COMPOSITION AND METHOD FOR THE DIAGNOSIS AND TREATMENT OF DISEASES ASSOCIATED WITH NEURITE DEGENERATION

RELATED APPLICATION INFORMATION

[0001] This application claims the benefit of U.S. Serial Number 61/591,324, filed on January 27, 2012, the contents of which are herein incorporated by reference in their entirety.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on January 25, 2013, is named 11423USO.txt and is 100,936 bytes in size.

FIELD OF THE INVENTION

[0003] The present invention relates to antibodies and methods of using the antibodies to treat and diagnose diseases associated with neurite degeneration, such as multiple sclerosis.

BACKGROUND

[0004] The early stages of many neurodegenerative diseases are characterized by neurite damage and compromised synaptic function. Neurite degeneration often leads to neuronal cell death and can impair the conduction of signals in the affected nerves, causing impairment in sensation, movement, cognition, or other functions depending on which nerves are involved. Neurite degeneration is also a pathological hallmark of multiple sclerosis ("MS"). MS is an autoimmune, neurodegenerative disease that affects about 350,000 people in the United States and is a major cause of nervous system disability or death in young adults. A common clinical condition in humans afflicted with MS is the degenerative formation of neural lesions resulting from extensive degradation of the myelin sheaths surrounding the axons of the neurons, and eventual degradation of the axons themselves. The demyelination that occurs in MS is believed to be initiated by the attack of protease enzymes on three major neurological proteins: myelin

basic protein (MBP), proteo-lipid protein (PLP) and myelin oligodendrocyte glycoprotein (MOG). Mechanistically, MS is an inflammatory demyelinating disease that is at least partially caused by an autoimmune response to myelin degradation products. Recent studies have emphasized the role of neurite and axonal injury in addition to the well known demyelation and inflammatory mechanisms.

[0005] Patients typically are diagnosed as having a neurite degenerative disease based on a combination of patient history and neurologic examination, including magnetic resonance imaging (MRI) of the brain and spinal cord, electrodiagnostic procedures (e.g., evoked potential tests such as visual evoked potentials, brain stem auditory evoked potentials, or somatosensory evoked potentials), and lumbar puncture to look for evidence of immunoglobulin synthesis in the cerebrospinal fluid.

[0006] Currently, there is no cure for diseases associated with neurite degeneration, so treatment typically involves management of symptoms and treatment of the frequency and severity of relapses.

SUMMARY OF THE INVENTION

[0007] In one aspect, the present invention is directed to an isolated antibody or antibody fragment thereof which binds to Repulsive Guidance Molecule a ("RGMa"). The antibody comprises a domain or region selected from (a) a variable heavy domain region comprising the amino acid sequence of SEQ ID NO:1, (b) a variable light domain region comprising the amino acid sequence of SEQ ID NO:5, (c) a variable heavy domain region comprising the amino acid sequence of SEQ ID NO:9, (d) a variable light domain region comprising the amino acid sequence of SEQ ID NO:13, (e) a variable heavy domain region comprising the amino acid sequence of SEQ ID NO:17, (f) a variable light domain region comprising the amino acid sequence of SEQ ID NO:21, (g) a variable heavy domain region comprising the amino acid sequence of SEQ ID NO:25, (h) a variable light domain region comprising the amino acid sequence of SEQ ID NO:29, (i) a variable heavy domain region comprising the amino acid sequence of SEQ ID NO:33, (j) a variable light domain region comprising the amino acid sequence of SEQ ID NO:37; (k) a variable heavy domain region comprising the amino acid sequence of SEQ ID NO:41; (l) a variable light domain region comprising the amino acid sequence of SEQ ID NO:45; (m) a variable heavy domain region comprising the amino acid

sequence of SEQ ID NO:49; (n) a variable light domain region comprising the amino acid sequence of SEQ ID NO:53, (o) a variable heavy domain region comprising the amino acid sequence of SEQ ID NO:57, (p) a variable light domain region comprising the amino acid sequence of SEQ ID NO:61, (q) a variable heavy domain region comprising the amino acid sequence of SEQ ID NO:152, (r) a variable light domain region comprising the amino acid sequence of SEQ ID NO:95, (s) a variable heavy domain region comprising the amino acid sequence of SEQ ID NO:99, (t) a variable light domain region comprising the amino acid sequence of SEQ ID NO:103, (u) a variable heavy domain region comprising the amino acid sequence of SEQ ID NO:107, (v) a variable light domain region comprising the amino acid sequence of SEQ ID NO:111, (w) a variable heavy domain region comprising the amino acid sequence of SEQ ID NO:115, (x) a variable light domain region comprising the amino acid sequence of SEQ ID NO:119, (y) a variable heavy domain region comprising the amino acid sequence of SEQ ID NO:123, (z) a variable light domain region comprising the amino acid sequence of SEQ ID NO:127, (aa) a variable heavy domain region comprising the amino acid sequence of SEQ ID NO:131, (bb) a variable light domain region comprising the amino acid sequence of SEQ ID NO:135, (cc) a variable light domain region comprising the amino acid sequence of SEQ ID NO:67, (dd) a variable light domain region comprising the amino acid sequence of SEQ ID NO:68, (ee) a variable light domain region comprising the amino acid sequence of SEQ ID NO:69 (ff) a variable light domain region comprising the amino acid sequence of SEQ ID NO:70, (gg) a variable light domain region comprising the amino acid sequence of SEQ ID NO:71, (hh) a variable light domain region comprising the amino acid sequence of SEQ ID NO:72, (ii) a variable light domain region comprising the amino acid sequence of SEQ ID NO:73, (jj) a variable heavy domain comprising the amino acid sequence of SEQ ID NO:1 and a variable light domain region comprising the amino acid sequence of SEQ ID NO:5, (kk) a variable heavy domain comprising the amino acid sequence of SEQ ID NO:9 and a variable light domain region comprising the amino acid sequence of SEQ ID NO:13, (ll) a variable heavy domain comprising the amino acid sequence of SEQ ID NO:17 and a variable light domain region comprising the amino acid sequence of SEQ ID NO:21, (mm) a variable heavy domain comprising the amino acid sequence of SEQ ID NO:25 and a variable light domain region comprising the amino acid sequence of SEQ ID NO:29, (nn) a variable heavy domain comprising the amino acid sequence of SEQ ID NO:33 and a variable light domain

region comprising the amino acid sequence of SEQ ID NO:37, (oo) a variably heavy domain comprising the amino acid sequence of SEQ ID NO:41 and a variable light domain region comprising the amino acid sequence of SEQ ID NO:45, (pp) a variable heavy domain comprising the amino acid sequence of SEQ ID NO:49 and a variable light domain region comprising the amino acid sequence of SEQ ID NO:53, (qq) a variable heavy domain comprising the amino acid sequence of SEQ ID NO:57 and a variable light domain region comprising the amino acid sequence of SEQ ID NO:61, (rr) a variable heavy domain region comprising the amino acid sequence of SEQ ID NO:152 and a variable light domain region comprising the amino acid sequence of SEQ ID NO:95, (ss) a variable heavy domain region comprising the amino acid sequence of SEQ ID NO:99 and a variable light domain region comprising the amino acid sequence of SEQ ID NO:103, (tt) a variable heavy domain region comprising the amino acid sequence of SEQ ID NO:107 and a variable light domain region comprising the amino acid sequence of SEQ ID NO:111, (uu) a variable heavy domain region comprising the amino acid sequence of SEQ ID NO:115 and a variable light domain region comprising the amino acid sequence of SEQ ID NO:119, (vv) a variable heavy domain region comprising the amino acid sequence of SEQ ID NO:123 and a variable light domain region comprising the amino acid sequence of SEQ ID NO:127, (ww) a variable heavy domain region comprising the amino acid sequence of SEQ ID NO:131 and a variable light domain region comprising the amino acid sequence of SEQ ID NO:135, (xx) a variable heavy chain comprising a complementarity determining region (CDR)1 comprising the amino acid sequence of SEQ ID NO:2, a CDR2 comprising the amino acid sequence of SEQ ID NO:3, and a CDR3 comprising the amino acid sequence of SEQ ID NO:4, (yy) a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:6, a CDR2 comprising the amino acid sequence of SEQ ID NO:7, and a CDR3 comprising the amino acid sequence of SEQ ID NO:8, (zz) a variable heavy chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:10, a CDR2 comprising the amino acid sequence of SEQ ID NO:11, and a CDR3 comprising the amino acid sequence of SEQ ID NO:12, (aaa) a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:14, a CDR2 comprising the amino acid sequence of SEQ ID NO:15, and a CDR3 comprising the amino acid sequence of SEQ ID NO:16, (bbb) a variable heavy chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:18, a CDR2 comprising the amino acid sequence of SEQ ID NO:19, and a CDR3

comprising the amino acid sequence of SEQ ID NO:20, (ccc) a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:22, a CDR2 comprising the amino acid sequence of SEQ ID NO:23, and a CDR3 comprising the amino acid sequence of SEQ ID NO:24, (ddd) a variable heavy chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:26, a CDR2 comprising the amino acid sequence of SEQ ID NO:27, and a CDR3 comprising the amino acid sequence of SEQ ID NO:28, (eee) a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:30, a CDR2 comprising the amino acid sequence of SEQ ID NO:31, and a CDR3 comprising the amino acid sequence of SEQ ID NO:32, (fff) a variable heavy chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:34, a CDR2 comprising the amino acid sequence of SEQ ID NO:35, and a CDR3 comprising the amino acid sequence of SEQ ID NO:36, (ggg) a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:38, a CDR2 comprising the amino acid sequence of SEQ ID NO:39, and a CDR3 comprising the amino acid sequence of SEQ ID NO:40, (hhh) a variable heavy chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:42, a CDR2 comprising the amino acid sequence of SEQ ID NO:43, and CDR3 comprising the amino acid sequence of SEQ ID NO:44; (iii) a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:46, a CDR2 comprising the amino acid sequence of SEQ ID NO:47, and a CDR3 comprising the amino acid sequence of SEQ ID NO:48; (jjj) a variable heavy chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:50, a CDR2 comprising the amino acid sequence of SEQ ID NO:51, and a CDR3 comprising the amino acid sequence of SEQ ID NO:52 and a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:54, a CDR2 comprising the amino acid sequence of SEQ ID NO:55, and a CDR3 comprising the amino acid sequence of SEQ ID NO:56, (kkk) a variable heavy chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:58, a CDR2 comprising the amino acid sequence of SEQ ID NO:59, and a CDR3 comprising the amino acid sequence of SEQ ID NO:60, (lll) a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:62, a CDR2 comprising the amino acid sequence of SEQ ID NO:63, and a CDR3 comprising the amino acid sequence of SEQ ID NO:64, (mmm) a variable heavy chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:92 or 153, a CDR2 comprising the amino acid sequence of SEQ ID NO:93 or 154, and a CDR3 comprising the amino acid sequence of SEQ ID NO:94 or 155, (nnn) a variable

light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:96 or 156, a CDR2 comprising the amino acid sequence of SEQ ID NO:97 or 157, and a CDR3 comprising the amino acid sequence of SEQ ID NO:98 or 158, (ooo) a variable heavy chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:100, a CDR2 comprising the amino acid sequence of SEQ ID NO:101, and a CDR3 comprising the amino acid sequence of SEQ ID NO:102, (ppp) a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:104, a CDR2 comprising the amino acid sequence of SEQ ID NO:105, and a CDR3 comprising the amino acid sequence of SEQ ID NO:106, (qqq) a variable heavy chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:108, a CDR2 comprising the amino acid sequence of SEQ ID NO:109, and a CDR3 comprising the amino acid sequence of SEQ ID NO:110, (rrr) a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:112, a CDR2 comprising the amino acid sequence of SEQ ID NO:113, and a CDR3 comprising the amino acid sequence of SEQ ID NO:114, (sss) a variable heavy chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:116, a CDR2 comprising the amino acid sequence of SEQ ID NO:117, and a CDR3 comprising the amino acid sequence of SEQ ID NO:118, (ttt) a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:120, a CDR2 comprising the amino acid sequence of SEQ ID NO:121, and a CDR3 comprising the amino acid sequence of SEQ ID NO:122, (uuu) a variable heavy chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:124, a CDR2 comprising the amino acid sequence of SEQ ID NO:125, and a CDR3 comprising the amino acid sequence of SEQ ID NO:126, (vvv) a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:128, a CDR2 comprising the amino acid sequence of SEQ ID NO:129, and a CDR3 comprising the amino acid sequence of SEQ ID NO:130, (www) a variable heavy chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:132, a CDR2 comprising the amino acid sequence of SEQ ID NO:133, and a CDR3 comprising the amino acid sequence of SEQ ID NO:134, (xxx) a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:136, a CDR2 comprising the amino acid sequence of SEQ ID NO:137, and a CDR3 comprising the amino acid sequence of SEQ ID NO:138, (yyy) a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:6, a CDR2 comprising the amino acid sequence of SEQ ID NO:7, and a CDR3 comprising the amino acid sequence of SEQ ID

NO:67, (zzz) a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:6, a CDR2 comprising the amino acid sequence of SEQ ID NO:7, and a CDR3 comprising the amino acid sequence of SEQ ID NO:68, (aaaa) a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:6, a CDR2 comprising the amino acid sequence of SEQ ID NO:7, and a CDR3 comprising the amino acid sequence of SEQ ID NO:69, (bbbb) a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:6, a CDR2 comprising the amino acid sequence of SEQ ID NO:7, and a CDR3 comprising the amino acid sequence of SEQ ID NO:70,(cccc) a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:6, a CDR2 comprising the amino acid sequence of SEQ ID NO:7, and a CDR3 comprising the amino acid sequence of SEQ ID NO:71, (dddd) a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:6, a CDR2 comprising the amino acid sequence of SEQ ID NO:7, and a CDR3 comprising the amino acid sequence of SEQ ID NO:72, (eeee) a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:6, a CDR2 comprising the amino acid sequence of SEQ ID NO:7, and a CDR3 comprising the amino acid sequence of SEQ ID NO:73, (ffff) a variable heavy chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:2, a CDR2 comprising the amino acid sequence of SEQ ID NO:3, and a CDR3 comprising the amino acid sequence of SEQ ID NO:4 and a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:6, a CDR2 comprising the amino acid sequence of SEQ ID NO:7, and a CDR3 comprising the amino acid sequence of SEQ ID NO:8, (gggg) a variable heavy chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:2, a CDR2 comprising the amino acid sequence of SEQ ID NO:3, and a CDR3 comprising the amino acid sequence of SEQ ID NO:4 and a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:6, a CDR2 comprising the amino acid sequence of SEQ ID NO:7, and a CDR3 comprising the amino acid sequence of SEQ ID NO:67, (hhhh) a variable heavy chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:92 or 153, a CDR2 comprising the amino acid sequence of SEQ ID NO:93 or 154, and a CDR3 comprising the amino acid sequence of SEQ ID NO:94 or 155 and a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:96 or 156, a CDR2 comprising the amino acid sequence of SEQ ID NO:97 or 157, and a CDR3 comprising the amino acid sequence of SEQ ID NO:98 or 158, (iiii) a variable heavy chain comprising a CDR1

comprising the amino acid sequence of SEQ ID NO:100, a CDR2 comprising the amino acid sequence of SEQ ID NO:101, and a CDR3 comprising the amino acid sequence of SEQ ID NO:102 and a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:104, a CDR2 comprising the amino acid sequence of SEQ ID NO:105, and a CDR3 comprising the amino acid sequence of SEQ ID NO:106, (jjjj) a variable heavy chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:108, a CDR2 comprising the amino acid sequence of SEQ ID NO:109, and a CDR3 comprising the amino acid sequence of SEQ ID NO:110 and a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:112, a CDR2 comprising the amino acid sequence of SEQ ID NO:113, and a CDR3 comprising the amino acid sequence of SEQ ID NO:114, (kkkk) a variable heavy chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:116, a CDR2 comprising the amino acid sequence of SEQ ID NO:117, and a CDR3 comprising the amino acid sequence of SEQ ID NO:118 and a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:120, a CDR2 comprising the amino acid sequence of SEQ ID NO:121, and a CDR3 comprising the amino acid sequence of SEQ ID NO:122, (llll) a variable heavy chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:124, a CDR2 comprising the amino acid sequence of SEQ ID NO:125, and a CDR3 comprising the amino acid sequence of SEQ ID NO:126 and a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:128, a CDR2 comprising the amino acid sequence of SEQ ID NO:129, and a CDR3 comprising the amino acid sequence of SEQ ID NO:130, (mmmm) a variable heavy chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:132, a CDR2 comprising the amino acid sequence of SEQ ID NO:133, and a CDR3 comprising the amino acid sequence of SEQ ID NO:134 and a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:136, a CDR2 comprising the amino acid sequence of SEQ ID NO:137, and a CDR3 comprising the amino acid sequence of SEQ ID NO:138, (nnnn) a variable heavy chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:2, a CDR2 comprising the amino acid sequence of SEQ ID NO:3, and a CDR3 comprising the amino acid sequence of SEQ ID NO:4 and a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:6, a CDR2 comprising the amino acid sequence of SEQ ID NO:7, and a CDR3 comprising the amino acid sequence of SEQ ID NO:68, (oooo) a variable heavy chain comprising a CDR 1 comprising the

amino acid sequence of SEQ ID NO:2, a CDR2 comprising the amino acid sequence of SEQ ID NO:3, and a CDR3 comprising the amino acid sequence of SEQ ID NO:4, and a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:6, a CDR2 comprising the amino acid sequence of SEQ ID NO:7, and a CDR3 comprising the amino acid sequence of SEQ ID NO:69, (pppp) a variable heavy chain comprising a CDR 1 comprising the amino acid sequence of SEQ ID NO:2, a CDR2 comprising the amino acid sequence of SEQ ID NO:3, and a CDR3 comprising the amino acid sequence of SEQ ID NO:4, and a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:6, a CDR2 comprising the amino acid sequence of SEQ ID NO:7, and a CDR3 comprising the amino acid sequence of SEQ ID NO:70, (qqqq) a variable heavy chain comprising a CDR 1 comprising the amino acid sequence of SEQ ID NO:2, a CDR2 comprising the amino acid sequence of SEQ ID NO:3, and a CDR3 comprising the amino acid sequence of SEQ ID NO:4, and a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:6, a CDR2 comprising the amino acid sequence of SEQ ID NO:7, and a CDR3 comprising the amino acid sequence of SEQ ID NO:71, (rrrr) a variable heavy chain comprising a CDR 1 comprising the amino acid sequence of SEQ ID NO:2, a CDR2 comprising the amino acid sequence of SEQ ID NO:3, and a CDR3 comprising the amino acid sequence of SEQ ID NO:4, and a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:6, a CDR2 comprising the amino acid sequence of SEQ ID NO:7, and a CDR3 comprising the amino acid sequence of SEQ ID NO:72, (ssss) a variable heavy chain comprising a CDR 1 comprising the amino acid sequence of SEQ ID NO:2, a CDR2 comprising the amino acid sequence of SEQ ID NO:3, and a CDR3 comprising the amino acid sequence of SEQ ID NO:4, and a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:6, a CDR2 comprising the amino acid sequence of SEQ ID NO:7, and a CDR3 comprising the amino acid sequence of SEQ ID NO:73, (tttt) a variable heavy chain comprising a CDR 1 comprising the amino acid sequence of SEQ ID NO:10, a CDR2 comprising the amino acid sequence of SEQ ID NO:11, and a CDR3 comprising the amino acid sequence of SEQ ID NO:12, and a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:14, a CDR2 comprising the amino acid sequence of SEQ ID NO:15, and a CDR3 comprising the amino acid sequence of SEQ ID NO:16, (uuuu) a variable heavy chain comprising a CDR 1 comprising the amino acid sequence of SEQ ID NO:18, a CDR2 comprising the amino acid sequence of SEQ ID

NO:19, and a CDR3 comprising the amino acid sequence of SEQ ID NO:20, and a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:22, a CDR2 comprising the amino acid sequence of SEQ ID NO:23, and a CDR3 comprising the amino acid sequence of SEQ ID NO:24, (vvvv) a variable heavy chain comprising a CDR 1 comprising the amino acid sequence of SEQ ID NO:26, a CDR2 comprising the amino acid sequence of SEQ ID NO:27, and a CDR3 comprising the amino acid sequence of SEQ ID NO:28, and a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:30, a CDR2 comprising the amino acid sequence of SEQ ID NO:31, and a CDR3 comprising the amino acid sequence of SEQ ID NO:32, (wwww) a variable heavy chain comprising a CDR 1 comprising the amino acid sequence of SEQ ID NO:34, a CDR2 comprising the amino acid sequence of SEQ ID NO:35, and a CDR3 comprising the amino acid sequence of SEQ ID NO:36, and a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:38, a CDR2 comprising the amino acid sequence of SEQ ID NO:39, and a CDR3 comprising the amino acid sequence of SEQ ID NO:40, (xxxx) a variable heavy domain chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:42, a CDR2 comprising the amino acid sequence of SEQ ID NO:43, and a CDR comprising the amino acid sequence of SEQ ID NO:44, and a variable light domain chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:46, a CDR2 comprising the amino acid sequence of SEQ ID NO:47, and a CDR3 comprising the amino acid sequence of SEQ ID NO:48; (yyyy) a variable heavy chain comprising a CDR 1 comprising the amino acid sequence of SEQ ID NO:50, a CDR2 comprising the amino acid sequence of SEQ ID NO:51, and a CDR3 comprising the amino acid sequence of SEQ ID NO:52, and a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:54, a CDR2 comprising the amino acid sequence of SEQ ID NO:55, and a CDR3 comprising the amino acid sequence of SEQ ID NO:56, (zzzz) a variable heavy chain comprising a CDR 1 comprising the amino acid sequence of SEQ ID NO:58, a CDR2 comprising the amino acid sequence of SEQ ID NO:59, and a CDR3 comprising the amino acid sequence of SEQ ID NO:60, and a variable light chain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:62, a CDR2 comprising the amino acid sequence of SEQ ID NO:63, and a CDR3 comprising the amino acid sequence of SEQ ID NO:64.

[0008] The isolated antibody or antibody fragment may be a human antibody, an immunoglobulin molecule, a disulfide linked Fv, a monoclonal antibody, an affinity matured, a scFv, a chimeric antibody, a single domain antibody, a CDR-grafted antibody, a diabody, a humanized antibody, a multispecific antibody, a Fab, a dual specific antibody, a DVD, a Fab', a bispecific antibody, a F(ab')2, or a Fv. The antibody or antibody fragment may be human. The antibody or antibody fragment may comprise a heavy chain immunoglobulin constant domain selected from the group consisting of a human IgM constant domain, a human IgG4 constant domain, a human IgG1 constant domain, a human IgE constant domain, a human IgG2 constant domain, a human IgG3 constant domain, or a human IgA constant domain. The human IgG1 constant domain may comprise, or consist of, SEQ ID NO:140.

[0009] The antibody or fragment thereof may comprise a variable heavy region comprising a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:9, SEQ ID NO:17, SEQ ID NO:25, SEQ ID NO:33, SEQ ID NO:41, SEQ ID NO:49, SEQ ID NO:57, SEQ ID NO:91, SEQ ID NO:99, SEQ ID NO:107, SEQ ID NO:115, SEQ ID NO:123, and SEQ ID NO:131.

[0010] The isolated antibody or antibody fragment may comprise a variable light region comprising a sequence selected from the group consisting of SEQ ID NO:5, SEQ ID NO:13, SEQ ID NO:21, SEQ ID NO:29, SEQ ID NO:37, SEQ ID NO:45, SEQ ID NO:53, SEQ ID NO:61, SEQ ID NO:95, SEQ ID NO:103, SEQ ID NO:111, SEQ ID NO:119, SEQ ID NO:127, and SEQ ID NO:135.

[0011] The isolated antibody or antibody fragment may comprise a variable light domain that comprises complementarity-determining region (CDR) residues SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8, or SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:67, SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:68, or SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:69, or SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:70, or SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:71, or SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:72 or SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:73, or SEQ ID NO:14, SEQ ID NO:15, and SEQ ID NO:16, or SEQ ID NO:22, SEQ ID NO:23, and SEQ ID NO:24, or SEQ ID NO:30, SEQ ID NO:31, and SEQ ID NO:32, or SEQ ID NO:38, SEQ ID NO:39, and SEQ ID NO:40, or SEQ ID NO:54, SEQ ID NO:55, and SEQ ID NO:56, or SEQ ID NO:62, SEQ ID NO:63, and SEQ ID NO:64, or SEQ ID NO:46, SEQ ID

NO:47, and SEQ ID NO:48, SEQ ID NO:96, SEQ ID NO:97, and SEQ ID NO:98, SEQ ID NO:104, SEQ ID NO:105, and SEQ ID NO:106, SEQ ID NO:112, SEQ ID NO:113, and SEQ ID NO:114, SEQ ID NO:120, SEQ ID NO:121, and SEQ ID NO:122, SEQ ID NO:128, SEQ ID NO:129, and SEQ ID NO:130, SEQ ID NO:136, SEQ ID NO:137, and SEQ ID NO:138, and SEQ ID NO: 156, and SEQ ID NO: 157, and SEQ ID NO: 158.

[0012] The isolated antibody or antibody fragment may comprise a variable heavy domain that comprises complementarity-determining region (CDR) residues SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NO:4, or SEQ ID NO:10, SEQ ID NO:11, and SEQ ID NO:12, or SEQ ID NO:18, SEQ ID NO:19, and SEQ ID NO:20, or SEQ ID NO:26, SEQ ID NO:27, and SEQ ID NO:28, or SEQ ID NO:34, SEQ ID NO:35, and SEQ ID NO:36, or SEQ ID NO:50, SEQ ID NO:51, and SEQ ID NO:52, or SEQ ID NO:58, SEQ ID NO:59, and SEQ ID NO:60, SEQ ID NO:42, SEQ ID NO:43, and SEQ ID NO:44, SEQ ID NO:92, SEQ ID NO:93, and SEQ ID NO:94, SEQ ID NO:100, SEQ ID NO:101, and SEQ ID NO:102, SEQ ID NO:108, SEQ ID NO:109, and SEQ ID NO:110, SEQ ID NO:116, SEQ ID NO:117, and SEQ ID NO:118, SEQ ID NO:124, SEQ ID NO:125, and SEQ ID NO:126, SEQ ID NO:132, SEQ ID NO:133, and SEQ ID NO:134, SEQ ID NO: 153, and SEQ ID NO: 154, and SEQ ID NO: 155.

[0013] The isolated antibody or antibody fragment may comprise a variable heavy domain that comprises complementarity-determining region (CDR) residues SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NO:4, and a variable light domain that comprises complementarity-determining region (CDR) residues SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8.

[0014] The isolated antibody or antibody fragment may comprise a variable heavy domain that comprises complementarity-determining region (CDR) residues SEQ ID NO:10, SEQ ID NO:11, and SEQ ID NO:12, and a variable light domain that comprises complementarity-determining region (CDR) residues SEQ ID NO:14, SEQ ID NO:15, and SEQ ID NO:16.

[0015] The isolated antibody or antibody fragment may comprise a variable heavy domain that comprises complementarity-determining region (CDR) residues SEQ ID NO:18, SEQ ID NO:19, and SEQ ID NO:20, and a variable light domain that comprises complementarity-determining region (CDR) residues SEQ ID NO:22, SEQ ID NO:23, and SEQ ID NO:24.

[0016] The isolated antibody or antibody fragment may comprise a variable heavy domain that comprises complementarity-determining region (CDR) residues SEQ ID NO:26, SEQ ID NO:27,

and SEQ ID NO:28, and a variable light domain that comprises complementarity-determining region (CDR) residues SEQ ID NO:30, SEQ ID NO:31, and SEQ ID NO:32.

[0017] The isolated antibody or antibody fragment may comprise a variable heavy domain that comprises complementarity-determining region (CDR) residues SEQ ID NO:34, SEQ ID NO:35, and SEQ ID NO:36, and a variable light domain that comprises complementarity-determining region (CDR) residues SEQ ID NO:38, SEQ ID NO:39, and SEQ ID NO:40.

[0018] The isolated antibody or antibody fragment may comprise a variable heavy domain that comprises complementarity-determining region (CDR) residues SEQ ID NO:50, SEQ ID NO:51, and SEQ ID NO:52, and a variable light domain that comprises complementarity-determining region (CDR) residues SEQ ID NO:54, SEQ ID NO:55, and SEQ ID NO:56.

[0019] The isolated antibody or antibody fragment may comprise a variable heavy domain that comprises complementarity-determining region (CDR) residues SEQ ID NO:58, SEQ ID NO:59, and SEQ ID NO:60, and a variable light domain that comprises complementarity-determining region (CDR) residues SEQ ID NO:62, SEQ ID NO:63, and SEQ ID NO:64.

[0020] The isolated antibody or antibody fragment may comprise a variable heavy domain that comprises complementarity-determining region (CDR) residues SEQ ID NO:42, SEQ ID NO:43, and SEQ ID NO:44, and a variable light domain that comprises complementarity-determining region (CDR) residues SEQ ID NO:46, SEQ ID NO:47, and SEQ ID NO:48.

[0021] The isolated antibody or antibody fragment may comprise a variable heavy domain that comprises complementarity-determining region (CDR) residues SEQ ID NO:92 or 153, SEQ ID NO:93 or 154, and SEQ ID NO:94 or 155, and a variable light domain that comprises complementarity-determining region (CDR) residues SEQ ID NO:96 or 156, SEQ ID NO:97 or 157, and SEQ ID NO:98 or 158.

[0022] The isolated antibody or antibody fragment may comprise a variable heavy domain that comprises complementarity-determining region (CDR) residues SEQ ID NO:100, SEQ ID NO:101, and SEQ ID NO:102, and a variable light domain that comprises complementarity-determining region (CDR) residues SEQ ID NO:104, SEQ ID NO:105, and SEQ ID NO:106.

[0023] The isolated antibody or antibody fragment may comprise a variable heavy domain that comprises complementarity-determining region (CDR) residues SEQ ID NO:108, SEQ ID

NO:109, and SEQ ID NO:110, and a variable light domain that comprises complementarity-determining region (CDR) residues SEQ ID NO:112, SEQ ID NO:113, and SEQ ID NO:114.

[0024] The isolated antibody or antibody fragment may comprise a variable heavy domain that comprises complementarity-determining region (CDR) residues SEQ ID NO:116, SEQ ID NO:117, and SEQ ID NO:118, and a variable light domain that comprises complementarity-determining region (CDR) residues SEQ ID NO:120, SEQ ID NO:121, and SEQ ID NO:122.

[0025] The isolated antibody or antibody fragment may comprise a variable heavy domain that comprises complementarity-determining region (CDR) residues SEQ ID NO:124, SEQ ID NO:125, and SEQ ID NO:126, and a variable light domain that comprises complementarity-determining region (CDR) residues SEQ ID NO:128, SEQ ID NO:129, and SEQ ID NO:130.

[0026] The isolated antibody or antibody fragment may comprise a variable heavy domain that comprises complementarity-determining region (CDR) residues SEQ ID NO:132, SEQ ID NO:133, and SEQ ID NO:134, and a variable light domain that comprises complementarity-determining region (CDR) residues SEQ ID NO:136, SEQ ID NO:137, and SEQ ID NO:138.

[0027] The isolated antibody or antibody fragment may comprise an agent selected from the group consisting of: an immunoadhesion molecule, an imaging agent, and a therapeutic agent. The imaging agent may be a radiolabel, an enzyme, a fluorescent label, a luminescent label, a bioluminescent label, a magnetic label, or biotin. The radiolabel may be 3H, 14C, 35S, 90Y, 99Tc, 111In, 125I, 131I, 177Lu, 166Ho, or 153Sm.

[0028] In another aspect, the present invention is directed to an antibody, or fragment thereof, that binds to the RGMa epitope PCKILKCNSEFWSATSGSHAPAS (hRGMa 47-69) (SEQ ID NO:79). The antibody that binds to the RGMa epitope PCKILKCNSEFWSATSGSHAPAS (hRGMa 47-69) (SEQ ID NO:79), may comprise a variable heavy domain that comprises complementarity-determining region (CDR) residues SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NO:4, and a variable light domain that comprises complementarity-determining region (CDR) residues SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8.

[0029] In another aspect, the present invention is directed to an isolated antibody or antibody fragment thereof which binds to Repulsive Guidance Molecule a ("RGMa"). The antibody or antibody fragment comprises a variable heavy domain that comprises three complementarity-

determining regions (CDR- H1, H2, and H3) corresponding to the following formulas, respectively:

Xaa1 – Xaa2 – Xaa3 – Xaa4 – Xaa5 (Formula 1-CDR-H1), wherein Xaa1 is an amino acid selected from the group consisting of S, D, E, N, G, and T; Xaa2 is an amino acid selected from the group consisting of H, Y, L, S, and Q; Xaa3 is an amino acid selected from the group consisting of G, D, A, T, and Y; Xaa4 is an amino acid selected from the group consisting of I, M, and W; and Xaa5 is an amino acid sequence from the group consisting of S, N, H, A, T, and Q;

Xaa1 – Xaa2 – Xaa3 – Xaa4 – Xaa5 – Xaa6 – Xaa7 – Xaa8 – Xaa9 – Xaa10 – Xaa11 – Xaa12 – Xaa13 – Xaa14 – Xaa15 – Xaa16 – (Xaa)n (Formula 2-CDR-H2), wherein n is 0 or 1, and

wherein Xaa1 is an amino acid selected from the group consisting of W, V, A, G, L, E, S, and N; Xaa2 is an amino acid selected from the group consisting of I, M, and F; Xaa3 is an amino acid selected from the group consisting of S, N, D, F, and Y; Xaa4 is an amino acid selected from the group consisting of P, Y, G, W, H, A, and S; Xaa5 is an amino acid selected from the group consisting of Y, N, D, E, S, K, G, and T; Xaa6 is an amino acid selected from the group consisting of S, G, D, T, and N; Xaa7 is an amino acid selected from the group consisting of G, S, I, E, N, and R; Xaa8 is an amino acid selected from the group consisting of N, L, R, S, T, and Y; Xaa9 is an amino acid selected from the group consisting of T, K, G, N, I, and Y; Xaa10 is an amino acid selected from the group consisting of N, G, Y, T, and K; Xaa11 is an amino acid selected from the group consisting of Y, F, N, and H; Xaa12 is an amino acid selected from the group consisting of A, T, V, P, L, and S; Xaa13 is an amino acid selected from the group consisting of Q, D, P, and S; Xaa14 is an amino acid selected from the group consisting of K, S, N, and L; Xaa15 is an amino acid selected from the group consisting of L, F, V, K, and R; Xaa16 is an amino acid selected from the group consisting of Q, K, R, and S; and Xaa17 is a glycine; and

Xaa1 – Xaa2 – Xaa3 – Xaa4 – Xaa5 – Xaa6 – (Xaa)n (Formula 3-CDR-H3), wherein n is 0 - 11, and

wherein Xaa1 is an amino acid selected from the group consisting of V, S, E, N, L, D, Q, and A; Xaa2 is an amino acid selected from the group consisting of G, T, R, Y, L, I, D, and S; Xaa3 is an amino acid selected from the group consisting of S, V, D, G, F, Y, P, M, C, L, and A; Xaa4 is

an amino acid selected from the group consisting of G, L, Y, N, E, K, A, and F; Xaa5 is an amino acid selected from the group consisting of P, S, Y, A, V, G, T, E, and W; Xaa6 is an amino acid selected from the group consisting of Y, V, S, L, D, G, H, and P; Xaa7 is an amino acid selected from the group consisting of Tyr, Asp, Gly, Ser, Phe, Leu, and Cys; Xaa8 is an amino acid selected from the group consisting of Tyr, Lys, Asp, Ala, and Gln; Xaa9 is an amino acid selected from the group consisting of Met, Glu, Phe, Leu, Ser, Thr, Pro, and Tyr; Xaa10 is an amino acid selected from the group consisting of Asp, Gly, Tyr, Ser, Leu, His, and Phe; Xaa11 is an amino acid selected from the group consisting of Val, Tyr, Leu, His, Gly, Trp, and Asp; Xaa12 is an amino acid selected from the group consisting of Tyr and Phe; Xaa13 is an amino acid selected from the group consisting of Tyr, Gly, and Asp; Xaa14 is an amino acid selected from the group consisting of Ala, Leu, Pro, and Tyr; Xaa15 is an amino acid selected from the group consisting of Met, Leu, and Phe; Xaa16 is an amino acid selected from the group consisting of Asp and Gly; and Xaa17 is an amino acid selected from the group consisting of an Val, Asp, and Tyr.

