DLL3 TARGETING TRISPECIFIC PROTEINS AND METHODS OF USE

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 63/591,718 filed October 19, 2023, and U.S. Provisional Application No. 63,574,506 filed April 04, 2024, the entire contents of which are incorporated by reference herein.

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

[0002] The contents of the electronic sequence listing (26003-WO-SEQLIST-25SEP2024.xml; Size: 1,712,128 bytes; and Date of Creation: September 25, 2024) are herein incorporated by reference in their entirety.

BACKGROUND OF THE INVENTION

[0003] The selective destruction of an individual cell or a specific cell type is often desirable in a variety of clinical settings. For example, it is a primary goal of cancer therapy to specifically destroy tumor cells, while leaving healthy cells and tissues intact and undamaged. One such method is by inducing an immune response against the tumor, to make immune effector cells such as natural killer (NK) cells or cytotoxic T lymphocytes (CTLs) attack and destroy tumor cells.

SUMMARY

[0004] Described herein is a method of treating cancer in a subject in need thereof, the method comprising administration of an effective amount of a DLL3 targeting trispecific protein to the subject, wherein the protein comprises (a) a first domain (A) which specifically binds to human CD3; (b) a second domain (B) which is a half-life extension domain; and (c) a third domain (C) which specifically binds to DLL3, wherein the domains are linked in the order H2N-(A)-(B)-(C)-COOH, or by linkers L1 and L2, and wherein the DLL3 targeting trispecific protein is administered according to a schedule comprising the following steps: (i) administration of a first dose of the DLL3 targeting trispecific protein, (ii) administration of a second dose of the DLL3 targeting trispecific protein, wherein the second dose is higher than the first dose, and (iii) administration of a third dose of the DLL3 targeting trispecific protein, wherein the third dose is higher than the second dose.

[0004a] In an aspect, provided herein is use of a DLL3 targeting trispecific protein in the manufacture of a medicament for treating cancer in a subject, wherein the protein comprises

(a) a first domain (A) which specifically binds to human CD3;

(b) a second domain (B) which is a half-life extension domain; and

(c) a third domain (C) which specifically binds to DLL3,

wherein the domains are linked in the order H_2N-(A)-(B)-(C)-COOH, or by linkers L1 and L2, and wherein the DLL3 targeting trispecific protein is to be administered according to a schedule comprising the following:

(i) administration of a first dose of the DLL3 targeting trispecific protein,

(ii) administration of a second dose of the DLL3 targeting trispecific protein, wherein the second dose is higher than the first dose, and

(iii) administration of a third dose of the DLL3 targeting trispecific protein, wherein the third dose is higher than the second dose.

[0005] In another example, provided herein is a DLL3 targeting trispecific protein for use in treatment of cancer in a subject in need thereof, the treatment comprising administration of an effective amount of a DLL3 targeting trispecific protein to the subject, wherein the protein comprises (a) a first domain (A) which specifically binds to human CD3; (b) a second domain (B) which is a half-life extension domain; and (c) a third domain (C) which specifically binds to

DLL3, wherein the domains are linked in the order H2N-(A)-(B)-(C)-COOH, or by linkers L1 and L2, and wherein the DLL3 targeting trispecific protein is administered according to a schedule comprising the following steps: (i) administration of a first dose of the DLL3 targeting trispecific protein, (ii) administration of a second dose of the DLL3 targeting trispecific protein, wherein the second dose is higher than the first dose, and (iii) administration of a third dose of the DLL3 targeting trispecific protein, wherein the third dose is higher than the second dose.

[0006] In some examples, the first dose is from about 0.015 mg to about 50 mg or from about 0.015 mg to about 3.6 mg. In some examples, the first dose is from about 1 mg to about 20 mg. In some examples, the first dose is from about 1 mg to about 10 mg. In some examples, the first dose is from about 1 mg to about 5 mg. In some examples, the first dose is from about 1 mg to about 3 mg. In some examples, the first dose is from about 0.5 mg to about 3.5 mg, from about 1 mg to about 3 mg, or about 2000 μg. In some examples, the first dose is from about 1.6 mg to about 5.6 mg, from about 2.6 mg to about 4.6 mg, or about 3600 μg. In some examples, the first dose is about 0.015 mg, about 1 mg, about 2 mg, or about 3.6 mg. In some examples, the first dose is about 0.015 mg. In some examples, the first dose is about 1 mg. In some examples, the first dose is about 2 mg. In some examples, the first dose is about 3.6 mg. In some examples, the first dose is administered for from about 1 week to about 36 weeks. In some examples, the first dose is administered for from about 1 week to about 27 weeks. In some examples, the first dose is administered for from about 1 week to about 18 weeks. In some examples, the first dose is administered for from about 1 week to about 9 weeks. In some examples, the first dose is administered once a day. In some examples, the first dose is administered twice a day. In some examples, the first dose is administered three times a day. In some examples, the first dose is administered five times a day. In some examples, the first dose is administered once a week. In some examples, the first dose is administered twice per week. In some examples, the first dose is administered every other week. In some examples, the first dose is administered every three weeks. In some examples, the first dose is administered intravenously, intraperitoneally, subcutaneously, intramuscularly, topically or intradermally. In some examples, the second dose is from about 1 mg to about 100 mg. In some examples, the second dose is from about 1 mg to about 50 mg. In some examples, the second dose is from about 50 mg to about 100 mg. In some examples, the second dose is from about 1 mg to about 13.4 mg, from about 2.2 mg to about 12.2 mg, or about 7.2 mg. In some examples, the second dose is from about 2 mg to about 22 mg, from about 7 mg to about 17 mg, or about 12 mg. In some examples, the second dose is from about 14 mg to about 34 mg, from about 19 mg to about 29 mg, or about 24 mg. In some examples, the second dose is about 3.6 mg,

