

with cytotoxics and differentiation agents. See, *e.g.*, Mottamal, M., *et al.*, *Molecules* 20:3898-3941 (2015).

**[0008]** Methylation of genomic DNA is an extensively characterized epigenetic modification. Methylation of DNA leads to transcriptional quiescence, resulting in gene silencing. Gravina G. L., *et al.*, *Mol. Cancer* 9:305-320 (2010). Methylation is facilitated by DNA methyltransferases (DNMTs), which catalyze the addition of a methyl group to the 5' carbon of cytosine residues. *Id.* Hypermethylated DNA is common in tumor and other cancer cells. A variety of DNA methyltransferase inhibitors (DNMTi (used herein both as the singular and the plural)) are currently in use or are being tested for treatment of various cancers. These include deoxyribonucleoside analogs such as azacytidine, and non-nucleoside analogs such as antisense oligonucleotides and small molecule enzyme inhibitors. *Id.*

## SUMMARY

**[0009]** This disclosure provides a method for inhibiting, delaying, or reducing malignant cell growth in a subject with cancer, where the method includes administering to the subject a combination therapy including an effective amount of an isolated antibody or antigen-binding fragment thereof that specifically binds to semaphorin-4D (SEMA4D) and an effective amount of an epigenetic modulating agent. According to the provided method, the anti-SEMA4D antibody or fragment thereof can inhibit SEMA4D interaction with its receptor, *e.g.*, Plexin-B1, Plexin-B2, or CD72. For example, according to the provided method the anti-SEMA4D antibody or fragment thereof can inhibit SEMA4D-mediated Plexin-B1 signal transduction.

**[0009a]** In one aspect, the present disclosure refers to the use of an isolated antibody or antigen-binding fragment in the manufacture of a medicament for use in combination with an epigenetic modulating agent for inhibiting, delaying, or reducing malignant cell growth in a subject with cancer, wherein the isolated antibody or antigen-binding fragment thereof specifically binds to semaphorin-4D (SEMA4D).

**[0010]** In certain examples, the anti-SEMA4D antibody or fragment thereof includes a variable heavy chain (VH) that includes VH CDRs 1-3 having the amino acid sequences SEQ ID NOS: 2, 3, and 4, respectively, and a variable light chain (VL) that includes VL CDRs 1-3 having the amino acid sequences SEQ ID NOS: 6, 7, and 8, respectively. In

certain examples the amino acid sequences of the VH and VL include, respectively, SEQ ID NO: 1 and SEQ ID NO: 5, or SEQ ID NO: 9 and SEQ ID NO: 10.

**[0011]** In certain examples, the epigenetic modulating agent can include a histone deacetylase (HDAC) inhibitor (HDACi), a DNA methyltransferase (DNMT) inhibitor (DNMTi), or any combination thereof.

**[0012]** In certain examples, the epigenetic modulating agent can include a histone deacetylase (HDAC) inhibitor (HDACi). In certain examples the HDAC can be a Class I HDAC, a Class IIA HDAC, a Class IIB HDAC, a Class IV HDAC, or any combination thereof, or the HDAC can include a zinc-containing catalytic domain. In certain examples, the HDACi can bind to the zinc-containing catalytic domain of the HDAC. In certain examples, the HDACi can include a chemical moiety selected from the group consisting of a hydroxamic acid or a salt thereof, a cyclic tetrapeptide, a depsipeptide, a benzamide, an electrophilic ketone, an aliphatic acid or a salt thereof, or any combination thereof. For example, in certain examples, the HDACi can be Vorinostat, Romidepsin, Chidamide, Panobinostat, Belinostat, Valproic acid or a salt thereof, Mocetinostat, Abexinostat, Entinostat, Pracinostat, Resminostat, Givinostat, Quisinostat, Kevetrin, CUDC-101, AR-42, Tefinostat (CHR-2845), CHR-3996, 4SC-202, CG200745, ACY-1215, ACY-241, any combination thereof, or any salt, crystal, amorphous structure, hydrate, derivative, metabolite, isomer, or prodrug thereof. In certain examples, the HDACi is Entinostat (Pyridin-3-ylmethyl N-[[4-[(2-aminophenyl)carbamoyl]phenyl]methyl]carbamate).

