can be the result of formation of bonds between particular functional groups or particular spatial fit (e.g., lock and key type) whereas nonspecific binding is usually the result of van der Waals forces. Specific binding does not however necessarily imply that a monoclonal antibody binds one and only one target.

[0209] The basic antibody structural unit is a tetramer of subunits. Each tetramer includes two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. This variable region is initially expressed linked to a cleavable signal peptide. The variable region without the signal peptide is sometimes referred to as a mature variable region. Thus, for example, a light chain mature variable region means a light chain variable region without the light chain signal peptide. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function.

[0210] Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, and define the antibody's isotype as IgG, IgM, IgA, IgD and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 10 or more amino acids. (See generally, Fundamental Immunology (Paul, W., ed., 2nd ed. Raven Press, N.Y., 1989, Ch. 7, incorporated by reference in its entirety for all purposes).

[0211] The mature variable regions of each light/heavy chain pair form the antibody binding site. Thus, an intact antibody has two binding sites. Except in bifunctional or bispecific antibodies, the two binding sites are the same. The chains all exhibit the same general structure of relatively conserved framework regions (FR) joined by three hypervariable regions, also called complementarity determining regions or CDRs. The CDRs from the two chains of each pair are aligned by the framework regions, enabling binding to a specific epitope. From N-terminal to C-terminal, both light and heavy chains comprise the domains 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), or Chothia & Lesk, J. Mol. Biol. 196:901-917 (1987); Chothia et al., Nature 342:878-883 (1989). Kabat also provides a widely used numbering convention (Kabat numbering) in which corresponding residues between different heavy chains or between different light chains are assigned the same number (e.g., H83 means position 83 by Kabat numbering in the mature heavy chain variable region; likewise position L36 means position 36 by Kabat numbering in the mature light chain variable region). Kabat

numbering is used throughout in referring to positions in the variable region of an antibody unless explicitly stated otherwise.

[0212] The term "antibody" includes intact antibodies and antigen binding fragments thereof. Typically, fragments compete with the intact antibody from which they were derived for specific binding to the target including separate heavy chains, light chains Fab, Fab', $F(ab')_2$, F(ab)c, diabodies, Dabs, nanobodies, and Fv. Fragments can be produced by recombinant DNA techniques, or by enzymatic or chemical separation of intact immunoglobulins.

[0213] The term "antibody" also includes a bispecific antibody, and/or a chimeric antibody, and/or a humanized antibody. A bispecific or bifunctional antibody is an artificial hybrid antibody having two different heavy/light chain pairs and two different binding sites (see, *e.g.*, Songsivilai and Lachmann, Clin. Exp. Immunol., 79:315-321 (1990); Kostelny et al., J. Immunol. 148:1547-53 (1992)). In some bispecific antibodies, the two different heavy/light chain pairs may include a humanized heavy chain/light chain pair and a heavy chain/light chain pair specific for a different epitope.

[0214] In some bispecific antibodies, one heavy chain light chain pair is a humanized antibody as further disclosed below and the heavy light chain pair is from an antibody that binds to a receptor expressed on the blood brain barrier, such as an insulin receptor, an insulin-like growth factor (IGF) receptor, a leptin receptor, or a lipoprotein receptor, or a transferrin receptor (Friden et al., PNAS 88:4771-4775, 1991; Friden et al., Science 259:373-377, 1993). Such a bispecific antibody can be transferred cross the blood brain barrier by receptor-mediated transcytosis. Brain uptake of the bispecific antibody can be further enhanced by engineering the bi-specific antibody to reduce its affinity to the blood brain barrier receptor. Reduced affinity for the receptor resulted in a broader distributioin in the brain (see, e.g., Atwal. et al. *Sci. Trans. Med.* **3,** 84ra43, 2011; Yu et al. *Sci. Trans. Med.* **3,** 84ra44, 2011).

[0215] Exemplary bispecific antibodies can also be (1) a dual-variable-domain antibody (DVD-Ig), where each light chain and heavy chain contains two variable domains in tandem through a short peptide linkage (Wu et al., Generation and Characterization of a Dual Variable Domain Immunoglobulin (DVD-Ig™) Molecule, In: Antibody Engineering, Springer Berlin Heidelberg (2010)); (2) a Tandab, which is a fusion of two single chain diabodies resulting in a tetravalent

bispecific antibody that has two binding sites for each of the target antigens; (3) a flexibody, which is a combination of scFvs with a diabody resulting in a multivalent molecule; (4) a so called "dock and lock" molecule, based on the "dimerization and docking domain" in Protein Kinase A, which, when applied to Fabs, can yield a trivalent bispecific binding protein consisting of two identical Fab fragments linked to a different Fab fragment; (5) a so-called Scorpion molecule, comprising, e.g., two scFvs fused to both termini of a human Fc-region. Examples of platforms useful for preparing bispecific antibodies include but are not limited to BiTE (Micromet), DART (MacroGenics), Fcab and Mab2 (F-star) , Fc-engineered IgGl (Xencor) or DuoBody (based on Fab arm exchange, Genmab).

[0216] The term "epitope" refers to a site on an antigen to which an antibody binds. An epitope can be formed from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of one or more proteins. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation. Methods of determining spatial conformation of epitopes include, for example, x-ray crystallography and 2-dimensional nuclear magnetic resonance. See, *e.g.*, Epitope Mapping Protocols, in Methods in Molecular Biology, Vol. 66, Glenn E. Morris, Ed. (1996).

[0217] An "antagonist" antibody or other binding agent is one which inhibits a biological activity of the antigen it binds. Such antibodies may substantially or completely inhibit the biological activity of the antigen.

[0218] The terms "biological activity" and "biologically active" with regard to MCAM refer to its ability to specifically bind its ligand (a laminin α4 chain, *e.g.*, the α4 chain of laminin 411) and/or to facilitate the infiltration of MCAM-expressing cells, *e.g.*, TH17 cells, into the CNS.

[0219] "Inhibit" means an agent decreases the biological activity of at least one target, for example MCAM. Such an inhibitor inhibits the activity of at least one target by at least about at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 95% or at least 100%.

[0220] A "subject" includes a human or other mammalian subject that receives either prophylactic or therapeutic treatment.

[0221] For purposes of classifying amino acids substitutions as conservative or nonconservative, amino acids are grouped as follows: Group I (hydrophobic side chains): met, ala, val, leu, ile; Group II (neutral hydrophilic side chains): cys, ser, thr; Group III (acidic side chains): asp, glu; Group IV (basic side chains): asn, gln, his, lys, arg; Group V (residues influencing chain orientation): gly, pro; and Group VI (aromatic side chains): trp, tyr, phe. Conservative substitutions involve substitutions between amino acids in the same class. Non-conservative substitutions constitute exchanging a member of one of these classes for a member of another.

[0222] Percentage sequence identities are determined with antibody sequences maximally aligned by the Kabat numbering convention. After alignment, if a subject antibody region (e.g., the entire mature variable region of a heavy or light chain) is being compared with the same region of a reference antibody, the percentage sequence identity between the subject and reference antibody regions is the number of positions occupied by the same amino acid in both the subject and reference antibody region divided by the total number of aligned positions of the two regions, with gaps not counted, multiplied by 100 to convert to percentage.

[0223] Compositions or methods "comprising" one or more recited elements may include other elements not specifically recited. For example, a composition that comprises antibody may contain the antibody alone or in combination with other ingredients.

[0224] Designation of a range of values includes all integers within or defining the range, and all subranges defined by integers within the range.

[0225] Unless otherwise apparent from the context, the term "about" encompasses values within a standard margin of error of measurement (SEM) of a stated value.

[0226] The terms "2120.4.19", "m2120", "mouse 2120" antibody refer to a rodent derived monoclonal antibody clone having a mature variable heavy chain corresponding to SEQ ID NO: 114 and a mature variable light chain corresponding to SEQ ID NO: 120. "Humanized 2120" or

"hu2120" refers to humanized variants of the 2120.4.19 clone. Statistical significance means $p \leq 0.05$.

DETAILED DESCRIPTION

General

[0227] The present application among other things dosage regimes, and monitoring regimes for patients treated with antibodies inhibiting MCAM interaction with lamin-alpha4. Methods of treatment with inhibitors of MCAM expression are also provided.

I. TARGET MOLECULES

[0228] Natural human wild-type MCAM (melanoma cell adhesion molecule, also known as CD146 and MUC18) is a protein of 646 amino acids having the following amino acid sequence:

MGLPRLVCAFLLAACCCCPRVAGVPGEAEQPAPELVEVEVGSTALLKCGLSQSQGNLS
HVDWFSVHKEKRTLIFRVRQGQGQSEPGEYEQRLSLQDRGATLALTQVTPQDERIFLCQ
GKRPRSQEYRIQLRVYKAPEEPNIQVNPLGIPVNSKEPEEVATCVGRNGYPIPQVIWYKN
GRPLKEEKNRVHIQSSQTVESSGLYTLQSILKAQLVKEDKDAQFYCELNYRLPSGNHMK
ESREVTVPVFYPTEKVWLEVEPVGMLKEGDRVEIRCLADGNPPPHFSISKQNPSTREAEE
ETTNDNGVLVLEPARKEHSGRYECQAWNLDTMISLLSEPQELLVNYVSDVRVSPAAPER
QEGSSLTLTCEAESSQDLEFQWLREETDQVLERGPVLQLHDLKREAGGGYRCVASVPSI
PGLNRTQLVKLAIFGPPWMAFKERKVWVKENMVLNLSCEASGHPRPTISWNVNGTASE
QDQDPQRVLSTLNVLVTPELLETGVECTASNDLGKNTSILFLELVNLTTLTPDSNTTTGL
STSTASPHTRANSTSTERKLPEPESRGVVIVAVIVCILVLAVLGAVLYFLYKKGKLPCRRS
GKQEITLPPSRKTELVVEVKSDKLPEEMGLLQGSSGDKRAPGDQGEKYIDLRH (SEQ ID NO: 11).

[0229] (GenBank database under Accession Number AAA20922.1 (CAA48332)). MCAM is a cell surface glycoprotein belonging to the immunoglobulin superfamily involved in cell adhesion, and in cohesion of the endothelial monolayer at intercellular junctions in vascular tissue. It also promotes tumor progression of many cancers, such as solid tumors, including melanoma and prostate cancer. It is known to interact in a homotypic/homophilic manner and

may also bind to other ligands. The human MCAM includes five immunoglobulin domains (1: amino acid residues 24-129; 2: amino acid residues 139-242; 3: amino acid residues 244-330; 4: amino acid residues 335-424; and 5: amino acid residues 430-510), shown as SEQ ID NOS:22-26. Approximately residues 24-559 constitute the extracellular domain.

[0230] Unless otherwise apparent from the context, reference to MCAM or its fragments includes the natural human wildtype amino acid sequences indicated above, and human allelic variants thereof.

[0231] Laminin α4 refers to one of the polypeptide chains found in laminin molecules, which are expressed in the basal lamina (of the basement membrane), a protein network foundation for most cells and organs. Laminins are known to bind to cell membranes through plasma membrane molecules and contribute to cell attachment. The laminin α4 chain typically forms a complex with a laminin β-chain, and a laminin γ-chain. The laminin α4 chain is found in numerous laminin molecules including laminin 411 (laminin 8 or α4β1γ1); laminin 421 (laminin 9 or α4β2γ1), and laminin 423 (laminin 14 or α4β2γ3). There are two main isoforms of the human laminin α4-chain: GenBank Accession Nos. NP001098676 and NP001098677 (SEQ ID NOS:27 and 28, respectively). "Laminin 411" refers to a trimeric polypeptide complex made up of three polypeptide subunits or chains: α4-chain, a β1-chain, and a γ1-chain.

III. Antibodies

A. Antibody specificity

[0232] The present methods employ antibodies to MCAM that fully or partially inhibits its binding to laminin-alpha 4. Although practice of the invention is not dependent on understanding of mechanism, it is believed such inhibition inhibits MCAM-expressing cells e.g.., a TH17 cells from infiltrating or migrating into a subject's tissue. Examples of such antibodies are described in WO2012/170071, WO2014/039975, WO2015/136470, WO2015/136469 and US 14/656,596, each incorporated by reference in its entirety.

[0233] Some such antibodies are humanized. A humanized antibody is a genetically engineered antibody in which the CDRs from a non-human "donor" antibody (i.e., 2120.4.19) are grafted into human "acceptor" antibody sequences (see, e.g., Queen, US 5,530,101 and

5,585,089; Winter, US 5,225,539, Carter, US 6,407,213, Adair, US 5,859,205 6,881,557, Foote, US 6,881,557). The acceptor antibody sequences can be, for example, a mature human antibody sequence, a composite of such sequences, a consensus sequence of human antibody sequences, or a germline region sequence. The human acceptor antibody sequences can optionally be selected from among the many known human antibody sequences to provide a high degree of sequence identity (e.g., 65-85% identity) between a human acceptor sequence variable region frameworks and corresponding variable region frameworks of a donor antibody chain. Thus, a humanized antibody is an antibody having some or all CDRs entirely or substantially from a donor antibody and variable region framework sequences and constant regions, if present, entirely or substantially from human antibody sequences. Similarly a humanized heavy chain has at least one, two and usually all three CDRs entirely or substantially from a donor antibody heavy chain, and a heavy chain variable region framework sequence and heavy chain constant region, if present, substantially from human heavy chain variable region framework and constant region sequences. Similarly a humanized light chain has at least one, two and usually all three CDRs entirely or substantially from a donor antibody light chain, and a light chain variable region framework sequence and light chain constant region, if present, substantially from human light chain variable region framework and constant region sequences. Other than nanobodies and dAbs, a humanized antibody comprises a humanized heavy chain and a humanized light chain. A CDR in a humanized antibody is substantially from a corresponding CDR in a non-human antibody when at least 85%, 90%, 95% or 100% of corresponding residues (as defined by Kabat) are identical between the respective CDRs, except CDRH1 can have up to two substitutions and CHDRH2 can have substitutions at positions H60-65. The variable region framework sequences of an antibody chain or the constant region of an antibody chain are substantially from a human variable region framework sequence or human constant region respectively when at least 85%, 90%, 95% or 100% of corresponding residues defined by Kabat are identical.

[0234] Although humanized antibodies often incorporate all six CDRs (preferably as defined by Kabat) from a mouse antibody, they can also be made with less than all CDRs (e.g., at least 3, 4, or 5 CDRs) from a mouse antibody (e.g., Pascalis et al., J. Immunol. 169:3076, 2002; Vajdos et al., Journal of Molecular Biology, 320: 415-428, 2002; Iwahashi et al., Mol. Immunol. 36:1079-1091, 1999; Tamura et al, Journal of Immunology, 164:1432-1441, 2000).

[0235] In some antibodies only part of the CDRs, namely the subset of CDR residues required for binding, termed the SDRs, are needed to retain binding in a humanized antibody. CDR residues not contacting antigen and not in the SDRs can be identified based on previous studies (for example residues H60-H65 in CDR H2 are often not required), from regions of Kabat CDRs lying outside Chothia hypervariable loops (Chothia, J. Mol. Biol. 196:901, 1987), by molecular modeling and/or empirically, or as described in Gonzales et al., Mol. Immunol. 41: 863, 2004. In such humanized antibodies at positions in which one or more donor CDR residues is absent or in which an entire donor CDR is omitted, the amino acid occupying the position can be an amino acid occupying the corresponding position (by Kabat numbering) in the acceptor antibody sequence. The number of such substitutions of acceptor for donor amino acids in the CDRs to include reflects a balance of competing considerations. Such substitutions are potentially advantageous in decreasing the number of mouse amino acids in a humanized antibody and consequently decreasing potential immunogenicity. However, substitutions can also cause changes of affinity, and significant reductions in affinity are preferably avoided. Positions for substitution within CDRs and amino acids to substitute can also be selected empirically.

[0236] The 2120.4.19 rat antibody against MCAM was disclosed in WO2014/039975 and is defined herein by SEQ ID NOs: 69-80. Residues 145, 167, 175, 206, 207, 216 and 225 of human MCAM contribute most to the epitope of the 2120.4.19 antibody (WO2015/136469 and WO2015/136470). Chimeric, veneered, and humanized forms of the 2120.4.19 antibody were also disclosed in the '975 application. The disclosed humanized forms are defined herein as SEQ ID NOs: 115-119, 121-123, 139-141, and 153. The disclosed forms including any permutation of a humanized heavy chain and humanized light chain represented by these SEQ ID NOS. can be used in some aspects of the present invention, such as pharmaceutical compositions and formulations.

[0237] Additional humanized forms of the 2120.4.19 antibody in which glutamine is substituted to glutamic acid at position 1 (Kabat numbering) of the heavy chain variable region (i.e. Q1E) are disclosed in US14/656,596 filed March 12, 2015 and herein. The Q1E substitution in the heavy chain variable region is a conservative substitution not expected to produce a substantial effect on the binding characteristics of the antibody, but which can improve antibody stability.

[0238] Unless otherwise apparent from the context, the following description includes the humanized antibodies disclosed in WO2014/039975 and the Q1E variants disclosed in US14/656,596.

[0239] Such antibodies include antibodies comprising a heavy chain variable region comprising Kabat CDR1 of SEQ ID NO: 78: GFSLTSNGVS; Kabat CDR2 of SEQ ID NO: 79: AISSGGTTYYNSAFKS; and Kabat CDR3 of SEQ ID NO: 80: RYGYGWYFDF. Some antibodies comprise a light chain variable region comprising Kabat CDR1 of SEQ ID NO: 73: KASQNIYNSLA; Kabat CDR2 of SEQ ID NO: 74: NANSLQT; and Kabat CDR3 of SEQ ID NO: 75: QQFYSGYT. Some such antibodies comprise an N32S substitution or an N32Q substitution in Kabat CDR1 of SEQ ID NO: 78, and some comprise a G33A substitution in Kabat CDR1 of SEQ ID NO: 78. These substitutions have been found to offer improved characteristics including an increase in antibody affinity and potency.

[0240] Other provided anti-MCAM antibodies are antibodies in which the mature heavy chain variable region has at least 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 161, and the mature light chain variable region has at least 90%, 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID NO: 123. Some such antibodies include three heavy chain and three light chain CDRs entirely or substantially identical to the CDR regions of the donor 2120.4.19 antibody. If not identical, CDRs preferably have substitutions at a type and position defined herein, such as in the previous paragraph. The CDR regions can be defined by any conventional definition (e.g., Chothia) but are preferably as defined by Kabat.

