USE OF SEMAPHORIN-4D BINDING MOLECULES FOR TREATING NEURODEGENERATIVE DISORDERS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This International Patent Application claims the benefit of U.S. provisional patent application No. 62/012,805, filed on June 16, 2014, U.S. Provisional Appl. No. 61/979,384, filed on April 14, 2014, and U.S. Provisional Appl. No. 61/893,814, filed on October 21, 2013, the contents of which are each hereby incorporated by reference in their entireties.

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

[0002] The content of the electronically submitted sequence listing in ASCII text file (Name 58008-137466seq-lstg.txt; Size: 32,256 bytes; and Date of Creation: October 20, 2014) filed with the application is incorporated herein by reference in its entirety.

BACKGROUND OF THE DISCLOSURE

[0003] Semaphorin 4D (SEMA4D), also known as CD100, is a transmembrane protein (*e.g.*, SEQ ID NO: 1 (human); SEQ ID NO: 2 (murine)) that belongs to the semaphorin gene family. SEMA4D is expressed on the cell surface as a homodimer, but upon cell activation SEMA4D can be released from the cell surface via proteolytic cleavage to generate sSEMA4D, a soluble form of the protein, which is also biologically active. See Suzuki *et al.*, *Nature Rev. Immunol. 3*:159-167 (2003); Kikutani *et al.*, *Nature Immunol. 9*:17-23 (2008).

[0004] SEMA4D is expressed at high levels in lymphoid organs, including the spleen, thymus, and lymph nodes, and in non-lymphoid organs, such as the brain, heart, and kidney. In lymphoid organs, SEMA4D is abundantly expressed on resting T cells but only weakly expressed on resting B cells and antigen-presenting cells

(APCs), such as dendritic cells (DCs). Its expression, however, is upregulated in these cells following activation by various immunological stimuli. The release of soluble SEMA4D from immune cells is also increased by cell activation.

[0005] SEMA4D has been implicated in the development of neurodegenerative disorders, autoimmune diseases, demyelinating diseases, and certain cancers. However, the effect of blocking SEMA4D signaling on the organization and function of the central nervous system (CNS) including brain and spinal cord and on behaviors controlled by the CNS remains to be elucidated. This is important because changes in the CNS have a profound influence on a subject's behavior and quality of life. In particular, such changes can impact a subject's neuropsychiatric behavior, cognitive behavior, and motor skills. There remains, therefore, a need for treatments for neurodegenerative disorders that alleviate the symptoms associated with the disorder.

SUMMARY

[0006] Methods for using semaphorin 4D binding molecules to alleviate symptoms in a subject having neurodegenerative disorders are disclosed herein. According to embodiments of the disclosure illustrated herein, there is provided a method for improving symptoms in a subject with a neurodegenerative disorder including administering to the subject an effective amount of an isolated binding molecule which specifically binds to semaphorin 4D (SEMA4D) and inhibits, suppresses, prevents, reverses or slows the effect of SEMA4D.

[0007] According to embodiments illustrated herein, there is provided a method of treating a subject with a neurodegenerative disorder including administering to the subject an effective amount of an isolated binding molecule which specifically binds to semaphorin 4D (SEMA4D), wherein the binding to SEMA4D acts to improve symptoms associated with the disorder.

[0007a] In one aspect, there is provided use of an effective amount of an isolated antibody or antigen-binding fragment thereof that specifically binds to semaphorin-4D (SEMA4D) in the preparation of a medicament for alleviation of symptoms in a subject having Huntington's disease (HD), wherein the antibody or antigen-binding fragment thereof inhibits SEMA4D interaction with a SEMA4D receptor, wherein the antibody or antigen-binding fragment thereof comprises a variable heavy chain (VH) comprising VHCDRs 1-3 comprising SEQ ID NOs: 6, 7, and 8, respectively, and a variable light chain (VL) comprising VLCDRs 1-3 comprising SEQ ID NOs: 14, 15, and 16, respectively.

[0008] Provided is use of an effective amount of an isolated binding molecule which specifically binds to semaphorin-4D (SEMA4D) in the preparation of a medicament for alleviating symptoms in a subject having a neurodegenerative disorder. Methods of alleviating symptoms in a subject having a neurodegenerative disorder are also provided, comprising administering to that subject an effective amount of an isolated binding molecule which specifically binds to semaphorin-4D (SEMA4D). In certain embodiments of the uses and methods, the binding molecule inhibits SEMA4D interaction with its receptor or a portion of its receptor. In certain embodiments of the uses and methods, the receptor is selected from the group consisting of Plexin-B1 and Plexin-

B2. In certain embodiments of the uses and methods, the binding molecule inhibits SEMA4D-mediated Plexin-B1 signal transduction. In certain embodiments of the uses and methods, the isolated binding molecule specifically binds to the same SEMA4D epitope as a reference monoclonal antibody selected from the group consisting of VX15/2503 or 67. In certain embodiments of the uses and methods, the isolated binding molecule competitively inhibits a reference monoclonal antibody selected from the group consisting of VX15/2503 or 67 from specifically binding to SEMA4D. In certain embodiments of the uses and methods, the isolated binding molecule comprises an antibody or antigen-binding fragment thereof. In certain embodiments of the uses and methods, the antibody or antigen-binding fragment thereof is monoclonal antibody VX15/2503 or 67. In certain embodiments of the uses and methods, the antibody or antigen-binding fragment thereof comprises a variable heavy chain (VH) comprising VHCDRs 1-3 comprising SEQ ID NOs 6, 7, and 8, respectively, and a variable light chain (VL) comprising VLCDRs 1-3 comprising SEQ ID NOs 14, 15, and 16, respectively. In certain embodiments of the uses and methods, the VH and VL comprise, respectively, SEQ ID NO: 9 and SEQ ID NO: 17 or SEQ ID NO: 10 and SEQ ID NO: 18. In certain embodiments of any of the aforementioned uses and methods, the neurodegenerative disorder is selected from a group consisting of Alzheimer's disease, Parkinson's disease, Huntington's disease, Down syndrome, ataxia, amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), HIV-related cognitive impairment, CNS Lupus, mild cognitive impairment, or a combination thereof. In certain embodiments of any of the aforementioned uses and methods, the neurodegenerative disorder is Alzheimer's disease or Huntington's disease. In certain embodiments of any one of the aforementioned uses and methods, the symptoms are selected from a group consisting of neuropsychiatric symptoms, cognitive symptoms, motor dysfunction, and any combination thereof. In certain embodiments of any of the aforementioned uses and methods, the neuropsychiatric symptoms are selected from a group consisting of reducing anxiety-like behavior, improving spatial memory, increasing locomotion, and any combination thereof.

[0009] Provided is use of an effective amount of an isolated binding molecule which specifically binds to SEMA4D in the preparation of a medicament for alleviating symptoms in a subject having a neurodegenerative disorder, wherein the binding molecule competitively inhibits a reference monoclonal antibody selected from the group consisting of VX15/2503 or 67 from specifically binding to SEMA4D. Methods of alleviating symptoms in a subject having a neurodegenerative disorder are also provided, comprising administering to that subject an effective amount of an isolated binding molecule which specifically binds to SEMA4D, wherein the binding molecule competitively inhibits a reference monoclonal antibody selected from the group consisting of VX15/2503 or 67 from specifically binding to SEMA4D.

In certain embodiments of the uses and methods, the binding molecule inhibits SEMA4D interaction with its receptor or a portion of its receptor. In certain embodiments of the uses and methods, the receptor is selected from the group consisting of Plexin-B1 and Plexin-B2. In certain embodiments of the uses and methods, the binding molecule inhibits SEMA4D-mediated Plexin-B1 signal transduction. In certain embodiments of the uses and methods, the isolated binding molecule comprises an antibody or antigen-binding fragment thereof. In certain embodiments of the uses and methods, the antibody or antigen-binding fragment thereof is monoclonal antibody VX15/2503 or 67. In certain embodiments of the uses and methods, the antibody or antigen-binding fragment thereof comprises a variable heavy chain (VH) comprising VHCDRs 1-3 comprising SEQ ID NOs 6, 7, and 8, respectively, and a variable light chain (VL) comprising VLCDRs 1-3 comprising SEQ ID NOs 14, 15, and 16, respectively. In certain embodiments of the uses and methods, the VH and VL comprise, respectively, SEQ ID NO: 9 and SEQ ID NO: 17 or SEQ ID NO: 10 and SEQ ID NO: 18. In certain embodiments of any of the aforementioned uses and methods, the neurodegenerative disorder is selected from a group consisting of Alzheimer's disease, Parkinson's disease, Huntington's disease, Down syndrome, ataxia, amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), HIV-related cognitive impairment, CNS Lupus, mild cognitive impairment, or a combination thereof. In certain embodiments of any of the aforementioned uses and methods, the neurodegenerative disorder is Alzheimer's disease or Huntington's disease. In certain embodiments of any one of the aforementioned uses and methods, the symptoms are selected from a group consisting of neuropsychiatric symptoms, cognitive symptoms, motor dysfunction, and any combination thereof. In certain embodiments of any of the aforementioned uses and methods, the neuropsychiatric symptoms are selected from a group consisting of reducing anxiety-like behavior, improving spatial memory, increasing locomotion, and any combination thereof.

[0010] Additional uses and methods of treating a subject having a neurodegenerative or neuroinflammatory disorder, or of effecting a desirable outcome in a subject having a neurodegenerative or neuroinflammatory disorder, are provided herein. Provided is use of an effective amount of an isolated binding molecule which specifically binds to semaphorin-4D (SEMA4D) in the preparation of a medicament for treating a subject having a neurodegenerative disorder, wherein the binding to SEMA4D acts to alleviate symptoms associated with the disorder. Methods of treating a subject having a neurodegenerative disorder are also provided, comprising administering to the subject an effective amount of an isolated binding molecule which specifically binds to semaphorin-4D (SEMA4D), wherein the binding to SEMA4D acts to alleviate symptoms associated with the disorder. Provided is use of an effective amount of an isolated binding molecule which specifically binds to SEMA4D in the preparation of a medicament for promoting myelination in a subject having a neurodegenerative disorder, wherein the binding molecule modulates astrocyte-mediated activity of oligodendrocyte-myelin function. Methods of

promoting myelination in a subject having a neurodegenerative disorder are also provided, comprising administering to that subject an effective amount of an isolated binding molecule which specifically binds to SEMA4D, wherein the binding molecule modulates astrocyte-mediated activity of oligodendrocyte-myelin function. Provided is use of an effective amount of an isolated binding molecule which specifically binds to SEMA4D in the preparation of a medicament for preventing neural cell death in a subject having a neurodegenerative disorder, wherein the binding molecule modulates astrocyte-mediated synaptic activity. Methods of preventing neural cell death in a subject having a neurodegenerative disorder are also provided, comprising administering to that subject an effective amount of an isolated binding molecule which specifically binds to SEMA4D, wherein the binding molecule modulates astrocyte-mediated synaptic activity. Provided is use of an effective amount of an isolated binding molecule which specifically binds to SEMA4D in the preparation of a medicament for preventing injury to the blood-brain barrier in a subject having a neuroinflammatory or neurodegenerative disorder, wherein the binding molecule modulates astrocyte-mediated maintenance of the integrity of the blood-brain barrier. Methods of preventing injury to the blood-brain barrier in a subject having a neuroinflammatory or neurodegenerative disorder are also provided, comprising administering to that subject an effective amount of an isolated binding molecule which specifically binds to SEMA4D, wherein the binding molecule modulates astrocyte-mediated maintenance of the integrity of the blood-brain barrier. Provided is use of an effective amount of an isolated binding molecule which specifically binds to SEMA4D in the preparation of a medicament for preventing astrocyte activation in a subject having, determined to have, or suspected of having a neuroinflammatory or neurodegenerative disorder, wherein the binding molecule modulates astrocyte-mediated maintenance of the integrity of the blood-brain barrier. Methods of preventing astrocyte activation in a subject having, determined to have, or suspected of having a neuroinflammatory or neurodegenerative disorder are also provided, comprising administering to that subject an effective amount of an isolated binding molecule which specifically binds to SEMA4D, wherein the binding molecule modulates astrocyte-mediated maintenance of the integrity of the blood-brain barrier. Provided is use of an effective amount of an isolated binding molecule which specifically binds to SEMA4D in the preparation of a medicament for maintaining or restoring astrocyte-mediated trophic support of oligodendrocyte precursor cells (OPCs) in a subject having, determined to have, or suspected of having a neuroinflammatory or neurodegenerative disease, wherein the binding molecule prevents retraction of astrocyte processes and chemotactic movement of OPCs towards regions of damage. Methods of maintaining or restoring astrocyte-mediated trophic support of oligodendrocyte precursor cells (OPCs) in a subject having, determined to have, or suspected of having a neuroinflammatory or

neurodegenerative disorder are also provided, comprising administering to that subject an effective amount of an isolated binding molecule which specifically binds to SEMA4D, wherein the binding molecule prevents retraction of astrocyte processes and chemotactic movement of OPCs toward regions of damage. Provided is use of an effective amount of an isolated binding molecule which specifically binds to SEMA4D in the preparation of a medicament for protecting inhibitory neurons from degeneration in early Alzheimer's disease in a subject having, determined to have, or suspected of having early Alzheimer's disease, wherein the binding molecule restores the number of somatostatin positive neurons, NYP-positive neurons, or both in the subject. Methods of protecting inhibitory neurons from degeneration in early Alzheimer's disease are also provided, comprising administering to a subject having, determined to have, or suspected of having early Alzheimer's disease an effective amount of an isolated binding molecule which specifically binds to SEMA4D, wherein the binding molecule restores the number of somatostatin positive neurons, NYP-positive neurons, or both in the subject. In certain embodiments of the aforementioned uses and methods, the binding molecule inhibits SEMA4D interaction with its receptor or a portion of its receptor. In certain embodiments of the aforementioned uses and methods, the receptor is selected from the group consisting of Plexin-B1 and Plexin-B2. In certain

embodiments of the aforementioned uses and methods, the binding molecule inhibits SEMA4D-mediated Plexin-B1 signal transduction. In certain embodiments of the aforementioned uses and methods, the isolated binding molecule specifically binds to the same SEMA4D epitope as a reference monoclonal antibody selected from the group consisting of VX15/2503 or 67. In certain embodiments of the aforementioned uses and methods, the isolated binding molecule competitively inhibits a reference monoclonal antibody selected from the group consisting of VX15/2503 or 67 from specifically binding to SEMA4D. In certain embodiments of the aforementioned uses and methods, the isolated binding molecule comprises an antibody or antigen-binding fragment thereof. In certain embodiments of the aforementioned uses and methods, the antibody or antigen-binding fragment thereof is monoclonal antibody VX15/2503 or 67. In certain embodiments of the aforementioned uses and methods, the antibody or antigen-binding fragment thereof comprises a variable heavy chain (VH) comprising VHCDRs 1-3 comprising SEQ ID NOs 6, 7, and 8, respectively, and a variable light chain (VL) comprising VLCDRs 1-3 comprising SEQ ID NOs 14, 15, and 16, respectively. In certain embodiments of the aforementioned uses and methods, the VH and VL comprise, respectively, SEQ ID NO: 9 and SEQ ID NO: 17 or SEQ ID NO: 10 and SEQ ID NO: 18. In certain embodiments of the aforementioned uses and methods, the neurodegenerative disorder is selected from a group consisting of Alzheimer's disease, Parkinson's disease, Huntington's disease, Down syndrome, ataxia, amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), HIV-related cognitive impairment, CNS Lupus, mild cognitive impairment, or a combination thereof. In certain embodiments of the aforementioned uses and methods, the neurodegenerative disorder is Alzheimer's disease or Huntington's disease. In certain embodiments of the aforementioned uses and methods, symptoms of the subject that are alleviated by the uses and methods are selected from a group consisting of neuropsychiatric symptoms, cognitive symptoms, motor dysfunction, and any combination thereof. In certain embodiments of the aforementioned uses and methods, the neuropsychiatric symptoms of the subject that are alleviated by the uses and methods are selected from a group consisting of reducing anxiety-like behavior, improving spatial memory, increasing locomotion, and any combination thereof. In certain embodiments of the aforementioned uses and methods, the subject is determined to have the neurodegenerative disorder by processing a sample or image from the subject.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

[0011] FIGURE 1: Schematic of experimental protocol described in the Examples.

[0012] FIGURES 2: *In vivo* CVN model measuring anxiety-like behavior in CVN mice treated with anti-SEMA4D antibody ("MAb 67") or control isotype ("MAb 2B8"). Total locomotion (FIG. 2A) and locomotion in the center of the open field (FIG. 2B).

[0013] FIGURE 3: *In vivo* CVN model measuring spatial memory in a radial-arm water maze (FIG. 3A) CVN mice treated with anti-SEMA4D antibody ("MAb 67") or control isotype (FIG. 3B).

[0014] FIGURE 4: *In vivo* CVN model measuring the density of GABAergic synapses and concentration of vesicular GABA transporter (VGAT) in CVN mice treated with anti-SEMA4D antibody ("MAb 67") or control isotype. VGAT positive vesicles (FIG. 4A) and VGAT staining intensity level per vesicle (FIG. 4B).

[0015] FIGURE 5: *In vivo* YAC128 model measuring anxiety-like behavior in mice treated with anti-SEMA4D antibody ("MAb 67") and control isotype. Entries into (FIG. 5A) and time spent in the center of the field (FIG. 5B).

[0016] FIGURE 6: *In vivo* YAC128 model measuring spatial memory in mice treated with anti-SEMA4D antibody ("MAb 67") and control isotype. Panel A=Trial 1 (FIG. 6A), and Panel B=Trial 2 (FIG. 6B).

[0017] FIGURE 7: *In vivo* YAC128 model measuring cortical (FIG. 7A) and corpus callosum (FIG. 7B) volume in mice treated with anti-SEMA4D antibody ("MAb 67") or control isotype.

[0018] FIGURE 8: *In vivo* YAC128 model measuring testicular degeneration in mice treated with anti-SEMA4D antibody ("MAb 67") or control isotype.

[0019] FIGURE 9A-P: Immunohistochemical analysis of cell types expressing SEMA4D, plexin-B1, and CD72 in normal rat spinal cord. Nkx2.2 (panel B) is an oligodendrocyte precursor cell marker, glial fibrillary acid protein (GFAP) (panel G) is an astrocytic cell marker, and Iba1 (panel N) is a microglial cell marker. Panels A, E, I, and M show merged images, and panels D, H, L, and P, show the same sections stained with DAPI to visualize cellular nuclei.