**[0013]** In certain examples, the epigenetic modulating agent can include a DNA methyltransferase (DNMT) inhibitor (DNMTi). In certain examples the DNMT can be DNMT1, DNMT-3a, DNMT-3b, or any combination thereof. In certain examples, the DNMTi can be a nucleoside analog, an antisense oligonucleotide, a small molecule enzyme inhibitor, or any combination thereof. For example, in certain examples the DNMTi can be azacytidine, decitabine, zebularine, SGI-110, epigallocatechin gallate, MG98, RG108, procainamide, hydralazine, any combination thereof, or any salt, crystal, amorphous structure, hydrate, derivative, metabolite, isomer, or prodrug thereof. In certain examples the DNMTi is azacytidine.

**[0014]** In a particular example of the provided method, the isolated antibody or antigen-binding fragment thereof that specifically binds to semaphorin-4D (SEMA4D) comprises a VH comprising the amino acid sequence SEQ ID NO: 1 and a VL comprising

the amino acid sequence SEQ ID NO: 5, and the epigenetic modulating agent comprises the HDACi Entinostat. In another particular example of the provided method, the isolated antibody or antigen-binding fragment thereof that specifically binds to semaphorin-4D (SEMA4D) comprises a VH comprising the amino acid sequence SEQ ID NO: 1 and a VL comprising the amino acid sequence SEQ ID NO: 5, and the epigenetic modulating agent comprises the DNMTi azacytidine.

**[0015]** According to the provided method, the antibody or antigen-binding fragment thereof and the epigenetic modulating agent can be administered separately, or they can be administered simultaneously.

**[0016]** In certain examples of the provided method, administration of the combination therapy can result in enhanced therapeutic efficacy relative to administration of the antibody or fragment thereof or the epigenetic modulating agent alone. For example, in certain examples the enhanced therapeutic efficacy is greater than an additive effect, *e.g.*, a synergistic effect.

**[0017]** In certain examples of the provided method, the cancer to be treated can be a solid tumor, a hematological malignancy, any metastasis thereof, or any combination thereof. In certain examples, the solid tumor can be, *e.g.*, a sarcoma, a carcinoma, a melanoma, any metastases thereof, or any combination thereof. More specifically, the solid tumor can be, *e.g.*, squamous cell carcinoma, adenocarcinoma, basal cell carcinoma, renal cell carcinoma, ductal carcinoma of the breast, soft tissue sarcoma, osteosarcoma, melanoma, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, cancer of the peritoneum, hepatocellular carcinoma, gastrointestinal cancer, gastric cancer, pancreatic cancer, neuroendocrine cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, brain cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, esophageal cancer, salivary gland carcinoma, kidney cancer, liver cancer, prostate cancer, vulval cancer, thyroid cancer, head and neck cancer, any metastases thereof, or any combination thereof.

**[0018]** In certain examples of the provided method, the cancer to be treated can be a hematologic malignancy or metastasis thereof. In certain examples, the hematologic malignancy can be leukemia, lymphoma, myeloma, acute myeloid leukemia, chronic myeloid leukemia, acute lymphocytic leukemia, chronic lymphocytic leukemia, hairy cell

leukemia, Hodgkin lymphoma, non-Hodgkin lymphoma, multiple myeloma, any metastases thereof, or any combination thereof.

**[0019]** In certain examples, the method provided herein can further include administration of an additional cancer therapy, *e.g.*, surgery, chemotherapy, radiation therapy, a cancer vaccine, administration of an immunostimulatory agent, adoptive T cell or antibody therapy, administration of an immune checkpoint blockade inhibitor, administration of a regulatory T cell (Treg) modulator, and a combination thereof.