[0241] Any of the above described antibodies can be humanized antibodies. Some humanized antibodies comprise a mature heavy chain variable region comprising the three Kabat CDRs of SEQ ID NO: 161 (which are the same as the CDRs of SEQ ID NO: 156) except that position 32 (Kabat numbering) can be N, S, or Q, and position 33 (Kabat numbering) can be G or A, and a mature light chain variable region comprising the three Kabat CDRs of SEQ ID NO: 123 (which are the same as the CDRs of SEQ ID NO: 120), preferably wherein the mature heavy chain variable region is at least 90% identical to SEQ ID NO: 161, and preferably wherein the mature light chain variable region is at least 90% identical to SEQ ID NO: 123. Any such antibody can have either Q or E (i.e., Q1E substitution) at position H1 by Kabat numbering.

[0242] The antibodies provided herein having a Q1E substitution in the mature heavy chain variable region include antibodies comprising a mature heavy chain variable region having the amino acid sequence of SEQ ID NO: 156 (i.e., 2120.4.19.Q1E), SEQ ID NO: 157, SEQ ID NO: 158, SEQ ID NO: 159, SEQ ID NO: 160, or SEQ ID NO: 161. Some such antibodies comprise a mature light chain variable region having the amino acid sequence designated SEQ ID NO: 120, SEQ ID NO: 121, SEQ ID NO: 122, or SEQ ID NO: 123. The mature heavy chain and light chain variable regions can be combined in any possible permutation. An exemplary combination is an antibody that comprises the mature heavy chain variable region having the amino acid sequence of SEQ ID NO: 161, and the mature light chain variable region having the amino acid sequence designated SEQ ID NO: 123. Forms of these antibodies without the Q1E substitution, such as have been described in PCT/US2013/058773, can also be used in some aspects of the invention, such as pharmaceutical compositions and formulations.

[0243] Further provided are antibodies in which the heavy chain mature variable region has at least 90%, 95%, 96%, 97%, 98%, 99% or 100 % sequence identity to the amino acid sequence of any of SEQ ID NO: 156 (i.e., 2120.4.19.Q1E), SEQ ID NO: 157, SEQ ID NO: 158, SEQ ID NO: 159, SEQ ID NO: 160, or SEQ ID NO: 161 and the light chain has at least 90%,95%, 96%, 97%, 98% or 99% sequence identity to any of SEQ ID NO: 120, SEQ ID NO: 121, SEQ ID NO: 122, or SEQ ID NO: 123. Such antibodies are preferably humanized. Any such antibody can have either Q or E (i.e., Q1E substitution) at position H1 by Kabat numbering.

[0244] Variants of disclosed SEQ ID NOs typically differ from the mature heavy chain and light chain variable region sequences by a small number (e.g., typically no more than 1, 2, 3, 5 or 10 in either the light chain or heavy chain mature variable region framework, or both) of replacements, deletions or insertions. Any changes are preferably conservative substitutions.

[0245] Also provided are chimeric and veneered forms of the 2120.4.19 antibody. A chimeric antibody is an antibody in which the mature variable regions of light and heavy chains of a non-human antibody (e.g., a mouse) are combined with human light and heavy chain constant regions. Such antibodies substantially or entirely retain the binding specificity of the mouse antibody, and are about two-thirds human sequence.

[0246] A veneered antibody is a type of humanized antibody that retains some and usually all of the CDRs and some of the non-human variable region framework residues of a non-human antibody but replaces other variable region framework residues that may contribute to B- or T-cell epitopes, for example exposed residues with residues from the corresponding positions of a human antibody sequence (Padlan, Mol. Immunol. 28:489, 1991). The result is an antibody in which the CDRs are entirely or substantially from a non-human antibody and the variable region frameworks of the non-human antibody are made more human-like by the substitutions.

[0247] Human antibodies against MCAM are provided by a variety of techniques described below. Some human antibodies are selected by competitive binding experiments, by the phage display method of Winter, above, or otherwise, to have the same epitope specificity as a particular non-human antibody, such as 2120.4.19. Human antibodies can also be screened for a particular epitope specificity by using only a fragment of MCAM as the target antigen, and/or by screening antibodies against a collection of deletion mutants of MCAM.

[0248] Methods for producing human antibodies include the trioma method of Oestberg et al., *Hybridoma* 2:361-367 (1983); Oestberg, U.S. Patent No. 4,634,664; and Engleman et al., US Patent 4,634,666, use of transgenic mice including human immunoglobulin genes (see, e.g., Lonberg et al., WO93/12227 (1993); US 5,877,397, US 5,874,299, US 5,814,318, US 5,789,650, US 5,770,429, US 5,661,016, US 5,633,425, US 5,625,126, US 5,569,825, US 5,545,806, *Nature* 148, 1547-1553 (1994), *Nature Biotechnology* 14, 826 (1996), Kucherlapati, WO 91/10741 (1991) and phage display methods (see, .e.g. Dower et al., WO 91/17271 and McCafferty et al., WO 92/01047, US 5,877,218, US 5,871,907, US 5,858,657, US 5,837,242, US 5,733,743 and US 5,565,332.

[0249] Chimeric, humanized (including veneered) and human antibodies are typically produced by recombinant expression as described above.

B. Methods of Screening Antibodies for Activity

[0250] The inhibitory activity of the MCAM antibodies described herein can be assayed by various methods including competitive binding assays with antibodies that bind the same or a

substantially similar epitope (e.g., m2120) and blocking of MCAM binding with its ligand, the laminin α4 chain of laminin 411.

[0251] For example, the activity of MCAM antibodies to inhibit the interaction between MCAM and the laminin α4 chain of laminin 411 can be screened as follows. MCAM-expressing cells are (a) incubating with a recombinant polypeptide comprising a laminin α4 chain, *e.g.*, an α4 chain of laminin 411, in the presence or absence of a candidate antibody; (b) monitoring the level of binding of the laminin α4 to the cells, e.g. by fluorescence microscopy or flow cytometry; and (c) identifying said candidate antibody as an inhibitor the MCAM/laminin α4 interaction if the level of laminin α4 binding is lower in the presence than in the absence of the candidate antibody. An alternate screening protocol involves the use of a population of cells expressing a laminin α4 chain, which can be incubated with MCAM, in the presence and absence of a candidate antibody, and binding of MCAM to the cell population monitored. If the binding of MCAM to the cell population in the presence of the candidate antibody is lower than in its absence, the candidate antibody is an MCAM antagonist.

[0252] Other methods of monitoring include fluorescence-activated cell sorting (FACS) and enzyme-linked immunosorbent assay (ELISA).

[0253] The MCAM antagonists identified based on their ability to inhibit the binding of MCAM to its ligand, e.g., a laminin α4 chain, are candidates for the treatment of inflammatory conditions characterized by infiltration of MCAM-expressing cells.

[0254] The inhibitory activity of an MCAM antibody can also be assessed *in vivo*. An example of a methodology for assessing the inhibitory activity of an MCAM antibody is with an experimental autoimmune encephalomyelitis (EAE) model. EAE is a disease that is generated in laboratory animals to produce symptoms similar to those of multiple sclerosis (MS) in humans. *See*, *e.g.*, Bauer et al., *Proc. Nat'l Acad. Sci. USA* 106: 1920-1925 (2009). EAE is generally produced by injecting animals with different proteins from the central nervous system of other animals, for example, extracts of myelin basic protein and whole spinal cord or brain tissue, or with T cells that specifically react to myelin. EAE is commonly used to follow the course of relapsing or progressive forms of MS. EAE has been served as a suitable animal model to both develop therapeutic agents for MS and study the specific disease processes of MS. *See, e.g.*,

Gold et al., *Brain* 129: 1953-1971 (2006); *see also* Steinman et al., *Ann. Neurol.* 60: 12-21 (2006).

[0255] The effects of MCAM blockade on disease progression can be examined in a therapeutic model of EAE in which TH17 polarization occurs *in vivo*. Mice are immunized with PLP 139-151 peptide to induce EAE. After disease onset, mice are treated intraperitoneally with either a candidate anti-MCAM antibody or isotype control, and every day thereafter. Mice are monitored daily and scored for in a blinded manner, and body weights were obtained every 2-3 days. A delay in relapse and significant reduction in symptom severity in mice treated with a candidate MCAM antibody is indicative of a successful candidate antibody.

C. Selection of Constant Region

[0256] The heavy and light chain variable regions of chimeric, veneered or humanized antibodies can be linked to at least a portion of a human constant region. The choice of constant region depends, in part, whether antibody-dependent cell-mediated cytotoxicity, antibody dependent cellular phagocytosis and/or complement dependent cytotoxicity are desired. For example, human isotopes IgG1 and IgG3 have complement-dependent cytotoxicity and human isotypes IgG2 and IgG4 do not. Human IgG1 and IgG3 also induce stronger cell mediated effector functions than human IgG2 and IgG4. Light chain constant regions can be lambda or kappa.

[0257] One or several amino acids at the amino or carboxy terminus of the light and/or heavy chain, such as the C-terminal lysine of the heavy chain, may be missing or derivatized in a proportion or all of the molecules. Substitutions can be made in the constant regions to reduce or increase effector function such as complement-mediated cytotoxicity or ADCC (see, e.g., Winter et al., US Patent No. 5,624,821; Tso et al., US Patent No. 5,834,597; and Lazar et al., Proc. Natl. Acad. Sci. USA 103:4005, 2006), or to prolong half-life in humans (see, e.g., Hinton et al., J. Biol. Chem. 279:6213, 2004). Exemplary substitutions include a Gln at position 250 and/or a Leu at position 428 (EU numbering is used in this paragraph for the constant region) for increasing the half-life of an antibody. Substitution at any or all of positions 234, 235, 236 and/or 237 reduce affinity for Fcγ receptors, particularly FcγRI receptor (*see, e.g.*, US 6,624,821). An alanine substitution at positions 234, 235, and 237 of human IgG1 can be used

for reducing effector functions. Some antibodies have alanine substitution at positions 234, 235 and 237 of human IgG1 for reducing effector functions. Optionally, positions 234, 236 and/or 237 in human IgG2 are substituted with alanine and position 235 with glutamine (see, e.g., US 5,624,821). In some antibodies, a mutation at one or more of positions 241, 264, 265, 270, 296, 297, 322, 329, and 331 by EU numbering of human IgG1 is used. In some antibodies, a mutation at one or more of positions 318, 320, and 322 by EU numbering of human IgG1 is used. In some antibodies, positions 234 and/or 235 are substituted with alanine and/or position 329 is substituted with glycine. In some antibodies, positions 234 and 235 are substituted with alanine, such as in SEQ ID NO: 172. In some antibodies, the isotype is human IgG2 or IgG4. An exemplary human light chain kappa constant region has the amino acid sequence of SEQ ID NO: 168. The N-terminal arginine of SEQ ID NO: 168 can be omitted, in which case light chain kappa constant region has the amino acid sequence of SEQ ID NO: 169. An exemplary human IgG1 heavy chain constant region has the amino acid sequence of SEQ ID NO: 170 (with or without the C-terminal lysine). Antibodies can be expressed as tetramers containing two light and two heavy chains, as separate heavy chains, light chains, as Fab, Fab', F(ab')2, and Fv, or as single chain antibodies in which heavy and light chain mature variable domains are linked through a spacer.

[0258] Human constant regions show allotypic variation and isoallotypic variation between different individuals, that is, the constant regions can differ in different individuals at one or more polymorphic positions. Isoallotypes differ from allotypes in that sera recognizing an isoallotype bind to a non-polymorphic region of a one or more other isotypes. Thus, for example, another heavy chain constant region is of IgG1 G1m3 allotype and has the amino acid sequence of SEQ ID NO: 171. Another heavy chain constant region has the amino acid sequence of SEQ ID NO: 171 except that it lacks the C-terminal lysine. Another heavy chain constant region has the amino acid sequence of SEQ ID NO: 172. Yet another heavy chain constant region has the amino acid sequence of SEQ ID NO: 172 except that it lacks the C-terminal lysine.

[0259] The invention further provides nucleic acids encoding any of the above constant regions. Optionally, such nucleic acids further encode a signal peptide and can be expressed with the signal peptide linked to the constant region.

D. Expression of Recombinant Antibodies

[0260] Antibodies can be produced by recombinant expression. Nucleic acids encoding the antibodies can be codon-optimized for expression in the desired cell-type (e.g., CHO or Sp2/0). Recombinant nucleic acid constructs typically include an expression control sequence operably linked to the coding sequences of antibody chains, including naturally-associated or heterologous promoter regions. The expression control sequences can be eukaryotic promoter systems in vectors capable of transforming or transfecting eukaryotic host cells. Once the vector has been incorporated into the appropriate host, the host is maintained under conditions suitable for high level expression of the nucleotide sequences, and the collection and purification of the crossreacting antibodies. The vector or vectors encoding the antibody chains can also contain a selectable gene, such as dihydrofolate reductase, to allow amplification of copy number of the nucleic acids encoding the antibody chains.

[0261] E. coli is a prokaryotic host particularly useful for expressing antibodies, particularly antibody fragments. Microbes, such as yeast are also useful for expression. Saccharomyces is an example of a yeast host, with suitable vectors having expression control sequences, an origin of replication, termination sequences and the like as desired. Typical promoters include 3-phosphoglycerate kinase and other glycolytic enzymes. Inducible yeast promoters include, among others, promoters from alcohol dehydrogenase, isocytochrome C, and enzymes responsible for maltose and galactose utilizations.

[0262] Mammalian cells can be used for expressing nucleotide segments encoding immunoglobulins or fragments thereof. See Winnacker, From Genes to Clones, (VCH Publishers, NY, 1987). A number of suitable host cell lines capable of secreting intact heterologous proteins have been developed in the art, and include CHO cell lines, various COS cell lines, HeLa cells, HEK293 cells, L cells, and non-antibody-producing myelomas including Sp2/0 and NS0. It can be advantageous to use nonhuman cells. Expression vectors for these cells can include expression control sequences, such as an origin of replication, a promoter, an enhancer (Queen et al., Immunol. Rev. 89:49 (1986)), and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences. Suitable expression control sequences are promoters derived from

endogenous genes, cytomegalovirus, SV40, adenovirus, bovine papillomavirus, and the like. See Co et al., J. Immunol. 148:1149 (1992).

[0263] Having introduced vector(s) encoding antibody heavy and light chains into cell culture, cell pools can be screened for growth productivity and product quality in serum-free media. Top-producing cell pools can then be subjected to FACS-based single-cell cloning to generate monoclonal lines. Specific productivities above 50 pg or 100 pg per cell per day, which correspond to product titers of greater than 7.5 g/L culture, can be advantageous. Antibodies produced by single cell clones can also be tested for turbidity, filtration properties, PAGE, IEF, UV scan, HP–SEC, carbohydrate-oligosaccharide mapping, mass spectrometry, and binding assay, such as ELISA or Biacore. A selected clone can then be banked in multiple vials and stored frozen for subsequent use.

[0264] Once expressed, antibodies can be purified according to standard procedures of the art, including protein A capture, column chromatography (e.g., hydrophobic interaction or ion exchange), low-pH for viral inactivation and the like (see generally, Scopes, Protein Purification (Springer-Verlag, NY, 1982)).

[0265] Methodology for commercial production of antibodies including codon optimization, selection of promoters, transcription elements, and terminators, serum-free single cell cloning, cell banking, use of selection markers for amplification of copy number, CHO terminator, serum free single cell cloning, improvement of protein titers (see, e.g., US 5,786,464, US 5,888,809, US 6,063,598, US 6,114,148, US 7,569,339, WO2004/050884, WO2005/019442, WO2008/012142, WO2008/012142, WO2008/107388, and WO2009/027471).

IV. INHIBITORS OF MCAM EXPRESSION

[0266] The Examples show that treatment with a naked (i.e., unconjugated) anti-MCAM antibody reduces expression of MCAM on the surface of CD4 T cells. Inhibitors of MCAM expression can achieve the same effect through a different mechanism. Agents that can be designed to inhibit expression of a gene (in this case human MCAM) include zinc finger proteins, talons, siRNA, anti-sense RNA and Cas9 enzyme directed by a guide RNA. Such

agents can bind to DNA interfering with transcription or bind to and/or cleave mRNA interfering with expression.

V. METHODS OF TREATMENT

[0267] The antibodies or other antagonists disclosed herein can be used for treating or effecting prophylaxis of subjects having an immune disorder, particularly an immune disorder mediated at least in part by TH17 cells, or having a cancer (e.g., meeting art-recognized criteria, such as those of the DSM-IV-TR or DSM-V) or at elevated risk relative to the general population of a disease disclosed herein. Elevated risk can be assessed from presence of one or more genetic or biochemical markers associated with the disease, or one or more symptoms consistent with the disease but insufficient to allow a definite diagnosis. Types of condition treatable by the methods include autoimmune diseases, neuroinflammatory diseases, other immune or inflammatory diseases, hypersensitivity disorders and cancer, particularly those mediated at least in part by TH17 cells or target cells expressing MCAM (e.g., a cancer cell expressing MCAM). Some conditions may fall in more than one category. For example, multiple sclerosis is an autoimmune disease and neuroinflammatory disease and chronic beryllium disease is an autoimmune disease and hypersensitivity disorder. Practice of the present methods does not necessarily depend on an understanding of mechanism.

[0268] The present methods can also be used for treatment or prophylaxis of autoimmune diseases include systemic autoimmune diseases, organ- or tissue-specific autoimmune diseases, and diseases that exhibit autoimmune-type expressions. In these diseases, the body develops a cellular and/or humoral immune response against one of its own antigens, leading to destruction of that antigen and potentially crippling and/or fatal consequences. The cellular response if present can be B-cell or T-cell or both. TH17 cells, a lineage T helper cells characterized by production of interleukin (IL)-17 and IL-22, have been reported to enter tissues to facilitate pathogenic autoimmune responses, including multiple sclerosis in humans and experimental autoimmune encephalomyelitis (EAE) in mice. See, e.g., Cua et al., Nature 421: 744-748 (2003); Ivonov et al., Cell 126: 1121-1133 (2006). TH17 cells may initiate or propagate an inflammatory response by their specific recruitment to and infiltration of tissue.

[0269] Examples of autoimmune diseases include Graves' disease, Hashimoto's thyroiditis, autoimmune polyglandular syndrome, insulin-dependent diabetes mellitus (type 1 diabetes), insulin-resistant diabetes mellitus (type 2 diabetes), immune-mediated infertility, autoimmune Addison's disease, pemphigus vulgaris, pemphigus foliaceus, dermatitis herpetiformis, autoimmune alopecia, vitiligo, autoimmune hemolytic anemia, idiopathic thrombocytopenic purpura, autoimmune thrombocytopenic purpura, pernicious anemia, myasthenia gravis, Guillain-Barre syndrome, stiff man syndrome, acute rheumatic fever, sympathetic ophthalmia, Goodpasture's syndrome, autoimmune uveitis, temporal artertis, Bechet's disease, inflammatory bowel diseases, Crohn's disease, ulcerative colitis, primary bilary cirrhosis, autoimmune hepatitis, autoimmune oophoritis, fibromyalgia, polymyositis, dermatomyostis, ankylosing spondylitis, giant cell arteritis, polymyalgia rheumatica (PMR), Takayashu arteritis, panniculitis, pemphigoid, vasculitis of unknown origin, anca negative vasculitis, anca positive vasculitis, systemic lupus erythematosus, psoriatic arthritis, rheumatoid arthritis, scleroderma, systemic necrotizing vasculitis, Wegener's granulomatosis, CREST syndrome, antiphospholipid syndrome, Sjogren's syndrome, eosinophilic gastroenteritis, atypical topical dermatitis, cardiomyopathy, post-infectious syndromes, postinfectious endomyocarditis, celiac disease, multiple sclerosis, sarcoidosis, Crohn’s disease, Behcet’s disease and psoriasis.