about 6 mg, about 7.2 mg, about 12 mg, about 24 mg, or about 36 mg. In some examples, the second dose is about 3.6 mg. In some examples, the second dose is about 6 mg. In some examples, the second dose is about 7.2 mg. In some examples, the second dose is about 12 mg. In some examples, the second dose is about 24 mg. In some examples, the second dose is about 36 mg. In some examples, the second dose is administered for from about 1 week to about 36 weeks. In some examples, the second dose is administered for from about 1 week to about 27 weeks. In some examples, the second dose is administered for from about 1 week to about 18 weeks. In some examples, the second dose is administered for from about 1 week to about 9 weeks. In some examples, the second dose is administered once a day. In some examples, the second dose is administered twice a day. In some examples, the second dose is administered three times a day. In some examples, the second dose is administered five times a day. In some examples, the second dose is administered once a week. In some examples, the second dose is administered twice per week. In some examples, the second dose is administered every other week. In some examples, the second dose is administered every three weeks. In some examples, the second dose is maintained to the end of the schedule after the administration of the first dose. In some examples, the second dose is administered intravenously, intraperitoneally, subcutaneously, intramuscularly, topically or intradermally. In some examples, the third dose is from about 1 mg to about 100 mg. the third dose is from about 1 mg to about 50 mg. In some examples, the third dose is from about 50 mg to about 100 mg. In some examples, the third dose is from about 2 mg to about 22 mg, from about 7 mg to about 17 mg, or about 12 mg. In some examples, the third dose is from about 14 mg to about 34 mg, from about 19 mg to about 29 mg, or about 24 mg. In some examples, the third dose is from about 38 mg to about 58 mg, from about 43 mg to about 53 mg, or about 48 mg. In some examples, the third dose is about 24 mg or about 36 mg. In some examples, the third dose is about 24 mg. In some examples, the third dose is about 36 mg. In some examples, the third dose is administered for from about 1 week to about 36 weeks. In some examples, the third dose is administered for from about 1 week to about 27 weeks. In some examples, the third dose is administered for from about 1 week to about 18 weeks. In some examples, the third dose is administered for from about 1 week to about 9 weeks. In some examples, the third dose is administered once a day. In some examples, the third dose is administered twice a day. In some examples, the third dose is administered three times a day. In some examples, the third dose is administered five times a day. In some examples, the third dose is administered once a week. In some examples, the third dose is administered twice per week. In some examples, the third dose is administered every other week. In some examples, the third

dose is administered every three weeks. In some examples, the third dose is maintained to the end of the schedule after the administration of the first dose. In some examples, the third dose is administered intravenously, intraperitoneally, subcutaneously, intramuscularly, topically or intradermally.

[0007] In some examples, the method further comprises administration of an effective amount of atezolizumab to the subject. In some examples, the dose of the atezolizumab is from about 50 mg to about 2500 mg, from about 200 mg to about 2200 mg, from about 400 mg to about 2000 mg, from about 600 mg to about 1900 mg, from about 800 mg to about 1700 mg, from about 1000 mg to about 1500 mg, from about 1100 mg to about 1400 mg, or from about 1200 mg to about 1300 mg. In some examples, the dose of the atezolizumab is from about 1480 mg to about 1880 mg, from about 1580 to about 1780 mg, or about 1680 mg. In some examples, the dose of the atezolizumab is about 1680 mg. In some examples, the atezolizumab is administered for from about 1 week to about 36 weeks. In some examples, the atezolizumab is administered for from about 1 week to about 27 weeks. In some examples, the atezolizumab is administered for from about 1 week to about 18 weeks. In some examples, the atezolizumab is administered for from about 1 week to about 9 weeks. In some examples, the atezolizumab is administered once a week. In some examples, the atezolizumab is administered twice per week. In some examples, the atezolizumab is administered every other week. In some examples, the atezolizumab is administered every three weeks. In some examples, the atezolizumab is administered every four weeks. In some examples, the atezolizumab is administered at a dose of about 1680 mg every four weeks. In some examples, the atezolizumab is administered every five weeks. In some examples, the administration of the atezolizumab begins before the step of the administration of the first dose, at the same time as the step of the administration of the first dose, after the step of the administration of the first dose and before the step of the administration of the second dose, at the same time as the step of the administration of the second dose, after the step of the administration of the second dose and before the step of the administration of the third dose, at the same time as the step of the administration of the third dose, or after the step of the administration of the third dose. In some examples, the administration of the atezolizumab is maintained to the completion of the administration of the first dose, to the completion of the administration of the second dose, or to the completion of the administration of the third dose. In some examples, the atezolizumab is administered intravenously, intraperitoneally, subcutaneously, intramuscularly, topically or intradermally.

