

different cell types that constitute the tumor stroma (Evans *et al.*, *Cancer Immunol. Res.* 3:689–701 (2015)).

[0005] SEMA4D is implicated in neurodegenerative disorders, autoimmune diseases, demyelinating diseases, and cancer. In the central nervous system (CNS), SEMA4D is expressed on, *e.g.*, infiltrating immune cells and oligodendrocyte precursor cells and its receptors are expressed on, *e.g.*, neurons, oligodendrocytes, astrocytes, and endothelial cells (Okuno, T., *et al.*, *J. Immunol.* 184:1499–1506 (2010)). SEMA4D can serve as an axonal guidance molecule (Swiercz *et al.*, *Neuron* 35:51-63 (2002)) and can mediate GABAergic and glutamatergic synapse development (Paradis *et al.*, *Neuron* 53:217-232 (2007)) among other activities.

[0006] SEMA4D has also been shown to play a role in the migration and differentiation of neuronal and oligodendrocyte precursor cells, CNS inflammation, and neurodegeneration. For example, SEMA4D-deficient mice are resistant to the development of experimental autoimmune encephalomyelitis (EAE) (Kumanogoh A *et al.*, *Immunity* 13:621-631 (2000)), and blockade of SEMA4D can inhibit microglial activation and neuroinflammation in EAE (Okuno, T., *et al.*, *J. Immunol.* 184:1499–1506 (2010)). Similarly, SEMA4D stimulation of endothelial cells can lead to production of the pro-inflammatory cytokine IL-8 (Yang, YH *et al.*, *PLoS One* 6:e25826 (2011)).

[0007] Recent publications have demonstrated the efficacy of anti-SEMA4D binding molecules, *e.g.*, antibodies or antigen-binding fragments thereof in treating both cancer and neuroinflammatory/neurodegenerative diseases. For example, recent phase I clinical trials with the anti-SEMA4D antibody VX15/2503 demonstrated safety and efficacy in treating solid tumors. See, *e.g.*, Patnaik, A., *et al.* *Clin. Cancer Res.* 22:827-836 (2016) and Southwell AL, *et al.*, *Neurobiol. Dis* 76:46-56 (2015). There remains a need, however, for additional anti-SEMA4D antibodies with modified and/or improved profiles, *e.g.*, with respect to binding characteristics, immunogenicity, and/or potency.

SUMMARY

[0008] This disclosure provides compositions and methods for treating diseases associated with semaphorin-4D (SEMA4D) pathology such as autoimmune diseases, inflammatory diseases, cancers, neuroinflammatory diseases and neurodegenerative disorders. According to examples of the disclosure illustrated herein, there is provided

antibodies or antigen-binding fragments thereof that specifically bind to SEMA4D. According to other examples of the disclosure illustrated herein, there is provided a method for treating a subject with an autoimmune disease, inflammatory disease, cancer, neuroinflammatory disease, and neurodegenerative disorder including administering to the subject an effective amount of an antibody or antigen-binding fragment which specifically binds to SEMA4D and neutralizes SEMA4D activity.

[0009] In certain examples, the antibody or antigen-binding fragment thereof that specifically binds to SEMA4D includes a heavy chain variable region (VH) and a light chain variable region (VL); wherein the VH includes complementarity determining regions (HCDRs) HCDR1, HCDR2, and HCDR3 that includes amino acid sequences identical or identical except for one, two, three, or four amino acid substitutions in one, two, or all three of the HCDRs to SEQ ID NO: 2, SEQ ID NO: 3, and SEQ ID NO: 4, respectively; and wherein the VL includes complementarity determining regions (LCDRs) LCDR1, LCDR2, and LCDR3 that includes amino acid sequences identical or identical except for one, two, three, or four amino acid substitutions in one, two, or all three of the LCDRs to SEQ ID NO: 6, SEQ ID NO: 7, and SEQ ID NO: 8, respectively. In one aspect, the present disclosure refers to an antibody or antigen-binding fragment thereof that specifically binds to semaphorin-4D (SEMA4D) comprising a heavy chain variable region (VH) and a light chain variable region (VL); wherein the VH comprises complementarity determining regions (HCDRs) HCDR1, HCDR2, and HCDR3 comprising amino acid sequences identical or identical except for one, two, three, or four amino acid substitutions in one, two, or all three of the HCDRs to SEQ ID NO: 2, SEQ ID NO: 3, and SEQ ID NO: 4, respectively; and wherein the VL comprises complementarity determining regions (LCDRs) LCDR1, LCDR2, and LCDR3 comprising amino acid sequences identical or identical except for one, two, three, or four amino acid substitutions in one, two, or all three of the LCDRs to SEQ ID NO: 6, SEQ ID NO: 7, and SEQ ID NO: 8, respectively.