[0270] Some exemplary diseases, which are believed to have an autoimmune etiology, and are treatable by the present methods include giant cell arteritis, polymyalgia rheumatica (PMR) and Takayasu’s arteritis. These diseases are characterized by infiltration of TH17 and TH1 cells into blood vessel walls. Cells expressing MCAM have been found to localize around blood vessels. Although practice of the methods is not dependent on understanding of mechanism, it is believed that in some methods antibodies or other antagonists function at least in part by inhibiting the interaction of MCAM expressed on T cells (e.g., TH17 cells) and laminin α4 chain, e.g., an α4 chain of laminin 411 expressed on the surface of an endothelial cell forming a blood vessel wall. Antibody-drug conjugates can have additional mechanisms of action including the cytotoxic or cytostatic effect of the linked agent, typically after uptake within the targeted cell. Antibody-drug conjugates may also induce macrophage toxicity.

[0271] Giant-cell arteritis (GCA or temporal arteritis or cranial arteritis or Horton disease) is an inflammatory disease of blood vessels most commonly involving large and medium arteries of

the head, predominantly the branches of the external carotid artery. The most serious complication is occlusion of the ophthalmic artery, which is a branch of the internal carotid. It can create a medical create a medical emergency which can cause irreversible ischemia and blindness if not treated promptly. GCA is conventionally treated with glucocorticoids, which reduce current symptoms of inflammation and prevent occlusion but has no effect on vessel wall pathology, resulting in subjects relapsing after recovery.

[0272] GGA onset occurs at age 50 onward with a mean age of diagnosis of 72. About 190,000 subjects present with the disease. This disease frequently manifests as an analgesic resistant headache (60-90%) and visual loss (12-40%). Symptoms show a dramatic initial response to glucocorticoids.

[0273] GCA is diagnosed from clinical presentation, patient characteristics, blood test markers of inflammation and most characteristically, presence of giant multinucleate cells in vessel wall biopsies most usually proximate to the internal elastic membrane.

[0274] GCA is caused by infiltration of TH17 and TH1 cells into a blood vessel wall. Histo-pathological lesions are observed in all layers of the artery leading to segmental and focal panarteritis with a polymorphic cell infiltrate that includes T cells, macrophages and multinucleated giant cells, a fragmented internal elastic lamina and intimal hyperplasia. TH17 cells are the precursors of giant cells (Samson Clin Exp Rheumatol. 2013 Jan-Feb;31(1 Suppl 75):S65-73. Epub 2013 Apr 19.). Therefore antibodies of the disclosure can inhibit GCA by inhibiting migration of TH17 or TH1 cells into blood vessel walls or conversion of TH17 cells to giant cells, among other mechanism. An animal model for GCA is available for testing antibodies. The animal model is formed by transferring human temporal arterial specimens subcutaneously into SCID mice (Deng et al., Circ. Res. 104, 488-495 (2009)). A cellular model is also available for analyzing the effect of potential drugs on migration and proliferation of smooth muscle cells in the vasculature. This models uses smooth muscle cells on a Matrigel™ matrix.

[0275] Polymyalgia rheumatica abbreviated as PMR, is a related condition to GCA, in which subjects have pain or stiffness, usually in the neck, shoulders, upper arms and hips, but which may occur all over the body. The pain can be very sudden, or can occur gradually over a period.

It may be caused by an inflammatory condition of blood vessels such as temporal arteritis. Elevated ESR and C-reactive protein are characteristic of PMR as is a rapid response to low dose corticosteroids, which are the conventional treatment for PMR.

[0276] PMR can occur concurrently or separately than GCA. Approximately 15% of patients with PMR develop giant cell arteritis (GCA), and 40-50% of patients with GCA have associated PMR. Most inflammation is at the level of the synovium and bursae, with MRI studies revealing periarticular inflammation as well as bursitis in the bursae associated with both the shoulder and hip girdles. Systemic macrophage and T-cell activation are characteristic of both GCA and PMR. Patients often have an elevated IL-6 level which is likely responsible for the systemic inflammatory response in both GCA and PMR. A decrease in the level of circulating IL-6 correlates with remission of clinical symptoms. As with GCA, age of onset is 50 year or later with a mean of 72 years.

[0277] Takayasu's arteritis is a rare type of vasculitis related to GCA. It is a form of large vessel granulomatous vasculitis with massive intimal fibrosis and vascular narrowing, affecting often young or middle-aged women of Asian descent. It mainly affects the aorta (the main blood vessel leaving the heart) and its branches, as well as the pulmonary arteries. Females are about 8–9 times more likely to be affected than males. Those with the disease often notice symptoms between 15 and 30 years of age Takayasu's arteritis can also lead to arm or chest pain and high blood pressure and eventually to heart failure or stroke. The goal of treatment is to relieve inflammation in the arteries and prevent potential complications. Signs and symptoms of Takayasu's arteritis include: arm or leg weakness or pain with use (claudication), Lightheadedness or dizziness, fainting, headaches, memory problems, trouble thinking, shortness of breath, visual problems, high blood pressure, difference in blood pressure between arms, a difficult-to-find or absent pulse in the wrists; too few red blood cells (anemia), chest pain, abdominal pain. The condition is characterized by segmental and patchy granulomatous inflammation of the aorta and its major derivative branches. This inflammation leads to arterial stenosis, thrombosis, and aneurysms. There is also irregular fibrosis of the blood vessels due to chronic vasculitis, leading to sometimes massive intimal fibrosis (fibrosis of the inner section of the blood vessels). Prominent narrowing due to inflammation, granuloma, and fibrosis is often seen in arterial studies such as magnetic resonance angiography (MRA), computed tomography

angiography (CTA), or arterial angiography (DSA). Treatments include corticosteroids, methotrexate, azathioprine, adalimumab, etenercept, infliximab and tocilizumab.

[0278] The present methods can also be used for treatment or prophylaxis of granulomatous lung diseases, such as sarcoidosis, chronic beryllium disease (CBD), Wegener granulomatosis, Blau syndrome and hot tub lung disease. Such diseases can be considered autoimmune disorders or hypersensitivity disorders. These diseases are characterized by infiltration of TH1 cells into the lungs. T helper cells expressing MCAM have been found in the lungs of patients with granulomatous lung diseases, but are predominantly Th1 and relatively few are Th17 cells. Although practice of the methods is not dependent on understanding of mechanism, it is believed that in some methods antibodies or other antagonists function at least in part by down-regulating MCAM, reducing the expression of MCAM on T cells, reducing the population of T cells expressing MCAM and/or by inhibiting the interaction of MCAM expressed on T cells (e.g., Th1 cells) and laminin α4 chain, *e.g.*, an α4 chain of laminin 411 expressed on the surface of an endothelial cell forming a blood vessel wall. Antibody-drug conjugates can have additional mechanisms of action including the cytotoxic or cytostatic effect of the linked agent, typically after uptake within the targeted cell. Antibody-drug conjugates may also induce macrophage toxicity.

[0279] In both sarcoidosis and chronic beryllium disease (CBD), antigen exposure leads to granuloma formation. Sarcoidosis affects multiple organs, predominantly the lungs, and develops because of exposure to an as yet unknown antigen. CBD predominantly affects the lungs and is clinically, radiologically and pathologically similar to sarcoidosis, with a known trigger, beryllium. CBD causes inflammation and sometimes scarring of the lung tissue and is immune-mediated. CBD typically develops in individuals who have experienced beryllium sensitization, which can occur, for example, when inhaling dust or fumes of beryllium, which is used in various industries including electronics, aerospace, dental atomic energy and defense. It is also possible for beryllium sensitization to occur if beryllium penetrates the skin.- Studies have shown that on average, 1-6% of exposed workers develop beryllium sensitization, although the rates can be much higher depending on exposure, for example, 16% among beryllium machinists. Beryllium sensitivity can develop soon after exposure or many year later (e.g., 30-40 years). Lung cancer rates are significantly higher in people exposed to beryllium. In addition,

when the respiratory system is damaged, strain is placed on the heart, leading to an enlarged heart and heart disease over time. As CBD progresses, symptoms include shortness of breath, a persistent dry cough, fatigue, night sweats, chest and joint pain, and loss of appetite. CBD is diagnosed from clinical presentation, breath sounds in the lungs, swollen lymph nodes, a bronchoscopy and/or an enlarged liver. In addition, people exposed to beryllium can be tested for sensitization with a beryllium lymphocyte proliferation blood test (BeLPT).

[0280] The present methods can also be used for treating graft versus host disease, an immune disease mediated at least in part by TH17 cells.

[0281] The present methods can be used to inhibit growth or metastasis of cancer, particularly cancers expressing MCAM. Cancers can be hematopoietic malignancies or solid tumors, i.e., masses of cells that result from excessive cell growth or proliferation, either benign or malignant, including pre-cancerous legions. Cancers can be benign, malignant, or metastatic. Metastatic cancer refers to a cancer that has spread from the place where it first started to another place in the body. Tumors formed by metastatic cancer cells are called a metastatic tumor or a metastasis, which is a term also used to refer to the process by which cancer cells spread to other parts of the body. In general, metastatic cancer has the same name and same type of cancer cells as the original, or primary, cancer. Examples of cancer include solid tumors, such as melanoma, carcinoma, blastoma, and sarcoma. Cancers also include hematologic malignancies, such as leukemia or lymphoid malignancies, such as lymphoma. More particular examples of such cancers include squamous cell cancer, lung cancer, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer, pancreatic cancer, glioma, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial cancer or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, as well as head and neck cancer.

[0282] Antibodies or inhibitors of expression are administered in an effective regime meaning a dosage, route of administration and frequency of administration that delays the onset, reduces the severity, inhibits further deterioration, and/or ameliorates at least one sign or symptom of a disease being treated (i.e., a positive treatment response). If a patient is already suffering from a

disorder, the regime can be referred to as a therapeutically effective regime. If the patient is at elevated risk of the disorder relative to the general population but is not yet experiencing symptoms, the regime can be referred to as a prophylactically effective regime. In some instances, therapeutic or prophylactic efficacy can be observed in an individual patient relative to historical controls or past experience in the same patient. In other instances, therapeutic or prophylactic efficacy can be demonstrated in a preclinical or clinical trial in a population of treated patients relative to a control population of untreated patients. The dosage depends on the condition of the patient and response to prior treatment, if any, whether the treatment is prophylactic or therapeutic and whether the disorder is acute or chronic, among other factors.

[0283] Exemplary dosages for an antibody are 0.3-50 mg /kg body weight. Exemplary dosage ranges include 0.3-1 mg/kg, 1-3 mg/kg, 3-10 mg/kg, 10-30 mg/kg and 30-50 mg/kg. Exemplary dosage regimes for an antibody includes 0.3 1, 3, 10, 30 or 50 mg/kg. Dosages of antibody are usually administered on multiple occasions separated by an interval. The interval can be, for example, 2-5 weeks, e.g., every 2 weeks, every four weeks, or every month. Dosages of antibodies can be administered on at least three occasions, at least ten occasions, at least until a positive treatment response is detected, at least until steady state levels of antibody in the plasma are reached (i.e., the same peaks and troughs between dosages within experimental errors), for at least one year, for at least 10 years or for the remaining life of the patient. For some patients, the same dose of antibody is administered at the same intervals, although in other patients the dose or frequency can be increased or decreased depending on treatment response and side effects observed.

[0284] Administration can be parenteral, intravenous, oral, subcutaneous, intra-arterial, intracranial, intrathecal, intraperitoneal, topical, intranasal or intramuscular. Preferred route of administration are intravenous infusion or subcutaneous administration. Intravenous administration can be, for example, by infusion over a period such as 30-90 min. Some exemplary regimes include 3-10 mg/kg every two weeks and 20-30 mg/kg of antibody every four weeks by intravenous infusion. However, other routes of administration can be performed, optionally with adjustment of the dose or frequency to achieve the same area under the curve as an intravenous infusion.

[0285] Treatment with antibodies or other inhibitors disclosed herein can be combined with other treatments effective against the disorder being treated. Combination treatments can be formulated for administered separately. Some examples of treatments useful for combination therapies include methotrexate, steroids, NSAIDs, or any combination thereof.

[0286] Additional therapeutic agents for treatment of multiple sclerosis include one or more of the following: teriflunomide, interferon beta-1a, interferon beta-1b, glatiramer acetate, fingolimod, and mitoxantrone, or a corticosteroid, such as prednisone, methylprednisolone, or dexamethasone.

[0287] Additional therapeutic agents for cancer include alkylating agents such as carmustine, chlorambucil, cisplatin, carboplatin, oxaliplatin, procarbazine, and cyclophosphamide; antimetabolites such as fluorouracil, floxuridine, fludarabine, gemcitabine, methotrexate and hydroxyurea; natural products including plant alkaloids and antibiotics such as bleomycin, doxorubicin, daunorubicin, idarubicin, etoposide, mitomycin, mitoxantrone, vinblastine, vincristine, and Taxol (paclitaxel) or related compounds such as Taxotere®; the topoisomerase 1 inhibitor irinotecan; temozolomide and Gliadel®, carmustine; and inhibitors of tyrosine kinases such as Gleevec®, Sutent® (sunitinib malate), Nexavar® (sorafenib) and Tarceva® (erlotinib) or Iressa® (gefitinib); inhibitors of angiogenesis; and monoclonal antibodies, including Herceptin® against the HER2 antigen; Avastin® against VEGF; or antibodies to the Epidermal Growth Factor (EGF) receptor such as Erbitux® (cetuximab) and Vectibix® (panitumumab).

[0288] Additional agents for treating Parkinson's disease include including levodopa, benzaseride, carbidopa, dopamine agonists, non-ergot dopamine agonists, catechol-O-methyl ("COMT") inhibitors such as, for example, entacopone or tolcopone, monoamine oxidase ("MAO") inhibitors, such as, for example, rasagaline, amantadine, or anticholinergic agents

VI. FORMULATIONS

[0289] Pharmaceutical compositions for parenteral administration are preferably sterile and substantially isotonic and manufactured under GMP conditions. Pharmaceutical compositions can be provided in unit dosage form (i.e., the dosage for a single administration). Pharmaceutical compositions can be formulated using one or more physiologically and pharmaceutically acceptable carriers, diluents, excipients or auxiliaries. The formulation

depends on the route of administration chosen. For injection, antibodies can be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiological saline or acetate buffer (to reduce discomfort at the site of injection). The solution can contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively antibodies can be in lyophilized form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

[0290] Formulations are provided comprise an antibody or other antagonist described herein, a buffer, one or more sugars and/or polyols and a surfactant, and have a pH within the range from about 5.5 to about 7. The formulations can be prepared for storage in liquid form or in lyophilized form. When stored in lyophilized form, the formulations can be reconstituted with a liquid (e.g., sterile water) to the concentrations and properties described herein. When a lyophilized composition is said to be reconstitutable by adding water to generate a formulation of specified component concentrations and pH, it is meant that the lyophilized formulation can be so reconstituted simply by addition of water (i.e., without supplying additional amounts of components or adding acid or base to change the pH). The concentrations and properties of a prelyophilized liquid formulation can also be in accordance with those described below if the lyophilized formulation is reconstituted to the same volume as the formulation prelyophilization. If the volume is different, then concentrations of formulations should be adjusted proportionally. For example, if the reconstituted volume is half the prelyophilization volume, then the concentrations of components in the prelyophilization formulation should be half the concentrations in the reconstituted formulation.

[0291] Optionally, the antibody is resuspended in a formulation as described below, temporarily frozen for storage prelyophilization, lyophilized, and reconstituted with water to the same concentrations as prelyophilization. Such a formulation should preferably stabilize the antibody throughout freezing, lyophilization, storage, and reconstitution as well as being suitable for parenteral administration. In an exemplary work flow, a purified antibody is resuspended at about 40 mg/mL in a formulation and stored frozen at -40°C in bags. Bags are thawed at room temperature for 3 hours and the contents are pooled. The formulation is sterile filtered through a 0.2 micron sterile filer. Vials are filled with 5.4 mL of the formulation and lyophilized. Lyophilized vials are stored at 2-8°C. Lyophilized vials are reconstituted by adding sterile water

(e.g., approximately 5.0 to 5.4 mL sterile water, depending on the formulation). Five mL of the reconstituted product is then added into the port of an IV bag containing 20-100 mL of normal saline, lactated Ringers solution, or 5% dextrose solution or the like for intravenous infusion into a patient.

[0292] Some formulations include a bulking agent, which may or may not be the same as the sugar/polyol component. Typically, the formulations are sterile, for example, as accomplished by sterile filtration using a 0.2 μm or a 0.22 μm filter. The formulations are also generally stable by low to undetectable levels of fragmentation and/or aggregation as further defined below on freezing and thawing. Still other formulations are stable following reconstitution of a lyophilized cake for at least three months at about 40°C. In some formulations, less than about 5% of the antibody is present as an aggregate in the formulation.

[0293] In some formulations, the antibody is present at a concentration within the range from about 5 mg/mL to about 100 mg/mL. In some formulations, the antibody is present at a concentration within the range from about 5 mg/mL to about 50 mg/mL. In some formulations, the antibody is present at a concentration within the range from about 25 mg/mL to about 50 mg/mL. For example, the antibody may be present at a concentration of about 35-45 mg/mL or about 40 mg/mL. The antibody may be present in a sterile liquid dosage form of about 50 mg/vial to about 500 mg/vial, or greater. The antibody may be present in a lyophilized dosage form of about 40 mg/vial to about 500 mg/vial. For example, the antibody may be present in a sterile liquid or lyophilized dosage form of about 250-350 mg/vial or about 200 mg/vial.