[0030] In another aspect, the present invention is directed to an isolated antibody or antibody fragment thereof which binds to Repulsive Guidance Molecule a ("RGMa"). The antibody or antibody fragment comprises a variable light domain that comprises three complementarity-determining regions (CDR-L1, L2 and L3) corresponding to the following formulas, respectively:

Xaa1 – Xaa2 – Xaa3 – Xaa4 – Xaa5 – Xaa6 – Xaa7 – Xaa8 – Xaa9 – Xaa10 – Xaa11 – (Xaa)n (Formula 1-CDR-L1), wherein n is 0-3, and

wherein Xaa1 is an amino acid selected from the group consisting of T, S, R, G, and Q; Xaa2 is an amino acid selected from the group consisting of G, L, and A; Xaa3 is an amino acid selected from the group consisting of T, D, S, N and A; Xaa4 is an amino acid selected from the group consisting of S, K, G, Q, N, and E; Xaa5 is an amino acid sequence from the group consisting of S, L, G, I , D, and P; Xaa6 is an amino acid selected from the group consisting of S, G, N, H, and I; Xaa7 is an amino acid selected from the group consisting of V, D, I, S, G and H; Xaa8 is an amino acid selected from the group consisting of G, K, A, S, I, N, T, and D; Xaa9 is an amino acid selected from the group consisting of D, Y, A, C, S, and F; Xaa10 is an amino acid selected from the group consisting of S, A, G, L, V, and N; Xaa11 is an amino acid selected from the

group consisting of I, C, Y, H, R, N, and S; Xaa12 is an amino acid selected from the group consisting of Tyr, Gly, Ala, and Val; Xaa13 is an amino acid selected from the group consisting of Val, and Asn; and Xaa14 is an amino acid selected from the group consisting of Ser and His;

Xaa1 – Xaa2 – Xaa3 – Xaa4 – Xaa5 – Xaa6 – Xaa7 – (Xaa)n (Formula 2-CDR-L2), wherein n is 0-4, and wherein Xaa1 is an amino acid selected from the group consisting of D, Q, G, V, Y, S and E; Xaa2 is an amino acid selected from the group consisting of V, D, N, and A; Xaa3 is an amino acid selected from the group consisting of T, S, Y, N, and K; Xaa4 is an amino acid selected from the group consisting of K, N, D, Q and T; Xaa5 is an amino acid selected from the group consisting of R, G, S, and L; Xaa6 is an amino acid selected from the group consisting of P, S, I, and E; Xaa7 is an amino acid selected from the group consisting of S, H, I, and T; Xaa8 is Asn; Xaa9 is Lys; and Xaa10 is Gly; Xaa11 is Asp; and

Xaa1 – Xaa2 – Xaa3 – Xaa4 – Xaa5 – Xaa6 – Xaa7 – Xaa8 – Xaa9 – (Xaa)n (Formula 3-CDR-L3), wherein n is 0-2, and wherein Xaa1 is an amino acid selected from the group consisting of C, Q, H, F, H, L, V, I, K, Y, and A; Xaa2 is an amino acid selected from the group consisting of S, A, T, Q, and V; Xaa3 is an amino acid selected from the group consisting of Y, W, and S; Xaa4 is an amino acid selected from the group consisting of A, D, G, S, H and Y; Xaa5 is an amino acid selected from the group consisting of G, S, N, P, D, V, and T; Xaa6 is an amino acid selected from the group consisting of I, T, S, G, L, F and Y; Xaa7 is an amino acid selected from the group consisting of D, T, L, I, P, and S; Xaa8 is an amino acid selected from the group consisting of T, G, R, Y, D, N, W, L, F and P; Xaa9 is an amino acid selected from the group consisting of L, V, G, T, and H; Xaa10 is an amino acid selected from the group consisting of Val, Tyr, and His; Xaa11 is Leu or Val.

[0031] In another aspect, the present invention is directed to an isolated antibody or antibody fragment thereof which binds to Repulsive Guidance Molecule a ("RGMa"), wherein the antibody or antibody fragment comprises a variable heavy domain that comprises three complementarity-determining regions (CDR-H1, H2, and H3) corresponding to the following formulas, respectively:

[0032] Xaa1 – Xaa2 – Xaa3 – Xaa4 – Xaa5 (Formula 1 – CDR-H1), wherein Xaa1 is an amino acid selected from the group consisting of S, D, E, N, G, and T; Xaa2 is an amino acid selected from the group consisting of H, Y, L, S, and Q; Xaa3 is an amino acid selected from the group

consisting of G, D, A, T, and Y; Xaa4 is an amino acid selected from the group consisting of I, M, and W; and Xaa5 is an amino acid sequence from the group consisting of S, N, H, A, T, and Q;

[0033] Xaa1 – Xaa2 – Xaa3 – Xaa4 – Xaa5 – Xaa6 – Xaa7 – Xaa8 – Xaa9 – Xaa10 – Xaa11 – Xaa12 – Xaa13 – Xaa14 – Xaa15 – Xaa16 – (Xaa)n (Formula 2 – CDR-H2), wherein n is 0 or 1, and

[0034] wherein Xaa1 is an amino acid selected from the group consisting of Y, V, A, G, L, G, S, and N; Xaa2 is an amino acid selected from the group consisting of I, M, and F; Xaa3 is an amino acid selected from the group consisting of S, N, D, F, and Y; Xaa4 is an amino acid selected from the group consisting of P, Y, G, W, H, A, and S; Xaa5 is an amino acid selected from the group consisting of Y, N, D, E, S, K, G, and T; Xaa6 is an amino acid selected from the group consisting of S, G, D, T, and N; Xaa7 is an amino acid selected from the group consisting of G, S, I, E, N, and R; Xaa8 is an amino acid selected from the group consisting of N, L, R, S, T, and Y; Xaa9 is an amino acid selected from the group consisting of T, K, G, N, I, and Y; Xaa10 is an amino acid selected from the group consisting of N, G, Y, T, and K; Xaa11 is an amino acid selected from the group consisting of Y, F, N, and H; Xaa12 is an amino acid selected from the group consisting of A, T, V, P, L, and S; Xaa13 is an amino acid selected from the group consisting of Q, D, P, and S; Xaa14 is an amino acid selected from the group consisting of K, S, N, and L; Xaa15 is an amino acid selected from the group consisting of L, F, V, K, and R; Xaa16 is an amino acid selected from the group consisting of Q, K, R, and S; and Xaa17 is a glycine; and

[0035] Xaa1 – Xaa2 – Xaa3 – Xaa4 – Xaa5 – Xaa6 – (Xaa)n (Formula 3 – CDR-H3), wherein n is 0 - 11, and

[0036] wherein Xaa1 is an amino acid selected from the group consisting of V, S, E, N, L, D, Q, and A; Xaa2 is an amino acid selected from the group consisting of G, T, R, Y, L, I, D, and S; Xaa3 is an amino acid selected from the group consisting of S, V, D, G, F, Y, P, M, C, L, and A; Xaa4 is an amino acid selected from the group consisting of G, L, Y, N, E, K, A, and F; Xaa5 is an amino acid selected from the group consisting of P, S, Y, A, V, G, T, E, and W; Xaa6 is an amino acid selected from the group consisting of Y, V, S, L, D, G, H, and P; Xaa7 is an amino acid selected from the group consisting of Tyr, Asp, Gly, Ser, Phe, Leu, and Cys; Xaa8 is an

amino acid selected from the group consisting of Tyr, Lys, Asp, Ala, and Gln; Xaa9 is an amino acid selected from the group consisting of Met, Glu, Phe, Leu, Ser, Thr, Pro, and Tyr; Xaa10 is an amino acid selected from the group consisting of Asp, Gly, Tyr, Ser, Leu, His, and Phe; Xaa11 is an amino acid selected from the group consisting of Val, Tyr, Leu, His, Gly, Trp, and Asp; Xaa12 is an amino acid selected from the group consisting of Tyr and Phe; Xaa13 is an amino acid selected from the group consisting of Tyr, Gly, and Asp; Xaa14 is an amino acid selected from the group consisting of Ala, Leu, Pro, and Tyr; Xaa15 is an amino acid selected from the group consisting of Met, Leu, and Phe; Xaa16 is an amino acid selected from the group consisting of Asp and Gly; and Xaa17 is an amino acid selected from the group consisting of an Val, Asp, and Tyr; and

wherein the antibody or antibody fragment also comprises a variable light domain that comprises three complementarity-determining regions (CDR-L1, L2, and L3) corresponding to the following formulas, respectively:

Xaa1 – Xaa2 – Xaa3 – Xaa4 – Xaa5 – Xaa6 – Xaa7 – Xaa8 – Xaa9 – Xaa10 – Xaa11 – (Xaa)n (Formula 1 –CDR-L1), wherein n is 0-3, and

wherein Xaa1 is an amino acid selected from the group consisting of T, S, R, G, and Q; Xaa2 is an amino acid selected from the group consisting of G, L, and A; Xaa3 is an amino acid selected from the group consisting of T, D, S, N and A; Xaa4 is an amino acid selected from the group consisting of S, K, G, Q, N, and E; Xaa5 is an amino acid sequence from the group consisting of S, L, G, I , D, and P; Xaa6 is an amino acid selected from the group consisting of S, G, N, H, and I; Xaa7 is an amino acid selected from the group consisting of V, D, I, S, G and H; Xaa8 is an amino acid selected from the group consisting of G, K, A, S, I, N, T, and D; Xaa9 is an amino acid selected from the group consisting of D, Y, A, C, S, and F; Xaa10 is an amino acid selected from the group consisting of S, A, G, L, V, and N; Xaa11 is an amino acid selected from the group consisting of I, C, Y, H, R, N, and S; Xaa12 is an amino acid selected from the group consisting of Tyr, Gly, Ala, and Val; Xaa13 is an amino acid selected from the group consisting of Val, and Asn; and Xaa14 is an amino acid selected from the group consisting of Ser and His;

Xaa1 – Xaa2 – Xaa3 – Xaa4 – Xaa5 – Xaa6 – Xaa7 – (Xaa)n (Formula 2 – CDR-L2), wherein n is 0-4, and wherein Xaa1 is an amino acid selected from the group consisting of D, Q, G, V, Y, S and E; Xaa2 is an amino acid selected from the group consisting of V, D, N, and A; Xaa3 is an

amino acid selected from the group consisting of T, S, Y, N, and K; Xaa4 is an amino acid selected from the group consisting of K, N, D, Q and T; Xaa5 is an amino acid selected from the group consisting of R, G, S, and L; Xaa6 is an amino acid selected from the group consisting of P, S, I, and E; Xaa7 is an amino acid selected from the group consisting of S, H, I, and T; Xaa8 is Asn; Xaa9 is Lys; and Xaa10 is Gly; Xaa11 is Asp; and

Xaa1 – Xaa2 – Xaa3 – Xaa4 – Xaa5 – Xaa6 – Xaa7 – Xaa8 – Xaa9 – (Xaa)n (Formula 3 – CDR-L3), wherein n is 0-2, and

wherein Xaa1 is an amino acid selected from the group consisting of C, Q, H, F, H, L, V, I, K, Y, and A; Xaa2 is an amino acid selected from the group consisting of S, A, T, Q, and V; Xaa3 is an amino acid selected from the group consisting of Y, W, and S; Xaa4 is an amino acid selected from the group consisting of A, D, G, S, H and Y; Xaa5 is an amino acid selected from the group consisting of G, S, N, P, D, V, and T; Xaa6 is an amino acid selected from the group consisting of I, T, S, G, L, F and Y; Xaa7 is an amino acid selected from the group consisting of D, T, L, I, P, and S; Xaa8 is an amino acid selected from the group consisting of T, G, R, Y, D, N, W, L, F and P; Xaa9 is an amino acid selected from the group consisting of L, V, G, T, and H; Xaa10 is an amino acid selected from the group consisting of Val, Tyr, and His; Xaa11 is Leu or Val.

[0037] In another aspect, the present invention is directed to a pharmaceutical composition that comprises the herein described antibody, antibody fragment, or mixture or derivative thereof.

[0037a] In another aspect there is provided use of a therapeutically, effective amount of the herein described antibody in the manufacture of a medicament for treating, preventing, modulating, or attenuating a disease or disorder associated with neurite degeneration.

[0038] In another aspect, the present invention is directed to a method of treating, preventing, modulating, or attenuating a disease or disorder associated with neurite degeneration, comprising administering to a subject in need thereof a therapeutically effective amount of the herein described antibody. The neurite degenerative disorder may be multiple sclerosis, Parkinson's disease; Alzheimer's disease; Huntington's disease; amyotrophic lateral sclerosis and other motoneuron diseases; Tay-Sachs disease; Niemann-Pick disease; Gaucher's disease; Hurler's syndrome; idiopathic inflammatory demyelinating diseases; vitamin B12 deficiency; central pontine myelinolysis; tabes dorsalis; transverse myelitis; Devic's disease, progressive multifocal leukoencephalopathy; optic neuritis; traumatic injury to the CNS; ischemic cerebral stroke; a retinopathy; such as glaucoma, diabetic retinopathy or age-dependent macular degeneration; and a leukodystrophy.

[0039] In another aspect, the present invention is directed to a method for determining whether a subject has a neurite degenerative disorder. The method may comprise measuring the level of RGMa in a sample from the subject; and comparing the level of RGMa in the sample with a normal control. An altered level of RGMa indicates that the subject has a neurite degenerative disorder. An increased level of RGMa as compared to the normal control, indicates that the subject has neurite degenerative disorder. The sample may be a blood sample or a serum sample or a cerebrospinal fluid sample. The step of measuring the level of RGMa in a sample, may be conducted with an immunoassay. The immunoassay may be an enzyme-linked immunosorbent assay (ELISA). The ELISA may be a sandwich ELISA.

[0040] In another aspect, the present invention is directed to an isolated antibody or antibody fragment comprising SEQ ID NOs:1-7 and 67.

[0041] In another aspect, the present invention is directed to an isolated antibody or antibody fragment comprising SEQ ID NOs:1-7 and 68.

[0042] In another aspect, the present invention is directed to an isolated antibody or antibody fragment comprising SEQ ID NOs:1-7 and 69.

[0043] In another aspect, the present invention is directed to an isolated antibody or antibody fragment comprising SEQ ID NOs:1-7 and 70.

[0044] In another aspect, the present invention is directed to an isolated antibody or antibody fragment comprising SEQ ID NOs:1-7 and 71.

[0045] In another aspect, the present invention is directed to an isolated antibody or antibody fragment comprising SEQ ID NOs:1-7 and 72.

[0046] In another aspect, the present invention is directed to an isolated antibody or antibody fragment comprising SEQ ID NOs:1-7 and 73.

In another aspect, the present invention is directed to an isolated monoclonal antibody or antibody fragment comprising SEQ ID NOs:1-7 and 67, 68, 69, 70, 71, 72, or 73, that binds to the RGMa epitope PCKILKCNSEFWSATSGSHAPAS (hRGMa 47-69) (SEQ ID NO:79).

[0047a] In another aspect, there is provided an isolated antibody or antibody fragment thereof which binds to Repulsive Guidance Molecule a ("RGMa"), wherein the antibody or antibody fragment comprises a heavy chain variable region comprising a complementarity determining region (CDR)1 comprising the amino acid sequence of SEQ ID NO:2, a CDR2 comprising the amino acid sequence of SEQ ID NO:3, and a CDR3 comprising the amino acid sequence of SEQ

ID NO:4, and a light chain variable region comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:6, a CDR2 comprising the amino acid sequence of SEQ ID NO:7, and a CDR3 comprising the amino acid sequence of SEQ ID NO:73.

[0047b] In another aspect, there is provided the use of the antibody or antibody fragment as disclosed herein in the manufacture of a medicament for the treatment, prevention, modulation, or attenuation of a disease or disorder associated with neurite degeneration.

[0047c] In another aspect, there is provided a method for determining whether a subject has a neurite degenerative disorder, comprising

(a) measuring the level of RGMa in a sample isolated from the subject, wherein the level of RGMa in the sample is determined using the antibody or antibody fragment as described herein; and

(b) comparing the level of RGMa in the sample with a normal control,

wherein an altered level of RGMa indicates that the subject has a neurite degenerative disorder.

BRIEF DESCRIPTION OF THE DRAWINGS

[0048] Figure 1 shows testing results from a neurite outgrowth assay using 50 μg/ml of a hRGMa fragment. This fragment encompasses the N-terminal amino acids 47-127 of SEQ ID NO:65 (See SEQ ID NO:139) and contains both high affinity neogenin- and the bone morphogenetic protein- interaction domains.

[0049] Figure 2 shows testing results from neurite outgrowth assay using 50 μg/ml of full length hRGMa.

[0050] Figure 3 shows a bar chart that is reflective of the level of in vivo regenerative growth of retinal ganglion cells axons perilesionally (0-500 μm) in the presence of antibody AE12-1.

[0051] Figure 4 shows a bar chart that is reflective of the level of in vivo regenerative growth of retinal ganglion cell axons (500-1000 μm) in the presence of antibody AE12-1 in direct comparison with human 5F9.23.

[0052] Figure 5 shows MS spectra from the reduced E1 fraction of trypsin/Asp-N excised E. coli hRGMa with AE12-1 mAb showing the +3 and +4 charge states (boxed) of two peptides corresponding to the sequences (SEQ ID NOS 74 and 80, respectively, in order of appearance) shown on the spectra. The peaks labeled with * are peptides that could not be assigned to the hRGMa antigen and may be related to the antibody.

[0053] Figure 6 shows MS (top) and MS/MS (bottom) spectra from denatured, reduced E1 fraction of hRGMa (MYC construct) with AE12-1 mAb excised with trypsin and Asp-N, confirming the sequence of the excised peptide as KAGSPCKILKCNSEFWSATSGSHAPAS (SEQ ID NO:81).

[0054] Figure 7 shows MS (top) and MS/MS (bottom) spectra from denatured, reduced E1 fraction of hRGMa (MYC construct) with AE12-1 mAb excised with trypsin and Asp-N, confirming the sequence of the excised peptide as AGSPCKILKCNSEFWSATSGSHAPAS (SEQ ID NO:89).

[0055] Figure 8 shows micrographs of nerve lesions in rats treated with a control antibody (hIgG at 10 mg/kg; see top panel), AE12-1 antibody at 1 mg/kg (see middle panel), or h5F9.23 antibody at 1 mg/kg (see bottom panel).

[0056] Figure 9 shows the results of an analysis of neurite outgrowth of SH-SY5Y cells on 96 well plates coated with fibronectin as a substrate after treatment with full length human RGMa and its neutralization by AE12-1 (left bar graph) and AE12-1-H (right bar graph). Antibody and full length hRGMa were added at the same time and subsequently cultures were incubated for 24 hours.

[0057] Figure 10 shows the results of an analysis of neurite outgrowth of SH-SY5Y cells on 96 well plates coated with fibronectin as a substrate after treatment with full length human RGMa and its neutralization by AE12-1-K (left bar graph) and AE12-1-F (right bar graph). Antibody and full length hRGMa wre added at the same time and subsequently cultures were incubated for 24 hours.

[0058] Figure 11 shows the results of an analysis of neurite outgrowth of SH-SY5Y cells on 96 well plates coated with fibbronectin as a substrate after treatment with full length human RGMa and its neutralization by AE-12-1-I (left bar graph) and AE-12-L (right bar graph). Antibody and full length hRGMa were added at the same time and the cultures were subsequently incubated for 24 hours.

[0059] Figure 12 shows the results of an analysis of neurite outgrowth of SH-SY5Y cells on 96 well plates coated with fibronectin as a substrate after treatment with full length human RGMa and its neutralization by AE12-1-V (left bar graph) and AE-12-1-Y (right bar graph). Antibody and full length hRGMa were added at the same time and the cultures were subsequently incubated for 24 hours.

[0060] Figure 13 shows the results of an RGMa binding assay on SH-SY5Y cells and primary neurons (HCA High Content Analysis) using AE12-6, AE12-15 and AE12-23.

[0061] Figure 14 shows the results of an RGMa binding inhibition assay on SH-SY5Y cells (via High Content Analysis (HCA)) using AE12-1 and the AE12-1 cysteine variants.

[0062] Figure 15 shows the neutralizing effect of AE12-1 and AE12-6 on RGMa neurite outgrowth repulsion in a neurite outgrowth assay on rat hippocampal primary neurons. r5F9 antibody is used as a control.

[0063] Figure 16 shows that three (3) RGMa selective monoclonal antibodies, specifically, AE12-1, AE12-1Y and h5F9.23, induce massive regeneration of GAP-43 positive fibers beyond the crush site as described in Example 9. Y-axis = number of axon bundles in the area 0-500 µm beyond crush site. *** $p < 0.001$: significance versus hIgG, ** $p < 0.01$: significance versus hIgG, * $p < 0.05$: significance versus human IgG.

[0064] Figure 17 shows that three (3) RGMa selective monoclonal antibodies, specifically, AE12-1, AE12-1Y and h5F9.23, increase the number of retinal axonal bundles, thereby protecting the retinal nerve fiber layer. Y-axis = number of fiber bundles in the retina as described in Example 10. ** $p < 0.01$: significance versus hIgG, * $p < 0.05$: significance versus hIgG.

[0065] Figure 18 shows that the RGMa mAbs AE12-1, AE12-1Y and h5F9.23 accelerate functional recovery in the spinal tEAE model as described in Example 11. Antibody treatment (iv) was started approximately one week after cytokine injection and was repeated once per week. Doses given were 10 mg/kg. *** $p < 0.001$: significance versus hIgG, ** $p < 0.01$: significance versus hIgG, * $p < 0.05$: significance versus hIgG.

[0066] Figure 19 shows that the RGMa mAbs AE12-1, AE12-1Y and ABT-207 (h5F9.23) increase GAP-43 and MBP area and decrease the inflammatory lesion in direct comparion with the human IgG control antibody as described in Example 11. *** $p < 0.001$: significance versus hIgG, ** $p < 0.01$: significance versus hIgG, * $p < 0.05$: significance versus hIgG.

[0067] Figure 20 shows that the RGMa mAb AE12-1Y-QL accelerated functional recovery in the spinal tEAE model at three different doses 0.1; 1 and 10 mg/kg given once per week intravenously as described in Example 11. Antibody treatment (iv) was started approximately one week after cytokine injection and was repeated once per week. *** $p < 0.001$: significance versus hIgG, ** $p < 0.01$: significance versus hIgG, * $p < 0.05$: significance versus hIgG.

DETAILED DESCRIPTION

[0068] The inventors have discovered new antibodies that bind to Repulsive Guidance Molecule a ("RGMa") and may be used to treat diseases related to neurite degeneration. Provided herein are specific and non-specific antibodies that are capable of attenuating clinical signs associated with diseases related to neurite degeneration.

1. Definitions

[0069] The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting. As used in the specification and the appended claims, the singular forms "a," "and" and "the" include plural references unless the context clearly dictates otherwise.

a. About

[0070] "About" as used herein may refer to approximately a +/- 10% variation from the stated value. It is to be understood that such a variation is always included in any given value provided herein, whether or not specific reference is made to it.

b. Affinity Matured Antibody

[0071] "Affinity Matured Antibody" is used herein to refer to an antibody with one or more alterations in one or more CDRs, which result in an improvement in the affinity (i.e. K_D, k_d or k_a) of the antibody for a target antigen compared to a parent antibody, which does not possess the alteration(s). Exemplary affinity matured antibodies will have nanomolar or even picomolar affinities for the target antigen. A variety of procedures for producing affinity matured antibodies are known in the art, including the screening of a combinatory antibody library that has been prepared using bio-display. For example, Marks et al., BioTechnology, 10: 779-783 (1992) describes affinity maturation by VH and VL domain shuffling. Random mutagenesis of CDR and/or framework residues is described by Barbas et al., Proc. Nat. Acad. Sci. USA, 91: 3809-3813 (1994); Schier et al., Gene, 169: 147-155 (1995); Yelton et al., J. Immunol., 155: 1994-2004 (1995); Jackson et al., J. Immunol., 154(7): 3310-3319 (1995); and Hawkins et al, J. Mol. Biol., 226: 889-896 (1992). Selective mutation at selective mutagenesis positions and at contact or hypermutation positions with an activity-enhancing amino acid residue is described in U.S. Pat. No. 6,914,128 B1.

c. Antibody and Antibodies

[0072] "Antibody" and "antibodies" as used herein refers to monoclonal antibodies, multispecific antibodies, human antibodies, humanized antibodies (fully or partially humanized), animal antibodies such as, but not limited to, a bird (for example, a duck or a goose), a shark, a whale, and a mammal, including a non-primate (for example, a cow, a pig, a camel, a llama, a horse, a goat, a rabbit, a sheep, a hamster, a guinea pig, a cat, a dog, a rat, a mouse, etc.) or a non-human primate (for example, a monkey, a chimpanzee, etc.), recombinant antibodies, chimeric antibodies, single-chain Fvs ("scFv"), single chain antibodies, single domain antibodies, Fab fragments, F(ab') fragments, $F(ab')_2$ fragments, disulfide-linked Fvs ("sdFv"), and anti-idiotypic ("anti-Id") antibodies, dual-domain antibodies, dual variable domain (DVD) or triple variable domain (TVD) antibodies (dual-variable domain immunoglobulins and methods for making them are described in Wu, C., et al., Nature Biotechnology, 25(11):1290-1297 (2007) and PCT International Application WO 2001/058956, the contents of each of which are herein incorporated by reference), and functionally active epitope-binding fragments of any of the above. In particular, antibodies include immunoglobulin molecules and immunologically active fragments of immunoglobulin molecules, namely, molecules that contain an analyte-binding site. Immunoglobulin molecules can be of any type (for example, IgG, IgE, IgM, IgD, IgA and IgY), class (for example, IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass. For simplicity sake, an antibody against an analyte is frequently referred to herein as being either an "anti-analyte antibody," or merely an "analyte antibody" (e.g., an anti-RGMa antibody or an RGMa antibody).

d. Antibody Fragment

[0073] "Antibody fragment" as used herein refers to a portion of an intact antibody comprising the antigen-binding site or variable region. The portion does not include the constant heavy chain domains (i.e. CH2, CH3 or CH4, depending on the antibody isotype) of the Fc region of the intact antibody. Examples of antibody fragments include, but are not limited to, Fab fragments, Fab' fragments, Fab'-SH fragments, $F(ab')_2$ fragments, Fd fragments, Fv fragments, diabodies, single-chain Fv (scFv) molecules, single-chain polypeptides containing only one light chain variable domain, single-chain polypeptides containing the three CDRs of the light-chain variable domain, single-chain polypeptides containing only one heavy chain variable region, and single-chain polypeptides containing the three CDRs of the heavy chain variable region.

e. **Binding Constants**

[0074] "Binding Constants" are described herein. The term "association rate constant," "k_{on}" or "k_a" as used herein, refers to the value indicating the binding rate of an antibody to its target antigen or the rate of complex formation between an antibody and antigen as shown by the equation below:

Antibody (Ab) + Antigen (Ag) $\rightarrow$ Ab-Ag.

[0075] The term "dissociation rate constant," "k_{off}" or "k_d" as used interchangeably herein, refers to the value indicating the dissociation rate of an antibody from its target antigen or separation of Ab-Ag complex over time into free antibody and antigen as shown by the equation below:

Antibody (Ab) + Antigen (Ag) $\leftarrow$ Ab-Ag.

[0076] Methods for determining association and dissociation rate constants are well known in the art. Using fluorescence-based techniques offers high sensitivity and the ability to examine samples in physiological buffers at equilibrium. Other experimental approaches and instruments such as a BIAcore® (biomolecular interaction analysis) assay can be used (e.g., instrument available from BIAcore International AB, a GE Healthcare company, Uppsala, Sweden). Additionally, a KinExA® (Kinetic Exclusion Assay) assay, available from Sapidyne Instruments (Boise, Idaho) can also be used.

[0077] The term "equilibrium dissociation constant" or "K_D" as used interchangeably, herein, refers to the value obtained by dividing the dissociation rate (k_{off}) by the association rate (k_{on}). The association rate, the dissociation rate and the equilibrium dissociation constant are used to represent the binding affinity of an antibody to an antigen.

f. **Binding Protein**

[0078] "Binding Protein" is used herein to refer to a monomeric or multimeric protein that binds to and forms a complex with a binding partner, such as, for example, a polypeptide, an antigen, a chemical compound or other molecule, or a substrate of any kind. A binding protein specifically binds a binding partner. Binding proteins include antibodies, as well as antigen-binding fragments thereof and other various forms and derivatives thereof as are known in the art and described herein below, and other molecules comprising one or more antigen-binding domains that bind to an antigen molecule or a particular site (epitope) on the antigen molecule.

Accordingly, a binding protein includes, but is not limited to, an antibody, a tetrameric immunoglobulin, an IgG molecule, an IgG_1 molecule, a monoclonal antibody, a chimeric antibody, a CDR-grafted antibody, a humanized antibody, an affinity matured antibody, and fragments of any such antibodies that retain the ability to bind to an antigen.

g. Bispecific Antibody

[0079] "Bispecific antibody" is used herein to refer to a full-length antibody that is generated by quadroma technology (see Milstein et al., Nature, 305(5934): 537-540 (1983)), by chemical conjugation of two different monoclonal antibodies (see, Staerz et al., Nature, 314(6012): 628-631 (1985)), or by knob-into-hole or similar approaches, which introduce mutations in the Fc region (see Holliger et al., Proc. Natl. Acad. Sci. USA, 90(14): 6444-6448 (1993)), resulting in multiple different immunoglobulin species of which only one is the functional bispecific antibody. A bispecific antibody binds one antigen (or epitope) on one of its two binding arms (one pair of HC/LC), and binds a different antigen (or epitope) on its second arm (a different pair of HC/LC). By this definition, a bispecific antibody has two distinct antigen-binding arms (in both specificity and CDR sequences), and is monovalent for each antigen to which it binds.

h. CDR

[0080] "CDR" is used herein to refer to the "complementarity determining region" within an antibody variable sequence. There are three CDRs in each of the variable regions of the heavy chain and the light chain, which are designated "CDR1", "CDR2", and "CDR3", for each of the variable regions. The term "CDR set" as used herein refers to a group of three CDRs that occur in a single variable region that binds the antigen. The exact boundaries of these CDRs have been defined differently according to different systems. The system described by Kabat (Kabat et al., Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, Md. (1987) and (1991)) not only provides an unambiguous residue numbering system applicable to any variable region of an antibody, but also provides precise residue boundaries defining the three CDRs. These CDRs may be referred to as "Kabat CDRs". Chothia and coworkers (Chothia and Lesk, J. Mol. Biol., 196: 901-917 (1987); and Chothia et al., Nature, 342: 877-883 (1989)) found that certain sub-portions within Kabat CDRs adopt nearly identical peptide backbone conformations, despite having great diversity at the level of amino acid sequence. These sub-portions were designated as "L1", "L2", and "L3", or "H1", "H2", and "H3", where the "L" and the "H" designate the light chain and the heavy chain regions, respectively. These regions may be

referred to as "Chothia CDRs", which have boundaries that overlap with Kabat CDRs. Other boundaries defining CDRs overlapping with the Kabat CDRs have been described by Padlan, FASEB J., 9: 133-139 (1995), and MacCallum, J. Mol. Biol., 262(5): 732-745 (1996). Still other CDR boundary definitions may not strictly follow one of the herein systems, but will nonetheless overlap with the Kabat CDRs, although they may be shortened or lengthened in light of prediction or experimental findings that particular residues or groups of residues or even entire CDRs do not significantly impact antigen binding. The methods used herein may utilize CDRs defined according to any of these systems, although certain embodiments use Kabat- or Chothia-defined CDRs.

i. **Component or Components**

[0081] "Component," "components," or "at least one component," refer generally to a capture antibody, a detection or conjugate calibrator, a control, a sensitivity panel, a container, a buffer, a diluent, a salt, an enzyme, a co-factor for an enzyme, a detection reagent, a pretreatment reagent/solution, a substrate (e.g., as a solution), a stop solution, and the like that can be included in a kit for assay of a test sample, such as a patient urine, serum or plasma sample, in accordance with the methods described herein and other methods known in the art. Some components can be in solution or lyophilized for reconstitution for use in an assay.

j. **Consensus or Consensus Sequence**

[0082] "Consensus" or "Consensus Sequence" as used herein refers to a synthetic nucleic acid sequence, or corresponding polypeptide sequence, constructed based on analysis of an alignment of multiple subtypes of a particular antigen. The sequence may be used to induce broad immunity against multiple subtypes or serotypes of a particular antigen. Synthetic antigens, such as fusion proteins, may be manipulated to consensus sequences (or consensus antigens).

k. **Control**

[0083] "Control" as used herein refers to a composition known to not contain an analyte of interest ("negative"), e.g., RGMa (such as membrane-associated RGMa, soluble RGMa, fragments of membrane-associated RGMa, fragments of soluble RGMa, variants of RGMa (membrane-associated or soluble RGMa) or any combinations thereof), or to contain an analyte of interest ("positive control"), e.g., RGMa (such as membrane-associated RGMa, soluble RGMa, fragments of membrane-associated RGMa, fragments of soluble RGMa, variants of

RGMa (membrane-associated or soluble RGMa) or any combinations thereof). A positive control can comprise a known concentration of RGMa. "Control," "positive control," and "calibrator" may be used interchangeably herein to refer to a composition comprising a known concentration of RGMa. A "positive control" can be used to establish assay performance characteristics and is a useful indicator of the integrity of reagents (e.g., analytes). A "normal control" may refer to a sample or a subject that is free from an iron-related disease or disorder.

l. Derivative

[0084] "Derivative" of an antibody as used herein may refer to an antibody having one or more modifications to its amino acid sequence when compared to a genuine or parent antibody and exhibit a modified domain structure. The derivative may still be able to adopt the typical domain configuration found in native antibodies, as well as an amino acid sequence, which is able to bind to targets (antigens) with specificity. Typical examples of antibody derivatives are antibodies coupled to other polypeptides, rearranged antibody domains or fragments of antibodies. The derivative may also comprise at least one further compound, e.g. a protein domain, said protein domain being linked by covalent or non-covalent bonds. The linkage can be based on genetic fusion according to the methods known in the art. The additional domain present in the fusion protein comprising the antibody employed in accordance with the invention may preferably be linked by a flexible linker, advantageously a peptide linker, wherein said peptide linker comprises plural, hydrophilic, peptide-bonded amino acids of a length sufficient to span the distance between the C-terminal end of the further protein domain and the N-terminal end of the antibody or vice versa. The antibody may be linked to an effector molecule having a conformation suitable for biological activity or selective binding to a solid support, a biologically active substance (e.g. a cytokine or growth hormone), a chemical agent, a peptide, a protein or a drug, for example.

m. Dual-Specific Antibody

[0085] "Dual-specific antibody" is used herein to refer to a full-length antibody that can bind two different antigens (or epitopes) in each of its two binding arms (a pair of HC/LC) (see PCT publication WO 02/02773). Accordingly a dual-specific binding protein has two identical antigen binding arms, with identical specificity and identical CDR sequences, and is bivalent for each antigen to which it binds.

n. Dual Variable Domain

[0086] "Dual variable domain" is used herein to refer to two or more antigen binding sites on a binding protein, which may be divalent (two antigen binding sites), tetravalent (four antigen binding sites), or multivalent binding proteins. DVDs may be monospecific, i.e., capable of binding one antigen (or one specific epitope), or multispecific, i.e., capable of binding two or more antigens (i.e., two or more epitopes of the same target antigen molecule or two or more epitopes of different target antigens). A preferred DVD binding protein comprises two heavy chain DVD polypeptides and two light chain DVD polypeptides and is referred to as a "DVD immunoglobulin" or "DVD-Ig". Such a DVD-Ig binding protein is thus tetrameric and reminiscent of an IgG molecule, but provides more antigen binding sites than an IgG molecule. Thus, each half of a tetrameric DVD-Ig molecule is reminiscent of one half of an IgG molecule and comprises a heavy chain DVD polypeptide and a light chain DVD polypeptide, but unlike a pair of heavy and light chains of an IgG molecule that provides a single antigen binding domain, a pair of heavy and light chains of a DVD-Ig provide two or more antigen binding sites.

[0087] Each antigen binding site of a DVD-Ig binding protein may be derived from a donor ("parental") monoclonal antibody and thus comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) with a total of six CDRs involved in antigen binding per antigen binding site. Accordingly, a DVD-Ig binding protein that binds two different epitopes (i.e., two different epitopes of two different antigen molecules or two different epitopes of the same antigen molecule) comprises an antigen binding site derived from a first parental monoclonal antibody and an antigen binding site of a second parental monoclonal antibody.