[0010] In certain examples, the anti-SEMA4D antibody or antigen-binding fragment thereof includes VH complementarity determining regions HCDR1, HCDR2, and HCDR3 that includes amino acid sequences identical to SEQ ID NO: 2, SEQ ID NO: 3, and SEQ ID NO: 4, respectively, and VL complementarity determining regions LCDR1, LCDR2, and LCDR3 that includes amino acid sequences identical to SEQ ID NO: 6, SEQ ID NO: 7, and SEQ ID NO: 8, respectively.

[0011] In certain examples, the anti-SEMA4D antibody or antigen-binding fragment thereof includes a VH that includes framework regions (HFWs) HFW1, HFW2, HFW3, and HFW4, and the VL that includes framework regions (LFWs) LFW1, LFW2, LFW3, and LFW4. In certain examples, the framework regions can be derived from a human antibody or from a non-human antibody.

[0012] In certain examples, the anti-SEMA4D antibody or antigen-binding fragment thereof includes a VH that includes the amino acid sequence SEQ ID NO: 1. In certain examples, the anti-SEMA4D antibody or antigen-binding fragment thereof includes a VL that includes the amino acid sequence SEQ ID NO: 5. In certain examples, the anti-SEMA4D antibody or antigen-binding fragment thereof includes a VH and VL that includes amino acid sequence SEQ ID NO: 1 and SEQ ID NO: 5, respectively.

[0013] In certain examples the VH of the anti-SEMA4D antibody or antigen-binding fragment thereof provided herein includes an amino acid sequence at least 80%, 85%, 90%, 95%, or 100% identical to SEQ ID NO: 1. In certain examples, the VL of the anti-SEMA4D antibody or antigen-binding fragment thereof provided herein includes an amino acid sequence at least 80%, 85%, 90%, 95%, or 100% identical to SEQ ID NO: 5. In certain examples the VH can include an amino acid sequence at least 80%, 85%, 90%, 95%, or 100% identical to SEQ ID NO: 1, and the VL can include an amino acid sequence at least 80%, 85%, 90%, 95%, or 100% identical to SEQ ID NO: 5. In one aspect, the present disclosure refers to an antibody or antigen-binding fragment thereof that specifically binds to semaphorin-4D (SEMA4D) comprising a heavy chain variable region (VH) and a light chain variable region (VL); wherein the VH comprises an amino acid sequence at least 80%, 85%, 90%, 95%, or 100% identical to SEQ ID NO: 1; or wherein the VL comprises an amino acid sequence at least 80%, 85%, 90%, 95%, or 100% identical to SEQ ID NO: 5; or wherein the VH comprises an amino acid sequence at least 80%, 85%, 90%, 95%, or 100% identical to SEQ ID NO: 1; and wherein the VL comprises an amino acid sequence at least 80%, 85%, 90%, 95%, or 100% identical to SEQ ID NO: 5.

[0014] In certain examples, the anti-SEMA4D antibody or fragment thereof provided herein includes a heavy chain constant region or fragment thereof fused to the C-terminus of the VH. In certain examples the heavy chain constant region or fragment thereof is a human heavy chain constant region. In certain examples the heavy chain constant region or fragment thereof can be a human IgG4 constant region. In certain examples the heavy chain constant region or fragment thereof is a non-human constant region. In certain examples, the heavy chain constant region or fragment thereof is a murine IgG1 constant region. In certain examples, the anti-SEMA4D antibody or fragment thereof includes a VH and VL, wherein the light chain constant region or fragment thereof is fused to the C-terminus of the VL. In certain examples, the anti-SEMA4D antibody or fragment thereof provided herein includes a light chain constant region or fragment thereof fused to the C-terminus of the VL. In certain examples the light chain constant region is a human light chain constant region, *e.g.*, a human lambda or human kappa light chain constant region. In certain examples the light chain constant region is a non-human light chain constant region, *e.g.*, a murine lambda light chain constant region.