[0008] In another example, the present disclosure provides a method of treating cancer in a subject in need thereof, the method comprising administration of an effective amount of a DLL3

targeting trispecific protein to the subject, wherein the protein comprises (a) a first domain (A) which specifically binds to human CD3; (b) a second domain (B) which is a half-life extension domain; and (c) a third domain (C) which specifically binds to DLL3, wherein the domains are linked in the order H2N-(A)-(B)-(C)-COOH, or by linkers L1 and L2; wherein the DLL3 targeting trispecific protein is administered according to a schedule comprising the following steps: (i) administration of a first dose of the DLL3 targeting trispecific protein, and (ii) administration of a second dose of the DLL3 targeting trispecific protein, wherein the second dose is higher than the first dose; and wherein the method further comprises administration of an effective amount of atezolizumab to the subject.

[0009] In another example, the present disclosure provides a DLL3 targeting trispecific protein for use in treatment of cancer in a subject in need thereof, the treatment comprising administration of an effective amount of a DLL3 targeting trispecific protein to the subject, wherein the protein comprises (a) a first domain (A) which specifically binds to human CD3; (b) a second domain (B) which is a half-life extension domain; and (c) a third domain (C) which specifically binds to DLL3, wherein the domains are linked in the order H2N-(A)-(B)-(C)-COOH, or by linkers L1 and L2; wherein the DLL3 targeting trispecific protein is administered according to a schedule comprising the following steps: (i) administration of a first dose of the DLL3 targeting trispecific protein, and (ii) administration of a second dose of the DLL3 targeting trispecific protein, wherein the second dose is higher than the first dose; and wherein the method further comprises administration of atezolizumab to the subject.

[0009a] In another aspect, provided herein is use of a DLL3 targeting trispecific protein in the manufacture of a medicament for treating cancer in a subject, wherein the protein comprises

(a) a first domain (A) which specifically binds to human CD3;

(b) a second domain (B) which is a half-life extension domain; and

(c) a third domain (C) which specifically binds to DLL3,

wherein the domains are linked in the order H_2N-(A)-(B)-(C)-COOH, or by linkers L1 and L2; wherein the DLL3 targeting trispecific protein is to be administered according to a schedule comprising the following:

(i) administration of a first dose of the DLL3 targeting trispecific protein, and

(ii) administration of a second dose of the DLL3 targeting trispecific protein, wherein the second dose is higher than the first dose; and

wherein an effective dose of atezolizumab is to be further administered to the subject.

[0010] In some examples, the first dose is from about 0.015 mg to about 50 mg or from about 0.015 mg to about 3.6 mg. In some examples, the first dose is from about 1 mg to about 20 mg. In some examples, the first dose is from about 1 mg to about 10

mg. In some examples, the first dose is from about 1 mg to about 5 mg. In some examples, the first dose is from about 1 mg to about 3 mg. In some examples, the first dose is from about 0.5 mg to about 3.5 mg, from about 1 mg to about 3 mg, or about 2000 µg. In some examples, the first dose is from about 1.6 mg to about 5.6 mg, from about 2.6 mg to about 4.6 mg, or about 3600 µg. In some examples, the first dose is about 1 mg. In some examples, the first dose is about 2 mg. In some examples, the first dose is administered for from about 1 week to about 36 weeks. In some examples, the first dose is administered for from about 1 week to about 27 weeks. In some examples, the first dose is administered for from about 1 week to about 18 weeks. In some examples, the first dose is administered for from about 1 week to about 9 weeks. In some examples, the first dose is administered once a day. In some examples, the first dose is administered twice a day. In some examples, the first dose is administered three times a day. In some examples, the first dose is administered five times a

day. In some examples, the first dose is administered once a week. In some examples, the first dose is administered twice per week. In some examples, the first dose is administered every other week. In some examples, the first dose is administered every three weeks. In some examples, the first dose is administered intravenously, intraperitoneally, subcutaneously, intramuscularly, topically or intradermally. In some examples, the second dose is from about 1 mg to about 100 mg. In some examples, the second dose is from about 1 mg to about 50 mg. In some examples, the second dose is from about 50 mg to about 100 mg. In some examples, the second dose is from about 1 mg to about 13.4 mg, from about 2.2 mg to about 12.2 mg, or about 7.2 mg. In some examples, the second dose is from about 2 mg to about 22 mg, from about 7 mg to about 17 mg, or about 12 mg. In some examples, the second dose is from about 14 mg to about 34 mg, from about 19 mg to about 29 mg, or about 24 mg. In some examples, the second dose is about 6 mg, about 12 mg, about 24 mg, or about 36 mg. In some examples, the second dose is about 6 mg. In some examples, the second dose is about 12 mg. In some examples, the second dose is about 24 mg. In some examples, the second dose is about 36 mg. In some examples, the second dose is administered for from about 1 week to about 36 weeks. In some examples, the second dose is administered for from about 1 week to about 27 weeks. In some examples, the second dose is administered for from about 1 week to about 18 weeks. In some examples, the second dose is administered for from about 1 week to about 9 weeks. In some examples, the second dose is administered once a day. In some examples, the second dose is administered twice a day. In some examples, the second dose is administered three times a day. In some examples, the second dose is administered five times a day. In some examples, the second dose is administered once a week. In some examples, the second dose is administered twice per week. In some examples, the second dose is administered every other week. In some examples, the second dose is administered every three weeks. In some examples, the second dose is maintained to the end of the schedule after the administration of the first dose. In some examples, the second dose is administered intravenously, intraperitoneally, subcutaneously, intramuscularly, topically or intradermally. In some examples, the dose of the atezolizumab is from about 50 mg to about 2500 mg, from about 200 mg to about 2200 mg, from about 400 mg to about 2000 mg, from about 600 mg to about 1900 mg, from about 800 mg to about 1700 mg, from about 1000 mg to about 1500 mg, from about 1100 mg to about 1400 mg, or from about 1200 mg to about 1300 mg. In some examples, the dose of the atezolizumab is from about 1480 mg to about 1880 mg, from about 1580 to about 1780 mg, or about 1680 mg. In some examples, the dose of the atezolizumab is about 1680 mg. In some examples, the atezolizumab is administered for

