(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
17 September 2015 (17.09.2015)

WIPO | PCT

(10) International Publication Number
WO 2015/136469 A1

(51) International Patent Classification:
C07K 16/30 (2006.01)

(21) International Application Number:
PCT/IB2015/051786

(22) International Filing Date:
12 March 2015 (12.03.2015)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

61/952,123	12 March 2014 (12.03.2014)	US
62/023,698	11 July 2014 (11.07.2014)	US
62/068,438	24 October 2014 (24.10.2014)	US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

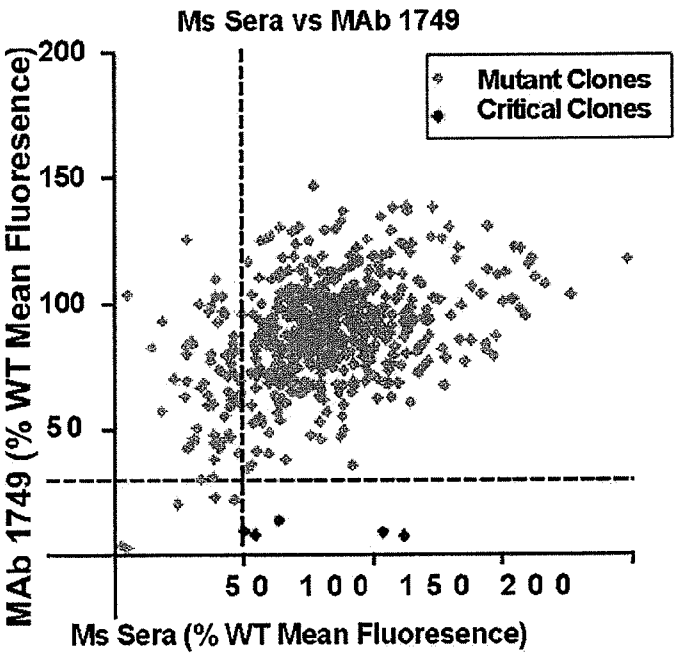
(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

[Continued on next page]

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

(57) Abstract: The invention provides anti-MCAM antibodies that inhibit the ability of human MCAM to bind a laminin alpha-4 chain. The invention also provides pharmaceutical compositions, methods of generating such antibodies, and their use in the manufacture of medicaments for treatment of neuroinflammatory disease, autoimmune disease, or cancer.

FIG.1



WO 2015/136469 A1

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

Published:

— *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-REFERENCES TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 61/952,123, filed March 12, 2014, U.S. Provisional Application No. 62/023,698, filed July 11, 2014, and U.S. Provisional Application No. 62/068,438, filed October 24, 2014, each of the aforementioned applications is incorporated in its entirety herein for all purposes.

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

[0002] The Sequence Listing written in file 459014SEQLIST.txt, created on March 4, 2015, for "ANTI-MCAM ANTIBODIES AND ASSOCIATED METHODS OF USE" is 148 kilobytes. 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 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 humanized antibodies comprising a mature heavy chain variable region comprising the three Kabat CDRs of SEQ ID NO:156, and being at least 97% identical to SEQ ID NO:156, and a mature light chain variable region comprising the three Kabat CDRs of SEQ ID NO:160, and being at least 97% identical to SEQ ID NO:160. In some antibodies, the mature heavy chain variable region is at least 98% or 99% identical to SEQ ID NO:156 and the mature light chain variable region is at least 98% or 99% identical to SEQ ID NO:160. In some antibodies, the mature heavy chain variable region has the amino acid

sequence of SEQ ID NO:156 and the mature light chain variable region has the amino acid sequence of SEQ ID NO:160. In some antibodies, position 93 (Kabat numbering) of the mature heavy chain variable region is occupied by T; position 42 (Kabat numbering) of the mature heavy chain variable region is occupied by E; position 43 (Kabat numbering) of the mature light chain variable region is occupied by S; position 9 (Kabat numbering) of the mature light heavy chain variable region is occupied by S; position 19 (Kabat numbering) of the mature light heavy chain variable region is occupied by V. In some antibodies, position 93 (Kabat numbering) of the mature heavy chain variable region is occupied by T; position 42 (Kabat numbering) of the mature heavy chain variable region is occupied by E; position 3 (Kabat numbering) of the mature heavy chain variable region is occupied by K, position 43 (Kabat numbering) of the mature light chain variable region is occupied by S; position 9 (Kabat numbering) of the mature light heavy chain variable region is occupied by S; position 19 (Kabat numbering) of the mature light heavy chain variable region is occupied by V. In some antibodies, the heavy chain constant region has the amino acid sequence of SEQ ID NO: 173 or 174 and/or the light chain constant region has the amino acid sequence of SEQ ID NO: 170 or 171.

[0005] Then ivention further provides anti-MCAM antibodies that bind to human MCAM (SEQ ID NO:11) at an epitope including amino acid residue 318. In some such antibodies, the epitope comprises amino acid residue 324. In some such antibodies, the epitope comprises amino acid residue 326. In some antibodies, the epitope comprises at least five contiguous amino acids residues of human MCAM including amino acid residue 318. In some such antibodies, the antibody is not an antibody selected from the group consisting of:

(a) clone 15 having a mature heavy chain variable region corresponding to SEQ ID NO:18 and a mature light chain variable region corresponding to SEQ ID NO:13;

(b) clone 17 having a mature heavy chain variable region corresponding to SEQ ID NO:7 and a mature light chain variable region corresponding to SEQ ID NO:2;

(c) 1174.1.3 having a mature heavy chain variable region corresponding to SEQ ID NO:35 and a mature light chain variable region corresponding to SEQ ID NO:30;

(d) 1414.1.2 having a mature heavy chain variable region corresponding to SEQ ID NO:45 and a mature light chain variable region corresponding to SEQ ID NO:40;

(e) 1415.1.1 having a mature heavy chain variable region corresponding to SEQ ID NO:55 and a mature light chain variable region corresponding to SEQ ID NO:50;

(f) 1749.1.3 having a mature heavy chain variable region corresponding to SEQ ID NO:65 and a mature light chain variable region corresponding to SEQ ID NO:60;

(g) 2120.4.19 having a mature heavy chain variable region corresponding to SEQ ID NO:77 and a mature light chain variable region corresponding to SEQ ID NO:70;

(h) 2107.4.10 having a mature heavy chain variable region corresponding to SEQ ID NO:89 and a mature light chain variable region corresponding to SEQ ID NO:84: and

(i) an antibody comprising CDRs substantially from the monoclonal antibodies 1174.1.3, 1414.1.2 , 1415.1.1, 1749.1.3, 2120.4.19, and 2107.4.10. In some such antibodies, the antibody is monoclonal. In some such antibodies, the antibody is chimeric, humanized, veneered, or human.

In some such antibodies, the antibody is not an antibody selected from the group consisting of:

(a) clone 15 having a mature heavy chain variable region corresponding to SEQ ID NO:18 and a mature light chain variable region corresponding to SEQ ID NO:13;

(b) clone 17 having a mature heavy chain variable region corresponding to SEQ ID NO:7 and a mature light chain variable region corresponding to SEQ ID NO:2;

(c) 1174.1.3 having a mature heavy chain variable region corresponding to SEQ ID NO:35 and a mature light chain variable region corresponding to SEQ ID NO:30;

(d) 1414.1.2 having a mature heavy chain variable region corresponding to SEQ ID NO:45 and a mature light chain variable region corresponding to SEQ ID NO:40;

(e) 1415.1.1 having a mature heavy chain variable region corresponding to SEQ ID NO:55 and a mature light chain variable region corresponding to SEQ ID NO:50;

(f) 1749.1.3 having a mature heavy chain variable region corresponding to SEQ ID NO:65 and a mature light chain variable region corresponding to SEQ ID NO:60;

(g) 2120.4.19 having a mature heavy chain variable region corresponding to SEQ ID NO:77 and a mature light chain variable region corresponding to SEQ ID NO:70, 71,or 72;

(h) 2107.4.10 having a mature heavy chain variable region corresponding to SEQ ID NO:89 and a mature light chain variable region corresponding to SEQ ID NO:82 or 84: and

(i) an antibody comprising CDRs substantially from the monoclonal antibodies 1174.1.3, 1414.1.2 , 1415.1.1, 1749.1.3, 2120.4.19, and 2107.4.10. In some such antibodies, the antibody is monoclonal. In some such antibodies, the antibody is chimeric, humanized, veneered, or human.

[0006] The invention further provides a pharmaceutical composition comprising any of the above-mentioned antibodies.

[0007] The invention further provides the use of any of the above-mentioned antibodies in the manufacture of a medicament for the treatment of an inflammatory disorder characterized by infiltration of MCAM-expressing cells into a site of inflammation in the body. Such an inflammatory disorder may be a central nervous system (CNS) inflammatory disorder characterized by infiltration of MCAM-expressing cells into the CNS.

[0008] The invention further provides the use of any of the above-mentioned antibodies in the manufacture of a medicament for the treatment of multiple sclerosis, Parkinson's disease, allergic contact dermatitis, psoriasis, psoriatic arthritis, rheumatoid arthritis, sarcoidosis, inflammatory bowel disease, Crohn's disease, or cancer (e.g. solid or haematologic tumors), such as melanoma.

[0009] The invention further provides a method of treating an inflammatory disorder characterized by infiltration of MCAM-expressing cells to a site of inflammation, the method comprising administering to a mammalian subject in need thereof an effective amount of any of the above-mentioned antibodies. In some methods, the disease is multiple sclerosis, Parkinson's disease, allergic contact dermatitis, psoriasis, psoriatic arthritis, rheumatoid arthritis, sarcoidosis, inflammatory bowel disease, Crohn's disease, or cancer (e.g. solid or haematologic tumors), such as melanoma. In some methods, the MCAM-expressing cells are TH17 cells. In some methods, the mammalian subject is a human. In some of the methods, the antibody inhibits the binding of

MCAM to a protein comprising a laminin α-4 chain. In some of the methods, the mammalian subject is a human. In some of the methods, the MCAM-expressing cells are TH17 cells.

[0010] The invention further provides an isolated peptide comprising an epitope for binding an anti-MCAM monoclonal antibody, wherein the peptide comprises 5-50 contiguous amino acid residues of human MCAM (SEQ ID NO:11) including amino acid residue 318. In some of these peptides, the peptide is linked to a carrier polypeptide. In some of these peptides, the peptide is combined with an adjuvant.

[0011] The invention further provides for a method of generating an antibody that inhibits binding of human MCAM to a laminin α-4 chain, comprising:

(a) immunizing a subject with a peptide described above;

(b) isolating B-cells from the subject, wherein the B-cells secrete antibodies;

(c) screening the antibodies to identify an antibody that inhibits binding of human MCAM to a laminin α-4 chain. In some of the methods, the method further comprises:

(d) fusing the B-cells with immortalized cells in culture to form monoclonal antibody-producing hybridoma cells;

(e) culturing the hybridoma cells; and,

(f) isolating monoclonal antibodies from culture.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] FIG. 1 depicts the identification of critical clones. The mean 1749.1.3 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

[0013] FIG. 2 depicts a homology model of human MCAM, indicating the location of five residues identified as potentially critical binding sites for 1749.1.3, including C272, Y318, C320, V340, and W377.

[0014] FIG. 3A depicts an alignment of the amino acid sequences of 1749.1.3 with the humanized 1749 light chain mature variable regions. ABA71407.1 and CAI99800.1 are the human acceptor V_L sequence. CDR regions according to Kabat definition are highlighted in gray.

[0015] FIG. 3B depicts an alignment of the amino acid sequences of 1749.1.3 with the humanized 1749 heavy chain mature variable regions. AAX82494.1 and ADX65676.1 are the human acceptor V_H sequence. CDR regions according to Kabat definition are highlighted in gray.

BRIEF DESCRIPTION OF THE SEQUENCES

[0016] SEQ ID NO:1 is the nucleic acid sequence encoding the mature light chain variable region of antibody clone 17.

[0017] SEQ ID NO:2 is the amino acid sequence of the mature light chain variable region of antibody clone 17.

[0018] SEQ ID NO:3 is the amino acid sequence of CDRL1 of the antibody clone 17.

[0019] SEQ ID NO:4 is the amino acid sequence of CDRL2 of the antibody clone 17.

[0020] SEQ ID NO:5 is the amino acid sequence of CDRL3 of the antibody clone 17.

[0021] SEQ ID NO:6 is the nucleic acid sequence encoding the mature heavy chain variable region of antibody clone 17.

[0022] SEQ ID NO:7 is the amino acid sequence of the mature heavy chain variable region of antibody clone 17.

[0023] SEQ ID NO:8 is the amino acid sequence of CDRH1 of the antibody clone 17.

[0024] SEQ ID NO:9 is the amino acid sequence of CDRH2 of the antibody clone 17.

[0025] SEQ ID NO:10 is the amino acid sequence of CDRH3 of the antibody clone 17.

[0026] SEQ ID NO:11 is the amino acid sequence of human MCAM Accession No. CAA48332.

[0027] SEQ ID NO:12 is the nucleic acid sequence encoding the mature light chain variable region of antibody clone 15.

[0028] SEQ ID NO:13 is the amino acid sequence of the mature light chain variable region of antibody clone 15.

[0029] SEQ ID NO:14 is the amino acid sequence of CDRL1 of the antibody clone 15.

[0030] SEQ ID NO:15 is the amino acid sequence of CDRL2 of the antibody clone 15.

[0031] SEQ ID NO:16 is the amino acid sequence of CDRL3 of the antibody clone 15.

[0032] SEQ ID NO:17 is the nucleic acid sequence encoding the mature heavy chain variable region of antibody clone 15.

[0033] SEQ ID NO:18 is the amino acid sequence of the mature heavy chain variable region of antibody clone 15.