[0015] In certain examples, the anti-SEMA4D antibody or fragment thereof provided herein can be an Fab fragment, an Fab' fragment, an F(ab')₂ fragment, an Fd fragment, a single-chain Fv fragment (scFv), or a disulfide-linked Fv fragment (sdFv). In certain examples the anti-SEMA4D antibody or fragment thereof can be multi-specific, *e.g.*, bispecific.

[0016] In certain examples, the anti-SEMA4D antibody or fragment thereof provided herein can specifically bind to human SEMA4D, mouse SEMA4D, rat SEMA4D, and/or non-human primate SEMA4D, *e.g.*, cynomolgus monkey SEMA4D and/or marmoset SEMA4D. In certain examples, the anti-SEMA4D antibody or fragment thereof provided herein can bind to SEMA4D, *e.g.*, human SEMA4D, mouse SEMA4D, rat SEMA4D,

and/or non-human primate SEMA4D, *e.g.*, cynomolgus monkey SEMA4D and/or marmoset SEMA4D, with an affinity characterized by a dissociation constant K_D no greater than 500 nM, 100 nM, 50.0 nM, 40.0 nM, 30.0 nM, 20.0 nM, 10.0 nM, 9.0 nM, 8.0 nM, 7.0 nM, 6.0 nM, 5.0 nM, 4.0 nM, 3.0 nM, 2.0 nM, 1.0 nM, 0.50 nM, 0.10 nM, 0.050 nM, 0.01 nM, 0.005 nM, or 0.001 nM

[0017] In certain examples, the anti-SEMA4D antibody or fragment thereof provided herein can inhibit SEMA4D from binding to a SEMA4D receptor, *e.g.*, Plexin-B1, Plexin-B2, CD72, or any combination thereof.

[0018] In certain examples, the anti-SEMA4D antibody or fragment thereof elicits minimal or no anti-antibody immune response upon administration to a subject.

[0019] In certain examples, the anti-SEMA4D antibody or fragment thereof includes a heterologous moiety fused or conjugated thereto. In certain examples, the heterologous moiety can be, *e.g.*, a polypeptide, a cytotoxic agent, a therapeutic agent, a prodrug, a lipid, a carbohydrate, a nucleic acid, a detectable label, a polymer, or any combination thereof. In certain examples, the heterologous moiety can include a binding molecule, an enzyme, a cytokine, a lymphokine, a hormonal peptide, or any combination thereof. In another example, the heterologous moiety can include a radionuclide, a biological toxin, an enzymatically active toxin, or any combination thereof. In another example, the heterologous moiety can include an enzyme, a fluorescent label, a chemiluminescent label, a bioluminescent label, a radioactive label, or any combination thereof.

[0020] In one aspect, the present disclosure refers to a composition comprising any of the antibody or fragment thereof as disclosed herein, and a carrier. In certain examples, the disclosure provides a pharmaceutical composition that includes the anti-SEMA4D antibody or fragment thereof provided herein, and a carrier.

[0021] In certain examples, the disclosure provides an isolated polynucleotide or combination of polynucleotides that includes one or more nucleic acid sequences encoding the anti-SEMA4D antibody or fragment thereof. In one aspect, the present disclosure refers to an isolated polynucleotide or combination of polynucleotides comprising one or more nucleic acid sequences encoding any of the antibody or fragment thereof as disclosed herein, or a subunit thereof. In another example, the polynucleotide or combination of polynucleotides includes a nucleic acid sequence that encodes the VH of the anti-SEMA4D antibody or fragment thereof. In another example, the polynucleotide or combination of polynucleotides includes a nucleic acid sequence that encodes the VL of the anti-SEMA4D antibody or fragment thereof. In another example, the polynucleotide or combination of polynucleotides includes a nucleic acid sequence that encodes the VH and VL of the anti-SEMA4D antibody or fragment thereof. In yet another example, the disclosure provides a vector that includes the polynucleotide that includes one or more nucleic acid sequences encoding the anti-SEMA4D antibody or fragment thereof. In

another example, the disclosure provides a host cell that includes the vector. In one aspect, the present disclosure refers to a host cell comprising the polynucleotide or combination of polynucleotides as disclosed herein, or a vector or vectors comprising the polynucleotide or combination of polynucleotides as disclosed herein. In another example, the disclosure provides a method of producing the anti-SEMA4D antibody or fragment thereof. In one aspect, the present disclosure refers to a method for producing any of the antibody or fragment thereof as disclosed herein, comprising culturing the host cell as disclosed herein and recovering the antibody or fragment thereof.