from about 1 week to about 36 weeks. In some examples, the atezolizumab is administered for from about 1 week to about 27 weeks. In some examples, the atezolizumab is administered for from about 1 week to about 18 weeks. In some examples, the atezolizumab is administered for from about 1 week to about 9 weeks. In some examples, the atezolizumab is administered once a week. In some examples, the atezolizumab is administered twice per week. In some examples, the atezolizumab is administered every other week. In some examples, the atezolizumab is administered every three weeks. In some examples, the atezolizumab is administered every four weeks. In some examples, the atezolizumab is administered at a dose of about 1680 mg every four weeks. In some examples, the atezolizumab is administered every five weeks. In some examples, the administration of the atezolizumab begins before the step of the administration of the first dose, at the same time as the step of the administration of the first dose, after the step of the administration of the first dose and before the step of the administration of the second dose, at the same time as the step of the administration of the second dose, after the step of the administration of the second dose and before the step of the administration of the third dose, at the same time as the step of the administration of the third dose, or after the step of the administration of the third dose. In some examples, the administration of the atezolizumab is maintained to the completion of the administration of the first dose, to the completion of the administration of the second dose, or to the completion of the administration of the third dose. In some examples, the atezolizumab is administered intravenously, intraperitoneally, subcutaneously, intramuscularly, topically or intradermally.

[0011] In some examples, the DLL3 targeting trispecific protein has an elimination half-time of at least 12 hours, at least 20 hours, at least 25 hours, at least 30 hours, at least 35 hours, at least 40 hours, at least 45 hours, at least 50 hours, or at least 100 hours. In some examples, the third domain comprises a VHH domain. In some examples, the VHH domain is human, humanized, affinity matured, or a combination thereof. In some examples, the third domain comprises one or more sequences selected from the group consisting of SEQ ID NO: 1-442. In some examples, the third domain comprises the amino acid sequence of SEQ ID NO: 432 or an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 432. In some examples, the third domain comprises the amino acid sequence of SEQ ID NO: 432.

[0012] In some examples, the first domain comprises a variable light chain and variable heavy chain each of which is capable of specifically binding to human CD3. In some examples, the first domain comprises the amino acid sequence of SEQ ID NO: 1793 or an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 1793. In some examples, the first domain comprises the amino acid sequence of SEQ ID NO: 1793. In

some examples, the first domain is humanized or human. In some examples, the second domain binds human serum albumin. In some examples, the second domain comprises a scFv, a variable heavy domain (VH), a variable light domain (VL), a peptide, a ligand, or a small molecule. In some examples, the second domain comprises the amino acid sequence of SEQ ID NO: 1774 or an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 1774. In some examples, the second domain comprises the amino acid sequence of SEQ ID NO: 1774. In some examples, linkers L1 and L2 are each independently $(GS)_n$ (SEQ ID NO: 1809), $(GGS)_n$ (SEQ ID NO: 1810), $(GGGS)_n$ (SEQ ID NO: 1811), $(GGSG)_n$ (SEQ ID NO: 1812), $(GGSGG)_n$ (SEQ ID NO: 1813), $(GGGGS)_n$ (SEQ ID NO: 1814), or GGGGSGGGS (SEQ ID NO: 1808), wherein n is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In some examples, linkers L1 and L2 are each independently $(GGGGS)_4$ (SEQ ID NO: 1817), $(GGGGS)_3$ (SEQ ID NO: 1818) or GGGGSGGGS (SEQ ID NO: 1808). In some examples, the domains are linked in the order H_2N-(C)-L1-(B)-L2-(A)-COOH. In some examples, the DLL3 targeting trispecific protein is less than about 80 kDa. In some examples, the DLL3 targeting trispecific protein is from about 50 to about 75 kDa. In some examples, the DLL3 targeting trispecific protein is less than about 60 kDa. In some examples, the DLL3 targeting trispecific protein comprises a sequence of SEQ ID NO: 1890 or 1891. In some examples, the DLL3 targeting trispecific protein comprises a sequence as set forth in SEQ ID NO: 1890. In some examples, the DLL3 targeting trispecific protein comprises a sequence as set forth in SEQ ID NO: 1891.