[0034] SEQ ID NO:19 is the amino acid sequence of CDRH1 of the antibody clone 15.

[0035] SEQ ID NO:20 is the amino acid sequence of CDRH2 of the antibody clone 15.

[0036] SEQ ID NO:21 is the amino acid sequence of CDRH3 of the antibody clone 15.

[0037] SEQ ID NO:22 is the amino acid sequence of human MCAM domain 1 (residues 19-129).

[0038] SEQ ID NO:23 is the amino acid sequence of human MCAM domain 2 (residues 139-242).

[0039] SEQ ID NO:24 is the amino acid sequence of human MCAM domain 3 (residues 244-321).

[0040] SEQ ID NO:25 is the amino acid sequence of human MCAM domain 4 (residues 355-424).

[0041] SEQ ID NO:26 is the amino acid sequence of human MCAM domain 5 (residues 430-510).

[0042] SEQ ID NO:27 is the amino acid sequence of an α4-chain isoform of human laminin 411 (Accession No. NP001098676).

[0043] SEQ ID NO:28 is the amino acid sequence of an α4-chain isoform of human laminin 411 (Accession No. CAA48332).

[0044] SEQ ID NO:29 is the nucleic acid sequence encoding the mature light chain variable region of antibody 1174.1.3.

[0045] SEQ ID NO:30 is the amino acid sequence of the mature light chain variable region of antibody 1174.1.3.

[0046] SEQ ID NO:31 is the amino acid sequence of CDRL1 of antibody 1174.1.3.

[0047] SEQ ID NO:32 is the amino acid sequence of CDRL2 of antibody 1174.1.3.

[0048] SEQ ID NO:33 is the amino acid sequence of CDRL3 of antibody 1174.1.3.

[0049] SEQ ID NO:34 is the nucleic acid sequence encoding the mature heavy chain variable region of antibody 1174.1.3.

[0050] SEQ ID NO:35 is the amino acid sequence of the mature heavy chain variable region of antibody 1174.1.3.

[0051] SEQ ID NO:36 is the amino acid sequence of CDRH1 of antibody 1174.1.3.

[0052] SEQ ID NO:37 is the amino acid sequence of CDRH2 of antibody 1174.1.3.

[0053] SEQ ID NO:38 is the amino acid sequence of CDRH3 of antibody 1174.1.3.

[0054] SEQ ID NO:39 is the nucleic acid sequence encoding the mature light chain variable region of antibody 1414.1.2.

[0055] SEQ ID NO:40 is the amino acid sequence of the mature light chain variable region of antibody 1414.1.2.

[0056] SEQ ID NO:41 is the amino acid sequence of CDRL1 of antibody 1414.1.2.

[0057] SEQ ID NO:42 is the amino acid sequence of CDRL2 of antibody 1414.1.2.

[0058] SEQ ID NO:43 is the amino acid sequence of CDRL3 of antibody 1414.1.2.

[0059] SEQ ID NO:44 is the nucleic acid sequence encoding the mature heavy chain variable region of antibody 1414.1.2.

[0060] SEQ ID NO:45 is the amino acid sequence of the mature heavy chain variable region of antibody 1414.1.2.

[0061] SEQ ID NO:46 is the amino acid sequence of CDRH1 of antibody 1414.1.2.

[0062] SEQ ID NO:47 is the amino acid sequence of CDRH2 of antibody 1414.1.2.

[0063] SEQ ID NO:48 is the amino acid sequence of CDRH3 of antibody 1414.1.2.

[0064] SEQ ID NO:49 is the nucleic acid sequence encoding the mature light chain variable region of antibody 1415.1.1.

[0065] SEQ ID NO:50 is the amino acid sequence of the mature light chain variable region of antibody 1415.1.1.

[0066] SEQ ID NO:51 is the amino acid sequence of CDRL1 of antibody 1415.1.1.

[0067] SEQ ID NO:52 is the amino acid sequence of CDRL2 of antibody 1415.1.1.

[0068] SEQ ID NO:53 is the amino acid sequence of CDRL3 of antibody 1415.1.1.

[0069] SEQ ID NO:54 is the nucleic acid sequence encoding the mature heavy chain variable region of antibody 1415.1.1.

[0070] SEQ ID NO:55 is the amino acid sequence of the mature heavy chain variable region of antibody 1415.1.1.

[0071] SEQ ID NO:56 is the amino acid sequence of CDRH1 of antibody 1415.1.1.

[0072] SEQ ID NO:57 is the amino acid sequence of CDRH2 of antibody 1415.1.1.

[0073] SEQ ID NO:58 is the amino acid sequence of CDRH3 of antibody 1415.1.1.

[0074] SEQ ID NO:59 is the nucleic acid sequence encoding the mature light chain variable region of antibody 1749.1.3.

[0075] SEQ ID NO:60 is the amino acid sequence of the mature light chain variable region of antibody 1749.1.3.

[0076] SEQ ID NO:61 is the amino acid sequence of CDRL1 of antibody 1749.1.3.

[0077] SEQ ID NO:62 is the amino acid sequence of CDRL2 of antibody 1749.1.3.

[0078] SEQ ID NO:63 is the amino acid sequence of CDRL3 of antibody 1749.1.3.

[0079] SEQ ID NO:64 is the nucleic acid sequence encoding the mature heavy chain variable region of antibody 1749.1.3.

[0080] SEQ ID NO:65 is the amino acid sequence of the mature heavy chain variable region of antibody 1749.1.3.

[0081] SEQ ID NO:66 is the amino acid sequence of CDRH1 of antibody 1749.1.3.

[0082] SEQ ID NO:67 is the amino acid sequence of CDRH2 of antibody 1749.1.3.

[0083] SEQ ID NO:68 is the amino acid sequence of CDRH3 of antibody 1749.1.3.

[0084] SEQ ID NO:69 is the nucleic acid sequence encoding a mature light chain variable region of antibody 2120.4.19.

[0085] 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.

[0086] SEQ ID NO:71 is the amino acid sequence of a mature light chain variable region of antibody 2120.4.19.

[0087] SEQ ID NO:72 is the amino acid sequence of a mature light chain variable region of antibody 2120.4.19.

[0088] SEQ ID NO:73 is the amino acid sequence of CDRL1 of antibody 2120.4.19.

[0089] SEQ ID NO:74 is the amino acid sequence of CDRL2 of antibody 2120.4.19.

[0090] SEQ ID NO:75 is the amino acid sequence of CDRL3 of antibody 2120.4.19.

[0091] SEQ ID NO:76 is the nucleic acid sequence encoding the mature heavy chain variable region of antibody 2120.4.19.

[0092] SEQ ID NO:77 is the amino acid sequence of the mature heavy chain variable region of antibody 2120.4.19.

[0093] SEQ ID NO:78 is the amino acid sequence of CDRH1 of antibody 2120.4.19.

[0094] SEQ ID NO:79 is the amino acid sequence of CDRH2 of antibody 2120.4.19.

[0095] SEQ ID NO:80 is the amino acid sequence of CDRH3 of antibody 2120.4.19.

[0096] SEQ ID NO:81 is a nucleic acid sequence encoding a mature light chain variable region of antibody 2107.4.10.

[0097] 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.

[0098] SEQ ID NO:83 is a nucleic acid sequence encoding a mature light chain variable region of antibody 2107.4.10.

[0099] 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.

[0100] SEQ ID NO:85 is the amino acid sequence of CDRL1 of antibody 2107.4.10.

[0101] SEQ ID NO:86 is the amino acid sequence of CDRL2 of antibody 2107.4.10.

[0102] SEQ ID NO:87 is the amino acid sequence of CDRL3 of antibody 2107.4.10.

[0103] SEQ ID NO:88 is the nucleic acid sequence encoding the mature heavy chain variable region of antibody 2107.4.10.

[0104] SEQ ID NO:89 is the amino acid sequence of the mature heavy chain variable region of antibody 2107.4.10.

[0105] SEQ ID NO:90 is the amino acid sequence of CDRH1 of antibody 2107.4.10.

[0106] SEQ ID NO:91 is the amino acid sequence of CDRH2 of antibody 2107.4.10.

[0107] SEQ ID NO:92 is the amino acid sequence of CDRH3 of antibody 2107.4.10.

[0108] SEQ ID NO:93 is the amino acid sequence of the mature heavy chain variable region of antibody 1749.1.3.

[0109] SEQ ID NO:94 is the amino acid sequence of the mature heavy chain variable region of humanized antibody 1749 version 1 (VH1).

[0110] SEQ ID NO:95 is the amino acid sequence of the mature heavy chain variable region of humanized antibody 1749 version 2 (VH2).

[0111] SEQ ID NO:96 is the amino acid sequence of the heavy chain variable framework donor U96282_VH.

[0112] SEQ ID NO:97 is the amino acid sequence of the mature light chain variable region of antibody 1749.1.3.

[0113] SEQ ID NO:98 is the amino acid sequence of the mature light chain variable region of humanized antibody 1749 version 1 (VL1).

[0114] SEQ ID NO:99 is the amino acid sequence of the mature light chain variable region of humanized antibody 1749 version 2 (VL2).

[0115] SEQ ID NO:100 is the amino acid sequence of the light chain variable framework donor X02990_VL.

[0116] SEQ ID NO:101 is the amino acid sequence of the mature heavy chain variable region of antibody 2107.4.10.18.

[0117] SEQ ID NO:102 is the amino acid sequence of the mature heavy chain variable region of humanized antibody 2107 version 1 (VH1).

[0118] SEQ ID NO:103 is the amino acid sequence of the mature heavy chain variable region of humanized antibody 2107 version 2 (VH2).

[0119] SEQ ID NO:104 is the amino acid sequence of the mature heavy chain variable region of humanized antibody 2107 version 3 (VH3).

[0120] SEQ ID NO:105 is the amino acid sequence of the mature heavy chain variable region of humanized antibody 2107 version 4A (VH4A).

[0121] SEQ ID NO:106 is the amino acid sequence of the mature heavy chain variable region of humanized antibody 2107 version 5A (VH5A).

[0122] SEQ ID NO:107 is the amino acid sequence of the mature heavy chain variable region of humanized antibody 2107 version 6 (VH6).

[0123] SEQ ID NO:108 is the amino acid sequence of the heavy chain variable framework donor AF062133_VH.

[0124] SEQ ID NO:109 is the amino acid sequence of the mature light chain variable region of antibody 2107.4.10.18.

[0125] SEQ ID NO:110 is the amino acid sequence of the mature light chain variable region of humanized antibody 2107 version 1 (VL1).

[0126] SEQ ID NO:111 is the amino acid sequence of the mature light chain variable region of humanized antibody 2107 version 2 (VL2).

[0127] SEQ ID NO:112 is the amino acid sequence of the mature light chain variable region of humanized antibody 2107 version 3 (VL3).

[0128] SEQ ID NO:113 is the amino acid sequence of the light chain variable framework donor U86803.

[0129] SEQ ID NO:114 is the amino acid sequence of the mature heavy chain variable region of antibody 2120.4.19.6.

[0130] SEQ ID NO:115 is the amino acid sequence of the mature heavy chain variable region of humanized antibody 2120 version 1 (VH1).

[0131] SEQ ID NO:116 is the amino acid sequence of the mature heavy chain variable region of humanized antibody 2120 version 2 (VH2).

[0132] SEQ ID NO:117 is the amino acid sequence of the mature heavy chain variable region of humanized antibody 2120 version 3 (VH3).

[0133] SEQ ID NO:118 is the amino acid sequence of the mature heavy chain variable region of humanized antibody 2120 version 4 (VH4).

[0134] SEQ ID NO:119 is the amino acid sequence of the mature heavy chain variable region of humanized antibody 2120 version 5 (VH5).

[0135] SEQ ID NO:120 is the amino acid sequence of the mature light chain variable region of antibody 2120.4.19.6.

[0136] SEQ ID NO:121 is the amino acid sequence of the mature light chain variable region of humanized antibody 2120 version 1 (VL1).

[0137] SEQ ID NO:122 is the amino acid sequence of the mature light chain variable region of humanized antibody 2120 version 2 (VL2).

[0138] SEQ ID NO:123 is the amino acid sequence of the mature light chain variable region of humanized antibody 2120 version 3 (VL3).

[0139] SEQ ID NO:124 is the amino acid sequence of the light chain variable framework donor X84343_VL.

[0140] SEQ ID NO:125 is the amino acid sequence of a humanized heavy chain framework region.

[0141] SEQ ID NO:126 is the amino acid sequence of a humanized heavy chain framework region.

[0142] SEQ ID NO:127 is the amino acid sequence of a humanized heavy chain framework region.

[0143] SEQ ID NO:128 is the amino acid sequence of a humanized heavy chain/light chain framework region.

[0144] SEQ ID NO:129 is the amino acid sequence of a humanized light chain framework region.

[0145] SEQ ID NO:130 is the amino acid sequence of a humanized light chain framework region.

[0146] SEQ ID NO:131 is the amino acid sequence of a humanized light chain framework region.

[0147] SEQ ID NO:132 is the amino acid sequence of a humanized light chain framework region.

[0148] SEQ ID NO:133 is the amino acid sequence of a humanized heavy chain framework region.

[0149] SEQ ID NO:134 is the amino acid sequence of a humanized heavy chain framework region.

[0150] SEQ ID NO:135 is the amino acid sequence of a humanized heavy chain framework region.

[0151] SEQ ID NO:136 is the amino acid sequence of a humanized heavy chain framework region.

[0152] SEQ ID NO:137 is the amino acid sequence of a humanized heavy chain framework region.

[0153] SEQ ID NO:138 is the amino acid sequence of a humanized heavy chain framework region.

[0154] SEQ ID NO:139 is the amino acid sequence of CDRH1 of humanized antibody 2120 version 3 (VH3).