[0022] In a certain example of the provided method, the anti-SEMA4D antibody or antigen-binding fragment neutralizes SEMA4D in a human subject.

[0023] In one aspect, the present disclosure refers to use of any of the antibody or fragment thereof as disclosed herein in the manufacture of a medicament for neutralizing SEMA4D in a human subject, wherein the human subject is in need of treatment for a disease or disorder selected from the group consisting of an autoimmune disease or disorder, an inflammatory disease or disorder, cancer, a neuroinflammatory disease or disorder, a neurodegenerative disease or disorder, or any combination thereof. According to examples illustrated herein, the disclosure provides a method for treating a subject with an autoimmune disease or disorder, an inflammatory disease or disorder, cancer, a neuroinflammatory disease or disorder, a neurodegenerative disease or disorder, or any combination thereof including administering to the subject an effective amount of an antibody or antigen-binding fragment which specifically binds to SEMA4D and neutralizes SEMA4D activity. In certain examples, the neuroinflammatory disease or disorder is multiple sclerosis. In certain examples, the neurodegenerative disease or disorder is stroke, 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 examples, the autoimmune disease or the inflammatory disease is arthritis. In certain examples, the autoimmune disease or the inflammatory disease is rheumatoid arthritis.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

[0024] **FIGURE 1A** compares binding of MAbD2517 and MAbVX15/2503 (both formulated in PBS) to human SEMA4D as measured by ELISA.

[0025] **FIGURE 1B** compares binding of MAbD2517 and MAbVX15/2503 (both formulated in PBS) to cynomolgus monkey SEMA4D as measured by ELISA.

[0026] **FIGURE 1C** compares binding of MAbD2517 and MAbVX15/2503 (both formulated in PBS) to marmoset SEMA4D as measured by ELISA.

[0027] **FIGURE 1D** compares binding of MAbD2517 and MAbVX15/2503 (both formulated in PBS) to mouse SEMA4D as measured by ELISA.

[0028] **FIGURE 2A** shows the ability MAbD2517 to block human SEMA4D from binding to 293PLXNB1 cells as compared to MAbVX15/2503.

[0029] **FIGURE 2B** shows the ability MAbD2517 to block cynomolgus monkey SEMA4D from binding to 293PLXNB1 cells as compared to MAbVX15/2503.

WHAT IS CLAIMED IS:

1. An antibody or antigen-binding fragment thereof that specifically binds to semaphorin-4D (SEMA4D) comprising a heavy chain variable region (VH) and a light chain variable region (VL); wherein the VH comprises complementarity determining regions (HCDRs) HCDR1, HCDR2, and HCDR3 comprising amino acid sequences identical or identical except for one, two, three, or four amino acid substitutions in one, two, or all three of the HCDRs to SEQ ID NO: 2, SEQ ID NO: 3, and SEQ ID NO: 4, respectively; and wherein the VL comprises complementarity determining regions (LCDRs) LCDR1, LCDR2, and LCDR3 comprising amino acid sequences identical or identical except for one, two, three, or four amino acid substitutions in one, two, or all three of the LCDRs to SEQ ID NO: 6, SEQ ID NO: 7, and SEQ ID NO: 8, respectively.
2. The antibody or fragment thereof of claim 1, wherein the HCDR1, HCDR2, and HCDR3 comprise amino acid sequences identical to SEQ ID NO: 2, SEQ ID NO: 3, and SEQ ID NO: 4, respectively, and wherein the LCDR1, LCDR2, and LCDR3 comprise amino acid sequences identical to SEQ ID NO: 6, SEQ ID NO: 7, and SEQ ID NO: 8, respectively.
3. The antibody or fragment thereof of claim 1 or claim 2, wherein the VH further comprises framework regions (HFWs) HFW1, HFW2, HFW3, and HFW4, and wherein the VL further comprises framework regions (LFWs) LFW1, LFW2, LFW3, and LFW4.
4. The antibody or fragment thereof of claim 3, wherein the framework regions are derived from a human antibody.
5. The antibody or fragment thereof of claim 3, wherein the framework regions are derived from a non-human antibody.