[0294] The formulation can comprise any of the antibodies described herein. In some formulations, the formulated antibody is an antibody comprising: (i) a mature heavy chain variable region comprising the three Kabat CDRs of SEQ ID NO: 161 except that position 32 (Kabat numbering) can be N, S, or Q, and position 33 (Kabat numbering) can be G or A, wherein the mature heavy chain variable region is at least 90% identical to SEQ ID NO: 161, and (ii) a mature light chain variable region comprising the three Kabat CDRs of SEQ ID NO: 123, and being at least 90% identical to SEQ ID NO: 123. In such formulations, position 1 (Kabat numbering) of the mature heavy chain variable region can be occupied by E. In some formulations, the mature heavy chain variable region has the amino acid sequence of SEQ ID

NO: 157, SEQ ID NO: 158, SEQ ID NO: 159, SEQ ID NO: 160, or SEQ ID NO: 161, and the mature light chain variable region has the amino acid sequence of SEQ ID NO: 121, SEQ ID NO: 122, or SEQ ID NO: 123. For example, in some formulations, the mature heavy chain variable region has the amino acid sequence of SEQ ID NO: 161 and the mature light chain variable region has the amino acid sequence of SEQ ID NO: 123.

[0295] In other formulations, the formulated antibody is an isolated anti-MCAM antibody described herein. In such a formulation, the isolated anti-MCAM antibody binds to human MCAM (SEQ ID NO: 11) at an epitope including amino acid residue 141.

[0296] Buffers are used in the disclosed formulations to achieve a suitable pH for the antibody, such as, for example, histidine, succinate, and citrate buffers. Some formulations have a pH within the range from about 5.5 to about 7, for example, a pH of 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, or 7.0. Some formulations have a pH of between about 5.5 to about 6.5. Some formulations have a pH of about 6.0 and other formulations have a pH of about 6.5. In some formulations, histidine buffer is present at a concentration within the range from about 10 mM to about 30 mM, for example, at a concentration of about 15-25 mM or about 20 mM.

[0297] Suitable sugars and/or polyols for the formulations include trehalose and sucrose, or a combination thereof. Sugars/polyols serve as bulking agents, lyoprotecting agent, and/or tonicity adjusting agents. For example, some formulations include trehalose present at a concentration within the range from about 200 mM to about 260 mM, or sucrose present at a concentration within the range from about 200 mM to about 260 mM. Some formulations include trehalose present at a concentration of about 220 mM. Other formulations include sucrose present at a concentration of about 220 mM. Some such formulations are characterized by an osmolality in the range of about 250-400, 300-400, or 300-350 mOsm/kg, such as, for example, 287 or 295 mOsm/kg.

[0298] Formulations can contain a surfactant to reduce antibody aggregation and absorption to surfaces. Suitable surfactants include polysorbate 20 present at a concentration within the range from about 0.005% to about 0.05% by weight. Polysorbate 20 protects against marked increases in aggregation or turbidity that would otherwise occur in formulations of antibodies. The

polysorbate 20 may be present at a concentration within the range from about 0.01% to about 0.05%. For example, the concentration can be 0.005%, 0.01%, 0.015%, 0.02%, 0.025%, 0.03%, 0.035%, 0.04%, 0.045%, or 0.05%. Alternatively, in some formulations, polysorbate 20 is present at a concentration within the range of about from about 0.05 g/L, 0.1 g/L, 0.15 g/L, 0.2 g/L, 0.25 g/L, 0.3 g/L, 0.35 g/L, 0.4 g/L, 0.45 g/L, or 0.5 g/L. Some formulations include polysorbate 20 at a concentration of 0.2 g/L.

[0299] An exemplary formulation (liquid, prelyophilization or reconstituted after lyophilization) is characterized by a pH within the range from about 5.5 to about 7 and includes: (a) an antibody described herein, at a concentration within the range from about 10 mg/mL to about 50 mg/mL; (b) a histidine buffer present at a concentration within the range from about 10 mM to about 30 mM; (c) one or more sugars and polyols ("sugar/polyol") selected from trehalose present at a concentration within the range from about 200 mM to about 260 mM, and sucrose present at a concentration within the range from about 200 mM to about 260 mM; and (d) polysorbate 20 present at a concentration within the range from about 0.005% to about 0.05% by weight. In one example, the formulation can include: (a) any antibody described herein; (b) a histidine buffer at a concentration of about 20 mM; (c) sucrose at a concentration of about 220 mM; (d) polysorbate 20 at a concentration of about 0.02%; and a pH of about 6.0. In another example, the formulation can include: (a) any antibody described herein; (b) a histidine buffer at a concentration of about 20 mM; (c) trehalose at a concentration of about 220 mM; (d) polysorbate 20 at a concentration of about 0.02%; and a pH of about 6.5.

[0300] Some lyophilized formulations include: (a) an antibody described herein; (b) histidine buffer; (c) trehalose or sucrose; and (d) polysorbate 20. The lyophilized formulation can include about 200 mg of the antibody. Some lyophilized formulations are capable of being reconstituted with sterile water. Some lyophilized formulations include 100-300 or 150-250 mg antibody, 10 to 20 or 14 to 16 mg of histidine, 300 to 450 or 350 to 400 mg sucrose, and 0.5 to 1.5 mg or 0.75 to 1.25 mg polysorbate 20. Other lyophilized formulations include 100 to 300 or 150 to 250 mg antibody, 10 to 20 or 14 to 16 mg of histidine, 360 to 500 or 400 to 450 mg trehalose dehydrate, and 0.5 to 1.5 mg or 0.75 to 1.25 mg polysorbate 20.

[0301] An exemplary lyophilized formulation includes 200 mg of an antibody, 15.5 mg of histidine, 376 mg sucrose, and 1 mg polysorbate 20. Another exemplary lyophilized formulation includes 200 mg of an antibody, 15.5 mg of histidine, 416 mg trehalose dihydrate, and 1 mg polysorbate 20. Some such formulations can be reconstituted to a volume of about 5 mL. Other lyophilized formulations include the same components in the same proportions as any disclosed in this paragraph but in different amounts (e.g., 400 mg antibody, 31 mg histidine, 752 mg sucrose, and 2 mg polysorbate 20).

[0302] Lyophilized formulations can be reconstituted to an antibody concentration of about 30-50 or 35-45 mg/mL, for example to about 40 mg/mL; (b) a histidine buffer present at a concentration of about 10-30 or 15-25 mM, for example about 20 mM; (c) sucrose or trehalose present at a concentration of about 160-330 or 200-260 mM, for example about 220 mM; (d) polysorbate 20 present at a concentration of about 0.1-0.3 or 0.15 to 0.25 g/L, for example about 0.2 g/L; and (e) a pH of about 5.5-6.5, for example about 6.0 (if sucrose is present) or 6.5 (if trehalose is present).

[0303] Liquid or reconstituted lyophilized formulations are preferably substantially isotonic, implying an osmolality of about 250-350 mOsm/kg water. Some formulations have an osmolality of 270-300 mOsm/kg. Some formulations have an osmolality of about 287 or about 295 mOsm/kg. Liquid or reconstituted lyophilized formulations can also be hypertonic > 350 mOsm/kg water or hypotonic (<250 mOsm/kg water).

[0304] Any of the formulations described can be made without pharmaceutical excipients, carriers or the like, other than those described as being components herein. Such a formulation can be described as consisting of the recited components, or consisting essentially of the recited components if insignificant amounts of other components not affecting the properties of the formulation are present. Formulations are preferably made under good manufacturing practices (GMP) approved or approvable by the FDA for preparation of drugs for administration to humans.

[0305] Also provided are antibody formulations having stability at 38°C-42°C (e.g., as assessed by high performance size exclusion chromatography (HPSEC)) for at least about 30 days, for at least about 3 months, or longer. Such formulations may also have stability at 20°C-24°C for at

least about 1 year, and/or stability at 2°C-4°C for at least about 3 years. Stability of lyophilized formulations is assessed for storage in the lyophilized state. A formulation is considered stable if, after incubation at one or more of these specified combinations of time and temperature, it meets the below definition for low to undetectable fragmentation and/or low to undetectable aggregation. More particularly, the disclosed formulations exhibit low to undetectable levels of antibody aggregation and/or fragmentation, or a low or undetectable increase in fragmentation and/or aggregation above an initial level (*e.g.*, less than about 5% aggregation). A formulation having low to undetectable levels of fragmentation contains at least about 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99%, of the total protein, for example, in a single peak as determined by hydrophobic interaction chromatography, or in two peaks (one corresponding to each of the antibody heavy chains and antibody light chains) by reduced Capillary Gel Electrophoresis (rCGE), representing the non-degraded antibody, and containing no other single peaks having more than 5%, more than 4%, more than 3%, more than 2%, more than 1%, or more than 0.5% of the total protein each. A formulation having low to undetectable levels of aggregation contains no more than about 15%, no more than about 10%, no more that about 5%, no more than about 4%, no more than about 3%, no more than about 2%, no more than about 1%, or no more than about 0.5% aggregation by weight protein, as measured by high performance size exclusion chromatography (HPSEC). For example, in some formulations, less than about 5% of the antibody is present as an aggregate. Stable formulations also show little or no loss of biological activity(ies) having, for example, binding affinity measurable by ELISAs and/or additional functional assay, that is at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% of an initial measurable value.

VII. MONITORING REGIMES

[0306] Patients receiving treatment with an anti-MCAM antibody can be monitored using the level of MCAM expressed by CD4 T cells or level of MCAM extracellular domain in blood as markers of a positive treatment response. A decreased level of MCAM expressed on CD4 T cells and/or an increased level of MCAM in blood indicates a positive treatment response. Although understanding of mechanism is not required for practice of the invention, it is believed that anti-MCAM antibodies reduce expression of MCAM on CD4 T cells by inducing isotype switching such that more MCAM is expressed as a soluble extracellular domain. MCAM on

CD4 cells can be detected by an immuno assay, employing an antibody that specifically binds to MCAM. The antibody should have a different epitope specificity than the anti-MCAM antibody used for treatment. In some methods, the assay employs two antibodies, one to recognize CD4 T cells, the other to detect MCAM expression. Dual color FACS provides a suitable format for such detection. Soluble MCAM can be detected in blood including whole blood or any fraction thereof, such as plasma or serum, also by immuno assay, such as ELISA. Preferably, such assays are performed before treatment and on one or more occasions after commencing treatment to determine whether levels of MCAM change responsive to treatment.

[0307] The level of change of MCAM if any responsive to treatment can be used as an indicator of whether to continue or change treatment. In general, patients experiencing a positive treatment response (i.e., decreased expression of MCAM on CD4 cells or increased expression in blood) are likely to continue receiving treatment with anti-MCAM antibody. Patients not experiencing a positive treatment response may have treatment changed by increasing the dose or increasing the frequency of administration, or particularly if such measures still do not result in a positive response to treatment, may have treatment discontinued. A positive response to treatment or otherwise maybe but one of several factors determined in setting future treatment, but in general a higher percentage of patients having a positive treatment response continue treatment with an anti-MCAM antibody than patients not having a positive treatment response.

[0308] All patent filings, websites, other publications, accession numbers and the like cited above or below are incorporated by reference in their entirety for all purposes to the same extent as if each individual item were specifically and individually indicated to be so incorporated by reference. If different versions of a sequence are associated with an accession number at different times, the version associated with the accession number at the effective filing date of this application is meant. The effective filing date means the earlier of the actual filing date or filing date of a priority application referring to the accession number if applicable. Likewise if different versions of a publication, website or the like are published at different times, the version most recently published at the effective filing date of the application is meant unless otherwise indicated. Any feature, step, element, embodiment, or aspect of the invention can be used in combination with any other unless specifically indicated otherwise. Although the present invention has been described in some detail by way of illustration and example for purposes of

clarity and understanding, it will be apparent that certain changes and modifications may be practiced within the scope of the appended claims.

EXAMPLES

Materials and Methods

Antibody generation / characterization

[0309] For the generation of antibodies capable of binding to murine MCAM, MCAM-Fc was generated by fusing the extracellular domain of murine MCAM to human IgG and produced in CHO cells using standard techniques. Lou/M rats were immunized with 100 µg of MCAM-Fc protein in CFA (1:1 volume). Rats were boosted two times at two week intervals with MCAM-Fc protein in incomplete Freund's adjuvant (IFA) (1:1 volume). Hybridomas were generated from immunized rats using standard protocols and clones were selected by Clonepix. CHO cells were transfected with the full length murine MCAM gene and selected for stable expression using neomycin and standard techniques. Parental CHO cells (MCAM negative) were fluorescently labeled with carboxyfluorescein succinimidyl ester (CFSE) using standard techniques and mixed at a 1:1 ratio with unlabeled MCAM transfected CHO cells. Hybridoma supernatants were incubated with this mixture of cells for 30 minutes and binding of potential MCAM specific antibodies was detected with a fluorescently labeled anti-rat secondary antibody (Jackson Immuno) by flow cytometry.

[0310] Supernatants from hybridomas that screened positive for MCAM specific antibodies were pre-incubated with fluorescently labeled mouse MCAM-Fc protein (5 µg/mL) for 30 minutes before addition to the laminin α4 expressing cell line WM2664 and neutralization of binding of the MCAM-Fc protein to the cell line was determined by flow cytometry.

[0311] For the generation of rat antibodies capable of binding to human MCAM, hMCAM-Fc was generated by fusing the extracellular domain of human MCAM to human IgG and produced in CHO cells using standard techniques. Lou/M rats were immunized with 250 µg of hMCAM-Fc protein in CFA (1:1 volume). Rats were boosted two times at two week intervals with hMCAM-Fc protein in incomplete Freund's adjuvant (IFA) (1:1 volume). Hybridomas were generated from immunized rats using standard protocols and clones were selected by Clonepix.

CHO cells were transfected with the full length human MCAM gene and selected for stable expression using neomycin and standard techniques. Parental CHO cells (MCAM negative) were fluorescently labeled with carboxyfluorescein succinimidyl ester (CFSE) using standard techniques and mixed at a 1:1 ratio with unlabeled human MCAM transfected CHO cells. Hybridoma supernatants were incubated with this mixture of cells for 30 minutes and binding of potential human MCAM specific antibodies was detected with a fluorescently labeled anti-rat secondary antibody (Jackson Immuno) by flow cytometry.

[0312] For the generation of mouse antibodies capable of binding to human MCAM, hMCAM-Fc was generated by fusing the extracellular domain of human MCAM to human IgG and produced in CHO cells using standard techniques. Balb/c mice were immunized with 50 μg of hMCAM-Fc protein in CFA (1:1 volume). Mice were boosted two times at two week intervals with hMCAM-Fc protein in incomplete Freund's adjuvant (IFA) (1:1 volume). Hybridomas were generated from immunized mice using standard protocols and clones were selected by Clonepix. CHO cells were transfected with the full length human MCAM gene and selected for stable expression using neomycin and standard techniques. Parental CHO cells (MCAM negative) were fluorescently labeled with carboxyfluorescein succinimidyl ester (CFSE) using standard techniques and mixed at a 1:1 ratio with unlabeled human MCAM transfected CHO cells. Hybridoma supernatants were incubated with this mixture of cells for 30 minutes and binding of potential human MCAM specific antibodies was detected with a fluorescently labeled anti-mouse secondary antibody (Jackson Immuno) by flow cytometry.

[0313] Supernatants from hybridomas that screened positive for human MCAM specific antibodies were pre-incubated with fluorescently labeled hMCAM-Fc protein (5 μg/mL) for 30 minutes before addition to the laminin α4 expressing cell line WM2664 and neutralization of binding of the hMCAM-Fc protein to the cell line was determined by flow cytometry.

Nucleic Acid and protein manipulation

[0314] For determination of CDRs, total RNA was isolated from hybridoma cells using RNAquous-4PCR kit (Ambion), and was used for cDNA synthesis. First and second strand cDNA was synthesized using methods modified from Marathon cDNA amplification (Clontech) with the cDNA adaptor ligated to the 5'-end of the obtained dscDNA. The reverse specific

primer was designed based on the specific antibody isotype constant region sequence for both heavy and light chains, and was used along with the adaptor primer in the PCR amplification of both VL and VH fragments using Pfu Ultra DNA polymerase (Stratagene). The amplified PCR product was cloned into pCR-Blunt-TOPO (Invitrogen), and the nucleotide sequence was determined. The sequences of the identified clones were compared for percent identity within the VL and VH sequences.

[0315] For determination of IL-17 concentrations in the supernatant, ELISA was performed using a commercial kit (R&D Systems).

Example 1. Generation of anti-MCAM monoclonal antibodies

[0316] Mouse and rat monoclonal antibodies directed against human MCAM protein were generated as described in Materials and Methods above. The specific binding between the monoclonal antibody and human MCAM was confirmed by assessing the monoclonal antibody's ability to bind to cells transfected with human MCAM. For this, untransfected cells were labeled with carboxyfluorescein succinimidyl ester (CFSE) and mixed with unlabeled human MCAM transfected cells. Untransfected cells could, therefore, be differentiated.

[0317] Using these techniques, 823 independent mouse fusions clones were isolated and shown to express an antibody capable of binding to human MCAM. Additionally, 152 independent rat fusions clones were isolated and shown to express an antibody capable of binding to human MCAM.

[0318] Next, the anti-human MCAM monoclonal antibodies were used to test their ability to block the binding of human MCAM to its ligand. Human MCAM-Fc protein (5 μg/mL) was pre-incubated with isotype control antibody, or 10 μg/mL of the test monoclonal antibody for 30 minutes in PBS. The mixture was added to healthy spinal cord tissue sections and subsequently characterized by fluorescence microscopy as described in Materials and Methods above. Furthermore, parental CHO cells (CHOK1) or CHO cells transfected with a human MCAM gene were preincubated with CHO culture media (DMEM), recombinant laminin 411 (10 μg/ml), or recombinant laminin 511 (*i.e.*, laminin 10 ($\alpha5\beta1\gamma1$)) (10 μg/ml) at 37°C for 45 minutes. Cells were washed, and specific binding of laminin 411, but not laminin 511, to MCAM was detected

with a pan-laminin antibody by flow cytometry. Pre-incubation of human MCAM transfected CHO cells with the anti-MCAM antibody (at 20 μg/ml), prior to laminin incubation, abolished the binding of human MCAM to laminin 411.

[0319] Using this technique, it was shown that 87 of the 823 independent mouse fusion clones and 26 of the 152 independent rat fusion clones described above expressed an antibody that was capable of blocking the interaction between human MCAM protein and its ligand, α-4 chain of laminin.

Example 2. Further characterization of anti-MCAM monoclonal antibodies

[0320] The 87 independent mouse fusion clones and 26 independent rat fusion clones described in Example 1 above as being capable of (i) binding to human MCAM, and (ii) blocking the interaction between human MCAM and the α-4 chain of laminin were further characterized as follows. First, IC50 quantitation for the ability of the monoclonal antibody to block the binding of human MCAM to the α-4 chain of laminin was determined as follows. CHO cells expressing human MCAM were incubated with an anti-human MCAM antibody (at various concentrations) for 30 minutes at 4 degrees Celsius. Unbound antibody was then washed away, and the cells were incubated with recombinant human laminin 411 at 20 ug/ml for 45 minutes at 37 degrees Celsius. Unbound laminin was then washed away, and the laminin bound to the surface of the cells was detected with fluorescently labeled anti-laminin antibodies. After washing, the amount of laminin bound to the surface was detected by flow cytometry, and IC50s were calculated based on the mean fluorescent intensity.