[0088] A description of the design, expression, and characterization of DVD-Ig binding molecules is provided in PCT Publication No. WO 2007/024715, U.S. Pat. No. 7,612,181, and Wu et al., Nature Biotech., 25: 1290-1297 (2007). A preferred example of such DVD-Ig molecules comprises a heavy chain that comprises the structural formula VD1-(X1)n-VD2-C-(X2)n, wherein VD1 is a first heavy chain variable domain, VD2 is a second heavy chain variable domain, C is a heavy chain constant domain, X1 is a linker with the proviso that it is not CH1, X2 is an Fc region, and n is 0 or 1, but preferably 1; and a light chain that comprises the structural formula VD1-(X1)n-VD2-C-(X2)n, wherein VD1 is a first light chain variable domain, VD2 is a second light chain variable domain, C is a light chain constant domain, X1 is a linker with the proviso that it is not CH1, and X2 does not comprise an Fc region; and n is 0 or 1, but

preferably 1. Such a DVD-Ig may comprise two such heavy chains and two such light chains, wherein each chain comprises variable domains linked in tandem without an intervening constant region between variable regions, wherein a heavy chain and a light chain associate to form tandem functional antigen binding sites, and a pair of heavy and light chains may associate with another pair of heavy and light chains to form a tetrameric binding protein with four functional antigen binding sites. In another example, a DVD-Ig molecule may comprise heavy and light chains that each comprise three variable domains (VD1, VD2, VD3) linked in tandem without an intervening constant region between variable domains, wherein a pair of heavy and light chains may associate to form three antigen binding sites, and wherein a pair of heavy and light chains may associate with another pair of heavy and light chains to form a tetrameric binding protein with six antigen binding sites.

[0089] In a preferred embodiment, a DVD-Ig binding protein according to the invention not only binds the same target molecules bound by its parental monoclonal antibodies, but also possesses one or more desirable properties of one or more of its parental monoclonal antibodies. Preferably, such an additional property is an antibody parameter of one or more of the parental monoclonal antibodies. Antibody parameters that may be contributed to a DVD-Ig binding protein from one or more of its parental monoclonal antibodies include, but are not limited to, antigen specificity, antigen affinity, potency, biological function, epitope recognition, protein stability, protein solubility, production efficiency, immunogenicity, pharmacokinetics, bioavailability, tissue cross reactivity, and orthologous antigen binding.

[0090] A DVD-Ig binding protein binds at least one epitope of RGMa. Non-limiting examples of a DVD-Ig binding protein include a DVD-Ig binding protein that binds one or more epitopes of RGMa, a DVD-Ig binding protein that binds an epitope of a human RGMa and an epitope of a RGMa of another species (for example, mouse), and a DVD-Ig binding protein that binds an epitope of a human RGMa and an epitope of another target molecule (for example, VEGFR2 or VEGFR1).

o. Epitope or Epitopes

[0091] "Epitope," or "epitopes," or "epitopes of interest" refer to a site(s) on any molecule that is recognized and can bind to a complementary site(s) on its specific binding partner. The molecule and specific binding partner are part of a specific binding pair. For example, an

epitope can be on a polypeptide, a protein, a hapten, a carbohydrate antigen (such as, but not limited to, glycolipids, glycoproteins or lipopolysaccharides), or a polysaccharide. Its specific binding partner can be, but is not limited to, an antibody.

p. **Framework or Framework Sequence**

[0092] "Framework" (FR) or "Framework sequence" as used herein may mean the remaining sequences of a variable region minus the CDRs. Because the exact definition of a CDR sequence can be determined by different systems (for example, see above), the meaning of a framework sequence is subject to correspondingly different interpretations. The six CDRs (CDR-L1, -L2, and -L3 of light chain and CDR-H1, -H2, and -H3 of heavy chain) also divide the framework regions on the light chain and the heavy chain into four sub-regions (FR1, FR2, FR3, and FR4) on each chain, in which CDR1 is positioned between FR1 and FR2, CDR2 between FR2 and FR3, and CDR3 between FR3 and FR4. Without specifying the particular sub-regions as FR1, FR2, FR3, or FR4, a framework region, as referred by others, represents the combined FRs within the variable region of a single, naturally occurring immunoglobulin chain. As used herein, a FR represents one of the four sub-regions, and FRs represents two or more of the four sub-regions constituting a framework region.

[0093] Human heavy chain and light chain FR sequences are known in the art that can be used as heavy chain and light chain "acceptor" framework sequences (or simply, "acceptor" sequences) to humanize a non-human antibody using techniques known in the art. In one embodiment, human heavy chain and light chain acceptor sequences are selected from the framework sequences listed in publicly available databases such as V-base (hypertext transfer protocol://vbase.mrc-cpe.cam.ac.uk/) or in the international ImMunoGeneTics® (IMGT®) information system (hypertext transfer protocol://imgt.cines.fr/texts/IMGTrepertoire/LocusGenes/).

q. **Functional Antigen Binding Site**

[0094] "Functional antigen binding site" as used herein may mean a site on a binding protein (e.g. an antibody) that is capable of binding a target antigen. The antigen binding affinity of the antigen binding site may not be as strong as the parent binding protein, e.g., parent antibody, from which the antigen binding site is derived, but the ability to bind antigen must be measurable using any one of a variety of methods known for evaluating protein, e.g., antibody, binding to an

antigen. Moreover, the antigen binding affinity of each of the antigen binding sites of a multivalent protein, e.g., multivalent antibody, herein need not be quantitatively the same.

r. Human Antibody

[0095] "Human antibody" as used herein may include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis in vitro or by somatic mutation in vivo). However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

s. Humanized Antibody

[0096] "Humanized antibody" is used herein to describe an antibody that comprises heavy and light chain variable region sequences from a non-human species (e.g. a mouse) but in which at least a portion of the VH and/or VL sequence has been altered to be more "human-like," i.e., more similar to human germline variable sequences. A "humanized antibody" is an antibody or a variant, derivative, analog, or fragment thereof, which immunospecifically binds to an antigen of interest and which comprises a framework (FR) region having substantially the amino acid sequence of a human antibody and a complementary determining region (CDR) having substantially the amino acid sequence of a non-human antibody. As used herein, the term "substantially" in the context of a CDR refers to a CDR having an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of a non-human antibody CDR. A humanized antibody comprises substantially all of at least one, and typically two, variable domains (Fab, Fab', $F(ab')_2$, FabC, Fv) in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin (i.e., donor antibody) and all or substantially all of the framework regions are those of a human immunoglobulin consensus sequence. In an embodiment, a humanized antibody also comprises at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. In some embodiments, a humanized antibody contains the light chain as well as at least the variable domain of a heavy chain. The antibody also may include the CH1, hinge, CH2, CH3, and CH4 regions of the heavy chain. In some embodiments, a humanized antibody only contains a humanized light chain. In some embodiments, a humanized antibody only

contains a humanized heavy chain. In specific embodiments, a humanized antibody only contains a humanized variable domain of a light chain and/or humanized heavy chain.

[0097] A humanized antibody can be selected from any class of immunoglobulins, including IgM, IgG, IgD, IgA, and IgE, and any isotype, including without limitation IgG1, IgG2, IgG3, and IgG4. A humanized antibody may comprise sequences from more than one class or isotype, and particular constant domains may be selected to optimize desired effector functions using techniques well-known in the art.

[0098] The framework regions and CDRs of a humanized antibody need not correspond precisely to the parental sequences, e.g., the donor antibody CDR or the consensus framework may be mutagenized by substitution, insertion, and/or deletion of at least one amino acid residue so that the CDR or framework residue at that site does not correspond to either the donor antibody or the consensus framework. In a preferred embodiment, such mutations, however, will not be extensive. Usually, at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% of the humanized antibody residues will correspond to those of the parental FR and CDR sequences. As used herein, the term "consensus framework" refers to the framework region in the consensus immunoglobulin sequence. As used herein, the term "consensus immunoglobulin sequence" refers to the sequence formed from the most frequently occurring amino acids (or nucleotides) in a family of related immunoglobulin sequences (see, e.g., Winnaker, From Genes to Clones (Verlagsgesellschaft, Weinheim, 1987)). A "consensus immunoglobulin sequence" may thus comprise a "consensus framework region(s)" and/or a "consensus CDR(s)". In a family of immunoglobulins, each position in the consensus sequence is occupied by the amino acid occurring most frequently at that position in the family. If two amino acids occur equally frequently, either can be included in the consensus sequence.

t. **Identical or Identity**

[0099] "Identical" or "identity," as used herein in the context of two or more polypeptide or polynucleotide sequences, can mean that the sequences have a specified percentage of residues that are the same over a specified region. The percentage can be calculated by optimally aligning the two sequences, comparing the two sequences over the specified region, determining the number of positions at which the identical residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of

positions in the specified region, and multiplying the result by 100 to yield the percentage of sequence identity. In cases where the two sequences are of different lengths or the alignment produces one or more staggered ends and the specified region of comparison includes only a single sequence, the residues of the single sequence are included in the denominator but not the numerator of the calculation.

u. Isolated Polynucleotide

[00100] "Isolated polynucleotide" as used herein may mean a polynucleotide (e.g. of genomic, cDNA, or synthetic origin, or a combination thereof) that, by virtue of its origin, the isolated polynucleotide is not associated with all or a portion of a polynucleotide with which the "isolated polynucleotide" is found in nature; is operably linked to a polynucleotide that it is not linked to in nature; or does not occur in nature as part of a larger sequence.

v. Label and Detectable Label

[00101] "Label" and "detectable label" as used herein refer to a moiety attached to an antibody or an analyte to render the reaction between the antibody and the analyte detectable, and the antibody or analyte so labeled is referred to as "detectably labeled." A label can produce a signal that is detectable by visual or instrumental means. Various labels include signal-producing substances, such as chromogens, fluorescent compounds, chemiluminescent compounds, radioactive compounds, and the like. Representative examples of labels include moieties that produce light, e.g., acridinium compounds, and moieties that produce fluorescence, e.g., fluorescein. Other labels are described herein. In this regard, the moiety, itself, may not be detectable but may become detectable upon reaction with yet another moiety. Use of the term "detectably labeled" is intended to encompass such labeling.

[0100] Any suitable detectable label as is known in the art can be used. For example, the detectable label can be a radioactive label (such as ^{3}H, ^{125}I, ^{35}S, ^{14}C, ^{32}P, and ^{33}P), an enzymatic label (such as horseradish peroxidase, alkaline peroxidase, glucose 6-phosphate dehydrogenase, and the like), a chemiluminescent label (such as acridinium esters, thioesters, or sulfonamides; luminol, isoluminol, phenanthridinium esters, and the like), a fluorescent label (such as fluorescein (e.g., 5-fluorescein, 6-carboxyfluorescein, 3'6-carboxyfluorescein, 5(6)-carboxyfluorescein, 6-hexachloro-fluorescein, 6-tetrachlorofluorescein, fluorescein isothiocyanate, and the like)), rhodamine, phycobiliproteins, R-phycoerythrin, quantum dots

(e.g., zinc sulfide-capped cadmium selenide), a thermometric label, or an immuno-polymerase chain reaction label. An introduction to labels, labeling procedures and detection of labels is found in Polak and Van Noorden, *Introduction to Immunocytochemistry,* 2nd ed., Springer Verlag, N.Y. (1997), and in Haugland, *Handbook of Fluorescent Probes and Research Chemicals* (1996), which is a combined handbook and catalogue published by Molecular Probes, Inc., Eugene, Oregon. A fluorescent label can be used in FPIA (see, e.g., U.S. Patent Nos. 5,593,896, 5,573,904, 5,496,925, 5,359,093, and 5,352,803, which are hereby incorporated by reference in their entireties). An acridinium compound can be used as a detectable label in a homogeneous chemiluminescent assay (see, e.g., Adamczyk et al., Bioorg. Med. Chem. Lett. 16: 1324-1328 (2006); Adamczyk et al., Bioorg. Med. Chem. Lett. 4: 2313-2317 (2004); Adamczyk et al., Biorg. Med. Chem. Lett. 14: 3917-3921 (2004); and Adamczyk et al., Org. Lett. 5: 3779-3782 (2003)).

[0101] In one aspect, the acridinium compound is an acridinium-9-carboxamide. Methods for preparing acridinium 9-carboxamides are described in Mattingly, J. Biolumin. Chemilumin. 6: 107-114 (1991); Adamczyk et al., J. Org. Chem. 63: 5636-5639 (1998); Adamczyk et al., Tetrahedron 55: 10899-10914 (1999); Adamczyk et al., Org. Lett. 1: 779-781 (1999); Adamczyk et al., Bioconjugate Chem. 11: 714-724 (2000); Mattingly et al., In *Luminescence Biotechnology: Instruments and Applications;* Dyke, K. V. Ed.; CRC Press: Boca Raton, pp. 77–105 (2002); Adamczyk et al., Org. Lett. 5: 3779-3782 (2003); and U.S. Pat. Nos. 5,468,646, 5,543,524 and 5,783,699 (each of which is incorporated herein by reference in its entirety for its teachings regarding same).

[0102] Another example of an acridinium compound is an acridinium-9-carboxylate aryl ester. An example of an acridinium-9-carboxylate aryl ester of formula II is 10-methyl-9-(phenoxycarbonyl)acridinium fluorosulfonate (available from Cayman Chemical, Ann Arbor, MI). Methods for preparing acridinium 9-carboxylate aryl esters are described in McCapra et al., Photochem. Photobiol. 4: 1111-21 (1965); Razavi et al., Luminescence 15: 245-249 (2000); Razavi et al., Luminescence 15: 239-244 (2000); and U.S. Patent No. 5,241,070 (each of which is incorporated herein by reference in its entirety for its teachings regarding same). Such acridinium-9-carboxylate aryl esters are efficient chemiluminescent indicators for hydrogen peroxide produced in the oxidation of an analyte by at least one oxidase in terms of the intensity of the signal and/or the rapidity of the signal. The course of the chemiluminescent emission for

the acridinium-9-carboxylate aryl ester is completed rapidly, i.e., in under 1 second, while the acridinium-9-carboxamide chemiluminescent emission extends over 2 seconds. Acridinium-9-carboxylate aryl ester, however, loses its chemiluminescent properties in the presence of protein. Therefore, its use requires the absence of protein during signal generation and detection. Methods for separating or removing proteins in the sample are well-known to those skilled in the art and include, but are not limited to, ultrafiltration, extraction, precipitation, dialysis, chromatography, and/or digestion (see, e.g., Wells, *High Throughput Bioanalytical Sample Preparation. Methods and Automation Strategies*, Elsevier (2003)). The amount of protein removed or separated from the test sample can be about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, or about 95%. Further details regarding acridinium-9-carboxylate aryl ester and its use are set forth in U.S. Pat. App. No. 11/697,835, filed April 9, 2007. Acridinium-9-carboxylate aryl esters can be dissolved in any suitable solvent, such as degassed anhydrous *N,N*-dimethylformamide (DMF) or aqueous sodium cholate.

w. Linking Sequence and Linking Peptide Sequence

[0103] "Linking sequence" or "linking peptide sequence" refers to a natural or artificial polypeptide sequence that is connected to one or more polypeptide sequences of interest (e.g., full-length, fragments, etc.). The term "connected" refers to the joining of the linking sequence to the polypeptide sequence of interest. Such polypeptide sequences are preferably joined by one or more peptide bonds. Linking sequences can have a length of from about 4 to about 50 amino acids. Preferably, the length of the linking sequence is from about 6 to about 30 amino acids. Natural linking sequences can be modified by amino acid substitutions, additions, or deletions to create artificial linking sequences. Exemplary linking sequences include, but are not limited to: (i) Histidine (His) tags, such as a 6X His tag (SEQ ID NO: 148), which has an amino acid sequence of HHHHHH (SEQ ID NO:148), are useful as linking sequences to facilitate the isolation and purification of polypeptides and antibodies of interest; (ii) Enterokinase cleavage sites, like His tags, are used in the isolation and purification of proteins and antibodies of interest. Often, enterokinase cleavage sites are used together with His tags in the isolation and purification of proteins and antibodies of interest. Various enterokinase cleavage sites are known in the art. Examples of enterokinase cleavage sites include, but are not limited to, the amino acid sequence of DDDDK (SEQ ID NO:149) and derivatives thereof (e.g., ADDDDK (SEQ ID

NO:150), etc.); (iii) Miscellaneous sequences can be used to link or connect the light and/or heavy chain variable regions of single chain variable region fragments. Examples of other linking sequences can be found in Bird et al., Science 242: 423-426 (1988); Huston et al., PNAS USA 85: 5879-5883 (1988); and McCafferty et al., Nature 348: 552-554 (1990). Linking sequences also can be modified for additional functions, such as attachment of drugs or attachment to solid supports. In the context of the present disclosure, the monoclonal antibody, for example, can contain a linking sequence, such as a His tag, an enterokinase cleavage site, or both.

x. **Multivalent Binding Protein**

[0104] "Multivalent binding protein" is used herein to refer to a binding protein comprising two or more antigen binding sites (also referred to herein as "antigen binding domains"). A multivalent binding protein is preferably engineered to have three or more antigen binding sites, and is generally not a naturally occurring antibody. The term "multispecific binding protein" refers to a binding protein that can bind two or more related or unrelated targets, including a binding protein capable of binding two or more different epitopes of the same target molecule.

y. **Predetermined Cutoff and Predetermined Level**

[0105] "Predetermined cutoff" and "predetermined level" refer generally to an assay cutoff value that is used to assess diagnostic/prognostic/therapeutic efficacy results by comparing the assay results against the predetermined cutoff/level, where the predetermined cutoff/level already has been linked or associated with various clinical parameters (e.g., severity of disease, progression/nonprogression/improvement, etc.). The present disclosure provides exemplary predetermined levels. However, it is well-known that cutoff values may vary depending on the nature of the immunoassay (e.g., antibodies employed, etc.). It further is well within the ordinary skill of one in the art to adapt the disclosure herein for other immunoassays to obtain immunoassay-specific cutoff values for those other immunoassays based on this disclosure. Whereas the precise value of the predetermined cutoff/level may vary between assays, the correlations as described herein should be generally applicable.

z. **Pretreatment Reagent**

[0106] "Pretreatment reagent," e.g., lysis, precipitation and/or solubilization reagent, as used in a diagnostic assay as described herein is one that lyses any cells and/or solubilizes any analyte

that is/are present in a test sample. Pretreatment is not necessary for all samples, as described further herein. Among other things, solubilizing the analyte (i.e., RGMa (such as membrane-associated RGMa, soluble RGMa, fragments of membrane-associated RGMa, fragments of soluble RGMa, variants of RGMa (membrane-associated or soluble RGMa) or any combinations thereof)) entails release of the analyte from any endogenous binding proteins present in the sample. A pretreatment reagent may be homogeneous (not requiring a separation step) or heterogeneous (requiring a separation step). With use of a heterogeneous pretreatment reagent there is removal of any precipitated analyte binding proteins from the test sample prior to proceeding to the next step of the assay. The pretreatment reagent optionally can comprise: (a) one or more solvents and salt, (b) one or more solvents, salt and detergent, (c) detergent, (d) detergent and salt, or (e) any reagent or combination of reagents appropriate for cell lysis and/or solubilization of analyte.

aa. Quality Control Reagents

[0107] "Quality control reagents" in the context of immunoassays and kits described herein, include, but are not limited to, calibrators, controls, and sensitivity panels. A "calibrator" or "standard" typically is used (e.g., one or more, such as a plurality) in order to establish calibration (standard) curves for interpolation of the concentration of an analyte, such as an antibody or an analyte. Alternatively, a single calibrator, which is near a predetermined positive/negative cutoff, can be used. Multiple calibrators (i.e., more than one calibrator or a varying amount of calibrator(s)) can be used in conjunction so as to comprise a "sensitivity panel."

bb. Recombinant Antibody and Recombinant Antibodies

[0108] "Recombinant antibody" and "recombinant antibodies" refer to antibodies prepared by one or more steps, including cloning nucleic acid sequences encoding all or a part of one or more monoclonal antibodies into an appropriate expression vector by recombinant techniques and subsequently expressing the antibody in an appropriate host cell. The terms include, but are not limited to, recombinantly produced monoclonal antibodies, chimeric antibodies, humanized antibodies (fully or partially humanized), multi-specific or multi-valent structures formed from antibody fragments, bifunctional antibodies, heteroconjugate Abs, DVD-Ig®s, and other antibodies as described in (i) herein. (Dual-variable domain immunoglobulins and methods for making them are described in Wu, C., et al., Nature Biotechnology, 25:1290-1297 (2007)). The

term "bifunctional antibody," as used herein, refers to an antibody that comprises a first arm having a specificity for one antigenic site and a second arm having a specificity for a different antigenic site, i.e., the bifunctional antibodies have a dual specificity.

cc. Sample, Test Sample, and Patient Sample

[0109] "Sample," "test sample," and "patient sample" may be used interchangeably herein. The sample, such as a sample of urine, serum, plasma, amniotic fluid, cerebrospinal fluid, placental cells or tissue, endothelial cells, leukocytes, or monocytes, can be used directly as obtained from a patient or can be pre-treated, such as by filtration, distillation, extraction, concentration, centrifugation, inactivation of interfering components, addition of reagents, and the like, to modify the character of the sample in some manner as discussed herein or otherwise as is known in the art.

dd. Series of Calibrating Compositions

[0110] "Series of calibrating compositions" refers to a plurality of compositions comprising a known concentration of Cys-CRGMa, wherein each of the compositions differs from the other compositions in the series by the concentration of Cys-CRGMa.

ee. Solid Phase

[0111] "Solid phase" refers to any material that is insoluble, or can be made insoluble by a subsequent reaction. The solid phase can be chosen for its intrinsic ability to attract and immobilize a capture agent. Alternatively, the solid phase can have affixed thereto a linking agent that has the ability to attract and immobilize the capture agent. The linking agent can, for example, include a charged substance that is oppositely charged with respect to the capture agent itself or to a charged substance conjugated to the capture agent. In general, the linking agent can be any binding partner (preferably specific) that is immobilized on (attached to) the solid phase and that has the ability to immobilize the capture agent through a binding reaction. The linking agent enables the indirect binding of the capture agent to a solid phase material before the performance of the assay or during the performance of the assay. The solid phase can, for example, be plastic, derivatized plastic, magnetic or non-magnetic metal, glass or silicon, including, for example, a test tube, microtiter well, sheet, bead, microparticle, chip, and other configurations known to those of ordinary skill in the art.

ff. Specific Binding

[0112] "Specific binding" or "specifically binding" as used herein may refer to the interaction of an antibody, a protein, or a peptide with a second chemical species, wherein the interaction is dependent upon the presence of a particular structure (e.g., an antigenic determinant or epitope) on the chemical species; for example, an antibody recognizes and binds to a specific protein structure rather than to proteins generally. If an antibody is specific for epitope "A", the presence of a molecule containing epitope A (or free, unlabeled A), in a reaction containing labeled "A" and the antibody, will reduce the amount of labeled A bound to the antibody.

gg. Specific Binding Partner

[0113] "Specific binding partner" is a member of a specific binding pair. A specific binding pair comprises two different molecules, which specifically bind to each other through chemical or physical means. Therefore, in addition to antigen and antibody specific binding pairs of common immunoassays, other specific binding pairs can include biotin and avidin (or streptavidin), carbohydrates and lectins, complementary nucleotide sequences, effector and receptor molecules, cofactors and enzymes, enzymes and enzyme inhibitors, and the like. Furthermore, specific binding pairs can include members that are analogs of the original specific binding members, for example, an analyte-analog. Immunoreactive specific binding members include antigens, antigen fragments, and antibodies, including monoclonal and polyclonal antibodies as well as complexes and fragments thereof, whether isolated or recombinantly produced.

hh. Stringent Conditions

[0114] "Stringent conditions" is used herein to describe hybridization to filter-bound DNA in 6x sodium chloride/sodium citrate (SSC) at about 45°C followed by one or more washes in 0.2xSSC/0.1% SDS at about 50-65°C. The term "under highly stringent conditions", refers to hybridization to filter-bound nucleic acid in 6xSSC at about 45°C followed by one or more washes in 0.1xSSC/0.2% SDS at about 68°C., or under other stringent hybridization conditions. See, for example, Ausubel, F. M. et al., eds., 1989, Current Protocols in Molecular Biology, Vol. I, Green Publishing Associates, Inc. and John Wiley & Sons, Inc., New York at pages 6.3.1-6.3.6 and 2.10.3.

ii. Treat, Treating or Treatment

[0115] "Treat", "treating" or "treatment" are each used interchangeably herein to describe reversing, alleviating, or inhibiting the progress of a disease, or one or more symptoms of such disease, to which such term applies. Depending on the condition of the subject, the term also refers to preventing a disease, and includes preventing the onset of a disease, or preventing the symptoms associated with a disease. A treatment may be either performed in an acute or chronic way. The term also refers to reducing the severity of a disease or symptoms associated with such disease prior to affliction with the disease. Such prevention or reduction of the severity of a disease prior to affliction refers to administration of an antibody or pharmaceutical composition of the present invention to a subject that is not at the time of administration afflicted with the disease. "Preventing" also refers to preventing the recurrence of a disease or of one or more symptoms associated with such disease. "Treatment" and "therapeutically," refer to the act of treating, as "treating" is defined above.

jj. Tracer

[0116] "Tracer" as used herein refers to an analyte or analyte fragment conjugated to a label, such as Cys-CRGMa conjugated to a fluorescein moiety, wherein the analyte conjugated to the label can effectively compete with the analyte for sites on an antibody specific for the analyte.

kk. Variant

[0117] "Variant" is used herein to describe a peptide or polypeptide that differs in amino acid sequence by the insertion, deletion, or conservative substitution of amino acids, but retain at least one biological activity. Representative examples of "biological activity" include the ability to be bound by a specific antibody or to promote an immune response. Variant is also used herein to describe a protein with an amino acid sequence that is substantially identical to a referenced protein with an amino acid sequence that retains at least one biological activity. A conservative substitution of an amino acid, i.e., replacing an amino acid with a different amino acid of similar properties (e.g., hydrophilicity, degree and distribution of charged regions) is recognized in the art as typically involving a minor change. These minor changes can be identified, in part, by considering the hydropathic index of amino acids, as understood in the art. Kyte et al., J. Mol. Biol. 157:105-132 (1982). The hydropathic index of an amino acid is based on a consideration of its hydrophobicity and charge. It is known in the art that amino acids of similar hydropathic

indexes can be substituted and still retain protein function. In one aspect, amino acids having hydropathic indexes of ±2 are substituted. The hydrophilicity of amino acids can also be used to reveal substitutions that would result in proteins retaining biological function. A consideration of the hydrophilicity of amino acids in the context of a peptide permits calculation of the greatest local average hydrophilicity of that peptide, a useful measure that has been reported to correlate well with antigenicity and immunogenicity. U.S. Patent No. 4,554,101, incorporated fully herein by reference. Substitution of amino acids having similar hydrophilicity values can result in peptides retaining biological activity, for example immunogenicity, as is understood in the art. Substitutions may be performed with amino acids having hydrophilicity values within ±2 of each other. Both the hyrophobicity index and the hydrophilicity value of amino acids are influenced by the particular side chain of that amino acid. Consistent with that observation, amino acid substitutions that are compatible with biological function are understood to depend on the relative similarity of the amino acids, and particularly the side chains of those amino acids, as revealed by the hydrophobicity, hydrophilicity, charge, size, and other properties. "Variant" also can be used to refer to an antigenically reactive fragment of an anti-RGMa antibody that differs from the corresponding fragment of anti-RGMa antibody in amino acid sequence but is still antigenically reactive and can compete with the corresponding fragment of anti-RGMa antibody for binding with RGMa. "Variant" also can be used to describe a polypeptide or a fragment thereof that has been differentially processed, such as by proteolysis, phosphorylation, or other post-translational modification, yet retains its antigen reactivity.

II. Vector

[0118] "Vector" is used herein to describe a nucleic acid molecule that can transport another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double-stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors can replicate autonomously in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant

expression vectors" (or simply, "expression vectors"). In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. "Plasmid" and "vector" may be used interchangeably as the plasmid is the most commonly used form of vector. However, other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions, can be used. In this regard, RNA versions of vectors (including RNA viral vectors) may also find use in the context of the present disclosure.

[0119] For the recitation of numeric ranges herein, each intervening number there between with the same degree of precision is explicitly contemplated. For example, for the range of 6-9, the numbers 7 and 8 are contemplated in addition to 6 and 9, and for the range 6.0-7.0, the number 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, and 7.0 are explicitly contemplated.

2. Anti-RGMa Antibodies

[0120] Provided herein are antibodies for use in methods of treating neurite degenerative diseases and disorders. Several of the herein described antibodies have been selected for binding to RGMa, while minimizing or eliminating reactivity with Repulsive Guidance Molecule c ("RGMc"). For example, see Table 4, wherein the PROfusion-derived monoclonal antibody AE12-1 and AE12-1 variants (AE12-1F, AE12-1H, AE12-1L, AE12-1V, AE12-1I, AE12-1K, and AE12-1Y) exhibit RGMa neutralizing activity without (low detection) cross reacting with RGMc. Because antibodies raised against RGMa can often cross-react with RGMc and, at high intravenous doses may result in iron accumulation in hepatocytes, the specific binding of the herein described antibodies for RGMa is of therapeutic benefit. Further, the high selectivity of these antibodies offers large therapeutic dose windows or ranges for treatment.

a. RGMa

[0121] Human RGMa, which can exist as a 450 amino acid protein with a predicted N-terminal signal peptide of 47 amino acids and a C-terminal GPI-attachment signal, was first proposed to regulate the guidance of retinal axons by binding to neogenin, a transmembrane protein that is also a receptor for netrins, which are secreted molecules that play a role in neuronal development and cell survival. In addition to regulating retinal axonal guidance, RGMa has been shown to inhibit axon growth in adult rats. See Yamashita et al., Current Opinion in Neurobiology (2007)17:1-6. Consistent with these mechanisms, RGMa expression increases after an injury to

the spinal cord, during which time inhibition of RGMa enhances axonal growth. See Kitayama et al., PLoS One, (2011) Vol.6 (9), pages 1-9; and Hata et al., J. Cell Biol. (2006)173:47-58. RGMa expression is also upregulated at the lesion site and in scar tissue of humans suffering from focal cerebral ischemia or traumatic brain injury. See Yamashita et al., Current Opinion in Neurobiology (2007)17:1-6; Schwab et al., Arch Neurol (2005)22:2134-2144; and Muramatsu et al., Nat. Medicine (2011) 17:488-94.

[0122] RGMa may have the following amino acid sequence:

MQPPRERLVV TGRAGWMGMG RGAGRSALGF WPTLAFLLCS FPAATSPCKI LKCNSEFWSA TSGSHA PASDDTPEFC AALRSYALCT RRTARTCRGD LAYHSAVHGI EDLMSQHNCS KDGPTSQPRL RTLPPAGDSQ ERSDSPEICH YEKSFHKHSA TPNYTHCGLF GDPHLRTFTD RFQTCKVQGA WPLIDNNYLN VQVTNTPVLP GSAATATSKL TIIFKNFQEC VDQKVYQAEM DELPAAFVDG SKNGGDKHGA NSLKITEKVS GQHVEIQAKY IGTTIVVRQV GRYLTFAVRM PEEVVNAVED WDSQGLYLCL RGCPLNQQID FQAFHTNAEG TGARRLAAAS PAPTAPETFP YETAVAKCKE KLPVEDLYYQ ACVFDLLTTG DVNFTLAAYY ALEDVKMLHS NKDKLHLYER TRDLPGRAAA GLPLAPRPLL GALVPLLALL PVFC (SEQ ID NO:65). The RGMa may be a fragment or variant of SEQ ID NO:65.

[0123] The fragment of RGMa may be between 5 and 425 amino acids, between 10 and 400 amino acids, between 50 and 350 amino acids, between 100 and 300 amino acids, between 150 and 250 amino acids, between 200 and 300 amino acids, or between 75 and 150 amino acids in length. The fragment may comprise a contiguous number of amino acids from SEQ ID NO:65.

[0124] The fragment of RGMa may have the following amino acid sequence:

PCKI LKCNSEFWSA TSGSHA PASDDTPEFC AALRSYALCT RRTARTCRGD LAYHSAVHGI EDLMSQHNCS KDGPTSQPRL RTLPPAGDSQ ERSDSPEICH YEKSFHKHSA TPNYTHCGLF GD (SEQ ID NO:66), which corresponds to amino acids 47-168 of SEQ ID NO:65. The RGMa fragment may be a fragment of SEQ ID NO:66. The RGMa fragment may be a variant of SEQ ID NO:66. The RGMa fragment may have the following RGMa sequence: PCKILKCNSEFWSATSGSHAPAS (SEQ ID NO:74).

[0125] RGMa may exist as a cell membrane bound form and/or as a soluble form.

b. RGMa – Recognizing Antibody

[0126] The antibody is an antibody that binds to RGMa, a fragment thereof, or a variant thereof. The antibody may be a fragment of the anti-RGMa antibody or a variant or a derivative thereof. The antibody may be a polyclonal or monoclonal antibody. The antibody may be a chimeric antibody, a single chain antibody, an affinity matured antibody, a human antibody, a humanized antibody, a fully human antibody or an antibody fragment, such as a Fab fragment, or a mixture thereof. Antibody fragments or derivatives may comprise $F(ab')_2$, Fv or scFv fragments. The antibody derivatives can be produced by peptidomimetics. Further, techniques described for the production of single chain antibodies can be adapted to produce single chain antibodies.

[0127] Human antibodies may be derived from phage-display technology or from transgenic mice that express human immmunoglobulin genes. The human antibody may be generated as a result of a human in vivo immune response and isolated. See, for example, Funaro et al., BMC Biotechnology, 2008(8):85. Therefore, the antibody may be a product of the human and not animal repertoire. Because it is of human origin, the risks of reactivity against self-antigens may be minimized. Alternatively, standard yeast display libraries and display technologies may be used to select and isolate human anti-RGMa antibodies. For example, libraries of naïve human single chain variable fragments (scFv) may be used to select human anti-RGMa antibodies. Transgenic animals may be used to express human antibodies.

[0128] Humanized antibodies may be antibody molecules from non-human species antibody that binds the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and framework regions from a human immunoglobulin molecule.

[0129] The antibody is distinguishable from known antibodies in that it possesses different biological function(s) than those known in the art. For example, not only do the antibodies of the invention recognize and bind RGMa, they are further characterized by having an additional biological activity, for example, the ability to attenuate clinical signs associated with diseases related to neurite degeneration.

[0130] The antibody may specifically bind to RGMa. The RGMa-specific RGMa antibody may comprise SEQ ID NOs:1 and 5; SEQ ID NOs:2-4 and 6-8; SEQ ID NOs:2-4, 6, 7, and 67; SEQ ID NOs:2-4, 6, 7, and 68; SEQ ID NOs:2-4, 6, 7, and 69; SEQ ID NOs:2-4, 6, 7, and 70; SEQ ID NOs:2-4, 6, 7, and 71; SEQ ID NOs:2-4, 6, 7, and 72; or SEQ ID NOs:2-4, 6, 7, and 73. The

antibody may bind to SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:74, or a fragment or variant thereof. The antibody may recognize and specifically bind an epitope present on a RGMa polypeptide or a variant as described above. The epitope may be SEQ ID NO:66, SEQ ID NO:74, or a variant thereof.

(1) Antibody Binding Characteristics

[0131] The antibody may immunospecifically bind to RGMa (SEQ ID NO:65), SEQ ID NO:66, SEQ ID NO:74, a fragment thereof, or a variant thereof and may have a k_{off} (or k_d) of at least $1.0x10^{-3}$ s^{-1}, of at least $1.0x10^{-4}$ s^{-1}, of at least $1.0x10^{-5}$ s^{-1}, of at least $1.0x10^{-6}$ s^{-1} or has a k_{off} (or k_d) ranging from $1.0x10^{-3}$ s^{-1} to $1.0x10^{-6}$ s^{-1}, from $1.0x10^{-3}$ s^{-1} to $1.0x10^{-5}$ s^{-1} or from $1.0x10^{-3}$ s^{-1} to $1.0x10^{-4}$ s^{-1}. The fragment may be SEQ ID NO:66 or SEQ ID NO:74.

[0132] The antibody may immunospecifically bind to RGMa (SEQ ID NO:65), SEQ ID NO:66, SEQ ID NO:74, a fragment thereof, or a variant thereof and has a k_{on} (or k_a) of at least $2.4x10^4$ $M^{-1}s^{-1}$, of at least about $2.5x10^4$ $M^{-1}s^{-1}$, of at least about $3.3x10^4$ $M^{-1}s^{-1}$, of at least about $5.0x10^4$ $M^{-1}s^{-1}$, of at least about $1.25x10^6$ $M^{-1}s^{-1}$ of at least about $1.35x10^6$ $M^{-1}s^{-1}$, of at least about $1.0x10^6$ $M^{-1}s^{-1}$, of at least about $1.0x10^7$ $M^{-1}s^{-1}$, or has a k_{on} (or k_a) ranging from about $5.0x10^4$ $M^{-1}s^{-1}$ to about $1.0x10^8$ $M^{-1}s^{-1}$, from about $3.3x10^4$ $M^{-1}s^{-1}$ to about $1.0x10^9$ $M^{-1}s^{-1}$, from about $2.5x10^4$ $M^{-1}s^{-1}$ to about $1.25x10^6$ $M^{-1}s^{-1}$, from about $2.4x10^4$ $M^{-1}s^{-1}$ to about $1.35x10^7$ $M^{-1}s^{-1}$. The fragment may be SEQ ID NO:66 or SEQ ID NO:74.