[0013] In some examples, the cancer is a tumorous disease, an autoimmune disease or an infection disease associated with DLL3. In some examples, the cancer is a neuroendocrine cancer, a prostate cancer, a lung cancer, a stomach cancer, a squamous cell carcinoma, a pancreatic cancer, a cholangiocarcinoma, a triple negative breast cancer or an ovarian cancer. In some examples, the cancer is a small cell lung cancer. In some examples, the cancer is a neuroendocrine prostate cancer. In some examples, the subject has one or more brain metastases (BMs). In some examples, the subject receives a further administration of radiotherapy. In some examples, the subject has a history of brain metastases. In some examples, the subject does not have a history of brain metastases. In some examples, the subject does not have CNS progression after administration of the effective amount of the DLL3 targeting trispecific protein. In some examples, the subject experiences a reduction of brain lesions after administration of the effective amount of the DLL3 targeting trispecific protein.

[0014] In another example, provided herein is a method of treating cancer in a subject in need thereof, the method comprising administration of gocatamig as a monotherapy to the subject at a dose of about 24 mg intravenously every three weeks. In some examples, the cancer is

associated with DLL3 expression. In some examples, the cancer is a neuroendocrine cancer, a prostate cancer, a lung cancer, a stomach cancer, a squamous cell carcinoma, a pancreatic cancer, a cholangiocarcinoma, a triple negative breast cancer or an ovarian cancer. In some examples, the cancer is a small cell lung cancer. In some examples, the cancer is a neuroendocrine prostate cancer.

[0014a] In another aspect, provided herein is use of gocatamig in the manufacture of a medicament for treating cancer in a subject, wherein the gocatamig is to be administered as a monotherapy to the subject at a dose of about 24 mg or 36 mg intravenously every three weeks.

[0014b] In another aspect, provided herein is use of gocatamig in the manufacture of a medicament for treating cancer in a subject, wherein the gocatamig is to be administered as a monotherapy to the subject according to a schedule comprising the following: (i) administration of a first dose of the gocatamig of 1 mg, (ii) administration of a second dose of gocatamig of 12 mg, and (iii) administration of a third dose of the gocatamig of 24 mg, wherein the schedule comprises a first dosing cycle comprising administration of the first dose to the subject on Day 1, administration of the second dose to the subject on Day 8, and administration of the third dose to the subject on Day 15, and wherein the schedule further comprises administration of a target dose to the subject every two weeks after administration of the third dose, wherein the target dose is 24 mg.

[0015] In another example, provided herein is gocatamig for use in a method of treating cancer in a subject in need thereof, the method comprising administration of gocatamig as a monotherapy to the subject at a dose of about 24 mg intravenously every three weeks. In some examples, the cancer is associated with DLL3 expression. In some examples, the cancer is a neuroendocrine cancer, a prostate cancer, a lung cancer, a stomach cancer, a squamous cell carcinoma, a pancreatic cancer, a cholangiocarcinoma, a triple negative breast cancer or an ovarian cancer. In some examples, the cancer is a small cell lung cancer. In some examples, the cancer is a neuroendocrine prostate cancer.

[0016] In another example, provided herein is a method of treating cancer in a subject in need thereof, the method comprising administration of gocatamig as a monotherapy to the subject at a dose of about 36 mg intravenously every three weeks. In some examples, the cancer is associated with DLL3 expression. In some examples, the cancer is a neuroendocrine cancer, a prostate cancer, a lung cancer, a stomach cancer, a squamous cell carcinoma, a pancreatic cancer, a cholangiocarcinoma, a triple negative breast cancer or an ovarian cancer. In some examples, the cancer is a small cell lung cancer. In some examples, the cancer is a neuroendocrine prostate cancer.

[0017] In another example, provided herein is gocatamig for use in a method of treating cancer in a subject in need thereof, the method comprising administration of gocatamig as a monotherapy to the subject at a dose of about 36 mg intravenously every three weeks. In some examples, the cancer is associated with DLL3 expression. In some examples, the cancer is a neuroendocrine cancer, a prostate cancer, a lung cancer, a stomach cancer, a squamous cell carcinoma, a pancreatic cancer, a cholangiocarcinoma, a triple negative breast cancer or an ovarian cancer. In some examples, the cancer is a small cell lung cancer. In some examples, the cancer is a neuroendocrine prostate cancer.

[0018] In some examples, the subject has small cell lung cancer (SCLC) which is relapsed/refractory following at least 1 prior line of systemic therapy that included platinum-based chemotherapy.

[0019] In some examples, the subject has neuroendocrine Prostate Cancer (NEPC; de novo or treatment-emergent) which is relapsed/refractory to standard systemic therapy.

[0020] In some examples, the subject has a high-grade neuroendocrine tumor type other than SCLC and NEPC, with at least one of the following: disease that is relapsed/refractory to standard systemic therapy; disease for which standard therapy does not exist; or disease for which standard therapy is not considered appropriate.