[0321] Using the above described assay, six independent anti-human MCAM monoclonal antibody clones were identified as binding to human MCAM and having the greatest ability to block the interaction between human MCAM expressed on the surface of cells and its binding ligand, human laminin 411. These six anti-MCAM monoclonal antibody clones are herein referred to as (i) the mouse anti-human MCAM monoclonal clones 1174.1.3, 1414.1.2, 1415.1.1, and 1749.1.3, and (ii) the rat anti-human MCAM monoclonal antibody clones 2120.4.19 and 2107.4.10. Amino acid and nucleic acid sequences of the heavy and light chains of these antibodies, and their hypervariable regions, are provided in SEQ ID NOs:29-92. More specifically, in the above assay, IC50s for the monoclonal antibody clones 1174.1.3, 1414.1.2,

1415.1.1, 1749.1.3, 2120.4.19, and 2107.4.10 were determined to be 0.469 ug/ml, 0.431 ug/ml, 0.307 ug/ml, 0.545 ug/ml, 0.888 ug/ml, and 0.290 ug/ml, respectively. Moreover, experiments performed to determine the specific binding affinity of each monoclonal antibody demonstrated that each was capable of binding to human MCAM protein with high affinity (data not shown). As such, each of these specific monoclonal antibodies was very capable of binding to human MCAM and inhibiting the interaction of cell-expressed human MCAM with its α-4 laminin binding ligand. In contrast, two control antibodies, a non-specific human IgG1 antibody and a previously described, fully human anti-MCAM antibody referred to as ABX-MA1 (e.g., see Mills et al., Cancer Res. 62:5106 (2002), and US Patent Nos. 6,924,360, 7,067,131, and 7,090,844) were both incapable of blocking the binding interaction between human MCAM and its laminin 411 counterpart. As such, the six specific monoclonal antibodies identified above possess the novel ability to both (i) bind with high affinity to human MCAM on the surface of living cells, and (ii) block the interaction of cell expressed human MCAM with a laminin protein comprising an α-4 laminin polypeptide chain.

Example 3. Generation of Humanized anti-MCAM 2120 Antibodies

[0322] Various humanized anti-MCAM antibodies were generated according to the following protocol. First, a three-dimensional molecular model of the variable regions was constructed using JN Biosciences' proprietary algorithm. Second, the framework amino acid residues important for the formation of the CDR structure or necessary for the binding to antigen were identified using the molecular model. In parallel, cDNA-derived human VH and VL amino acid sequences with high homology to the VH and VL amino acid sequences, respectively, were selected. Lastly, CDR sequences together with framework amino acid residues important for CDR structure or antigen binding were grafted from VH and VL into the corresponding selected human framework sequences.

[0323] Figure 3 depicts the alignment of various 2120 heavy and light chain sequences. Residue numbering is according to Kabat numbering. Different mutations to the framework (FR) amino acid residues involved in CDR formation and antigen binding were identified depending upon the version of antibody.

[0324] Exemplary mutations of the 2120 antibodies are depicted in Fig. 3A (boxed residues in CDR-H1 (S30T), between CDR-H1 and CDR-H2 (I37V and L48I), and between CDR-H2 and CDR-H3 (K71R) affect CDR contact; and S30T, I37V, L48I, and K71R mutations combined with an additional mutation after CDR-H2 (T68S) affect CDR contact); and Fig. 3B (boxed residues between CDR-L1 and CDR-L2 (L46V and Y49F) and between CDR-L2 and CDR-L3 (V58I) affect CDR contact; boxed residues between CDR-L1 and CDR-L2 (L46V and Y49F) affect CDR contact; and L46V, Y49F, and V58I mutations combined with an additional mutation before CDR-L1 (T22N) affect antibody/antigen interaction).

[0325] Several versions of each chain were designed (standard vs. aggressive or conservative). For those antibodies that contained N-deamidation motifs (NG), mutations to the asparagines or glycine were introduced into the standard version. The various humanized V regions were synthesized with a heterologous signal sequence and cloned into expression vectors containing human CK (VL) or human IgG1 (VH).

[0326] The heavy and light chain plasmids were co-transfected into 293F cells with the FreeStyle™ MAX transfection regent (Invitrogen) according to the manufacturer's protocol. The expressed antibody was purified with protein A PhyTip columns (Phynexus) and quantified via OD280.

[0327] The apparent affinities of the humanized antibodies were compared to the parental rodent or chimeric antibody in a competitive ELISA according to the following protocol.

[0328] ELISA plates were coated with recombinant hMCAM-His, and blocked with casein buffer to prevent non-specific binding. Biotinylated rodent or chimeric antibody was added at a subsaturating concentration, in the presence or absence of 3x increasing concentrations of unlabeled competitor (humanized antibody, rodent, or chimeric). After washing to remove unbound antibody, streptavidin HRP was added to allow detection of the biotinylated antibody. The ELISA was developed with TMB substrate and the OD450 was measured. The IC50 of the unlabeled competitor was determined using the GraphPad Prism5 software.

[0329] Table 5 summarizes the design of humanized sequences.

Table 5

2120	Donor Framework	Mutations
VH1	AF062133 IGHV2-26*01	S30T*, I37V, L48I and K71R
VH2	AF062133 IGHV2-26*01	VH1 mutations +T68S
VH3	AF062133 IGHV2-26*01	VH1 mutations +N32S
VH4	AF062133 IGHV2-26*01	VH1 mutations +N32Q
VH5	AF062133 IGHV2-26*01	VH1 mutations +G33A
VL1	X84343 IGKV1-39*01	L46V, Y49F and V58I
VL2	X84343 IGKV1-39*01	L46V, Y49F
VL3	X84343 IGKV1-39*01	VL1 + T22N

[0330] The heavy and light chain plasmids were co-transfected into 293F cells with the FreeStyle™ MAX transfection regent (Invitrogen) according to the manufacturer's protocol. The expressed antibody was purified with protein A PhyTip columns (Phynexus) and quantified via OD280.

[0331] The apparent affinities of the humanized antibodies were compared to the parental rodent or chimeric antibody in a competitive ELISA according to the following protocol:

[0332] ELISA plates were coated with recombinant hMCAM-His, and blocked with casein buffer to prevent non-specific binding. Biotinylated rodent or chimeric antibody was added at a subsaturating concentration, in the presence or absence of 3x increasing concentrations of unlabeled competitor (humanized antibody, rodent, or chimeric). After washing to remove unbound antibody, streptavidin HRP was added to allow detection of the biotinylated antibody. The ELISA was developed with TMB substrate and the OD450 was measured. The IC50 of the unlabeled competitor was determined using the GraphPad Prism5 software.

[0333] The affinities were measured using the ForteBio Octet Red. Anti-human Fc sensors were used to capture the humanized antibodies, and several concentrations of hMCAMHis analyte were used to determine the affinity using a 1:1 fitting model.

[0334] The potencies of the antibodies were measured in the laminin/FACS assay according to the following protocol: recombinant laminin 411 (Biolaminate) was added to hMCAM expressing CHO cells in the presence or absence of varying concentrations of the humanized, rodent, or chimeric antibodies. Following incubation for 30-45 minutes, the cells were washed and anti-laminin conjugated to AF650 (NovusBio) was added to detect the bound laminin. The cells were run on a flow cytometer to measure the laminin binding signal.

[0335] Table 6 provides the constructs used for transfection.

Table 6

Construct	**Description**
h2120_VH1	**Standard**
h2120_VH2	**Conservative**
h2120_VH3	**Standard+N-S**
h2120_VH4	Standard+N-Q
h2120_VH5	Standard+G-A
h2120_VL1	Standard
h2120_VL2	Aggressive
h2120_VL3	**Conservative**

[0336] Table 7 describes the specific transfection experiments.

Table 7

Transfection--round 1	
h2120_VH1+h2120_VL3	Standard VH + conservative VL
h2120_VH2+h2120_VL3	Conservative VH + conservative VL
h2120_VH3+h2120_VL3	N-S deamidate VH + conservative VL

Transfection--round 2	
h2120_VH4+h2120_VL3	N-Q deamidate VH + conservative VL
h2120_VH5+h2120_VL3	G-A deamidate VH + conservative VL
h2120_VH1+h2120_VL1	Standard VH + standard VL
h2120_VH1+h2120_VL2	Standard VH + aggressive VL

[0337] Table 8 shows the relative affinities of the humanized antibodies compared to the rodent parent as measured by ForteBio and competitive ELISA, as well as the expression levels for the first round of transfections.

Table 8

	Forte		ELISA		
	Expt. #1	Expt. #2	Expt. #1	Expt. #2	
Transfection--round 1	Fold over rodent	Fold over rodent	Fold over rodent	Fold over rodent	Expression level
rodent 2120	1.00	1.00	1.00	1.00	
h2120_VH1+h2120_VL3	5.64	6.21	2.23	2.42	22mg/L
h2120_VH2+h2120_VL3	6.57	6.43	1.93	2.62	16mg/L
h2120_VH3+h2120_VL3	16.14		3.47		22mg/L
chimeric 2120			0.97	1.72	

[0338] Table 9 shows the measured affinity by ForteBio, competitive ELISA, and functional blocking data (laminin/FACS assay) compared to the rodent parent, as well as the expression levels, from the second round of transfections.

Table 9

	Forte	Forte		Blocking		
			ELISA	Expt#1	Expt#2	
Transfection--round 2	Fold over rodent	Fold over rodent	Fold over rodent	Fold over rodent	Fold over rodent	Expression level
h2120_VH4+h2120_VL3	17.4		5.0	3.8	5.6	15mgL
h2120_VH5+h2120_VL3	**1.1**	**1.2**	**2.4**	**1.2**	1.5	**22mg/L**
h2120_VH1+h2120_VL1	8.8		3.1	2.0	3.5	17mg/L
h2120_VH1+h2120_VL2	10.8		3.1	4.6	12.6	2mg/L
h2120_VH1+h2120_VL3	5.9	5.8	1.8	1.7	2.8	22mg/L
rodent 2120	1.0	1.0	1.0	1.0	1.0	

[0339] Overall, the data demonstrates that the various 2120 humanized antibodies have a >5x reduction in affinity as measured by ForteBio, and most have a >2-3x reduction in apparent affinity and potency as measured by the competitive ELISA and laminin blocking assay, with the exception of VH5VL3 (G-A N-deamidation mutant VH/conservative VL), which had a <2x reduction in affinity and potency.

[0340] Certain candidate antibodies were re-expressed and tested for their affinity by ForteBio and their IC_{50}. The results are provided in Table 10 below.

Table 10

	Forte kD	Blocking IC50	Expression
h2120VH5VL3	1.3	0.7	12.7mg/L

Example 4. Modification of humanized 2120 antibodies

[0341] Utilizing the DNA manipulation methods described above and according to Liu et al. *JBC*. 286:11211-7, 2011, variants of the rat and humanized versions of the 2120.4.19 antibody

mature heavy chain variable regions were constructed. Variants of 2120.4.19, h2120VH1, h2120VH2, h2120VH3, h2120VH4, and h2120VH5 were constructed having a glutamine to glutamic acid substitution at position H1 (Kabat numbering) (Fig. 4A). These variants are referred to as 2120.4.19.Q1E, h2120VH1.Q1E, h2120VH2.Q1E, h2120VH3.Q1E, h2120VH4.Q1E, and h2120VH5.Q1E and are shown in SEQ ID NOS: 156-161. The humanized versions identified by SEQ ID NOs: 157-161 are depicted in the alignment in Fig. 4A. Various rat and humanized antibodies can be constructed using the modified variable heavy chains, including: h2120VH1.Q1E+h2120VL1; h2120VH1.Q1E +h2120VL2; h2120VH1.Q1E +h2120VL3; h2120VH2.Q1E +h2120VL1; h2120VH2.Q1E +h2120VL2; h2120VH2.Q1E +h2120VL3; h2120VH3.Q1E +h2120VL1; h2120VH3.Q1E +h2120VL2; h2120VH3.Q1E +h2120VL3; h2120VH4.Q1E +h2120VL1; h2120VH4.Q1E +h2120VL2; h2120VH4.Q1E +h2120VL3; h2120VH5.Q1E +h2120VL1; h2120VH5.Q1E +h2120VL2; and h2120VH5.Q1E +h2120VL3.

Example 5. MCAM expression is dose- and time-dependently downregulated by PRX003 treatment in hMCAM transgenic mice

[0342] Whole blood samples were analyzed using FACS with gating for NK1.1 and NK cells after single and the final of 4-weekly doses of PRX003.

[0343] In samples taken from one homozygous hMCAM transgenic mouse before or after (at 6 and 96 hours) injection of PRX003 (250 mg/kg), MCAM expression levels and the percentage of $MCAM^+$ cells were time-dependently decreased (FIGS. 5A-C).

[0344] Expression and subsequent reduction of MCAM were measured on NK cells by flow cytometry and were monitored over 7 days. After a single PRX003 dose, a reduction in MCAM expression on NK cells was apparent within the first 6 hours, and maximum MCAM reduction (<30% of baseline) was achieved by 48 hours in all dose groups. MCAM expression returned as the concentration of PRX003 fell to subsaturation levels in serum and approached full recovery at the 10-mg/kg dose (n=32 mice/dose) (FIGS 6A & B).

[0345] After the last of 4 weekly doses of PRX003, time to maximum reduction of MCAM was more rapid at the middle and high PRX003 doses, possibly because of sustained PRX003 exposure and continued partial downregulation through the 168-hour dosing interval with

repeated administration. MCAM expression on NK cells varied normally over time in hMCAM transgenic mice treated with vehicle alone (n=32 mice/dose) (FIGS 6A & B).

Example 6. PRX003 selectively downregulates MCAM and does not affect the viability of MCAM$^+$ cells

[0346] Heterozygous hMCAM transgenic mice, with both mMCAM and hMCAM on the surfaces of NK cells, were used to assess whether the loss of MCAM expression resulted from deletion of the cells.

[0347] PRX003 resulted in hMCAM downregulation (FIG. 7 A) but did not affect the level of mMCAM (FIG. 7 B), indicating that these cells remain viable in circulation. Conversely, clone 15 downregulates mMCAM without affecting levels of hMCAM.

Example 7. Dynamic changes in soluble MCAM induced by PRX003 are tightly coupled with cellular downregulation of MCAM

[0348] After a single dose of PRX003, serum soluble MCAM increased in a dose-dependent fashion in magnitude and duration.

[0349] Although baseline values were not determined, levels of soluble MCAM appeared to rise within 6 hours of dose administration and continued to increase approximately threefold to fourfold over mean vehicle control values at peak levels. This increase appeared to be reversible in nearly all dose groups and generally corresponded to PRX003 concentration and clearance from serum (FIG. 8).

Example 8. Anti-MCAM lowers inflammation and clinical score in a mouse model of EAE, known to involve TH17 cells.

[0350] Two days after the onset of EAE (12-14 days after immunization) and each day thereafter, the animals received either clone 15 neutralizing antibody or isotype control antibody. Mice treated with anti-MCAM had a less severe form of EAE (FIG 9A).

[0351] Anti-MCAM treatment also reduced the numbers of infiltrating MCAM$^+$ T cells in the central nervous system (CNS) detected histologically (FIG. 9B) and MCAM$^+$/CD4$^+$ T cells isolated from the CNS and analyzed by flow cytometry (FIG 9C).

Example 9. Nonclinical safety in hMCAM transgenic mice

[0352] MCAM is expressed primarily on circulating NK cells in the hMCAM transgenic mouse and is expressed at a low level on T cells and neutrophils, a pattern that differs from that seen in humans (because of an unknown mechanism). However, the off-target tissue cross-reactivity profile (e.g, PRX003 binding to vascular, adipocyte, and smooth muscle cells) in hMCAM transgenic mice appears similar to that seen in humans and allows for supplemental safety assessments of PRX003 for potential effects of binding off-target tissues with repeated administration.

[0353] Weekly intravenous injection of PRX003 in $hMCAM^{+/+}$ transgenic mice once weekly for 4 weeks was well tolerated at all dose levels (10, 50, and 250 mg/kg/week).

[0354] Neither PRX003-related change in any of the safety end points evaluated (clinical observation, clinical pathology, gross and microscopic pathology) nor target organ toxicity was observed.

[0355] Although antidrug antibodies were detected in several repeat-dose animals, systemic exposure occurred throughout the dosing interval.

[0356] Consequently, the no-observed-adverse-effect-level (NOAEL) for PRX003 in $hMCAM^{+/+}$ transgenic mice was the highest dose level tested (250 mg/kg/week).

Example 9. Nonclinical safety in hMCAM cynomolgus monkeys

[0357] A 3-month GLP repeat-dose toxicology study in cynomolgus monkeys was conducted.

[0358] Intravenous injection of PRX003 once every 2 weeks was well tolerated at all dose levels tested (10, 50 and 300 mg/kg/dose).

[0359] Dose-related increases in serum PRX003 exposure were maintained to the end of the treatment period.

[0360] No PRX003 treatment-related adverse changes occurred in any of the study parameters evaluated.

[0361] Consequently, the NOAEL for PRX003 in cynomolgus monkeys was the highest dose level tested (300 mg/kg/dose).

Example 10. Pharmacokinetic and Pharmacodynamic Study of PRX003 in Chimpanzee

[0362] The objective of this study was to model the pharmacokinetics and pharmacodynamics relationship of PRX003 (humanized anti-MCAM antibody with mature heavy chain variable region of SEQ ID NO: 161 and mature light chain variable region of SEQ ID NO: 123) and human IgG1 kappa isotype) exposure with MCAM saturation on circulating T cells following single IV infusions of PRX003 (0, 1.5, 5, 15 and 50 mg/kg) in chimpanzees.

[0363] Method: A total of 9 chimpanzees (4 males and 5 females) were dosed on Day 1 via slow IV infusion of vehicle or PRX003.

Table 11

Group	Test Article	Dose Level (mg/kg)	Dose Volume (mL/kg)	Dose Conc. (mg/mL)
1	Vehicle Control	0	1.25	0
2	PRX003	1.5	1.25	1.2
3	PRX003	5	1.25	4.0
4	PRX003	15	1.25	12
5	PRX003	50	1.25	40

[0364] Results: Prescreening for CD4+MCAM+ T Cells. Twenty chimpanzees were prescreened to select 9 animals for enrollment based on an appropriate population of circulating CD4+MCAM+ T cells, along with other factors including health status and housing social order. Among the 9 animals selected, the number of CD4+MCAM+ T cells ranged from 7 to 15% of the total CD3+CD4+ T cell population. An additional prestudy collection from the enrolled animals at Day -7 confirmed the prescreening values.