[0155] SEQ ID NO:140 is the amino acid sequence of CDRH1 of humanized antibody 2120 version 4 (VH4).

[0156] SEQ ID NO:141 is the amino acid sequence of CDRH1 of humanized antibody 2120 version 5 (VH5).

[0157] SEQ ID NO:142 is the amino acid sequence of a humanized light chain framework region.

[0158] SEQ ID NO:143 is the amino acid sequence of a humanized light chain framework region.

[0159] SEQ ID NO:144 is the amino acid sequence of a humanized light chain framework region.

[0160] SEQ ID NO:145 is the amino acid sequence of a humanized light chain framework region.

[0161] SEQ ID NO:146 is the amino acid sequence of a humanized light chain framework region.

[0162] SEQ ID NO:147 is the amino acid sequence of a humanized light chain framework region.

[0163] SEQ ID NO:148 is the amino acid sequence of a humanized light chain framework region.

[0164] SEQ ID NO:149 is the amino acid sequence of a humanized light chain framework region.

[0165] SEQ ID NO:150 is the amino acid sequence of a humanized light chain framework region.

[0166] SEQ ID NO:151 is the amino acid sequence of CDRH1 of humanized antibody 2107 version 1 (VH1).

[0167] SEQ ID NO:152 is the amino acid sequence of CDRH1 of humanized antibody 2107 version 4 (VH4).

[0168] SEQ ID NO:153 is the amino acid sequence of CDRH3 of humanized antibody 2120 version 1-5 (VH1-VH5).

[0169] SEQ ID NO:154 is the amino acid sequence of a humanized light chain framework region.

[0170] SEQ ID NO:155 is the amino acid sequence of a humanized heavy chain framework region.

[0171] SEQ ID NO:156 is the amino acid sequence of the mature heavy chain variable region of humanized antibody 1749 version 3 (VH3).

[0172] SEQ ID NO:157 is the amino acid sequence of the mouse heavy chain variable region structure template PBD#1HILVH.

[0173] SEQ ID NO:158 is the amino acid sequence of the heavy chain variable acceptor framework ACC#AAX82494.1.

[0174] SEQ ID NO:159 is the amino acid sequence of the heavy chain variable acceptor framework ACC#ADX65676.1.

[0175] SEQ ID NO:160 is the amino acid sequence of the mature light chain variable region of humanized antibody 1749 version 3 (VL3).

[0176] SEQ ID NO:161 is the amino acid sequence of the mouse light chain variable region structure template PDB#2LTQVL.

[0177] SEQ ID NO:162 is the amino acid sequence of the light chain variable acceptor framework ACC#ABA71407.1.

[0178] SEQ ID NO:163 is the amino acid sequence of the light chain variable acceptor framework CAI99800.1.

[0179] 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.

[0180] 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.

[0181] 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.

[0182] 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.

[0183] SEQ ID NO:168 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.

[0184] SEQ ID NO:169 is the amino acid sequence of the exemplary signal peptide encoded by the nucleic acid sequence of SEQ ID NO:168.

[0185] SEQ ID NO:170 is the amino acid sequence of a humanized 1749 light chain constant region, with Arginine at the N-terminus.

[0186] SEQ ID NO:171 is the amino acid sequence of a humanized 1749 light chain constant region, without Arginine at the N-terminus.

[0187] SEQ ID NO:172 is the amino acid sequence of a humanized 1749 heavy chain constant region.

[0188] SEQ ID NO:173 is the amino acid sequence of a BIP version heavy chain G1m3 allotype constant region.

[0189] SEQ ID NO:174 is the amino acid sequence of a BIP version heavy chain G1m3 allotype constant region.

[0190] SEQ ID NO:175 is the amino acid sequence of a mature light chain region of humanized antibody 1749 version 3 (VL3 + light chain constant region).

[0191] SEQ ID NO:176 is the amino acid sequence of a mature heavy chain region of humanized antibody 1749 version 3 (VH3 + BIP version heavy chain G1m3 allotype constant region).

[0192] SEQ ID NO:177 is the amino acid sequence of a mature heavy chain region of humanized antibody 1749 version 3 (VH3 + BIP version heavy chain G1m3 allotype constant region).

[0193] SEQ ID NO:178 is the amino acid sequence of the mature heavy chain variable region of humanized antibody 2107 version 4B (VH4B).

[0194] SEQ ID NO:179 is the amino acid sequence of the mature heavy chain variable region of humanized antibody 2107 version 5B (VH5B).

DEFINITIONS

[0195] 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.

[0196] 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.

[0197] 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.

[0198] 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).

[0199] 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.

[0200] 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.

[0201] 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.

[0202] 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).

[0203] 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).

[0204] 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).

[0205] 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.

[0206] 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.

[0207] "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 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 95% or at least about 100%.

[0208] A "subject" includes a human or other mammalian subject that receives either prophylactic or therapeutic treatment.

[0209] 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.

[0210] 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.

[0211] Compositions or methods "comprising" one or more recited elements may include other elements not specifically recited. For example, a composition that comprises an antibody may contain the antibody alone or in combination with other ingredients.

[0212] Designation of a range of values includes all integers within or defining the range, and all subranges defined by integers within the range.

[0213] Unless otherwise apparent from the context, the term "about" encompasses values within a standard margin of error of measurement (SEM) of a stated value.

[0214] Statistical significance means $p \leq 0.05$.

DETAILED DESCRIPTION

I. GENERAL

[0215] Antibodies with the useful property of inhibiting MCAM binding to the laminin α4 chain of laminin 411 are disclosed in WO/2012/170071 and PCT/US2013/058773. The present

application among other things (a) provides new humanized forms of the 1749.1.3 antibody, (b) maps the epitopes to which the 1749.1.3 antibody binds, and (c) provides antibodies binding to the same epitope.

[0216] The terms "1749.1.3", "m1749", or "mouse 1749" antibody refer to a mouse derived monoclonal antibody clone having a mature variable heavy chain corresponding to SEQ ID NO:93 and a mature variable light chain corresponding to SEQ ID NO:97. "Humanized 1749" or "hu1749" refers humanized variants of the 1749.1.3 clone. The humanized variant of 1749 having a mature heavy chain variable region corresponding to SEQ ID NO:156 and a mature light chain variable region corresponding to SEQ ID NO:160 is referred to herein as "hu1749VH3VL3".

II. TARGET MOLECULES

[0217] Natural human wildtype MCAM (melanoma cell adhesion molecule, also known as CD146 and MUC18) is a peptide 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).

(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 19-129; 2: amino acid residues 139-242; 3: amino acid residues 244-321; 4: amino acid residues 335-424; and 5: amino acid residues 430-510), shown as SEQ ID NOs:22-26.

[0218] 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.

[0219] 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 CAA48332 (SEQ ID NOs:27 and 28). "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.

[0220] Antagonist against MCAM include antibodies, fusion proteins of receptors or ligands to an IgG constant region other biologic binding molecules, and small molecules. Antibodies can be monoclonal or polyclonal. Antibodies can be nonhuman, such as mouse or rat, nonhuman primate or can be human. Antibodies can be chimeric, veneered, humanized, primatized and the like.

[0221] An MCAM antagonist refers to an antagonist that fully or partially inhibits the ability of MCAM (i) to specifically bind its ligand: a laminin α4 chain, *e.g.*, the α4 chain of laminin 411; and/or (ii) to facilitate an MCAM-expressing cell, *e.g.*, a TH17 cell, to infiltrate into or migrate to a subject's tissue. MCAM antagonists include antibodies or other antagonists binding to MCAM or to its ligand laminin alpha 4.

III. ANTIBODIES

A. Humanized Forms of Anti-MCAM Antibody 1749

[0222] A humanized antibody is a genetically engineered antibody in which the CDRs from a non-human "donor" antibody are grafted into human "acceptor" antibody sequences (see, e.g., Queen et al., US 5,530,101 and 5,585,089; Winter et al., US 5,225,539; Carter, US 6,407,213; Adair, US 5,859,205 and 6,881,557; and Foote, US 6,881,557). The acceptor antibody sequences can be, for example, a mature human antibody variable region sequence, a composite of such sequences, a consensus sequence of human antibody variable region sequences (e.g., light and heavy chain variable region consensus sequences of Kabat, 1991, supra), or a germline variable region sequence.

[0223] Examples of an acceptor sequence for the heavy chain are the human mature heavy chain variable regions with NCBI accession codes AAX82494.1 (GI:62421461) and/or ADX65676.1 (GI:323432073). Preferably a composite of these acceptors is used, as is the case in the present examples. These acceptor sequences include two CDRs having the same canonical form and the same length CDR-H3 with a kinked base as m1749 heavy chain and AAX82494.1 has a 91% sequence identity and ADX65676.1 has an 83% sequence identity in the heavy chain variable region framework. For the light chain, examples of an acceptor sequence are the light chain mature variable regions with NCBI accession codes ABA71407.1 (GI:77379502) and/or CAI99800.1 (GI:98956324). Preferably a composite of these sequences is used, as is the case in the present examples. These acceptor sequences include three CDRs having the same canonical form as a m1749 light chain and ABA71407.1 has an 85% sequence identity and CAI99800.1 has an 83% sequence identity in the light chain variable region framework.

[0224] The invention provides humanized antibodies having three light chain and three heavy chain CDRs as defined by Kabat entirely or substantially from the donor m1749 antibody and mature variable region framework sequences and constant regions, if present, entirely or substantially from human antibody sequences. Likewise a humanized heavy chain is a heavy chain having three heavy chain CDRs as defined by Kabat entirely or substantially from the heavy chain of the m1749 antibody, and a mature heavy chain variable sequence and heavy chain constant region sequence, if present, entirely or substantially from human antibody heavy chain

sequence. Likewise a humanized light chain is a light chain having three light chain CDRs as defined by Kabat entirely or substantially from the light chain of the m1749 antibody, and a mature light chain variable sequence and light chain constant region sequence, if present, entirely or substantially from human antibody light chain sequence. Some antibodies comprise a humanized heavy chain comprising Kabat CDR1 of SEQ ID NO:66; SYIMS; Kabat CDR2 of SEQ ID NO:67: TISSGGSSTYYPDSVKG; Kabat CDR3 of SEQ ID NO:68: DDDYDVKVFAY. Some antibodies comprise a humanized light chain comprising Kabat CDR1 of SEQ ID NO:61: KSSRSLLNSRIRKNYLA; Kabat CDR2 of SEQ ID NO:62: WASTRES; Kabat CDR3 of SEQ ID NO:63: KQSYNLLT. Some antibodies comprise a humanized heavy chain comprising the three Kabat CDRs of SEQ ID NOs:66, 67, and 68, and a humanized light chain comprising the three Kabat CDRs of SEQ ID NOs:61, 62, and 63. A CDR is substantially from m1749 if at least 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% of residues are identical to the corresponding residues in the corresponding CDR of m1749 except for CHRH2 Kabat positions 60-65 can be substituted. The mature variable region framework sequences of an antibody chain or the constant region sequence of an antibody chain are substantially from a human mature variable region framework sequence or human constant region sequence respectively when at least 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% of corresponding residues defined by Kabat are identical.

[0225] Certain amino acids from the human mature variable region framework residues can be selected for substitution based on their possible influence on CDR conformation and/or binding to antigen, mediating interaction between heavy and light chains, interaction with the constant region, being a site for desired or undesired post-translational modification, being an unusual residue for its position in a human variable region sequence and therefore potentially immunogenic, among other reasons. The following six variable region framework positions were considered as candidates for substitutions for one or more of these reasons as further specified in the Examples (D9S, A19V, P43S, Q3K, G42E, A93T).

[0226] Here as elsewhere, the first-mentioned residue is the residue of a humanized antibody formed by grafting Kabat CDRs into a human acceptor framework, and the second-mentioned residue is a residue being considered for replacing such residue. Thus within variable region

frameworks, the first mentioned residue is human and within CDRs the first mentioned residue is mouse (e.g., C97S).

[0227] Amino acid substitutions can be made in the CDRs. One possible variation is to substitute certain residues in the CDRs of the m1749 antibody with corresponding residues from human CDRs sequences, typically from the CDRs of the human acceptor sequences used in designing the exemplified humanized antibodies. 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.

[0228] One reason for performing a substitution within a CDR is that a mouse residue is a site of posttranslational modification that may interfere with expression or assembly of an antibody.

[0229] The invention provides variants of the humanized 1749 antibody in which the humanized heavy chain mature variable region shows at least 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO:156 and the humanized light chain mature variable region shows at least 90%, 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID NO:160. Some such humanized antibodies include three heavy and three light chain CDRs entirely or substantially identical to the CDR regions of hu1749, which are the same as those of the mouse donor

antibody. The CDR regions can be defined by any conventional definition (e.g., Chothia) but are preferably as defined by Kabat.

[0230] The humanized 1749 antibody in which the humanized heavy chain mature variable region is SEQ ID NO:156 and the humanized light chain mature variable region is SEQ ID NO:160 is refered to as 1749VH3VL3. Some variants of the humanized 1749VH3VL3 antibody retain some or all of the backmutations in hu1749VH3VL3. In other words, at least 1, 2, 3, 4, 5, or preferably all 6 of the following are present: H3 is occupied by K, H42 is occupied by E, H93 is occupied by T, L9 is occupied by S, L19 is occupied by V, and L43 is occupied by S.

[0231] In addition to retaining at least 1, 2, 3, 4, 5, or preferably all 6 of the backmutations of hu1749VH3VL3, humanized 1749 antibodies may also contain additional backmutations in the variable region frameworks. Examples of such backmutations include include H1 occupied by D, H10 occupied by D, H13 occupied by K, H19 occupied by K, H113 occupied by A, L5 occupied by S, L15 occupied by A, L18 occupied by K, L21 occupied by M, L63 occupied by T, L78 occupied by V, L83 occupied by L, L100 occupied by A, L104 occupied by L, and/or L106 occupied by L. For selection of backmutations for a therapeutic or diagnostic product, one should take into account the degree to which they in general do not improve affinity and the degree to which introducing more mouse residues may give increased risk of immunogenicity.