[0021] In some examples, the subject has a histologically or cytologically confirmed malignancy associated with expression of Delta-like Canonical Notch Ligand 3 (DLL3).

[0022] In another example, provided herein is a pharmaceutical unit dose comprising 0.015 mg, 1 mg, 2 mg, 3.6 mg, 6 mg, 7 mg, 7.2 mg, 12 mg, 24 mg, 36 mg, or 48 mg of gocatamig. In some examples, the pharmaceutical unit dose comprises 0.015 mg of gocatamig. In some examples, the pharmaceutical unit dose comprises 1 mg of gocatamig. In some examples, the pharmaceutical unit dose comprises 2 mg of gocatamig. In some examples, the pharmaceutical unit dose comprises 3.6 mg of gocatamig. In some examples, the pharmaceutical unit dose comprises 6 mg of gocatamig. In some examples, the pharmaceutical unit dose comprises 7.2 mg of gocatamig. In some examples, the pharmaceutical unit dose comprises 12 mg of gocatamig. In some examples, the pharmaceutical unit dose comprises 24 mg of gocatamig. In some examples, the pharmaceutical unit dose comprises 36 mg of gocatamig. In some examples, the pharmaceutical unit dose comprises 48 mg of gocatamig.

[0023] In some examples, the unit dose is in a single-dose prefilled vial for intravenous administration.

[0024] In another example, provided herein is the use of any one of the pharmaceutical unit doses disclosed herein in the manufacture of a medicament for treating cancer in a subject in need thereof. In some examples, the cancer is associated with DLL3 expression. In some examples, the cancer is a neuroendocrine cancer, a prostate cancer, a lung cancer, a stomach cancer, a squamous cell carcinoma, a pancreatic cancer, a cholangiocarcinoma, a triple negative breast cancer or an ovarian cancer. In some examples, the cancer is a small cell lung cancer. In some examples, the subject has small cell lung cancer (SCLC) which is relapsed/refractory following at least 1 prior line of systemic therapy that included platinum-based chemotherapy. In some examples, the cancer is a neuroendocrine prostate cancer. In some examples, the subject has neuroendocrine prostate cancer which is relapsed/refractory to standard systemic therapy. In some examples, the subject has a high-grade neuroendocrine tumor type other than SCLC and NEPC, with at least one of the following: disease that is relapsed/refractory to standard systemic therapy; disease for which standard therapy does not exist; or disease for which standard therapy is not considered appropriate. In

some examples, the subject has a histologically or cytologically confirmed malignancy associated with expression of Delta-like Canonical Notch Ligand 3 (DLL3).

INCORPORATION BY REFERENCE

[0025] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0026] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0027] **Fig. 1** illustrates the various domains of an exemplary DLL3 targeting trispecific protein of this disclosure.

[0028] **Fig. 2** illustrates results of a T cell dependent cellular cytotoxicity (TDCC) assay on DMS-153 cells, using exemplary DLL3 targeting trispecific proteins containing DLL3 binding domains of this disclosure, DH18, DH11, DH67, and DH56.

[0029] **Fig. 3** illustrates results of a TDCC assay on DMS-153 cells, using exemplary DLL3 targeting trispecific proteins containing exemplary DLL3 binding domains of this disclosure DH2, DH43, DH10, and DH6.

[0030] **Fig. 4** illustrates results of a TDCC assay on DMS-153 cells, using exemplary DLL3 targeting trispecific proteins containing exemplary DLL3 binding domains of this disclosure DH82, DH23, DH89, and DH17.

[0031] **Fig. 5** illustrates results of a TDCC assay on DMS-153 cells, using exemplary DLL3 targeting trispecific proteins containing exemplary DLL3 binding domains of this disclosure DH83, DH12, DH61, and DH29.

[0032] **Fig. 6** illustrates results of a TDCC assay on DMS-153 cells, using exemplary DLL3 targeting trispecific proteins containing exemplary DLL3 binding domains of this disclosure DH58, and DH70, and a control trispecific protein.

[0033] **Fig. 7** illustrates results of a TDCC assay on DMS-153 cells, using exemplary affinity matured DLL3 targeting trispecific proteins containing exemplary DLL3 targeting domains of this disclosure 1A011, 2E05, 1H012, 2E02, and 1C03.

CLAIMS

WHAT IS CLAIMED IS:

1. Use of a DLL3 targeting trispecific protein in the manufacture of a medicament for treating cancer in a subject, wherein the protein comprises

(a) a first domain (A) which specifically binds to human CD3;

(b) a second domain (B) which is a half-life extension domain; and

(c) a third domain (C) which specifically binds to DLL3,

wherein the domains are linked in the order H_2N-(A)-(B)-(C)-COOH, or by linkers L1 and L2, and wherein the DLL3 targeting trispecific protein is to be administered according to a schedule comprising the following:

(i) administration of a first dose of the DLL3 targeting trispecific protein,

(ii) administration of a second dose of the DLL3 targeting trispecific protein, wherein the second dose is higher than the first dose, and

(iii) administration of a third dose of the DLL3 targeting trispecific protein, wherein the third dose is higher than the second dose.