(2) Antibody Structure

(a) Heavy Chain and Light Chain CDRs

[0133] The antibody may immunospecifically bind to RGMa (SEQ ID NO:65), SEQ ID NO:66, SEQ ID NO:74, a fragment thereof, or a variant thereof and comprise a variable heavy chain and/or variable light chain shown in Table 1. The antibody may immunospecifically bind to RGMa, a fragment thereof, or a variant thereof and comprise one or more of the heavy chain or light chain CDR sequences also shown in Table 1. The light chain of the antibody may be a kappa chain or a lambda chain. For example, see Table 1.

[0134] Provided herein is an isolated nucleic acid encoding an antibody that immunospecifically binds to RGMa, a fragment thereof, or a variant thereof. The isolated nucleic acid may comprise a nucleotide sequence that hybridizes, under stringent conditions, to the nucleic acid molecule

that encodes an antibody comprising the heavy chain or light chain CDR sequences shown in Table 1.

Table 1 List of Amino Acid Sequences of VH and VL Regions of Fully Human Anti-RGMa Monoclonal Antibodies (AE12-1 to AE12-8, AE12-13, AE12-15, AE12-20, AE12-21, AE12-23, and AE12-24)

PROTEIN REGION	SEQ ID NO.	SEQUENCE
AE12-1 (VH)	1	EVQLVQSGAEVKKPGASVKVSCKAS GYTFTSHGISWVRQAPGQGLDWMG WISPYSGNTNYAQKLQGRVTMTTD TSTSTAYMELSSLRSEDTAVYYCAR VGSGPYYYMDVWGQGTLVTVSS
AE12-1 (VH) CDR-H1	2	SHGIS
AE12-1 (VH) CDR-H2	3	WISPYSGNTNYAQKLQG
AE12-1 (VH) CDR-H3	4	VGSGPYYYMDV
AE12-1 (VL) (Lambda chain)	5	QSALTQPRSVSGSPGQSVTISCTG TSSSVGDSIYVSWYQQHPGKAPK LMLYDVTKRPSGVPDRFSGSKSG NTASLTISGLQAEDEADYYCCSY AGTDTLFGGGTKVTVL
AE12-1 (VL) CDR-L1	6	TGTSSSVGDSIYVS
AE12-1 (VL) CDR-L2	7	DVTKRPS
AE12-1 (VL) CDR-L3	8	CSYAGTDTL
AE12-2 (VH)	9	EVQLVQSGAEVKKPGASVKVSC KASGYTFTSYDINWVRQATGQG LEWMGWMNPNSGNTGYAQKFQ GRVTMTRNTSISTAYMELSSLRSE DTAVYYCARSTSLSVWGQGTLVT

		VSS
AE12-2 (VH) CDR-H1	10	SYDIN
AE12-2 (VH) CDR-H2	11	WMNPNSGNTGYAQKFQG
AE12-2 (VH) CDR-H3	12	STSLSV
AE12-2 (VL) (Lambda chain)	13	SYELTQPPSVSVSPGQTASITCSGD KLGDKYACWYQQKPGQSPVLVIY QDSKRPSGIPKRFSGSNSGDTATLT ISGTQAMDEADYYCQAWDSSTGV FGPGTKVTVL
AE12-2 (VL) CDR-L1	14	SGDKLGDKYAC
AE12-2 (VL) CDR-L2	15	QDSKRPS
AE12-2 (VL) CDR-L3	16	QAWDSSTGV
AE12-3 (VH)	17	EVQLVESGGGLVQPGRSLRLSCA ASGFTFDDYAMHWVRQAPGKGL EWVAVISYDGSNKYYADSVKGR FTISRDNSKNTLYLQMNSLRAED TAVYYCARERVYSSGKEGYYYG MDVWGQGTMVTVSS
AE12-3 (VH) CDR-H1	18	DYAMH
AE12-3 (VH) CDR-H2	19	VISYDGSNKYYADSVKG
AE12-3 (VH) CDR-H3	20	ERVYSSGKEGYYYGMDV
AE12-3 (VL) (Lambda chain)	21	QSGLTQPPSVSAAPGQRVTISCTG SGSNIGAGYGVHWYQQLPATAPK ILIYGDYNRPSGVPDRFSGSRSGTS ASLTITGLQAEDEADYYCQSYDNS LRGVLFGGGTKLTVL
AE12-3 (VL) CDR-L1	22	TGSGSNIGAGYGVH
AE12-3 (VL) CDR-L2	23	GDYNRPS
AE12-3 (VL) CDR-L3	24	QSYDNSLRGVL
AE12-4 (VH)	25	EVQLVESGGGVVQPGTSL

		RLSCAASGFPFSSYGMHW VRQAPGKGLEWVAAISG DGILKYYTDSVKGRFTISRD NSKNTLYLQMNNLSGEDTG LYYCARNYDNSLDYWGQGTL VTVSS
AE12-4 (VH) CDR-H1	26	SYGMH
AE12-4 (VH) CDR-H2	27	AISGDGILKYYTDSVKG
AE12-4 (VH) CDR-H3	28	NYDNSLDY
AE12-4 (VL) (Lambda chain)	29	QPVLTQSPSVSASLGASVKV TCTLSSGHSAYAIAWHQQQP EKGPRYLMKVNSDGSHNK GDGVPDRFSGSSSGAERYL IISGLQSEDEADYYCQTWG PGIRVFGGGTKLTVL
AE12-4 (VL) CDR-L1	30	TLSSGHSAYAIA
AE12-4 (VL) CDR-L2	31	VNSDGSHNKGD
AE12-4 (VL) CDR-L3	32	QTWGPGIRV
AE12-5 (VH)	33	EVQLVQSGAEVKKPGASVKVSCK VSGHSLSELTIHWVRQAPGKGLEW MGGFDPEDGRGTYAPNFRGRVTM TEDTSTDTAYMELSGLRSEDAAVY YCATLLGEYDSYFDLWGRGTLVTV SS
AE12-5 (VH) CDR-H1	34	ELTIH
AE12-5 (VH) CDR-H2	35	GFDPEDGRGTYAPNFRG
AE12-5 (VH) CDR-H3	36	LLGEYDSYFDL
AE12-5 (VL) (Kappa chain)	37	DVVMTQSPDFQSVTPEDKVTITCR ASQSIGSCLHWYQQKPDQSPKLLI KYASQSISGVPSRFSGSGSGTDFTL TINSLEAEDAATYYCHQSSSLPYT FGQGTKLEIK

AE12-5 (VL) CDR-L1	38	RASQSIGSCLH
AE12-5 (VL) CDR-L2	39	YASQSIS
AE12-5 (VL) CDR-L3	40	HQSSSLPYT
AE12-6 (VH)	41	EVQLVQSGAEVKKPGASVKVSCKASG YIFTNYDIAWVRQAPGQGLEWMGWM NPDSGNTGFVQKFKGRVTATSNTDITT AYMELSSLTSEDTAVYYCARDRFGSGY DLDHWGQGTLVTVSS
AE12-6 (VH) CDR-H1	42	NYDIA
AE12-6 (VH) CDR-H2	43	WMNPDSGNTGFVQKFKG
AE12-6 (VH) CDR-H3	44	DRFGSGYDLDH
AE12-6 (VL) (Lambda chain)	45	SYELTQPPSVSVAPGQTARITCGGNNIGS KSVHWYQQKPGQAPVLVVYDDSDRPSG IPERFSGSNSGNTATLTISRVEAGDEADY YCQVWGSSSDHYVFGTGTKVTVL
AE12-6 (VL) CDR-L1	46	GGNNIGSKSVH
AE12-6 (VL) CDR-L2	47	DDSDRPS
AE12-6 (VL) CDR-L3	48	QVWGSSSDHYV
AE12-7 (VH)	49	EVQLVESGGGLVQPGGSLRLSCAASGF TSSSYAMTWVRQAPGKGLEWVSGISGS GESTYYADSVKGRFTISRDNSKNTLYLQ MNSLRVEDTAIYYCARQGYGAHDYWG QGTLVTVSS
AE12-7 (VH) CDR-H1	50	SYAMT
AE12-7 (VH) CDR-H2	51	GISGSGESTYYADSVKG
AE12-7 (VH) CDR-H3	52	QGYGAHDY
AE12-7 (VL) (Lambda chain)	53	QSVLTQPPSASGTPGQRVTISCSGASSNVG SNRVNWYQQFPGMAPKLLIYSNNQRPSGV PDRFSGSKSGTSASLAISGLQSEDEADYYCA AWDDSLNGYVFGTGTKVTVL
AE12-7 (VL) CDR-L1	54	SGASSNVGSNRVN
AE12-7 (VL) CDR-L2	55	SNNQRPS

AE12-7 (VL) CDR-L3	56	AAWDDSLNGYV
AE12-8 (VH)	57	EVQLLESGGGLVKPGGSLRLSCAASGFTFD DYAMHWVRQAPGKGLEWVSLISWDGG STYYADSVKGRFTISRDNSKNSLYLQMN SLRAEDTALYYCAKDIPKVGGYSYGYG ALGYWGQGTPVTVSS
AE12-8 (VH) CDR-H1	58	DYAMH
AE12-8 (VH) CDR-H2	59	LISWDGGSTYYADSVKG
AE12-8 (VH) CDR-H3	60	DIPKVGGYSYGYGALGY
AE12-8 (VL) (Lambda chain)	61	SYELTQPPSVSVAPGQTARITCGGNNIGD ISVHWYQQKSGQAPMLVVHDDSDRPSG IPERFSGSNSGSSATLTISRVEAGDEAD YHCQVWDSGSGHHVFGTGTKVTVL
AE12-8 (VL) CDR-L1	62	GGNNIGDISVH
AE12-8 (VL) CDR-L2	63	DDSDRPS
AE12-8 (VL) CDR-L3	64	QVWDSGSGHHV
AE12-13 (VH)	91	EVQLQESGAGLLKPSETLSLTCAVYGG SFSGYYWSWIRQPPGKGLEWIGEINHS GSTNYNPSLKSRVTISVDTSKNQFSLKL SSVTAADTAVYYCARDDGAGVFDLW GRGTLVTVSS
AE12-13 (VH) CDR-H1	92	GYYWS
AE12-13 (VH) CDR-H2	93	EINHSGSTNYNPSLKS
AE12-13 (VH) CDR-H3	94	DDGAGVFDL
AE12-13 (VL) (Kappa chain)	95	DIQLTQSPSSLSASVGDGVTITCQASQ DISNYLNWYQQKPGKAPKLLIYDASN LETGVPSRFSGSGSGTFFTLTINNLQPE DFATYYCQQSGNTPWTFGQGTKVEINR
AE12-13 (VL) CDR-L1	96	QASQDISNYLN

AE12-13 (VL) CDR-L2	97	DASNLET
AE12-13 (VL) CDR-L3	98	QQSGNTPWT
AE12-15 (VH)	99	EVQLVQSGAEVKEPGASVKVSCKA SGYTFTDYYIQWVRQAPGHGLEWM GWINPKTGGTNYLQKFQGRVTMTR DTSTRTAYMELSSLRSDDTAFYYCV REDMNTVLATSWFDPWGQGTLVTVSS
AE12-15 (VH) CDR-H1	100	DYYIQ
AE12-15 (VH) CDR-H2	101	WINPKTGGTNYLQKFQG
AE12-15 (VH) CDR-H3	102	EDMNTVLATSWFDP
AE12-15 (VL) (Lambda chain)	103	SYELTQPPSVSVSPGQTARITCSGNQ LGHKFASWYQQKPGQSPVVVIYED KKRPSGIPERFSGSNSGNTATLTISG TQAMDEADYYCQVWDVITDHYVF GTGTKVTVLG
AE12-15 (VL) CDR-L1	104	SGNQLGHKFAS
AE12-15 (VL) CDR-L2	105	EDKKRPS
AE12-15 (VL) CDR-L3	106	QVWDVITDHYV
AE12-20 (VH)	107	EVQLVQSGSEVKKPGASVKLSCKT SGYTFTNSAIHWVRQAPGQRLEWM GWINAGNGNTKYSQKFQGRVTITR DTSASTAYMELSSLRSEDTAVYYCA WAYCGGDCYSLDYWGQGTLVTVSS
AE12-20 (VH) CDR-H1	108	NSAIH
AE12-20 (VH) CDR-H2	109	WINAGNGNTKYSQKFQG
AE12-20 (VH) CDR-H3	110	AYCGGDCYSLDY
AE12-20 (VL) (Kappa chain)	111	DIQVTQSPSSLAASVGDRVTITCQAS QDISNYLNWYQQRPGKAPKLLIYDA SNLETGVPPRFSGDGSGTHFSFTITNV

		QPEDVGTYYCQQYDSLPLTFGQGTR LEIKR
AE12-20 (VL) CDR-L1	112	QASQDISNYLN
AE12-20 (VL) CDR-L2	113	DASNLET
AE12-20 (VL) CDR-L3	114	QQYDSLPLT
AE12-21 (VH)	115	EVQLLESGGDLVRPGGSLRLTCEGSG FNFFTQTIHWVRQAPGKGLEWVASIS SDSNYIYHADSLKGRFTVSRDNAQDS VFLQMNSLRVEDTAVYYCARDILLEP LAPHYYYGLDVWGQGTTVTVSS
AE12-21 (VH) CDR-H1	116	TQTIH
AE12-21 (VH) CDR-H2	117	SISSDSNYIYHADSLKG
AE12-21 (VH) CDR-H3	118	DILLEPLAPHYYYGLDV
AE12-21 (VL) (Kappa chain)	119	DIQVTQSPSSLSASVGDRVTITCRAS QPISTYVNWYQQKPGKAPKLLIYDA STLEIGVPSRISGSGSGTDFTFTISSLQ PEDIATYYCQQYDNFPLTFGGGTKV DIKR
AE12-21 (VL) CDR-L1	120	RASQPISTYVN
AE12-21 (VL) CDR-L2	121	DASTLEI
AE12-21 (VL) CDR-L3	122	QQYDNFPLT
AE12-23 (VH)	123	EVQLQESGPGLVKPSETLSLTCTVSG GSISGYYWSWIRQSPGKGLEWIGEIFH TGRTYYNPSLRSRLTISVDTSKNQFSL KLSSLTAADTAVYYCARDSAFGSFD YWGQGTLVTVSS
AE12-23 (VH) CDR-H1	124	GYYWS
AE12-23 (VH) CDR-H2	125	EIFHTGRTYYNPSLRS
AE12-23 (VH) CDR-H3	126	DSAFGSFDY
AE12-23 (VL) (Kappa chain)	127	DIRVTQSPSSLSASVGDRVTITCQAN EDISIYLNWYQQRPGKAPKLLIYDA SNLETGVPSRFSGSGSGTDFTFTISS

		LQPEDFATYYCQQYHTYPFTFGGGT KVDIKR
AE12-23 (VL) CDR-L1	128	QANEDISIYLN
AE12-23 (VL) CDR-L2	129	DASNLET
AE12-23 (VL) CDR-L3	130	QQYHTYPFT
AE12-24 (VH)	131	EVQLQESGPGLVKPSETLSLTCNVSG GSISSYYWSWIRQPPGKGLEWIGNIYY SGSTNYNPSLKSRVTISVDTSKSQFSLK LSSVTAADTAVYYCARALDFWSGQY FDYWGQGTLVTVSS
AE12-24 (VH) CDR-H1	132	SYYWS
AE12-24 (VH) CDR-H2	133	NIYYSGSTNYNPSLKS
AE12-24 (VH) CDR-H3	134	ALDFWSGQYFDY
AE12-24 (VL) (Kappa chain)	135	DIVMTQTPSSLSASVGDRVTITCQASQD ISDYLNWYQQKPGKAPKLLIYDASTLE SGVPSRFSGSGSGTDFTLTISGLQPEDF ATYYCQQSYSIPPTFGPGTRLEIKR
AE12-24 (VL) CDR-L1	136	QASQDISDYLN
AE12-24 (VL) CDR-L2	137	DASTLES
AE12-24 (VL) CDR-L3	138	QQSYSIPPT

[0135] The antibody or variant or derivative thereof may contain one or more amino acid sequences that are greater than 95%, 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, or 50% identical to one or more of SEQ ID NOs:1-64 and 67-73. The antibody or variant or derivative thereof may be encoded by one or more nucleic acid sequences that are greater than 95%, 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, or 50% identical to one or more of SEQ ID NOs:1-64 and 67-73. Polypeptide identity and homology can be determined, for example, by the algorithm described in the report: Wilbur, W. J. and Lipman, D. J. Proc. Natl. Acad. Sci. USA 80, 726-730 (1983). The herein described antibody, variant or derivative thereof may be encoded by a nucleic acid that hybridizes under stringent conditions with the complement of one or more of SEQ ID NOs:3-42. The herein described antibody, variant or derivative thereof may be encoded by a nucleic acid that hybridizes under highly stringent conditions with the complement of one or more nucleic acids that encode one or more of SEQ ID NOs:1-64 and 67-73.

[0136] The antibody may comprise SEQ ID NO:8, wherein the Cys residue of SEQ ID NO:8 is substituted for another amino acid. The antibody may comprise SEQ ID NOs:1 and 5, or 2-4 and 6-8, wherein the Cys residue of SEQ ID NO:8 is substituted for another amino acid, or wherein the Cys residue at position 91 of SEQ ID NO:5 is substituted with another amino acid. The Cys residue at position 91 of SEQ ID NO:5 may be substituted with a phenylalanine, a histidine, a leucine, a valine, an isoleucine, a lysine, or a tyrosine, for example. The Cys residue of SEQ ID NO:8 may be substituted with a phenylalanine (see SEQ ID NO:67), a histidine (see SEQ ID NO:68), a leucine (see SEQ ID NO:69), a valine (see SEQ ID NO:70), an isoleucine (see SEQ ID NO:71), a lysine (see SEQ ID NO:72), or a tyrosine (see SEQ ID NO:73), for example. See Table 2.

[0137] The antibody may be an IgG, IgE, IgM, IgD, IgA and IgY molecule class (for example, IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass. For example, the antibody may be an IgG1 molecule having the following constant region sequence:
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE**AA**G
GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ
YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD
KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:140).

[0138] The above constant region in SEQ ID NO:140 contains two (2) mutations of the wildtype constant region sequence at positions 234 and 235. Specifically, these mutations are leucine to alanine changes at each of positions 234 and 235 (which are referred to as the "LLAA" mutations). These mutations are shown above in bold and underlining. The purpose of these mutations is to eliminate the effector function.

[0139] Alternatively, an IgG1 molecule can have the above constant region sequence (SEQ ID NO:140) containing one or more mutations. For example, the constant region sequence of SEQ ID NO:140 may containing a mutation at amino acid 250 where threonine is replaced with glutamine (SEQ ID NO:141), a mutation at amino acid 428 where methionine is replaced with leucine (SEQ ID NO:142) or mutations at amino acid 250 where threonine is replaced with

glutamine and a mutation at amino acid 428 where methionine is replaced with leucine (SEQ ID NO:143) as shown below in Table 2A.

[0140] Alternatively, an IgG1 molecule can contain a heavy chain comprising: AE12-1 (VH) CDR-H1 (SEQ ID NO:2), AE12-1 (VH) CDR-H2 (SEQ ID NO:3), AE12-1 (VH) CDR-H3 (SEQ ID NO:4) and a light chain comprising: AE12-1 (VL) CDR-L1 (SEQ ID NO:6), AE12-1 (VL) CDR-L2 (SEQ ID NO:7) and AE12-1-V (VL) CDR-L3 (SEQ ID NO:70) and a constant sequence of SEQ ID NO:143 as shown below in Table 2B (this antibody is referred to as AE12-1V-QL and has a light chain sequence of SEQ ID NO:144 and a heavy chain sequence of SEQ ID NO: 145).

[0141] Alternatively, an IgG1 molecule can contain a heavy chain comprising: AE12-1 (VH) CDR-H1 (SEQ ID NO:2), AE12-1 (VH) CDR-H2 (SEQ ID NO:3), AE12-1 (VH) CDR-H3 (SEQ ID NO:4) and a light chain comprising: AE12-1 (VL) CDR-L1 (SEQ ID NO:6), AE12-1 (VL) CDR-L2 (SEQ ID NO:7) and AE12-1-Y (VL) CDR-L3 (SEQ ID NO:73) and a constant sequence of SEQ ID NO:143 as shown below in Table 2B (this antibody is referred to as AE12-1Y-QL and has a light chain sequence of SEQ ID NO:146 and a heavy chain sequence of SEQ ID NO: 147).

Table 2

PROTEIN REGION	SEQ ID NO.	SEQUENCE
AE12-1-F (VL) CDR-L3	67	FSYAGTDTL
AE12-1-H (VL) CDR-L3	68	HSYAGTDTL
AE12-1-L (VL) CDR-L3	69	LSYAGTDTL
AE12-1-V (VL) CDR-L3	70	VSYAGTDTL
AE12-1-I (VL) CDR-L3	71	ISYAGTDTL
AE12-1-K (VL) CDR-L3	72	KSYAGTDTL
AE12-1-Y (VL) CDR-L3	73	YSYAGTDTL

Table 2A

Amino acid Mutation	SEQ ID NO:	SEQUENCE
None	140	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN SGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFP PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCL VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
T250Q	141	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN SGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFP PKPKDQLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCL VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
M428L	142	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN SGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFP PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCL VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL TVDKSRWQQGNVFSCSVLHEALHNHYTQKSLSLSPGK
T250Q and M428L	143	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN SGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFP PKPKDQLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCL VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL TVDKSRWQQGNVFSCSVLHEALHNHYTQKSLSLSPGK

Table 2B

PROTEIN	SEQ	SEQUENCE

REGION	ID NO:	
AE12-1V-QL Light chain (CDR's underlined and mutations bolded)	144	QSALTQPRSVSGSPGQSVTISCTGTSSSVGDSIYVSWYQQHPGKAPK LMLYDVTKRPSGVPDRFSGSKSGNTASLTISGLQAEDEADYYCVSYA GTDTLFGGGTKVTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDF YPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKS HRSYSCQVTHEGSTVEKTVAPTECS*
AE12-1V-QL Heavy chain (CDR's underlined and mutations bolded)	145	EVQLVQSGAEVKKPGASVKVSCKASGYTFTSHGISWVRQAPGQGLDW MGWISPYSGNTNYAQKLQGRVTMTTDTSTSTAYMELSSLRSEDTAVY YCARVGSGPYYYMDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV TVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEA AGGPSVFLFPPKPKDQLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA PIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VLHEALHNHYTQKSLSLSPGK*
AE12-1Y-QL Light chain (CDR's underlined and mutations bolded)	146	QSALTQPRSVSGSPGQSVTISCTGTSSSVGDSIYVSWYQQHPGKAP KLMLYDVTKRPSGVPDRFSGSKSGNTASLTISGLQAEDEADYYCYS YAGTDTLFGGGTKVTVLGQPKAAPSVTLFPPSSEELQANKATLVCLI SDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPE QWKSHRSYSCQVTHEGSTVEKTVAPTECS*
AE12-1Y-QL Heavy chain (CDR's underlined and mutations bolded)	147	EVQLVQSGAEVKKPGASVKVSCKASGYTFTSHGISWVRQAPGQGLDWM GWISPYSGNTNYAQKLQGRVTMTTDTSTSTAYMELSSLRSEDTAVYYC ARVGSGPYYYMDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAAL GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVF LFPPKPKDQLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVLHEALHNHYTQKSL SLSPGK*

[0142] The antibody or antibody fragment may comprise a variable heavy domain that comprises three complementarity-determining regions (CDR-H1, H2, and H3) corresponding to the following formulas, respectively:

Xaa1 – Xaa2 – Xaa3 – Xaa4 – Xaa5 (Formula 1-CDR-H1), wherein Xaa1 is an amino acid selected from the group consisting of S, D, E, N, G, and T; Xaa2 is an amino acid selected from the group consisting of H, Y, L, S, and Q; Xaa3 is an amino acid selected from the group consisting of G, D, A, T, and Y; Xaa4 is an amino acid selected from the group consisting of I, M, and W; and Xaa5 is an amino acid sequence from the group consisting of S, N, H, A, T, and Q;

Xaa1 – Xaa2 – Xaa3 – Xaa4 – Xaa5 – Xaa6 – Xaa7 – Xaa8 – Xaa9 – Xaa10 – Xaa11 – Xaa12 – Xaa13 – Xaa14 – Xaa15 – Xaa16 – (Xaa)n (Formula 2-CDR-H2), wherein n is 0 or 1, and

wherein Xaa1 is an amino acid selected from the group consisting of W, V, A, G, L, E, S, and N; Xaa2 is an amino acid selected from the group consisting of I, M, and F; Xaa3 is an amino acid selected from the group consisting of S, N, D, F, and Y; Xaa4 is an amino acid selected from the group consisting of P, Y, G, W, H, A, and S; Xaa5 is an amino acid selected from the group consisting of Y, N, D, E, S, K, G, and T; Xaa6 is an amino acid selected from the group consisting of S, G, D, T, and N; Xaa7 is an amino acid selected from the group consisting of G, S, I, E, N, and R; Xaa8 is an amino acid selected from the group consisting of N, L, R, S, T, and Y; Xaa9 is an amino acid selected from the group consisting of T, K, G, N, I, and Y; Xaa10 is an amino acid selected from the group consisting of N, G, Y, T, and K; Xaa11 is an amino acid selected from the group consisting of Y, F, N, and H; Xaa12 is an amino acid selected from the group consisting of A, T, V, P, L, and S; Xaa13 is an amino acid selected from the group consisting of Q, D, P, and S; Xaa14 is an amino acid selected from the group consisting of K, S, N, and L; Xaa15 is an amino acid selected from the group consisting of L, F, V, K, and R; Xaa16 is an amino acid selected from the group consisting of Q, K, R, and S; and Xaa17 is a glycine; and

Xaa1 – Xaa2 – Xaa3 – Xaa4 – Xaa5 – Xaa6 – (Xaa)n (Formula 3-CDR-H3), wherein n is 0 - 11, and

wherein Xaa1 is an amino acid selected from the group consisting of V, S, E, N, L, D, Q, and A; Xaa2 is an amino acid selected from the group consisting of G, T, R, Y, L, I, D, and S; Xaa3 is an amino acid selected from the group consisting of S, V, D, G, F, Y, P, M, C, L, and A; Xaa4 is an amino acid selected from the group consisting of G, L, Y, N, E, K, A, and F; Xaa5 is an amino acid selected from the group consisting of P, S, Y, A, V, G, T, E, and W; Xaa6 is an

amino acid selected from the group consisting of Y, V, S, L, D, G, H, and P; Xaa7 is an amino acid selected from the group consisting of Tyr, Asp, Gly, Ser, Phe, Leu, and Cys; Xaa8 is an amino acid selected from the group consisting of Tyr, Lys, Asp, Ala, and Gln; Xaa9 is an amino acid selected from the group consisting of Met, Glu, Phe, Leu, Ser, Thr, Pro, and Tyr; Xaa10 is an amino acid selected from the group consisting of Asp, Gly, Tyr, Ser, Leu, His, and Phe; Xaa11 is an amino acid selected from the group consisting of Val, Tyr, Leu, His, Gly, Trp, and Asp; Xaa12 is an amino acid selected from the group consisting of Tyr and Phe; Xaa13 is an amino acid selected from the group consisting of Tyr, Gly, and Asp; Xaa14 is an amino acid selected from the group consisting of Ala, Leu, Pro, and Tyr; Xaa15 is an amino acid selected from the group consisting of Met, Leu, and Phe; Xaa16 is an amino acid selected from the group consisting of Asp and Gly; and Xaa17 is an amino acid selected from the group consisting of an Val, Asp, and Tyr.

[0143] The isolated antibody or antibody fragment thereof may comprise a variable light domain that comprises three complementarity-determining regions (CDR-L1, L2, and L3) corresponding to the following formulas, respectively:

Xaa1 – Xaa2 – Xaa3 – Xaa4 – Xaa5 – Xaa6 – Xaa7 – Xaa8 – Xaa9 – Xaa10 – Xaa11 – (Xaa)n (Formula 1-CDR-L1), wherein n is 0-3, and

wherein Xaa1 is an amino acid selected from the group consisting of T, S, R, G, and Q; Xaa2 is an amino acid selected from the group consisting of G, L, and A; Xaa3 is an amino acid selected from the group consisting of T, D, S, N and A; Xaa4 is an amino acid selected from the group consisting of S, K, G, Q, N, and E; Xaa5 is an amino acid sequence from the group consisting of S, L, G, I , D, and P; Xaa6 is an amino acid selected from the group consisting of S, G, N, H, and I; Xaa7 is an amino acid selected from the group consisting of V, D, I, S, G and H; Xaa8 is an amino acid selected from the group consisting of G, K, A, S, I, N, T, and D; Xaa9 is an amino acid selected from the group consisting of D, Y, A, C, S, and F; Xaa10 is an amino acid selected from the group consisting of S, A, G, L, V, and N; Xaa11 is an amino acid selected from the group consisting of I, C, Y, H, R, N, and S; Xaa12 is an amino acid selected from the group consisting of Tyr, Gly, Ala, and Val; Xaa13 is an amino acid selected from the group consisting of Val, and Asn; and Xaa14 is an amino acid selected from the group consisting of Ser and His;

Xaa1 – Xaa2 – Xaa3 – Xaa4 – Xaa5 – Xaa6 – Xaa7 – (Xaa)n (Formula 2-CDR-L2), wherein n is 0-4, and wherein Xaa1 is an amino acid selected from the group consisting of D, Q, G, V, Y, S and E; Xaa2 is an amino acid selected from the group consisting of V, D, N, and A; Xaa3 is an amino acid selected from the group consisting of T, S, Y, N, and K; Xaa4 is an amino acid selected from the group consisting of K, N, D, Q and T; Xaa5 is an amino acid selected from the group consisting of R, G, S, and L; Xaa6 is an amino acid selected from the group consisting of P, S, I, and E; Xaa7 is an amino acid selected from the group consisting of S, H, I, and T; Xaa8 is Asn; Xaa9 is Lys; and Xaa10 is Gly; Xaa11 is Asp; and

Xaa1 – Xaa2 – Xaa3 – Xaa4 – Xaa5 – Xaa6 – Xaa7 – Xaa8 – Xaa9 – (Xaa)n (Formula 3-CDR-L3), wherein n is 0-2, and

wherein Xaa1 is an amino acid selected from the group consisting of C, Q, H, F, H, L, V, I, K, Y, and A; Xaa2 is an amino acid selected from the group consisting of S, A, T, Q, and V; Xaa3 is an amino acid selected from the group consisting of Y, W, and S; Xaa4 is an amino acid selected from the group consisting of A, D, G, S, H and Y; Xaa5 is an amino acid selected from the group consisting of G, S, N, P, D, V, and T; Xaa6 is an amino acid selected from the group consisting of I, T, S, G, L, F and Y; Xaa7 is an amino acid selected from the group consisting of D, T, L, I, P, and S; Xaa8 is an amino acid selected from the group consisting of T, G, R, Y, D, N, W, L, F and P; Xaa9 is an amino acid selected from the group consisting of L, V, G, T, and H; Xaa10 is an amino acid selected from the group consisting of Val, Tyr, and His; Xaa11 is Leu or Val.

[0144] The antibody or antibody fragment comprises a variable heavy domain that comprises three complementarity-determining regions (CDR-H1, H2, and H3) corresponding to the following formulas, respectively:

Xaa1 – Xaa2 – Xaa3 – Xaa4 – Xaa5 (Formula 1 – CDR-H1), wherein Xaa1 is an amino acid selected from the group consisting of S, D, E, N, G, and T; Xaa2 is an amino acid selected from the group consisting of H, Y, L, S, and Q; Xaa3 is an amino acid selected from the group consisting of G, D, A, T, and Y; Xaa4 is an amino acid selected from the group consisting of I, M, and W; and Xaa5 is an amino acid sequence from the group consisting of S, N, H, A, T, and Q;

Xaa1 – Xaa2 – Xaa3 – Xaa4 – Xaa5 – Xaa6 – Xaa7 – Xaa8 – Xaa9 – Xaa10 – Xaa11 – Xaa12 – Xaa13 – Xaa14 – Xaa15 – Xaa16 – (Xaa)n (Formula 2 – CDR-H2), wherein n is 0 or 1, and

wherein Xaa1 is an amino acid selected from the group consisting of Y, V, A, G, L, G, S, and N; Xaa2 is an amino acid selected from the group consisting of I, M, and F; Xaa3 is an amino acid selected from the group consisting of S, N, D, F, and Y; Xaa4 is an amino acid selected from the group consisting of P, Y, G, W, H, A, and S; Xaa5 is an amino acid selected from the group consisting of Y, N, D, E, S, K, G, and T; Xaa6 is an amino acid selected from the group consisting of S, G, D, T, and N; Xaa7 is an amino acid selected from the group consisting of G, S, I, E, N, and R; Xaa8 is an amino acid selected from the group consisting of N, L, R, S, T, and Y; Xaa9 is an amino acid selected from the group consisting of T, K, G, N, I, and Y; Xaa10 is an amino acid selected from the group consisting of N, G, Y, T, and K; Xaa11 is an amino acid selected from the group consisting of Y, F, N, and H; Xaa12 is an amino acid selected from the group consisting of A, T, V, P, L, and S; Xaa13 is an amino acid selected from the group consisting of Q, D, P, and S; Xaa14 is an amino acid selected from the group consisting of K, S, N, and L; Xaa15 is an amino acid selected from the group consisting of L, F, V, K, and R; Xaa16 is an amino acid selected from the group consisting of Q, K, R, and S; and Xaa17 is a glycine; and

Xaa1 – Xaa2 – Xaa3 – Xaa4 – Xaa5 – Xaa6 – (Xaa)n (Formula 3 – CDR-H3), wherein n is 0 - 11, and

wherein Xaa1 is an amino acid selected from the group consisting of V, S, E, N, L, D, Q, and A; Xaa2 is an amino acid selected from the group consisting of G, T, R, Y, L, I, D, and S; Xaa3 is an amino acid selected from the group consisting of S, V, D, G, F, Y, P, M, C, L, and A; Xaa4 is an amino acid selected from the group consisting of G, L, Y, N, E, K, A, and F; Xaa5 is an amino acid selected from the group consisting of P, S, Y, A, V, G, T, E, and W; Xaa6 is an amino acid selected from the group consisting of Y, V, S, L, D, G, H, and P; Xaa7 is an amino acid selected from the group consisting of Tyr, Asp, Gly, Ser, Phe, Leu, and Cys; Xaa8 is an amino acid selected from the group consisting of Tyr, Lys, Asp, Ala, and Gln; Xaa9 is an amino acid selected from the group consisting of Met, Glu, Phe, Leu, Ser, Thr, Pro, and Tyr; Xaa10 is an amino acid selected from the group consisting of Asp, Gly, Tyr, Ser, Leu, His, and Phe; Xaa11 is an amino acid selected from the group consisting of Val, Tyr, Leu, His, Gly, Trp, and Asp; Xaa12 is an amino acid selected from the group consisting of Tyr and Phe; Xaa13 is an amino acid selected from the group consisting of Tyr, Gly, and Asp; Xaa14 is an amino acid selected from the group consisting of Ala, Leu, Pro, and Tyr; Xaa15 is an amino acid selected

from the group consisting of Met, Leu, and Phe; Xaa16 is an amino acid selected from the group consisting of Asp and Gly; and Xaa17 is an amino acid selected from the group consisting of an Val, Asp, and Tyr; and

wherein the antibody or antibody fragment also comprises a variable light domain that comprises three complementarity-determining regions (CDR-L1, L2, and L3) corresponding to the following formulas, respectively:

Xaa1 – Xaa2 – Xaa3 – Xaa4 – Xaa5 – Xaa6 – Xaa7 – Xaa8 – Xaa9 – Xaa10 – Xaa11 – (Xaa)n (Formula 1 –CDR-L1), wherein n is 0-3, and

wherein Xaa1 is an amino acid selected from the group consisting of T, S, R, G, and Q; Xaa2 is an amino acid selected from the group consisting of G, L, and A; Xaa3 is an amino acid selected from the group consisting of T, D, S, N and A; Xaa4 is an amino acid selected from the group consisting of S, K, G, Q, N, and E; Xaa5 is an amino acid sequence from the group consisting of S, L, G, I , D, and P; Xaa6 is an amino acid selected from the group consisting of S, G, N, H, and I; Xaa7 is an amino acid selected from the group consisting of V, D, I, S, G and H; Xaa8 is an amino acid selected from the group consisting of G, K, A, S, I, N, T, and D; Xaa9 is an amino acid selected from the group consisting of D, Y, A, C, S, and F; Xaa10 is an amino acid selected from the group consisting of S, A, G, L, V, and N; Xaa11 is an amino acid selected from the group consisting of I, C, Y, H, R, N, and S; Xaa12 is an amino acid selected from the group consisting of Tyr, Gly, Ala, and Val; Xaa13 is an amino acid selected from the group consisting of Val, and Asn; and Xaa14 is an amino acid selected from the group consisting of Ser and His;

Xaa1 – Xaa2 – Xaa3 – Xaa4 – Xaa5 – Xaa6 – Xaa7 – (Xaa)n (Formula 2 – CDR-L2), wherein n is 0-4, and wherein Xaa1 is an amino acid selected from the group consisting of D, Q, G, V, Y, S and E; Xaa2 is an amino acid selected from the group consisting of V, D, N, and A; Xaa3 is an amino acid selected from the group consisting of T, S, Y, N, and K; Xaa4 is an amino acid selected from the group consisting of K, N, D, Q and T; Xaa5 is an amino acid selected from the group consisting of R, G, S, and L; Xaa6 is an amino acid selected from the group consisting of P, S, I, and E; Xaa7 is an amino acid selected from the group consisting of S, H, I, and T; Xaa8 is Asn; Xaa9 is Lys; and Xaa10 is Gly; Xaa11 is Asp; and

Xaa1 – Xaa2 – Xaa3 – Xaa4 – Xaa5 – Xaa6 – Xaa7 – Xaa8 – Xaa9 – (Xaa)n (Formula 3 – CDR-L3), wherein n is 0-2, and

wherein Xaa1 is an amino acid selected from the group consisting of C, Q, H, F, H, L, V, I, K, Y, and A; Xaa2 is an amino acid selected from the group consisting of S, A, T, Q, and V; Xaa3 is an amino acid selected from the group consisting of Y, W, and S; Xaa4 is an amino acid selected from the group consisting of A, D, G, S, H and Y; Xaa5 is an amino acid selected from the group consisting of G, S, N, P, D, V, and T; Xaa6 is an amino acid selected from the group consisting of I, T, S, G, L, F and Y; Xaa7 is an amino acid selected from the group consisting of D, T, L, I, P, and S; Xaa8 is an amino acid selected from the group consisting of T, G, R, Y, D, N, W, L, F and P; Xaa9 is an amino acid selected from the group consisting of L, V, G, T, and H; Xaa10 is an amino acid selected from the group consisting of Val, Tyr, and His; Xaa11 is Leu or Val.