**[0020]** In another aspect, this disclosure further provides a pharmaceutical composition that includes an effective amount of an isolated antibody or antigen-binding fragment thereof that specifically binds to semaphorin-4D (SEMA4D) and an effective amount of an epigenetic modulating agent. In certain examples, the pharmaceutical composition can further include a carrier, an excipient, or any combination thereof.

**[0021]** In certain examples, the antibody or fragment thereof of the provided composition can include a variable heavy chain (VH) comprising VH CDRs 1-3 comprising SEQ ID NOS: 2, 3, and 4, respectively, and a variable light chain (VL) comprising VL CDRs 1-3 comprising SEQ ID NOS: 6, 7, and 8, respectively. In certain examples, the antibody or fragment thereof of the provided composition can include a VH and VL that include, respectively, SEQ ID NO: 1 and SEQ ID NO: 5, or SEQ ID NO: 9 and SEQ ID NO: 10.

**[0022]** In certain examples, the epigenetic modulating agent included in the provided pharmaceutical composition can include a histone deacetylase (HDAC) inhibitor (HDACi), a DNA methyltransferase (DNMT) inhibitor (DNMTi), or any combination thereof.

**[0023]** Where the composition includes a HDACi, the HDACi can be, *e.g.*, Vorinostat, Romidepsin, Chidamide, Panobinostat, Belinostat, Valproic acid or a salt thereof, Mocetinostat, Abexinostat, Entinostat, Pracinostat, Resminostat, Givinostat, Quisinostat, Kevetrin, CUDC-101, AR-42, Tefinostat (CHR-2845), CHR-3996, 4SC-202, CG200745, ACY-1215, ACY-241, any combination thereof, or any salt, crystal, amorphous structure, hydrate, derivative, metabolite, isomer, or prodrug thereof. In certain examples the HDACi can be Entinostat (Pyridin-3-ylmethyl N-[[4-[(2-aminophenyl)carbamoyl]phenyl]methyl]carbamate).

**[0024]** Where the composition includes a DNMTi, the DNMTi can be, e.g., azacytidine, decitabine, zebularine, SGI-110, epigallocatechin gallate, MG98, RG108, procainamide, hydralazine, any combination thereof, or any salt, crystal, amorphous structure, hydrate, derivative, metabolite, isomer, or prodrug thereof. In certain examples the DNMTi can be azacytidine.

**[0025]** In a particular aspect, the disclosure provides a pharmaceutical composition that includes an effective amount of an isolated antibody or antigen-binding fragment thereof that specifically binds to semaphorin-4D (SEMA4D), where the antibody or fragment thereof includes the VH amino acid sequence SEQ ID NO: 1 and the VL amino acid sequence SEQ ID NO: 5; and an effective amount of the HDACi Entinostat. In another particular aspect, the disclosure provides a pharmaceutical composition that includes an effective amount of an isolated antibody or antigen-binding fragment thereof that specifically binds to semaphorin-4D (SEMA4D), where the antibody or fragment thereof includes the VH amino acid sequence SEQ ID NO: 1 and the VL amino acid sequence SEQ ID NO: 5; and an effective amount of the DNMTi azacytidine.

## BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

**[0026]** FIGURE 1 shows the experimental design for combination therapy experiments in Examples 1 and 2.

**[0027]** FIGURE 2A shows the mean tumor volume over time for the various treatments in Example 1.

**[0028]** FIGURE 2B shows percent survival over time for the various treatments in Example 1. Statistical significance was determined using Mantel Cox Log Rank test. Prism reports results as non-significant (ns) at  $P > 0.05$ , significant (symbolized by “\*”) at  $0.01 < P \leq 0.05$ , very significant (“\*\*”) at  $0.001 < P \leq 0.01$ , and extremely significant (“\*\*\*\*”) at  $P \leq 0.001$  or (“\*\*\*\*\*”) at  $P \leq 0.0001$ .