[0232] In any of the above antibodies, other amino acid substitutions can be made in the mature variable region framework, for example, in residues not in contact with the CDRs. Often the replacements made in the variant humanized sequences are conservative with respect to the replaced amino acids.

B. Selection of Constant Region

[0233] 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.

[0234] 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 reduces 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:174. 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:170. The N-terminal arginine of SEQ ID NO:170 can be omitted, in which case light chain kappa constant region has the amino acid sequence of SEQ ID NO:171. An exemplary human IgG1 heavy chain constant region has the amino acid sequence of SEQ ID NO:172 (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.

[0235] 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:173. Another heavy chain constant region has the amino acid sequence of SEQ ID NO:173 except that it lacks the C-terminal lysine. Another heavy chain constant region has the amino acid sequence of SEQ ID NO:174. Yet another heavy chain constant region has the amino acid sequence of SEQ ID NO:174 except that it lacks the C-terminal lysine.

[0236] 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.

C. Expression of Recombinant Antibodies

[0237] 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.

[0238] 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.

[0239] 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).

[0240] 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.

[0241] 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)).

[0242] 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).

D. Nucleic Acids

[0243] The invention further provides nucleic acids encoding any of the heavy and light chains described above. Typically, the nucleic acids also encode a signal peptide fused to the mature heavy and light chains (e.g., signal peptides having amino acid sequences of SEQ ID NOs:165, 167, and 169 that can be encoded by SEQ ID NOs:164, 166, and 168). Coding sequences on nucleic acids can be in operable linkage with regulatory sequences to ensure expression of the coding sequences, such as a promoter, enhancer, ribosome binding site, transcription termination signal and the like. The nucleic acids encoding heavy and light chains can occur in isolated form or can be cloned into one or more vectors. The nucleic acids can be synthesized by for example, solid state synthesis or PCR of overlapping oligonucleotides. Nucleic acids encoding heavy and light chains can be joined as one contiguous nucleic acid, e.g., within an expression vector, or can be separate, e.g., each cloned into its own expression vector.

E. Characterization of MCAM Epitopes for Antibody Binding and Production of Antibodies That Bind the Same

1. MCAM Epitopes for Antibody Binding

[0244] The invention provides monoclonal antibodies that bind to specific epitopes within the human MCAM protein. Some antibodies of the invention bind to the same or overlapping epitope as antibody designated 1749.1.3 (m1749).

[0245] The invention provides antibodies that bind to the same or overlapping epitope as antibody designated m1749. Mutations at residues 272, 318, 320, 340, and 377 of MCAM disrupts specific binding of m1749 (e.g., <30% binding to mutant MCAM compared to a positive control wild type MCAM as described as the examples). Because relatively few residues affect binding and the residues are spaced more broadly than a typical linear epitope (e.g., 3-20 contiguous amino acids), these results provide an indication that m1749 binds to a

conformational epitope. Alternatively, one or more of the residues affecting binding may do so allosterically without direct contact with the antibody.

[0246] Antibodies binding to an epitope including one or more of residues 272, 318, 320, 324, 326, 340, and 377 of MCAM, and particularly to an epitope including one or more of residues 318, 324, and 326, are likely to share useful inhibitory properties with m1749. Thus, antibodies whose specific binding is inhibited by mutagenesis of one or more or residues 318, 324, and 326 and particularly residue 318 of MCAM are likely to share similar properties to m1749. Some such antibodies bind to an epitope that includes or consists of residue 318, 324, and/or 326 of MCAM. The epitope can be linear, such as an epitope (e.g., 2-5, 3-5, 3-10, 3-15, 3-20, 5-10, 5-15, 5-20, 5-30, 5-40, 5-50, 5-60, or 5-70 contiguous amino acids) including 1, 2, or 3 of the specified amino acids (318, 324, and 326) or be conformational including or consisting of 1, 2, or 3 of the specified amino acids.

2. The Generation of Antibodies That Bind Specific MCAM Epitopes

[0247] Some antibodies of the invention bind to the same or overlapping epitope as the m1749 antibody. The production of other non-human monoclonal antibodies, e.g., murine, guinea pig, primate, rabbit or rat, against human MCAM can be accomplished by, for example, immunizing the animal with human MCAM or a peptide fragment thereof including the desired epitope (the "immunogen"), and screening resulting antibodies for binding to MCAM, optionally in competition with m1749 (See Harlow & Lane, *Antibodies, A Laboratory Manual* (CSHP NY, 1988) incorporated by reference for all purposes). Optionally, the immunogen is conjugated to carrier molecule. Optionally, the immunogen is administered with an adjuvant. Several types of adjuvant can be used as described below. Complete Freund's adjuvant followed by incomplete adjuvant is preferred for immunization of laboratory animals. Rabbits or guinea pigs are typically used for making polyclonal antibodies. Mice are typically used for making monoclonal antibodies. Antibodies are screened for specific binding to a desired epitope within MCAM.

[0248] The invention provides peptide fragments of MCAM that are used to create antibodies directed to the above described epitopes. Examples of such peptides include a peptide that is between 2-5, 3-5, 3-10, 3-15, 3-20, 5-10, 5-15, 5-20, 5-30, 5-40, 5-50, 5-60, or 5-70 contiguous amino acids in length and includes at least one of amino acids residues 318, 324, and

326 of MCAM. In some of these peptides, the peptide includes all three of amino acid residue 318, 324, and 326.

[0249] Immunogens may be conjugated to carrier molecules, typically a carrier polypeptide, and thus help elicit an immune response against the fragment conjugated to the carrier. A single agent can be linked to a single carrier, multiple copies of an agent can be linked to multiple copies of a carrier, which are in turn linked to each other, multiple copies of an agent can be linked to a single copy of a carrier, or a single copy of an agent can be linked to multiple copies of a carrier, or different carriers. Suitable carriers include serum albumins, keyhole limpet hemocyanin, immunoglobulin molecules, thyroglobulin, ovalbumin, tetanus toxoid, or a toxoid from other pathogenic bacteria, such as diphtheria (*e.g.*, CRM_{197}), *E. coli,* cholera, or *H. pylori*, or an attenuated toxin derivative.

[0250] Immunogens are often administered with pharmaceutically acceptable adjuvants. The adjuvant increases the titer of induced antibodies and/or the binding affinity of induced antibodies relative to the situation if the peptide were used alone. A variety of adjuvants can be used in combination with an immunogenic fragment of MCAM, to elicit an immune response. Preferred adjuvants augment the intrinsic response to an immunogen without causing conformational changes in the immunogen that affect the qualitative form of the response. Preferred adjuvants include aluminum hydroxide and aluminum phosphate, 3 De-O-acylated monophosphoryl lipid A (MPL™) (*see* GB 2220211 (RIBI ImmunoChem Research Inc., Hamilton, Montana, now part of Corixa). Stimulon™ QS-21 is a triterpene glycoside or saponin isolated from the bark of the Quillaja Saponaria Molina tree found in South America (*see* Kensil *et al.*, in *Vaccine Design: The Subunit and Adjuvant Approach* (eds. Powell & Newman, Plenum Press, NY, 1995); US 5,057,540), (Aquila BioPharmaceuticals, Framingham, MA; now Antigenics, Inc., New York, NY). Other adjuvants are oil in water emulsions (such as squalene or peanut oil), optionally in combination with immune stimulants, such as monophosphoryl lipid A (*see* Stoute *et al.*, *N. Engl. J. Med.* 336, 86-91 (1997)), pluronic polymers, and killed mycobacteria. Another adjuvant is CpG (WO 98/40100). Adjuvants can be administered as a component of a therapeutic composition with an active agent or can be administered separately, before, concurrently with, or after administration of the therapeutic agent.

3. Types of Antibodies

[0251] Antibodies can be monoclonal or polyclonal. Antibodies can be nonhuman, such as mouse or rat, nonhuman primate or can be human. Antibodies can be chimeric, veneered, humanized, primatized and the like.

[0252] Monoclonal antibodies are humanized using the methods described above and the methods described in 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.

[0253] The invention further provides chimeric and veneered forms of non-human antibodies that bind specifically to the MCAM epitopes described above.

[0254] 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.

[0255] 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.

[0256] 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 mouse antibody, such as one of the mouse monoclonals described in the examples. 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.

[0257] 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.

[0258] Chimeric, humanized (including veneered) and human antibodies are typically produced by recombinant expression as described above.

[0259] The invention further provides non-antibody binding molecules. Non-antibody binding molecules include, for example, anticalins, which are based upon the lipocalin scaffold, a protein structure characterized by a rigid beta-barrel that supports four hypervariable loops which form the ligand binding site. Novel binding specificities are engineered by targeted random mutagenesis in the loop regions, in combination with functional display and guided selection (Skerra (2008) FEBS J. 275: 2677-2683). Other suitable scaffolds may include, for example, adnectins, or monobodies, based on the tenth extracellular domain of human fibronectin III (Koide and Koide (2007) Methods Mol. Biol. 352: 95-109); affibodies, based on the Z domain of staphylococcal protein A (Nygren et al. (2008) FEBS J. 275: 2668-2676)); DARPins, based on ankyrin repeat proteins (Stumpp et al. (2008) Drug. Discov. Today 13: 695-701); fynomers, based on the SH3 domain of the human Fyn protein kinase (Grabulovski et al. (2007) J. Biol. Chem. 282: 3196-3204); affitins, based on Sac7d from Sulfolobus acidolarius (Krehenbrink et al. (2008) J. Mol. Biol. 383: 1058-1068); affilins, based on human y-B-crystallin (Ebersbach et al. (2007) J. Mol. Biol. 372: 172-185); avimers, based on the A domains of membrane receptor proteins (Silverman et al. (2005) Biotechnol. 23: 1556-1561); cysteine-rich knottin peptides (Kolmar (2008) FEBS J. 275: 2684-2690); and engineered Kunitz-type inhibitors (Nixon and Wood (2006) Curr. Opin. Drug. Discov. Dev. 9: 261-268). For review, see Gebauer and Skerra (2009) Curr. Opin. Chem. Biol. 13: 245-255.

[0260] In some of these antibodies, the antibody is not any one of the antibodies or antibodies including CDRs (as defined by Kabat, Chothia, or a composite thereof) entirely or substantially from the antibodies described in WO/2012/170071 and PCT/US2013/058773, particularly the antibodies designated clone 15 (defined by SEQ ID NOs:12-21) and clone 17 (defined by SEQ ID NOs:1-10) in WO/2012/170071 and the mouse anti-human MCAM monoclonal clones designated 1174.1.3, 1414.1.2, 1415.1.1, and 1749.1.3, and the rat anti-human MCAM monoclonal antibody clones designated 2120.4.19 and 2107.4.10 described in PCT/US2013/058773.

4. Methods of Screening Antibodies for Activity

[0261] The inhibitory activity of the MCAM antibodies described herein can be assayed by any method known in the art, including competitive binding assays with antibodies that bind the same or a substantially similar epitope (e.g., m1749) and blocking of MCAM binding with its ligand, the laminin α4 chain of laminin 411.

[0262] For example, the activity of MCAM antibodies to block 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.

[0263] Other methods of monitoring include fluorescence-activated cell sorting (FACS) and enzyme-linked immunosorbent assay (ELISA).

[0264] 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.

[0265] 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).

[0266] 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.

F. Conjugated Antibodies

[0267] Conjugated antibodies that specifically bind to MCAM can be useful in targeting cancer or tumor cells for destruction or in targeting cells involved in autoimmune diseases or neuroinflammatory diseases. Such antibodies can also be useful in targeting any disease mediated at least in part by expression of MCAM. For example, such antibodies can be conjugated with other therapeutic agents, other proteins, other antibodies, and/or detectable labels. *See* WO 03/057838; US 8,455,622. Such therapeutic agents can be any agent that can be

used to treat, combat, ameliorate, prevent, or improve an unwanted condition or disease in a patient, such as an autoimmune disease, a neuroinflammatory disease, or a cancer. Therapeutic agents can include cytotoxic agents, cytostatic agents, radiotherapeutic agents, immunomodulators, or any biologically active agents that facilitate or enhance the activity of the antibody. A cytotoxic agent can be any agent that is toxic to a cell. A cytostatic agent can be any agent that inhibits cell proliferation. An immunomodulator can be any agent that stimulates or inhibits the development or maintenance of an immunologic response. A radiotherapeutic agent can be any molecule or compound that emits radiation. If such therapeutic agents are coupled to an MCAM-specific antibody, such as the antibodies described herein, the coupled therapeutic agents will have a specific affinity for MCAM-expressing cells (e.g., immune cells, such as TH17-expressing cells, or cancer cells, such as malignant melanocytes) over other cells. Consequently, administration of the conjugated antibodies directly targets MCAM-expressing cells with minimal effects on other surrounding cells and tissue. This can be particularly useful for therapeutic agents that are too toxic to be administered on their own. In addition, smaller quantities of the therapeutic agents can be used.

[0268] Antibodies can be modified to act as immunotoxins. *See, e.g.*, U.S. Patent No. 5,194,594. For example, ricin, a cellular toxin derived from plants, can be coupled to antibodies by using the bifunctional reagents S-acetylmercaptosuccinic anhydride for the antibody and succinimidyl 3-(2-pyridyldithio)propionate for ricin. *See* Pietersz *et al., Cancer Res.* 48(16):4469-4476 (1998). The coupling results in loss of B-chain binding activity of ricin, while impairing neither the toxic potential of the A-chain of ricin nor the activity of the antibody. Similarly, saporin, an inhibitor of ribosomal assembly, can be coupled to antibodies via a disulfide bond between chemically inserted sulfhydryl groups. *See* Polito *et al., Leukemia* 18:1215-1222 (2004).