[0020] FIGURE 10: DAB immunohistochemical analysis of amyloid pathology and glial activation in normal (top three panels) and CVN (bottom three panels) mice.

Subiculum sections were stained for amyloid-beta 1-42 (left panels), the microglial cell marker Iba1 (middle panels) and the astrocytic cell marker GFAP.

[0021] FIGURES 11A-11B: Characterization and expression patterns of plexin-B1 and plexin-B2 receptors in the CVN Alzheimer's disease mouse model. FIG. 11A shows immunohistochemical analysis of plexin-B1 expression in normal (top panels) and CVN (bottom panels) mice. Brain sections were stained for plexin-B1, and GFAP, as well as DAPI to visualize cellular nuclei. FIG. 11B shows expression levels of plexin-B1 (left graph) and plexin-B2 (right graph) following inhibition of SEMA4D signaling.

[0022] FIGURE 12: Immunohistochemical analysis of plexin-B2 expression in normal (top panels) and YAC128 (bottom panels) mice. Brain sections were stained for plexin-B2, and GFAP, as well as DAPI to visualize cellular nuclei.

[0023] FIGURE 13: Schematic representation of the roles SEMA4D signaling can play in the regulation of astrocyte function in health and disease. Left Panel: Plexin+ (shaded region of astrocyte exterior surface) astrocytic processes interdigitate between SEMA4D+ NIKX2.2+ oligodendrocyte precursor cells (OPCs) and provide trophic support (SEMA4D+ shown as shaded region of OPC exterior surface). In CNS disease, activated astrocytes upregulate Plexin expression and retract processes via SEMA4D signaling. Locally, this results in diminished trophic support and increased chemotaxis-driven OPC movement toward regions of damage, while lack of astrocytic support at lesion site impedes remyelination. Center Panel: In CNS disease, astrocytic activation leads to upregulation of Plexin (shaded region of astrocyte exterior surface) expression, increased SEMA4D signaling and process retraction, which results in a loss of neuronal axon guidance, decreased trophic support, and/or dysregulated glutamate uptake/release. Ultimately, depending upon severity of disease stimulus, synapse loss and subsequent excitotoxic neuron death can occur. Right Panel: CNS disease-induced astrocyte activation increases SEMA4D signaling through Plexin (shaded region of astrocyte exterior surface), which leads to a retraction of astrocytic foot processes as evidenced by redistribution of aquaporin-4. This results in dysregulation and permeability of the BBB, thereby facilitating endothelial inflammation and subsequent leukocyte entry into the CNS.

[0024] FIGURE 14: Immunohistochemical analysis showing SEMA4D-expressing OPCs oriented in close association with GFAP+ astrocytic processes in normal rats. Brain sections were stained for SEMA4D (OPCs), and GFAP (astrocytes), as well as DAPI to visualize cellular nuclei.

[0025] FIGURE 15 A, B, C, D: *In* vivo CVN model measuring somatostatin- (panel A), neuropeptide-Y (NPY)- (panel B), NPY receptor 1 (NPY1R) (panel C), or NPY receptor 2 (NPY2R) (panel D) positive signaling within the subiculum or dentate gyrus, respectively, in CVM mice treated with anti-SEMA4D antibody ("MAb 67") or control isotype. Error bars indicate standard error. "*"=$p<0.05$ and "***"=$p<0.005$ by 1-way ANOVA with Bonferroni's Multiple Comparison Test.

[0026] FIGURE 16: Immunohistochemical analysis of aquaporin-4 expression patterns in normal and CVN mice.

[0027] FIGURE 17: *In vitro* DIV-BBB model measuring integrity of the blood-brain barrier upon addition of anti-SEMA4D monoclonal antibody VX15/2503.

[0028] FIGURES 18A-18B: Immunocytochemical analysis showing astrocyte activation in rat astrocytes. FIG. 18A shows immunocytochemical analysis of astrocyte activation as reflected in the relative increase in GFAP positive area in cultured rat astrocytes treated with SEMA4D in isolation or following pretreatment with thioacetamide (TAA). FIG. 18B shows immunocytochemical analysis of astrocyte activation as reflected in the ratio of F-actin to G-actin in cultured rat astrocytes treated with SEMA4D in isolation or in combination with prostaglandin D2. Error bars represent standard deviation. "*"=$P<0.05$ by one-way ANOVA with Bonferroni's Multiple Comparison Test.

DETAILED DESCRIPTION OF THE DISCLOSURE

I. Definitions

[0029] It is to be noted that the term "a" or "an" entity refers to one or more of that entity; for example, "an anti-SEMA4D antibody" is understood to represent one or more anti-SEMA4D antibodies. As such, the terms "a" (or "an"), "one or more," and "at least one" can be used interchangeably herein.

[0030] Furthermore, "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus,

the term and/or" as used in a phrase such as "A and/or B" herein is intended to include "A and B," "A or B," "A" (alone), and "B" (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following embodiments: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

[0031] Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure is related. For example, the Concise Dictionary of Biomedicine and Molecular Biology, Juo, Pei-Show, 2nd ed., 2002, CRC Press; The Dictionary of Cell and Molecular Biology, 3rd ed., 1999, Academic Press; and the Oxford Dictionary Of Biochemistry And Molecular Biology, Revised, 2000, Oxford University Press, provide one of skill with a general dictionary of many of the terms used in this disclosure.

[0032] Units, prefixes, and symbols are denoted in their Système International de Unites (SI) accepted form. Numeric ranges are inclusive of the numbers defining the range. Unless otherwise indicated, amino acid sequences are written left to right in amino to carboxy orientation. The headings provided herein are not limitations of the various aspects or aspects of the disclosure, which can be had by reference to the specification as a whole. Accordingly, the terms defined immediately below are more fully defined by reference to the specification in its entirety.

[0033] As used herein, the term “non-naturally occurring” substance, composition, entity, and/or any combination of substances, compositions, or entities, or any grammatical variants thereof, is a conditional term that explicitly excludes, but only excludes, those forms of the substance, composition, entity, and/or any combination of substances, compositions, or entities that are well-understood by persons of ordinary skill in the art as being “naturally-occurring,” or that are, or might be at any time, determined or interpreted by a judge or an administrative or judicial body to be, “naturally-occurring.”

[0034] As used herein, the term “neurodegenerative disorder” or “neurodegenerative disease” refers to a central nervous system (CNS) disorder that is characterized by the death of neurons in one or more regions of the nervous system and the subsequent functional impairment of the affected parties. Examples of neurodegenerative disorders include, without limitation, Alzheimer's disease, Parkinson's disease,

Huntington's disease, Down syndrome, ataxia, amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), HIV-related cognitive impairment (HAND, HIV-Associated Neurocognitive Disorder), CNS Lupus and mild cognitive impairment. Neurodegenerative diseases have an enormous impact on the lives of affected individuals and their families as well as society as a whole.

[0035] As used herein, the term "Alzheimer's disease" refers to a progressive disease initially manifesting itself with partial amnesia, and later restlessness, disorientation, aphasia, agnosia or apraxia (cognitive decline), dementia and sometimes euphoria or depressions. The disease typically starts at 40 to 90 years of age and predominantly affects females. As to its prevalence, estimations are about 13 % of the population above 65 years age.

[0036] As used herein, the term "Huntington's Disease" refers to a neurodegenerative disease, which is due to expansion of a poly-glutamine tract at the N-terminus of the protein huntingtin (expressed by the HTT gene) where the expansion can be more than 35-40 repetitions of the amino acid glutamine in the mutated protein (mHTT). The disease presents with progressive neuronal death in different brain areas, including toxicity in medium-sized spiny neurons of the striatum that determines the appearance of the classic motor incoordination and movements such as "Chorea". The mechanism of action of mHTT has been described as both gain and loss of function compared with the wild-type protein and involves the acquisition or loss of competence to interact with various proteins in different cellular compartments.

[0037] The term "therapeutically effective amount" refers to an amount of an antibody, polypeptide, polynucleotide, small organic molecule, or other drug effective to "treat" a disease or disorder in a subject or mammal. In the case of a neurodegenerative disorder, the therapeutically effective amount of the drug can alleviate symptoms of the disorder; decrease, reduce, retard or stop the incidence of symptoms; decrease, reduce, retard the severity of symptoms; inhibit, *e.g.*, suppress, retard, prevent, stop, or reverse the manifestation of symptoms; relieve to some extent one or more of the symptoms associated with the disorder; reduce morbidity and mortality; improve quality of life; or a combination of such effects.

[0038] The term "symptoms" as referred to herein refer to, *e.g.*, 1) neuropsychiatric symptoms, 2) cognitive symptoms, and 3) motor dysfunction. Examples of

neuropsychiatric symptoms include, for instance, anxiety-like behavior. Examples of cognitive symptoms include, for instance, learning and memory deficits. Examples of motor dysfunction include, for instance, locomotion.

[0039] Terms such as "treating" or "treatment" or "to treat" or "alleviating" or "to alleviate" or "improving" or "to improve" refer to both 1) therapeutic measures that cure, slow down, lessen symptoms of, reverse, and/or halt progression of a diagnosed pathologic condition or disorder and 2) prophylactic or preventative measures that prevent and/or slow the development of a targeted pathologic condition or disorder. Thus those in need of treatment include those already with the disorder; those prone to have the disorder; and those in whom the disorder is to be prevented. Beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilization *(i.e.*, not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment. Those in need of treatment include those already with the condition or disorder as well as those prone to have the condition or disorder or those in which the condition or disorder is to be prevented.

[0040] By "subject" or "individual" or "animal" or "patient" or "mammal," is meant any subject, particularly a mammalian subject, for whom diagnosis, prognosis, or therapy is desired. Mammalian subjects include humans, domestic animals, farm animals, and zoo, sports, or pet animals such as dogs, cats, guinea pigs, rabbits, rats, mice, horses, cattle, cows, bears, and so on.

[0041] As used herein, phrases such as "a subject that would benefit from administration of an anti-SEMA4D antibody" and "an animal in need of treatment" includes subjects, such as mammalian subjects, that would benefit from administration of an anti-SEMA4D antibody or other SEMA4D binding molecule used, *e.g.*, for detection of a SEMA4D polypeptide (*e.g.*, for a diagnostic procedure) and/or from treatment, *i.e.*, palliation or prevention of a disease, with an anti-SEMA4D antibody or other SEMA4D binding molecule.

[0042] A "binding molecule" or "antigen binding molecule" of the present disclosure refers in its broadest sense to a molecule that specifically binds an antigenic determinant. In one embodiment, the binding molecule specifically binds to

SEMA4D, *e.g.*, to a transmembrane SEMA4D polypeptide of about 150 kDa or a soluble SEMA4D polypeptide of about 120 kDa (commonly referred to as sSEMA4D). In another embodiment, a binding molecule of the disclosure is an antibody or an antigen binding fragment thereof. In another embodiment, a binding molecule of the disclosure comprises at least one heavy or light chain CDR of an antibody molecule. In another embodiment, a binding molecule of the disclosure comprises at least two CDRs from one or more antibody molecules. In another embodiment, a binding molecule of the disclosure comprises at least three CDRs from one or more antibody molecules. In another embodiment, a binding molecule of the disclosure comprises at least four CDRs from one or more antibody molecules. In another embodiment, a binding molecule of the disclosure comprises at least five CDRs from one or more antibody molecules. In another embodiment, a binding molecule of the disclosure comprises at least six CDRs from one or more antibody molecules.

[0043] The present disclosure is directed to a method of alleviating symptoms in a subject having a neurodegenerative disorder, comprising administering to the subject an anti-SEMA4D binding molecule, *e.g.,* an antibody, or antigen-binding fragment, variant, or derivative thereof. Unless specifically referring to full-sized antibodies such as naturally occurring antibodies, the term "anti-SEMA4D antibody" encompasses full-sized antibodies as well as antigen-binding fragments, variants, analogs, or derivatives of such antibodies, *e.g.*, naturally occurring antibody or immunoglobulin molecules or engineered antibody molecules or fragments that bind antigen in a manner similar to antibody molecules.

[0044] As used herein, "human" or "fully human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulins and that do not express endogenous immunoglobulins, as described infra and, for example, in U.S. Pat. No. 5,939,598 by Kucherlapati *et al.* "Human" or "fully human" antibodies also include antibodies comprising at least the variable domain of a heavy chain, or at least the variable domains of a heavy chain and a light chain, where the variable domain(s) have the amino acid sequence of human immunoglobulin variable domain(s).

[0045] "Human" or "fully human" antibodies also include "human" or "fully human" antibodies, as described above, that comprise, consist essentially of, or consist of, variants (including derivatives) of antibody molecules (*e.g.*, the VH regions and/or VL regions) described herein, which antibodies or fragments thereof immunospecifically bind to a SEMA4D polypeptide or fragment or variant thereof. Standard techniques known to those of skill in the art can be used to introduce mutations in the nucleotide sequence encoding a human anti-SEMA4D antibody, including, but not limited to, site-directed mutagenesis and PCR-mediated mutagenesis which result in amino acid substitutions. In some embodiments, the variants (including derivatives) encode less than 50 amino acid substitutions, less than 40 amino acid substitutions, less than 30 amino acid substitutions, less than 25 amino acid substitutions, less than 20 amino acid substitutions, less than 15 amino acid substitutions, less than 10 amino acid substitutions, less than 5 amino acid substitutions, less than 4 amino acid substitutions, less than 3 amino acid substitutions, or less than 2 amino acid substitutions relative to the reference VH region, VHCDR1, VHCDR2, VHCDR3, VL region, VLCDR1, VLCDR2, or VLCDR3.

[0046] In certain embodiments, the amino acid substitutions are conservative amino acid substitutions, discussed further below. Alternatively, mutations can be introduced randomly along all or part of the coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for biological activity to identify mutants that retain activity (*e.g.*, the ability to bind a SEMA4D polypeptide, *e.g.*, human, murine, or both human and murine SEMA4D). Such variants (or derivatives thereof) of "human" or "fully human" antibodies can also be referred to as human or fully human antibodies that are "optimized" or "optimized for antigen binding" and include antibodies that have improved affinity to antigen.

[0047] The terms "antibody" and "immunoglobulin" are used interchangeably herein. An antibody or immunoglobulin comprises at least the variable domain of a heavy chain, and normally comprises at least the variable domains of a heavy chain and a light chain. Basic immunoglobulin structures in vertebrate systems are relatively well understood. See, *e.g.*, Harlow *et al.* (1988) Antibodies: A Laboratory Manual (2nd ed.; Cold Spring Harbor Laboratory Press).

[0048] As used herein, the term "immunoglobulin" comprises various broad classes of polypeptides that can be distinguished biochemically. Those skilled in the art will appreciate that heavy chains are classified as gamma, mu, alpha, delta, or epsilon, (γ, μ, α, δ, ε) with some subclasses among them (*e.g.*, γ1-γ4). It is the nature of this chain that determines the "class" of the antibody as IgG, IgM, IgA IgG, or IgE, respectively. The immunoglobulin subclasses (isotypes) *e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, etc. are well characterized and are known to confer functional specialization. Modified versions of each of these classes and isotypes are readily discernable to the skilled artisan in view of the instant disclosure and, accordingly, are within the scope of the instant disclosure. All immunoglobulin classes are clearly within the scope of the present disclosure, the following discussion will generally be directed to the IgG class of immunoglobulin molecules. With regard to IgG, a standard immunoglobulin molecule comprises two identical light chain polypeptides of molecular weight approximately 23,000 Daltons, and two identical heavy chain polypeptides of molecular weight 53,000-70,000. The four chains are typically joined by disulfide bonds in a "Y" configuration wherein the light chains bracket the heavy chains starting at the mouth of the "Y" and continuing through the variable region.

[0049] Light chains are classified as either kappa or lambda (κ, λ). Each heavy chain class can be bound with either a kappa or lambda light chain. In general, the light and heavy chains are covalently bonded to each other, and the "tail" portions of the two heavy chains are bonded to each other by covalent disulfide linkages or non-covalent linkages when the immunoglobulins are generated either by hybridomas, B cells or genetically engineered host cells. In the heavy chain, the amino acid sequences run from an N-terminus at the forked ends of the Y configuration to the C-terminus at the bottom of each chain.

[0050] Both the light and heavy chains are divided into regions of structural and functional homology. The terms "constant" and "variable" are used functionally. In this regard, it will be appreciated that the variable domains of both the light (VL or VK) and heavy (VH) chain portions determine antigen recognition and specificity. Conversely, the constant domains of the light chain (CL) and the heavy chain (typically CH1, CH2 or CH3) confer important biological properties such as secretion, transplacental mobility, Fc receptor binding, complement binding, and the

like. By convention the numbering of the constant region domains increases as they become more distal from the antigen binding site or amino-terminus of the antibody. The N-terminal portion is a variable region and at the C-terminal portion is a constant region; the CH3 and CL domains typically comprise the carboxy-terminus of the heavy and light chain, respectively.

[0051] As indicated above, the variable region allows the antibody to selectively recognize and specifically bind epitopes on antigens. That is, the VL domain and VH domain, or subset of the complementarity determining regions (CDRs) within these variable domains, of an antibody combine to form the variable region that defines a three dimensional antigen binding site. This quaternary antibody structure forms the antigen binding site present at the end of each arm of the Y. More specifically, the antigen binding site is defined by three CDRs on each of the VH and VL chains. In some instances, *e.g.*, certain immunoglobulin molecules derived from camelid species or engineered based on camelid immunoglobulins, a complete immunoglobulin molecule can consist of heavy chains only, with no light chains. See, *e.g.*, Hamers-Casterman *et al.*, *Nature 363*:446-448 (1993).

[0052] In naturally occurring antibodies, the six "complementarity determining regions" or "CDRs" present in each antigen binding domain are short, non-contiguous sequences of amino acids that are specifically positioned to form the antigen binding domain as the antibody assumes its three dimensional configuration in an aqueous environment. The remainder of the amino acids in the antigen binding domains, referred to as "framework" regions, show less inter-molecular variability. The framework regions largely adopt a β-sheet conformation and the CDRs form loops that connect, and in some cases form part of, the β-sheet structure. Thus, framework regions act to form a scaffold that provides for positioning the CDRs in correct orientation by inter-chain, non-covalent interactions. The antigen binding domain formed by the positioned CDRs defines a surface complementary to the epitope on the immunoreactive antigen. This complementary surface promotes the non-covalent binding of the antibody to its cognate epitope. The amino acids comprising the CDRs and the framework regions, respectively, can be readily identified for any given heavy or light chain variable domain by one of ordinary skill in the art, since they have been precisely defined (see below).