[0145] In Formula 1-CDR-L1, if n is 1, then the formula will be as follows:

Xaa1 – Xaa2 – Xaa3 – Xaa4 – Xaa5 – Xaa6 – Xaa7 – Xaa8 – Xaa9 – Xaa10 – Xaa11 – Xaa12.

In Formula 1-CDR-L1, if n is 2, then the formula will be as follows:

Xaa1 – Xaa2 – Xaa3 – Xaa4 – Xaa5 – Xaa6 – Xaa7 – Xaa8 – Xaa9 – Xaa10 – Xaa11 – Xaa12 – Xaa13.

In Formula 1-CDR-L1, if n is 3, then the formula will be as follows:

Xaa1 – Xaa2 – Xaa3 – Xaa4 – Xaa5 – Xaa6 – Xaa7 – Xaa8 – Xaa9 – Xaa10 – Xaa11 – Xaa12 – Xaa13 – Xaa14.

In Formula 2-CDR-L2, if n is 1, then the formula will be as follows:

Xaa1 – Xaa2 – Xaa3 – Xaa4 – Xaa5 – Xaa6 – Xaa7 – Xaa8.

In Formula 2-CDR-L2, if n is 2, then the formula will be as follows:

Xaa1 – Xaa2 – Xaa3 – Xaa4 – Xaa5 – Xaa6 – Xaa7 – Xaa8 – Xaa9.

In Formula 2-CDR-L2, if n is 3, then the formula will be as follows:

Xaa1 – Xaa2 – Xaa3 – Xaa4 – Xaa5 – Xaa6 – Xaa7 – Xaa8 – Xaa9 – Xaa10.

In Formula 2-CDR-L2, if n is 4, then the formula will be as follows:

Xaa1 – Xaa2 – Xaa3 – Xaa4 – Xaa5 – Xaa6 – Xaa7 – Xaa8 – Xaa9 – Xaa10 – Xaa11.

In Formula 3-CDR-L3, if n is 1, then the formula will be as follows:

Xaa1 – Xaa2 – Xaa3 – Xaa4 – Xaa5 – Xaa6 – Xaa7 – Xaa8 – Xaa9 – Xaa10.

In Formula 3-CDR-L3, if n is 2, then the formula will be as follows:

Xaa1 – Xaa2 – Xaa3 – Xaa4 – Xaa5 – Xaa6 – Xaa7 – Xaa8 – Xaa9 – Xaa10 – Xaa11.

In Formula 2-CDR-H2, if n is 1, then the formula will be as follows:

Xaa1 – Xaa2 – Xaa3 – Xaa4 – Xaa5 – Xaa6 – Xaa7 – Xaa8 – Xaa9 – Xaa10 – Xaa11 – Xaa12 – Xaa13 – Xaa14 – Xaa15 – Xaa16 – Xaa17.

In Formula 3-CDR-H3, if n is 1, then the formula will be as follows:

Xaa1 – Xaa2 – Xaa3 – Xaa4 – Xaa5 – Xaa6 – Xaa7.

In Formula 3-CDR-H3, if n is 2, then the formula will be as follows:

Xaa1 – Xaa2 – Xaa3 – Xaa4 – Xaa5 – Xaa6 – Xaa7 – Xaa8.

In Formula 3-CDR-H3, if n is 3, then the formula will be as follows:

Xaa1 – Xaa2 – Xaa3 – Xaa4 – Xaa5 – Xaa6 – Xaa7 – Xaa8 – Xaa9.

In Formula 3-CDR-H3, if n is 4, then the formula will be as follows:

Xaa1 – Xaa2 – Xaa3 – Xaa4 – Xaa5 – Xaa6 – Xaa7 – Xaa8 – Xaa9 – Xaa10.

In Formula 3-CDR-H3, if n is 5, then the formula will be as follows:

Xaa1 – Xaa2 – Xaa3 – Xaa4 – Xaa5 – Xaa6 – Xaa7 – Xaa8 – Xaa9 – Xaa10 – Xaa11.

In Formula 3-CDR-H3, if n is 6, then the formula will be as follows:

Xaa1 – Xaa2 – Xaa3 – Xaa4 – Xaa5 – Xaa6 – Xaa7 – Xaa8 – Xaa9 – Xaa10 – Xaa11 – Xaa12.

In Formula 3-CDR-H3, if n is 7, then the formula will be as follows:

Xaa1 – Xaa2 – Xaa3 – Xaa4 – Xaa5 – Xaa6 – Xaa7 – Xaa8 – Xaa9 – Xaa10 – Xaa11 – Xaa12 – Xaa13.

In Formula 3-CDR-H3, if n is 8, then the formula will be as follows:

Xaa1 – Xaa2 – Xaa3 – Xaa4 – Xaa5 – Xaa6 – Xaa7 – Xaa8 – Xaa9 – Xaa10 – Xaa11 – Xaa12 – Xaa13 – Xaa14.

In Formula 3-CDR-H3, if n is 9, then the formula will be as follows:

Xaa1 – Xaa2 – Xaa3 – Xaa4 – Xaa5 – Xaa6 – Xaa7 – Xaa8 – Xaa9 – Xaa10 – Xaa11 – Xaa12 – Xaa13 – Xaa14 – Xaa15.

In Formula 3-CDR-H3, if n is 10, then the formula will be as follows:

Xaa1 – Xaa2 – Xaa3 – Xaa4 – Xaa5 – Xaa6 – Xaa7 – Xaa8 – Xaa9 – Xaa10 – Xaa11 – Xaa12 – Xaa13 – Xaa14 – Xaa15 – Xaa16.

In Formula 3-CDR-H3, if n is 11, then the formula will be as follows:

Xaa1 – Xaa2 – Xaa3 – Xaa4 – Xaa5 – Xaa6 – Xaa7 – Xaa8 – Xaa9 – Xaa10 – Xaa11 – Xaa12 – Xaa13 – Xaa14 – Xaa15 –Xaa16 – Xaa17.

c. Antibody Preparation/Production

[0146] Antibodies may be prepared by any of a variety of techniques. In general, antibodies can be produced by cell culture techniques, including the generation of monoclonal antibodies via conventional techniques, or via transfection of antibody genes, heavy chains and/or light chains into suitable bacterial or mammalian cell hosts, in order to allow for the production of antibodies, wherein the antibodies may be recombinant. The various forms of the term "transfection" are intended to encompass a wide variety of techniques commonly used for the introduction of exogenous DNA into a prokaryotic or eukaryotic host cell, e.g., electroporation, calcium-phosphate precipitation, DEAE-dextran transfection and the like. Although it is possible to express the antibodies of the invention in either prokaryotic or eukaryotic host cells, expression of antibodies in eukaryotic cells is preferable, and most preferable in mammalian host cells, because such eukaryotic cells (and in particular mammalian cells) are more likely than prokaryotic cells to assemble and secrete a properly folded and immunologically active antibody.

[0147] Exemplary mammalian host cells for expressing the recombinant antibodies of the invention include Chinese Hamster Ovary (CHO cells) (including dhfr-CHO cells, described in Urlaub and Chasin, Proc. Natl. Acad. Sci. USA, 77: 4216-4220 (1980)), used with a DHFR selectable marker, e.g., as described in Kaufman and Sharp, J. Mol. Biol., 159: 601-621 (1982), NS0 myeloma cells, COS cells, and SP2 cells. When recombinant expression vectors encoding antibody genes are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or, more preferably, secretion of the antibody into the culture medium in which the

host cells are grown. Antibodies can be recovered from the culture medium using standard protein purification methods.

[0148] Host cells can also be used to produce functional antibody fragments, such as Fab fragments or scFv molecules. It will be understood that variations on the above procedure are within the scope of the present invention. For example, it may be desirable to transfect a host cell with DNA encoding functional fragments of either the light chain and/or the heavy chain of an antibody of this invention. Recombinant DNA technology may also be used to remove some, or all, of the DNA encoding either or both of the light and heavy chains that is not necessary for binding to the antigens of interest. The molecules expressed from such truncated DNA molecules are also encompassed by the antibodies of the invention. In addition, bifunctional antibodies may be produced in which one heavy and one light chain are an antibody of the invention (i.e., binds human RGMa) and the other heavy and light chain are specific for an antigen other than human RGMa by crosslinking an antibody of the invention to a second antibody by standard chemical crosslinking methods.

[0149] In a preferred system for recombinant expression of an antibody, or antigen-binding portion thereof, of the invention, a recombinant expression vector encoding both the antibody heavy chain and the antibody light chain is introduced into dhfr-CHO cells by calcium phosphate-mediated transfection. Within the recombinant expression vector, the antibody heavy and light chain genes are each operatively linked to CMV enhancer/AdMLP promoter regulatory elements to drive high levels of transcription of the genes. The recombinant expression vector also carries a DHFR gene, which allows for selection of CHO cells that have been transfected with the vector using methotrexate selection/amplification. The selected transformant host cells are cultured to allow for expression of the antibody heavy and light chains and intact antibody is recovered from the culture medium. Standard molecular biology techniques are used to prepare the recombinant expression vector, transfect the host cells, select for transformants, culture the host cells and recover the antibody from the culture medium. Still further the invention provides a method of synthesizing a recombinant antibody of the invention by culturing a host cell of the invention in a suitable culture medium until a recombinant antibody of the invention is synthesized. The method can further comprise isolating the recombinant antibody from the culture medium.

[0150] Methods of preparing monoclonal antibodies involve the preparation of immortal cell lines capable of producing antibodies having the desired specificity. Such cell lines may be produced from spleen cells obtained from an immunized animal. The animal may be immunized with RGMa or a fragment and/or variant thereof. For example, any of SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:74, a fragment of SEQ ID NO:65, SEQ ID NO:66 or SEQ ID NO:74, or a variant of SEQ ID NO:65, SEQ ID NO:66 SEQ ID NO:74 may be used to immunize the animal. The peptide used to immunize the animal may comprise amino acids encoding human Fc, for example the fragment crystallizable region or tail region of human antibody. The spleen cells may then be immortalized by, for example, fusion with a myeloma cell fusion partner. A variety of fusion techniques may be employed. For example, the spleen cells and myeloma cells may be combined with a nonionic detergent for a few minutes and then plated at low density on a selective medium that supports that growth of hybrid cells, but not myeloma cells. One such technique uses hypoxanthine, aminopterin, thymidine (HAT) selection. After a sufficient time, usually about 1 to 2 weeks, colonies of hybrids are observed. Single colonies are selected and their culture supernatants tested for binding activity against the polypeptide. Hybridomas having high reactivity and specificity may be used.

[0151] Monoclonal antibodies may be isolated from the supernatants of growing hybridoma colonies. In addition, various techniques may be employed to enhance the yield, such as injection of the hybridoma cell line into the peritoneal cavity of a suitable vertebrate host, such as a mouse. Monoclonal antibodies may then be harvested from the ascites fluid or the blood. Contaminants may be removed from the antibodies by conventional techniques, such as chromatography, gel filtration, precipitation, and extraction. Affinity chromatography is an example of a method that can be used in a process to purify the antibodies.

[0152] The proteolytic enzyme papain preferentially cleaves IgG molecules to yield several fragments, two of which (the F(ab) fragments) each comprise a covalent heterodimer that includes an intact antigen-binding site. The enzyme pepsin is able to cleave IgG molecules to provide several fragments, including the F(ab')2 fragment, which comprises both antigen-binding sites.

[0153] The Fv fragment can be produced by preferential proteolytic cleavage of an IgM, and on rare occasions IgG or IgA immunoglobulin molecules. The Fv fragment may be derived using

recombinant techniques. The Fv fragment includes a non-covalent VH::VL heterodimer including an antigen-binding site which retains much of the antigen recognition and binding capabiltites of the native antibody molecule.

[0154] The antibody, antibody fragment, or derivative may comprise a heavy chain and a light chain complementarity determining region ("CDR") set, respectively interposed between a heavy chain and a light chain framework ("FR") set which provide support to the CDRs and define the spatial relationship of the CDRs relative to each other. The CDR set may contain three hypervariable regions of a heavy or light chain V region. Proceeding from the N-terminus of a heavy or light chain, these regions are denoted as "CDR1," "CDR2," and "CDR3," respectively. An antigen-binding site, therefore, may include six CDRs, comprising the CDR set from each of a heavy and a light chain V region. A polypeptide comprising a single CDR, (e.g., a CDR1, CDR2 or CDR3) may be referred to as a "molecular recognition unit." Crystallographic analyses of antigen-antibody complexes have demonstrated that the amino acid residues of CDRs form extensive contact with bound antigen, wherein the most extensive antigen contact is with the heavy chain CDR3. Thus, the molecular recognition units may be primarily responsible for the specificity of an antigen-binding site. In general, the CDR residues are directly and most substantially involved in influencing antigen binding.

[0155] Other suitable methods of producing or isolating antibodies of the requisite specificity can be used, including, but not limited to, methods that select recombinant antibody from a peptide or protein library (e.g., but not limited to, a bacteriophage, ribosome, oligonucleotide, RNA, cDNA, yeast or the like, display library); e.g., as available from various commercial vendors such as Cambridge Antibody Technologies (Cambridgeshire, UK), MorphoSys (Martinsreid/Planegg, Del.), Biovation (Aberdeen, Scotland, UK) BioInvent (Lund, Sweden), using methods known in the art. See U.S. Pat. Nos. 4,704,692; 5,723,323; 5,763,192; 5,814,476; 5,817,483; 5,824,514; 5,976,862. Alternative methods rely upon immunization of transgenic animals (e.g., SCID mice, Nguyen et al. (1997) Microbiol. Immunol. 41:901-907; Sandhu et al. (1996) Crit. Rev. Biotechnol. 16:95-118; Eren et al. (1998) Immunol. 93:154-161) that are capable of producing a repertoire of human antibodies, as known in the art and/or as described herein. Such techniques, include, but are not limited to, ribosome display (Hanes et al. (1997) Proc. Natl. Acad. Sci. USA, 94:4937-4942; Hanes et al. (1998) Proc. Natl. Acad. Sci. USA, 95:14130-14135); single cell antibody producing technologies (e.g., selected lymphocyte

antibody method ("SLAM") (U.S. Pat. No. 5,627,052, Wen et al. (1987) J. Immunol. 17:887-892; Babcook et al. (1996) Proc. Natl. Acad. Sci. USA 93:7843-7848); gel microdroplet and flow cytometry (Powell et al. (1990) Biotechnol. 8:333-337; One Cell Systems, (Cambridge, Mass).; Gray et al. (1995) J. Imm. Meth. 182:155-163; Kenny et al. (1995) Bio/Technol. 13:787-790); B-cell selection (Steenbakkers et al. (1994) Molec. Biol. Reports 19:125-134 (1994)).

[0156] An affinity matured antibody may be produced by any one of a number of procedures that are known in the art. For example, see Marks et al., BioTechnology, 10: 779-783 (1992) describes affinity maturation by VH and VL domain shuffling. Random mutagenesis of CDR and/or framework residues is described by Barbas et al., Proc. Nat. Acad. Sci. USA, 91: 3809-3813 (1994); Schier et al., Gene, 169: 147-155 (1995); Yelton et al., J. Immunol., 155: 1994-2004 (1995); Jackson et al., J. Immunol., 154(7): 3310-3319 (1995); Hawkins et al, J. Mol. Biol., 226: 889-896 (1992). Selective mutation at selective mutagenesis positions and at contact or hypermutation positions with an activity enhancing amino acid residue is described in U.S. Pat. No. 6,914,128 B1.

[0157] Antibody variants of the present invention can also be prepared using delivering a polynucleotide encoding an antibody of this invention to a suitable host such as to provide transgenic animals or mammals, such as goats, cows, horses, sheep, and the like, that produce such antibodies in their milk. These methods are known in the art and are described for example in U.S. Pat. Nos. 5,827,690; 5,849,992; 4,873,316; 5,849,992; 5,994,616; 5,565,362; and 5,304,489.

[0158] Antibody variants also can be prepared by delivering a polynucleotide of this invention to provide transgenic plants and cultured plant cells (e.g., but not limited to tobacco, maize, and duckweed) that produce such antibodies, specified portions or variants in the plant parts or in cells cultured therefrom. For example, Cramer et al. (1999) Curr. Top. Microbol. Immunol. 240:95-118 and references cited therein, describe the production of transgenic tobacco leaves expressing large amounts of recombinant proteins, e.g., using an inducible promoter. Transgenic maize have been used to express mammalian proteins at commercial production levels, with biological activities equivalent to those produced in other recombinant systems or purified from natural sources. See, e.g., Hood et al., Adv. Exp. Med. Biol. (1999) 464:127-147 and references cited therein. Antibody variants have also been produced in large amounts from transgenic plant

seeds including antibody fragments, such as single chain antibodies (scFv's), including tobacco seeds and potato tubers. See, e.g., Conrad et al. (1998) Plant Mol. Biol. 38:101-109 and reference cited therein. Thus, antibodies of the present invention can also be produced using transgenic plants, according to known methods.

[0159] Antibody derivatives can be produced, for example, by adding exogenous sequences to modify immunogenicity or reduce, enhance or modify binding, affinity, on-rate, off-rate, avidity, specificity, half-life, or any other suitable characteristic. Generally part or all of the non-human or human CDR sequences are maintained while the non-human sequences of the variable and constant regions are replaced with human or other amino acids.

[0160] Small antibody fragments may be diabodies having two antigen-binding sites, wherein fragments comprise a heavy chain variable domain (VH) connected to a light chain variable domain (VL) in the same polypeptide chain (VH VL). See for example, EP 404,097; WO 93/11161; and Hollinger et al., (1993) Proc. Natl. Acad. Sci. USA 90:6444-6448. By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. See also, U.S. Pat. No. 6,632,926 to Chen et al. which is hereby incorporated by reference in its entirety and discloses antibody variants that have one or more amino acids inserted into a hypervariable region of the parent antibody and a binding affinity for a target antigen which is at least about two fold stronger than the binding affinity of the parent antibody for the antigen.

[0161] The antibody may be a linear antibody. The procedure for making a linear antibody is known in the art and described in Zapata et al. (1995) Protein Eng. 8(10):1057-1062. Briefly, these antibodies comprise a pair of tandem Fd segments (VH-CH1-VH-CH1) which form a pair of antigen binding regions. Linear antibodies can be bispecific or monospecific.

[0162] The antibodies may be recovered and purified from recombinant cell cultures by known methods including, but not limited to, protein A purification, ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. High performance liquid chromatography ("HPLC") can also be used for purification.

[0163] It may be useful to detectably or therapeutically label the antibody. Methods for conjugating antibodies to these agents are known in the art. For the purpose of illustration only, antibodies can be labeled with a detectable moiety such as a radioactive atom, a chromophore, a fluorophore, or the like. Such labeled antibodies can be used for diagnostic techniques, either in vivo, or in an isolated test sample. Antibodies can also be conjugated, for example, to a pharmaceutical agent, such as chemotherapeutic drug or a toxin. They can be linked to a cytokine, to a ligand, to another antibody. Suitable agents for coupling to antibodies to achieve an anti-tumor effect include cytokines, such as interleukin 2 (IL-2) and Tumor Necrosis Factor (TNF); photosensitizers, for use in photodynamic therapy, including aluminum (III) phthalocyanine tetrasulfonate, hematoporphyrin, and phthalocyanine; radionuclides, such as iodine-131 (131I), yttrium-90 (90Y), bismuth-212 (212Bi), bismuth-213 (213Bi), technetium-99m (99mTc), rhenium-186 (186Re), and rhenium-188 (188Re); antibiotics, such as doxorubicin, adriamycin, daunorubicin, methotrexate, daunomycin, neocarzinostatin, and carboplatin; bacterial, plant, and other toxins, such as diphtheria toxin, pseudomonas exotoxin A, staphylococcal enterotoxin A, abrin-A toxin, ricin A (deglycosylated ricin A and native ricin A), TGF-alpha toxin, cytotoxin from chinese cobra (naja naja atra), and gelonin (a plant toxin); ribosome inactivating proteins from plants, bacteria and fungi, such as restrictocin (a ribosome inactivating protein produced by Aspergillus restrictus), saporin (a ribosome inactivating protein from Saponaria officinalis), and RNase; tyrosine kinase inhibitors; ly207702 (a difluorinated purine nucleoside); liposomes containing anti cystic agents (e.g., antisense oligonucleotides, plasmids which encode for toxins, methotrexate, etc.); and other antibodies or antibody fragments, such as F(ab).

[0164] The antibodies can be sequenced and replicated by recombinant or synthetic means. They also can be further sequenced down to the linear sequence of nucleotides that encode them. Accordingly, this invention provides these polynucleotides, alone or in combination with a carrier, vector or host cell as described above, that encode a sequence of an antibody of this invention.

[0165] Antibody production via the use of hybridoma technology, the selected lymphocyte antibody method (SLAM), transgenic animals, and recombinant antibody libraries is described in more detail below.

(1) Anti-RGMa Monoclonal Antibodies Using Hybridoma Technology

[0166] Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al., Antibodies: A Laboratory Manual, second edition, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1988); Hammerling, et al., In Monoclonal Antibodies and T-Cell Hybridomas, (Elsevier, N.Y., 1981). It is also noted that the term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

[0167] In an embodiment, the present invention provides methods of generating monoclonal antibodies as well as antibodies produced by the method. The method may comprise culturing a hybridoma cell secreting an antibody of the invention wherein, preferably, the hybridoma is generated by fusing splenocytes isolated from an animal, e.g., a rat or a mouse, immunized with RGMa with myeloma cells and then screening the hybridomas resulting from the fusion for hybridoma clones that secrete an antibody able to bind a polypeptide of the invention. Briefly, rats can be immunized with an RGMa antigen. In a preferred embodiment, the RGMa antigen is administered with an adjuvant to stimulate the immune response. Such adjuvants include complete or incomplete Freund's adjuvant, RIBI (muramyl dipeptides) or ISCOM (immunostimulating complexes). Such adjuvants may protect the polypeptide from rapid dispersal by sequestering it in a local deposit, or they may contain substances that stimulate the host to secrete factors that are chemotactic for macrophages and other components of the immune system. Preferably, if a polypeptide is being administered, the immunization schedule will involve two or more administrations of the polypeptide, spread out over several weeks; however, a single administration of the polypeptide may also be used.

[0168] After immunization of an animal with an RGMa antigen, antibodies and/or antibody-producing cells may be obtained from the animal. An anti-RGMa antibody-containing serum is obtained from the animal by bleeding or sacrificing the animal. The serum may be used as it is obtained from the animal, an immunoglobulin fraction may be obtained from the serum, or the

anti-RGMa antibodies may be purified from the serum. Serum or immunoglobulins obtained in this manner are polyclonal, thus having a heterogeneous array of properties.

[0169] Once an immune response is detected, e.g., antibodies specific for the antigen RGMa are detected in the rat serum, the rat spleen is harvested and splenocytes isolated. The splenocytes are then fused by well-known techniques to any suitable myeloma cells, for example cells from cell line SP20 available from the American Type Culture Collection (ATCC, Manassas, Va., US). Hybridomas are selected and cloned by limited dilution. The hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding RGMa. Ascites fluid, which generally contains high levels of antibodies, can be generated by immunizing rats with positive hybridoma clones.

[0170] In another embodiment, antibody-producing immortalized hybridomas may be prepared from the immunized animal. After immunization, the animal is sacrificed and the splenic B cells are fused to immortalized myeloma cells as is well known in the art. See, e.g., Harlow and Lane, supra. In a preferred embodiment, the myeloma cells do not secrete immunoglobulin polypeptides (a non-secretory cell line). After fusion and antibiotic selection, the hybridomas are screened using RGMa, or a portion thereof, or a cell expressing RGMa. In a preferred embodiment, the initial screening is performed using an enzyme-linked immunosorbent assay (ELISA) or a radioimmunoassay (RIA), preferably an ELISA. An example of ELISA screening is provided in PCT Publication No. WO 00/37504.

[0171] Anti-RGMa antibody-producing hybridomas are selected, cloned, and further screened for desirable characteristics, including robust hybridoma growth, high antibody production, and desirable antibody characteristics, as discussed further below. Hybridomas may be cultured and expanded in vivo in syngeneic animals, in animals that lack an immune system, e.g., nude mice, or in cell culture in vitro. Methods of selecting, cloning and expanding hybridomas are well known to those of ordinary skill in the art.

[0172] In a preferred embodiment, hybridomas are rat hybridomas. In another embodiment, hybridomas are produced in a non-human, non-rat species such as mice, sheep, pigs, goats, cattle, or horses. In yet another preferred embodiment, the hybridomas are human hybridomas, in which a human non-secretory myeloma is fused with a human cell expressing an anti-RGMa antibody.

[0173] Antibody fragments that recognize specific epitopes may be generated by known techniques. For example, Fab and F(ab')2 fragments of the invention may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce two identical Fab fragments) or pepsin (to produce a $F(ab')_2$ fragment). A F(ab')2 fragment of an IgG molecule retains the two antigen-binding sites of the larger ("parent") IgG molecule, including both light chains (containing the variable light chain and constant light chain regions), the CH1 domains of the heavy chains, and a disulfide-forming hinge region of the parent IgG molecule. Accordingly, a F(ab')2 fragment is still capable of crosslinking antigen molecules like the parent IgG molecule.

(2) Anti-RGMa Monoclonal Antibodies Using SLAM.

[0174] In another aspect of the invention, recombinant antibodies are generated from single, isolated lymphocytes using a procedure referred to in the art as the selected lymphocyte antibody method (SLAM), as described in U.S. Pat. No. 5,627,052; PCT Publication No. WO 92/02551; and Babcook et al., Proc. Natl. Acad. Sci. USA, 93: 7843-7848 (1996). In this method, single cells secreting antibodies of interest, e.g., lymphocytes derived from any one of the immunized animals are screened using an antigen-specific hemolytic plaque assay, wherein the antigen RGMa, a subunit of RGMa, or a fragment thereof, is coupled to sheep red blood cells using a linker, such as biotin, and used to identify single cells that secrete antibodies with specificity for RGMa. Following identification of antibody-secreting cells of interest, heavy- and light-chain variable region cDNAs are rescued from the cells by reverse transcriptase-PCR (RT-PCR) and these variable regions can then be expressed, in the context of appropriate immunoglobulin constant regions (e.g., human constant regions), in mammalian host cells, such as COS or CHO cells. The host cells transfected with the amplified immunoglobulin sequences, derived from in vivo selected lymphocytes, can then undergo further analysis and selection in vitro, for example, by panning the transfected cells to isolate cells expressing antibodies to RGMa. The amplified immunoglobulin sequences further can be manipulated in vitro, such as by in vitro affinity maturation method. See, for example, PCT Publication No. WO 97/29131 and PCT Publication No. WO 00/56772.

(3) Anti-RGMa Monoclonal Antibodies Using Transgenic Animals.

[0175] In another embodiment of the invention, antibodies are produced by immunizing a non-human animal comprising some, or all, of the human immunoglobulin locus with a RGMa

antigen. In an embodiment, the non-human animal is a XENOMOUSE® transgenic mouse, an engineered mouse strain that comprises large fragments of the human immunoglobulin loci and is deficient in mouse antibody production. See, e.g., Green et al., Nature Genetics, 7: 13-21 (1994) and U.S. Pat. Nos. 5,916,771; 5,939,598; 5,985,615; 5,998,209; 6,075,181; 6,091,001; 6,114,598; and 6,130,364. See also PCT Publication Nos. WO 91/10741; WO 94/02602; WO 96/34096; WO 96/33735; WO 98/16654; WO 98/24893; WO 98/50433; WO 99/45031; WO 99/53049; WO 00/09560; and WO 00/37504. The XENOMOUSE® transgenic mouse produces an adult-like human repertoire of fully human antibodies, and generates antigen-specific human monoclonal antibodies. The XENOMOUSE® transgenic mouse contains approximately 80% of the human antibody repertoire through introduction of megabase sized, germline configuration YAC fragments of the human heavy chain loci and x light chain loci. See Mendez et al., Nature Genetics, 15: 146-156 (1997), Green and Jakobovits, J. Exp. Med., 188: 483-495 (1998), the disclosures of which are hereby incorporated by reference.

(4) Anti-RGMa Monoclonal Antibodies Using Recombinant Antibody Libraries.

[0176] In vitro methods also can be used to make the antibodies of the invention, wherein an antibody library is screened to identify an antibody having the desired RGMa-binding specificity. Methods for such screening of recombinant antibody libraries are well known in the art and include methods described in, for example, U.S. Pat. No. 5,223,409 (Ladner et al.); PCT Publication No. WO 92/18619 (Kang et al.); PCT Publication No. WO 91/17271 (Dower et al.); PCT Publication No. WO 92/20791 (Winter et al.); PCT Publication No. WO 92/15679 (Markland et al.); PCT Publication No. WO 93/01288 (Breitling et al.); PCT Publication No. WO 92/01047 (McCafferty et al.); PCT Publication No. WO 92/09690 (Garrard et al.); Fuchs et al., Bio/Technology, 9: 1369-1372 (1991); Hay et al., Hum. Antibod. Hybridomas, 3: 81-85 (1992); Huse et al., Science, 246: 1275-1281 (1989); McCafferty et al., Nature, 348: 552-554 (1990); Griffiths et al., EMBO J., 12: 725-734 (1993); Hawkins et al., J. Mol. Biol., 226: 889-896 (1992); Clackson et al., Nature, 352: 624-628 (1991); Gram et al., Proc. Natl. Acad. Sci. USA, 89: 3576-3580 (1992); Garrard et al., Bio/Technology, 9: 1373-1377 (1991); Hoogenboom et al., Nucl. Acids Res., 19: 4133-4137 (1991); Barbas et al., Proc. Natl. Acad. Sci. USA, 88: 7978-7982 (1991); US Patent Application Publication No. 2003/0186374; and PCT Publication No. WO 97/29131, the contents of each of which are incorporated herein by reference.

[0177] The recombinant antibody library may be from a subject immunized with RGMa, or a portion of RGMa. Alternatively, the recombinant antibody library may be from a naive subject, i.e., one who has not been immunized with RGMa, such as a human antibody library from a human subject who has not been immunized with human RGMa. Antibodies of the invention are selected by screening the recombinant antibody library with the peptide comprising human RGMa to thereby select those antibodies that recognize RGMa. Methods for conducting such screening and selection are well known in the art, such as described in the references in the preceding paragraph. To select antibodies of the invention having particular binding affinities for RGMa, such as those that dissociate from human RGMa with a particular K_{off} rate constant, the art-known method of surface plasmon resonance can be used to select antibodies having the desired K_{off} rate constant. To select antibodies of the invention having a particular neutralizing activity for hRGMa, such as those with a particular IC_{50}, standard methods known in the art for assessing the inhibition of RGMa activity may be used.

[0178] In one aspect, the invention pertains to an isolated antibody, or an antigen-binding portion thereof, that binds human RGMa. Preferably, the antibody is a neutralizing antibody. In various embodiments, the antibody is a recombinant antibody or a monoclonal antibody.

[0179] For example, antibodies of the present invention can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. Such phage can be utilized to display antigen-binding domains expressed from a repertoire or combinatorial antibody library (e.g., human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, e.g., using labeled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv, or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkmann et al., J. Immunol. Methods, 182: 41-50 (1995); Ames et al., J. Immunol. Methods, 184:177-186 (1995); Kettleborough et al., Eur. J. Immunol., 24: 952-958 (1994); Persic et al., Gene, 187: 9-18 (1997); Burton et al., Advances in Immunology, 57: 191-280 (1994); PCT Publication No. WO 92/01047; PCT Publication Nos. WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619;

WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Pat. Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743; and 5,969,108.

[0180] As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies including human antibodies or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described in detail below. For example, techniques to recombinantly produce Fab, Fab', and $F(ab')_2$ fragments can also be employed using methods known in the art such as those disclosed in PCT publication No. WO 92/22324; Mullinax et al., BioTechniques, 12(6): 864-869 (1992); Sawai et al., Am. J. Reprod. Immunol., 34: 26-34 (1995); and Better et al., Science, 240: 1041-1043 (1988). Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Pat. Nos. 4,946,778 and 5,258,498; Huston et al., Methods in Enzymology, 203: 46-88 (1991); Shu et al., Proc. Natl. Acad. Sci. USA, 90: 7995-7999 (1993); and Skerra et al., Science, 240: 1038-1041 (1988).

[0181] Alternative to screening of recombinant antibody libraries by phage display, other methodologies known in the art for screening large combinatorial libraries can be applied to the identification of antibodies of the invention. One type of alternative expression system is one in which the recombinant antibody library is expressed as RNA-protein fusions, as described in PCT Publication No. WO 98/31700 (Szostak and Roberts), and in Roberts and Szostak, Proc. Natl. Acad. Sci. USA, 94: 12297-12302 (1997). In this system, a covalent fusion is created between an mRNA and the peptide or protein that it encodes by in vitro translation of synthetic mRNAs that carry puromycin, a peptidyl acceptor antibiotic, at their 3' end. Thus, a specific mRNA can be enriched from a complex mixture of mRNAs (e.g., a combinatorial library) based on the properties of the encoded peptide or protein, e.g., antibody, or portion thereof, such as binding of the antibody, or portion thereof, to the dual specificity antigen. Nucleic acid sequences encoding antibodies, or portions thereof, recovered from screening of such libraries can be expressed by recombinant means as described above (e.g., in mammalian host cells) and, moreover, can be subjected to further affinity maturation by either additional rounds of screening of mRNA-peptide fusions in which mutations have been introduced into the originally selected

sequence(s), or by other methods for affinity maturation in vitro of recombinant antibodies, as described above. A preferred example of this methodology, is PROfusion display technology.

[0182] In another approach the antibodies of the present invention can also be generated using yeast display methods known in the art. In yeast display methods, genetic methods are used to tether antibody domains to the yeast cell wall and display them on the surface of yeast. In particular, such yeast can be utilized to display antigen-binding domains expressed from a repertoire or combinatorial antibody library (e.g., human or murine). Examples of yeast display methods that can be used to make the antibodies of the present invention include those disclosed in U.S. Pat. No. 6,699,658 (Wittrup et al.) incorporated herein by reference.

d. Production of Recombinant RGMa Antibodies

[0183] Antibodies of the present invention may be produced by any of a number of techniques known in the art. For example, expression from host cells, wherein expression vector(s) encoding the heavy and light chains is (are) transfected into a host cell by standard techniques. The various forms of the term "transfection" are intended to encompass a wide variety of techniques commonly used for the introduction of exogenous DNA into a prokaryotic or eukaryotic host cell, e.g., electroporation, calcium-phosphate precipitation, DEAE-dextran transfection and the like. Although it is possible to express the antibodies of the invention in either prokaryotic or eukaryotic host cells, expression of antibodies in eukaryotic cells is preferable, and most preferable in mammalian host cells, because such eukaryotic cells (and in particular mammalian cells) are more likely than prokaryotic cells to assemble and secrete a properly folded and immunologically active antibody.