[0269] Radioisotopes can also be linked to antibodies. Preferred radioisotopes include yttrium^{90} (90Y), indium^{111} (111In), ^{131}I, ^{99}mTc, radiosilver-111, radiosilver-199, and Bismuth^{213}. Linkage of radioisotopes to antibodies may be performed with conventional bifunction chelates. For radiosilver-11 and radiosilver-199 linkage, sulfur-based linkers may be used. *See* Hazra *et al., Cell Biophys.* 24-25:1-7 (1994). Linkage of silver radioisotopes may involve reducing the immunoglobulin with ascorbic acid. For radioisotopes such as 111In and 90Y, ibritumomab

tiuxetan can be used and will react with such isotopes to form 111In-ibritumomab tiuxetan and 90Y-ibritumomab tiuxetan, respectively. *See* Witzig, *Cancer Chemother. Pharmacol.*, 48 Suppl 1:S91-S95 (2001).

[0270] Other therapeutic agents may also be linked to antibodies. Therapeutic agents are usually cytotoxic or cytostatic. For example, antibodies can be conjugated with toxic chemotherapeutic drugs such as maytansine, geldanamycin, tubulin inhibitors, such as auristatins, or minor groove binding agents, such as calicheamicin. Other representative therapeutic agents include agents known to be useful for treatment, management, or amelioration of an autoimmune disease, a neuroinflammatory disease, or a cancer, or symptoms of an autoimmune disease, a neuroinflammatory disease, or a cancer. Examples of such therapeutic agents are disclosed elsewhere herein.

[0271] Antibodies can also be coupled with other proteins. For example, antibodies can be coupled with Fynomers. Fynomers are small binding proteins (*e.g.*, 7 kDa) derived from the human Fyn SH3 domain. They can be stable and soluble, and they can lack cysteine residues and disulfide bonds. Fynomers can be engineered to bind to target molecules with the same affinity and specificity as antibodies. They are suitable for creating multi-specific fusion proteins based on antibodies. For example, Fynomers can be fused to N-terminal and/or C-terminal ends of antibodies to create bi- and tri-specific FynomAbs with different architectures. Fynomers can be selected using Fynomer libraries through screening technologies using FACS, Biacore, and cell-based assays that allow efficient selection of Fynomers with optimal properties. Examples of Fynomers are disclosed in Grabulovski *et al.*, *J. Biol. Chem.* 282:3196-3204 (2007); Bertschinger *et al.*, *Protein Eng. Des. Sel.* 20:57-68 (2007); Schlatter *et al.*, *MAbs.* 4:497-508 (2011); Banner *et al.*, *Acta. Crystallogr. D. Biol. Crystallogr.* 69(Pt6):1124-1137 (2013); and Brack *et al.*, *Mol. Cancer Ther.* 13:2030-2039 (2014).

[0272] The antibodies disclosed herein can also be coupled or conjugated to one or more other antibodies (*e.g.*, to form antibody heteroconjugates). Such other antibodies can bind to different epitopes within MCAM or can bind to a different target antigen.

[0273] Antibodies can also be coupled with a detectable label. Such antibodies can be used, for example, for diagnosing of an autoimmune disease, a neuroinflammatory disease, or a cancer,

for monitoring progression of an autoimmune disease, a neuroinflammatory disease, or a cancer, and/or for assessing efficacy of treatment. Such antibodies can be useful for performing such determinations in subjects having or being susceptible to an autoimmune disease, a neuroinflammatory disease, or a cancer, or in appropriate biological samples obtained from such subjects. Representative detectable labels that may be coupled or linked to an antibody include various enzymes, such as horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; prosthetic groups, such streptavidin/biotin and avidin/biotin; fluorescent materials, such as umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; luminescent materials, such as luminol; bioluminescent materials, such as luciferase, luciferin, and aequorin; radioactive materials, such as radiosilver-111, radiosilver-199, Bismuth^{213}, iodine (^{131}I, ^{125}I, ^{123}I, ^{121}I,), carbon (^{14}C), sulfur (^{5}S), tritium (^{3}H), indium (^{115}In, ^{113}In, ^{112}In, ^{111}In,), technetium (^{99}Tc), thallium (^{201}Ti), gallium (^{68}Ga, ^{67}Ga), palladium (^{103}Pd), molybdenum (^{99}Mo), xenon (^{133}Xe), fluorine (^{18}F), ^{153}Sm, ^{177}Lu, ^{159}Gd, ^{149}Pm, ^{140}La, ^{175}Yb, ^{166}Ho, ^{90}Y, ^{47}Sc, ^{186}Re, ^{188}Re, ^{142}Pr, ^{105}Rh, ^{97}Ru, ^{68}Ge, ^{57}Co, ^{65}Zn, ^{85}Sr, ^{32}P, ^{153}Gd, ^{169}Yb, ^{51}Cr, ^{54}Mn, ^{75}Se, ^{113}Sn, and ^{117}Tin; positron emitting metals using various positron emission tomographies; nonradioactive paramagnetic metal ions; and molecules that are radiolabelled or conjugated to specific radioisotopes.

[0274] Therapeutic agents, other proteins, other antibodies, and/or detectable labels may be coupled or conjugated, directly or indirectly through an intermediate (*e.g.*, a linker), to a murine, chimeric, veneered, or humanized antibody using techniques known in the art. *See e.g.*, Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy," in Monoclonal Antibodies And Cancer Therapy, Reisfeld *et al.* (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom *et al.*, "Antibodies For Drug Delivery," in Controlled Drug Delivery (2nd Ed.), Robinson *et al.* (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review," in Monoclonal Antibodies 84: Biological And Clinical Applications, Pinchera *et al.* (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy," in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin *et al.* (eds.), pp. 303-16 (Academic Press 1985); and Thorpe *et al.*, *Immunol. Rev.*, 62:119-58 (1982). Suitable linkers include, for example, cleavable and non-cleavable linkers. Different linkers that release the drugs under acidic or reducing conditions or on exposure to specific proteases can be employed.

Likewise, different linkers that release the coupled therapeutic agents, proteins, antibodies, and/or detectable labels under acidic or reducing conditions, on exposure to specific proteases, or under other defined conditions can be employed.

IV. METHODS OF TREATMENT AND PHARMACEUTICAL COMPOSITIONS

[0275] The antibodies or other antagonists of the invention can be used for treating or effecting prophylaxis of subjects having (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 an autoimmune disease, neuroinflammatory disease and cancer among others. 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. The above mentioned categories or disease are not necessarily mutually exclusive of one another; for example, multiple sclerosis can be classified as neuroinflammatory or autoimmune. Some specific exemplary diseases treatable by the present methods include multiple sclerosis, Parkinson's disease, allergic contact dermatitis, psoriasis, psoriatic arthritis, rheumatoid arthritis, sarcoidosis, inflammatory bowel disease, Crohn's disease, and cancer, particularly, solid tumors, such as melanoma. 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. 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 tumor-associated macrophage toxicity.

[0276] Neuroinflammatory conditions are characterized by CNS inflammation and/or cell/tissue damage. The indicia can include increased glial activation, increased pro-inflammatory cytokine/chemokine levels (e.g., TNFα, INFγ, IL-1β), increased blood-brain-barrier permeability, and/or increased immune cell (e.g., leukocyte) recruitment/invasion to the CNS. The neuroinflammation is often chronic associated with chronic activation of cells of the immune system (*i.e.*, autoimmune-associated neuroinflammation) but can alternatively or additional have acute episodes.

[0277] Multiple sclerosis is a preferred disease for treatment in any of its at least four subtypes. Relapsing-remitting MS (RR-MS) is the most common form of MS and is characterized by clearly defined exacerbations/relapses (acute attacks) followed by partial or complete recovery. There is no disease progression between the relapse periods. Initially (at the time of diagnosis) RR-MS represents about 85% of all newly diagnosed subjects. The definition of relapse requires the new symptom or sign to be present for at least 24 hours, to not be associated with a fever or intercurrent illness (such as the "flu" or a urinary tract infection), because an elevated body temperature can unmask silent or old lesions.

[0278] Primary progressive (PP-MS) is continuous from the beginning without clear relapses. There can be plateaus (periods of stabilization). 10-15% of all MS subjects are in this group and it tends to occur in older aged individuals. The female to male ratio is equal in this group, unlike other forms where females predominant by about 2:1. Also PP-MS tends to present with fewer cerebral MRI changes and more myelopathy/spinal cord related changes.

[0279] A secondary progressive form (SP-MS) starts as a RR-MS and later steady progression occurs with or without relapses. Approximately 50% of relapsing-remitting subjects progress to the secondary progressive form.

[0280] A progressive relapsing form (PR-MS), occurring in about 5% of individuals, is progressive from the onset with superimposed relapses (with or without recovery).

[0281] Diagnosis of MS is usually based on a medical history, a neurologic exam and various tests, including magnetic resonance imaging (MRI), evoked potentials (EP) and spinal fluid analysis. A definitive diagnosis of MS requires evidence of damage in at least two separate areas of the central nervous system (CNS), which includes the brain, spinal cord and optic nerves and evidence that the damage occurred at least one month apart and exclusion of all other possible diagnoses. As well as therapeutically treating subjects having a diagnosis of MS by art-recognized criteria, the present methods can also be used prophylactically to treat individually having at least one sign or symptom of MS placing them at increased risk of progression to MS compared with the general population of healthy individuals. For example, the methods can be used to treat individuals who have had one attack (also called a relapse or an exacerbation) of MS-like symptoms — referred to as a clinically-isolated syndrome (CIS), who may or may not

go on to develop MS. Individuals at risk of developing MS can also be identified by presence of an antibody to the protein KIR4.1 in their serum, among other methods.

[0282] Neuroinflammatory disease also includes Parkinson's disease. Symptoms of Parkinson's disease include tremor (e.g., trembling in hands, arms, legs, jaw, and face); rigidity or stiffness of the limbs and trunk; bradykinesia or slowness of movement; postural instability or impaired balance and coordination; depression and other emotional changes; difficulty in swallowing, chewing, and speaking; urinary problems or constipation; skin problems; sleep disruptions. Parkinson's disease can be diagnosed from such symptoms, and/or brain scans and/or other tests to rule out other diseases.

[0283] The present methods can be used to inhibit growth or metastasis of cancer. 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.

[0284] 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.

[0285] 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 arteritis, Bechet's disease, inflammatory bowel diseases, Crohn's disease, ulcerative colitis, primary biliary cirrhosis, autoimmune hepatitis, autoimmune oophoritis, fibromyalgia, polymyositis, dermatomyositis, ankylosing spondylitis, 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, and psoriasis.

[0286] Antibodies or other antagonists 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 (e.g., cancer). 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.

[0287] Exemplary dosages for an antibody are 0.1-20, or 0.5-5 mg/kg body weight (e.g., 0.5, 1, 2, 3, 4 or 5 mg/kg) or 10-1500 mg as a fixed dosage. 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.

[0288] Administration can be parenteral, intravenous, oral, subcutaneous, intra-arterial, intracranial, intrathecal, intraperitoneal, topical, intranasal or intramuscular. Administration into the systemic circulation by intravenous or subcutaneous administration is preferred. Intravenous administration can be, for example, by infusion over a period such as 30-90 min.

[0289] The frequency of administration depends on the half-life of the antibody in the circulation, the condition of the patient and the route of administration among other factors. The frequency can be daily, weekly, monthly, quarterly, or at irregular intervals in response to changes in the patient's condition or progression of the disorder being treated. An exemplary frequency for intravenous administration is between weekly and quarterly over a continuous cause of treatment, although more or less frequent dosing is also possible. For subcutaneous administration, an exemplary dosing frequency is daily to monthly, although more or less frequent dosing is also possible.

[0290] The number of dosages administered depends on whether the disorder is acute or chronic and the response of the disorder to the treatment. For acute disorders or acute exacerbations of a chronic disorder, between 1 and 10 doses are often sufficient. Sometimes a single bolus dose, optionally in divided form, is sufficient for an acute disorder or acute exacerbation of a chronic disorder. Treatment can be repeated for recurrence of an acute disorder or acute exacerbation. For chronic disorders, an antibody can be administered at regular intervals, e.g., weekly, fortnightly, monthly, quarterly, every six months for at least 1, 5 or 10 years, or the life of the patient.

[0291] 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.

[0292] Treatment with antibodies of the invention can be combined with other treatments effective against the disorder being treated. Combination treatments can be formulated for administered separately. 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.

[0293] 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 TaxotereRTM.; the topoisomerase 1 inhibitor irinotecan; temozolomide and GliadelRTM, carmustine; and inhibitors of tyrosine kinases such as Gleevec.RTM., Sutent.RTM. (sunitinib malate), Nexavar.RTM. (sorafenib) and Tarceva.RTM. (erlotinib) or Iressa.RTM. (gefitinib); inhibitors of angiogenesis; and monoclonal antibodies, including Herceptin.TM. against the HER2 antigen; Avastin.RTM. against VEGF; or antibodies to the Epidermal Growth Factor (EGF) receptor such as Erbitux.RTM. (cetuximab) and Vectibix.RTM. (panitumumab).

[0294] 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

V. KITS

[0295] The invention further provides kits (e.g., containers) comprising the MCAM antibodies or other antagonists of the invention and related materials, such as instructions for use (e.g., package insert). The instructions for use may contain, for example, instructions for administration of the MCAM antagonists and optionally one or more additional agents. The containers of MCAM antagonist(s) may be unit doses, bulk packages (e.g., multi-dose packages), or sub-unit doses.