[0053] In the case where there are two or more definitions of a term that is used and/or accepted within the art, the definition of the term as used herein is intended to include all such meanings unless explicitly stated to the contrary. A specific example is the use of the term "complementarity determining region" ("CDR") to describe the non-contiguous antigen combining sites found within the variable region of both heavy and light chain polypeptides. This particular region has been described by Kabat *et al.* (1983) U.S. Dept. of Health and Human Services, "Sequences of Proteins of Immunological Interest" and by Chothia and Lesk, *J. Mol. Biol. 196*:901-917 (1987), which are incorporated herein by reference, where the definitions include overlapping or subsets of amino acid residues when compared against each other. Nevertheless, application of either definition to refer to a CDR of an antibody or variants thereof is intended to be within the scope of the term as defined and used herein. The appropriate amino acid residues that encompass the CDRs as defined by each of the above cited references are set forth below in Table 1 as a comparison. The exact residue numbers that encompass a particular CDR will vary depending on the sequence and size of the CDR. Those skilled in the art can routinely determine which residues comprise a particular CDR given the variable region amino acid sequence of the antibody.

Table 1. CDR Definitions[1]

	Kabat	**Chothia**
VH CDR1	31-35	26-32
VH CDR2	50-65	52-58
VH CDR3	95-102	95-102
VL CDR1	24-34	26-32
VL CDR2	50-56	50-52
VL CDR3	89-97	91-96

[1]Numbering of all CDR definitions in Table 1 is according to the numbering conventions set forth by Kabat *et al.* (see below).

[0054] Kabat *et al.* also defined a numbering system for variable domain sequences that is applicable to any antibody. One of ordinary skill in the art can unambiguously assign this system of "Kabat numbering" to any variable domain sequence, without reliance on any experimental data beyond the sequence itself. As used herein, "Kabat numbering" refers to the numbering system set forth by Kabat *et al.* (1983) U.S. Dept. of Health and Human Services, "Sequence of Proteins of Immunological Interest." Unless otherwise specified, references to the numbering of specific amino acid

residue positions in an anti-SEMA4D antibody or antigen-binding fragment, variant, or derivative thereof of the present disclosure are according to the Kabat numbering system.

[0055] Antibodies or antigen-binding fragments, variants, or derivatives thereof of the disclosure include, but are not limited to, polyclonal, monoclonal, multispecific and bispecific in which at least one arm is specific for SEMA4D, human, humanized, primatized, or chimeric antibodies, single-chain antibodies, epitope-binding fragments, *e.g.*, Fab, Fab' and $F(ab')_2$, Fd, Fvs, single-chain Fvs (scFv), disulfide-linked Fvs (sdFv), fragments comprising either a VL or VH domain, fragments produced by a Fab expression library, and anti-idiotypic (anti-Id) antibodies (including, *e.g.*, anti-Id antibodies to anti-SEMA4D antibodies disclosed herein). ScFv molecules are known in the art and are described, *e.g.*, in U.S. Pat. No. 5,892,019. Immunoglobulin or antibody molecules of the disclosure can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA, and IgY), class (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2, etc.), or subclass of immunoglobulin molecule.

[0056] As used herein, the term "heavy chain portion" includes amino acid sequences derived from an immunoglobulin heavy chain. In certain embodiments, a polypeptide comprising a heavy chain portion comprises at least one of: a VH domain, a CH1 domain, a hinge (*e.g.*, upper, middle, and/or lower hinge region) domain, a CH2 domain, a CH3 domain, or a variant or fragment thereof. For example, a binding polypeptide for use in the disclosure can comprise a polypeptide chain comprising a CH1 domain; a polypeptide chain comprising a CH1 domain, at least a portion of a hinge domain, and a CH2 domain; a polypeptide chain comprising a CH1 domain and a CH3 domain; a polypeptide chain comprising a CH1 domain, at least a portion of a hinge domain, and a CH3 domain, or a polypeptide chain comprising a CH1 domain, at least a portion of a hinge domain, a CH2 domain, and a CH3 domain. In another embodiment, a polypeptide of the disclosure comprises a polypeptide chain comprising a CH3 domain. Further, a binding polypeptide for use in the disclosure can lack at least a portion of a CH2 domain (*e.g.*, all or part of a CH2 domain). As set forth above, it will be understood by one of ordinary skill in the art that these domains (*e.g.*, the heavy chain portions) can be modified such that they vary in amino acid sequence from the naturally occurring immunoglobulin molecule.

[0057] In certain anti-SEMA4D antibodies, or antigen-binding fragments, variants, or derivatives thereof disclosed herein, the heavy chain portions of one polypeptide chain of a multimer are identical to those on a second polypeptide chain of the multimer. Alternatively, heavy chain portion-containing monomers of the disclosure are not identical. For example, each monomer can comprise a different target binding site, forming, for example, a bispecific antibody.

[0058] The heavy chain portions of a binding molecule for use in the methods disclosed herein can be derived from different immunoglobulin molecules. For example, a heavy chain portion of a polypeptide can comprise a C_{H1} domain derived from an IgG1 molecule and a hinge region derived from an IgG3 molecule. In another example, a heavy chain portion can comprise a hinge region derived, in part, from an IgG1 molecule and, in part, from an IgG3 molecule. In another example, a heavy chain portion can comprise a chimeric hinge derived, in part, from an IgG1 molecule and, in part, from an IgG4 molecule.

[0059] As used herein, the term "light chain portion" includes amino acid sequences derived from an immunoglobulin light chain, *e.g.*, a kappa or lambda light chain. In some aspects, the light chain portion comprises at least one of a VL or CL domain.

[0060] Anti-SEMA4D antibodies, or antigen-binding fragments, variants, or derivatives thereof disclosed herein can be described or specified in terms of the epitope(s) or portion(s) of an antigen, *e.g.*, a target polypeptide disclosed herein (*e.g.*, SEMA4D) that they recognize or specifically bind. The portion of a target polypeptide that specifically interacts with the antigen binding domain of an antibody is an "epitope," or an "antigenic determinant." A target polypeptide can comprise a single epitope, but typically comprises at least two epitopes, and can include any number of epitopes, depending on the size, conformation, and type of antigen. Furthermore, it should be noted that an "epitope" on a target polypeptide can be or can include non-polypeptide elements, *e.g.*, an epitope can include a carbohydrate side chain.

[0061] The minimum size of a peptide or polypeptide epitope for an antibody is thought to be about four to five amino acids. Peptide or polypeptide epitopes can contain, *e.g.*, at least seven, at least nine or between at least about 15 to about 30 amino acids. Since a CDR can recognize an antigenic peptide or polypeptide in its tertiary form, the amino acids comprising an epitope need not be contiguous, and in

some cases, can be on separate peptide chains. A peptide or polypeptide epitope recognized by anti-SEMA4D antibodies of the present disclosure can contain a sequence of at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 15, at least 20, at least 25, or between about 15 to about 30 contiguous or non-contiguous amino acids of SEMA4D.

[0062] By "specifically binds," it is generally meant that an antibody binds to an epitope via its antigen binding domain, and that the binding entails some complementarity between the antigen binding domain and the epitope. According to this definition, an antibody is said to "specifically bind" to an epitope when it binds to that epitope, via its antigen binding domain more readily than it would bind to a random, unrelated epitope. The term "specificity" is used herein to qualify the relative affinity by which a certain antibody binds to a certain epitope. For example, antibody "A" can be deemed to have a higher specificity for a given epitope than antibody "B," or antibody "A" can be said to bind to epitope "C" with a higher specificity than it has for related epitope "D."

[0063] By "preferentially binds," it is meant that the antibody specifically binds to an epitope more readily than it would bind to a related, similar, homologous, or analogous epitope. Thus, an antibody that "preferentially binds" to a given epitope would more likely bind to that epitope than to a related epitope, even though such an antibody can cross-react with the related epitope.

[0064] By way of non-limiting example, an antibody can be considered to bind a first epitope preferentially if it binds the first epitope with a dissociation constant (K_D) that is less than the antibody's K_D for the second epitope. In another non-limiting example, an antibody can be considered to bind a first antigen preferentially if it binds the first epitope with an affinity that is at least one order of magnitude less than the antibody's K_D for the second epitope. In another non-limiting example, an antibody can be considered to bind a first epitope preferentially if it binds the first epitope with an affinity that is at least two orders of magnitude less than the antibody's K_D for the second epitope.

[0065] In another non-limiting example, an antibody can be considered to bind a first epitope preferentially if it binds the first epitope with an off rate (k(off)) that is less than the antibody's k(off) for the second epitope. In another non-limiting example, an antibody can be considered to bind a first epitope preferentially if it binds

the first epitope with an affinity that is at least one order of magnitude less than the antibody's k(off) for the second epitope. In another non-limiting example, an antibody can be considered to bind a first epitope preferentially if it binds the first epitope with an affinity that is at least two orders of magnitude less than the antibody's k(off) for the second epitope. An antibody or antigen-binding fragment, variant, or derivative disclosed herein can be said to bind a target polypeptide disclosed herein (*e.g.*, SEMA4D, *e.g.*, human, murine, or both human and murine SEMA4D) or a fragment or variant thereof with an off rate (k(off)) of less than or equal to 5 X 10^{-2} sec^{-1}, 10^{-2} sec^{-1}, 5 X 10^{-3} sec^{-1} or 10^{-3} sec^{-1}. In certain aspects, an antibody of the disclosure can be said to bind a target polypeptide disclosed herein (*e.g.*, SEMA4D, *e.g.*, human, murine, or both human and murine SEMA4D) or a fragment or variant thereof with an off rate (k(off)) less than or equal to 5 X 10^{-4} sec^{-1}, 10^{-4} sec^{-1}, 5 X 10^{-5} sec^{-1}, or 10^{-5} sec^{-1}, 5 X 10^{-6} sec^{-1}, 10^{-6} sec^{-1}, 5 X 10^{-7} sec^{-1} or 10^{-7} sec^{-1}.

[0066] An antibody or antigen-binding fragment, variant, or derivative disclosed herein can be said to bind a target polypeptide disclosed herein (*e.g.*, SEMA4D, *e.g.*, human, murine, or both human and murine SEMA4D) or a fragment or variant thereof with an on rate (k(on)) of greater than or equal to 10^3 M^{-1} sec^{-1}, 5 X 10^3 M^{-1} sec^{-1}, 10^4 M^{-1} sec^{-1} or 5 X 10^4 M^{-1} sec^{-1}. In some embodiments, an antibody of the disclosure cab be said to bind a target polypeptide disclosed herein (*e.g.*, SEMA4D, *e.g.*, human, murine, or both human and murine SEMA4D) or a fragment or variant thereof with an on rate (k(on)) greater than or equal to 10^5 M^{-1} sec^{-1}, 5 X 10^5 M^{-1} sec^{-1}, 10^6 M^{-1} sec^{-1}, or 5 X 10^6 M^{-1} sec^{-1} or 10^7 M^{-1} sec^{-1}.

[0067] An antibody is said to competitively inhibit binding of a reference antibody to a given epitope if it preferentially binds to that epitope to the extent that it blocks, to some degree, binding of the reference antibody to the epitope. Competitive inhibition can be determined by any method known in the art, for example, competition ELISA assays. An antibody can be said to competitively inhibit binding of the reference antibody to a given epitope by at least 90%, at least 80%, at least 70%, at least 60%, or at least 50%.

[0068] As used herein, the term "affinity" refers to a measure of the strength of the binding of an individual epitope with the CDR of an immunoglobulin molecule. See, *e.g.*, Harlow *et al.* (1988) Antibodies: A Laboratory Manual (Cold Spring Harbor Laboratory Press, 2nd ed.) pages 27-28. As used herein, the term "avidity" refers to

the overall stability of the complex between a population of immunoglobulins and an antigen, that is, the functional combining strength of an immunoglobulin mixture with the antigen. See, *e.g.*, Harlow at pages 29-34. Avidity is related to both the affinity of individual immunoglobulin molecules in the population with specific epitopes, and also the valencies of the immunoglobulins and the antigen. For example, the interaction between a bivalent monoclonal antibody and an antigen with a highly repeating epitope structure, such as a polymer, would be one of high avidity.

[0069] Anti-SEMA4D antibodies or antigen-binding fragments, variants, or derivatives thereof of the disclosure can also be described or specified in terms of their cross-reactivity. As used herein, the term "cross-reactivity" refers to the ability of an antibody, specific for one antigen, to react with a second antigen; a measure of relatedness between two different antigenic substances. Thus, an antibody is cross reactive if it binds to an epitope other than the one that induced its formation. The cross reactive epitope generally contains many of the same complementary structural features as the inducing epitope, and in some cases, can actually fit better than the original.

[0070] For example, certain antibodies have some degree of cross-reactivity, in that they bind related, but non-identical epitopes, *e.g.*, epitopes with at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, and at least 50% identity (as calculated using methods known in the art and described herein) to a reference epitope. An antibody can be said to have little or no cross-reactivity if it does not bind epitopes with less than 95%, less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, and less than 50% identity (as calculated using methods known in the art and described herein) to a reference epitope. An antibody can be deemed "highly specific" for a certain epitope, if it does not bind any other analog, ortholog, or homolog of that epitope.

[0071] Anti-SEMA4D binding molecules, *e.g.*, antibodies or antigen-binding fragments, variants or derivatives thereof, of the disclosure can also be described or specified in terms of their binding affinity to a polypeptide of the disclosure, *e.g.*, SEMA4D, *e.g.*, human, murine, or both human and murine SEMA4D. In certain aspects, the binding affinities include those with a dissociation constant or Kd less than 5×10^{-2} M, 10^{-2} M, 5×10^{-3} M, 10^{-3} M, 5×10^{-4} M, 10^{-4} M, 5×10^{-5} M, 10^{-5} M, 5

x 10^{-6} M, 10^{-6} M, 5 x 10^{-7} M, 10^{-7} M, 5 x 10^{-8} M, 10^{-8} M, 5 x 10^{-9} M, 10^{-9} M, 5 x 10^{-10} M, 10^{-10} M, 5 x 10^{-11} M, 10^{-11} M, 5 x 10^{-12} M, 10^{-12} M, 5 x 10^{-13} M, 10^{-13} M, 5 x 10^{-14} M, 10^{-14} M, 5 x 10^{-15} M, or 10^{-15} M. In certain embodiments, the anti-SEMA4D binding molecule, *e.g.*, an antibody or antigen binding fragment thereof, of the disclosure binds human SEMA4D with a Kd of about 5 x 10^{-9} to about 6 x 10^{-9}. In another embodiment, the anti-SEMA4D binding molecule, *e.g.*, an antibody or antigen binding fragment thereof, of the disclosure binds murine SEMA4D with a Kd of about 1 x 10^{-9} to about 2 x 10^{-9}.

[0072] As used herein, the term "chimeric antibody" will be held to mean any antibody wherein the immunoreactive region or site is obtained or derived from a first species and the constant region (which can be intact, partial or modified in accordance with the instant disclosure) is obtained from a second species. In certain embodiments the target binding region or site will be from a non-human source (*e.g.*, mouse or primate) and the constant region is human.

[0073] As used herein, the term "engineered antibody" refers to an antibody in which the variable domain in either the heavy or light chain or both is altered by at least partial replacement of one or more CDRs from an antibody of known specificity and, if necessary, by partial framework region replacement and sequence changing. Although the CDRs can be derived from an antibody of the same class or even subclass as the antibody from which the framework regions are derived, it is envisaged that the CDRs will be derived from an antibody of different class, or from an antibody from a different species. An engineered antibody in which one or more "donor" CDRs from a non-human antibody of known specificity is grafted into a human heavy or light chain framework region is referred to herein as a "humanized antibody." It is not always necessary to replace all of the CDRs with the complete CDRs from the donor variable domain to transfer the antigen binding capacity of one variable domain to another. Rather, one can transfer just those residues needed to maintain the activity of the target binding site need be transferred.

[0074] It is further recognized that the framework regions within the variable domain in a heavy or light chain, or both, of a humanized antibody can comprise solely residues of human origin, in which case these framework regions of the humanized antibody are referred to as "fully human framework regions" (for example, MAbs 1515/2503 or 67, disclosed in U.S. Patent Appl. Publication No. US

2010/0285036 A1 as MAb 2503, incorporated herein by reference in its entirety). Alternatively, one or more residues of the framework region(s) of the donor variable domain can be engineered within the corresponding position of the human framework region(s) of a variable domain in a heavy or light chain, or both, of a humanized antibody if necessary to maintain proper binding or to enhance binding to the SEMA4D antigen. A human framework region that has been engineered in this manner would thus comprise a mixture of human and donor framework residues, and is referred to herein as a "partially human framework region."

[0075] For example, humanization of an anti-SEMA4D antibody can be essentially performed following the method of Winter and co-workers (Jones *et al.*, *Nature 321*:522-525 (1986); Riechmann *et al.*, *Nature 332*:323-327 (1988); Verhoeyen *et al.*, *Science 239*:1534-1536 (1988)), by substituting rodent or mutant rodent CDRs or CDR sequences for the corresponding sequences of a human anti-SEMA4D antibody. See also U.S. Pat. Nos. 5,225,539; 5,585,089; 5,693,761; 5,693,762; 5,859,205; herein incorporated by reference. The resulting humanized anti-SEMA4D antibody would comprise at least one rodent or mutant rodent CDR within the fully human framework regions of the variable domain of the heavy and/or light chain of the humanized antibody. In some instances, residues within the framework regions of one or more variable domains of the humanized anti-SEMA4D antibody are replaced by corresponding non-human (for example, rodent) residues (see, for example, U.S. Pat. Nos. 5,585,089; 5,693,761; 5,693,762; and 6,180,370), in which case the resulting humanized anti-SEMA4D antibody would comprise partially human framework regions within the variable domain of the heavy and/or light chain.

[0076] Furthermore, humanized antibodies can comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance (*e.g.*, to obtain desired affinity). In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDRs correspond to those of a non-human immunoglobulin and all or substantially all of the framework regions are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details see Jones *et al.*, *Nature 331*:522-525 (1986); Riechmann *et al.*, *Nature 332*:323-329 (1988); and Presta, *Curr.*

Op. Struct. Biol. 2:593-596 (1992); herein incorporated by reference. Accordingly, such "humanized" antibodies can include antibodies wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some framework residues are substituted by residues from analogous sites in rodent antibodies. See, for example, U.S. Pat. Nos. 5,225,539; 5,585,089; 5,693,761; 5,693,762; 5,859,205. See also U.S. Pat. No. 6,180,370, and International Publication No. WO 01/27160, where humanized antibodies and techniques for producing humanized antibodies having improved affinity for a predetermined antigen are disclosed.