**[0029]** FIGURE 2C is a bar graph showing the percent of complete tumor regression (tumor volume  $< 50 \text{ mm}^3$ ) for the various treatments in Example 1. Statistical significance was determined using Fisher’s exact test, Prism reports results as non-significant (ns) at  $P > 0.05$ , significant (symbolized by “\*”) at  $0.01 < P \leq 0.05$ , very significant (“\*\*”) at  $0.001 < P \leq 0.01$ , and extremely significant (“\*\*\*\*”) at  $P \leq 0.001$  or (“\*\*\*\*\*”) at  $P \leq 0.0001$ .

**[0030]** FIGURE 2D shows the same data as FIGURE 2C, stratified as to whether or not the tumor exceeded at least  $100 \text{ mm}^3$  before regressing.

**[0031]** FIGURE 3A shows mean tumor volume over time for the various treatments in Example 2. Statistical significance was determined using 2-way ANOVA. Prism reports results as non-significant (ns) at  $P > 0.05$ , significant (symbolized by “\*”) at  $0.01 < P \leq 0.05$ , very significant (“\*\*”) at  $0.001 < P \leq 0.01$

**[0032]** FIGURE 3B shows tumor volume for individual control animals over time in Example 2.

Claims:

1. The use of an isolated antibody or antigen-binding fragment in the manufacture of a medicament for use in combination with an epigenetic modulating agent for inhibiting, delaying, or reducing malignant cell growth in a subject with cancer, wherein the isolated antibody or antigen-binding fragment thereof specifically binds to semaphorin-4D (SEMA4D).
2. The use of claim 1, wherein the antibody or fragment thereof inhibits SEMA4D interaction with its receptor.
3. The use of claim 2, wherein the receptor is Plexin-B1, Plexin-B2, or CD72.
4. The use of any one of claims 1 to 3, wherein the antibody or fragment thereof inhibits SEMA4D-mediated Plexin-B1 signal transduction.
5. The use of any one of claims 1 to 4, wherein the antibody or fragment thereof comprises a variable heavy chain (VH) comprising VH CDRs 1-3 comprising SEQ ID NOS: 2, 3, and 4, respectively, and a variable light chain (VL) comprising VL CDRs 1-3 comprising SEQ ID NOS: 6, 7, and 8, respectively.
6. The use of claim 5, wherein the VH and VL comprise, respectively, SEQ ID NO: 1 and SEQ ID NO: 5, or SEQ ID NO: 9 and SEQ ID NO: 10.
7. The use of any one of claims 1 to 6, wherein the epigenetic modulating agent comprises a histone deacetylase (HDAC) inhibitor (HDACi), a DNA methyltransferase (DNMT) inhibitor (DNMTi), or any combination thereof.
8. The use of claim 7, wherein the epigenetic modulating agent comprises a histone deacetylase (HDAC) inhibitor (HDACi).
9. The use of claim 8, wherein the HDAC comprises a Class I HDAC, a Class IIA HDAC, a Class IIB HDAC, a Class IV HDAC, or any combination thereof, or wherein the HDAC comprises a zinc-containing catalytic domain.

10. The use of claim 9, wherein the HDACi can bind to the zinc-containing catalytic domain of the HDAC.

11. The use of any one of claims 8 to 10, wherein the HDACi comprises a chemical moiety selected from the group consisting of a hydroxamic acid or a salt thereof, a cyclic tetrapeptide, a depsipeptide, a benzamide, an electrophilic ketone, an aliphatic acid or a salt thereof, or any combination thereof.

12. The use of any one of claims 8 to 11, wherein the HDACi comprises Vorinostat, Romidepsin, Chidamide, Panobinostat, Belinostat, Valproic acid or a salt thereof, Mocetinostat, Abexinostat, Entinostat, Pracinostat, Resminostat, Givinostat, Quisinostat, Kevetrin, CUDC-101, AR-42, Tefinostat (CHR-2845), CHR-3996, 4SC-202, CG200745, ACY-1215, ACY-241, any combination thereof, or any salt, crystal, amorphous structure, hydrate, derivative, metabolite, isomer, or prodrug thereof.