[0296] Package insert refers to instructions customarily included in commercial packages of therapeutic products that contain information about the indications, usage, dosage, administration, contraindications and/or warnings concerning the use of such therapeutic products

[0297] Kits can also include a second container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It can also include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

[0298] 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

[0299] 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.

[0300] 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.

[0301] 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.

[0302] 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.

[0303] 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

[0304] 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.

[0305] 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

[0306] 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.

[0307] 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.

[0308] 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.

[0309] 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

[0310] 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.

[0311] 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. Domain binding analysis for anti-MCAM monoclonal antibodies

[0312] ForteBio analysis was employed to determine the location of the antigen epitope on the human MCAM protein that is recognized and bound by monoclonal antibody clones 1174.1.3, 1414.1.2, 1415.1.1, 1749.1.3, 2120.4.19, and 2107.4.10. The following protocol was used: ForteBio anti-human IgG Fc biosensors were used to immobilize various MCAMhFc domains including full length MCAMhFc protein on to biosensor surface. These sensors were dipped into the MCAM specific 1174.1.3, 1414.1.2, 1415.1.1, 1749.1.3, 2120.4.19, or 2107.4.10 antibody for detection of binding to these domains or full length protein. After loading these samples into a black 96 well plate, the Octet Red was programmed as follows: 60 seconds for baseline #1; 180 seconds for loading various domains; 60 seconds for baseline #2; 180 seconds for association of antibody to domain; and 240 seconds for dissociation of antibody from domain.

Reagents and supplies used:

1. MCAMhFc final concentration @ 5 ug/ml
2. antibody clones 1174.1.3, 1414.1.2, 1415.1.1, 1749.1.3, 2120.4.19, and 2107.4.10 clones @ 5 ug/ml
3. ForteBio anti-human IgG Fc Capture (AHC) biosensors for kinetics experiments, cat# 18-5060
4. Block 96 well plate from Greiner Bio-one, cat# 655209
5. ForteBio Octet Red machine
6. Fresh tissue culture medium, DMEM with 20% FCS, was used as buffer for dilution

The results from these analyses are as follows.

[0313] Monoclonal antibody clones 1174.1.3, 1414.1.2, 1415.1.1, and 1749.1.3 were all shown to bind to an antigenic epitope found on domain 3 of the human MCAM protein, defined specifically by amino acids 244-321 (SEQ ID NO:24) of the human MCAM protein. These monoclonal antibodies were not capable of binding to human MCAM domain 1 (namely amino acids 19-129, SEQ ID NO:22), domain 2 (namely amino acids 139-242, SEQ ID NO:23), or the combination of domains 1 and 2 (namely, amino acids 19-242). Hence, monoclonal antibody clones 1174.1.3, 1414.1.2, 1415.1.1, and 1749.1.3 define a novel antigenic epitope located within domain 3 of the human MCAM protein.

[0314] Monoclonal antibody clones 2120.4.19, and 2107.4.10 were each shown to bind to an antigenic epitope defined by the combination of human MCAM domains 1 (namely amino acids 19-129, SEQ ID NO:22), and domain 2 (namely amino acids 139-242, SEQ ID NO:23). Neither of these two monoclonal antibodies bound to human MCAM domain 1 by itself. Hence, monoclonal antibody clones 2120.4.19 and 2107.4.10 define a novel antigenic epitope determined by the presence of both human MCAM protein domains 1 and 2.

[0315] In contrast to the above, the previously described fully human anti-MCAM antibody ABX-MA1 binds to a different antigenic epitope than those described above, namely an antigenic epitope that is fully defined and encompassed within human MCAM domain 1 only.

[0316] Given these results, since each of monoclonal antibody clones 1174.1.3, 1414.1.2, 1415.1.1, 1749.1.3, 2120.4.19, and 2107.4.10 are capable of both (i) binding to human MCAM, and (ii) blocking the interaction between human MCAM and an α-4 laminin containing protein, whereas the ABX-MA1 antibody is capable of only binding to human MCAM, but not blocking the interaction between human MCAM and an α-4 laminin containing protein, these results demonstrate that human MCAM domain 2, human MCAM domain 3, and the combination thereof play a role in the binding interaction with α-4 laminin chain. Given this, it is clear that antibodies which bind to human MCAM domain 2, human MCAM domain 3, and/or the combination thereof would find use as agents capable of blocking the interaction between human MCAM and α-4 laminin and, thereby, find use for inhibiting the various consequences described herein resulting from that interaction. In contrast, antibodies that bind to an antigenic epitope defined solely by human MCAM domain 1 (such as the ABX-MA1 antibody described herein) are not useful for blocking the MCAM / α-4 laminin interaction and its various downstream biological consequences.

Example 4. Shotgun Mutagenesis Epitope Mapping

[0317] Various amino acid residue of interest for anti-MCAM antibody binding were identified using shotgun mutagenesis and high-throughput cellular expression technology that enables the expression and analysis of large libraries of mutated target proteins within eukaryotic cells. Every residue in the human MCAM protein was individually mutated to an alanine, or other specified residue, to assay changes in function. Proteins were expressed within standard mammalian cell lines.

[0318] Table 1 shows a summary of the reagents and methods used to generate the shotgun mutagenesis library.

Table 1

Parental plasmid	hsMCAM-V5/HIS6 (Accession # NP 006491)
Final library size	528 mutant clones plus 17 additional site-directed mutants
Mutation Strategy	Alanine Scanning Mutagenesis
Cell type	BHK-S
Epitope Tag	C-terminal V5/HIS6

[0319] Full-length human MCAM was successfully codon-optimized, synthesized, and subcloned into a mammalian high-expression vector. This parental construct was then sequence-verified and validated for mammalian cell expression by immunodetection methods.

[0320] Detection of 1749.1.3 antibody and mouse sera binding to MCAM by immunofluorescence was successfully optimized for the high-throughput shotgun mutagenesis format. Serial dilutions of each primary antibody were tested with a single dilution of secondary antibody in a 384-well format. Antibodies were tested for detection of 293T and BHK cells expressing human MCAM. Optimal assay conditions were selected for screening the complete mutation library.

[0321] The MCAM mutation library was created and sequence verified, consisting of 545 clones (528/536 alanine mutants and 17/17 site-directed mutants), each bearing either a single residue substitution to alanine (alanine residues are substituted to serine) or a specified residue. Residues 35, 66, 161, 261, 342, 380, 414, and 435 are not represented in the library. The mutation library was screened in triplicate by immunodetection for binding to mouse sera. This validates cell surface expression for each mutant clone.

[0322] Multiple rounds of optimization were performed to determine conditions that are suitable for mapping. The following variables were evaluated: multiple laminin concentrations and anti-laminin secondary antibody concentrations, various blocking buffers to reduce nonspecific binding, multiple cell types, and multiple washing steps.

[0323] The mutation library was screened in triplicate by immunodetection for binding to the 1749.1.3 antibody. Reactivity was quantified for each mutant to identify point mutants that exhibit loss of binding.

[0324] Monoclonal antibody and sera reactivity were quantified for each mutant clone to identify point mutants that exhibit loss of binding without impacting surface expression. The critical residues for each antibody were identified by comparison of the monoclonal antibody binding profile to the sera binding profile of each mutant clone.

[0325] BHK cells were transfected with either wild-type (WT) MCAM or vector alone in a 384-well format, followed by immunodetection. Serial dilutions of each antibody (beginning with 4 µg/ml) were tested for immunoreactivity against WT or vector alone (Table 2). Each point represents the average of four replicates.

Table 2

Primary Ab conc (ug/mL)	MAb 1749.1.3		Ms Sera		Ms Sera Conc (ug/mL)
	S/B	Z'	S/B	Z'	
4.00	13.11	0.69	6.49	0.19	1:100
2.00	27.98	0.58	7.69	0.53	1:200
1.00	27.92	0.76	8.32	0.74	1:400
0.50	40.47	0.68	7.91	0.55	1:800
0.25	33.53	0.72	11.65	0.50	1:1600
0.13	29.95	0.79	16.29	0.50	1:3200
0.06	18.22	0.34	10.87	0.54	1:6400
0.03	10.41	0.62	10.22	0.39	1:12800
0.02	4.91	0.79	7.29	-0.19	1:25600
0.00	0.31	-4.83	1.77	-5.95	0.00

[0326] Optimal screening conditions for the immunodetection and epitope mapping of 1749.1.3 and Ms Sera were determined. Using these conditions, each antibody demonstrated a robust signal, high signal-to-background values, and low variability between replicates. These data indicate that these conditions are suitable for successful high-throughput epitope mapping.

Final screening concentrations of 0.5 μg/mL for 1749.1.3 and a 1:800 dilution of the Ms Sera were used. Secondary antibodies from Jackson ImmunoResearch were used at 1:400 for MAb and sera detection. Table 3 shows the experimental parameters optimized for high-throughput immunodetection.

Table 3

Experimental Parameter	**MAb 1749.1.3**	**Ms Sera**
Cells Fixative Blocking Buffer	BHK-S 4% PFA 10% Goat Serum	BHK-S 4% PFA 10% Goat Serum
Primary Ab	1749	Sera
Ab name Target Optimal Conc. Incubation (RT)	MCAM 0.5 ug/ml 60 min	MCAM 1:800 dilution 60 min
Secondary Ab Target	Mouse IgG	Mouse IgG
Optimal Conc. Incubation Manufacturer Cat # Antibody ID	1:400 (3.75 ug/ml) 30 min Jackson/ImmunoResearch 115-545-003 Alexa Fluor® 488-AffiniPure Goat Anti-Mouse IgG (H+L)	1:400 (3.75 ug/ ml) 30 min Jackson/ImmunoResearch 115-545-003 Alexa Fluor® 488-AffiniPure Goat Anti-Mouse IgG (H+L)
Washes Signal:Background	PBS (CA^{2+},Mg^{2+} free) 40:1	PBS (CA^{2+},Mg^{2+} free) 8:1

[0327] The mutation library was assayed for surface expression (mouse sera binding) and monoclonal antibody binding, in triplicate. Each raw data point was background-subtracted and normalized to the wild type MCAM reactivity values. The results are shown in Fig. 1. The mean monoclonal antibody binding value for each clone is plotted as a function of its mean surface expression value (Fig. 1, gray diamonds). Thresholds of <30% monoclonal antibody reactivity and >50% mouse sera binding were applied to identify clones (Fig. 1, black diamonds) that were negative for monoclonal antibody binding but positive for surface expression.

[0328] Critical residues for 1749.1.3 were identified by evaluating the mean monoclonal antibody reactivity of each clone compared to its overall surface expression (average serum reactivity). Residues involved in antibody binding were identified as those that were negative for monoclonal antibody binding (<30% WT) but positive for surface expression (>50% WT) (Table 4). The mean reactivity (and standard deviation) were determined for each critical residue.

Table 4

Residue ID	Mutations	MAb 1749.1.3	Ms Sera
272	**C272A**	7.6 (4.7)	54.5 (55.8)
318	**Y318A**	7.1 (2.4)	111.7 (9.2)
320	**C320A**	9.3 (11.2)	50 (54.6)
340	**V340A**	8.7 (8.3)	103.8 (71.3)
377	**W377A**	13.7 (10.3)	63.4 (18.9)

[0329] The critical amino acids identified by shotgun mutagenesis mapping suggest binding sites for the 1749.1.3 antibody. The data indicate that 1749.1.3 binds a conformationally complex epitope at the third Ig domain of MCAM.

[0330] Critical residues appear largely dependent upon structural stabilization contributed by disulfide bonds of the second and/or third Ig domains. Mutation to either cysteine 272 or 320 abolishes antibody binding, suggesting the shared disulfide bond of the third Ig domain plays a significant role in stabilizing the epitope.

Example 5. Confirmatory MCAM Epitope Mapping for Antibody and Laminin Binding

[0331] In order to identify 1749.1.3 binding sites on human MCAM, a homology model of human MCAM Ig3 was built up on pdb 3KVQ_A, 3V2A_R, 2IEP_A and 2YD1_A by using Schrodinger Maestro (Fig. 2). Twenty point mutants based on the structure information and shotgun mutagenesis information were designed and generated. These mutants were displayed on mammalian cells and FACS was used to test the binding of 1749.1.3 and laminin α-4 to the MCAM mutants. Three MCAM single mutants, I141A, D216A and Y318A, demonstrated a

complete loss of laminin α-4 binding. The Y318A mutant demonstrated a complete loss of 1749.1.3 binding.

[0332] To further confirm the data, stable cell lines expressing I141A, P145V, D216A and Y318A respectively were generated. Fortebio assays were performed with the purified proteins as described above. The control ABX-MA1 antibody bound to wild type MCAM and the MCAM mutants. The 1749.1.3 antibody did not show significant binding to the MCAM Y318A mutant.

Example 6. Humanization of 1749.1.3 antibodies

[0333] The starting point or donor antibody for humanization is the mouse antibody 1749 produced by a hybridoma described in WO/2012/170071 and PCT/US2013/058773. The mature heavy chain variable amino acid and nucleic acid sequence of mature m1749 are provided as SEQ ID NOS:93 and 64, respectively. The mature light chain variable amino acid and nucleic acid sequences of mature m1749 are provided as SEQ ID NOS:97 and 59, respectively. The heavy chain CDR1, CDR2, and CDR3 amino acid sequences are provided as SEQ ID NOs:66, 67, and 68, respectively. The light chain CDR1, CDR2, and CDR3 amino acid sequences are provided as SEQ ID NOs:61, 62, and 63, respectively. Kabat numbering is used throughout in this Example.