[0184] Exemplary mammalian host cells for expressing the recombinant antibodies of the invention include Chinese Hamster Ovary (CHO cells) (including dhfr-CHO cells, described in Urlaub and Chasin, Proc. Natl. Acad. Sci. USA, 77: 4216-4220 (1980), used with a DHFR selectable marker, e.g., as described in Kaufman and Sharp, J. Mol. Biol., 159: 601-621 (1982), NS0 myeloma cells, COS cells, and SP2 cells. When recombinant expression vectors encoding antibody genes are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or, more preferably, secretion of the antibody into the culture medium in which the

host cells are grown. Antibodies can be recovered from the culture medium using standard protein purification methods.

[0185] Host cells can also be used to produce functional antibody fragments, such as Fab fragments or scFv molecules. It will be understood that variations on the above procedure are within the scope of the present invention. For example, it may be desirable to transfect a host cell with DNA encoding functional fragments of either the light chain and/or the heavy chain of an antibody of this invention. Recombinant DNA technology may also be used to remove some, or all, of the DNA encoding either or both of the light and heavy chains that is not necessary for binding to the antigens of interest. The molecules expressed from such truncated DNA molecules are also encompassed by the antibodies of the invention. In addition, bifunctional antibodies may be produced in which one heavy and one light chain are an antibody of the invention (i.e., binds human RGMa) and the other heavy and light chain are specific for an antigen other than human RGMa by crosslinking an antibody of the invention to a second antibody by standard chemical crosslinking methods.

[0186] In a preferred system for recombinant expression of an antibody, or antigen-binding portion thereof, of the invention, a recombinant expression vector encoding both the antibody heavy chain and the antibody light chain is introduced into dhfr-CHO cells by calcium phosphate-mediated transfection. Within the recombinant expression vector, the antibody heavy and light chain genes are each operatively linked to CMV enhancer/AdMLP promoter regulatory elements to drive high levels of transcription of the genes. The recombinant expression vector also carries a DHFR gene, which allows for selection of CHO cells that have been transfected with the vector using methotrexate selection/amplification. The selected transformant host cells are cultured to allow for expression of the antibody heavy and light chains and intact antibody is recovered from the culture medium. Standard molecular biology techniques are used to prepare the recombinant expression vector, transfect the host cells, select for transformants, culture the host cells and recover the antibody from the culture medium. Still further the invention provides a method of synthesizing a recombinant antibody of the invention by culturing a host cell of the invention in a suitable culture medium until a recombinant antibody of the invention is synthesized. The method can further comprise isolating the recombinant antibody from the culture medium.

(a) Humanized Antibody

[0187] The humanized antibody may be an antibody or a variant, derivative, analog or portion thereof which immunospecifically binds to an antigen of interest and which comprises a framework (FR) region having substantially the amino acid sequence of a human antibody and a complementary determining region (CDR) having substantially the amino acid sequence of a non-human antibody. The humanized antibody may be from a non-human species antibody that binds the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and framework regions from a human immunoglobulin molecule.

[0188] As used herein, the term "substantially" in the context of a CDR refers to a CDR having an amino acid sequence at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of a non-human antibody CDR. A humanized antibody comprises substantially all of at least one, and typically two, variable domains (Fab, Fab', F(ab')2, FabC, Fv) in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin (i.e., donor antibody) and all or substantially all of the framework regions are those of a human immunoglobulin consensus sequence. According to one aspect, a humanized antibody also comprises at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. In some embodiments, a humanized antibody contains both the light chain as well as at least the variable domain of a heavy chain. The antibody also may include the CH1, hinge, CH2, CH3, and CH4 regions of the heavy chain. In some embodiments, a humanized antibody only contains a humanized light chain. In some embodiments, a humanized antibody only contains a humanized heavy chain. In specific embodiments, a humanized antibody only contains a humanized variable domain of a light chain and/or of a heavy chain.

[0189] The humanized antibody can be selected from any class of immunoglobulins, including IgM, IgG, IgD, IgA and IgE, and any isotype, including without limitation IgG 1, IgG2, IgG3 and IgG4. The humanized antibody may comprise sequences from more than one class or isotype, and particular constant domains may be selected to optimize desired effector functions using techniques well-known in the art.

[0190] The framework and CDR regions of a humanized antibody need not correspond precisely to the parental sequences, e.g., the donor antibody CDR or the consensus framework may be

mutagenized by substitution, insertion and/or deletion of at least one amino acid residue so that the CDR or framework residue at that site does not correspond to either the donor antibody or the consensus framework. In one embodiment, such mutations, however, will not be extensive. Usually, at least 90%, at least 95%, at least 98%, or at least 99% of the humanized antibody residues will correspond to those of the parental FR and CDR sequences. As used herein, the term "consensus framework" refers to the framework region in the consensus immunoglobulin sequence. As used herein, the term "consensus immunoglobulin sequence" refers to the sequence formed from the most frequently occurring amino acids (or nucleotides) in a family of related immunoglobulin sequences (See e.g., Winnaker, From Genes to Clones (Verlagsgesellschaft, Weinheim, Germany 1987)). In a family of immunoglobulins, each position in the consensus sequence is occupied by the amino acid occuring most frequently at that position in the family. If two amino acids occur equally frequently, either can be included in the consensus sequence.

[0191] The humanized antibody may be designed to minimize unwanted immunological response toward rodent anti-human antibodies, which limits the duration and effectiveness of therapeutic applications of those moieties in human recipients. The humanized antibody may have one or more amino acid residues introduced into it from a source that is non-human. These non-human residues are often referred to as "import" residues, which are typically taken from a variable domain. Humanization may be performed by substituting hypervariable region sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. For example, see U.S. Patent No. 4,816,567, the contents of which are herein incorporated by reference. The humanized antibody may be a human antibody in which some hypervariable region residues, and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies. Humanization or engineering of antibodies of the present invention can be performed using any known method, such as but not limited to those described in U.S. Pat. Nos. 5,723,323; 5,976,862; 5,824,514; 5,817,483; 5,814,476; 5,763,192; 5,723,323; 5,766,886; 5,714,352; 6,204,023; 6,180,370; 5,693,762; 5,530,101; 5,585,089; 5,225,539; and 4,816,567.

[0192] The humanized antibody may retain high affinity for RGMa and other favorable biological properties. The humanized antibody may be prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models

of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available. Computer programs are available that illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the recipient and import sequences so that the desired antibody characteristics, such as increased affinity for RGMa, is achieved. In general, the hypervariable region residues may be directly and most substantially involved in influencing antigen binding.

[0193] As an alternative to humanization, human antibodies (also referred to herein as "fully human antibodies") can be generated. For example, it is possible to isolate human antibodies from libararies via PROfusion and/or yeast related technologies. See Examples provided below. It is also possible to produce transgenic animals (e.g. mice that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, the homozygous deletion of the antibody heavy-chain joining region (J_H) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. The humanized or fully human antibodies may be prepared according to the methods described in U.S. Patent Nos. 5,770,429; 5,833,985; 5,837,243; 5,922,845; 6,017,517; 6,096,311; 6,111,166; 6,270,765; 6,303,755; 6,365,116; 6,410,690; 6,682,928; and 6,984,720, the contents each of which are herein incorporated by reference.

3. Pharmaceutical Compositions

[0194] The antibody may be a component in a pharmaceutical composition. The pharmaceutical composition may also contain a pharmaceutically acceptable carrier. The pharmaceutical compositions comprising antibodies of the invention are for use in, but not limited to, diagnosing, detecting, or monitoring a disorder, in preventing, treating, managing, or ameliorating of a disorder or one or more symptoms thereof, and/or in research. In a specific embodiment, a composition comprises one or more antibodies of the invention. In another embodiment, the pharmaceutical composition comprises one or more antibodies of the invention and one or more prophylactic or therapeutic agents other than antibodies of the invention for

treating a disorder in which activity of a targeted RGMa is detrimental. In a further embodiment, the prophylactic or therapeutic agents are known to be useful for, or have been, or are currently being used in the prevention, treatment, management, or amelioration of a disorder, or one or more symptoms thereof. In accordance with these embodiments, the composition may further comprise of a carrier, diluent or excipient.

[0195] The antibodies of the invention can be incorporated into pharmaceutical compositions suitable for administration to a subject. Typically, the pharmaceutical composition comprises an antibody of the invention and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Examples of pharmaceutically acceptable carriers include one or more of water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Pharmaceutically acceptable carriers may further comprise minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the antibody.

[0196] In a further embodiment, the pharmaceutical composition comprises at least one additional therapeutic agent for treating a disorder as disclosed herein.

[0197] Various delivery systems are known and can be used to administer one or more antibodies of the invention or the combination of one or more antibodies of the invention and a prophylactic agent or therapeutic agent useful for preventing, managing, treating, or ameliorating a disorder or one or more symptoms thereof, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the antibody or antibody fragment, receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of administering a prophylactic or therapeutic agent of the invention include, but are not limited to, parenteral administration (e.g., intradermal, intramuscular, intraperitoneal, intravenous and subcutaneous), epidurala administration, intratumoral administration, and mucosal administration (e.g., intranasal and oral routes). In addition, pulmonary administration can be employed, e.g.,

by use of an inhaler or nebulizer, and formulation with an aerosolizing agent. See, e.g., U.S. Pat. Nos. 6,019,968; 5,985,320; 5,985,309; 5,934,272; 5,874,064; 5,855,913; 5,290,540; and 4,880,078; and PCT Publication Nos. WO 92/19244; WO97/32572; WO97/44013; WO98/31346; and WO99/66903, each of which is incorporated herein by reference in their entireties. In one embodiment, an antibody of the invention, combination therapy, or a composition of the invention is administered using Alkermes AIR® pulmonary drug delivery technology (Alkermes, Inc., Cambridge, Mass.). In a specific embodiment, prophylactic or therapeutic agents of the invention are administered intramuscularly, intravenously, intratumorally, orally, intranasally, pulmonary, or subcutaneously. The prophylactic or therapeutic agents may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local.

[0198] In a specific embodiment, it may be desirable to administer the antibodies of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion, by injection, or by means of an implant, said implant being of a porous or non-porous material, including membranes and matrices, such as sialastic membranes, polymers, fibrous matrices (e.g., Tissuel®), or collagen matrices. In one embodiment, an effective amount of one or more antibodies of the invention is administered locally to the affected area to a subject to prevent, treat, manage, and/or ameliorate a disorder or a symptom thereof. In another embodiment, an effective amount of one or more antibodies of the invention is administered locally to the affected area in combination with an effective amount of one or more therapies (e.g., one or more prophylactic or therapeutic agents) other than an antibody of the invention of a subject to prevent, treat, manage, and/or ameliorate a disorder or one or more symptoms thereof.

[0199] In another embodiment, the antibody can be delivered in a controlled release or sustained release system. In one embodiment, a pump may be used to achieve controlled or sustained release (see Langer, supra; Sefton, 1987, CRC Crit. Ref. Biomed. Eng. 14:20; Buchwald et al., 1980, Surgery 88:507; Saudek et al., 1989, N. Engl. J. Med. 321:574). In another embodiment, polymeric materials can be used to achieve controlled or sustained release of the therapies of the invention (see e.g., Medical Applications of Controlled Release, Langer and Wise (eds.), CRC

Pres., Boca Raton, Fla. (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, 1983, J., Macromol. Sci. Rev. Macromol. Chem. 23:61; see also Levy et al., 1985, Science 228:190; During et al., 1989, Ann. Neurol. 25:351; Howard et al., 1989, J. Neurosurg. 7 1:105); U.S. Pat. No. 5,679,377; U.S. Pat. No. 5,916,597; U. S. Pat. No. 5,912,015; U.S. Pat. No. 5,989,463; U.S. Pat. No. 5,128,326; PCT Publication No. WO99/15154; and PCT Publication No. WO99/20253. Examples of polymers used in sustained release formulations include, but are not limited to, poly(2-hydroxy ethyl methacry-late), poly(methyl methacrylate), poly(acrylic acid), poly(ethylene-co-vinyl acetate), poly(methacrylic acid), polyglycolides (PLG), polyanhydrides, poly(N- vinyl pyrrolidone), poly(vinyl alcohol), polyacrylamide, poly(ethylene glycol), polylactides (PLA), poly(lactide-co-glycolides) (PLGA), and polyorthoesters. In a particular embodiment, the polymer used in a sustained release formulation is inert, free of leachable impurities, stable on storage, sterile, and biodegradable. In yet another embodiment, a controlled or sustained release system can be placed in proximity of the prophylactic or therapeutic target, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138 (1984)).

[0200] Controlled release systems are discussed in the review by Langer (1990, Science 249:1527-1533). Any technique known to one of skill in the art can be used to produce sustained release formulations comprising one or more antibodies of the invention. See, e.g., U. S. Pat. No. 4,526, 938, PCT publication WO91/05548, PCT publication WO96/20698, Ning et al., 1996, "Intratumoral Radioimmunotheraphy of a Human Colon Cancer Xenograft Using a Sustained-Release Gel," Radiotherapy &Oncology 39:179-189; Song et al., 1995, "Antibody Mediated Lung Targeting of Long- Circulating Emulsions," PDA Journal of Pharmaceutical Science & Technology 50:372-397; Cleek et al., 1997, "Biodegradable Polymeric Carriers for a bFGF Antibody for Cardiovascular Application," Pro. Int'l. Symp. Control. Rel. Bioact. Mater. 24:853-854; and Lam et al., 1997, "Microencapsulation of Recombinant Humanized Monoclonal Antibody for Local Delivery," Proc. Int'l. Symp. Control Rel. Bioact. Mater. 24:759- 760, each of which is incorporated herein by reference in their entireties.

[0201] In a specific embodiment, where the composition of the invention is a nucleic acid encoding an antibody, the nucleic acid can be administered in vivo to promote expression of its encoded antibody, by constructing it as part of an appropriate nucleic acid expression vector and

administering it so that it becomes intracellular, e.g., by use of a retroviral vector (see U. S. Pat. No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see, e.g., Joliot et al., 1991, Proc. Natl. Acad. Sci. USA 88:1864-1868). Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression by homologous recombination.

[0202] A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include, but are not limited to, parenteral, e.g., intravenous, intradermal, subcutaneous, oral, intranasal (e.g., inhalation), transdermal (e.g., topical), transmucosal, and rectal administration. In a specific embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous, subcutaneous, intramuscular, oral, intranasal, or topical administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection.

[0203] If the compositions of the invention are to be administered topically, the compositions can be formulated in the form of an ointment, cream, transdermal patch, lotion, gel, shampoo, spray, aerosol, solution, emulsion, or other form well-known to one of skill in the art. See, e.g., Remington's Pharmaceutical Sciences and Introduction to Pharmaceutical Dosage Forms, 19th ed., Mack Pub. Co., Easton, Pa. (1995). For non-sprayable topical dosage forms, viscous to semi-solid or solid forms comprising a carrier or one or more excipients compatible with topical application and having a dynamic viscosity greater than water are typically employed. Suitable formulations include, without limitation, solutions, suspensions, emulsions, creams, ointments, powders, liniments, salves, and the like, which are, if desired, sterilized or mixed with auxiliary agents (e.g., preservatives, stabilizers, wetting agents, buffers, or salts) for influencing various properties, such as, for example, osmotic pressure. Other suitable topical dosage forms include sprayable aerosol preparations wherein the active ingredient, for example in combination with a solid or liquid inert carrier, is packaged in a mixture with a pressurized volatile (e.g., a gaseous propellant, such as freon) or in a squeeze bottle. Moisturizers or humectants can also be added to

pharmaceutical compositions and dosage forms if desired. Examples of such additional ingredients are well-known in the art.

[0204] If the method of the invention comprises intranasal administration of a composition, the composition can be formulated in an aerosol form, spray, mist or in the form of drops. In particular, prophylactic or therapeutic agents for use according to the present invention can be conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebuliser, with the use of a suitable propellant (e.g., dichlorodifluoromethane, trichloro-fluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas). In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges (composed of, e.g., gelatin) for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

[0205] If the method of the invention comprises oral administration, compositions can be formulated orally in the form of tablets, capsules, cachets, gelcaps, solutions, suspensions, and the like. Tablets or capsules can be prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g., pregelatinised maize starch, polyvinylpyrrolidone, or hydroxypropyl methylcellulose); fillers (e.g., lactose, microcrystalline cellulose, or calcium hydrogen phosphate); lubricants (e.g., magnesium stearate, talc, or silica); disintegrants (e.g., potato starch or sodium starch glycolate); or wetting agents (e.g., sodium lauryl sulphate). The tablets may be coated by methods well-known in the art. Liquid preparations for oral administration may take the form of, but not limited to, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, cellulose derivatives, or hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., almond oil, oily esters, ethyl alcohol, or fractionated vegetable oils); and preservatives (e.g., methyl or propyl-p- hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring, and sweetening agents as appropriate. Preparations for oral administration may be suitably formulated for slow release, controlled release, or sustained release of a prophylactic or therapeutic agent(s).

[0206] The method of the invention may comprise pulmonary administration, e.g., by use of an inhaler or nebulizer, of a composition formulated with an aerosolizing agent. See, e.g., U.S. Pat. Nos. 6,019, 968; 5,985, 320; 5, 985,309; 5,934,272; 5,874,064; 5,855,913; 5,290,540; and 4,880,078; and PCT Publication Nos. WO 92/19244; WO 97/32572; WO 97/44013; WO 98/31346; and WO 99/66903, each of which is incorporated herein by reference their entireties. In a specific embodiment, an antibody of the invention, combination therapy, and/or composition of the invention is administered using Alkermes AIR® pulmonary drug delivery technology (Alkermes, Inc., Cambridge, Mass.).

[0207] The method of the invention may comprise administration of a composition formulated for parenteral administration by injection (e.g., by bolus injection or continuous infusion). Formulations for injection may be presented in unit dosage form (e.g., in ampoules or in multi-dose containers) with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle (e.g., sterile pyrogen-free water) before use. The methods of the invention may additionally comprise of administration of compositions formulated as depot preparations. Such long acting formulations may be administered by implantation (e.g., subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compositions may be formulated with suitable polymeric or hydrophobic materials (e.g., as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives (e.g., as a sparingly soluble salt).

[0208] The methods of the invention encompass administration of compositions formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

[0209] Generally, the ingredients of compositions are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the mode of administration is infusion, composition can be dispensed with

an infusion bottle containing sterile pharmaceutical grade water or saline. Where the mode of administration is by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[0210] In particular, the invention also provides that one or more of the antibodies, or pharmaceutical compositions, of the invention is packaged in a hermetically sealed container such as an ampoule or sachette indicating the quantity of the antibody. In one embodiment, one or more of the antibodies, or pharmaceutical compositions of the invention is supplied as a dry sterilized lyophilized powder or water free concentrate in a hermetically sealed container and can be reconstituted (e.g., with water or saline) to the appropriate concentration for administration to a subject. In one embodiment, one or more of the antibodies or pharmaceutical compositions of the invention is supplied as a dry sterile lyophilized powder in a hermetically sealed container at a unit dosage of at least 5 mg, for example at least 10 mg, at least 15 mg, at least 25 mg, at least 35 mg, at least 45 mg, at least 50 mg, at least 75 mg, or at least 100 mg. The lyophilized antibodies or pharmaceutical compositions of the invention should be stored at between 2°C and 8°C. in its original container and the antibodies, or pharmaceutical compositions of the invention should be administered within 1 week, for example within 5 days, within 72 hours, within 48 hours, within 24 hours, within 12 hours, within 6 hours, within 5 hours, within 3 hours, or within 1 hour after being reconstituted. In an alternative embodiment, one or more of the antibodies or pharmaceutical compositions of the invention is supplied in liquid form in a hermetically sealed container indicating the quantity and concentration of the antibody. In a further embodiment, the liquid form of the administered composition is supplied in a hermetically sealed container at least 0.25 mg/ml, for example at least 0.5 mg/ml, at least 1 mg/ml, at least 2.5 mg/ml, at least 5 mg/ml, at least 8 mg/ml, at least 10 mg/ml, at least 15 mg/ml, at least 25 mg/ml, at least 50 mg/ml, at least 75 mg/ml or at least 100 mg/ml. The liquid form should be stored at between 2°C and 8°C in its original container.

[0211] The antibodies of the invention can be incorporated into a pharmaceutical composition suitable for parenteral administration. In one aspect, antibodies will be prepared as an injectable solution containing 0.1-250 mg/ml antibody. The injectable solution can be composed of either a liquid or lyophilized dosage form in a flint or amber vial, ampule or pre-filled syringe. The buffer can be L-histidine (1-50 mM), optimally 5-10 mM, at pH 5.0 to 7.0 (optimally pH 6.0). Other suitable buffers include but are not limited to, sodium succinate, sodium citrate, sodium

phosphate or potassium phosphate. Sodium chloride can be used to modify the tonicity of the solution at a concentration of 0-300 mM (optimally 150 mM for a liquid dosage form). Cryoprotectants can be included for a lyophilized dosage form, principally 0-10% sucrose (optimally 0.5-1.0%). Other suitable cryoprotectants include trehalose and lactose. Bulking agents can be included for a lyophilized dosage form, principally 1-10% mannitol (optimally 2-4%). Stabilizers can be used in both liquid and lyophilized dosage forms, principally 1-50 mM L-Methionine (optimally 5-10 mM). Other suitable bulking agents include glycine, arginine, can be included as 0-0.05% polysorbate-80 (optimally 0.005-0.01%). Additional surfactants include but are not limited to polysorbate 20 and BRIJ surfactants. The pharmaceutical composition comprising the antibodies of the invention prepared as an injectable solution for parenteral administration, can further comprise an agent useful as an adjuvant, such as those used to increase the absorption, or dispersion of the antibody. A particularly useful adjuvant is hyaluronidase, such as Hylenex® (recombinant human hyaluronidase). Addition of hyaluronidase in the injectable solution improves human bioavailability following parenteral administration, particularly subcutaneous administration. It also allows for greater injection site volumes (i.e. greater than 1 ml) with less pain and discomfort, and minimum incidence of injection site reactions. (See International Appln. Publication No. WO 04/078140 and U.S. Patent Appln. Publication No. US2006104968, incorporated herein by reference.)

[0212] The compositions of this invention may be in a variety of forms. These include, for example, liquid, semi-solid and solid dosage forms, such as liquid solutions (e.g., injectable and infusible solutions), dispersions or suspensions, tablets, pills, powders, liposomes and suppositories. The preferred form depends on the intended mode of administration and therapeutic application. Compositions can be in the form of injectable or infusible solutions, such as compositions similar to those used for passive immunization of humans with other antibodies. In one embodiment, the antibody is administered by intravenous infusion or injection. In another embodiment, the antibody is administered by intramuscular or subcutaneous injection.

[0213] Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable to high drug concentration. Sterile injectable solutions can be prepared by incorporating the active compound (i.e., a binding protein, e.g. an antibody, of the present invention) in the required amount in an appropriate

solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile, lyophilized powders for the preparation of sterile injectable solutions, methods of preparation comprise vacuum drying and spray-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including, in the composition, an agent that delays absorption, for example, monostearate salts and gelatin.

[0214] The antibodies of the present invention can be administered by a variety of methods known in the art. For many therapeutic applications, the route/mode of administration may be subcutaneous injection, intravenous injection or infusion. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. In certain embodiments, the active compound may be prepared with a carrier that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art. See, e.g., Sustained and Controlled Release Drug Delivery Systems, J.R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

[0215] In certain embodiments, an antibody of the invention may be orally administered, for example, with an inert diluent or an assimilable edible carrier. The antibody (and other ingredients, if desired) may also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into the subject's diet. For oral therapeutic administration, the antibody may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. To administer an antibody of the invention by other than parenteral administration, it may be necessary to coat the antibody with, or co-administer the antibody with, a material to prevent its inactivation.

[0216] Supplementary active compounds can also be incorporated into the compositions. In certain embodiments, an antibody of the invention is coformulated with and/or coadministered with one or more additional therapeutic agents that are useful for treating disorders or diseases described herein. For example, an anti-RGMa antibody of the invention may be coformulated and/or coadministered with one or more additional antibodies that bind other targets (e.g., antibodies that bind other soluble antigens or that bind cell surface molecules). Furthermore, one or more antibodies of the invention may be used in combination with two or more of the foregoing therapeutic agents. Such combination therapies may advantageously utilize lower dosages of the administered therapeutic agents, thus avoiding possible toxicities or complications associated with the various monotherapies.

[0217] In certain embodiments, an antibody of the invention is linked to a half-life extending vehicle known in the art. Such vehicles include, but are not limited to, the Fc domain, polyethylene glycol, and dextran. Such vehicles are described, e.g., in U.S. Application Serial No. 09/428,082 and published PCT Application No. WO 99/25044, which are hereby incorporated by reference for any purpose.

[0218] In a specific embodiment, nucleic acid sequences comprising nucleotide sequences encoding an antibody of the invention are administered to treat, prevent, manage, or ameliorate a disorder or one or more symptoms thereof by way of gene therapy. Gene therapy refers to therapy performed by the administration to a subject of an expressed or expressible nucleic acid. In this embodiment of the invention, the nucleic acids produce their encoded antibody of the invention that mediates a prophylactic or therapeutic effect.

[0219] Any of the methods for gene therapy available in the art can be used according to the present invention. For general reviews of the methods of gene therapy, see Goldspiel et al., 1993, Clinical Pharmacy 12:488-505; Wu and Wu, 1991, Biotherapy 3:87-95; Tolstoshev, 1993, Ann. Rev. Pharmacol. Toxicol. 32:573-596; Mulligan, Science 260:926- 932 (1993); and Morgan and Anderson, 1993, Ann. Rev. Biochem. 62:191-217; May, 1993, TIBTECH 11(5):155-215. Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); and Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press,

NY (1990). Detailed description of various methods of gene therapy are disclosed in US20050042664 A1 which is incorporated herein by reference.

[0220] Antibodies of the invention can be used alone or in combination to treat diseases or conditions associated with neurite degeneration, such as multiple sclerosis, Alzheimer's disease, Down syndrome, dementia, Parkinson's disease, a traumatic injury to the central nervous system, or any other disease or condition associated with RGMa.

[0221] It should be understood that the antibodies of the invention can be used alone or in combination with one or more additional agents, e.g., a therapeutic agent (for example, a small molecule or biologic), said additional agent being selected by the skilled artisan for its intended purpose. For example, the additional therapeutic agent may be an immunosuppressant or an agent that treats one or more symptoms associated with multiple sclerosis. The additional drug may be a beta interferon. Beta interferons, such as Avonex, Betaseron, Extavia and Rebif, may slow the rate at which multiple sclerosis symptoms worsen over time. The additional agent may be Glatiramer (Copaxone), which may block the immune system's attack on myelin. The additional agent may be Fingolimod (Gilenya), which may trap immune cells in lymph nodes. The additional agent may be Natalizumab (Tysabri), which may interfere with the movement of potentially damaging immune cells from the bloodstream to the brain and spinal cord. The additional drug may be Mitoxantrone (Novantrone), which is an immunosuppressant drug.

[0222] The additional therapeutic agent can be a "cognitive enhancing drug," which is a drug that improves impaired human cognitive abilities of the brain (namely, thinking, learning, and memory). Cognitive enhancing drugs work by altering the availability of neurochemicals (e.g., neurotransmitters, enzymes, and hormones), by improving oxygen supply, by stimulating nerve growth, or by inhibiting nerve damage. Examples of cognitive enhancing drugs include a compound that increases the activity of acetylcholine such as, but not limited to, an acetylcholine receptor agonist (e.g., a nicotinic α-7 receptor agonist or allosteric modulator, an α4β2 nicotinic receptor agonist or allosteric modulators), an acetylcholinesterase inhibitor (e.g., donepezil, rivastigmine, and galantamine), a butyrylcholinesterase inhibitor, an N-methyl-D-aspartate (NMDA) receptor antagonist (e.g., memantine), an activity-dependent neuroprotective protein (ADNP) agonist, a serotonin 5-HT1A receptor agonist (e.g., xaliproden), a 5-HT4 receptor agonist, a 5-HT6 receptor antagonist, a serotonin 1A receptor antagonist, a histamine H3 receptor

antagonist, a calpain inhibitor, a vascular endothelial growth factor (VEGF) protein or agonist, a trophic growth factor, an anti-apoptotic compound, an AMPA-type glutamate receptor activator, a L-type or N-type calcium channel blocker or modulator, a potassium channel blocker, a hypoxia inducible factor (HIF) activator, a HIF prolyl 4-hydroxylase inhibitor, an anti-inflammatory agent, an inhibitor of amyloid Aβ peptide or amyloid plaque, an inhibitor of tau hyperphosphorylation, a phosphodiesterase 5 inhibitor (e.g., tadalafil, sildenafil), a phosphodiesterase 4 inhibitor, a monoamine oxidase inhibitor, or pharmaceutically acceptable salt thereof. Specific examples of such cognitive enhancing drugs include, but are not limited to, cholinesterase inhibitors such as donepezil (Aricept®), rivastigmine (Exelon®), galanthamine (Reminyl®), N-methyl-D-aspartate antagonists such as memantine (Namenda®). At least one cognitive enhancing drug can be administered simultaneously with the antibodies of the present invention or sequentially with the antibodies of the present invention (and in any order) including those agents currently recognized, or in the future being recognized, as useful to treat the disease or condition being treated by an antibody of the present invention). Additionally, it is believed that the combinations described herein may have additive or synergistic effects when used in the abovedescribed treatment. The additional agent also can be an agent that imparts a beneficial attribute to the therapeutic composition, e.g., an agent that affects the viscosity of the composition.

[0223] It should further be understood that the combinations are those combinations useful for their intended purpose. The agents set forth above are for illustrative purposes and not intended to be limiting. The combinations can comprise an antibody and at least one additional agent selected from the lists below. The combination can also include more than one additional agent, e.g., two or three additional agents if the combination is such that the formed composition can perform its intended function.

[0224] The pharmaceutical compositions may include a "therapeutically effective amount" or a "prophylactically effective amount" of an antibody. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of the antibody may be determined by a person skilled in the art and may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the antibody to elicit a desired response in the individual. A therapeutically effective amount is also one in which toxic or detrimental effects, if

any, of the antibody are outweighed by the therapeutically beneficial effects. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

[0225] Dosage regimens may be adjusted to provide the optimum desired response (e.g., a therapeutic or prophylactic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic or prophylactic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

[0226] An exemplary, non-limiting range for a therapeutically or prophylactically effective amount of the antibody is a dose of between 0.1 and 200 mg/kg, for example between 0.1 and 10 mg/kg. The therapeutically or prophylactically effective amount of the antibody may be between 1 and 200 mg/kg, 10 and 200 mg/kg, 20 and 200 mg/kg, 50 and 200 mg/kg, 75 and 200 mg/kg, 100 and 200 mg/kg, 150 and 200 mg/kg, 50 and 100 mg/kg, 5 and 10 mg/kg, or 1 and 10 mg/kg. It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated. Further, the antibody dose may be determined by a person skilled in the art and may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the antibody to elicit a desired response in the individual. The dose is also one in which toxic or detrimental effects, if any, of the antibody are outweighed by the therapeutically beneficial effects. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the

compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

4. Method of Treating, Preventing, Modulating or Attenuating a Disease Associated with Neurite Degeneration

[0227] In any subject, an assessment may be made as to whether the subject has a neurite degenerative disorder. The assessment may indicate an appropriate course of therapy, such as preventative therapy, maintenance therapy, or modulative therapy. Accordingly, provided herein is a method of treating, preventing, modulating, or attenuating a disease/disorder of neurite degeneration. The antibody may be administered to a subject in need thereof. The antibody may be administered in a therapeutically effective amount.

[0228] In general, the dosage of administered antibodies will vary depending upon such factors as the patient's age, weight, height, sex, general medical condition and previous medical history. Typically, it is desirable to provide the recipient with a dosage of antibody component, immunoconjugate or fusion protein which is in the range of from about 1 pg/kg to 10 mg/kg (amount of agent/body weight of patient), although a lower or higher dosage also may be administered as circumstances dictate. Dosage regimens may be adjusted to provide the optimum desired response (e.g., a therapeutic or prophylactic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be tested; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the present invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic or prophylactic effect to be achieved and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

[0229] An exemplary, non-limiting range for a therapeutically or prophylactically effective amount of an antibody or antibody portion of the invention is 0.1-20 mg/kg, more preferably 0.5-10 mg/kg. It is to be noted that dosage values may vary with the type and severity of the

condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

[0230] Administration of antibodies to a patient can be intravenous, intraarterial, intraperitoneal, intramuscular, subcutaneous, intrapleural, intrathecal, intraocular, intravitreal, by perfusion through a regional catheter, or by direct intralesional injection. When administering therapeutic proteins by injection, the administration may be by continuous infusion or by single or multiple boluses. Intravenous injection provides a useful mode of administration due to the thoroughness of the circulation in rapidly distributing antibodies. The antibody may be administered orally, for example, with an inert diluent or an assimilable edible carrier. The antibody and other ingredients, if desired, may be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, buccal tablets, troches, capsules, elixirs, sspensions, syrups, wafers, and the like.

[0231] Anti-RGMa antibodies may be administered at low protein doses, such as 20 milligrams to 2 grams protein per dose, given once, or repeatedly, parenterally. Alternatively, the antibodies may be administered in doses of 20 to 1000 milligrams protein per dose, or 20 to 500 milligrams protein per dose, or 20 to 100 milligrams protein per dose.

[0232] The antibodies may be administered alone or they may be conjugated to liposomes, and can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby the antibodies are combined in a mixture with a pharmaceutically acceptable carrier. A "pharmaceutically acceptable carrier" may be tolerated by a recipient patient. Sterile phosphate-buffered saline is one example of a pharmaceutically acceptable carrier. Other suitable carriers are well known to those in the art. See, for example, REMINGTON'S PHARMACEUTICAL SCIENCES, 19th Ed. (1995).

[0233] For purposes of therapy, antibodies are administered to a patient in a therapeutically effective amount in a pharmaceutically acceptable carrier. A "therapeutically effective amount" is one that is physiologically significant. The antibody is physiologically significant if its presence results in a detectable change in the physiology of a recipient patient. In the present context, the antibody may be physiologically significant if its presence results in, for example,

decreased interferon-γ (INF- γ), interleukin-2 (IL-2), IL-4 and/or IL-17 secretion from $CD4^+$ T cells. An agent is physiologically significant if its presence results in, for example, reduced proliferative responses and/or pro-inflammatory cytokine expression in peripheral blood mononuclear cells (PBMCs).

[0234] Additional treatment methods may be employed to control the duration of action of an antibody in a therapeutic application. Control release preparations can be prepared through the use of polymers to complex or adsorb the antibody. For example, biocompatible polymers include matrices of poly(ethylene-co-vinyl acetate) and matrices of a polyanhydride copolymer of a stearic acid dimer and sebacic acid. Sherwood et al., Bio/Technology 10:1446 (1992). The rate of release of an antibody from such a matrix depends upon the molecular weight of the protein, the amount of antibody within the matrix, and the size of dispersed particles. Saltzman et al., Biophys. J. 55:163 (1989); Sherwood et al., supra. Other solid dosage forms are described in REMINGTON'S PHARMACEUTICAL SCIENCES, 19th ed. (1995).

a. Neurite Degenerative Disorders/Diseases

[0235] The neurite disorder/disease may be any disease or disorder in which there is neurite damage and compromised synaptic function. This damage and compromised function may result from nerves lacking sufficient myelination and/or axon transection. The neurite degenerative disorder or disease may be multiple sclerosis, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis and other motoneuron diseases, huntington's disease, Tay-Sachs disease, Niemann-Pick disease, Gaucher's disease, Hurler's syndrome, idiopathic inflammatory demyelinating diseases, vitamin B12 deficiency, central pontine myelinolysis, tabes dorsalis, transverse myelitis, Devic's disease, progressive multifocal leukoencephalopathy, optic neuritis and other retinopathies associated with neurite degeneration, such as glaucoma, diabetic neuropathy and age-dependent macular degeneration, traumatic injury to the central nervous system, or leukodystrophies for example. The neurite degenerative disorder or disease may result from nerve fibers lacking sufficient wrapping of layers of tissue composed of a fat (lipoprotein) called myelin. These layers form the myelin sheath. The myelin sheath may enable electrical impulses to be conducted along the nerve fiber with speed and accuracy. When the myelin sheath is damaged or is missing, nerves do not conduct electrical impulses normally. Sometimes, as a result of the damaged or missing myelin sheath, the nerve fibers may also be damaged.