13. The use of claim 12, wherein the HDACi is Entinostat (Pyridin-3-ylmethyl N-[[4-[(2-aminophenyl)carbamoyl]phenyl]methyl]carbamate).

14. The use of claim 7, wherein the epigenetic modulating agent comprises a DNA methyltransferase (DNMT) inhibitor (DNMTi).

15. The use of claim 14, wherein the DNMT comprises DNMT1, DNMT-3a, DNMT-3b, or any combination thereof.

16. The use of claim 14 or claim 15, wherein the DNMTi is a nucleoside analog, an antisense oligonucleotide, a small molecule enzyme inhibitor, or any combination thereof.

17. The use of any one of claims 14 to 16, wherein the DNMTi comprises azacytidine, decitabine, zebularine, SGI-110, epigallocatechin gallate, MG98, RG108, procainamide, hydralazine, any combination thereof, or any salt, crystal, amorphous structure, hydrate, derivative, metabolite, isomer, or prodrug thereof.

18. The use of claim 17, wherein the DNMTi is azacytidine.

19. The use of any one of claims 1 to 18, wherein the antibody or antigen-binding fragment thereof and the epigenetic modulating agent are to be administered separately or simultaneously.

20. The use of any one of claims 1 to 19, wherein administration of the combination of the antibody or fragment thereof and the epigenetic modulating agent results in enhanced therapeutic efficacy relative to administration of the antibody or fragment thereof or the epigenetic modulating agent alone.

21. The use of claim 20, wherein the enhanced therapeutic efficacy is greater than an additive effect.

22. The use of any one of claims 1 to 21, wherein the cancer comprises a solid tumor, a hematological malignancy, any metastasis thereof, or any combination thereof.

23. The use of claim 22, wherein the cancer is a solid tumor or metastasis thereof.

24. The use of claim 23, wherein the solid tumor is a sarcoma, a carcinoma, a melanoma, any metastases thereof, or any combination thereof.

25. The use of claim 24, wherein the solid tumor is squamous cell carcinoma, adenocarcinoma, basal cell carcinoma, renal cell carcinoma, ductal carcinoma of the breast, soft tissue sarcoma, osteosarcoma, melanoma, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, cancer of the peritoneum, hepatocellular carcinoma, gastrointestinal cancer, gastric cancer, pancreatic cancer, neuroendocrine cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, brain cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, esophageal cancer, salivary gland carcinoma, kidney cancer, liver cancer, prostate cancer, vulval cancer, thyroid cancer, head and neck cancer, any metastases thereof, or any combination thereof.

26. The use of claim 22, wherein the cancer is a hematologic malignancy or metastasis thereof.

27. The use of claim 26, wherein the hematologic malignancy is leukemia, lymphoma, myeloma, acute myeloid leukemia, chronic myeloid leukemia, acute lymphocytic

leukemia, chronic lymphocytic leukemia, hairy cell leukemia, Hodgkin lymphoma, non-Hodgkin lymphoma, multiple myeloma, any metastases thereof, or any combination thereof.

28. The use of any one of claims 1 to 27, further comprising that an additional cancer therapy is to be administered.

29. The use of claim 28, wherein the additional therapy comprises surgery, chemotherapy, radiation therapy, a cancer vaccine, administration of an immunostimulatory agent, adoptive T cell or antibody therapy, administration of an immune checkpoint blockade inhibitor, administration of a regulatory T cell (Treg) modulator, and a combination thereof.

30. The use of any one of claims 1 to 13 or 19 to 29, wherein:

(a) the isolated antibody or antigen-binding fragment thereof that specifically binds to semaphorin-4D (SEMA4D) comprises a VH comprising the amino acid sequence SEQ ID NO: 1 and a VL comprising the amino acid sequence SEQ ID NO: 5, and

(b) the epigenetic modulating agent comprises the HDACi Entinostat.