[0077] As used herein, the term "healthcare provider" refers to individuals or institutions that directly interact and administer to living subjects, *e.g.*, human patients. Non-limiting examples of healthcare providers include doctors, nurses, technicians, therapist, pharmacists, counselors, alternative medicine practitioners, medical facilities, doctor's offices, hospitals, emergency rooms, clinics, urgent care centers, alternative medicine clinics/facilities, and any other entity providing general and/or specialized treatment, assessment, maintenance, therapy, medication, and/or advice relating to all, or any portion of, a patient's state of health, including but not limited to general medical, specialized medical, surgical, and/or any other type of treatment, assessment, maintenance, therapy, medication and/or advice.

[0078] As used herein, the term "healthcare benefits provider" encompasses individual parties, organizations, or groups providing, presenting, offering, paying for in whole or in part, or being otherwise associated with giving a patient access to one or more healthcare benefits, benefit plans, health insurance, and/or healthcare expense account programs.

[0079] As used herein, the term "clinical laboratory" refers to a facility for the examination or processing of materials or images derived from a living subject, *e.g.*, a human being. Non-limiting examples of processing include biological, biochemical, serological, chemical, immunohematological, hematological, biophysical, cytological, pathological, genetic, image based, or other examination of materials derived from the human body or of any or all of the human body for the purpose of providing information, *e.g.*, for the diagnosis, prevention, or treatment of any disease or impairment of, or the assessment of the health of living subjects, *e.g.*,

human beings. These examinations can also include procedures to collect or otherwise obtain an image, a sample, prepare, determine, measure, or otherwise describe the presence or absence of various substances in the body of a living subject, *e.g.*, a human being, or a sample obtained from the body of a living subject, *e.g.*, a human being.

II. Target Polypeptide Description

[0080] As used herein, the terms "Semaphorin 4D," "SEMA4D" and "SEMA4D polypeptide" are used interchangeably, as are "SEMA4D" and "Sema4D." In certain embodiments, SEMA4D is expressed on the surface of or secreted by a cell. In another embodiment, SEMA4D is membrane bound. In another embodiments, SEMA4D is soluble, *e.g.*, sSEMA4D. In other embodiments, SEMA4D can include a full-sized SEMA4D or a fragment thereof, or a SEMA4D variant polypeptide, wherein the fragment of SEMA4D or SEMA4D variant polypeptide retains some or all functional properties of the full-sized SEMA4D.

[0081] The full-sized human SEMA4D protein is a homodimeric transmembrane protein consisting of two polypeptide chains of 150 kDa. SEMA4D belongs to the semaphorin family of cell surface receptors and is also referred to as CD100. Both human and mouse SEMA4D/Sema4D are proteolytically cleaved from their transmembrane form to generate 120-kDa soluble forms, indicating the existence of two Sema4D isoforms (Kumanogoh *et al.*, *J. Cell Science* *116*(7):3464 (2003)). Semaphorins consist of soluble and membrane-bound proteins that were originally defined as axonal-guidance factors during development which play an important role in establishing precise connections between neurons and their appropriate target. Structurally considered a class IV semaphorin, SEMA4D consists of an amino-terminal signal sequence followed by a characteristic 'Sema' domain, which contains 17 conserved cysteine residues, an Ig-like domain, a lysine-rich stretch, a hydrophobic transmembrane region, and a cytoplasmic tail.

[0082] A polypeptide chain of SEMA4D can include a signal sequence of about 13 amino acids and further includes a semaphorin domain of about 512 amino acids, an immunoglobulin-like (Ig-like) domain of about 65 amino acids, a lysine-rich stretch of 104 amino acids, a hydrophobic transmembrane region of about 19 amino acids, and a cytoplasmic tail of 110 amino acids. A consensus site for tyrosine

phosphorylation in the cytoplasmic tail supports the predicted association of SEMA4D with a tyrosine kinase (Schlossman, *et al.*, Eds. (1995) Leucocyte Typing V (Oxford University Press, Oxford).

[0083] SEMA4D is known to have at least three functional receptors, Plexin-B1, Plexin-B2 and CD72. One of the receptors, Plexin-B1, is expressed in non-lymphoid tissues and has been shown to be a high affinity (1 nM) receptor for SEMA4D (Tamagnone *et al.*, *Cell 99*:71-80 (1999)). SEMA4D stimulation of Plexin-B1 signaling has been shown to induce growth cone collapse of neurons, and to induce process extension collapse and apoptosis of oligodendrocytes (Giraudon *et al.*, *J. Immunol. 172*:1246-1255 (2004); Giraudon *et al.*, *NeuroMolecular Med. 7*:207-216 (2005)). After binding to SEMA4D, Plexin-B1 signaling mediates the inactivation of R-Ras, leading to a decrease in the integrin mediated attachment to the extracellular matrix, as well as to activation of RhoA, leading to reorganization of the cytoskeleton and cell migration. See Kruger *et al.*, *Nature Rev. Mol. Cell Biol. 6*:789-800 (2005); Pasterkamp, *TRENDS in Cell Biology 15*:61-64 (2005)). Plexin-B2, on the other hand, has an intermediate affinity for SEMA4D and recent reports indicate that Plexin-B2 regulates migration of cortical neurons and proliferation and migration of neuroblasts in the adult subventricular zone (Azzarelli *et al,. Nat Commun* 2014 Feb 27, 5:3405, DOI: 10.1038/ncomms4405; and Saha *et al., J. Neuroscience,* 2012 November 21, 32(47):16892–16905). .

[0084] In lymphoid tissues CD72 is utilized as a low affinity (300nM) SEMA4D receptor (Kumanogoh *et al.*, *Immunity 13*:621-631 (2000)). B cells and APCs express CD72, and anti-CD72 antibodies have many of the same effects as sSEMA4D, such as enhancement of CD40-induced B cell responses and B cell shedding of CD23. CD72 is thought to act as a negative regulator of B cell responses by recruiting the tyrosine phosphatase SHP-1, which can associate with many inhibitory receptors. Interaction of SEMA4D with CD72 results in the dissociation of SHP-1, and the loss of this negative activation signal. SEMA4D has been reported to promote T cell stimulation and B cell aggregation and survival *in vitro*. The addition of SEMA4D-expressing cells or sSEMA4D enhances CD40-induced B cell proliferation and immunoglobulin production *in vitro*, and accelerates *in vivo* antibody responses (Ishida *et al.*, *Inter. Immunol. 15*:1027-1034 (2003); Kumanogoh and H. Kukutani, *Trends in Immunol. 22*:670-676 (2001)). sSEMA4D enhances the CD40 induced

maturation of dendritic cells (DCs), including up-regulation of costimulatory molecules and increased secretion of IL-12. In addition, sSEMA4D can inhibit immune cell migration, which can be reversed by addition of blocking anti-SEMA4D antibodies (Elhabazi *et al.*, *J. Immunol.* 166:4341-4347 (2001); Delaire *et al.*, *J. Immunol.* 166:4348-4354 (2001)).

[0085] Sema4D is expressed at high levels in lymphoid organs, including the spleen, thymus, and lymph nodes, and in non-lymphoid organs, such as the brain, heart, and kidney. In lymphoid organs, Sema4D is abundantly expressed on resting T cells but only weakly expressed on resting B cells and antigen-presenting cells (APCs), such as DCs. Cellular activation increases the surface expression of SEMA4D as well as the generation of soluble SEMA4D (sSEMA4D).

[0086] The expression pattern of SEMA4D suggests that it plays an important physiological as well as pathological role in the immune system. SEMA4D has been shown to promote B cell activation, aggregation and survival; enhance CD40-induced proliferation and antibody production; enhance antibody response to T cell dependent antigens; increase T cell proliferation; enhance dendritic cell maturation and ability to stimulate T cells; and is directly implicated in demyelination and axonal degeneration (Shi *et al.*, *Immunity 13*:633-642 (2000); Kumanogoh *et al.*, *J Immunol 169*:1175-1181 (2002); and Watanabe *et al.*, *J Immunol 167*:4321-4328 (2001)).

[0087] SEMA4D knock out (SEMA4D-/-) mice have provided additional evidence that SEMA4D plays an important role in both humoral and cellular immune responses. There are no known major abnormalities of non-lymphoid tissues in SEMA4D-/- mice. DCs from the SEMA4D-/- mice have poor allostimulatory ability and show defects in expression of costimulatory molecules, which can be rescued by the addition of sSEMA4D. Mice deficient in SEMA4D (SEMA4D-/-) fail to develop experimental autoimmune encephalomyelitis induced by myelin oligodendrocyte glycoprotein peptide, because myelin oligodendrocyte glycoprotein-specific T cells are poorly generated in the absence of SEMA4D (Kumanogoh *et al.*, *J Immunol 169*:1175-1181 (2002)). A significant amount of soluble SEMA4D is also detected in the sera of autoimmunity-prone MRL/lpr mice (model of systemic autoimmune diseases such as SLE), but not in normal mice. Further, the levels of sSEMA4D correlate with levels of auto-antibodies and increase with age (Wang *et al.*, *Blood 97*:3498-3504 (2001)). Soluble SEMA4D has also been shown to accumulate in the

cerebral spinal fluid and sera of patients with demyelinating disease, and sSEMA4D induces apoptosis of human pluripotent neural precursors (Dev cells), and both inhibits process extension and induces apoptosis of rat oligodendrocytes *in vitro* (Giraudon *et al.*, *J Immunol 172*(2):1246-1255 (2004)). This apoptosis was blocked by an anti-SEMA4D MAb.

III. Anti-SEMA4D Antibodies

[0088] Antibodies that bind SEMA4D have been described in the art. See, for example, US Patent No. 8,496,938, US Publ. Nos. 2008/0219971 A1, US 2010/0285036 A1, and US 2006/0233793 A1, International Patent Applications WO 93/14125, WO 2008/100995, and WO 2010/129917, and Herold *et al., Int. Immunol. 7*(1): 1-8 (1995), each of which is herein incorporated in its entirety by reference.

[0089] The disclosure generally relates to a method of alleviating symptoms in a subject having a neuroinflammatory or neurodegenerative disorder, *e.g.*, a human patient, comprising administration of an antibody which specifically binds to SEMA4D, or an antigen-binding fragment, variant, or derivative thereof. In certain embodiments, the antibody blocks the interaction of SEMA4D with one or more of its receptors, *e.g.*, Plexin-B1. Anti-SEMA4D antibodies having these properties can be used in the methods provided herein. Antibodies that can be used include, but are not limited to MAbs VX15/2503, 67, and 76 and antigen-binding fragments, variants, or derivatives thereof which are fully described in US 2010/0285036 A1. Additional antibodies which can be used in the methods provided herein include the BD16 and BB18 antibodies described in US 2006/0233793 A1 as well as antigen-binding fragments, variants, or derivatives thereof; or any of MAb 301, MAb 1893, MAb 657, MAb 1807, MAb 1656, MAb 1808, Mab 59, MAb 2191, MAb 2274, MAb 2275, MAb 2276, MAb 2277, MAb 2278, MAb 2279, MAb 2280, MAb 2281, MAb 2282, MAb 2283, MAb 2284, and MAb 2285, as well as any fragments, variants or derivatives thereof as described in US 2008/0219971 A1. In certain embodiments an anti-SEMA4D antibody for use in the methods provided herein binds human, murine, or both human and murine SEMA4D. Also useful are antibodies which bind to the same epitope as any of the aforementioned antibodies and/or antibodies which competitively inhibit any of the aforementioned antibodies.

[0090] In certain embodiments, an anti-SEMA4D antibody or antigen-binding fragment, variant, or derivative thereof useful in the methods provided herein has an amino acid sequence that has at least about 80%, about 85%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, or about 95% sequence identity to the amino acid sequence for a reference anti-SEMA4D antibody molecule, for example those described above. In a further embodiment, the binding molecule shares at least about 96%, about 97%, about 98%, about 99%, or 100% sequence identity to a reference antibody.

[0091] In another embodiment, an anti-SEMA4D antibody or antigen-binding fragment, variant, or derivative thereof useful in the methods provided herein comprises, consists essentially of, or consists of an immunoglobulin heavy chain variable domain (VH domain), where at least one of the CDRs of the VH domain has an amino acid sequence that is at least about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99%, or identical to CDR1, CDR2 or CDR3 of SEQ ID NO: 9 or 10.

[0092] In another embodiment, an anti-SEMA4D antibody or antigen-binding fragment, variant, or derivative thereof useful in the methods provided herein comprises, consists essentially of, or consists of an immunoglobulin heavy chain variable domain (VH domain), where at least one of the CDRs of the VH domain has an amino acid sequence that is at least about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99%, or identical to SEQ ID NO: 6, SEQ ID NO: 7, or SEQ ID NO: 8.

[0093] In another embodiment, an anti-SEMA4D antibody or antigen-binding fragment, variant, or derivative thereof useful in the methods provided herein comprises, consists essentially of, or consists of an immunoglobulin heavy chain variable domain (VH domain), where at least one of the CDRs of the VH domain has an amino acid sequence identical, except for 1, 2, 3, 4, or 5 conservative amino acid substitutions, to SEQ ID NO: 6, SEQ ID NO: 7, or SEQ ID NO: 8.

[0094] In another embodiment, an anti-SEMA4D antibody or antigen-binding fragment, variant, or derivative thereof useful in the methods provided herein comprises, consists essentially of, or consists of a VH domain that has an amino acid sequence that is at least about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or

100% identical to SEQ ID NO: 9 or SEQ ID NO: 10, wherein an anti-SEMA4D antibody comprising the encoded VH domain specifically or preferentially binds to SEMA4D.

[0095] In another embodiment, an anti-SEMA4D antibody or antigen-binding fragment, variant, or derivative thereof useful in the methods provided herein comprises, consists essentially of, or consists of an immunoglobulin light chain variable domain (VL domain), where at least one of the CDRs of the VL domain has an amino acid sequence that is at least about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99%, or identical to CDR1, CDR2 or CDR3 of SEQ ID NO: 17 or 18.

[0096] In another embodiment, an anti-SEMA4D antibody or antigen-binding fragment, variant, or derivative thereof useful in the methods provided herein comprises, consists essentially of, or consists of an immunoglobulin light chain variable domain (VL domain), where at least one of the CDRs of the VL domain has an amino acid sequence that is at least about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99%, or identical to SEQ ID NO: 14, SEQ ID NO: 15, or SEQ ID NO: 16.

[0097] In another embodiment, an anti-SEMA4D antibody or antigen-binding fragment, variant, or derivative thereof useful in the methods provided herein comprises, consists essentially of, or consists of an immunoglobulin light chain variable domain (VL domain), where at least one of the CDRs of the VL domain has an amino acid sequence identical, except for 1, 2, 3, 4, or 5 conservative amino acid substitutions, to SEQ ID NO: 14, SEQ ID NO: 15, or SEQ ID NO: 16.

[0098] In a further embodiment, an anti-SEMA4D antibody or antigen-binding fragment, variant, or derivative thereof useful in the methods provided herein comprises, consists essentially of, or consists of a VL domain that has an amino acid sequence that is at least about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or 100% identical to SEQ ID NO: 17 or SEQ ID NO: 18, wherein an anti-SEMA4D antibody comprising the encoded VL domain specifically or preferentially binds to SEMA4D.

[0099] Also included for use in the methods provided herein are polypeptides encoding anti-SEMA4D antibodies, or antigen-binding fragments, variants, or

derivatives thereof as described herein, polynucleotides encoding such polypeptides, vectors comprising such polynucleotides, and host cells comprising such vectors or polynucleotides, all for producing anti-SEMA4D antibodies, or antigen-binding fragments, variants, or derivatives thereof for use in the methods described herein.

[0100] Suitable biologically active variants of the anti-SEMA4D antibodies of the disclosure can be used in the methods of the present disclosure. Such variants will retain the desired binding properties of the parent anti-SEMA4D antibody. Methods for making antibody variants are generally available in the art.

[0101] Methods for mutagenesis and nucleotide sequence alterations are well known in the art. See, for example, Walker and Gaastra, eds. (1983) Techniques in Molecular Biology (MacMillan Publishing Company, New York); Kunkel, Proc. *Natl. Acad. Sci. USA 82*:488-492 (1985); Kunkel *et al.*, *Methods Enzymol. 154*:367-382 (1987); Sambrook *et al.* (1989) Molecular Cloning: A Laboratory Manual (Cold Spring Harbor, N.Y.); U.S. Pat. No. 4,873,192; and the references cited therein; herein incorporated by reference. Guidance as to appropriate amino acid substitutions that do not affect biological activity of the polypeptide of interest can be found in the model of Dayhoff *et al.* (1978) in Atlas of Protein Sequence and Structure (Natl. Biomed. Res. Found., Washington, D.C.), pp. 345-352, herein incorporated by reference in its entirety. The model of Dayhoff *et al.* uses the Point Accepted Mutation (PAM) amino acid similarity matrix (PAM 250 matrix) to determine suitable conservative amino acid substitutions. In certain embodiments, conservative substitutions, such as exchanging one amino acid with another having similar properties can be used. Examples of conservative amino acid substitutions as taught by the PAM 250 matrix of the Dayhoff *et al.* model include, but are not limited to, Gly↔Ala, Val↔Ile↔Leu, Asp↔Glu, Lys↔Arg, Asn↔Gln, and Phe↔Trp↔Tyr.

[0102] In constructing variants of the anti-SEMA4D binding molecule, *e.g.*, an antibody or antigen-binding fragment thereof, polypeptides of interest, modifications are made such that variants continue to possess the desired properties, *e.g.*, being capable of specifically binding to a SEMA4D, *e.g.*, human, murine, or both human and murine SEMA4D, *e.g.*, expressed on the surface of or secreted by a cell and having SEMA4D blocking activity, as described herein. Obviously, any mutations made in the DNA encoding the variant polypeptide must not place the sequence out of reading frame and in certain embodiments will not create complementary regions that

could produce secondary mRNA structure. See EP Patent Application Publication No. 75,444.