[0236] Young infants may normally lack mature myelin sheaths. As a result, their movements are jerky, uncoordinated, and awkward. As myelin sheaths develop, movements become smoother, more purposeful, and more coordinated. However, myelin sheaths do not develop normally in children with certain diseases, such as Tay-Sachs disease, Niemann-Pick disease, Gaucher's disease, and Hurler's syndrome.

[0237] In adults, the myelin sheath can be destroyed by stroke, inflammation, immune disorders, metabolic disorders, and nutritional deficiencies (such as a lack of vitamin B12). Poisons, drugs (such as the antibiotic ethambutol), and excessive use of alcohol can damage or destroy the myelin sheath. If the sheath is able to repair and regenerate itself, normal nerve function may return. However, if the sheath is severely damaged, the underlying nerve fiber can die. Because nerve fibers in the central nervous system (brain and spinal cord) rarely regenerate, such damage is irreversible.

[0238] Some neurite degenerative disorders that cause demyelination affect mainly the central nervous system. Others affect mainly nerves in other parts of the body. Neurite degenerative disorders that cause demyelination in the central nervous system and have no known cause are called primary demyelinating disorders. Multiple sclerosis is the most common of these disorders.

(1) Multiple Sclerosis

[0239] The clinical course of MS may be divided into four major categories (or subtypes): relapsing remitting (RRMS), secondary progressive (SPMS), primary progressive (PMS) and progressive relapsing (PRMS). Patients who have clinical relapses every few months or years with intervening periods of clinically stability define RRMS. RRMS may be twice more common in females than males in the second or third decade of life. In contrast to RRMS, patients with SPMS display progressive deterioration between relapses. RRMS patients may convert to SPMS over time characterized by a gradual decline in neurological function. Approximately 15% of MS patients have PPMS characterized by late onset and an unrelenting deterioration of neurological function from disease onset. Benign MS is arbitrarily defined as those RRMS patients who after more than 15 years following initial diagnosis are still mobile and show only mild deficits (Expanded Disability Status Scale [EDSS]). Typically, these patients

show little or no progression after their initial attack and require no therapeutic intervention; however, it is not possible to diagnose this form of MS until 5 years from MS onset.

(2) Parkinson's Disease

[0240] Parkinson's disease is widespread throughout the Western hemisphere and was first reported by physician James Parkinson in 1817. Parkinson's disease may be first detected as a tremor in a limb, and may ultimately progress to include three other manifestations: (i) rigidity, which is characterized by "cog wheel" like movement and "lead pipe" rigidity; (ii) bradykinesia or slowness in movement, and (iii) postural instability associated with a stooped stance and an impaired gait. These altered movements are features of a motor dysfunction, but in addition there can also be a mental impairment in as many as 40% of all Parkinson's patients.

[0241] Parkinson's disease may be caused by a deficient state of levodopamine in the brain. More specifically, levodopamine may induce dyskinesis in Parkinson's patients and result of denervation of the substantia nigra. To date, medical science has not found a substrate that would allow an injectable form of levodopa to reach the brain and successfully cross the blood brain barrier. The current dopamine replacement therapy is aimed at either direct replacement or mimicking the action at the dopamine receptor sites in the brain. While the levodopa therapy may create some beneficial changes initially, those changes may wane over time, and produce other problems such as severe sleep disturbance, dyskinesias, and constant nausea. Medical approaches to Parkinson's disease include surgical destruction of the tissue of the brain and the insertion of microelectrodes (deep brain electrical stimulation) to affected portions of the brain. The insertion of electrodes has the advantage of being reversible. These interventions, however, are generally transient and neither produces a permanent change in the Parkinsonian state nor reverses the effects of the disease.

[0242] Parkinson's disease may be a multifactor, neurodegenerative disorder, which evolves due to excessive oxidation. The substantia nigra is susceptible to oxidative damage which supports this theory of the formation of Parkinson's disease. Abnormalities of the oxidative phosphorylation impair the mitochondria of the substantia nigra, and intensify free radical generation.

(3) Injury from Reactive Oxygen Species (Free Radicals)

[0243] Reactive oxygen species (ROS) may attack several types of tissues and chronic exposure to ROS may attenuate various biological functions and increase the risk of several types of serious disorders, including neurite degenerative disorders and diseases. ROS can attack neurons and induce cell death. For example, treatment of neurons with low concentrations of hydrogen peroxide may induce neurite injury by influencing detrimental changes in neurite morphology, sometimes called neurite beading. Neurite beading may be one of the early events of neuronal degeneration prior to induction of death of hydrogen peroxide-treated neurons.

(4) Alzheimer's Disease

[0244] Alzheimer's disease (AD) is the major cause of dementia in the elderly. Although rare genetic forms of AD exist, most patients are classified as having sporadic AD, since no family history is usually identified. Pathologically, AD is characterized by neuronal and synaptic degeneration with an increased number of senile plaques and neurofibrillary tangles compared to non-demented individuals of comparable age.

[0245] The senile plaques, characteristic of Alzheimer's disease, are composed of a central core of aggregated beta-amyloid, a breakdown product of amyloid precursor protein (APP). The neurofibrillary tangles are insoluble intracellular thread-like structures made up of a hyperphosphorylated form of a protein called tau, which is associated with microtubules.

[0246] Post-mortem slices of brain tissue of victims of Alzheimer's disease exhibit the presence of amyloid in the form of proteinaceous extracellular cores of the neuritic plaques that are characteristic of AD. The amyloid cores of these neuritic plaques are composed of a protein called β-amyloid that is arranged in a predominately beta-pleated sheet configuration. Mori et al., Journal of Biological Chemistry 267: 17082 (1992); Kirschner et al., PNAS 83: 503 (1986). Neuritic plaques are an early and invariant aspect of the disease. Mann et al., J. Neurol. Sci. 89: 169; Mann, Mech. Ageing Dev. 31: 213 (1985); Terry et al., J. Neuropathol. Exp. Neurol 46: 262 (1987).

[0247] The initial deposition of Aβ may occur before clinical symptoms are noticeable. The currently recommended "minimum microscopic criteria" for the diagnosis of AD is based on the number of neuritic plaques found in brain. Khachaturian, Arch. Neurol., supra (1985). Unfortunately, assessment of neuritic plaque counts must be delayed until after death.

[0248] Amyloid-containing neuritic plaques are a prominent feature of selective areas of the brain in AD as well as Down's Syndrome and in persons homozygous for the apolipoprotein E4 allele who are very likely to develop AD. Corder et al., Science 261: 921 (1993); Divry, P., J. Neurol. Psych. 27: 643-657 (1927); Wisniewski et al., in Zimmerman, H. M. (ed.): PROGRESS IN NEUROPATHOLOGY (Grune and Stratton, N.Y. 1973) pp. 1-26. Brain amyloid is readily demonstrated by staining brain sections with thioflavin S or Congo red. Puchtler et al., J. Histochem. Cytochem. 10: 35 (1962). Congo red stained amyloid is characterized by a dichroic appearance, exhibiting a yellow-green polarization color. The dichroic binding is the result of the beta-pleated sheet structure of the amyloid proteins. Glenner, G. N. Eng. J. Med. 302: 1283 (1980). A detailed discussion of the biochemistry and histochemistry of amyloid can be found in Glenner, N. Eng. J. Med., 302: 1333 (1980).

(5) Traumatic Injury to Central Nervous System

[0249] The incidence of traumatic brain injury (TBI) in the United States is conservatively estimated to be more than 2 million persons annually with approximately 500,000 hospitalizations. Of these, about 70,000 to 90,000 head injury survivors are permanently disabled.

[0250] Neural pathways in the central nervous system of a subject are at risk if neurons are subjected to mechanical or chemical trauma or to neuropathic degeneration sufficient to put neurons at risk of dying. A host of neuropathies, some of which affect only a subpopulation or a system of neurons in the peripheral or central nervous systems have been identified to date. The neuropathies, which may affect the neurons themselves or the associated glial cells, may result from cellular metabolic dysfunction, infection, exposure to toxic agents, autoimmunity dysfunction, malnutrition or ischemia. In some cases the cellular dysfunction is thought to induce cell death directly. In other cases, the neuropathy may induce sufficient tissue necrosis to stimulate the body's immune/inflammatory system and the mechanisms of the body's immune response to the initial neural injury then destroys the neurons and the pathway defined by these neurons.

b. Subject

[0251] The subject may be a mammal, which may be a human. The subject may have, or be at risk of developing a neurite degenerative disorder or disease. The subject may already be undergoing treatment for a neurite degenerative disorder or disease.

5. Method of Diagnosis

[0252] Provided herein is a method for determining whether a subject has neurite degenerative disease or disorder. The level of membrane associated RGMa may be measured and compared to a level of RGMa in a control sample. The control sample may be from a normal tissue. An altered level of RGMa as compared to the control may indicate that the subject has a neurite degenerative disease or disorder. For example, an increased level of RGMa, as compared to a normal control, may indicate that the subject has a neurite degenerative disease or disorder. The level of RGMa may be measured using the herein described antibodies.

a. Sample

[0253] The sample may be any tissue sample from the subject. The sample may comprise protein from the subject. The sample may be blood serum, plasma, or a tissue biopsy. The sample may be used directly as obtained from the subject or following pretreatment to modify a characteristic of the sample. Pretreatment may include extraction, concentration, inactivation of interfering components, and/or the addition of reagents.

[0254] Any cell type, tisse, or bodily fluid may be utilized to obtain a sample. Such cell types, tissues, and fluid may include sections of tissues such as biopsy and autopsy samples, frozen sections taken for histologic purposes, blood, plasma, serum sputum, stool, tears, mucus, saliva, hair, and skin. Cell types and tissues may also include lymph fluid, ascetic fluid, gynecological fluid, urine, peritoneal fluid, cerebrospinal fluid, a fluid collected by vaginal rinsing, or a fluid collected by vaginal flushing. A tissue or cell type may be provided by removing a sample of cells from an animal, but can also be accomplished by using previously isolated cells (e.g. isolated by another person, at another time, and/or for another purpose). Archival tissues, such as those having treatment or outcome history, may also be used. Protein purification may not be necessary.

b. RGMa Detection

[0255] The presence or amount of RGMa present in a body sample may be readily determined by, for example, mass spectrometry, immunoassays or immunohistochemistry (e.g. with sections from tissue biopsies) using the herein described antibodies (monoclonal or polyclonal) or fragments thereof against RGMa. Anti-RGMa antibodies and fragments thereof can be produced as described above. Other methods of detection include those described in, for example, U.S. Patent Nos. 6,143,576; 6,113,855; 6,019,944; 5,985,579; 5,947,124; 5,939,272; 5,922,615; 5,885,527; 5,851,776; 5,824,799; 5,679,526; 5,525,524; and 5,480,792, each of which is hereby incorporated by reference in its entirety.

(1) Immunoassay

[0256] RGMa, and/or peptides thereof, may be analyzed using an immunoassay. The presence or amount of RGMa can be determined using the herein described antibodies and detecting specific binding to RGMa. For example, the antibody, or fragment thereof, may specifically bind to a polypeptide comprising SEQ ID NO:65, or a fragment thereof. The antibody, or fragment thereof, may specifically bind to a polypeptide comprising SEQ ID NO:66, or a fragment thereof.

[0257] Any immunoassay may be utilized. The immunoassay may be an enzyme-linked immunoassay (ELISA), radioimmunoassay (RIA), a competitive inhibition assay, such as forward or reverse competitive inhibition assays, a fluorescence polarization assay, or a competitive binding assay, for example. The ELISA may be a sandwich ELISA. Specific immunological binding of the antibody to the RGMa can be detected via direct labels, such as fluorescent or luminescent tags, metals and radionuclides attached to the antibody or via indirect labels, such as alkaline phosphatase or horseradish peroxidase.

[0258] The use of immobilized antibodies or fragments thereof may be incorporated into the immunoassay. The antibodies may be immobilized onto a variety of supports, such as magnetic or chromatographic matrix particles, the surface of an assay plate (such as microtiter wells), pieces of a solid substrate material, and the like. An assay strip can be prepared by coating the antibody or plurality of antibodies in an array on a solid support. This strip can then be dipped into the test biological sample and then processed quickly through washes and detection steps to generate a measurable signal, such as a colored spot.

(a) Sandwich ELISA

[0259] The Sandwich ELISA measures the amount of antigen between two layers of antibodies (i.e. capture and a detection antibody). The RGMa to be measured may contain at least two antigenic sites capable of binding to antibody. Either monoclonal or polyclonal antibodies may be used as the capture and detection antibodies in the sandwich ELISA.

[0260] Generally, at least two antibodies are employed to separate and quantify RGMa or RGMa fragment in a test sample. More specifically, the at least two antibodies bind to certain epitopes of RGMa or RGMa fragment forming an immune complex which is referred to as a "sandwich". One or more antibodies can be used to capture the RGMa or RGMa fragment in the test sample (these antibodies are frequently referred to as a "capture" antibody or "capture" antibodies) and one or more antibodies is used to bind a detectable (namely, quantifiable) label to the sandwich (these antibodies are frequently referred to as the "detection" antibody or "detection" antibodies). In a sandwich assay, both antibodies binding to their epitope may not be diminished by the binding of any other antibody in the assay to its respective epitope. In other words, antibodies may be selected so that the one or more first antibodies brought into contact with a test sample suspected of containing RGMa or RGMa fragment do not bind to all or part of an epitope recognized by the second or subsequent antibodies, thereby interfering with the ability of the one or more second detection antibodies to bind to the RGMa or RGMa fragment.

[0261] The antibodies may be used as a first antibody in said immunoassay. Preferably, the antibody immunospecifically binds to epitopes comprising at least three contiguous (3) amino acids of SEQ ID NOs:65, 66 or 74. In addition to the antibodies of the present invention, said immunoassay may comprise a second antibody that immunospecifically binds to epitopes having an amino acid sequence comprising at least three contiguous (3) amino acids of SEQ ID NO:65, 66 or 74, wherein the contiguous (3) amino acids to which the second antibody binds is different from the contiguous (3) amino acids to which the first antibody binds.

[0262] In a preferred embodiment, a test sample suspected of containing RGMa or RGMa fragment can be contacted with at least one first capture antibody (or antibodies) and at least one second detection antibodies either simultaneously or sequentially. In the sandwich assay format, a test sample suspected of containing RGMa or RGMa fragment is first brought into contact with the at least one first capture antibody that specifically binds to a particular epitope under

conditions which allow the formation of a first antibody-RGMa complex. If more than one capture antibody is used, a first multiple capture antibody-RGMa complex is formed. In a sandwich assay, the antibodies, preferably, the at least one capture antibody, are used in molar excess amounts of the maximum amount of RGMa or RGMa fragment expected in the test sample. For example, from about 5 µg/ml to about 1 mg/ml of antibody per ml of microparticle coating buffer may be used.

[0263] Optionally, prior to contacting the test sample with the at least one first capture antibody, the at least one first capture antibody can be bound to a solid support which facilitates the separation the first antibody-RGMa complex from the test sample. Any solid support known in the art can be used, including but not limited to, solid supports made out of polymeric materials in the forms of wells, tubes or beads. The antibody (or antibodies) can be bound to the solid support by adsorption, by covalent bonding using a chemical coupling agent or by other means known in the art, provided that such binding does not interfere with the ability of the antibody to bind RGMa or RGMa fragment. Moreover, if necessary, the solid support can be derivatized to allow reactivity with various functional groups on the antibody. Such derivatization requires the use of certain coupling agents such as, but not limited to, maleic anhydride, N-hydroxysuccinimide and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide.

[0264] After the test sample suspected of containing RGMa or RGMa fragment is brought into contact with the at least one first capture antibody, the test sample is incubated in order to allow for the formation of a first capture antibody (or multiple antibody)-RGMa complex. The incubation can be carried out at a pH of from about 4.5 to about 10.0, at a temperature of from about 2°C to about 45°C, and for a period from at least about one (1) minute to about eighteen (18) hours, from about 2-6 minutes, or from about 3-4 minutes.

[0265] After formation of the first/multiple capture antibody-RGMa complex, the complex is then contacted with at least one second detection antibody (under conditions which allow for the formation of a first/multiple antibody-RGMa-second antibody complex). If the first antibody-RGMa complex is contacted with more than one detection antibody, then a first/multiple capture antibody-RGMa-multiple antibody detection complex is formed. As with first antibody, when the at least second (and subsequent) antibody is brought into contact with the first antibody-RGMa complex, a period of incubation under conditions similar to those described above is

required for the formation of the first/multiple antibody-RGMa-second/multiple antibody complex. Preferably, at least one second antibody contains a detectable label. The detectable label can be bound to the at least one second antibody prior to, simultaneously with or after the formation of the first/multiple antibody-RGMa-second/multiple antibody complex. Any detectable label known in the art can be used.

(b) Forward Competitive Inhibition

[0266] In a forward competitive format, an aliquot of labeled RGMa, RGMa fragment or RGMa variant thereof of a known concentration is used to compete with RGMa or RGMa fragment in a test sample for binding to RGMa antibody (such as an antibody of the present invention).

[0267] In a forward competition assay, an immobilized antibody (such as an antibody of the present invention) can either be sequentially or simultaneously contacted with the test sample and a labeled RGMa, RGMa fragment or RGMa variant thereof. The RGMa peptide, RGMa fragment or RGMa variant can be labeled with any detectable label, including those detectable labels discussed above in connection with the anti-RGMa antibodies. In this assay, the antibody can be immobilized on to a solid support. Alternatively, the antibody can be coupled to an antibody, such as an antispecies antibody, that has been immobilized on to a solid support, such as a microparticle.

[0268] The labeled RGMa peptide, RGMa fragment or RGMa variant, the test sample and the antibody are incubated under conditions similar to those described above in connection with the sandwich assay format. Two different species of antibody-RGMa complexes may then be generated. Specifically, one of the antibody-RGMa complexes generated contains a detectable label while the other antibody-RGMa complex does not contain a detectable label. The antibody-RGMa complex can be, but does not have to be, separated from the remainder of the test sample prior to quantification of the detectable label. Regardless of whether the antibody-RGMa complex is separated from the remainder of the test sample, the amount of detectable label in the antibody-RGMa complex is then quantified. The concentration of RGMa or RGMa fragment in the test sample can then be determined by comparing the quantity of detectable label in the antibody-RGMa complex to a standard curve. The standard curve can be generated using serial dilutions of RGMa or RGMa fragment of known concentration, by mass spectrometry, gravimetrically and by other techniques known in the art.

[0269] The antibody-RGMa complex can be separated from the test sample by binding the antibody to a solid support, such as the solid supports discussed above in connection with the sandwich assay format, and then removing the remainder of the test sample from contact with the solid support.

(c) Reverse Competition Assay

[0270] In a reverse competition assay, an immobilized RGMa peptide, RGMa fragment or RGMa variant thereof can either be sequentially or simultaneously contacted with a test sample and at least one labeled antibody. Preferably, the antibody specifically binds to an epitope having an amino acid sequence comprising at least three contiguous (3) amino acids of SEQ ID NO:65 or 66. The RGMa peptide, RGMa fragment or RGMa variant can be bound to a solid support, such as the solid supports discussed above in connection with the sandwich assay format. The RGMa peptide fragment may have an amino acid sequence that contains SEQ ID NO:65 or 66.

[0271] The immobilized RGMa peptide, RGMa peptide fragment or RGMa variant thereof, test sample and at least one labeled antibody are incubated under conditions similar to those described above in connection with the sandwich assay format. Two different species RGMa-antibody complexes are then generated. Specifically, one of the RGMa-antibody complexes generated is immobilized and contains a detectable label while the other RGMa-antibody complex is not immobilized and contains a detectable label. The non-immobilized RGMa-antibody complex and the remainder of the test sample are removed from the presence of the immobilized RGMa-antibody complex through techniques known in the art, such as washing. Once the non-immobilized RGMa antibody complex is removed, the amount of detectable label in the immobilized RGMa-antibody complex is then quantified. The concentration of RGMa or RGMa fragment in the test sample can then be determined by comparing the quantity of detectable label in the RGMa-complex to a standard curve. The standard curve can be generated using serial dilutions of RGMa or RGMa fragment of known concentration, by mass spectrometry, gravimetrically and by other techniques known in the art.

(d) Fluorescence Polarization

[0272] In a fluorescence polarization assay, an antibody or functionally active fragment thereof may first contacted with an unlabeled test sample suspected of containing RGMa or a RGMa fragment thereof to form an unlabeled RGMa-antibody complex. The unlabeled RGMa-antibody

complex is then contacted with a fluorescently labeled RGMa, RGMa fragment or RGMa variant thereof. The labeled RGMa, RGMa fragment or RGMa variant competes with any unlabeled RGMa or RGMa fragment in the test sample for binding to the antibody or functionally active fragment thereof. The amount of labeled RGMa-antibody complex formed is determined and the amount of RGMa in the test sample determined via use of a standard curve.

[0273] The antibody used in a fluorescence polarization assay specifically binds to an epitope having an amino acid sequence comprising at least three (3) amino acids from SEQ ID NO:65 or SEQ ID NO:66 or SEQ ID NO:74.

[0274] The antibody, labeled RGMa peptide, RGMa peptide fragment or RGMa variant thereof and test sample and at least one labeled antibody may be incubated under conditions similar to those described above in connection with the sandwich immunoassay.

[0275] Alternatively, an antibody or functionally active fragment thereof may be simultaneously contacted with a fluorescently labeled RGMa, RGMa fragment or RGMa variant thereof and an unlabeled test sample suspected of containing RGMa or RGMa fragment thereof to form both labeled RGMa-antibody complexes and unlabeled RGMa-antibody complexes. The amount of labeled RGMa-antibody complex formed is determined and the amount of RGMa in the test sample determined via use of a standard curve.

[0276] Alternatively, an antibody or functionally active fragment thereof is first contacted with a fluorescently labeled RGMa, RGMa fragment or RGMa variant thereof to form a labeled RGMa-antibody complex. The labeled BNP-antibody complex is then contacted with an unlabeled test sample suspected of containing RGMa or an RGMa fragment thereof. Any unlabeled RGMa or RGMa fragment in the test sample competes with the labeled RGMa, RGMa fragment or RGMa variantfor binding to the antibody or functionally active fragment thereof. The amount of labeled RGMa-antibody complex formed is determined the amount of RGMa in the test sample determined via use of a standard curve. The antibody used in this immunoassay specifically binds to an epitope having an amino acid sequence comprising at least three contiguous (3) amino acids of SEQ ID NOs:65, 66 or 74.

(e) Mass Spectrometry

[0277] Mass spectrometry (MS) analysis may be used alone or in combination with other methods. Other methods include immunoassays and those described above to detect specific

polynucleotides. The mass spectrometry method may be used to determine the presence and/or quantity of one or more biomarkers. MS analysis may comprise matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF) MS analysis, such as, for example, directed –spot MALDI-TOF or liquid chromatography MALDI-TOF mass spectrometry analysis. In some embodiments, the MS analysis comprises electrospray ionization (ESI) MS, such as liquid chromatography (LC) ESI-MS. Mass analysis can be accomplished using commercially-available spectrometers. Methods for utilizing MS analysis, including MALDI-TOF MS and ESI-MS, to detect the presence and quantity of biomarker peptides in biological samples may be used. See, for example, U.S. Patent Nos. 6,925,389; 6,989,100; and 6,890,763 for guidance, each of which is incorporated herein by reference.

c. Control

[0278] It may be desirable to include a control sample. The control sample may be analyzed concurrently with the sample from the subject as described above. The results obtained from the subject sample can be compared to the results obtained from the control sample. Standard curves may be provided, with which assay results for the biological sample may be compared. Such standard curves present levels of marker as a function of assay units, i.e. fluorescent signal intensity, if a fluorescent label is used. Using samples taken from multiple donors, standard curves can be provided for control levels of the RGMa in normal tissue, as well as for "at-risk" levels of the RGMa in tissue taken from donors, who may have one or more of the characteristics set forth above.

6. Kit

[0279] Provided herein is a kit, which may be used for treating or diagnosing a subject. The kit may comprise the antibody and a means for administering the antibody. The kit can further comprise instructions for using the kit and conducting the analysis, monitoring, or treatment.

[0280] The kit may also comprise one or more containers, such as vials or bottles, with each container containing a separate reagent. The kit may further comprise written instructions, which may describe how to perform or interpret an analysis, monitoring, treatment, or method described herein.

[0281] The present invention has multiple aspects, illustrated by the following non-limiting examples.

EXAMPLES

Example 1

Anti-RGMa Human Monoclonal Antibody Production and Isolation

[0282] Using PROfusion mRNA display technology, pooled human spleen, tonsil, PBMC and lymph node antibody libraries were selected through eight rounds against RGMa antigens: 100nM biotin-labeled human or rat RGMa. PROfusion technology is described in U.S. Patent Application Publication Nos: 20100099103 and 20100105569, the contents each of which are herein incorporated by reference. Selected sc-Fv fragments were reformatted into fully human IgGs. Following screening of IgGs in RGMa-based ELISAs, AE12-1 through AE12-8 were identified as positive binders to human and rat RGMa.

[0283] Antibodies AE12-13, AE12-15, AE12-20, AE12-21, AE12-23, and AE12-24 are fully human anti-RGMa antibodes identified from large naïve human scFv yeast libraries selected against human RGMa using standard yeast display technologies. 2 rounds of Magnetic-activated cell sorting (MACS) and 4 rounds of Fluorescence-activated cell sorting (FACS) were performed on the libraries using 100 nM biotinylated human RGMa as the selection antigen. For the last round of sorting, cells were also negatively selected against a human RGMc-Fc antigen. Selected sc-Fv fragments were reformatted into fully human IgGs. Following screening of IgGs in human RGMa ELISA, AE12-13, -15, -20, -21, -23, and -24 were identified as positive binders to human RGMa. AE12-13, AE12-15 and AE12-23 also cross-reacted to human RGMc as evaluated by ELISA.

Example 2

Antibody Characterization

[0284] The 8 PROfusion mAbs (AE12-1, AE12-2, AE12-3, AE12-4, AE12-5, AE12-6, AE12-7, and AE12-8), were tested by direct binding ELISAs to examine binding to human RGMa (hRGMa) and rat RGMa and cross-reactivity to hRGMc. hRGMa competition ELISA was employed to test if any of these mAbs would compete h5F9.23 for binding to hRGMa. h5F9.23 is a humanized anti-RGMa lead mAb derived from rat hybridoma and known to bind the N-terminal domain of RGMa. H5F9.23 has the following sequences:

VH h5F9.23	151	EVQLVESGGGLVQPGGSLRLSCAASGFTFS NYGMNWIRQAPGKGLEWIGMIYYDSSEKHYA DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAV YYCAKGTTPDYWGQGTMVTVSS
VL h5F9.23	152	DVVLTQSPLSLPVTLGQPASISCRSSQSLEY SDGYTFLEWFQQRPGQSPRLLIYEVSNRFSG VPDRFSGSGSGTDFTLKISRVEAEDVGVYY CFQATHDPLTFGQGTKLEIKR
VH h5F9.23 CDR-H1	153	NYGMN
VH h5F9.23 CDR-H2	154	MIYYDSSEKHYADSVKG
VH h5F9.23 CDR-H3	155	GTTPDY
VL h5F9.23 CDR-L1	156	RSSQSLEYSDGYTFLE
VL h5F9.23 CDR-L2	157	EVSNRFS
VL h5F9.23 CDR-L3	158	FQATHDPLT

Neogenin or BMP-2/BMP-4 competition ELISA was employed to determine if these mAbs would block hRGMa binding to its receptor neogenin or BMP-2/BMP-4.

[0285] Based on ELISA data, all 8 PROfusion mAbs bound to human and rat RGMa (Table 3). For RGMc binding in ELISA, 3 mAbs (AE12-6, -7 and -8) showed binding to hRGMc, AE12-4 showed weak binding at high concentrations, and the other 4 mAbs (AE12-1, -2, -3 and -5) showed no binding to hRGMc at concentrations up to 100 nM. In hRGMa competition ELISA, AE12-1, AE12-3 and AE12-6 were able to compete with h5F9.23 in binding to hRGMa, suggesting that the binding epitopes of these 3 mAbs are near or overlap with h5F9.23 epitope. Dot blotting assay with hRGMa fragments showed that AE12-1 and AE12-6 bound the N-terminal fragment, AE12-2 and AE12-4 bound the C-terminal fragment, and the other 4 Abs did not show any detectable binding signal. For blocking hRGMa binding to neogenin in competition ELISA, only AE12-5 and AE12-6 showed blocking activity comparative to or better than h5F9.23, AE12-1 and AE12-4 showed weak inhibition, and AE12-2, -3, -7, and -8 showed no inhibition at concentrations up to 100 nM. In BMP-2/BMP-4 competition ELISA, only AE12-1, AE12-4 and AE12-6 blocked hRGMa binding to BMP-2/BMP-4.

[0286] The PROfusion mAbs were further tested in cell-based binding assays for their ability to block hRGMa binding to neuronal cells. In MSD-based cell binding assay in which cells were incubated with biotinylated hRGMa-Fc at room temperature and the hRGMa binding was detected by streptavidin-Sulfo-Tag, only AE12-1 and AE12-6 blocked hRGMa binding to human SH-SY5Y neuronal cells, similar to h5F9.23 (Table 3).

[0287] However, in a high content screening (HCS) assay, in which cells were incubated with hRGMa-Fc at 37°C and the hRGMa binding was detected by Cy3-labeled anti-Fc Ab and calculated with high content fluorescent image analysis, only AE12-6 among PROfusion antibodies showed strong inhibition on RGMa binding to both SH-SY5Y neuronal cells and rat hippocampal primary neurons (Table 3, Figure 13). Also shown in Figure 13, AE12-15 and AE12-23, the antibodies derived from naïve human scFv yeast libraries, inhibited hRGMa binding to cells.

[0288] The BMP responsive element (BRE) was constructed using overlapping oligos based on the sequence described by Korchynskyi and ten Dijke (J. Biol. Chem. 2002, 277:4883), and cloned into a basic luciferase reporter vector pGL4.27 [luc2P/minP/Hygro] (Promega) to generate a BRE luciferase reporter construct. RGM family members (RGMa, RGMb and RGMc) are coreceptors for BMP signaling. Both RGMa and RGMc BMP reporter assays were established by cotransfection of 293HEK cells with BMP reporter plasmid and RGMa or RGMc expression plasmid, and used to screen mAbs for neutralizing activity towards RGMa and RGMc. In RGMa BMP reporter assay, AE12-1 and AE12-6 neutralized RGMa activity, consistent with data from MSD-based cell binding assay (Table 3). In the RGMc BMP reporter assay, AE12-6 neutralized RGMc activity, whereas AE12-1 did not. Thus, AE12-1 is a neutralizing mAb specific to RGMa.

[0289] The PROfusion mAbs were further tested for their ability to neutralize RGMa in a chemotaxis assay with SH-SY5Y neuronal cells. In this assay, RGMa acts as a repulsive molecule to inhibit cell chemotaxis. AE12-1 showed strong neutralizing activity towards hRGMa (Table 3). AE12-4 and AE12-6 showed some neutralizing activity.

[0290] AE12-1 was tested in neurite outgrowth assay with human SH-SY5Y neuronal cells. In this assay, RGMa, either full-length or N-terminal fragment, inhibits neurite outgrowth. Consistent with its functional activity in RGMa BMP reporter assay and chemotaxis assay,

AE12-1 exhibited strong neutralizing activity towards either full-length hRGMa or N-terminal fragment (Table 3).

[0291] BIAcore analysis of AE12-1 on hRGMc and human, cynomolgus (cyno) monkey and rat RGMa demonstrated that AE12-1 did not bind hRGMc but exhibited good cross-reactivity to human, cyno and rat RGMa with comparable affinity. See Table 4.

Table 3

Clone→	AE12-1	AE12-2	AE12-3	AE12-4	AE12-5	AE12-6	AE12-7	AE12-8	h5F9.23
hRGMa binding (ELISA)	++	++	++	+	+	++	+	+	++
Rat RGMa binding (ELISA)	++	++	++	+	+	++	+	+	++
hRGMc-His ELISA	-	-	-	+/-	-	++	+	+	++
Compete with h5F9.23 for binding to hRGMa (ELISA)	+	-	+	-	-	+	-	-	
Mapping hRGMa fragments	N	C	[a]Neg	C	[a]Neg	N	[a]Neg	[a]Neg	N
Compete with biot-hRGMa-Fc for binding to neo-His (ELISA)	+/-	-	-	+/-	++	+++	-	-	++
[b] Block hRGMa-Fc binding to SH-SY5Y cells (MSD)	++	-	-	-	-	++	-	-	++
[c] Block hRGMa-Fc	[d](-)	-	-	-	+	+++	-	-	++

binding to SH-SY5Y cells (HCS)									
Compete with FL-RGMa-Fc for binding to BMP-2 (ELISA)	+++	-	-	++	-	++	-	-	++
Compete with FL-RGMa-Fc for binding to BMP-4 (ELISA)	+++	-	-	++	-	++	[e]?	-	++
Neutralize hRGMa in RGMa BMP reporter assay	++	-	-	-	-	++	-	-	++
Neutralize hRGMc in RGMc BMP reporter assay	-	-	-	-	-	++	-	-	++
Neuralize hRGMa in Chemotaxis with SH-SY5Y cells	+++	-	-	+	-	+	-	-	++
Neutralize hRGMa in neurite outgrowth assay	+++								++

[0292] With respect to Table 3, "[a]Neg" corresponds to negative binding with all fragments tested in dot blotting. "[b]MSD" corresponds to using biotinylated hRGMa-Fc complexed with streptavidin-Sulfo-Tag, and incubation with cells at room temperature (RT). "[c]HCS" corresponds to using hRGMa-Fc complexed with Cy3-labeled anti-Fc Ab, and inbation with cells at 37°C. "[d]AE12-1" corresponds to a dramatically enhanced RGMa-Fc binding to cells, in contrast to inhibiting biotin-RGMa-Fc binding to SH-SY5Y cells by MSD. "[e]?" corresponds to

data that is inconclusive for AE12-7. "[d]AE12-4" – the concentration of AE12-4 in the chemotaxis assay is inversely correlated with neutralizing activity.

[0293] In a BMP-responsive reporter assay in which RGMa or RGMc enhances BMP signaling by interacting with BMPs, an antibody comprising SEQ ID NOs:1 and 5 (AE12-1) blocked RGMa activity but not RGMc activity, consistent with its functional antagonism and binding specificity for RGMa.

[0294] Figures 13 and 14 illustrate the neutralizing effects of the specified antibodies on RGMa binding to neuronal cells using a live cell binding assay on SH-SY5Y cells and rat hippocampal primary neurons. Fc-tagged RGMa and Cy3-labeled anti-Fc antibodies are complexed at 4°C for 60 minutes, followed by an incubation of the complex with a blocking antibody at room temperature for 10 minutes. The RGMa-Cy3 + antibody complex is then added to cells together with Hoechsts 33342 for 30 minutes at 37°C to allow binding onto the cells. The cell are then washed twice in culture medium and fixed with PFH. Cell imaging is performed with the BD Pathway and images analyzed with the Definiens Architect software.

[0295] As mentioned above, AE12-6, AE12-15, and AE12-23 blocked binding of RGMa to SH-SY5Y cells and primary neurons. See Figure 13. In the HCS assay, AE12-1 did not inhibit binding of RGMa on SH-SY5Y cells. See Figure 14. The highest concentrations of AE12-1 enhanced RGMa-Fc binding to cells, whereas in the lower concentrations the levels were equal to control RGMa binding levels. This is in contrast to inhibiting biotin-RGMa-Fc binding to SH-SY5Y cells by MSD (MSD corresponds to using biotinylated hRGMa-Fc complexed with streptavidin-Sulfo-Tag, and incubation with cells at room temperature). The difference between the MSD and the HCS assay may be due to different assay conditions.

[0296] Figure 15 shows the neutralization effects on RGMa repulsion by r5F9 (control), AE12-1, and AE12-6 in a neurite outgrowth assay. 6500 rat hippocampal primary neurons per well were plated on poly-l-lysine coated 96-well imaging plates. The cells were treated for 24 hours with RGMa fragment 47-127 of SEQ ID NO:65 (SEQ ID NO:139) in combination with anti-RGMa antibodies. The cells were fixed and stained with BIII-tubulin using a neurite outgrowth kit protocol from Millipore. Images were acquired with a BD Pathway and were analyzed with Definiens Architect to measure the neurite outgrowth per neuron.

Example 3

Antibody Variants and Binding Data

[0297] Table 4 shows that by substituting for the Cys residue in AE12-1 VL CDR3 (SEQ ID NO:8), one can generate variants having improved affinity to hRGMa. See SEQ ID NOs:67-73. For example, see Table 4, wherein antibody clone AE12-1-Y showed at least a 10-fold increased affinity to hRGMa and AE12-1-F showed a 5-fold increased affinity to hRGMa. Others showed comparable affinity as the parental AE12-1. All variants blocked hRGMa binding to SH-SY5Y cells in MSD-based cell binding assay, neutralized RGMa but not RGMc activity in BMP reporter assays, and exhibited high thermal stability and good solubility in preformulation studies.