[0365] Serum PRX003 Levels. Blood was collected by venipuncture predose and at 5 minutes, 8, 24, 48, 96, 168, 240, 336, and weekly thereafter up to 1680 hours (10 weeks) post dose on

Day 1 for pharmacokinetics evaluation. None of the serum samples from the vehicle control group contained quantifiable serum concentrations of PRX003. All animals in the 1.5, 5, 15 and 50 mg/kg dose groups were exposed to PRX003 following a single IV infusion. Serum PRX003 concentrations in all treated animals were below the limit of quantitation (BLQ) by the 1344-hour (8-week) time point.

[0366] Serum ADA levels. Serum anti-PRX003 antibody (ADA) assessments are summarized in Table 12. Serum samples from the 1512-hour time point were initially screened for ADA levels because there was no measurable PRX003 in serum from any animal at this time point to interfere with the assay. A low titer (1:100) of ADA was confirmed in one treated animal (5 mg/kg male) at this time point. In selected earlier time points (predose, 504, 1008 hours) in this animal, also with no quantifiable PRX003 to interfere, no ADA titers were detected and there was no apparent meaningful effect on exposures or PK profile.

Table 12

Dose Group (mg/kg)	Animal Number	OD (1512 hrs)	Screen Positive*	OD Confirmation	Confirmed Positive*	Titer
0	1F	0.050	No	-	-	-
1.5	2M	0.050	No	-	-	-
	2F	0.063	Yes	0.051	No	-
5	3M	0.199	Yes	0.105	Yes	1:100
	3F	0.095	Yes	0.053	No	-
15	4M	0.069	Yes	0.041	No	-
	4F	0.049	No	-	-	-
50	5M	0.054	No	-	-	-
	5F	0.048	No	-	-	-
Confirmed ADA Animal	**OD (Predose)**	**Confirmed Positive***	**OD (504 hrs)**	**Confirmed Positive***	**OD (1008 hrs)**	**Confirmed Positive***
3M	.0.051	No	0.047	No	0.053	No

ADA = anti-drug antibody; F = female; M = male; OD = optical density (1:50 screening dilution; cutpoint = 0.055)

* OD > 0.055

[0367] PRX003 Pharmacokinetics. Individual free serum PRX003 concentrations in chimpanzees were plotted over time in. Dose-dependent, non-linear kinetics was observed over the dose range tested in chimpanzees. Loss of exposure (BLQ = 0.08 µg/mL) occurred within 1-2 weeks following IV administration of the low doses (1.5 and 5 mg/kg). Loss of exposure at 15 mg/kg occurred after 3 weeks while exposures at the 50 mg/kg high dose were maintained for 6-7 weeks. Consistent with the route of administration, peak concentrations of PRX003 were generally observed at the first sampling time point (5 minutes or 0.0833 hours) post end-of-infusion, except in one 15 mg/kg male (Tmax = 8 hours). Consistent with the observed nonlinear kinetics, there were generally greater than proportional increases in exposure with increasing dose, mainly with respect to AUC, where a nearly 200-fold increase was observed with a 33-fold increase from the low (1.5 mg/kg) to high (50 mg/kg) dose. No apparent gender differences in systemic exposure were observed.

[0368] MCAM Occupancy and Expression on Circulating T Cells. Initial evaluation of MCAM occupancy revealed saturable binding by PRX003 (i.e. no labeled PRX003 signal by FACS) on circulating T cells during the first 24 hours in all dose groups, beginning at 5 minutes (0.083 hours) post end-of-infusion (EOI), which corresponded with serum PRX003 levels (>30 µg/mL) that were well above experimental concentrations (1-3 µg/mL) required to saturate MCAM in chimpanzee whole blood, *in vitro*. Through the first 24 hours post EOI, a concurrent time-dependent loss of signal from a labeled non-competing anti-MCAM commercial antibody (P1H12) was observed, indicating a reduction of MCAM expression on the cell surface. For this reason, the expression and subsequent reduction of MCAM were measured independently on CD4+ memory T cells with a more sensitive FACS assay and monitored over time to establish the relationship of PRX003 exposure with reduction/recovery of MCAM expression as a potential PD biomarker of PRX003 activity.

[0369] Both time to maximum reduction in MCAM expression (<10% of baseline) on CD4+ memory T cells and duration of reduction were dose-dependent. Maximum MCAM reduction was achieved by 48 hours at the low dose compared to nearly 2 weeks at the high dose, with duration of effect lasting several days up to 4 weeks, respectively, before recovery back to baseline MCAM expression levels. The vehicle-treated control animal shows normal variability of MCAM expression on CD4+ memory T cells over time. As seen with occupancy and

consistent with MCAM saturation levels *in vitro*, serum PRX003 levels above ~1 µg/mL were generally required for maximum reduction of MCAM on CD4+ memory T cells. Recovery of MCAM was observed as soon as serum PRX003 levels dropped below the predicted saturation concentration and took approximately 2 weeks to return to baseline MCAM expression levels in all dose groups.

[0370] Serum Soluble MCAM levels. Serum soluble MCAM increased in a dose-dependent fashion, with respect to both magnitude and duration, following a single IV infusion of PRX003. Levels of soluble MCAM began to rise in all dose groups by 8 hours, and continued to increase up to ~150% over baseline levels by 48 hours at the low dose and up to ~400% by 840 hours (5 weeks) at the high dose. Within 2-3 weeks after peak levels were observed, soluble MCAM had returned to baseline levels, which generally corresponded with serum PRX003 at or near subsaturating concentrations for binding MCAM+ T cells. The vehicle-treated control animal shows normal variability of soluble MCAM levels.

[0371] MCAM Expression in Skin. Skin punch biopsies (5 mm diameter, area above pectoralis muscle) were taken from all animals at 672 hours following a single IV infusion of vehicle or PRX003 to assess MCAM expression in various cell types, including endothelium, smooth muscle and adipose. Despite dose-dependent reductions in levels of MCAM expression on circulating T cells at this time point, no treatment differences in MCAM levels were detected on non-circulating skin cells, even at the highest dose level.

Example 11. Selection of Doses and Dosing Schedule

[0372] The PK/PD model for MCAM expression that was developed in the chimpanzee was used to estimate human doses that 1) maintain a partial MCAM expression inhibition with an every other week (q2wk) dose interval; 2) maintain full MCAM expression inhibition with a q2wk dose interval, and 3) maintain full MCAM expression inhibition with an every fourth week (q4wk) dose interval. A clinically pragmatic dose regimen can be within this range.

[0373] The model demonstrates that 3 mg/kg-q2wk, 10 mg/kg-q2wk, and 25 mg/kg-q4wk achieve these respective PD targets. These predictions form the basis for a clinical trial dose range that extends up to 30 mg/kg. Nonclinical safety supports this planned dose range.

[0374] The no-observed-adverse-effect-level (NOAEL) in cynomolgus monkeys provides a dose multiple of 1000-fold over the starting dose of 0.3 mg/kg and 10-fold over the highest dose planned of 30 mg/kg PRX003 in the current study." Half-log doses were added in between to arrive at 1 mg/kg, 3 mg/kg and 10 mg/kg in addition to 0.3 mg/kg and 30 mg/kg.

Example 12. Single Ascending Dose Study of PRX003 in Healthy Subjects

[0375] This single ascending dose study is to determine safety, tolerability, pharmacokinetics, and immunogenicity of PRX003.

[0376] Method: Randomized, double-blind, placebo-controlled, single ascending dose-escalation study of PRX003 administered by intravenous infusion over approximately 60 minutes in healthy subjects. Patients enrolled in the trial met all of the following criteria:

1. Healthy subjects
2. Body mass index (B-MI) between 18-32 kg/m2 with a minimum weight of 45 kg
3. Female subjects must be surgically sterile or post-menopausal or if of child-bearing potential must use contraception
4. Male subjects and their partners of childbearing potential must use contraception

[0377] Anyone of the following criteria excluded a patient from being enrolled in the trial:

1. Positive test for drug of abuse
2. Past or current history of alcohol abuse
3. Positive for TB, hepatitis B, hepatitis C or HIV infection

[0378] Based on preclinical models of markers of target engagement, doses were selected to evaluate a range of PD responses spanning from submaximal responses of short duration to maximal responses with durations that meet or exceed the target dose interval of 4 weeks. Five escalating dose cohorts received 0.3, 1.0, 3.0, 10 and 30 mg/kg of PRX003 or placebo and were monitored in an inpatient unit for 24 hours and by periodic follow-up for 12 weeks. This study

provides data on the safety, tolerability, pharmacokinetics, pharmacodynamics and immunogenicity of PRX003 in healthy human subjects.

Example 13: Multiple Ascending Dose Study of PRX003 in Subjects with Psoriasis

[0379] This study determines safety, tolerability, pharmacokinetics, and immunogenicity of PRX003.

[0380] Methods: Randomized, Double-blind, Placebo-controlled, Multiple Ascending Dose Study of PRX003 Administered by Intravenous Infusion in Subjects with Psoriasis. Patients enrolled in the trial met all of the following criteria:

1. Male or female, 18 to 80 years of age (inclusive), body weight range of ≥ 45 kg (99 lb) to ≤ 120 kg (264 lb) and a body mass index (BMI) of 18 - 35 kg/m2
2. Provide written informed consent
3. PASI score of ≥12
4. Plaque psoriasis covering ≥10% of BSA
5. s-PGA score of 3 or 4
6. Able to perform all protocol-specified assessments and comply with the study visit schedule
7. Female subjects who are not postmenopausal or surgically sterile must use physician approved contraception for at least 60 days prior to Baseline (Day 1/Visit 2) to 12 weeks following the last study drug administration. Unless they are at least 2 years postmenopausal or surgically sterile, women must have a pregnancy test with follicle stimulating hormone (FSH) >40 IU/L and estradiol <20 pg/mL (unless on hormone-replacement therapy). Women of childbearing potential must be non lactating and have a negative serum pregnancy test (beta human chorionic gonadotropin [β HCG]) at Screening (Visit 1).

8. If male, must be surgically sterile or must agree to use physician-approved contraception from Baseline (Day 1/Visit 2) to 12 weeks following the last study drug administration

[0381] Anyone of the following criteria excluded a patient from being enrolled in the trial:

1. Presents with psoriasis that is predominantly guttate, erythrodermic, inverse, pustular or palmo-plantar, or an unstable form of psoriasis

2. Receipt of any of the following within the specified time frame prior to Baseline (Day 1/Visit 2):

    - Topical psoriasis treatments (other than low-potency topical corticosteroids or emollients, which are permitted during the study) within 2 weeks

    - Systemic (nonbiologic) psoriasis treatments within 4 weeks or 5 half-lives (whichever is longer)

    - Biologic psoriasis treatments within 12 weeks or 5 half-lives (whichever is longer)

    - Drugs that appear to have a strong causal relationship to psoriasis (e.g., beta-blockers and lithium) within 4 weeks or 5 half-lives (whichever is longer)

    - Phototherapy within 4 weeks

3. Participation in recreational sunbathing or use of a sun-bed (e.g., tanning salon) within 7 days prior to Baseline (Day 1)

4. Any major medical illness or unstable medical condition that, in the opinion of the Investigator or Sponsor, may interfere with the subject's ability to comply with study procedures or abide by study restrictions, or with the ability to interpret safety data, including, but not limited to:

- Within 5 years of Screening (Visit 1)
- History of cancer with the exception of fully excised non-melanoma skin cancer
- History of stroke
- History of epilepsy or seizure disorder other than febrile seizures as a child, or any seizure or loss of consciousness
- History of or active autoimmune disease (other than psoriasis or PsA)
- Within 2 years of Screening (Visit 1)
- Myocardial infarction
- Clinically significant cardiovascular disease including any of the following: unstable angina, decompensated congestive heart failure, clinically significant arrhythmias
- Vascular diseases, including, but not limited to, blood clotting disorders, atherosclerosis, aneurysms, and renal artery disease

5. Hypotension (systolic blood pressure [BP] ≤85 millimeters of mercury [mmHg]) at Baseline (Day 1/Visit 2) predose or a known history or documentation of hypotension on more than one occasion within 3 months prior to Baseline (Day 1/Visit 2)

6. Uncontrolled hypertension as indicated by a resting systolic BP ≥150 mmHg or diastolic BP ≥95 mmHg at Screening (Visit 1) or Baseline (Day 1/Visit 2) predose or a known history or documentation of uncontrolled hypertension on more than one occasion within 3 months prior to Baseline (Day 1/Visit 2)

7. Clinically significant systemic infection (e.g., chronic or acute infection, urinary tract infection, upper respiratory infection) within 30 days of Baseline (Day 1/ Visit 2), or a history or presence of recurrent or chronic infection (e.g., viral

infections [including hepatitis B or C, human immunodeficiency virus (HIV)], bacterial infections, systemic fungal infections, or syphilis)

8. History of any inflammatory bowel disease

9. Any current psychiatric diagnosis according to Diagnostic and Statistical Manual of Mental Disorders IV Text Revision (DSM-IV-TR) that may interfere with the subject's ability to perform the study and all assessments (e.g., alcohol or drug-related abuse or alcohol dependence, or alcohol or drug-related dementia, major depression, developmental disability, schizophrenia, bipolar disorder). Note: Subjects with adequately controlled depression for at least 6 months are not excluded; however, suicidal ideation or attempt at any time within the past year is exclusionary.

10. A positive tuberculosis skin test (TST) during Screening (Visit 1) or a positive Interferon Release Assay Test during Screening for subjects with a history of Bacillus Calmette Guérin (BCG) vaccination. Note: In the event a subject has had a TST within 3 months before Baseline (Day 1/Visit 2), this does not need to be repeated during screening and the previous result can be carried forward and used in this study. Prior standard treatment for latent tuberculosis and prior exposure to tuberculosis with subsequent standard prophylactic treatment is allowed if recent (within < 30 days) negative chest X-ray.

11. Any of the following laboratory abnormalities at Screening (Visit 1):

    - Total bilirubin (unless attributed to Gilbert's syndrome) >1.5 times the upper limit of normal (× ULN), alanine aminotransferase (ALT) or aspartate aminotransferase (AST) >2 × ULN

    - Serum creatinine >133 µmol/L (1.5 mg/dL)

    - Hemoglobin <11.5 g/dL for males or <10.0 g/dL for females, absolute neutrophil count of <1500/µL (with the exception of a documented history of a chronic benign neutropenia), or platelet count of <120,000/µL

12. Use of an investigational product or device or participation in a drug research study within a period of 30 days (or 5 half-lives of the drug, whichever is longer) prior to Screening (Visit 1); for investigational products or drug research studies relating to psoriasis or arthritis, the duration will be extended to 12 weeks (or 5 half-lives of the drug, whichever is longer) prior to Screening (Visit 1)

13. Allergy to any of the components of PRX003 such as histidine, sucrose and polysorbate 20

14. Receipt of any vaccine (with the exception of seasonal influenza) within 30 days prior to Screening (Visit 1)

15. Donation of >500 mL of blood within 3 months prior to Screening (Visit

[0382] Treatment at 1, 3, 10, or 30 mg/kg was administered three times at intervals of 28 days.

Example 14: Formulation of the PRX003 Drug Product

[0383] Description: The PRX003 drug product is supplied as a lyophilized powder for solution for infusion. It is a white to yellowish powder, presented in a Type I glass vial with a nominal fill volume of 5 mL. PRX003 drug product is to be reconstituted with water for injection (WFI) to a concentration of 40 mg/mL in a buffered, isotonic, preservative-free solution. The formulation is composed of 20 mM Histidine buffer, 220 mM Sucrose and 0.2 g/L Polysorbate 20 at pH 6.0. The container closure system consists of a Type I 20/25 mL (20R) glass vial, elastomeric stopper suitable for lyophilization, and aluminum seal with white colored plastic button.

[0384] Composition: The qualitative and quantitative composition of a unit vial of the PRX003 drug product (200 mg/vial) is described in Table 13.

Table 13

Components	Grade	Concentration [mmol/L]	Concentration [g/L]	Nominal amount [mg/vial] V = 5 mL	Function
PRX003	-	0.276	40.0	200	Drug Substance
L-Histidine	USP, Ph. Eur.	9.0	1.40	6.98	Buffer component
L-Histidine HCl monohydrate	USP, Ph. Eur.	11.0	2.31	11.55	Buffer component
Sucrose	USP, Ph. Eur.	220	75.3	376.53	Stabilizer and tonicity agent
Polysorbate 20	USP, Ph. Eur.	N/A	0.20	1.00	Surfactant and stabilizer

[0385] Overfill: An overfill of at least 0.2 mL is applied and the vials are filled to a target fill volume of 5.4 mL ± 0.2 mL to permit an extractable dose of 200 mg per vial, upon reconstitution of the lyophilized cake with 5 mL WFI. In this case the concentration is approximately 40 mg/mL.

WHAT IS CLAIMED IS:

1. A method of treating or effecting prophylaxis of a patient having or at risk of a neuroinflammatory, autoimmune disease, immune disease mediated by TH17 cells, or cancer, comprising administering to the patient an effective regime of an antibody comprising a mature heavy chain variable region at least 90% identical to SEQ ID NO: 161, and a mature light chain variable region at least 90% identical to SEQ ID NO: 123, wherein the effective regime comprises a dose of 0.3 to 50 mg/kg administered on multiple occasions at intervals of 2-5 weeks.

2. The method of claim 1, wherein the antibody is a human IgG1 kappa antibody comprising a mature heavy chain variable region of SEQ ID NO: 161 and a mature light chain variable region of SEQ ID NO: 123.

3. The method of claim 1 or 2, wherein the antibody is administered as a component of a formulation comprising

(a) the antibody at a concentration within a range from about 10 mM to about 50 mM;

(b) one or more sugars and polyols ("sugar/polyol") selected from:

(i) sucrose present at a concentration within the range from about 200 mM to about 260 mM; and

(ii) trehalose present at a concentration within the range from about 200 mM to about 260 mM ;

(c) histidine at 10-30 mM; and

(d) polysorbate 20 present at a concentration within the range from about 0.005% to about 0.05% by weight;

wherein the pharmaceutical formulation is characterized by a pH within the range from about 5.5 to about 7.

4. The method of claim 3, wherein the formulation comprises the antibody at 40 mg/ml, histidine at 20 mM, sucrose at 220 mM, polysorbate at 0.2 g/L and pH 6.

5. The method of any preceding claim, wherein the antibody is administered intravenously.

6. The method of any preceding claim, wherein the interval is four weeks.

7. The method of any preceding claim, wherein the dose is administered at least three times.

8. The method of any preceding claim, wherein the dose is administered at least ten times.

9. The method of any preceding claim, wherein the dose is administered at least until a steady state intravenous concentration is obtained.

10. The method of any preceding claim, wherein the dose is administered for the rest of the patient's life.

11. The method of any preceding claim, wherein the same dose is administered at the same interval on the multiple occasions.