[0103] Methods for measuring anti-SEMA4D binding molecule, *e.g.*, an antibody or antigen-binding fragment, variant, or derivative thereof, binding specificity include, but are not limited to, standard competitive binding assays, assays for monitoring immunoglobulin secretion by T cells or B cells, T cell proliferation assays, apoptosis assays, ELISA assays, and the like. See, for example, such assays disclosed in WO 93/14125; Shi *et al.*, *Immunity 13*:633-642 (2000); Kumanogoh *et al.*, *J Immunol 169*:1175-1181 (2002); Watanabe *et al.*, *J Immunol 167*:4321-4328 (2001); Wang *et al.*, *Blood 97*:3498-3504 (2001); and Giraudon *et al.*, *J Immunol 172*(2):1246-1255 (2004), all of which are herein incorporated by reference.

[0104] When discussed herein whether any particular polypeptide, including the constant regions, CDRs, VH domains, or VL domains disclosed herein, is at least about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or even about 100% identical to another polypeptide, the % identity can be determined using methods and computer programs/software known in the art such as, but not limited to, the BESTFIT program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, Wis. 53711). BESTFIT uses the local homology algorithm of Smith and Waterman (1981) Adv. Appl. Math. 2:482-489, to find the best segment of homology between two sequences. When using BESTFIT or any other sequence alignment program to determine whether a particular sequence is, for example, 95% identical to a reference sequence according to the present disclosure, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference polypeptide sequence and that gaps in homology of up to 5% of the total number of amino acids in the reference sequence are allowed.

[0105] For purposes of the present disclosure, percent sequence identity can be determined using the Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 2, BLOSUM matrix of 62. The Smith-Waterman homology search algorithm is taught in Smith and Waterman (1981) Adv. Appl. Math. 2:482-489. A variant can, for example, differ from a reference anti-SEMA4D antibody (*e.g.*, MAb VX15/2503, 67, or 76) by as few

as 1 to 15 amino acid residues, as few as 1 to 10 amino acid residues, such as 6-10, as few as 5, as few as 4, 3, 2, or even 1 amino acid residue.

[0106] Percentage of "sequence identity" can also be determined by comparing two optimally aligned sequences over a comparison window. In order to optimally align sequences for comparison, the portion of a polynucleotide or polypeptide sequence in the comparison window can comprise additions or deletions termed gaps while the reference sequence is kept constant. An optimal alignment is that alignment which, even with gaps, produces the greatest possible number of "identical" positions between the reference and comparator sequences. Percentage "sequence identity" between two sequences can be determined using the version of the program "BLAST 2 Sequences" which was available from the National Center for Biotechnology Information as of September 1, 2004, which program incorporates the programs BLASTN (for nucleotide sequence comparison) and BLASTP (for polypeptide sequence comparison), which programs are based on the algorithm of Karlin and Altschul (*Proc. Natl. Acad. Sci. USA* 90(12):5873-5877, 1993). When utilizing "BLAST 2 Sequences," parameters that were default parameters as of September 1, 2004, can be used for word size (3), open gap penalty (11), extension gap penalty (1), gap drop-off (50), expect value (10) and any other required parameter including but not limited to matrix option.

[0107] The constant region of an anti-SEMA4D antibody can be mutated to alter effector function in a number of ways. For example, see U.S. Pat. No. 6,737,056B1 and U.S. Patent Application Publication No. 2004/0132101A1, which disclose Fc mutations that optimize antibody binding to Fc receptors.

[0108] In certain anti-SEMA4D antibodies or fragments, variants or derivatives thereof useful in the methods provided herein, the Fc portion can be mutated to decrease effector function using techniques known in the art. For example, the deletion or inactivation (through point mutations or other means) of a constant region domain can reduce Fc receptor binding of the circulating modified antibody thereby increasing tumor localization. In other cases, constant region modifications consistent with the instant disclosure moderate complement binding and thus reduce the serum half-life. Yet other modifications of the constant region can be used to modify disulfide linkages or oligosaccharide moieties that allow for enhanced localization due to increased antigen specificity or antibody flexibility. The resulting

physiological profile, bioavailability and other biochemical effects of the modifications, such as tumor localization, biodistribution and serum half-life, can easily be measured and quantified using well known immunological techniques without undue experimentation. Anti-SEMA4D antibodies for use in the methods provided herein include derivatives that are modified, *e.g.*, by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from specifically binding to its cognate epitope. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, *e.g.*, by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications can be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, etc. Additionally, the derivative can contain one or more non-classical amino acids.

[0109] A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a side chain with a similar charge. Families of amino acid residues having side chains with similar charges have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Alternatively, mutations can be introduced randomly along all or part of the coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for biological activity to identify mutants that retain activity (*e.g.*, the ability to bind an anti-SEMA4D polypeptide, to block SEMA4D interaction with its receptor, or to alleviate symptoms associated with a neurodegenerative disorder in a patient).

[0110] For example, it is possible to introduce mutations only in framework regions or only in CDR regions of an antibody molecule. Introduced mutations can be silent or neutral missense mutations, *i.e.*, have no, or little, effect on an antibody's ability to bind antigen. These types of mutations can be useful to optimize codon usage, or

improve a hybridoma's antibody production. Alternatively, non-neutral missense mutations can alter an antibody's ability to bind antigen. One of skill in the art would be able to design and test mutant molecules with desired properties such as no alteration in antigen binding activity or alteration in binding activity (*e.g.*, improvements in antigen binding activity or change in antibody specificity). Following mutagenesis, the encoded protein can routinely be expressed and the functional and/or biological activity of the encoded protein, (*e.g.*, ability to immunospecifically bind at least one epitope of a SEMA4D polypeptide) can be determined using techniques described herein or by routinely modifying techniques known in the art.

[0111] In certain embodiments, the anti-SEMA4D antibodies for use in the methods provided herein comprise at least one optimized complementarity-determining region (CDR). By "optimized CDR" is intended that the CDR has been modified and optimized to improve binding affinity and/or anti-SEMA4D activity that is imparted to an anti-SEMA4D antibody comprising the optimized CDR. "Anti-SEMA4D activity" or "SEMA4D blocking activity" can include activity which modulates one or more of the following activities associated with SEMA4D: B cell activation, aggregation and survival; CD40-induced proliferation and antibody production; antibody response to T cell dependent antigens; T cell or other immune cell proliferation; dendritic cell maturation; demyelination and axonal degeneration; apoptosis of pluripotent neural precursors and/or oligodendrocytes; induction of endothelial cell migration; inhibition of spontaneous monocyte migration; binding to cell surface Plexin-B1 or other receptor, or any other activity associated with soluble SEMA4D or SEMA4D that is expressed on the surface of SEMA4D+ cells. Anti-SEMA4D activity can also be attributed to a decrease in incidence or severity of diseases associated with SEMA4D expression, including, but not limited to, certain types of cancers including lymphomas, autoimmune diseases, inflammatory diseases including central nervous system (CNS) and peripheral nervous system (PNS) inflammatory diseases, transplant rejections, and invasive angiogenesis. Examples of optimized antibodies based on murine anti-SEMA4D MAbs BD16 and BB18, were described in US Publ. No. 2008/0219971 A1, International Patent Application WO 93/14125 and Herold *et al.*, *Int. Immunol.* 7(1): 1-8 (1995), each of which are herein incorporated by reference in their entirety. The modifications can involve replacement

of amino acid residues within the CDR such that an anti-SEMA4D antibody retains specificity for the SEMA4D antigen and has improved binding affinity and/or improved anti-SEMA4D activity.

IV. Astrocytes

[0112] Astrocytes are specialized glial cells that perform many essential complex functions in the healthy CNS, including regulation of blood flow, fluid/ion/pH/neurotransmitter homeostasis, synapse formation/function, energy and metabolism, and blood-brain barrier maintenance (Barres BA (2008) The mystery and magic of glia: a perspective on their roles in health and disease. Neuron 60:430–440.) Importantly, astrocytes respond to CNS injury through a process referred to as reactive astrogliosis, which serves as a major pathological hallmark of neuroinflammatory and neurodegenerative diseases. Increasing evidence points towards the potential of reactive astrogliosis to play either primary or contributing roles in CNS disorders via loss of normal astrocyte functions or gain of abnormal activities. Given their central role in many CNS diseases, there is a significant need to identify and rigorously test new molecular targets that restore normal astrocyte function to effectively slow or even reverse disease progression. There are several potential pathways through which astrocytes can impact CNS diseases.

[0113] ***Astrocytes and OPC Support.*** Demyelination that occurs in neuroinflammatory diseases, such as Multiple Sclerosis, is associated with marked destruction and loss of cells comprising the oligodendrocyte lineage (Ozawa K, *et al.* Patterns of oligodendroglia pathology in multiple sclerosis. Brain. 1994;117:1311-1322.). Endogenous remyelination mechanisms fail during the recovery phase in part because of the inability of OPCs to fully differentiate into mature myelinating oligodendrocytes (Wolswijk G. Oligodendrocyte survival, loss and birth in lesions of chronic-stage multiple sclerosis. Brain. 2000;123:105-115.). Data obtained from other experimentally induced demyelination models indicate that newly maturing OPCs, in contrast to surviving mature oligodendrocytes, are required for remyelination during the recovery phase (Levine JM, Reynolds R. Activation and proliferation of endogenous oligodendrocyte precursor cells during ethidium bromide-induced demyelination. Exp Neurol. 1999;160:333-347). Astrocytes have been shown to play a significant role in supporting the function and viability of the oligodendrocyte

lineage. For example, Talbott and colleagues showed that in ethidium bromide-induced demyelinated lesions, astrocytes are required for Nkx2.2+/Olig2+ OPCs to fully differentiate into oligodendrocytes and carry out remyelination (Exp Neurol. 2005 Mar;192(1):11-24. Endogenous Nkx2.2+/Olig2+ oligodendrocyte precursor cells fail to remyelinate the demyelinated adult rat spinal cord in the absence of astrocytes. Talbott JF, Loy DN, Liu Y, Qiu MS, Bunge MB, Rao MS, Whittemore SR). Arai and Lo demonstrated *in vitro* that astrocytes provide soluble trophic factor support to OPCs that protect these cells from increased oxidative stress (Arai, K. and Lo, E. H. (2010), Astrocytes protect oligodendrocyte precursor cells via MEK/ERK and PI3K/Akt signaling. J. Neurosci. Res., 88: 758–763. doi: 10.1002/jnr.22256). Others have shown that inhibition of astrocyte activation in the settings of experimental autoimmune encephalomyelitis, experimental optic neuritis, and spinal cord injury leads to improved remyelination profiles and functional outcome measures (Brambilla R, Persaud T, Hu X, Karmally S, Shestopalov VI, Dvoriantchikova G, Ivanov D, Nathanson L, Barnum SR, Bethea JR. 2009. Transgenic inhibition of astroglial NF-kappa B improves functional outcome in experimental autoimmune encephalomyelitis by suppressing chronic central nervous system inflammation. J Immunol 182:2628–2640; Brambilla R, Dvoriantchikova G, Barakat D, Ivanov D, Bethea JR, Shestopalov VI. 2012. Transgenic inhibition of astroglial NF-kappaB protects from optic nerve damage and retinal ganglion cell loss in experimental optic neuritis. J Neuroinflammation 9:213; Brambilla R, Bracchi-Ricard V, Hu WH, Frydel B, Bramwell A, Karmally S, Green EJ, Bethea JR. 2005. Inhibition of astroglial nuclear factor kappaB reduces inflammation and improves functional recovery after spinal cord injury. J Exp Med 202:145–156).

[0114] Given the role that astrocytes play in facilitation of OPC survival and function, the juxtaposition of SEMA4D-expressing OPCs and SEMA4D receptor-expressing astrocytes identified here suggests that disease-related activation of astrocytes with associated upregulation of plexin-B receptors and SEMA4D signaling have profound effects on OPC function.

[0115] ***Astrocytes and Neuronal Support.*** Accumulating evidence indicates that astrocytes play direct roles in synaptic transmission through the regulated release of synaptically active molecules including glutamate, purines (ATP and adenosine), GABA, and D-serine (reviewed by Halassa MM, Fellin T, Haydon PG (2007), The

tripartite synapse: roles for gliotransmission in health and disease. Trends Mol Med 13:54–63; Nedergaard M, Ransom B, Goldman SA (2003) New roles for astrocytes: redefining the functional architecture of the brain. Trends Neurosci 26:523–530). The release of such gliotransmitters occurs in response to changes in neuronal synaptic activity, involves astrocyte excitability as reflected by increases in astrocyte calcium signaling, and can alter neuronal excitability (Halassa MM, Fellin T, Haydon PG (2007), The tripartite synapse: roles for gliotransmission in health and disease. Trends Mol Med 13:54–63; Nedergaard M, Ransom B, Goldman SA (2003) New roles for astrocytes: redefining the functional architecture of the brain. Trends Neurosci 26:523–530). In addition to having direct effects on synaptic activity via the release of gliotransmitters, astrocytes have the potential to exert powerful and long-term influences on synaptic function through the release of growth factors and related molecules (Barres BA (2008) The mystery and magic of glia: a perspective on their roles in health and disease. Neuron 60:430–440).

[0116] ***Astrocytes and BBB Integrity.*** Astrocytes play an essential role in formation of the blood-brain barrier (BBB) and in regulating transport across the BBB, a homeostatic process critical for proper neuronal function. The BBB is a highly complex brain endothelial structure of the differentiated neurovascular system comprised of pericytes, astrocytes, and endothelial cells. BBB compromise has been implicated in a number of neurodegenerative diseases, including meningitis, brain edema, epilepsy, Alzheimer's disease (AD), Parkinson's disease (PD), stroke, amyotrophic lateral sclerosis (ALS), and Multiple Sclerosis (MS; reviewed by Zlokovic BV. Neurovascular pathways to neurodegeneration in Alzheimer's disease and other disorders. Nat Rev Neurosci. 2011;12:723-738).

[0117] Astrocytes are "polarized" cells in that they extend specialized membranous processes comprised of unique cellular machinery and membrane components that interact with specific cell types. For example, astrocytic processes proximal to cerebral microvessels or pia are characterized by a high density of the water channel, aquaporin 4 (Aqp4) (Neely JD, Amiry-Moghaddam M, Ottersen OP, Froehner SC, Agre P, Adams ME (2001) Syntrophin-dependent expression and localization of Aquaporin-4 water channel protein. Proc Natl Acad Sci U S A 98, 14108-14113; Amiry-Moghaddam M, Otsuka T, Hurn PD, Traystman RJ, Haug FM, Froehner SC, Adams ME, Neely JD, Agre P, Ottersen OP, Bhardwaj A (2003) An alpha-

syntrophin-dependent pool of AQP4 in astroglial end-feet confers bidirectional water flow between blood and brain. Proc Natl Acad Sci U S A 100, 2106-2111.). In contrast, astrocytic processes facing synaptic regions are enriched in glutamate transporters, while the density of Aqp4 is comparatively low (Nielsen S, Nagelhus EA, Amiry-Moghaddam M, Bourque C, Agre P, Ottersen OP (1997) Specialized membrane domains for water transport in glial cells: High-resolution immunogold cytochemistry of aquaporin-4 in rat brain. J Neurosci 17, 171-180; Chaudhry FA, Lehre KP, van Lookeren Campagne M, Ottersen OP, Danbolt NC, Storm-Mathisen J (1995) Glutamate transporters in glial plasma membranes: Highly differentiated localizations revealed by quantitative ultrastructural immunocytochemistry. Neuron 15, 711-720). Interestingly, astrocytic polarization is disrupted in a brain undergoing neurodegeneration. For example, in the setting of AD, Aqp4 staining intensities significantly decrease in regions with significant amyloid plaque burden. In fact, Yang and colleagues showed that the accumulation of amyloid pathology in tg-ArcSwe AD mice is coupled temporally and spatially to loss of astrocyte polarization (J Alzheimer's Dis. 2011;27(4):711-22. doi: 10.3233/JAD-2011-110725; Loss of astrocyte polarization in the tg-ArcSwe mouse model of Alzheimer's disease. Yang JL, Lunde LK, Nuntagij P, Oguchi T, Camassa LM, Nilsson LN, Lannfelt L, Xu Y, Amiry-Moghaddam M, Ottersen OP, Torp R.).

[0118] ***Role of SEMA4D Signaling in Promoting Astrocyte Activation.*** Given the association of SEMA4D receptor expression and the astrocyte activation marker GFAP, there exists the possibility that SEMA4D signaling can potentiate astrocyte activation, thereby providing a "feed-forward" mechanism during disease states. To examine the effects of SEMA4D on astrocyte activation, primary cultures of rat astrocytes were generated and treated with SEMA4D in isolation or in combination with thioacetamide (TAA) (Example 6 and Figure 18A below), a well-known hepatotoxic and hepatocarcinogenic agent that has been shown to induce plexin-B1 expression *in vivo* (Lim, J. S., Jeong, S. Y., Hwang, J. Y., Park, H. J., Cho, J. W., & Yoon, S. (2006), or prostaglandin D2 (Example 6 and Figure 18B below), a known activation factor produced by microglia in the CNS, Toxicogenomics Analysis on Thioacetamide-induced Hepatotoxicity in Mice. MOLECULAR & CELLULAR TOXICOLOGY, 2(2), 126-133.).

V. Treatment Methods Using Therapeutic Anti-SEMA4D Antibodies

[0119] Methods of the disclosure are directed to the use of anti-SEMA4D binding molecules, *e.g.*, antibodies, including antigen-binding fragments, variants, and derivatives thereof, to treat a subject having a neurodegenerative disorder. In certain embodiments the endothelial cells express a SEMA4D receptor, in others the neuronal cells express a SEMA4D receptor, and in others both endothelial and neuronal cells express a SEMA4D receptor. In certain embodiments the receptor is Plexin-B1. Though the following discussion refers to administration of an anti-SEMA4D antibody, the methods described herein are also applicable to the antigen-binding fragments, variants, and derivatives of these anti-SEMA4D antibodies or other biologics or small molecules that retain the desired properties of the anti-SEMA4D antibodies of the disclosure, *e.g.*, capable of specifically binding SEMA4D, *e.g.*, human, mouse, or human and mouse SEMA4D, having SEMA4D neutralizing activity, and/or blocking the interaction of SEMA-4D with its receptor, *e.g.*, Plexin-B1. In another embodiment, the methods refers to administration of an anti-SEMA4D antibody, the methods described herein can also refer to the administration of anti-Plexin-B1 or anti-Plexin-B2 binding molecules that are capable of specifically binding Plexin-B1 and/or Plexin-B2 and blocking the interaction of SEMA-4D with one or both of its Plexin receptors, *e.g.*, Plexin-B1 and/or Plexin-B2.