[0334] The variable kappa (Vk) of m1749 belongs to mouse Kabat subgroup 1 which corresponds to human Kabat subgroup 4. The variable heavy (Vh) of m1749 belongs to mouse Kabat subgroup 3d which corresponds to human Kabat subgroup 3 (Kabat et al. Sequences of Proteins of Immunological Interest, Fifth Edition. NIH Publication No. 91-3242, 1991). The 17 residue CDR-L1 belongs to canonical class 3, the 7 residue CDR-L2 belongs to canonical class 1, the 8 residue CDR-L3 belongs to canonical class 3 in Vk (Martin & Thornton, J Mol Biol. 263:800-15, 1996). The 5 residue CDR-H1 belongs to canonical class 1, the 17 residue CDR-H2 belongs to canonical class 1 or 3 (Martin & Thornton, J Mol Biol. 263:800-15, 1996). The CDR-H3 has no canonical classes, but the 11 residue loop probably has a kinked base according to the rules of Shirai et al., FEBS Lett. 455:188-97 (1999).

[0335] The residues at the interface between the Vk and Vh domains are the ones commonly found. A search was made over the protein sequences in the PDB database (Deshpande et al.,

Nucleic Acids Res. 33: D233-7, 2005) to find structures which would provide a rough structural model of 1749. The antibody against integral membrane protein DsbB in *E. coli* because has good overall sequence similarity to m1749 Vk, retaining the same canonical structures for the loops. The X-ray crystal structure of the anti-DsbB antibody (pdb code 2LTQ; Tang et al., J. Mol. Biol. 425:1670-82, 2013; SEQ ID NO:161) was used for the Vk structure in the modeling. The antibody directed against a peptide immunogen from influenza virus hemagglutinin has good overall sequence similarity to 1749 Vh structure. It also has a CDR-H3 of a similar length with a kinked base. The structure of the antibody directed against a peptide immunogen from influenza virus hemagglutinin (1HIL; Rini et al., Science 255: 959-65, 1992; SEQ ID NO:157) has reasonable resolution (2.0A), and was used for the Vh structure in the modeling. In addition, CDRs-H1 and H2 of 1H1L have the same canonical structures for CDR-H1 and CDR-H2 as that of 1749 Vh. BioLuminate® was used to model a rough structure of 1749Fv.

[0336] A search of the non-redundant protein sequence database from NCBI allowed selection of suitable human frameworks into which to graft the murine CDRs. For Vk, two human kappa light chains were chosen, the first with NCBI accession code ABA71407.1 (GI:77379502 ; SEQ ID NO:162) (Manske et al., Clin. Immunol. 120:106-20, 2006) and the second with NCBI accession code CAI99800.1 (GI:98956324; SEQ ID NO:163) (Su et al., J. Immunol. 181:1264-71,2008). This has the same canonical classes for CDR-L1, L2, and L3. ABA71407.1 has a sequence identity of 85% in the light chain variable region framework to murine 1749 light chain. CAI99800.1 has a sequence identity of 83% in the light chain variable region framework to murine 1749 light chain.

[0337] For Vh, two human Ig heavy chains were chosen, the first with NCBI accession code AAX82494.1 (GI:62421461; SEQ ID NO:158) (Lundquist, Infect. Immun. 74:3222-31, 2006) and the second with NCBI accession code ADX65676.1 (GI:323432073; SEQ ID NO:159) (unpublished). It shares the canonical form of 1749 CDR-H1 and H2, and H3 is 11 residues long with a predicted kinked base. AAX82494.1 has a sequence identity of 91% in the variable region framework to murine 1749 heavy chain. ADX65676.1 has a sequence identity of 83% in the variable region framework to murine 1749 heavy chain.

[0338] A humanized light chain variable region variant and a humanized heavy chain variable region variant were constructed containing the above substitutions (Hu1749VHv3; SEQ

ID NO:156, and Hu1749VLv3; SEQ ID NO:160) (Figs. 3A & B). The amino acids at H3, H42, H93, L9, L19, L43 in Hu1749VHv3 and Hu1749VLv3 are listed in Table 5.

Table 5

Kabat Numbering of Some Framework Residues for Backmutation in Humanized 1749 Antibodies

Kabat Residue #	Linear Residue #	ABA71407.1 light chain	CAI99800.1 light chain	AAX82494.1 heavy chain	ADX65676.1 heavy chain	Mouse 1749	Hu1749VLv3	Hu1749VHv 3
H3	3	-	-	Q	Q	K	-	K
H42	42	-	-	D	G	E	-	E
H93	97	-	-	A	A	T	-	T
L9	9	D	D	-	-	S	S	-
L19	19	A	A	-	-	V	V	-
L43	49	P	P	-	-	S	S	-

[0339] The rationales for selection of the above positions as candidates for substitution are as follows.

[0340] Q3K (here as elsewhere for framework backmutations, the first mentioned residue is the human residue and the second the mouse residue): K contacts Y102 in CDRH3. Therefore, it should be maintained in the framework.

[0341] G42E: E has similar side chain as D in human acceptor AAX82494.1. E is more frequent than D in humans. This backmutation contributes to protein stability.

[0342] A93T: This position is a Vk/Vh interface residue.

[0343] D9S: This residue does not contact or affect CDRs and/or interface. The frequency of S is greater than D in human framework regions.

[0344] A19V: The frequency of V and A are similar in human framework regions.

[0345] P43S: S contacts two interface residues in VH: Y91 and W103. Therefore, it is critical and should to be maintained in the framework.

[0346] >Hu1749VHv3

EVKLVESGGGLVQPGGSLRLSCAASGFTFS**SYIMS**WVRQTPEKRLEWVA**TISSGGSSTY YPDSVKG**RFTISRDNAKNTLYLQMSSLKSEDTAMYYCTR**DDDYDVKVFAY**WGQGTLV TVSS

[0347] >Hu1749VLv3

DIVMTQSPSSLAVSLGERVTINC**KSSRSLLNSRIRKNYLA**WYQQKPGQSPKLLIY**WAST RES**GVPDRFSGSGSGTDFTLTISSLQAEDVAVYYC**KQSYNLLT**FGQGTKVEIKR

Example 7. Characterization of variant the humanized 1749H3L3 antibody

[0348] Binding kinetics of the humanized 1749 antibody comprising the heavy chain Hu1749VHv3 and the light chain Hu1749VLv3 have been characterized.

[0349] Binding kinetic of humanized 1749 antibodies were measured by bio-layer interferometry (BLI) using a ForteBio Octet QK instrument (ForteBio, Menlo Park, CA). Detailed binding kinetic parameters (association rate, apparent ka, dissociation rate, apparent kd, and affinity constant, apparent KD) were determined for chimeric 1749 and humanized 1749 antibodies (Table 6). Apparent ka, apparent kd and apparent K_D are binding kinetic parameters obtained using ForteBio assay formats.

[0350] The hu1749H3L3 variant was found to give the lowest dissociation constant (highest association constant), the same as 1749.1.3 within the SEM.

Table 6

Binding Kinetic Parameters of Murine 1749, Chimeric 1749, and Humanized 1749 Antibodies

Antibody	**Apparent K_D M**	**Apparent K_a ($M^{-1}s^{-1}$)**	**Apparent K_d (s^{-1})**
Mouse 1749	2.86E-10	1.41E+6	4.02E-04
Chimeric 1749	2.26E-10	1.94E+6	4.39E-04
Human 1749VH3VL3 (Hu1749VHv3 and Hu1749VLv3)	2.21E-10	1.99E+6	4.40E-04

[0351] In addition, Analysis with Dynamic Light Scattering (DLS) shows a level of polydiversity (%PD) of the h1749H3L3 antibody similar to that of the parental m1749 antibody (Table 7). Dynamic Light Scattering measurements were taken in a Wyatt DynaPro Nanostar Dynamic Light Scattering instrument, in 10 microliter size volumes within a quartz cuvette. All measurements were obtained at 37°C with each measurement having 10 acquisitions with an acquisition time of 5 seconds. Regularization was done by the Wyatt Technology Dynamics 7.0 software using a Rayleigh Spheres model.

Table 7

DLS Analysis of 1749 Variants

mAb	%Pd
h1749 WT	68.8
h1749 P43S	64.6
m1749	10.2
ch1749	19.6
h1749H3L3	22.0

WHAT IS CLAIMED IS:

1. A humanized antibody comprising:

(a) a mature heavy chain variable region comprising the three Kabat CDRs of SEQ ID NO:156, and being at least 97% identical to SEQ ID NO:156; and

(b) a mature light chain variable region comprising the three Kabat CDRs of SEQ ID NO:160, and being at least 97% identical to SEQ ID NO:160.

2. The humanized antibody of claim 1, wherein the mature heavy chain variable region is at least 98% identical to SEQ ID NO:156 and the mature light chain variable region is at least 97% identical to SEQ ID NO:160.

3. The humanized antibody of claim 1, wherein the mature heavy chain variable region is at least 99% identical to SEQ ID NO:156 and the mature light chain variable region is at least 97% identical to SEQ ID NO:160.

4. The humanized antibody of claim 1, wherein the mature heavy chain variable region has the amino acid sequence of SEQ ID NO:156 and the mature light chain variable region is at 97% identical to SEQ ID NO:160.

5. The humanized antibody of claim 1, wherein the mature heavy chain variable region is at least 97% identical to SEQ ID NO:156 and the mature light chain variable region is at least 98% identical to SEQ ID NO:160.

6. The humanized antibody of claim 1, wherein the mature heavy chain variable region is at least 97% identical to SEQ ID NO:156 and the mature light chain variable region is at least 99% identical to SEQ ID NO:160.

7. The humanized antibody of claim 1, wherein the mature heavy chain variable region is at least 97% identical to SEQ ID NO:156 and the mature light chain variable region has the amino acid sequence of SEQ ID NO:160.

8. The humanized antibody of claim 1, wherein the mature heavy chain variable region is at least 98% identical to SEQ ID NO:156 and the mature light chain variable region is at least 98% identical to SEQ ID NO:160.

9. The humanized antibody of claim 1, wherein the mature heavy chain variable region is at least 99% identical to SEQ ID NO:156 and the mature light chain variable region is at least 99% identical to SEQ ID NO:160.

10. The humanized antibody of claim 1, wherein the mature heavy chain variable region has the amino acid sequence of SEQ ID NO:156 and the mature light chain variable region has the amino acid sequence of SEQ ID NO:160.

11. The humanized antibody of any one of claims 1-10 or 44-46, further provided that position 3 (Kabat numbering) of the mature heavy chain variable region is occupied by K.

12. The humanized antibody of any one of claims 1-11 or 44-46, further provided that position 93 (Kabat numbering) of the mature heavy chain variable region is occupied by T.

13. The humanized antibody of any one of claims 1-12 or 44-46, further provided that position 42 (Kabat numbering) of the mature heavy chain variable region is occupied by E.

14. The humanized antibody of any one of claims 1-13 or 44-46, further provided that position 43 (Kabat numbering) of the mature light chain variable region is occupied by S.

15. The humanized antibody of any one of claims 1-14 or 44-46, further provided that position 9 (Kabat numbering) of the mature light heavy chain variable region is occupied by S.

16. The humanized antibody of any one of claims 1-15 or 44-46, further provided that position 19 (Kabat numbering) of the mature light heavy chain variable region is occupied by V.

17. The humanized antibody of any one of claims 1-16 or 44-46, which is an antigen-binding fragment.

18. An isolated anti-MCAM antibody that binds to human MCAM (SEQ ID NO:11) at an epitope including amino acid residue 318.

19. The isolated anti-MCAM antibody of claim 18, wherein the epitope comprises amino acid residue 324.

20. The isolated anti-MCAM antibody of claim 18 or claim 19, wherein the epitope comprises amino acid residue 326.

21. The isolated anti-MCAM antibody of any one of claims 18-20 that includes at least five contiguous residues of MCAM including amino acid residue 318.

22. The isolated anti-MCAM antibody of any one of claims 18-21, wherein the antibody is not monoclonal antibody 1749.1.3 or an antibody comprising CDRs substantially from monoclonal antibody 1749.1.3.

23. The isolated anti-MCAM antibody of any one of claims 18-22, wherein the antibody is monoclonal.

24. The isolated anti-MCAM antibody of any one of claims 18-23, wherein the antibody is chimeric, humanized, veneered, or human.

25. The isolated anti-MCAM antibody of any one of claims 18-23, wherein the antibody is an antigen-binding fragment.

26. A pharmaceutical composition comprising the humanized antibody or the isolated anti-MCAM antibody of any one of claims 1-25 or 44-46.

27. Use of the humanized antibody or the isolated anti-MCAM antibody of any one of claims 1-25 or 44-46, in the manufacture of a medicament for the treatment of an inflammatory disorder in a mammalian subject, characterized by infiltration of MCAM-expressing cells into a site of inflammation in the body.

28. Use of the humanized antibody or the isolated anti-MCAM antibody of any one of claims 1-25 or 44-46, in the manufacture of a medicament for the treatment of a central nervous system (CNS) inflammatory disorder in a mammalian subject, characterized by infiltration of MCAM-expressing cells into the CNS.

29. Use of the humanized antibody or the isolated anti-MCAM antibody of any one of claims 1-25 or 44-46, in the manufacture of a medicament for the treatment of multiple sclerosis in a mammalian subject.

30. Use of the humanized antibody or the isolated anti-MCAM antibody of any one of claims 1-25 or 44-46, in the manufacture of a medicament for the treatment of psoriasis in a mammalian subject.

31. Use of the humanized antibody or the isolated anti-MCAM antibody of any one of claims 1-25 or 44-46, in the manufacture of a medicament for the treatment of a solid tumor, such as melanoma in a mammalian subject.

32. Use of the humanized antibody or the isolated anti-MCAM antibody of any one of claims 1-25 or 44-46, in the manufacture of a medicament for the treatment of sarcoidosis in a mammalian subject.

33. Use of the humanized antibody or the isolated anti-MCAM antibody of any one of claims 1-25 or 44-46, in the manufacture of a medicament for the treatment of psoriatic arthritis in a mammalian subject.