12. The method of any preceding claim, wherein the dose is 0.3, 1.0, 3.0, 10, 30 or 50 mg/kg.

13. The method of any one of claims 1-11, wherein the dose is 0.3-1 mg/kg.

14. The method of any one of claims 1-11, wherein the dose is 1-3 mg/kg.

15. The method of any one of claims 1-11, wherein the dose is 3-10 mg/kg.

16. The method of any one of claims 1-11, wherein the dose is 10-30 mg/kg.

17. The method of any one of claims 1-11, wherein the dose is 30-50 mg/kg.

18. The method of any one of claims 1-11, wherein the dose is 3-10 mg/kg every two weeks administered intravenously.

19. The method of any one of claims 1-11 wherein the dose is 20-30 mg/kg every four weeks administered intravenously.

20. The method of any preceding claim, wherein the disease is multiple sclerosis.

21. The method of any one of claims 1-18 wherein the disease is psoriasis, psoriatic arthritis, Crohn's disease or rheumatoid arthritis.

22. The method of any one of claims 1-18, wherein the disease is Behcet's disease, Giant Cell Arteritis, Polymyalgia Rheumatica, or Takayasu's Arteritis.

23. The method of any one of claim 1-18 wherein the disease is a granulomatous lung disease, such as chronic beryllium disease.

24. The method of any one of claim 1-18, wherein the disease is glioblastoma.

25. The method of any one of claims 1-18, wherein the disease is graft versus host disease.

26. The method of any preceding claim, wherein the antibody is administered in combination with methotrexate, a steroid, a nonsteroidal anti-inflammatory drug, or a combination thereof.

27. The method of any one of claims 1-21, wherein the antibody is administered as a monotherapy.

28. A method of treating or effecting prophylaxis of a patient having or at risk of a neuroinflammatory or autoimmune disease or immune disease mediated at least in part by TH17 cells or cancer comprising administering an inhibitor of expression of MCAM.

29. The method of claim 28, wherein the inhibitor is a zinc finger protein, a transcriptional activator like effector (TALE), an siRNA, an anti-sense RNA, a ribozyme, or Cas9 enzyme and a guide RNA that directs the Cas9 enzyme to binding human MCAM DNA.

30. A method of monitoring patients having or at risk of a neuroinflammatory, autoimmune disease, immune disease mediated at least in part by TH17 cells, or cancer undergoing treatment with an antibody that specifically binds human MCAM and inhibits its interaction with laminin-alpha4, comprising measuring a level of MCAM expressed by CD4 T-cells of the patients or a level of MCAM extracellular domain in a blood sample of the patients, wherein a reduction of a level of MCAM expressed by CD4 T cells or an increase in MCAM extracellular in the blood responsive to treatment indicates a positive response to treatment.

31. The method of claim 30, wherein some patients show a positive response to treatment and some patients do not show a positive response to treatment, and a higher percentage of patients showing a positive response to treatment continue to undergo treatment with the antibody than patients not showing a positive response to treatment.

32. A method of treating or effecting prophylaxis of a patient having or at risk of psoriasis, psoriatic arthritis, Crohn's disease, rheumatoid arthritis, graft versus host disease, a granulomatous lung disease, giant cell arteritis, or glioblastoma comprising administering to the patient an effective regime of pharmaceutical composition, wherein:

a. the pharmaceutical composition has a pH of about 6 and comprises about

   i. 40 mg/ml of an antibody comprising a mature heavy chain variable region of SEQ ID NO: 161 and a mature light chain variable region of SEQ ID NO: 123,

   ii. 20 mM histidine,

   iii. 220 mM sucrose, and

   iv. 0.02% polysorbate 20;

b. the effective regime comprises a dose of 1.0, 3.0 or 10-30 mg/kg administered intravenously every 2-5 weeks.

33. The method of claim 32, wherein the disease is psoriatic arthritis.

34. The method of claim 32, wherein the disease is psoriasis.

35. The method of claim 32, wherein the disease is Crohn's disease.

36. The method of claim 32, wherein the disease is rheumatoid arthritis.

37. The method of claim 32, wherein the disease is graft versus host disease.

38. The method of claim 32, wherein the disease is chronic beryllium disease.

39. The method of claim 32, wherein the disease is giant cell arteritis.

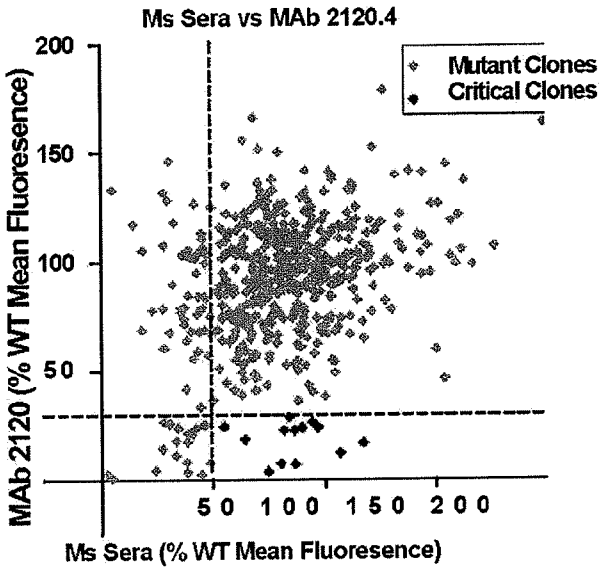
40. The method of claim 32, wherein the disease is glioblastoma.

41. The method of any one of claims 32-40, wherein the dose is 3 mg/kg and is administered about every four weeks.

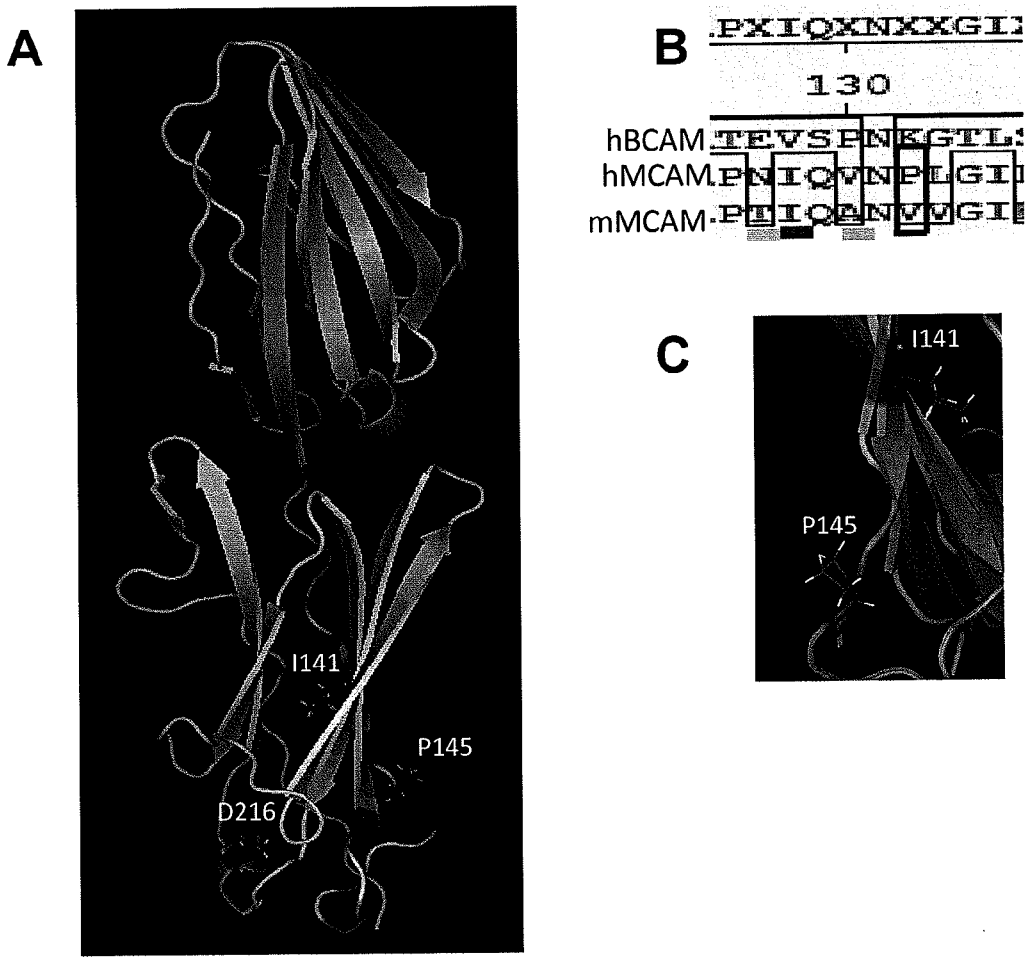
42. The method of any one of claims 32-40, wherein the dose is 10 mg/kg and is administered about every four weeks.

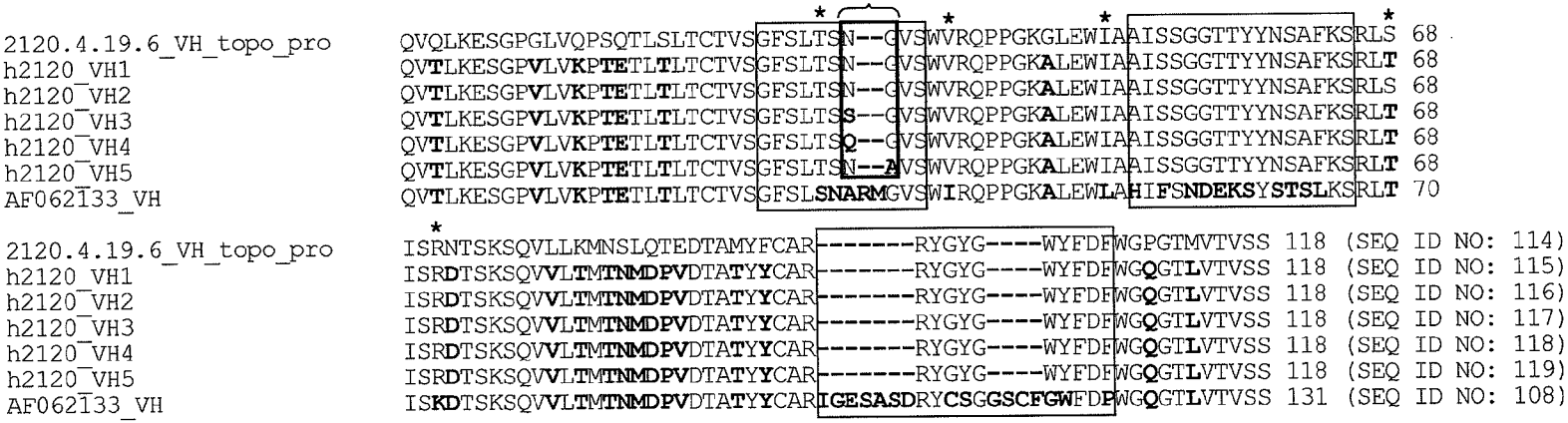
43. The method of any one of claims 32-40, wherein the dose is 30 mg/kg and is administered about every four weeks.

FIG.1


Ms Sera vs MAb 2120.4
Mutant Clones
Critical Clones
MAb 2120 (% WT Mean Fluoresence)
200
150
100
50
50 100 150 200
Ms Sera (% WT Mean Fluoresence)

FIGS. 2A-C

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h2120_VH2 QVTLKESGPVLVKPTETLTLTCTVSGFSLTSN--GVSWVRQPPGKALEWIAAISSGGTTYYNSAFKSRLS 68
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h2120_VH5 QVTLKESGPVLVKPTETLTLTCTVSGFSLTSN--AVSWVRQPPGKALEWIAAISSGGTTYYNSAFKSRLT 68
AF062133_VH QVTLKESGPVLVKPTETLTLTCTVSGFSLSNARMGVSWIRQPPGKALEWLAHIFSNDEKSYSTSLKSRLT 70
2120.4.19.6_VH_topo_pro ISRNTSKSQVLLKMNSLQTEDTAMYFCAR-------RYGYG----WYFDFWGPGTMVTVSS 118 (SEQ ID NO: 114)
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h2120_VH4 ISRDTSKSQVVLTMTNMDPVDTATYYCAR-------RYGYG----WYFDFWGQGTLVTVSS 118 (SEQ ID NO: 118)
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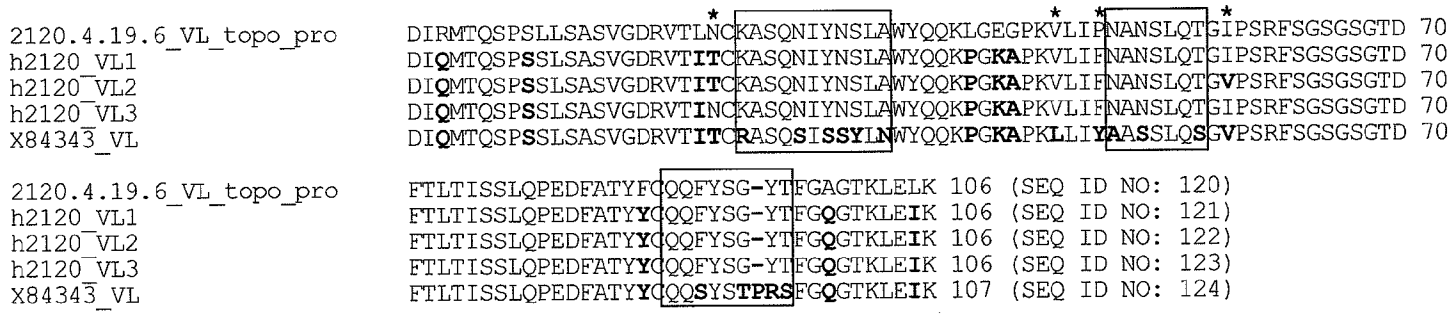
AF062133_VH ISKDTSKSQVVLTMTNMDPVDTATYYCARIGESASDRYCSGGSCFGWFDFWGQGTLVTVSS 131 (SEQ ID NO: 108)

FIG. 3A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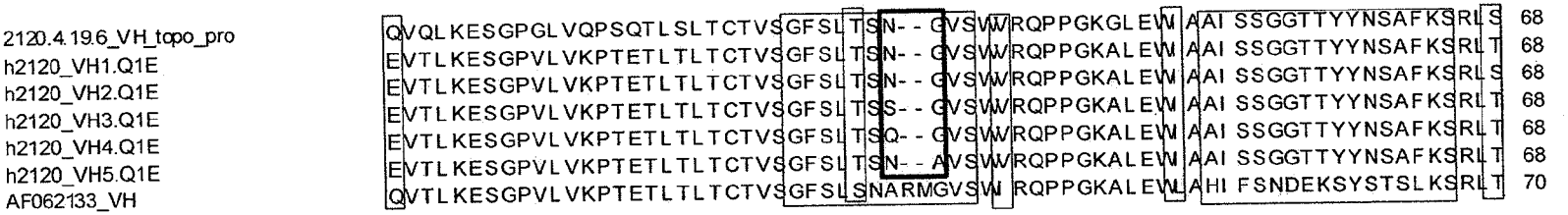
h2120_VL2 DIQMTQSPSSLSASVGDRVTITCKASQNIYNSLAWYQQKPGKAPKVLIFNANSLQTGVPSRFSGSGSGTD 70
h2120_VL3 DIQMTQSPSSLSASVGDRVTINCKASQNIYNSLAWYQQKPGKAPKVLIFNANSLQTGIPSRFSGSGSGTD 70
X84343_VL DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTD 70
2120.4.19.6_VL_topo_pro FTLTISSLQPEDFATYFCQQFYSG-YTFGAGTKLELK 106 (SEQ ID NO: 120)
h2120_VL1 FTLTISSLQPEDFATYYCQQFYSG-YTFGQGTKLEIK 106 (SEQ ID NO: 121)
h2120_VL2 FTLTISSLQPEDFATYYCQQFYSG-YTFGQGTKLEIK 106 (SEQ ID NO: 122)
h2120_VL3 FTLTISSLQPEDFATYYCQQFYSG-YTFGQGTKLEIK 106 (SEQ ID NO: 123)
X84343_VL FTLTISSLQPEDFATYYCQQSYSTPRSFGQGTKLEIK 107 (SEQ ID NO: 124)