[0120] In one embodiment, treatment includes the application or administration of an anti-SEMA4D binding molecule, *e.g.*, an antibody or antigen binding fragment thereof or other biologic or small molecule that binds and neutralizes SEMA4D as described herein to a patient, where the patient has, or has the risk of developing a neurodegenerative disorder. In another embodiment, treatment is also intended to include the application or administration of a pharmaceutical composition comprising the anti-SEMA4D binding molecule, *e.g.*, an antibody or antigen binding fragment thereof to a patient, where the patient has, or has the risk of developing a neurodegenerative disorder.

[0121] The anti-SEMA4D binding molecules, *e.g.*, antibodies or binding fragments thereof as described herein are useful for the treatment of various neurodegenerative disorders. In some embodiments, treatment of a neurodegenerative disorder is intended to induce an improvement in the symptoms associated with the disorder. In

other embodiments, treatment of a neurodegenerative disorder is intended to reduce, retard or stop an increase in symptom manifestations. In other embodiments, treatment of a neurodegenerative disorder is intended to inhibit, *e.g.*, suppress, retard, prevent, stop, or reverse a manifestation of symptoms. In other embodiments, treatment of a neurodegenerative disorder is intended to relieve to some extent one or more of the symptoms associated with the disorder. In these situations, the symptoms can be neuropsychiatric symptoms, cognitive symptoms, and/or motor dysfunction. In other embodiments, treatment of a neurodegenerative disorder is intended to reduce morbidity and mortality. In other embodiments, treatment of a neurodegenerative disorder is intended to improve quality of life.

[0122] In one embodiment, the disclosure relates to the use of anti-SEMA4D binding molecules, *e.g.*, antibodies or antigen-binding fragments, variants, or derivatives thereof, as a medicament, in particular for use in the treatment of neurodegenerative disorders to improve the symptoms associated with the disorder.

[0123] In accordance with the methods of the present disclosure, at least one anti-SEMA4D binding molecule, *e.g.*, an antibody or antigen binding fragment, variant, or derivative thereof, or other biologic or small molecule as defined elsewhere herein can be used to promote a positive therapeutic response with respect to the neurodegenerative disorder. A "positive therapeutic response" with respect to the neurodegenerative disorder is intended to include an improvement in the symptoms associated with the disorder. Such positive therapeutic responses are not limited to the route of administration and can comprise administration to the donor, the donor tissue (such as for example organ perfusion), the host, any combination thereof, and the like. In particular, the methods provided herein are directed to inhibiting, preventing, reducing, alleviating, or lessening the progression of a neurodegenerative disorder in a patient. Thus, for example, an improvement in the disorder can be characterized as an absence of clinically observable symptoms, a decrease in the incidence of clinically observable symptoms, or a change in the clinically observable symptoms.

[0124] Activities that change the symptoms associated with neurodegenerative disorders can be detected and measured using *in vivo* mouse models. In certain embodiments, a CVN mouse model can be employed. The CVN mouse incorporates mutations of Aβ precursor protein that are characteristic of familial Alzheimer's disease (AD) in three independent lineages together with a mutation that reproduces

some of the conditions of brain inflammation associated with AD (Colton *et al.*, J Alzheimer's Dis.15:571–587, 2008; Van Nostrand *et al.*, Stroke 41:S135-S138, 2010). The CVN model displays some of the primary pathologies associated with Alzheimer's disease: Aβ plaques, hyperphosphorylated tau causing neurofibrillary tangles and cell death (neuronal loss), and consistent spatial memory impairment and neurovascular deficits. In comparison to other mouse mutants used for modeling Alzheimer's disease, the CVN Mouse shows more Alzheimer related pathologies at an earlier age. In other embodiments, the YAC128 mouse model of Huntington's Disease (HD) can be employed. YAC128 mice express the full-length mutant human huntingtin gene (mHTT) and accurately recapitulate many of the signs and symptoms of HD. It should be appreciated that people skilled in the art will recognize that other models have been described and usefully employed for studies of disease mechanisms and treatment of symptoms in neurodegenerative disorders in the literature and that the present disclosure should not be limited to any one particular model.

[0125] The anti-SEMA4D binding molecules, *e.g.*, antibodies or antigen binding fragments, variants, or derivatives thereof or other biologics or small molecules can be used in combination with at least one or more other treatments for neurodegenerative disorders; where the additional therapy is administered prior to, during, or subsequent to the anti-SEMA4D binding molecule, *e.g.*, antibody or antigen binding fragment, variant, or derivative thereof, therapy. Thus, where the combined therapies comprise administration of an anti-SEMA4D binding molecule, *e.g.*, an antibody or antigen binding fragment, variant, or derivative thereof, in combination with administration of another therapeutic agent, the methods of the disclosure encompass coadministration, using separate formulations or a single pharmaceutical formulation, with simultaneous or consecutive administration in either order.

[0126] To apply the methods and systems of the disclosure in certain embodiments, samples or images from a patient can be obtained before or, after, or both before and after the administration of a therapy comprising an effective amount of an isolated binding molecule that specifically binds to Semaphorin-4D (SEMA4D), to a subject determined to have a neurodegenerative disorder, or to a subject suspected of having a neurodegenerative disorder. In some cases, successive samples or images can be obtained from the patient after therapy has commenced, or after therapy has ceased,

or both before and after therapy. Samples or images can, for example, be requested by a healthcare provider (*e.g.*, a doctor) or healthcare benefits provider, obtained and/or processed by the same or a different healthcare provider (*e.g.*, a nurse, a hospital) or a clinical laboratory, and after processing, the results can be forwarded to yet another healthcare provider, healthcare benefits provider, or the patient. Similarly, the measuring/determination of one or more scores, comparisons between scores, evaluation of the scores and treatment decisions can be performed by one or more healthcare providers, healthcare benefits providers, and/or clinical laboratories.

[0127] In certain aspects of any of the aforementioned procedures, the neurodegenerative disorder is selected from a group consisting of Alzheimer's disease, Parkinson's disease, Huntington's disease, Down syndrome, ataxia, amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), HIV-related cognitive impairment, CNS Lupus, mild cognitive impairment, or a combination thereof. In certain aspects of any of the aforementioned procedures, the neurodegenerative disorder is Alzheimer's disease or Huntington's disease,

[0128] In some aspects, a healthcare provider can administer or instruct another healthcare provider to administer a therapy comprising an effective amount of an isolated binding molecule that specifically binds to Semaphorin-4D (SEMA4D), where the subject has, is determined to have, or is suspected to have, a neurodegenerative disorder. A healthcare provider can implement or instruct another healthcare provider or patient to perform the following actions: obtain a sample or image, process a sample or image, submit a sample or image, receive a sample or image, transfer a sample or image, analyze or measure a sample or image, quantify a sample or image, provide the results obtained after analyzing/measuring/quantifying a sample or image, receive the results obtained after analyzing/measuring/quantifying a sample or image, compare/score the results obtained after analyzing/measuring/quantifying one or more samples or images, provide the comparison/score from one or more samples, obtain the comparison/score from one or more samples or images, administer a therapy, *e.g.*, an effective amount of an isolated binding molecule that specifically binds to Semaphorin-4D (SEMA4D), commence the administration of a therapy, cease the administration of a therapy, continue the administration of a therapy, temporarily interrupt the administration of a therapy, increase the amount of an administered therapeutic agent, decrease the amount of an

administered therapeutic agent, continue the administration of an amount of a therapeutic agent, increase the frequency of administration of a therapeutic agent, decrease the frequency of administration of a therapeutic agent, maintain the same dosing frequency on a therapeutic agent, replace a therapy or therapeutic agent by at least another therapy or therapeutic agent, combine a therapy or therapeutic agent with at least another therapy or additional therapeutic agent.

[0129] In some aspects, a healthcare benefits provider can authorize or deny, for example, collection of a sample, processing of a sample, submission of a sample, receipt of a sample, transfer of a sample, analysis or measurement a sample, quantification a sample, provision of results obtained after analyzing/measuring/quantifying a sample, transfer of results obtained after analyzing/measuring/quantifying a sample, comparison/scoring of results obtained after analyzing/measuring/quantifying one or more samples, transfer of the comparison/score from one or more samples, administration of a therapy or therapeutic agent, commencement of the administration of a therapy or therapeutic agent, cessation of the administration of a therapy or therapeutic agent, continuation of the administration of a therapy or therapeutic agent, temporary interruption of the administration of a therapy or therapeutic agent, increase of the amount of administered therapeutic agent, decrease of the amount of administered therapeutic agent, continuation of the administration of an amount of a therapeutic agent, increase in the frequency of administration of a therapeutic agent, decrease in the frequency of administration of a therapeutic agent, maintain the same dosing frequency on a therapeutic agent, replace a therapy or therapeutic agent by at least another therapy or therapeutic agent, or combine a therapy or therapeutic agent with at least another therapy or additional therapeutic agent.

[0130] In certain aspects of any of the aforementioned procedures, the neurodegenerative disorder is selected from a group consisting of Alzheimer's disease, Parkinson's disease, Huntington's disease, Down syndrome, ataxia, amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), HIV-related cognitive impairment, CNS Lupus, mild cognitive impairment, or a combination thereof. In certain aspects of any of the aforementioned procedures, the neurodegenerative disorder is Alzheimer's disease or Huntington's disease.

[0131] In addition, a healthcare benefits provider can, *e.g.*, authorize or deny the prescription of a therapy, authorize or deny coverage for therapy, authorize or deny reimbursement for the cost of therapy, determine or deny eligibility for therapy, etc.

[0132] In some aspects, a clinical laboratory can, for example, collect or obtain a sample, process a sample, submit a sample, receive a sample, transfer a sample, analyze or measure a sample, quantify a sample, provide the results obtained after analyzing/measuring/quantifying a sample, receive the results obtained after analyzing/measuring/quantifying a sample, compare/score the results obtained after analyzing/measuring/quantifying one or more samples, provide the comparison/score from one or more samples, obtain the comparison/score from one or more samples, or other related activities.

[0133] In certain aspects of any of the aforementioned procedures, the neurodegenerative disorder is selected from a group consisting of Alzheimer's disease, Parkinson's disease, Huntington's disease, Down syndrome, ataxia, amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), HIV-related cognitive impairment, CNS Lupus, mild cognitive impairment, or a combination thereof. In certain aspects of any of the aforementioned procedures, the neurodegenerative disorder is Alzheimer's disease or Huntington's disease.

[0134] In certain aspects, any of the aforementioned procedures can be used to determine if a subject has a neurodegenerative disorder. In certain aspects, the neurodegenerative disorder is selected from a group consisting of Alzheimer's disease, Parkinson's disease, Huntington's disease, Down syndrome, ataxia, amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), HIV-related cognitive impairment, CNS Lupus, mild cognitive impairment, or a combination thereof. In certain aspects of any of the aforementioned procedures, the neurodegenerative disorder is Alzheimer's disease or Huntington's disease,

[0135] In some aspects, a healthcare provider, clinical laboratory, or other entity can, for example, collect or obtain an image, process an image, submit an image, receive an image, transfer an image, analyze or measure an image, quantify an image, provide the results obtained after analyzing/measuring/quantifying an image, receive the results obtained after analyzing/measuring/quantifying an image, compare/score the results obtained after analyzing/measuring/quantifying one or more images, provide the comparison/score from one or more images, obtain the comparison/score from one

or more images, or other related activities. Images that can be used in such aspects include, but are not limited to, images obtained by angiography, ultrasound , computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET), optical coherence tomography (OCT), near-infrared spectroscopy (NIRS), and NIR fluorescence. In certain embodiments, imaging techniques that have been described in the literature can be used (Tardif et al. Circ Cardiovasc Imaging 4:319-333 (2011)).

VI. Pharmaceutical Compositions and Administration Methods

[0136] Methods of preparing and administering anti-SEMA4D binding molecules, *e.g.*, antibodies, or antigen-binding fragments, variants, or derivatives thereof to a subject in need thereof are well known to or are readily determined by those skilled in the art. The route of administration of the anti-SEMA4D binding molecule, *e.g*, antibody, or antigen-binding fragment, variant, or derivative thereof, can be, for example, oral, parenteral, by inhalation or topical. The term parenteral as used herein includes, *e.g.*, intravenous, intraarterial, intraperitoneal, intramuscular, subcutaneous, rectal, or vaginal administration. While all these forms of administration are clearly contemplated as being within the scope of the disclosure, an example of a form for administration would be a solution for injection, in particular for intravenous or intraarterial injection or drip. A suitable pharmaceutical composition for injection can comprise a buffer (*e.g.* acetate, phosphate or citrate buffer), a surfactant (*e.g.* polysorbate), optionally a stabilizer agent (*e.g.* human albumin), etc. However, in other methods compatible with the teachings herein, anti-SEMA4D binding molecules, *e.g.*, antibodies, or antigen-binding fragments, variants, or derivatives thereof can be delivered directly to the site of the adverse cellular population thereby increasing the exposure of the diseased tissue to the therapeutic agent.

[0137] As discussed herein, anti-SEMA4D binding molecules, *e.g.*, antibodies, or antigen-binding fragments, variants, or derivatives thereof can be administered in a pharmaceutically effective amount for the *in vivo* treatment of neurodegenerative disorders. In this regard, it will be appreciated that the disclosed binding molecules can be formulated so as to facilitate administration and promote stability of the active agent. In certain embodiments, pharmaceutical compositions in accordance with the present disclosure comprise a pharmaceutically acceptable, non-toxic, sterile carrier

such as physiological saline, non-toxic buffers, preservatives and the like. For the purposes of the instant application, a pharmaceutically effective amount of an anti-SEMA4D binding molecule, *e.g.*, an antibody, or antigen-binding fragment, variant, or derivative thereof, shall be held to mean an amount sufficient to achieve effective binding to a target and to achieve a benefit, *e.g.*, improve the symptoms associated with a neurodegenerative disorder.

[0138] The pharmaceutical compositions used in this disclosure comprise pharmaceutically acceptable carriers, including, *e.g.*, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol, and wool fat.

[0139] Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include, *e.g.*, water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. In the subject disclosure, pharmaceutically acceptable carriers include, but are not limited to, 0.01-0.1 M, *e.g.*, about 0.05 M phosphate buffer or 0.8% saline. Other common parenteral vehicles include sodium phosphate solutions, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers, such as those based on Ringer's dextrose, and the like. Preservatives and other additives can also be present such as, for example, antimicrobials, antioxidants, chelating agents, and inert gases and the like.

[0140] More particularly, pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In such cases, the composition must be sterile and should be fluid to the

extent that easy syringability exists. It should be stable under the conditions of manufacture and storage and can be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (*e.g.*, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity 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. Suitable formulations for use in the therapeutic methods disclosed herein are described in Remington's Pharmaceutical Sciences (Mack Publishing Co.) 16th ed. (1980).

[0141] Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal and the like. In many cases, isotonic agents can be included, for example, sugars, polyalcohols, such as mannitol, sorbitol, or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

[0142] In any case, sterile injectable solutions can be prepared by incorporating an active compound (*e.g.*, an anti-SEMA4D antibody, or antigen-binding fragment, variant, or derivative thereof, by itself or in combination with other active agents) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated herein, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle, which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation include vacuum drying and freeze-drying, which yield a powder of an active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The preparations for injections are processed, filled into containers such as ampoules, bags, bottles, syringes or vials, and sealed under aseptic conditions according to methods known in the art. Further, the preparations can be packaged and sold in the form of a kit. Such articles of manufacture can have labels or package inserts

indicating that the associated compositions are useful for treating a subject suffering from, or predisposed to a disease or disorder.

[0143] Parenteral formulations can be a single bolus dose, an infusion or a loading bolus dose followed with a maintenance dose. These compositions can be administered at specific fixed or variable intervals, *e.g.*, once a day, or on an "as needed" basis.

[0144] Certain pharmaceutical compositions used in this disclosure can be orally administered in an acceptable dosage form including, *e.g.*, capsules, tablets, aqueous suspensions or solutions. Certain pharmaceutical compositions also can be administered by nasal aerosol or inhalation. Such compositions can be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, and/or other conventional solubilizing or dispersing agents.

[0145] The amount of an anti-SEMA4D binding molecule, *e.g.*, antibody, or fragment, variant, or derivative thereof, to be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. The composition can be administered as a single dose, multiple doses or over an established period of time in an infusion. Dosage regimens also can be adjusted to provide the optimum desired response (*e.g.*, a therapeutic or prophylactic response).

[0146] In keeping with the scope of the present disclosure, anti-SEMA4D antibodies, or antigen-binding fragments, variants, or derivatives thereof can be administered to a human or other animal in accordance with the aforementioned methods of treatment in an amount sufficient to produce a therapeutic effect. The anti-SEMA4D antibodies, or antigen-binding fragments, variants or derivatives thereof can be administered to such human or other animal in a conventional dosage form prepared by combining the antibody of the disclosure with a conventional pharmaceutically acceptable carrier or diluent according to known techniques. It will be recognized by one of skill in the art that the form and character of the pharmaceutically acceptable carrier or diluent is dictated by the amount of active ingredient with which it is to be combined, the route of administration and other well-known variables. Those skilled in the art will further appreciate that a cocktail comprising one or more species of anti-SEMA4D binding molecules, *e.g.*, antibodies,

or antigen-binding fragments, variants, or derivatives thereof, of the disclosure can be used.

[0147] By "therapeutically effective dose or amount" or "effective amount" is intended an amount of anti-SEMA4D binding molecule, *e.g.*, antibody or antigen binding fragment, variant, or derivative thereof, that when administered brings about a positive therapeutic response with respect to treatment of a patient with a disease to be treated. In the case of a neurodegenerative disorder, a positive therapeutic response can alleviate symptoms of the disorder; decrease, reduce, retard or stop the incidence of symptoms; decrease, reduce, retard the severity of symptoms; inhibit, *e.g.*, suppress, retard, prevent, stop, or reverse the manifestation of symptoms; relieve to some extent one or more of the symptoms associated with the disorder; reduce morbidity and mortality; improve quality of life; or a combination of such effects.

[0148] Therapeutically effective doses of the compositions of the present disclosure, for the decrease in the incidence of symptoms, vary depending upon many different factors, including means of administration, target site, physiological state of the patient, whether the patient is human or an animal, other medications administered, and whether treatment is prophylactic or therapeutic. In certain embodiments the patient is a human, but non-human mammals including transgenic mammals can also be treated. Treatment dosages can be titrated using routine methods known to those of skill in the art to optimize safety and efficacy.