31. The use of any one of claims 1 to 7 or 14 to 29, wherein:

(a) the isolated antibody or antigen-binding fragment thereof that specifically binds to semaphorin-4D (SEMA4D) comprises a comprising the VH amino acid sequence SEQ ID NO: 1 and a VL comprising the amino acid sequence SEQ ID NO: 5, and

(b) the epigenetic modulating agent comprises the DNMTi azacytidine.

32. A pharmaceutical composition comprising an effective amount of an isolated antibody or antigen-binding fragment thereof that specifically binds to semaphorin-4D (SEMA4D) and an effective amount of an epigenetic modulating agent.

33. The pharmaceutical composition of claim 32, further comprising a carrier, an excipient, or any combination thereof.

34. The pharmaceutical composition of claim 32 or claim 33, wherein the antibody or fragment thereof comprises a variable heavy chain (VH) comprising VH CDRs 1-3 comprising

SEQ ID NOS: 2, 3, and 4, respectively, and a variable light chain (VL) comprising VL CDRs 1-3 comprising SEQ ID NOS: 6, 7, and 8, respectively.

35. The pharmaceutical composition of claim 34, wherein the VH and VL comprise, respectively, SEQ ID NO: 1 and SEQ ID NO: 5, or SEQ ID NO: 9 and SEQ ID NO: 10.

36. The pharmaceutical composition of any one of claims 32 to 35, wherein the epigenetic modulating agent comprises a histone deacetylase (HDAC) inhibitor (HDACi), a DNA methyltransferase (DNMT) inhibitor (DNMTi), or any combination thereof.

37. The pharmaceutical composition of claim 36, wherein the epigenetic modulating agent comprises a histone deacetylase (HDAC) inhibitor (HDACi).

38. The pharmaceutical composition of claim 37, wherein the HDACi comprises Vorinostat, Romidepsin, Chidamide, Panobinostat, Belinostat, Valproic acid or a salt thereof, Mocetinostat, Abexinostat, Entinostat, Pracinostat, Resminostat, Givinostat, Quisinostat, Kevetrin, CUDC-101, AR-42, Tefinostat (CHR-2845), CHR-3996, 4SC-202, CG200745, ACY-1215, ACY-241, any combination thereof, or any salt, crystal, amorphous structure, hydrate, derivative, metabolite, isomer, or prodrug thereof.

39. The pharmaceutical composition of claim 38, wherein the HDACi is Entinostat (Pyridin-3-ylmethyl N-[[4-[(2-aminophenyl)carbamoyl]phenyl]methyl]carbamate).

40. The pharmaceutical composition of claim 36, wherein the epigenetic modulating agent comprises a DNA methyltransferase (DNMT) inhibitor (DNMTi).

41. The pharmaceutical composition of claim 40, wherein the DNMTi comprises azacytidine, decitabine, zebularine, SGI-110, epigallocatechin gallate, MG98, RG108, procainamide, hydralazine, any combination thereof, or any salt, crystal, amorphous structure, hydrate, derivative, metabolite, isomer, or prodrug thereof.

42. The pharmaceutical composition of claim 41, wherein the DNMTi is azacytidine.

43. A pharmaceutical composition comprising:

- (a) an effective amount of an isolated antibody or antigen-binding fragment thereof that specifically binds to semaphorin-4D (SEMA4D), wherein the antibody or fragment thereof comprises the VH amino acid sequence SEQ ID NO: 1 and the VL amino acid sequence SEQ ID NO: 5, and
  - (b) an effective amount of the HDACi Entinostat.
44. A pharmaceutical composition comprising:
- (a) an effective amount of an isolated antibody or antigen-binding fragment thereof that specifically binds to semaphorin-4D (SEMA4D), wherein the antibody or fragment thereof comprises the VH amino acid sequence SEQ ID NO: 1 and the VL amino acid sequence SEQ ID NO: 5, and
  - (b) an effective amount of the DNMTi azacytidine.