FIG. 3B

FIG. 4A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h2120_VH3.Q1E
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h2120_VH5.Q1E
AF062133_VH
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EVTLKESGPVLVKPTETLTLTCTVSGFSLTSN--AVSWVRQPPGKALEWLAAISSGGTTYYNSAFKSRLT 68
QVTLKESGPVLVKPTETLTLTCTVSGFSLSNARMGVSWIRQPPGKALEWLAHIFSNDEKSYSTSLKSRLT 70
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ISRDTSKSQVVLTMTNMDPVDTATYYCAR-------RYGYG---WYFDFWGQGTLVTVSS 118
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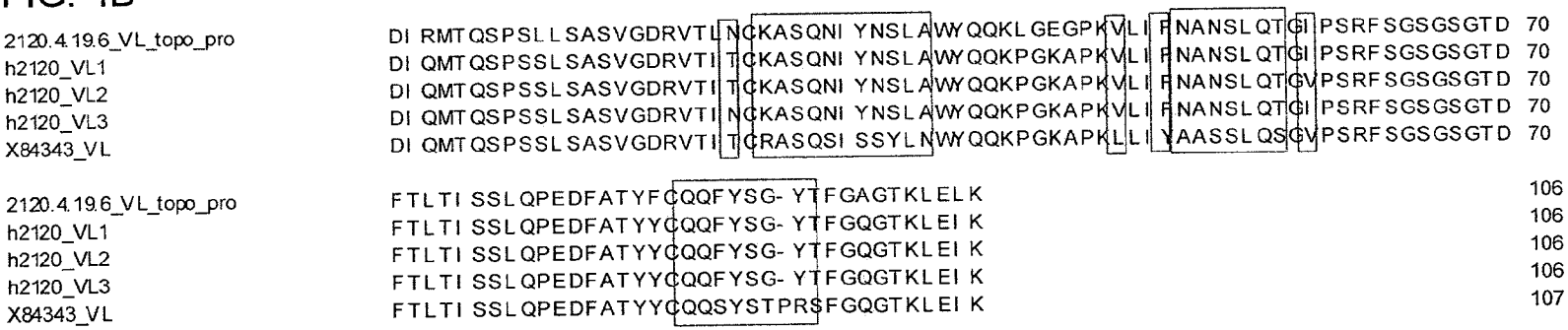
FIG. 4B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h2120_VL2
h2120_VL3
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DIQMTQSPSSLSASVGDRVTITCKASQNIYNSLAWYQQKPGKAPKVLIFNANSLQTGIPSRFSGSGSGTD 70
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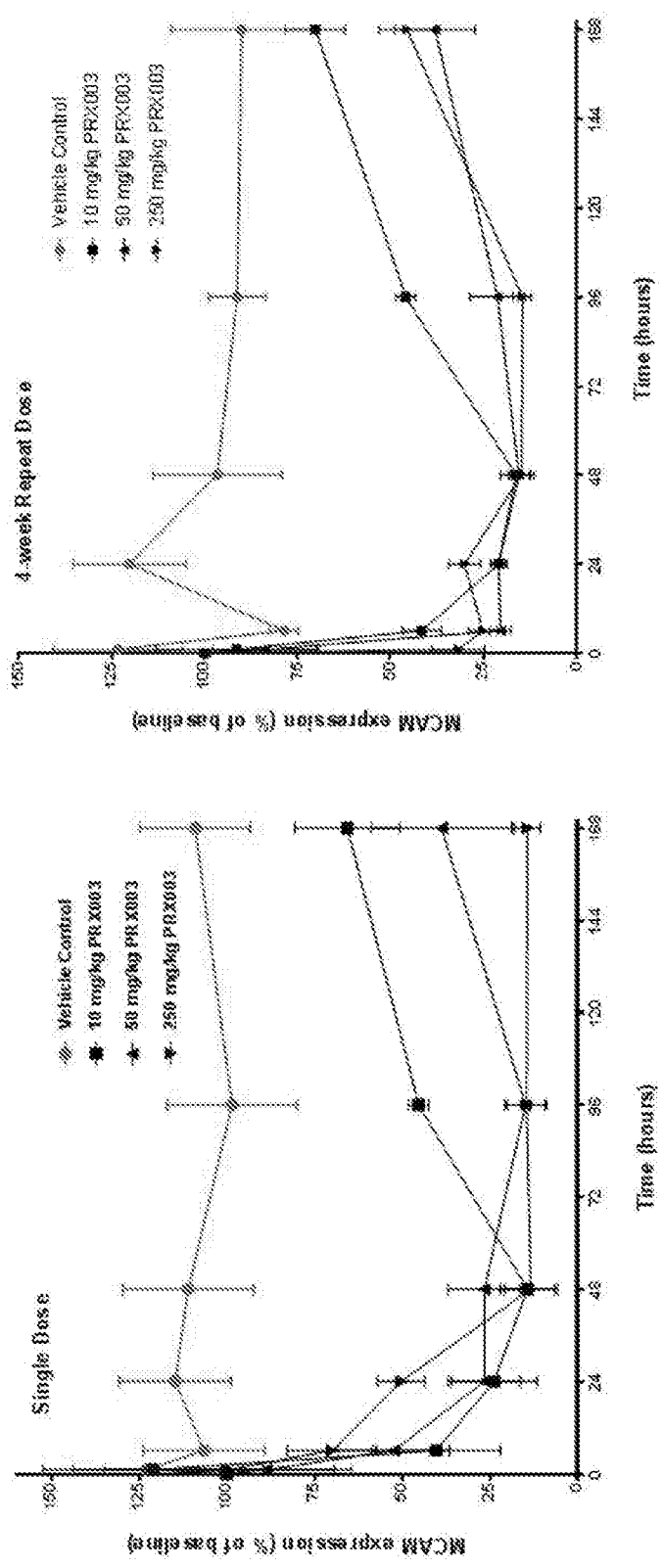
FTLTISSLQPEDFATYYCQQFYSG-YTFGQGTKLEIK 106
FTLTISSLQPEDFATYYCQQSYSTPRSFGQGTKLEIK 107

FIGS. 5A-C

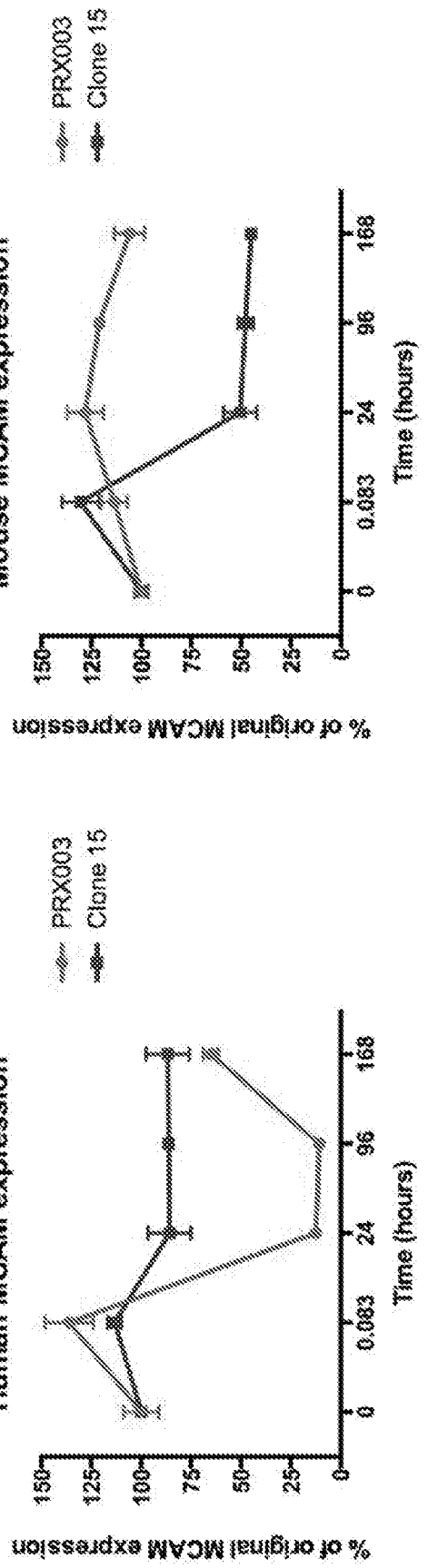
FSC-A, forward scatter A; hMCAM, human melanoma cell adhesion molecule; MFI, mean fluorescence intensity; PE, phycoerythrin.

FIGS. 6A-B



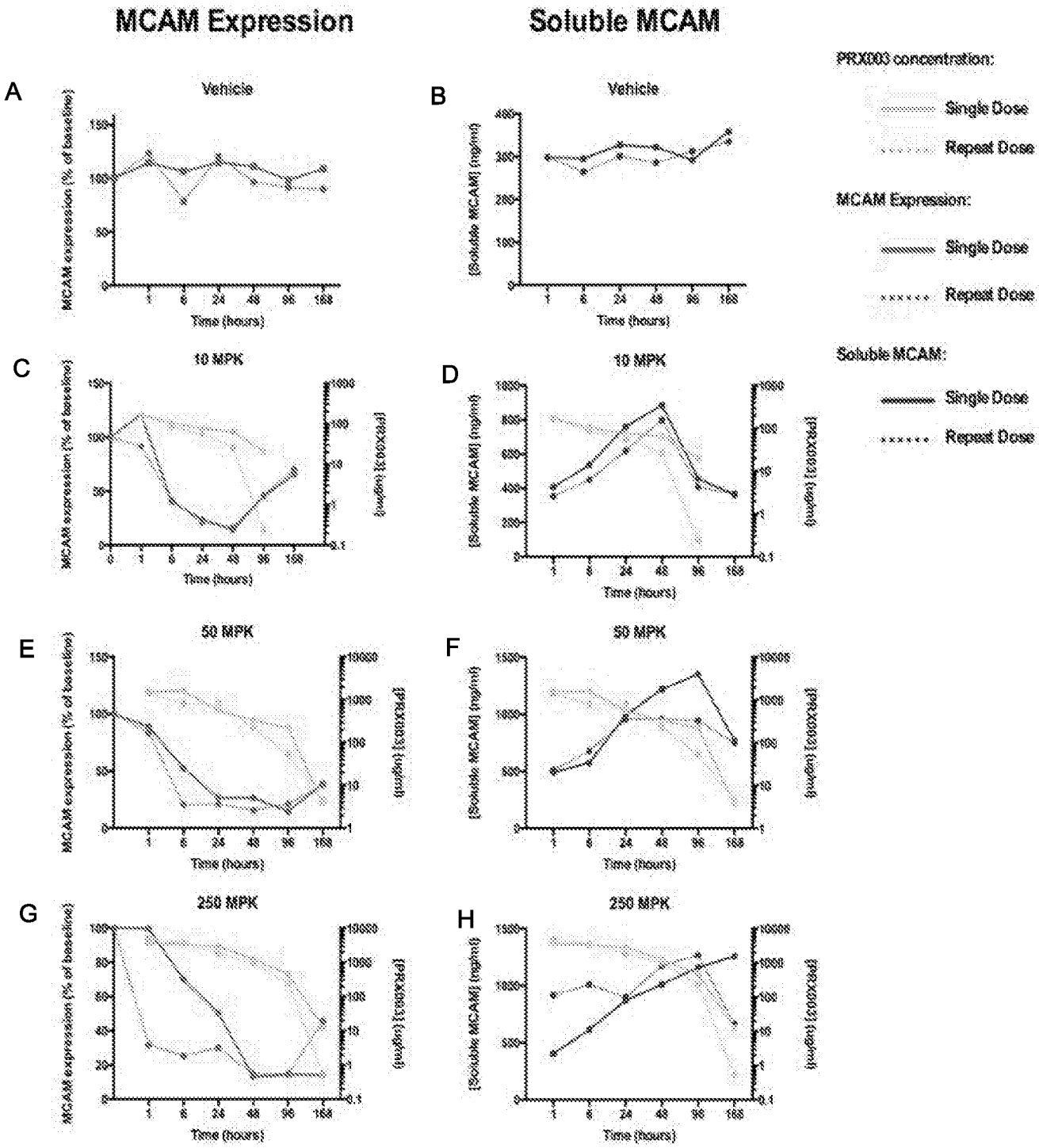
FSC-A, forward scatter A; hMCAM, human melanoma cell adhesion molecule; MFI, mean fluorescence intensity; PE, phycoerythrin.

FIGS. 7A-B



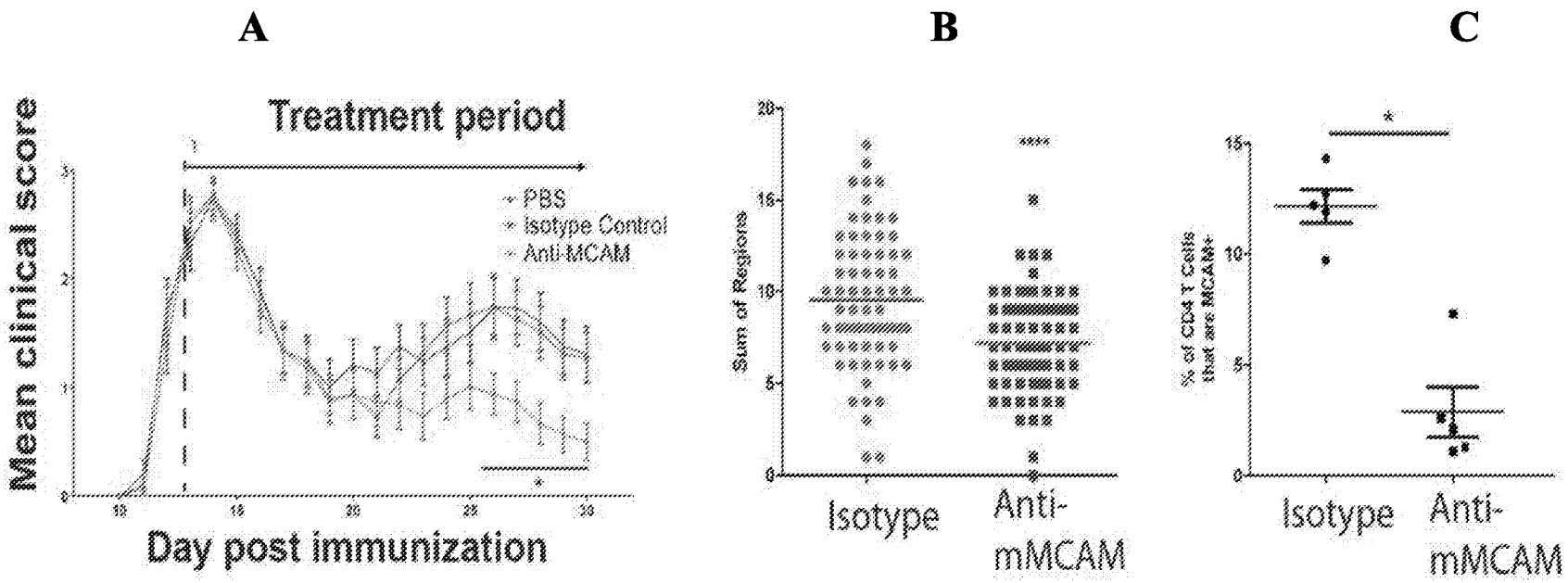
FSC-A, forward scatter A; hMCAM, human melanoma cell adhesion molecule; MFI, mean fluorescence intensity; PE, phycoerythrin. MCAM, melanoma cell adhesion molecule.

FIG. 8



MCAM, melanoma cell adhesion molecule; MPK, mg/kg.

FIGS. 9A-C



EAE, experimental autoimmune encephalomyelitis; MCAM, melanoma cell adhesion molecule; PBS, phosphate-buffered saline; SEM, standard error of the mean.

*$P < 0.05$ by Wilcoxon nonparametric test. Data represent the mean of 15 mice ± SEM.

†$P < 0.0001$. Each dot represents the sum of scored regions.

‡$P < 0.05$, where each dot represents samples from a single mouse.

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2017/051264

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/395 C07K16/30
ADD.

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

B. FIELDS SEARCHED

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

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, EMBASE, BIOSIS, Sequence Search, 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	WO 2015/136470 A1 (PROTHENA BIOSCIENCES LTD [IE]) 17 September 2015 (2015-09-17) cited in the application	1-29, 32-43
Y	paragraphs [0003], [0303], [0304], [0306]; claims 13, 53, 57-65; sequences 123, 161	30,31

X	WO 2015/136468 A1 (PROTHENA BIOSCIENCES LTD [IE]; UNIV MUNSTER [DE]) 17 September 2015 (2015-09-17) paragraphs [0360], [0365], [0370]; claims 1, 9, 11, 28; sequences 86, 100,	1-29, 32-43

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

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
31 July 2017	08/08/2017

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	Saame, Tina

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2014/039975 A2 (NEOTOPE BIOSCIENCES LTD [IE]; FLANAGAN KENNETH [US]; BAKER JEANNE [US]) 13 March 2014 (2014-03-13) cited in the application	28,29
A	claims 38, 45,47; sequences 119, 123	1-27, 32-43

X	WO 2012/170071 A1 (ELAN PHARMACEUTICAS INC [US]; FLANAGAN KENNETH [US]; JOHNSTON JENNIFER) 13 December 2012 (2012-12-13) cited in the application	28,29
A	the whole document abstract	1-27, 32-43

X	HONGXIA DUAN ET AL: "Targeting endothelial CD146 attenuates neuroinflammation by limiting lymphocyte extravasation to the CNS", SCIENTIFIC REPORTS, vol. 3, no. 1, 18 April 2013 (2013-04-18), XP55394070, DOI: 10.1038/srep01687 page 3, column 1 - column 2 page 3, column 1	28,29

A	Kapaettu Satyamoorthy ET AL: "Mel-CAM-speci c genetic suppressor elements inhibit melanoma growth and invasion through loss of gap junctional communication", , 2 August 2001 (2001-08-02), XP55394065, Retrieved from the Internet: URL:http://www.nature.com/onc/journal/v20/n34/pdf/1204616a.pdf [retrieved on 2017-07-26] abstract	28,29

Y	C. LAROCHELLE ET AL: "Melanoma cell adhesion molecule identifies encephalitogenic T lymphocytes and promotes their recruitment to the central nervous system", BRAIN, vol. 135, no. 10, 13 September 2012 (2012-09-13), pages 2906-2924, XP055201643, ISSN: 0006-8950, DOI: 10.1093/brain/aws212 page 2914	30,31

	-/--	

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	PRADEEP K DAGUR ET AL: "MCAM-expressing CD4T cells in peripheral blood secrete IL-17A and are significantly elevated in inflammatory autoimmune diseases", JOURNAL OF AUTOIMMUNITY, LONDON, GB, vol. 37, no. 4, 7 September 2011 (2011-09-07), pages 319-327, XP028119862, ISSN: 0896-8411, DOI: 10.1016/J.JAUT.2011.09.003 [retrieved on 2011-09-13] abstract -----	30,31

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2017/051264

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

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

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

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

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

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

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

see additional sheet

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

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

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

1-27, 30-43(completely); 28, 29(partially)

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

Remark on Protest

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

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

☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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

1. claims: 1-27, 32-43

A method of treating or effecting prophylaxis of a patient having or at risk of a neuroinflammatory or autoimmune disease or immune disease mediated by TH17 cells, or cancer, comprising administering to the patient an effective regime of an antibody comprising a mature heavy chain variable region at least 90% identical to SEQ ID NO: 161, and a mature light chain variable region at least 90% identical to SEQ ID NO: 123, wherein the effective regime comprises a dose of 0.3 to 50 mg/kg administered on multiple occasions at intervals of 2-5 weeks; a method of treating or effecting prophylaxis of a patient having or at risk of psoriasis, psoriatic arthritis, Crohn's disease, rheumatoid arthritis, graft versus host disease, a granulomatous lung disease, giant cell arteritis, or glioblastoma comprising administering to the patient an effective regime of pharmaceutical composition, wherein the pharmaceutical composition has a pH of about 6 and comprises about 40 mg/ml of an antibody comprising a mature heavy chain variable region of SEQ ID No 161 and a mature light chain variable region of SEQ ID No 123, 20 mM histidine, 220 mM sucrose, and 0.02% polysorbate 20; and wherein the effective regime comprises a dose of 1.0, 3.0 or 10-30 mg/kg administered intravenously every 2-5 weeks.

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2. claims: 28, 29(partially)

A method of treating or effecting prophylaxis of a patient having or at risk of a neuroinflammatory or autoimmune disease or immune disease mediated at least in part by TH17 cells , comprising administering an inhibitor of expression of MCAM.

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3. claims: 28, 29(partially)

A method of treating or effecting prophylaxis of a patient having or at risk of a cancer comprising administering an inhibitor of expression of MCAM.

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4. claims: 30, 31

A method of monitoring patients having or at risk of a neuroinflammatory or autoimmune disease or immune disease mediated at least in part by TH17 cells, or cancer undergoing treatment with an antibody that specifically binds human MCAM and inhibits its interaction with laminin-alpha4, comprising measuring a level of MCAM expressed by CD4 T-cells of the patients or a level of MCAM

extracellular domain in a blood sample of the patients, wherein a reduction of a level of MCAM expressed by CD4 T cells or an increase in MCAM extracellular in the blood responsive to treatment indicates a positive response to treatment.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2017/051264

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