34. Use of the humanized antibody or the isolated anti-MCAM antibody of any one of claims 1-25 or 44-46, in the manufacture of a medicament for the treatment of Parkinson's disease in a mammalian subject.

35. Use of the humanized antibody or the isolated anti-MCAM antibody of any one of claims 1-25 or 44-46, in the manufacture of a medicament for the treatment of allergic contact dermatitis in a mammalian subject.

36. A method for treating an inflammatory disorder characterized by infiltration of MCAM-expressing cells to a site of inflammation, the method comprising

administering to a mammalian subject in need thereof an effective amount of the humanized antibody or the isolated anti-MCAM antibody of any one of claims 1-25 or 44-46.

37. The method of claim 36, wherein the MCAM-expressing cells are TH17 cells.

38. The method or use of any one of claims 27-37, wherein the mammalian subject is a human.

39. An isolated peptide comprising an epitope for binding an MCAM monoclonal antibody, wherein the peptide comprises 5-50 contiguous amino acid residues of human MCAM (SEQ ID NO:11) including amino acid residue 318.

40. The isolated peptide of claim 39, wherein the peptide is linked to a carrier polypeptide.

41. The isolated peptide of claim 39 or claim 40, wherein the peptide is combined with an adjuvant.

42. A method of generating an antibody that inhibits binding of human MCAM to a laminin α-4 chain, comprising:

(a) immunizing a subject with a peptide as defined by any one of claims 39-41;

(b) isolating B-cells from the subject, wherein the B-cells secrete antibodies; and

(c) screening the antibodies to identify an antibody that inhibits binding of human MCAM to a laminin α-4 chain.

43. The method of claim 42, further comprising:

(d) fusing the B-cells with immortalized cells in culture to form monoclonal antibody-producing hybridoma cells;

(e) culturing the hybridoma cells; and,

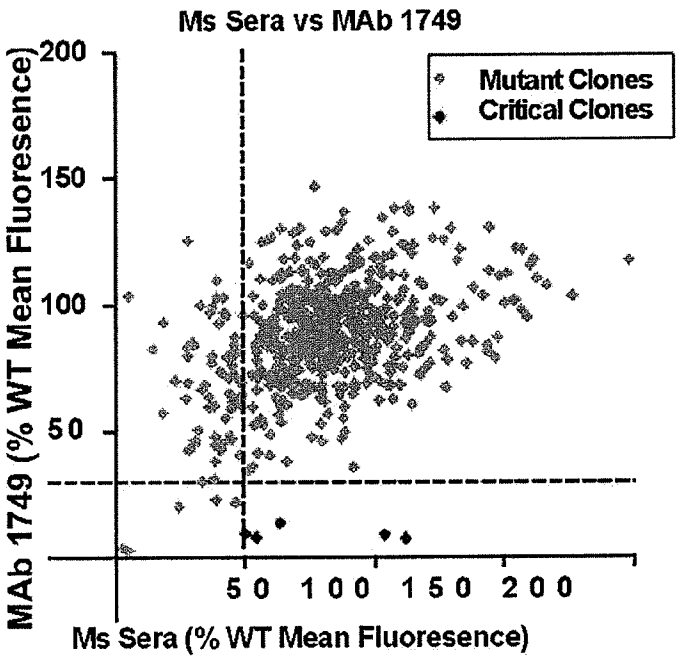
(f) isolating monoclonal antibodies from culture.

44. The humanized antibody of claim 1, wherein the mature heavy chain variable region has the amino acid sequence of SEQ ID NO:156 and the mature light chain variable region has the amino acid sequence of SEQ ID NO:160, and wherein the humanized antibody comprises a heavy chain constant region having the amino acid sequence of SEQ ID NO:173 and a light chain constant region having the amino acid sequence of SEQ ID NO:170.

45. The humanized antibody of claim 1, wherein the mature heavy chain variable region has the amino acid sequence of SEQ ID NO:156 and the mature light chain variable region has the amino acid sequence of SEQ ID NO:160, and wherein the humanized antibody comprises a heavy chain constant region having the amino acid sequence of SEQ ID NO:173 and a light chain constant region having the amino acid sequence of SEQ ID NO:171.

46. The humanized antibody of claim 1, wherein the mature heavy chain variable region has the amino acid sequence of SEQ ID NO:156 and the mature light chain variable region has the amino acid sequence of SEQ ID NO:160, and wherein the humanized antibody comprises a heavy chain constant region having the amino acid sequence of SEQ ID NO:174 and a light chain constant region having the amino acid sequence of SEQ ID NO:171.

FIG.1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Critical Clones
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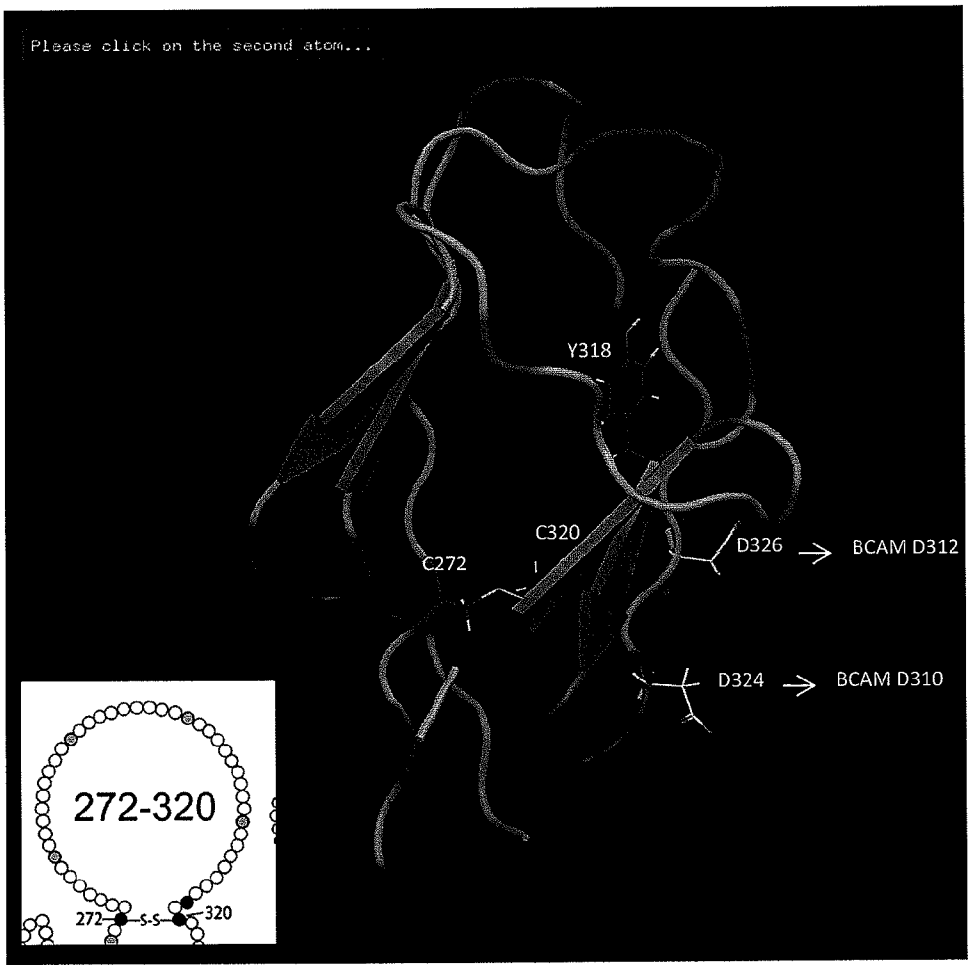
150
100
50
50 100 150 200
Ms Sera (% WT Mean Fluoresence)


Please click on the second atom...
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BCAM D310
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272
S-S
320

FIG.2

Figure 3A

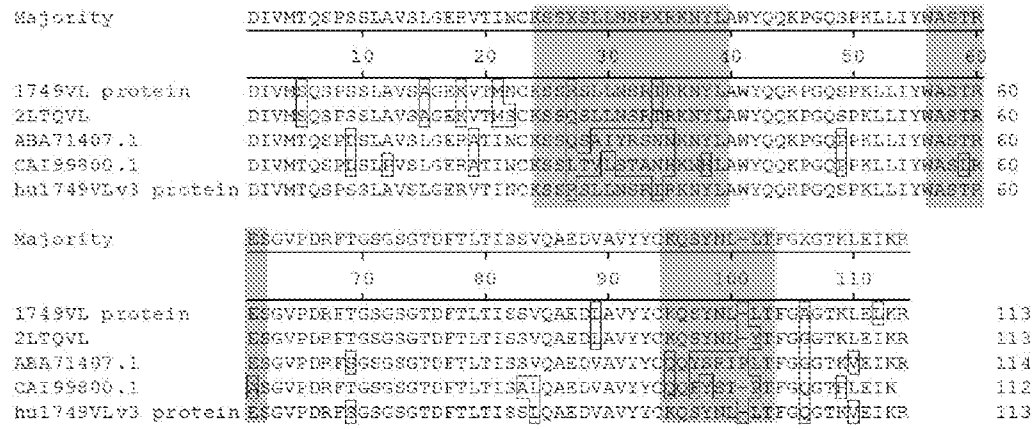
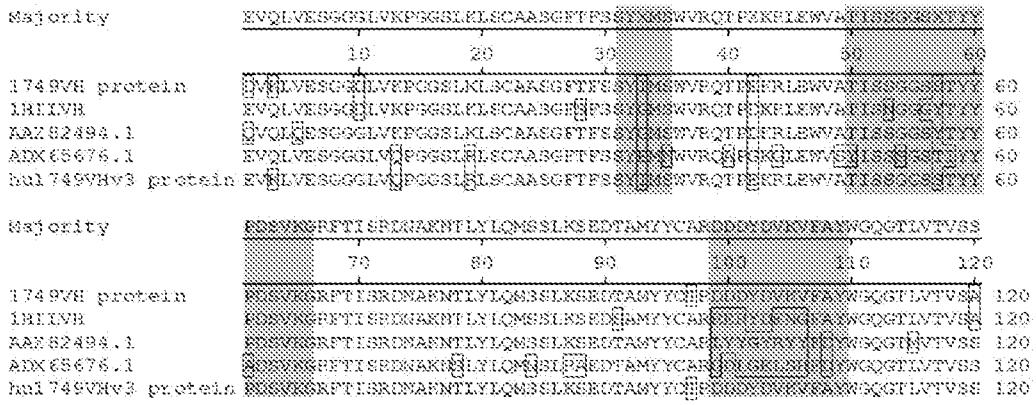


Figure 3B



Decoration 'Decoration $1': Box residues that differ from the Consensus.

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2015/051786

A. CLASSIFICATION OF SUBJECT MATTER
INV. 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)
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, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KEN FLANAGAN ET AL: "Laminin-411 Is a Vascular Ligand for MCAM and Facilitates TH17 Cell Entry into the CNS", PLOS ONE, vol. 7, no. 7, 6 July 2012 (2012-07-06), page e40443, XP055191302, DOI: 10.1371/journal.pone.0040443 p.2, col.1, 2 ----- -/--	1-17, 26-38, 44-46

[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

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 August 2015	26/08/2015

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	Chapman, Rob

3

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	YING ZHANG ET AL: "Generation and Characterization of a Panel of Monoclonal Antibodies Against Distinct Epitopes of Human CD146", HYBRIDOMA, vol. 27, no. 5, 1 October 2008 (2008-10-01), pages 345-352, XP055155690, ISSN: 1554-0014, DOI: 10.1089/hyb.2008.0034 Fig.1, 2 and antibody mAb AA98	1-41
X	WO 2012/170071 A1 (ELAN PHARMACEUTICAS INC [US]; FLANAGAN KENNETH [US]; JOHNSTON JENNIFER) 13 December 2012 (2012-12-13) cited in the application The whole document, in particular, Sections 3 and 4 (p.17-23), Example 5 and p.32, li.1-31	1-46
X	P. BU ET AL: "Anti-CD146 monoclonal antibody AA98 inhibits angiogenesis via suppression of nuclear factor- B activation", MOLECULAR CANCER THERAPEUTICS, vol. 5, no. 11, 1 November 2006 (2006-11-01), pages 2872-2878, XP055191240, ISSN: 1535-7163, DOI: 10.1158/1535-7163.MCT-06-0260 Fig.7	18-38
X,P	WO 2014/039975 A2 (NEOTOPE BIOSCIENCES LTD [IE]; FLANAGAN KENNETH [US]; BAKER JEANNE [US]) 13 March 2014 (2014-03-13) The whole document, in particular Example 13	1-46
T	XING LEI ET AL: "The multifaceted role of CD146/MCAM in the promotion of melanoma progression", CANCER CELL INTERNATIONAL, BIOMED CENTRAL, LONDON, GB, vol. 15, no. 1, 4 February 2015 (2015-02-04), page 3, XP021213056, ISSN: 1475-2867, DOI: 10.1186/S12935-014-0147-Z The whole document, in particular, Figure 1.	

3

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2015/051786

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2012170071 A1	13-12-2012	CA 2836373 A1	13-12-2012
		EP 2718327 A1	16-04-2014
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		US 2014227292 A1	14-08-2014
		WO 2014039975 A2	13-03-2014

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2015/051786

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

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

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

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

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

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

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

see additional sheet

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

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

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

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

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: 1-17, 44-46(completely); 26-38(partially)

   Humanised anti-MCAM/CD146/MUC16 antibodies capable of blocking the interaction between MCAM and an alpha-4 laminin, and related subject-matter

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2. claims: 18-25(completely); 26-38(partially)

   An anti-MCAM/CD146/MUC16 antibody that binds to an MCAM epitope comprising position 318, and related subject-matter

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3. claims: 39-43

   Methods and materials for generating an anti-MCAM/CD146/MUC16 antagonist

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