[0149] The amount of at least one anti-SEMA4D binding molecule, *e.g.*, antibody or binding fragment, variant, or derivative thereof, to be administered is readily determined by one of ordinary skill in the art without undue experimentation given the present disclosure. Factors influencing the mode of administration and the respective amount of at least one anti-SEMA4D binding molecule, *e.g.*, antibody, antigen-binding fragment, variant or derivative thereof include, but are not limited to, the severity of the disease, the history of the disease, and the age, height, weight, health, and physical condition of the individual undergoing therapy. Similarly, the amount of anti-SEMA4D binding molecule, *e.g.*, antibody, or fragment, variant, or derivative thereof, to be administered will be dependent upon the mode of administration and whether the subject will undergo a single dose or multiple doses of this agent.

[0150] The disclosure also provides for the use of an anti-SEMA4D binding molecule, *e.g.*, antibody of the disclosure, or antigen-binding fragment, variant, or derivative thereof, in the manufacture of a medicament for treating a subject for treating a neurodegenerative disorder, wherein the medicament is used in a subject that has been pretreated with at least one other therapy. By "pretreated" or "pretreatment" is intended the subject has received one or more other therapies (*e.g.*, been treated with at least one other neurodegenerative therapy) prior to receiving the medicament comprising the anti-SEMA4D binding molecule, *e.g.*, antibody or antigen-binding fragment, variant, or derivative thereof. "Pretreated" or "pretreatment" includes subjects that have been treated with at least one other therapy within 2 years, within 18 months, within 1 year, within 6 months, within 2 months, within 6 weeks, within 1 month, within 4 weeks, within 3 weeks, within 2 weeks, within 1 week, within 6 days, within 5 days, within 4 days, within 3 days, within 2 days, or even within 1 day prior to initiation of treatment with the medicament comprising the anti-SEMA4D binding molecule, for example, the monoclonal antibodies VX15/2503, 67, or 76 disclosed herein, or antigen-binding fragment, variant, or derivative thereof. It is not necessary that the subject was a responder to pretreatment with the prior therapy or therapies. Thus, the subject that receives the medicament comprising the anti-SEMA4D binding molecule, *e.g.*, an antibody or antigen-binding fragment, variant, or derivative thereof could have responded, or could have failed to respond, to pretreatment with the prior therapy, or to one or more of the prior therapies where pretreatment comprised multiple therapies.

[0151] The practice of the present disclosure will employ, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature. See, for example, Sambrook *et al.*, ed. (1989) Molecular Cloning A Laboratory Manual (2nd ed.; Cold Spring Harbor Laboratory Press); Sambrook *et al.*, ed. (1992) Molecular Cloning: A Laboratory Manual, (Cold Springs Harbor Laboratory, NY); D. N. Glover ed., (1985) DNA Cloning, Volumes I and II; Gait, ed. (1984) Oligonucleotide Synthesis; Mullis *et al.* U.S. Pat. No. 4,683,195; Hames and Higgins, eds. (1984) Nucleic Acid Hybridization; Hames and Higgins, eds. (1984) Transcription And Translation; Freshney (1987) Culture Of Animal Cells (Alan R.

Liss, Inc.); Immobilized Cells And Enzymes (IRL Press) (1986); Perbal (1984) A Practical Guide To Molecular Cloning; the treatise, Methods In Enzymology (Academic Press, Inc., N.Y.); Miller and Calos eds. (1987) Gene Transfer Vectors For Mammalian Cells, (Cold Spring Harbor Laboratory); Wu *et al.*, eds., Methods In Enzymology, Vols. 154 and 155; Mayer and Walker, eds. (1987) Immunochemical Methods In Cell And Molecular Biology (Academic Press, London); Weir and Blackwell, eds., (1986) Handbook Of Experimental Immunology, Volumes I-IV; Manipulating the Mouse Embryo, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., (1986); and in Ausubel *et al.* (1989) Current Protocols in Molecular Biology (John Wiley and Sons, Baltimore, Md.).

[0152] General principles of antibody engineering are set forth in Borrebaeck, ed. (1995) Antibody Engineering (2nd ed.; Oxford Univ. Press). General principles of protein engineering are set forth in Rickwood *et al.*, eds. (1995) Protein Engineering, A Practical Approach (IRL Press at Oxford Univ. Press, Oxford, Eng.). General principles of antibodies and antibody-hapten binding are set forth in: Nisonoff (1984) Molecular Immunology (2nd ed.; Sinauer Associates, Sunderland, Mass.); and Steward (1984) Antibodies, Their Structure and Function (Chapman and Hall, New York, N.Y.). Additionally, standard methods in immunology known in the art and not specifically described are generally followed as in Current Protocols in Immunology, John Wiley & Sons, New York; Stites *et al.*, eds. (1994) Basic and Clinical Immunology (8th ed; Appleton & Lange, Norwalk, Conn.) and Mishell and Shiigi (eds) (1980) Selected Methods in Cellular Immunology (W.H. Freeman and Co., NY).

[0153] Standard reference works setting forth general principles of immunology include Current Protocols in Immunology, John Wiley & Sons, New York; Klein (1982) J., Immunology: The Science of Self-Nonself Discrimination (John Wiley & Sons, NY); Kennett *et al.*, eds. (1980) Monoclonal Antibodies, Hybridoma: A New Dimension in Biological Analyses (Plenum Press, NY); Campbell (1984) "Monoclonal Antibody Technology" in Laboratory Techniques in Biochemistry and Molecular Biology, ed. Burden *et al.*, (Elsevere, Amsterdam); Goldsby *et al.*, eds. (2000) Kuby Immunology (4th ed.; H. Freemand & Co.); Roitt *et al.* (2001) Immunology (6th ed.; London: Mosby); Abbas *et al.* (2005) Cellular and Molecular Immunology (5th ed.; Elsevier Health Sciences Division); Kontermann and Dubel

(2001) Antibody Engineering (Springer Verlang); Sambrook and Russell (2001) Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Press); Lewin (2003) Genes VIII (Prentice Hall 2003); Harlow and Lane (1988) Antibodies: A Laboratory Manual (Cold Spring Harbor Press); Dieffenbach and Dveksler (2003) PCR Primer (Cold Spring Harbor Press).

[0154] All of the references cited above, as well as all references cited herein, are incorporated herein by reference in their entireties.

[0155] The following examples are offered by way of illustration and not by way of limitation.

EXAMPLES

Example 1: Testing the effect of an anti-SEMA4D binding molecule, *e.g.*, an antibody or antigen-binding fragment, variant, or derivative thereof, *e.g.*, VX15/2503, 67, or 76 on Alzheimer's disease (AD) in the CVN Mouse Model

[0156] **Experimental Design.** The CVN model was used to study the effect of anti-SEMA4D antibody (*e.g.*, MAb 67) on the pathologies and symptoms associated with AD. The CVN mouse incorporates mutations of human Aβ precursor protein that are characteristic of familial Alzheimer's disease (AD) in three independent lineages together with a deletion of a gene (nitric oxide synthase-2) to promote neuroinflammatory mechanisms associated with AD (Colton *et al.*, *J Alzheimers Dis.* 15:571–587, 2008; Van Nostrand *et al.*, *Stroke* 41:S135-S138, 2010).

[0157] The basic experimental design is shown in FIG. 1. Alzheimer's disease prone CVN mice (obtained from Charles River) were used to test the effect of an anti-SEMA4D binding molecule on AD. At 10 weeks of age, the mice were bled to obtain baseline serology levels. Between 10-12 weeks of age, the mice underwent behavioral pretesting to ensure they were capable of participating in the study. Following randomization, the CVN mice were treated weekly with anti-SEMA4D (Mab-67) or isotype control (MAb 2B8) antibody (30 mg/kg, i.v.) from week 26 to 38 at which time they were administered several behavioral tests. The behavioral tests were the open field test and the radial arm water maze.

[0158] **Open Field Test** - Exploratory activity of the animal is studied in open field test at 10 and 38 weeks of age for possible treatment induced hypo- or hyperactivity

(control test) or other effect. Mice are brought to the experimental room for at least 30 min acclimation to the experimental room conditions prior to testing. Activity chambers (Med Associates Inc, St Albans, VT; 27 x 27 x 20.3 cm) are equipped with IR beams. Mice are placed in the center of the chamber and their behavior is recorded for 30 min in 5-minute bins. Quantitative analysis is performed on the following five dependent measures: total locomotion, locomotion in the center of the open field, rearing rate in the center, total rearing frequency and velocity. Animals are tested at low-stress conditions where the light is lowered to approximately 10-30 lux of red light.

[0159] **Radial Arm Water Maze** -At 11 and 39 weeks of age mice are brought to the experimental room for at least 30 min acclimation to the experimental room conditions prior to testing. Two-day radial-arm water maze has been described in detail previously (Alamed *et al.* 2006). Briefly, a six-arm maze is submerged in a pool of water, and a platform is placed at the end of one arm. The mouse receives 15 trials per day for 2 d and on each trial is started in a different arm while the arm containing the platform remains the same for each mouse. The platform location of which remains constant over the 2 d for each mouse at a given age, but this location changes for each mouse between the 11 and 40 weeks of age testing time point. Using visual cues around the room, the mouse learns the position of the escape platform. The first 10 trials are considered training and alternate between a visible and a hidden platform. The final trials for day 1 and all trials on day 2 use a hidden platform. The number of errors (incorrect arm entries) was counted over a 1 min period. The errors are averaged over three trials resulting in 10 blocks for the 2 d period.

[0160] Following the conclusion of behavioral testing, mice were sacrificed, and brain tissues were processed for formalin-fixed paraffin-embedded (FFPE) immunohistochemistry. In view of reports of a role for SEMA4D in the induction of inhibitory GABAergic synapses (Kuzirian *et al.*, *J Neuroscience*, 33:8961– 8973 • 8961, 2013) the density of vesicles and intensity of expression of Vesicular GABA Transporter (VGAT) was determined in the Dentate Gyrus of treated CVN mice. The Dentate Gyrus is one of a few major centers of continued neurogenesis in the adult CNS and is thought to play a role in memory formation and retention. For all tests, statistical analysis was performed using the 2-way ANOVA test.

[0161] **Anti-SEMA4D reduces anxiety-like behavior.** Exploratory activity was studied in groups of 12 AD prone CVN mice treated with anti-SEMA4D or isotype control. An open field test was administered at 38 weeks of age for possible treatment-induced effects on locomotor activity and anxiety-like behavior. Mice were placed in the center of a lighted chamber and their behavior was recorded for 30 min in six 5-minute time bins. Quantitative analysis was performed for total locomotion (FIG. 2A) and locomotion in the center of the open field (FIG. 2B), which, as is known in the art, is a measure of anxiety-related behavior.

[0162] The results showed that AD prone CVN mice treated weekly with anti-SEMA4D antibody manifest greater open field exploration and less anxiety-like behavior (incursions into the center of the field) than mice treated with control MAb 2B8. These results are shown in FIG. 2.

[0163] **Anti-SEMA4D improves spatial memory.** At 39 weeks of age, CVN mice treated with anti-SEMA4D or 2B8 isotype control (n=9-13/group) were tested over 2-days in a radial-arm water maze (Alamed *et al.* 2006, shown in FIG. 3A). Briefly, each mouse received 15 trials per day (3 trials per block) on each of two consecutive days. Each trial was started from a different arm, while the arm containing the platform remained the same for each trial. Trial blocks on Day 1 alternated between a visible and hidden platform for training purposes. All trials on Day 2 were performed with a hidden platform to assess spatial memory. Day 1 was a training/learning period and on day 2 the latency to find the platform was recorded.

[0164] The results showed that anti-SEMA4D antibody (MAb 67) administration leads to a measurable decrease in latency suggesting improved spatial memory as compared to the control (MAb 2B8-treated) cohort. The results are shown in FIG. 3B.

[0165] **Anti-SEMA4D decreases GABAergic Synapses.** FFPE brain tissue sections from Mab-67 or MAb-2B8 treated CVN and WT mice (n=9-13/group) were stained with anti-VGAT antibody to detect GABAergic synaptic vesicles. Percentages of VGAT-positive vesicle signal and VGAT signal intensities per vesicle were quantified within the dentate gyrus of all animals and normalized to total dentate gyrus area scanned.

[0166] The results showed that anti-SEMA4D antibody treatment of CVN AD mice leads to a trend of decreasing density of VGAT positive vesicles (FIG. 4A) and a statistically significant decrease in VGAT staining intensity level per vesicle (FIG.

4B), a finding that suggests a role for SEMA4D in modulating GABAergic signaling *in vivo* and can provide mechanistic insight into the behavioral effects observed in MAb 67 treated CVN mice. GABAergic signaling is associated with a heterogeneous class of inhibitory neurons. As demonstrated in FIG. 15 of Example 6 below, more significant effects of treatment with anti-SEMA4D antibody on density of inhibitory neurons are observed when analysis is focused on the subset of somatostatin-, NPY-, or NPY2R-positive neurons.

Example 2: Testing the effect of an anti-SEMA4D binding molecule, *e.g.*, an antibody or antigen-binding fragment, variant, or derivative thereof, *e.g.*, MAbs VX15/2503 or 67 on Huntington's disease (HD) in the YAC128 mouse model

[0167] **Experimental Design.** A second experiment employing an *in vivo* YAC128 model was performed to study the effect of anti-SEMA4D antibody on the pathologies and symptoms associated with HD. The basic experimental design was similar to that shown in Example 1, and FIG. 1, above, but MAb (antibody) dosing in this case was performed weekly from week 6 to week 47 with between 13 and 22 YAC128 or WT mice per group. The YAC128 mice were bred and maintained at University of British Columbia, Centre for Molecular Medicine and Therapeutics.

[0168] **Anti-SEMA4D reduces anxiety-like behavior in the YAC128 Mouse Model.** To assess anxiety during open-field exploration, MAb-treated mice were placed in the lower left corner of a 50 × 50 cm open grey acrylic box with 20 cm tall sides in a room brightly lit by fluorescent ceiling lights. Open-field activity was recorded for 10 min by a ceiling-mounted video camera. Entries into (FIG. 5A) and time spent in the center of the field (FIG. 5B) were scored as a measure of anxiety-like behavior.

[0169] In contrast to control treated animals where YAC128 mice manifest greater anxiety-like behavior in open field exploration than wild type (WT) mice, there is no difference between WT and YAC128 mice treated with anti-SEMA4D antibody (MAb 67) indicating that MAb-67 ameliorates anxiety-like behavior in YAC128 mice. These results are shown in FIG. 5.

[0170] **Anti-SEMA4D improves spatial memory in the YAC128 mouse model.** To assess preference for a known object in a novel location, two different novel objects were placed in the upper left and right hand corners of an open field box.

Anti-SEMA4D-treated mice were introduced to the box in the lower left corner and recorded for 5 minutes (min) by a ceiling-mounted video camera, during which time the number of investigations of the two novel objects were scored (Trial 1, FIG. 6A). Mice were then removed from the box for 5 min, and the object at the top right corner of the box was moved to the lower right corner of the box. Mice were reintroduced to the box and recorded for an additional 5 min (Trial 2, FIG. 6B). The percentage of the investigations, or nose pokes, to the target object (the one in the new location) relative to all nose pokes was computed.

[0171] In contrast to control or Mab 67-treated wild type (WT) animals that preferentially explore an object in a novel location in Trial 2, control-treated YAC128 mice do not recognize or show preference for the object in a novel location. Treatment with anti-SEMA4D antibody restores normal novel object preference in YAC128 mice ($p<0.01$). This suggests that spatial memory in YAC128 mice was improved by Mab-67 treatment so that they recognized that an object was in a novel location. The results are shown in FIG. 6.

[0172] **Anti-SEMA4D prevents cortical and corpus callosum degeneration in YAC128 mice.** Free-floating brain tissue sections from 12 month-old MAb-treated YAC128 and WT mice (n=13-21/group) were stained with anti-NeuN antibody. Cortical (FIG. 7A) and corpus callosum (FIG. 7B) volumes were determined by tracing the perimeter of the defined structure in serial sections using StereoInvestigator software (Microbrightfield) and volumes were determined using the Cavalieri principle.

[0173] The results show that treatment with anti-SEMA4D antibody inhibits the normal disease related reduction in cortical and corpus callosum volume in YAC128 mice at 12 months of age. The results are shown in FIG. 7.

[0174] **Mab 67 Prevents Testicular Degeneration in YAC128 mice.** Testicular degeneration is observed in male HD patients and is recapitulated in male YAC128 mice. As shown in FIG. 8, treatment with anti-SEMA4D antibody prevents testicular degeneration in 12 month-old YAC128 mice. It is possible that the effects of disease and anti-SEMA4D antibody on both brain and testis reflect a common dependence on intracellular actin-dependent transport mechanisms in the normal function of these tissues.

Example 3: Examining expression patterns of SEMA4D, plexin-B1, plexin-B2 and CD72 in the rat CNS

[0175] To visualize the cell types within the CNS that express SEMA4D and its receptors plexin-B1, plexin-B2, and CD72, co-immunohistochemistry was performed on coronal spinal cord sections from naïve rats (FIG. 9A-P). Co-staining for the oligodendrocyte precursor cell marker, Nkx2.2, and SEMA4D (FIG. 9A-C), plexin-B1 and the astrocytic marker, GFAP (FIG. 9E-G), plexin-B1 and CD72 (FIG. 9I-K), and plexin-B1 and the microglial marker, Iba1 (FIG. 9M-O) was performed on spinal cord sections from naïve rats. In addition, all sections were stained with DAPI to visualize cellular nuclei (FIG. 9D, H, L, and P). Slides were imaged at 60X magnification using an EXi-Aqua-14 bit camera coupled to an Olympus Ix50 microscope.

[0176] The results in FIG. 9 show that within the normal CNS, SEMA4D is robustly expressed on Nkx2.2-positive oligodendrocyte precursors, while its receptors, plexin-B1, plexin-B2 (data not shown), and CD72, are expressed on multiple cell types and are especially prominent on Glial Fibrillary Acidic Protein (GFAP)-positive astrocytes.