2. The use of claim 1, wherein:

(i) the first dose is from about 0.015 mg to about 50 mg or from about 0.015 mg to about 3.6 mg;

(ii) the first dose is to be administered for from about 1 week to about 36 weeks;

(iii) the first dose is to be administered once a week, every other week, or every three weeks;

(iv) the first dose is to be administered intravenously, intraperitoneally, subcutaneously, intramuscularly, topically or intradermally;

(v) the second dose is from about 1 mg to about 100 mg;

(vi) the second dose is to be administered for from about 1 week to about 36 weeks;

(vii) the second dose is to be administered once a week, every other week, or every three weeks;

(viii) the second dose is to be maintained to the end of the schedule after the administration of the first dose;

(ix) the second dose is to be administered intravenously, intraperitoneally, subcutaneously, intramuscularly, topically or intradermally;

(x) the third dose is from about 1 mg to about 100 mg;

(xi) the third dose is to be administered for from about 1 week to about 36 weeks;

(xii) the third dose is to be administered once a week, every other week, or every three weeks;

(xiii) the third dose is to be maintained to the end of the schedule after the administration of the first dose; and/or

(xiv) the third dose is to be administered intravenously, intraperitoneally, subcutaneously, intramuscularly, topically or intradermally.

3. The use of claim 1 or 2, wherein:

(i) the first dose is about 0.015 mg, about 1 mg, about 2 mg, or about 3.6 mg;

(ii) the second dose is about 3.6 mg, about 6 mg, about 7.2 mg, about 12 mg, about 24 mg, or about 36 mg; and/or

(iii) the third dose is about 24 mg or about 36 mg.

4. The use of any one of claims 1-3, wherein the first dose is 1 mg, the second dose is 12 mg, and the third dose is 24 mg.

5. The use of any one of claims 1-4, wherein an effective dose of atezolizumab is to be further administered to the subject.

6. The use of claim 5, wherein

(i) the dose of atezolizumab is about 1680 mg;

(ii) the atezolizumab is to be administered for from about 1 week to about 36 weeks;

(iii) the atezolizumab is to be administered every week, every other week, every three weeks, or every four weeks;

(iv) the administration of the atezolizumab begins before the administration of the first dose, at the same time as the administration of the first dose, after the administration of the first dose and before the administration of the second dose, at the same time as the administration of the second dose, after the administration of the second dose and before the administration of the third dose, at the same time as the administration of the third dose, or after the administration of the third dose;

(v) the administration of the atezolizumab is to be maintained to the completion of the administration of the first dose, to the completion of the administration of the second dose, or to the completion of the administration of the third dose; and/or

(vi) the atezolizumab is to be administered intravenously, intraperitoneally, subcutaneously, intramuscularly, topically or intradermally.

7. Use of a DLL3 targeting trispecific protein in the manufacture of a medicament for treating cancer in a subject, wherein the protein comprises

(a) a first domain (A) which specifically binds to human CD3;

(b) a second domain (B) which is a half-life extension domain; and

(c) a third domain (C) which specifically binds to DLL3,

wherein the domains are linked in the order H_2N-(A)-(B)-(C)-COOH, or by linkers L1 and L2;

wherein the DLL3 targeting trispecific protein is to be administered according to a schedule comprising the following:

(i) administration of a first dose of the DLL3 targeting trispecific protein, and

(ii) administration of a second dose of the DLL3 targeting trispecific protein, wherein the second dose is higher than the first dose; and

wherein an effective dose of atezolizumab is to be further administered to the subject.

8. The use of claim 7, wherein:

(i) the first dose is from about 0.015 mg to about 50 mg;

(ii) the first dose is to be administered for from about 1 week to about 36 weeks;

(iii) the first dose is to be administered once a week, every other week, or every three weeks;

(iv) the first dose is to be administered intravenously, intraperitoneally, subcutaneously, intramuscularly, topically or intradermally;

(v) the second dose is from about 1 mg to about 100 mg;

(vi) the second dose is to be administered for from about 1 week to about 36 weeks;

(vii) the second dose is to be administered once a week, every other week, or every three weeks;

(viii) the second dose is to be administered intravenously, intraperitoneally, subcutaneously, intramuscularly, topically or intradermally;

(ix) the dose of the atezolizumab is from about 1480 mg to about 1880 mg, from about 1580 mg to about 1780 mg, or about 1680 mg;

(x) the atezolizumab is to be administered for from about 1 week to about 36 weeks;

(xi) the atezolizumab is to be administered every week, every other week, every three weeks, or every four weeks;

(xii) the administration of the atezolizumab begins before the administration of the first dose, at the same time as the administration of the first dose, after the administration of the first dose and before the administration of the second dose, or at the same time as the administration of the second dose;

(xiii) the administration of the atezolizumab is to be maintained to the completion of the administration of the first dose, or to the completion of the administration of the second dose; and/or

(xiv) the atezolizumab is to be administered intravenously, intraperitoneally, subcutaneously, intramuscularly, topically or intradermally.