6. The antibody or fragment thereof of any one of claims 1 to 4, wherein the VH comprises the amino acid sequence SEQ ID NO: 1; or wherein the VL comprises the amino acid sequence SEQ ID NO: 5; or , wherein the VH comprises the amino acid sequence SEQ ID NO: 1 and the VL comprises the amino acid sequence SEQ ID NO: 5.

7. An antibody or antigen-binding fragment thereof that specifically binds to semaphorin-4D (SEMA4D) comprising a heavy chain variable region (VH) and a light chain variable region (VL); wherein the VH comprises an amino acid sequence at least 80%, 85%, 90%, 95%, or 100% identical to SEQ ID NO: 1; or wherein the VL comprises an amino acid sequence at least 80%, 85%, 90%, 95%, or 100% identical to SEQ ID NO: 5; or wherein the VH comprises an amino acid sequence at least 80%, 85%, 90%, 95%, or 100% identical to SEQ ID NO: 1; and wherein the VL comprises an amino acid sequence at least 80%, 85%, 90%, 95%, or 100% identical to SEQ ID NO: 5.

8. The antibody or fragment thereof of any one of claims 1 to 7, further comprising a heavy chain constant region or fragment thereof fused to the C-terminus of the VH, wherein the constant region or fragment thereof is a human or non-human heavy chain constant region.

9. The antibody or fragment thereof of claim 8, wherein the heavy chain constant region or fragment thereof is a human IgG4 constant region.

10. The antibody or fragment thereof of claim 8, wherein the heavy chain constant region or fragment thereof is a murine IgG1 constant region.

11. The antibody or fragment thereof of any one of claims 1 to 10, further comprising a light chain constant region or fragment thereof fused to the C-terminus of the VL, wherein the constant region or fragment thereof is a human or non-human light chain constant region.

12. The antibody or fragment thereof of claim 11, wherein the light chain constant region or fragment thereof is a human lambda light chain constant region.
13. The antibody or fragment thereof of claim 11, wherein the light chain constant region or fragment thereof is a murine lambda light chain constant region.
14. The antibody or fragment thereof of any one of claims 1 to 13, which is an Fab fragment, an Fab' fragment, an F(ab')₂ fragment, an Fd fragment, a single-chain Fv fragment (scFv), or a disulfide-linked Fv fragment (sdFv).
15. The antibody or fragment thereof of any one of claims 1 to 14, which is multispecific.
16. The antibody of any one of claims 1 to 15, which can specifically bind to human SEMA4D, mouse SEMA4D, rat SEMA4D, or non-human primate SEMA4D.
17. The antibody or fragment thereof of any one of claims 1 to 16, which can inhibit SEMA4D from binding to a SEMA4D receptor.
18. The antibody or fragment thereof of claim 17, wherein the SEMA4D receptor is Plexin-B1, Plexin-B2, CD72, or a combination thereof.
19. A composition comprising the antibody or fragment thereof of any one of claims 1 to 18, and a carrier.
20. An isolated polynucleotide or combination of polynucleotides comprising one or more nucleic acid sequences encoding the antibody or fragment thereof of any one of claims 1 to 18, or a subunit thereof.
21. A host cell comprising the polynucleotide or combination of polynucleotides of claim 20, or a vector or vectors comprising the polynucleotide or combination of polynucleotides of claim 20.

22. A method for producing the antibody or fragment thereof of any one of claims 1 to 18, comprising culturing the host cell of claim 21 and recovering the antibody or fragment thereof.

23. Use of the antibody or fragment thereof according to any one of claims 1-18 in the manufacture of a medicament for neutralizing SEMA4D in a human subject, wherein the human subject is in need of treatment for a disease or disorder selected from the group consisting of an autoimmune disease or disorder, an inflammatory disease or disorder, cancer, a neuroinflammatory disease or disorder, a neurodegenerative disease or disorder, or any combination thereof.

24. The use of claim 23, wherein the disease or disorder is stroke, 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, multiple sclerosis, arthritis, rheumatoid arthritis or a combination thereof.