Example 4: Characterizing the expression patterns of plexin-B1 and plexin-B2 receptors in the CVN Alzheimer's disease mouse model

[0177] Homozygous bigenic CVN AD mice exhibit classical amyloid pathology and glial activation in the subiculum as compared to age-matched wild-type mice. Expression patterns of plexin-B1 were examined in the CVN mouse model of Alzheimer's disease. CVN (also known as APPSwDI/NOS2-/-) bigenic mice harbor the amyloid precursor protein Swedish-Dutch-Iowa mutant (APPSwDI) transgene and a targeted "null" mutation of the nitric oxide synthase 2 (Nos2, or inducible NOS, iNOS) locus. At 41 weeks of age, CVN and wild-type control mice were sacrificed and processed for DAB immunohistochemistry. The results are shown in FIG. 10, with the wild-type mouse sections in the top panels and the CVN mouse sections in the bottom panels). Sections were separately stained for amyloid-beta 1-42 peptide (top and bottom panels at left), microglial marker Iba1 (top and bottom center panels), or astrocyte marker GFAP (top and bottom panels at right). Slides were imaged at

20X magnification using a Retiga QICAM-12 bit camera coupled to an Olympus Ix50 microscope.

[0178] The results in FIG. 10 show that homozygous bigenic CVN mice (APPSwDI/NOS2-/-) develop classic amyloid pathology, microglial activation, and astrogliosis (bottom panels).

[0179] **Activated astrocytes in CVN AD mice exhibit enhanced plexin-B1 expression as compared to age-matched wild-type mice.** At 41 weeks of age, CVN and wild-type control mice were sacrificed and processed for fluorescent co-immunohistochemistry (FIG. 11A). Sections were separately stained for the SEMA4D receptor plexin-B1 and astrocyte marker GFAP, and DAPI to visualize cellular nuclei. Composite images are shown in the left-most panels. Slides were imaged at 60X magnification using an EXi-Aqua-14 bit camera coupled to an Olympus Ix50 microscope.

[0180] As shown in FIG. 11A, co-immunohistochemical analyses of plexin-B1 (second panels from left) and GFAP-positive astrocytes (third panels from left) within the brains of CVN and age-matched wild-type mice demonstrates that astrocytic activation, as evidenced by increased GFAP marker staining, positively correlates with enhanced co-registered plexin-B1 expression, suggesting SEMA4D/plexin signaling participates in the process of astrocyte activation.

[0181] **Inhibiting SEMA4D signaling in CVN AD mice restores plexin-B2 expression as compared to age-matched wild-type mice.** To determine if blocking SEMA4D signaling in CVN AD mice would impact the expression of plexin-B1 and/or its alternate cognate receptor, plexin-B2, in brain regions affected early in AD pathogenesis, 26 week-old CVN and wild-type control mice were injected weekly with 30 mg/kg anti-SEMA4D monoclonal antibody (67-2) or control IgG (2B8) intravenously for 13 weeks. At 41 weeks of age, mice were sacrificed and brain tissue sections from MAb-treated mice were stained with anti-plexin-B1 or anti-plexin-B2 to detect whether anti-SEMA4D treatment altered cognate receptor expression in the setting of ongoing AD-related pathogenesis. Percentage of plexin-B1 and plexin-B2-positive signals were quantified within the subiculum of all animals and normalized to total subiculum area scanned.

[0182] As shown in FIG. 11B, anti-SEMA4D antibody treatment of CVN AD mice lead to a restoration in plexin-B2 (right graph) staining intensity levels to those

quantified in WT control mice, but no significant change in plexin-B1 levels (left graph) relative to age-matched CVN AD control mice. These results suggest antibody-mediated SEMA4D inhibition selectively leads to destabilization of plexin-B2 and/or impedes a SEMA4D-driven feed-forward mechanism that selectively promotes plexin-B2 expression.

Example 5: Characterizing the expression patterns of plexin-B2 receptor in a YAC128 Huntington's disease mouse model

[0183] YAC128 mice express the full-length mutant human huntingtin gene (mHTT) and accurately recapitulate many of the signs and symptoms of HD (see also Example 2). Activated astrocytes in YAC128 Huntington disease mice exhibit enhanced plexin-B2 expression as compared to age-matched wild-type mice. At 12 months of age, YAC128 mice and wild-type control mice were sacrificed and processed for fluorescent co-immunohistochemistry (FIG. 12). Sections were co-stained for plexin-B2 (Plexin-B2, third panels from left), astrocyte marker GFAP (second panels from left) and DAPI to visualize cellular nuclei. Composite images are shown in the left-most panels. Slides were imaged at 60X magnification using an EXi-Aqua-14 bit camera coupled to an Olympus Ix50 microscope.

[0184] As shown in FIG. 12, co-immunohistochemical analyses of plexin-B2 and GFAP-positive astrocytes within the brains of YAC128 and wild-type mice demonstrates that astrocytic activation, as evidenced by increased GFAP marker staining, again positively correlates with co-registered SEMA4D receptor expression. Plexin-B2, whose best characterized ligand is SEMA4C, also has an intermediate affinity for SEMA4D (Azzarelli R, et al. An antagonistic interaction between PlexinB2 and Rnd3 controls RhoA activity and cortical neuron migration. *Nature Commun.* 2014; DOI:10.1038/ncomms4405)

Example 6: Examining the mechanisms by which SEMA4D signaling can modulate astrocyte function

[0185] The correlation between enhanced SEMA4D receptor expression and astrocytic activation in the setting of both AD and HD neurodegenerative disease suggests that SEMA4D signaling plays a role in astrocyte function and/or dysfunction. While not wishing to be bound by theory, this example provides evidence that SEMA4D signaling can participate in the regulation of astrocytic

function during responses to CNS injury, whether from acute or chronic stimuli. A schematicmodel supported by the data is depicted in FIG. 13, which shows three mechanisms by which SEMA4D signaling can potentially modulate astrocyte function. These three mechanisms are discussed below.

[0186] **Role of astrocytes and OPC support.** Plexin+ astrocytic processes interdigitate between SEMA4D+ NKX2.2+ oligodendrocyte precursor cells (OPCs) and provide trophic support. In CNS disease, activated astrocytes upregulate Plexin expression and retract processes via SEMA4D signaling. Locally, this loss of astrocyte:OPC proximity can result in diminished trophic support and increased chemotaxis-driven OPC movement toward regions of damage, while lack of astrocytic support at lesion site can impede OPC differentiation and remyelination.

[0187] To test this hypothesis, wild-type control rats were sacrificed and spinal cords were processed for fluorescent co-immunohistochemistry (FIG. 14). Sections were co-stained for SEMA4D (second panel from left), astrocyte marker GFAP (third panel from left) and DAPI to visualize cellular nuclei (right panel). Composite images are shown in the left-most panels and the dotted box depicts a 1.67X magnified inset below. Slides were imaged at 60X magnification using an EXi-Aqua-14 bit camera coupled to an Olympus Ix50 microscope.

[0188] As shown in FIG. 14, SEMA4D-expressing OPC are oriented in close proximity with GFAP+ astrocyte processes. Given the role that astrocytes play in facilitation of OPC survival and function, the juxtaposition of SEMA4D-expressing OPCs and SEMA4D receptor-expressing astrocytes suggests that disease-related activation of astrocytes with associated upregulation of plexin-B receptors and SEMA4D signaling can affect OPC function.

[0189] **Role of astrocytes in neuronal support.** In CNS disease, astrocytic activation leads to upregulation of Plexin expression, increased SEMA4D signaling and process retraction, which results in a loss of neuronal axon guidance, decreased trophic support, and/or dysregulated glutamate uptake/release. Ultimately, depending upon severity of disease stimulus, synapse loss and subsequent excitotoxic neuronal death can occur.

[0190] To determine if blocking SEMA4D signaling in CVN AD mice would impact synaptic marker expression in brain regions affected early in AD pathogenesis, 26 week-old CVN and wild-type control mice were injected weekly with 30 mg/kg

anti-SEMA4D monoclonal antibody (67-2) or control IgG (2B8) intravenously for 13 weeks. At 41 weeks of age, mice were sacrificed and brain tissue sections from MAb-treated mice were stained with anti-somatostatin antibody, anti-Neuropeptide-Y (NPY), anti-NPY receptor 1 (NPY1R), or anti-NPY receptor 2 (NPY2R) to detect specific subsets of inhibitory neurons that degenerate in early AD. Percentages of somatostatin-positive signal were quantified within the subiculum, and NPY-, NPY1R-, or NPY2R-positive signal were quantified within the dentate gyrus for all animals and normalized, respectively, to total subiculum or dentate gyrus area scanned. The results are shown in FIG. 15 A-D.

[0191] FIGS. 15A-15D show that anti-SEMA4D antibody treatment of CVN AD mice leads to a restoration in somatostatin (panel A), NPY (panel B), and NPY2R (panel D) staining intensity levels to levels characteristic of wild-type mice. Interestingly, agonists of NPY1R are reported to reduce stress and anxiety, while antagonists specific for NPY2R reduce stress and anxiety (Markus Heilig. The NPY system in stress, anxiety and depression. Neuropeptides 38 (2004) 213–224). As shown in FIG. 2 above, CVN mice treated with anti-SEMA4D exhibited reduced severity in anxiety in open field tests, findings that correlated with normalized (lower) NPY2R levels, while NPY1R levels were unchanged by anti-SEMA4D MAb treatment and remained higher than wild-type mice. Hence, these changes in NPY receptor levels are concordant with reduced anxiety behaviors observed in anti-SEMA4D treated CVN mice, a finding that further supports a role for SEMA4D in modulation of neurotransmission *in vivo*. It is noteworthy that downregulation of the inhibitory NPY neurotransmitter seen in the CVN AD model has also been reported in cerebral cortex of patients with Alzheimer's disease (Beal, *et al., Ann. Neurol.* 20, 282–288 (1986).

[0192] **Role of astrocytes in maintaining blood-brain barrier integrity.** As discussed elsewhere herein, astrocytic processes proximal to cerebral microvessels or pia are characterized by a high density of the water channel, aquaporin 4 (Aqp4). Astrocytic processes facing synaptic regions are enriched in glutamate transporters, where the density of Aqp4 is comparatively low. CNS disease-induced astrocyte activation increases SEMA4D signaling through Plexin, which leads to a retraction of astrocytic foot processes as evidenced by redistribution of aquaporin-4. This results in dysregulation and permeability of the BBB, thereby facilitating endothelial

inflammation and subsequent leukocyte entry into the CNS. In the setting of AD, Aqp4 staining intensities significantly decrease in regions with significant amyloid plaque burden.

[0193] To measure aquaporin-4 expression patterns in CVN AD mice as compared to age-matched wild-type mice, CVN and wild-type control mice were sacrificed at 41 weeks of age and processed for fluorescent co-immunohistochemistry. The results are shown in FIG. 16, with wild-type mice in the top panels and CVN mice in the lower panels. Sections were co-stained for aquaporin-4 (second panels from left), astrocyte marker GFAP (third panels from left) and DAPI (right panels) to visualize cellular nuclei. Composite images are shown in the left-most panels. Slides were imaged at 60X magnification using an EXi-Aqua-14 bit camera coupled to an Olympus Ix50 microscope.

[0194] As shown in FIG. 16, Aqp4 staining in age-matched CVN mice revealed a significant shift towards a diffuse pattern. This is in contrast to co-immunohistochemical analyses of Aqp4 and GFAP-positive astrocytes within the brains of wild-type mice, which demonstrate Aqp4 staining pattern in the subiculum that is restricted to areas proximal to microvasculature. This alteration in Aqp4 distribution, or loss in polarity, correlates with high astrocyte activation, as evidenced by increased intensity in GFAP staining. Given the strong co-registration in plexin-B1 staining in activated astrocytes (FIG. 11) and the role of SEMA4D/plexin-B1 signaling in cellular process retraction, these data suggest that SEMA4D signaling can play a role in the alteration of astrocyte polarity at the BBB interface during disease.

[0195] To analyze the impact of SEMA4D on BBB, a dynamic *in vitro* blood-brain barrier (DIV-BBB) model was employed. Briefly, the model consists of hollow polypropylene fibers that contain transcapillary 2 to 4-μm diameter pores. The fibers were connected to a pulsatile pump that facilitates continuous flow of media, and experimental compounds through the fibers and normal stimulation of endothelial flow receptors. Human brain endothelial cells were inoculated into the luminal compartment and allowed to adhere to and coat the inside walls of the fibers, while human astrocytes were seeded into the abluminal compartment bathing the outside surface of the fibers. The endothelial cells and astrocytes interact across the membrane to induce formation of a barrier with tight junctions between endothelial cells. The integrity of this barrier can be monitored continuously by measurement of

trans-endothelial electrical resistance (TEER). Human brain endothelial cells were inoculated into the luminal compartment and allowed to adhere to the polypropylene fibers, and human astrocytes were seeded separately on the abluminal surface of the fibers. At peak TEER (approximately 14 days *in vitro*), 0.5, 5, and 50μg/ml recombinant SEMA4D was added successively at 12-h intervals. At 36 h after initial SEMA4D exposure, 250 μg/ml control IgG (MAb 2955; 1 DIV-BBB unit) or anti-SEMA4D (VX15/2503; 2 DIV-BBB units) was added and TEER measured for another 132 h. The results are shown in FIG. 17. Error bars represent standard deviation. The data shown are representative of three independent experiments, with each demonstrating similar effects of SEMA4D and antibody on DIV-BBB integrity.

[0196] As shown in FIG. 17, the breakdown of BBB was reversed within 24 h by the addition of anti-SEMA4D antibody (VX15/2503). Introduction of a control recombinant protein did not result in a decrease in TEER (data not shown). Moreover, introduction of control IgG antibody (MAb 2955) did not affect SEMA4D-induced BBB compromise.

[0197] These data suggest that CNS disease-induced astrocyte activation increases SEMA4D signaling through plexin-B1 and/or plexin-B2 upregulation, which leads to a retraction of astrocytic foot processes as evidenced by redistribution of aquaporin-4. This results in dysregulation and permeability of the BBB, thereby facilitating endothelial inflammation and subsequent leukocyte entry into the CNS.

[0198] **Role of SEMA4D Signaling in Promoting Astrocyte Activation.** Given the association of SEMA4D receptor expression and the astrocyte activation marker GFAP, there exists the possibility that SEMA4D signaling can potentiate astrocyte activation, thereby providing a "feed-forward" mechanism during disease states. To examine the effects of SEMA4D on astrocyte activation, primary cultures of rat astrocytes were generated and treated with SEMA4D in isolation or in combination with thioacetamide (TAA) pretreatment, a well known hepatotoxic and hepatocarcinogenic agent that has been shown to induce plexin-B1 expression *in vivo* (Lim, J. S., *et al.*, (2006). Mol. Cell. Tox., 2(2), 126-133). Rat primary astrocytes were pretreated for 4 h with TAA followed by soluble SEMA4D for 24 h. Cells were then fixed and stained for GFAP and scanned at 20x magnification. Error bars represent standard deviation. "*"=$p<0.05$ by 1-way ANOVA with Bonferroni's Multiple Comparison Test.

[0199] As shown in FIG. 18A, a significant enhancement in GFAP, an activation marker for astrocytes, was observed upon addition of SEMA4D to cells pretreated with TAA, suggesting that SEMA4D signaling enhances astrocyte activation.

[0200] In a second set of *in vitro* studies, primary rat astrocytes were cultured and treated with or without prostaglandin D2, a known activation factor produced by microglia in the CNS, followed by an 8 or 24-h exposure to recombinant SEMA4D protein. Cells were fixed, processed for phalloidin (F-actin) and Dnase (G-actin) histochemistry, and F-actin/G-actin area ratios were calculated for each treatment condition. Error bars represent standard deviation. "**"=$p<0.01$ by 2-way ANOVA with Bonferroni's Multiple Comparison Test.

[0201] As shown in FIG. 18B, PGD2-activated astrocytes exposed to recombinant SEMA4D undergo a globular to filamentous transition in their actin cytoskeleton that is indicative of SEMA4D/Plexin-mediated signaling and a heightened astrocyte activation state.

[0202] Many modifications and other embodiments of the disclosures set forth herein will come to mind to one skilled in the art to which these disclosures pertain having the benefit of the teachings presented in the foregoing descriptions and the associated drawings. Therefore, it is to be understood that the disclosures are not to be limited to the specific embodiments disclosed and that modifications and other embodiments are intended to be included within the scope of the appended claims and list of embodiments disclosed herein. Although specific terms are employed herein, they are used in a generic and descriptive sense only and not for purposes of limitation.

WHAT IS CLAIMED IS:

1. Use of an effective amount of an isolated antibody or antigen-binding fragment thereof that specifically binds to semaphorin-4D (SEMA4D) in the preparation of a medicament for alleviation of symptoms in a subject having Huntington's disease (HD), wherein the antibody or antigen-binding fragment thereof inhibits SEMA4D interaction with a SEMA4D receptor, wherein the antibody or antigen-binding fragment thereof comprises a variable heavy chain (VH) comprising VHCDRs 1-3 comprising SEQ ID NOs: 6, 7, and 8, respectively, and a variable light chain (VL) comprising VLCDRs 1-3 comprising SEQ ID NOs: 14, 15, and 16, respectively.

2. The use of claim 1, wherein the SEMA4D receptor is selected from the group consisting of Plexin-B1, Plexin-B2, and CD72.

3. The use of claim 1 or claim 2, wherein the VH and VL comprise, respectively, SEQ ID NO: 9 and SEQ ID NO: 17 or SEQ ID NO: 10 and SEQ ID NO: 18.

4. The use of any one of claims 1 to 3, wherein the symptoms are selected from a group consisting of neuropsychiatric symptoms, cognitive symptoms, motor dysfunction, and any combination thereof.

5. The use of claim 4, wherein the neuropsychiatric symptoms are selected from a group consisting of anxiety-like behavior, reduced spatial memory, impaired locomotion, and any combination thereof.

6. The use of any one of claims 1 to 5, wherein the subject is human.

7. The use of any one of claims 1 to 6, wherein the antibody or antigen-binding fragment thereof is to be administered to the subject in combination with at least one or more other treatments for neurodegenerative disorders, and wherein the other treatment is to be administered prior to, during, or subsequent to administration of the antibody or antigen-binding fragment thereof to the subject.

8. The use of any one of claims 1 to 7, wherein the antibody or antigen-binding fragment thereof inhibits SEMA4D-mediated Plexin-B1 signal transduction.

9. The use of any one of claims 1 to 8, wherein the antibody or antigen-binding fragment thereof inhibits SEMA4D-mediated Plexin-B2 signal transduction.