9. The use of any one of claims 1-8, wherein:

(i) the DLL3 targeting trispecific protein has an elimination half-time of at least 12 hours, at least 20 hours, at least 25 hours, at least 30 hours, at least 35 hours, at least 40 hours, at least 45 hours, at least 50 hours, or at least 100 hours;

(ii) the third domain comprises a VHH domain;

(iii) the third domain comprises one or more sequences selected from the group consisting of SEQ ID NO: 1-442;

(iv) the first domain comprises a variable light chain and variable heavy chain each of which is capable of specifically binding to human CD3;

(v) the second domain binds human serum albumin;

(vi) linkers L1 and L2 are each, independently, (GS)n (SEQ ID NO: 1809), (GGS)n (SEQ ID NO: 1810), (GGGS)n (SEQ ID NO: 1811), (GGSG)n (SEQ ID NO: 1812), (GGSGG)n (SEQ ID NO: 1813), (GGGGS)n (SEQ ID NO: 1814), or GGGGSGGGS (SEQ ID NO: 1808), wherein n is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10;

(vii) the domains are linked in the order H2N-(C)-L1-(B)-L2-(A)-COOH or H2N-(A)-L1-(B)-L2-(C)-COOH; and/or

(viii) the DLL3 targeting trispecific protein is less than about 80 kDa.

10. The use of any one of claims 1-9, wherein:

(i) the third domain comprises the amino acid sequence of SEQ ID NO: 432;

(ii) the first domain comprises the amino acid sequence of SEQ ID NO: 1793; and/or

(iii) the second domain comprises the amino acid sequence of SEQ ID NO: 1774.

11. The use of any one of claims 1-10, wherein the DLL3 targeting trispecific protein comprises a sequence as set forth in SEQ ID NO: 1890.

12. The use of any one of claims 1-11, wherein:

(i) the cancer is associated with DLL3 expression; and/or

(ii) the cancer is a neuroendocrine cancer, a prostate cancer, a lung cancer, a stomach cancer, a squamous cell carcinoma, a pancreatic cancer, a cholangiocarcinoma, a triple negative breast cancer or an ovarian cancer.

13. The use of any one of claims 1-12, wherein the cancer is a small cell lung cancer.

14. The use of any one of claims 1-12, wherein the cancer is a neuroendocrine prostate cancer.

15. The use of any one of claims 1-14, wherein:

(i) the subject has one or more brain metastases;

(ii) an effective amount of radiotherapy is to be further administered to the subject;

(iii) the subject has a history of brain metastases or the subject does not have a history of brain metastases;

(iv) the subject does not have CNS progression after administration of the effective amount of the DLL3 targeting trispecific protein; and/or

(v) the subject experiences a reduction of brain lesions after administration of the effective amount of the DLL3 targeting trispecific protein.

16. Use of gocatamig in the manufacture of a medicament for treating cancer in a subject, wherein the gocatamig is to be administered as a monotherapy to the subject at a dose of about 24 mg or 36 mg intravenously every three weeks.

17. The use of claim 16, wherein:

(i) the cancer is associated with DLL3 expression;

(ii) the cancer is a neuroendocrine cancer, a prostate cancer, a lung cancer, a stomach cancer, a squamous cell carcinoma, a pancreatic cancer, a cholangiocarcinoma, a triple negative breast cancer or an ovarian cancer;

(iii) the subject has small cell lung cancer (SCLC) which is relapsed/refractory following at least 1 prior line of systemic therapy that included platinum-based chemotherapy;

(iv) the subject has neuroendocrine prostate cancer which is relapsed/refractory to standard systemic therapy;

(v) the subject has a high-grade neuroendocrine tumor type other than SCLC and NEPC, with at least one of the following: disease that is relapsed/refractory to standard systemic therapy; disease for which standard therapy does not exist; or disease for which standard therapy is not considered appropriate; or

(vi) the subject has a histologically or cytologically confirmed malignancy associated with expression of Delta-like Canonical Notch Ligand 3 (DLL3).

18. The use of any one of claims 1-17, wherein the medicament is formulated as a pharmaceutical unit dose comprising 0.015 mg, 1 mg, 2 mg, 3.6 mg, 6 mg, 7 mg, 7.2 mg, 12 mg, 24 mg, 36 mg, or 48 mg of gocatamig.

19. The use of claim 18, wherein the unit dose is in a single-dose prefilled vial for intravenous administration.

20. The use of claim 4, wherein the schedule comprises a first dosing cycle comprising administration of the first dose to the subject on Day 1, administration of the second dose to the subject on Day 8, and administration of the third dose to the subject on Day 15.

21. The use of claim 20, wherein the schedule further comprises administration of a target dose to the subject every two weeks after administration of the third dose, wherein the target dose is equal to the third dose.

22. Use of gocatamig in the manufacture of a medicament for treating cancer in a subject, wherein the gocatamig is to be administered as a monotherapy to the subject according to a schedule comprising the following: (i) administration of a first dose of the gocatamig of 1 mg, (ii) administration of a second dose of gocatamig of 12 mg, and (iii) administration of a third dose of the gocatamig of 24 mg, wherein the schedule comprises a first dosing cycle comprising administration of the first dose to the subject on Day 1, administration of the second dose to the subject on Day 8, and administration of the third dose to the subject on Day 15, and wherein the schedule further comprises administration of a target dose to the subject every two weeks after administration of the third dose, wherein the target dose is 24 mg.