Table 4

Ab		AE12-1	AE12-1-F	AE12-1-H	AE12-1-L	AE12-1-V	AE12-1-I	AE12-1-K	AE12-1-Y
hRGMa binding (ELISA)		++	++	++	++	++	+++	+++	+++
Cyno RGMa binding (ELISA)		++	+++	+++	+++	+++	+++	+++	+++
hRGMa-His	ka (M-1s-1)	$3.1x10^4$	$2.7x10^4$	$3.2x10^4$	$3.8x10^4$	$2.5x10^5$	$3x10^4$	$3.4x10^4$	$2.2x10^4$
	kd (s-1)	$2.3x10^{-4}$	$3.9x10^{-5}$	$1.2x10^{-4}$	$2.5x10^{-4}$	$1.5x10^{-4}$	$1.3x10^{-4}$	$3.0x10^{-4}$	$1.3x10^{-5}$
	Kd (nM)	7.3	1.4	3.8	6.6	5.9	4.5	8.8	0.6
Cyno RGMa-His	ka (M-1s-1)	$1.9x10^5$	$4.4x10^4$	$4.7x10^4$	$6.2x10^4$	$1.1x10^5$	$9.9x10^4$	$5.1x10^4$	$4x10^4$
	kd (s-1)	$1.7x10^{-3}$	$2.5x10^{-4}$	$6.3x10^{-4}$	$6.7x10^{-4}$	$9.9x10^{-4}$	$8.7x10^{-4}$	$4.7x10^{-4}$	$2x10^{-4}$
	Kd (nM)	8.8	5.4	13.4	10.1	9.2	8.9	9.3	4.9
Rat RGMa-His	ka (M-1s-1)	$2.6x10^4$	$2.6x10^4$	$2.9x10^4$	$3.2x10^4$	$2.2x10^5$	$2.8x10^4$	$1.6x10^4$	$1.4x10^4$
	kd (s-1)	$4.8x10^{-4}$	$1.4x10^{-4}$	$2.5x10^{-4}$	$3.9x10^{-4}$	$3.2x10^{-4}$	$3.2x10^{-4}$	$2.8x10^{-4}$	$6.9x10^{-5}$
	Kd (nM)	19	5.2	8.6	12	15	12	17	5
hRGMc-His	BIAcore binding	-	-	-	-	-	-	-	-
Block hRGMa-Fc binding to SH-SY5Y cells (MSD)		++	++	++	++	++	++	++	++
Neutralize RGMa in BRE luc assay		++	++	++	++	++	++	++	++
Neutralize RGMc in BRE luc assay		-	-	-	-	-	-	-	-
Tier 1 solubility/stability		++	++	++	++	++	++	++	++

Example 4
Neurite Outgrowth

[0298] As shown in Figures 1, 2 and 15, AE12-1 compeletely neutralized full length hRGMa and a fragment of hRGMa, as shown on SH-SY5Y cells and rat hippocampal primary neurons. This fragment of hRGMa corresponds to amino acids 47-127 of SEQ ID NO:65 as shown here: PCKI LKCNSEFWSA TSGSHAPASD DTPEFCAALR SYALCTRRTA RTCRGDLAYH SAVHGIEDLM SQHNCSKDGP TSQPRLR (SEQ ID NO:139).

[0299] Further neurite outgrowth experiments were performed to assess the effects of AE12-1 as well as AE12-1 variants, wherein the antibody comprises SEQ ID NOs:1 and 5, or 2-4 and 6-8, wherein the Cys residue of SEQ ID NO:8 is substituted for another amino acid, or wherein the Cys residue at position 91 of SEQ ID NO:5 is substituted with another amino acid (i.e. AE12-1-F, AE12-1-H, AE12-1-L, AE12-1-V, AE12-1-I, AE12-1-K, and AE12-1-Y). See Figures 9-12, wherein inhibition by the described antibody (24 hours incubation) on neurite outgrowth of SH-SY5Y cells treated with FL hRGMa are shown.

Example 5
In Vivo Studies

[0300] As shown in Figures 3 and 4, AE12-1 enhanced regenerative growth of retinal ganglion cell axons perilesionally (0-500 μm) (n = 3-5 rats/group). See Figure 3. Antibody AE12-1 also enhanced regenerative growth of retinal ganglion cell axons into areas further away from the lesion (500-1000 μm) (n = 3-5 rats/group).

Example 6
Rat Optic Nerve Crush Experiments

[0301] AE12-1 was active in rat optic nerve crush experiments. See Figure 8. A unilateral optic nerve crush lesion was done in male Wistar rats, 2-4 mm behind the eye. Rats were followed for 6 weeks (8 groups, n = 6) and antibodies were administered once per week intravenously at either10 mg/kg, 1 mg/kg, or 0.1 mg/kg. The hIgG1 control was administered intravenously, once per week at 10 mg/kg (n = 6 rats). In AE12-1 treated rats regenerating fibers were able to grow

beyond the optic nerve crush lesion, whereas in control antibody hIgG1-treated rats, regenerating fibers accumulate at the lesion due to their inability to overcome the lesions. See Figure 8.

Example 7

Epitope Mapping of Human RGMa (hRGMa) with Monoclonal Antibody AE12-1

[0302] Epitope mapping studies were undertaken for monoclonal antibody AE12-1. Data suggested that the epitope for AE12-1 is located in the N-terminal region of RGMa. Several constructs of hRGMa were employed to attempt to determine the epitope for AE12-1. These constructs included:

[0303] pelB-M-[RGMA(47-168)]-6His ("6His" disclosed as SEQ ID NO: 148) (E.coli) produced recombinantly. Antigen is 0.41 mg/mL in ChemTag#16211, S100 buffer, pH8, 25mM Tris, 100mM NaCl, 1mM DTT, 10% (v/v) glycerol. Sequence of this first antigen construct is: MKYLL PTAAA GLLLL AAQPA MAMPC KILKC NSEFW SATSG SHAPA SDDTP EFCAA LRSYA LCTRR TARTC RGDLA YHSAV HGIED LMSQH NCSKD GPTSQ PRLRT LPPAG DSQER SDSPE ICHYE KSFHK HSATP NYTHC GLFGD HHHHHH (SEQ ID NO:75).

[0304] [IgK-leader]-AttB1-[hRGMA(47-422)]-AttB2-MYC-6His ("6His" disclosed as SEQ ID NO: 148) produced recombinantly, 0.85 mg/ml in PBS. Sequence of this second antigen construct is: METDT LLLWV LLLWV PGSTG DAAQP ARRAR RTKLG TELGS TSPVW WNSAD ITSLY KKAGS PCKIL KCNSE FWSAT SGSHA PASDD TPEFC AALRS YALCT RRTAR TCRGD LAYHS AVHGI EDLMS QHNCS KDGPT SQPRL RTLPP AGDSQ ERSDS PEICH YEKSF HKHSA TPNYT HCGLF GDPHL RTFTD RFQTC KVQGA WPLID NNYLN VQVTN TPVLP GSAAT ATSKL TIIFK FNQEC VDQKV YQAEM DELPA AFVDG SKNGG DKHGA NSLKI TEKVS GQHVE IQAKY IGTTI VVRQV GRYLT FAVRM PEEVV NAVED WDSQG LYLCL RGCPL NQQID FQAFH TNAEG TGARR LAAAS PAPTA PETFP YETAV AKCKE KLPVE DLYYQ ACVFD LLTTG DVNFT LAAYY ALEDV KMLHS NKDKL HLYER TRDLP GNPAF LYKVV ISSTV AAARG GPEQK LISEE DLNSA VDHHH HHH (SEQ ID NO:76).

[0305] [IgK-leader]-AttB1-[hRGMA(47-168)]-Xa-[hIgG L Fc (257-481)] (mammalian construct), produced recombinantly, 1.18 mg/mL, in PBS. Sequence of this third antigen

construct is: METDT LLLWV LLLWV PGSTG DAAQP ARRAR RTKLP CKILK CNSEF WSATS GSHAP ASDDT PEFCA ALRSY ALCTR RTART CRGDL AYHSA VHGIE DLMSQ

[0306] HNCSK DGPTS QPRLR TLPPA GDSQE RSDSP EICHY EKSFH KHSAT PNYTH CGLFG DLNSA DIEGR MDPPC PAPEL LGGPS VFLFP PKPKD TLMIS RTPEV TCVVV DVSHE DPEVK FNWYV DGVEV HNAKT KPREE QYNST YRVVS VLTVL HQDWL NGKEY KCKVS NKALP APIEK TISKA KGQPR EPQVY TLPPS REEMT KNQVS LTCLV KGFYP SDIAV EWESN GQPEN NYKTT PPVLD SDGSF FLYSK LTVDK SRWQQ GNVFS CSVMH EALHN HYTQK SLSLS PGK (SEQ ID NO:77).

[0307] All of the antigens used contain the amino acid sequence of RGMa (47-168), wherein the numbering used to identify sequence positions correspond to the numbering of the parent protein. The sequence of hRGMa (47-168) is: PCKI LKCNS EFWSA TSGSH APASD DTPEF CAALR SYALC TRRTA RTCRG DLAYH SAVHG IEDLM SQHNC SKDGP TSQPR LRTLP PAGDS QERSD SPEIC HYEKS FHKHS ATPNY THCGL FGD (SEQ ID NO:78).

[0308] The buffers used for excising the epitopes were as follows:

Buffer A: 100 mM $NaHCO_3$, 500 mM NaCl, pH 8;

Buffer B: 100 mM $NaHCO_3$, 100 mM NaCl, pH 8;

Buffer C: 100 mM NaOAc, 500 mM NaCl, pH 4; and

Buffer D: 100 mM Tris-HCl, 500 mM NaCl, pH 8.

[0309] The monoclonal antibody was immobilized as follows. Twenty milligrams of CNBr-activated Sepharose beads (GE Healthcare, Uppsala Sweden) was weighed into a compact reaction column (USB Corp., Cleveland, OH) with a 35 μm frit and washed 3 times with 200 μl of 1 mM HCl, followed by washing 3 times with 200 μl of Buffer A.

[0310] Approximately 5-6 nmol of the AE12-1 mAb solution was dialyzed against PBS using a 10,000 MWCO Slide-A-Lyzer mini dialysis unit (Pierce, Rockford, IL) for approximately 40 minutes to remove the histidine buffer which would interfere with antibody binding to Sepharose. The dialyzed mAb solution was added to the activated resin and allowed to mix on a rotator (Mix-All Laboratory Tube Mixer, Torrey Pines Scientific, San Marcos, CA) for 4 hours at room temperature. After binding, the resin flow-through was collected, and the resin was washed three times with 200 μl Buffer A. The resin was suspended in 200 μl of Buffer D, and rotated at room temperature for 1 hour to block excess binding sites in the resin. The Buffer D

solution was flushed out and the resin was washed alternately with 200 µl of Buffer C and Buffer D (low/high pH washing), a total of three times each. The resin was washed three times with 200 µl of Buffer B, to make it ready for coupling with antigen.

[0311] Immobilized AE12-1 monoclonal antibody was coupled to hRGMa. Compact reaction columns (CRC) were prepared for the antigen coupling by washing the 35 µm frit of the CRC three times with 200 µl of Buffer B. The resin with the bound antibody was mixed gently to re-suspend the resin homogeneously, and 50 µl of the resin was placed in each prepared CRC. The resin was washed three times with 200 µl Buffer B. Approximately 1.5 nmol of hRGMa antigen was added to the resin with enough Buffer B to make a total volume of at least 200 µl. Prior to antigen coupling, the E. coli produced antigen was dialyzed against PBS buffer for approximately 30 minutes using a 3500 MWCO Slide-A-Lyzer mini-dialysis unit to remove DTT from the antigen buffer. The antigen/resin mixture was allowed to mix on a rotator for 4 hours at room temperature. The flow-through (FT) was collected, and the resin was washed three times with 200 µl of Buffer B.

[0312] The epitopes were excised using trypsin and endoproteinase Asp-N. The resin containing the immobilized antibody/antigen complex was suspended in 200 µl of Buffer B. A vial with 20 µg of trypsin (Promega, Madison, WI) was dissolved in 100 µl of the resuspension buffer (50 mM HOAc), for a concentration of 0.2 µg/µl, and a 2 µg vial of endoproteinase Asp-N (Roche) was dissolved in 50 µl water (0.04 µg/µl). The antigen cleavages were performed with 1:100 ratios (w/w) enzyme:antigen. The reaction proceeded for 4-6 hours with rotation at room temperature.

[0313] After digestion, the FT was collected and the resin was washed twice with 200 µl of Buffer B, with collection of each wash separately (Wash 1 and 2), 200 µl of Buffer A (Wash 3), and then 200 µl of Buffer B (Wash 4). The antigen peptides that were bound to the antibody were eluted from the resin with three 200 µl aliquots of 2% formic acid, and each elution was collected separately (Elution 1, 2 and 3). The Elution1 fraction was analyzed by mass spectrometry (LC-MS/MS) to determine the epitope region.

[0314] The Elution 1 fractions that were collected after digestion were analyzed by LC-ESI-MS/MS (positive ion) using an Agilent (Santa Clara, CA) 1100 capillary HPLC loading pump and 1200 nano-HPLC gradient pump, with a Chip Cube (40 nL enrichment column, 75 µm x 43 mm analytical column, C8 ZORBAX chip) interfaced to an Agilent 6510 QTOF MS. Injections

of up to 7 µl were used, and MS/MS was performed on the top 3 ions meeting the specified MS signal criteria.

[0315] Initial MS analysis of the epitope excison fractions (Elution 1) indicated the presence of large peptide species that could not be matched to expected proteolytic peptides due to the likely presence of disulfide linked peptides. To reduce disulfide bonds, 10 µl aliquots were pH adjusted to pH~8 using diluted NaOH, and reduced in 5 mM dithiothreitol (DTT) at 37°C for 30 minutes before re-analysis by MS. Some fractions required denaturation to achieve reduction, in which case the aliquot was diluted in an equal volume of 8M guanidine hydrochloride, 100 mM Tris (pH 8) before addition of DTT.

[0316] LC-ESI-MS/MS analysis of all Elution 1 fractions of the enzymatic digest of AE12-1 mAb coupled with the various constructs of hRGMa indicated the presence of several large peptide species with molecular weights in the range of 8.5 – 12 kDa. The masses and fragmentation of the large species were not sufficient to identify the peptides. In order to identify the eptitope peptides, reduction of the disulfide bonds was necessary. In all cases, the MS signal intensity of the peptides decreased significantly after reduction, in some cases being undetectable unless reduced in the presence of a denaturant.

[0317] After reduction of the Elution 1 fraction with DTT, the fractions were reanalyzed by LC-MS/MS. In the excision experiment using E. coli produced hRGMa, most of the peaks observed in the ion chromatogram were single charged species, many related to polymers or other additives. Two peptides were identified as being related to the hRGMa construct used. See Figure 5. The first was a peptide with a monoisotopic molecular weight of 2420.2 Da. The molecular weight of the peptide and the masses of a few fragments observed in the MS/MS spectra (not shown) are consistent with the sequence PCKILKCNSEFWSATSGSHAPAS (hRGMa 47-69) (SEQ ID NO:79) although the assignment was inconsistent with the enzyme specificity. The second potential epitope peptide with a monoisotopic molecular weight of 2551.2 Da revealed only 2-3 identifiable MS/MS fragments that were consistent with the sequence MPCKILKCNSEFWSATSGSHAPAS (SEQ ID NO:80). The enzyme specificity was not consistent; however, since the molecular weight of the starting antigen did not match the calculated mass of the full sequence, it is possible that the antigen has N-terminal heterogeneity that would account for the apparent inconsistent enzyme specificity.

[0318] Using the second antigen construct no peptides could be observed in the MS spectrum after DTT reduction. MS analysis after reduction under denaturing conditions did reveal peptides among the singly charged, background ions. Four peptides from the antigen were identified in the denatured and reduced E1 fraction. The first antigen-related peptide had a monoisotopic molecular weight of 2763.3 Da, which, along with the MS/MS fragmentation, was consistent with the sequence KAGSPCKILKCNSEFWSATSGSHAPAS (SEQ ID NO:81) (hRGMa 47-69 with 4 additional N-terminal residues). The spectra associated with this peptide are shown in Figure 6. A very low intensity peptide signal in the same spectrum (MW 2878.4 Da; +4 at m/z 720.84, marked with an asterisk in Figure 6) was consistent with the sequence KAGSPCKILKCNSEFWSATSGSHAPPASD (SEQ ID NO:82). A peptide of MW 2635.2, shown in Figure 7, was consistent with the sequence AGSPCKILKCNSEFWSATSGSHAPPAS (SEQ ID NO:90). An additional peptide at m/z 688.82 (most abundant isotope, +4 charge state), marked with an asterisk in Figure 7, co-eluted with the MW 2635.2 peptide. The limited MS/MS data obtained on this low intensity component was consistent with the sequence AGSPCKILKCNSEFWSATSGSHAPPASD (SEQ ID NO:83) (MW 2750.3 Da).

[0319] The third construct of hRGMa was used to try to confirm the eptiope with AE12-1. In the Elution 1 fraction that was DTT-reduced, very little reduction was observed, but one peptide could be identified as being related to hRGMa antigen and consistent with the results from the other antigen constructs. The peptide was observed at m/z 691.60 (+ 4 charge state), giving it a monoisotopic molecular weight of 2762.4 Da. Limited MS/MS data obtained for this peptide (not shown) suggests the sequence as TKLPCKILKCNSEFWSATSGSHAPAS (SEQ ID NO:84). Other peptides that were observed in the MS spectrum that could be assigned as being related to the region of hRGMa (47-168) included DSPEICHYEK (SEQ ID NO:85); GDLAYHSAVHGIE (SEQ ID NO:86); DLAYHSAVHGIE (SEQ ID NO:87); and DDTPEFCAALR (SEQ ID NO:88).

[0320] In the Elution 1 fractions (trypsin/Asp-N digestion) from the epitope excision experiment of hRGMa bound to AE12-1, the peptide hRGMa (47-69) was identified from three constructs of antigen. The peptide identified as the epitope for hRGMa with AE12-1 is: PCKILKCNSEFWSATSGSHAPAS (hRGMa 47-69) (SEQ ID NO:79).

Example 8
TOXICOLOGY STUDIES

[0321] Because iron accumulation in hepatocytes and the decrease of iron in the spleen may result from RGMc neutralization, the toxicokinetics and tolerability of the herein described RGMa-selective monoclonal antibodies were studied. The studies are expected to show that iron accumulation in hepatocytes and iron depletion in the spleen will not occur when the RGMa-selective monoclonal antibodies are administered to Sprague-Dawley rats.

Example 9
RGMa-Selective Monoclonal Antibodies AE12-1 and AE12-1Y, like Humanized Monoclonal Antibody 5F9, induce regeneration of crushed, damaged optic nerve axons in a rat model of optic nerve injury

[0322] The Optic Nerve Crush (also referred to as "Optic Nerve Injury") model provides an animal model to test various substances that stimulate regeneration of the optic nerve fibers and reduce the massive cell death of retinal ganglion cells.

[0323] The experiments were carried out in adult male Wistar rats obtained from Charles River (D) Laboratories (Germany). The animals are kept in single cages on a 12:12 hour light/dark cycle with food and water *ad libitum*. The optic nerve crush is always performed only at the left eye by minimal anterior surgery as described in P. Monnier et al., *J. Neurosci.*, 31:10494-10505 (2011), the contents of which are herein incorporated by reference, and follows human anterior visual surgical methods. Before and during the operation, the procedure animals are anesthetized by inhalation anesthesia using Sevoflurane (Abbott GmbH Co. & KG, Delkenheim, Germany) and are fixed on the operation table by using jaw clamp and adhesive tape for the limbs. A drop in body temperature is prevented by mounting animals on a heating pad. For anterior crush surgery of the rat optic nerve, the left eye is carefully freed of ligament and connective tissue. As a first step, a microsurgical cut (2–3 mm) of the adjacent tissue in the outer corner of the eye is performed. As a next step, the optic nerve is exposed by using a pair of forceps to move to the side the eye muscles and lacrimal gland, thus sparing it. As a further step, the meninges were longitudinally opened by using microscissors to expose the optic nerve. This results in a higher mobility of the eye and enables lateral rotation of the eye and access to its left optic nerve. The optic nerve is injured approximately 1-3 mm behind the eye, using a pair of forceps set to

provide a fixed maximum pressure for 10-20 seconds. Special care is taken not to damage the vascular supply to the eye.

[0324] After minimal invasive surgery, the animals are placed on paper towels in the clean cage placed on the warmer to control the body temperature until they start to move. An ointment which contains antibiotic (Gentamytrex, Dr. Mann Pharma) is applied onto the eye to avoid bacterial infection and drying-out of the sclera.

[0325] Carprofen (Rimadyl, 5 mg/kg) is applied intraperitoneally for postoperative pain therapy directly after surgery and then twice per day for a 3 day period. The animals are observed and controlled regularly for several hours directly after surgery and in the next 2-4 days to make sure that all the animals survived and recovered from anesthesia and surgery.

[0326] The above described modified anterior optic nerve crush approach has significant advantages in comparison with the standard optic nerve crush procedure which originates from the posterior part of the eye. Specifically, in the procedure described herein, no large open wounds are generated which require suturing and the infection risk of the very small wounds is significantly reduced. In addition, as a result of the lower time required for the crush (the above described anterior method is approximately 3 times faster than the posterior method known in the art) animals suffer less and are thus less stressed. Moreover, the amount of pain suffered by the animals is significantly reduced and animals recover at a much rates and much more quickly.

Systemic administration of antibodies

[0327] For systemic antibody delivery, male Wistar rats were treated systemically intravenously, (iv) with a humanized RGMa and RGMc-blocking 5F9 antibody (h5F9) (n = 8 animals) (humanized antibody 5F9 is described in U.S. Patent Publication No. 2010/0028340, the contents of which are herein incorporated by reference), with an RGMa-selective, human antibody, AE12-1, described herein, and with a closely related RGMa mAb, AE12-1Y, also described herein and with a human isotope control antibody (hIgG) (n = 8 animals). Rats were injected once per week intraveneously with 10 mg/kg of antibody given and injections were started immediately after optic nerve crush. All rats received 5 injections and animals were euthanized 6 weeks after crush injury. Experimenters were blinded and tissue isolation, processing, preparation of sections and quantitative analysis was done as described in P. Monnier et al., *J. Neurosci.*, 31:10494-10505 (2011) and Koeberle et al., *Neuroscience*, 169:495-504 (2010), the

contents of each of which are herein incorporated by reference. Composite images of rat optic nerves were prepared, the crush site was identified and GAP-43 positive fibers extending beyond the crush site for 500 μm were counted. As shown in Figure 16, all three RGMa antibodies - h5F9, AE12-1 and AE12-1Y induced significant regenerative growth beyond the crush site, in contrast to control animals treated with hIgG.

Example 10

RGMa-selective Monoclonal Antibodies AE12-1 and AE12-1Y like Humanized Antibody 5F9, protect the retinal nerve fiber layer (RNLF) from degeneration

[0328] In order to observe protection of RNLF degeneration, a new laboratory assay method was used. This method is based on explanting and analyzing adult rat retina from the eyes of rats with optic nerve crush and systemically treated with antibody 5F9, AE12-1, AE2-1Y and control antibody, human IgG. This method is an adaptation of the methods described by P. Monnier et al., *J. Neurosci.*, 31:10494-10505 (2011) and Koeberle et al., *Neuroscience*, 169:495-504 (2010). P. Monnier et al., *J. Neurosci.*, 31:10494-10505 (2011) and Koeberle et al., *Neuroscience*, 169:495-504 (2010). Adult male Wistar rates obtained from Charles River Laboratories (Germany) were injected once per week intraveneously with 10 mg/kg of antibody given and injections were started immediately after optic nerve crush. All rats received 5 injections and animals were euthanized 6 weeks after crush injury.

Retina preparation and immunofluorescent staining:

[0329] Animals were deep anesthetized with Sevoflurane (8%; Abbott GmbH Co. & KG, Delkenheim, Germany), then immediately sacrificed by opening the ribcage and perfused with 4% paraformaldehyde (PFA) solution through the left heart ventricle. The eyes were dissected with the connective tissue adjusted and placed in 4% PFA until preparation of retina takes place.

[0330] The preparation of the retina was performed in Hank's Balanced Salt Solution (HBSS, Magnesium- and Calcium-free; Invitrogen, #14170070). The eye was fixed at the connective tissue by tweezers and a round cut was made in sclera just around the cornea.

The half-sphere of retina was cut in four points, opened and spread on a gray nitrocellulose membrane (Sartorius, #13006-50-N). If necessary, the membrane with retina on it was allowed to air dry for 5-10 seconds

Thereafter, the retina on membrane was placed in 10% neutral phosphate-buffered formaldehyde solution (pH 7.3; Fisher Scientific, #F/1520/21) at +4°C until immunofluorescent staining was performed. Staining is performed according to the protocol described below.

[0331] The retina preparation was washed with TBS, followed by blocking and permeabilization with 5 % BSA, 1 % Triton X-100 in TBS for 30 minutes and then washed again with TBS. Primary antibodies, namely, monoclonal Ab TUJ-1, a mouse anti-ß III Tubulin Ab, AbCam, # ab14545;1:500 dilution, in TBS, 5 % BSA, was added for 1 hour at room temperature in the dark followed by washing with TBS, 0.1 % Tween 20. Next, a secondary antibody, namely, Donkey anti-mouse Cy3; Jackson ImmunoResearch (Dianova) 715-165-151, 1:1000 dilution, and Bisbenzimid (50 µg/ml 1:100 dilution in TBS, 5 % BSA) were added for 1 hour at room temperature in the dark followed by washing with TBS, 0.1 % Tween 20, and the subsequent washing with desalted H_2O. The preparation was then mounted with Fluoromount G and stored at +4°C in the dark.

Quantitative analysis of the protective effect of RGMa antibodies on the retinal nerve fiber layer in the eye (the RNFL)

[0332] Using the Axiovision software, randomly selected images (n = 12) of each retina were chosen and the number of nerve fibers determined for each image. For these experiments, 5 – 8 retinae with crushed optic nerves were used for each group: the h5F9 MAb group, the hIgG control MAb group and the AE12.1 and AE12-1Y MAb groups. Data analysis and statistical analysis was performed using the GraphPad prism program.

[0333] The results are illustrated in Figure 17. Specifically, a significantly higher number of nerve fiber bundles is observed in retinae of animals systemically treated with RGMa antibodies of the present invention when compared to hIgG control antibody treated animals.

Example 11
RGMa antibodies accelerate functional recovery in a focal spinal Experimental Autoimmune Encephalomyelitis (EAE) Model

[0334] Kerschensteiner et al. (*Am. J. Pathol.* 164: 1455 – 69, 2004) developed a focal, localized EAE model where large inflammatory lesions are not spread randomly in spinal cord, brain and optic nerve but can be selectively induced in either in the spinal cord or in other brain areas. Using this focal or targeted EAE model, large inflammatory lesions, very similar to MS spinal

cord lesions, are induced in the dorsal columns of the spinal cord, affecting the corticospinal tract. In this model, rats are first immunized with the myelin protein MOG. Two to three weeks after immunization, MOG antibody titers were determined and animals with a positive immune response were injected with a cytokine mixture (TNFα 250 ng, IFNg 150 U) locally at thoracical level 8 (T8). Within one week after cytokine injection, the rats developed hindlimb motor defects, tail paralysis and gait disturbances reaching an EAE score of 2.5. Four weeks after cytokine injection, this score improved to an EAE score of 1 (Kerschensteiner et al., *Am. J. Pathol.* 164: 1455 – 69, 2004).

[0335] Female Lewis rats were injected subcutaneously with 75 µl of MOG (75 µg, 1-125 aa, BlueSky Biotech, Worcester, MA) dissolved in saline and then emulsified with 75 µl of Incomplete Freund's Adjuvans (IFA, Sigma, #F5506). Right before injection and then every 7-8th day, blood samples were taken from animals to analyze the samples for MOG antibodies.

[0336] Two or three weeks after MOG immunization, blood was collected from immunized rats and an ELISA was performed to detect MOG-specific antibodies. Immunization results in induction of MOG antibodies in more than 90% of immunized rats. In this strain however, MOG antibody induction did not result in any disease symptoms. Lewis rats only developed motor deficits after local injection of 2 inflammatory cytokines (TNF α, IFNg) into the thoracical spinal cord at thoracical level 8 (T8).

[0337] For cytokine injection, rats were subject to Inhalation anesthesia with Sevoflurane (8%; Abbott GmbH Co. & KG, Delkenheim, Germany) and a laminectomy was performed by a standard procedure. Specifically, the skin on the back of the rat was shaved and disinfected with 70% of ethanol, then the shaved area was wiped with the prodine and a 2-3 cm cut was made with scalpel starting approx. from T3-4 till T11-12. The superficial fat was separated with fine scissors from the muscles and the muscles were cut by the middle line along the spine from one side. The gap between T8 and T9 was located and T8 was cleared from adjacent tissue. A microdrill was used to make a round hole approximately 1-2 mm in diameter in T8 and small tipped forceps were used to remove the periosteum and any bone fragments. Next, the dura mater was removed with micro scissors and a stereotactic injection was done with a very thin glass capillary connected to a 10 µl Hamilton syringe with Luer Tip (LT) and filled in with the mineral oil (Sigma Aldrich).

[0338] Using an automatic injector, the capillary was filled with 3 µl of cytokine mixture in PBS or just PBS with traces of Evans blue. A four times (4x) higher dose of TNFα (1000 ng) and the same dose of IFNγ (150 U) was used as reported by Kerschensteiner et al. (2004). The four times higher dose resulted in a significant extension of the recovery process in vehicle or control treated rats.

[0339] In the next step, a glass capillary was inserted to a depth of 0.7 mm and 2 µl of the cytokine mixture were injected in the middle of spinal cord at (T8) during a 5 minute-period using an automatic injector. After applying Lidocaine and closing the wound, rats were treated with an analgesic drug Rimadyl (directly after surgery and daily for another 3-4 days). Rats were then placed in a clean page on paper towels and were kept in the warmth until they awoke.

[0340] Rats developed first symptoms within one week after cytokine injection. Antibody treatment was started on day 7 or 8 after cytokine application. A hIgG control antibody and several different RGMa antibodies (namely, AE12-1, AE12-1Y and humanized 5F9.23 (h5f9.23 which is described in U.S. Patent Publication No. 2010/0028340)) were used and rats were treated once per week via the intravenous route. EAE scoring was done daily and scores were documented. Individuals conducting the experiments were blinded for the different treatment groups. After a period of 27 – 29 days post cytokine administration, the animals were killed, spinal cords were isolated and analyzed for expression of the following proteins: GAP-43 (regeneration marker), CD68 (inflammatory marker for activated microglia cells and macrophages) and MPB (myelin basic protein, a marker for remyelination or preserved myelin tracts). The area of these markers was measured, analyzed and statistically evaluated using one way ANOVA and Bonferroni significance test. As shown in Figure 18, all three RGMa antibodies accelerated functional recovery in the spinal tEAE model.

[0341] In the spinal tEAE model, all RGMa antibodies showed very similar regeneration- and neuroprotection stimulating activity. The RGMa –selective antibodies AE12-1 and AE12-1Y showed similar activity compared with h5F9.23, which neutralizes both RGMa and RGMc. Neutralization of RGMc does not seem to be required for efficacy. Therefore, to better understand the mechanism of action of all three RGMa mAbs in the focal spinal EAE model, several markers were evaluated in serial sections of spinal cords of antibody treated rats AE12-1Y, AE12-1 and h5F9.23 increased the regeneration area (GAP-43), the remyelination area

(myelin basic protein (MBP)) and decreased the inflammatory CD68 (CD68-positive) area around the spinal lesion site (See, Figure 19).

[0342] The RGMa-selective antibody AE12-1Y-QL was tested to determine what doses of this antibody are active in this spinal tEAE model. Specifically, 4 different antibody doses (namely, (0.01, 0.1, 1, 10 mg/k were given IV systemically once per week to the rats. AE12-1Y-QL showed significant activity at 0.1, 1 and 10 mg/kg (Figure 20). However, a dose of 0.01 mg/kg did not show efficacy.

Claims

1. An isolated antibody or antibody fragment thereof which binds to Repulsive Guidance Molecule a ("RGMa"), wherein the antibody or antibody fragment comprises a heavy chain variable region comprising a complementarity determining region (CDR)1 comprising the amino acid sequence of SEQ ID NO:2, a CDR2 comprising the amino acid sequence of SEQ ID NO:3, and a CDR3 comprising the amino acid sequence of SEQ ID NO:4, and a light chain variable region comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:6, a CDR2 comprising the amino acid sequence of SEQ ID NO:7, and a CDR3 comprising the amino acid sequence of SEQ ID NO:73.

2. The isolated antibody or antibody fragment of claim 1, wherein the antibody or antibody fragment is selected from the group consisting of a human antibody, an immunoglobulin molecule, a disulfide linked Fv, a monoclonal antibody, an affinity matured antibody, a scFv, a chimeric antibody, a single domain antibody, a CDR-grafted antibody, a diabody, a humanized antibody, a multispecific antibody, a Fab, a dual specific antibody, a DVD, a Fab', a bispecific antibody, a F(ab')2, and a Fv.

3. The isolated antibody or antibody fragment of claim 1 or claim 2, wherein the antibody or antibody fragment is human.

4. The isolated antibody or antibody fragment of any one of claims 1-3, wherein the antibody or antibody fragment comprises a heavy chain immunoglobulin constant domain selected from the group consisting of a human IgM constant domain, a human IgG4 constant domain, a human IgG1 constant domain, a human IgE constant domain, a human IgG2 constant domain, a human igG3 constant domain, and a human IgA constant domain.

5. The isolated antibody or antibody fragment of claim 4, wherein the human IgG1 constant domain comprises the amino acid sequence of SEQ ID NO: 140, 141, 142, or 143.

6. The isolated antibody or antibody fragment of claim 4, wherein the human IgG1 constant domain comprises the amino acid sequence of SEQ ID NO: 143.

7. The isolated antibody or antibody fragment of claim 6, wherein the antibody or antibody fragment comprises a light chain amino acid sequence as set forth in SEQ ID NO: 146 and a variable heavy chain amino acid sequence as set forth in SEQ ID NO: 147.

8. The antibody or antibody fragment of any one of claims 1-7, wherein the antibody or antibody fragment further comprises an agent selected from the group consisting of: an immunoadhesion molecule, an imaging agent, and a therapeutic agent, wherein

(a) the imaging agent is selected from the group consisting of a radiolabel, an enzyme, a fluorescent label, a luminescent label, a bioluminescent label, a magnetic label, and biotin, and

(b) the radiolabel is selected from the group consisting of 3H, 14C, 35S, 90Y, 99Tc, 111In, 125I, 131I, 177Lu, 166Ho, and 153Sm.

9. A pharmaceutical composition comprising the antibody, antibody fragment, mixture or derivative thereof of any one of claims 1-8.

10. Use of the antibody or antibody fragment of any one of claims 1-8 in the manufacture of a medicament for the treatment, prevention, modulation, or attenuation of a disease or disorder associated with neurite degeneration.

11. The use of claim 10, wherein the disease or disorder associated with neurite degeneration is selected from the group consisting of multiple sclerosis, Parkinson's disease, Alzheimer's disease, Tay-Sachs disease, Niemann-Pick disease, Gaucher's disease, Hurler's syndrome, Huntington's disease, amyotrophic lateral sclerosis, idiopathic inflammatory demyelinating diseases, vitamin B12 deficiency, central pontine myelinolysis, tabes dorsalis, transverse myelitis, Devic's disease, progressive multifocal leukoencephalopathy, optic neuritis, traumatic injury to the CNS, , an ischemic cerebral stroke, glaucoma, diabetic retinopathy, age-dependent macular degeneration, and a leukodystrophy.

12. A method for determining whether a subject has a neurite degenerative disorder, comprising

(a) measuring the level of RGMa in a sample isolated from the subject, wherein the level of RGMa in the sample is determined using the antibody or antibody fragment of any one of claims 1-8; and

(b) comparing the level of RGMa in the sample with a normal control,

wherein an altered level of RGMa indicates that the subject has a neurite degenerative disorder.

13. The method of claim 12, wherein an increased level of RGMa as compared to the normal control indicates that the subject has neurite degenerative disorder.

14. The method of claim 12 or claim 13, wherein the sample is selected from the group consisting of a blood sample, a cerebrospinal fluid sample, and a serum sample.

15. The method of any one of claims 12-14, wherein step (a) is an immunoassay.