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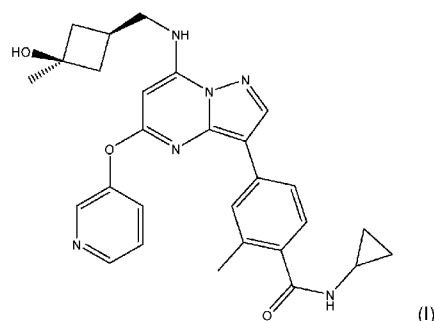
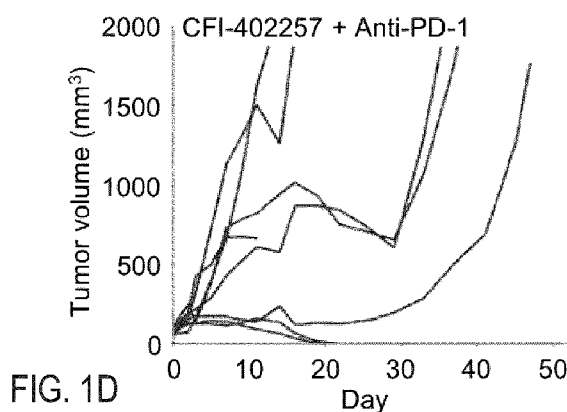
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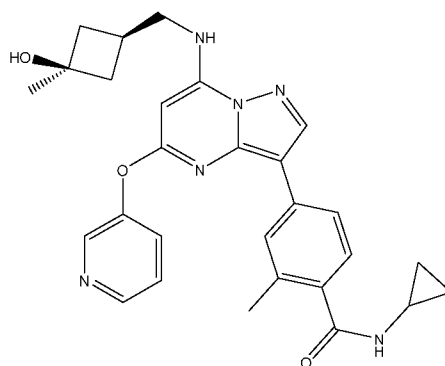
(57) Abstract: Provided herein are methods of treating cancer using an effective amount of a compound represented by the formula: (I) or a pharmaceutically acceptable salt thereof and an effective amount of an immune checkpoint inhibitor. Also provided are compositions comprising the same compound represented by the formula shown above or a pharmaceutically acceptable salt thereof and an immune checkpoint inhibitor.

COMBINATION THERAPIES FOR INHIBITION OF TTK PROTEIN KINASE**CROSS REFERENCE TO A RELATED APPLICATION**

[0001] This reference claims priority to International Patent Application No. PCT/CA2017/050920, filed August 1, 2017, which is incorporated herein by reference in its entirety.

BACKGROUND

[0002] CFI-402257 is a compound represented by the formula:



that inhibits TTK protein kinase (also known as Monopolar spindle 1 or Mps1) activity. TTK is a core protein of the spindle assembly checkpoint (SAC), which is a signaling cascade that prevents chromosome missegregation by arresting the cell cycle in mitosis until all chromosomes are properly attached to the mitotic spindle. Thus, the SAC ensures healthy proliferation and precise division in cells. Many cancer cells have a weakened SAC response that may contribute to malignancy. However, more severe disabling of the SAC generates a level of chromosome instability that exceeds the adaption capacity of cancer cells, and is a potential anticancer strategy. Many tumors are shown to over-express TTK, which correlates with worse prognosis. Inhibition of TTK activity in cancer cells causes severe chromosome missegregation, cell death and inhibited tumor growth.

[0003] CFI-402257 is presently undergoing a phase I clinical trial (ClinicalTrials.gov ID NCT02792465) with patients having advanced solid cancers. Given the potency and selectivity of CFI-402257 in inhibiting a critical mitotic checkpoint protein, it would be advantageous to further enhance the efficacy of this drug candidate in cancer treatment.

SUMMARY

[0004] It has now been found by the inventors of the present application that the administration of the compound CFI-402257 or a pharmaceutically acceptable salt thereof and an immune checkpoint inhibitor synergistically treats cancer. *See e.g.*, FIGS. 1D and 2D, which both illustrate complete tumor regression in the syngeneic CT26 mouse colon carcinoma model upon administration of a combination of the compound CFI-402257 or a pharmaceutically acceptable salt thereof and the rat IgG2a anti-PD-1 antibody. Importantly, when the animals in which complete regression have occurred are re-challenged by inoculation with the same cancer cells, tumors did not grow in any of these animals, thereby indicating that immunity to the cancer cells has been generated by the CFI-402257-anti-PD-1-antibody combination therapy. Furthermore, animals that are subjected to this combination therapy do not suffer from any significant body weight loss, thereby indicating that both agents at at least the administered dosages are well-tolerated.

[0005] Based on these results, provided herein are methods of treating cancer in a subject, by administering to the subject an effective amount of the compound CFI-402257 or a pharmaceutically acceptable salt thereof and an effective amount of an immune checkpoint inhibitor as described herein.

[0006] Also provided herein are pharmaceutical compositions comprising the compound CFI-402257 or a pharmaceutically acceptable thereof and an immune checkpoint inhibitor as described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0007] FIG. 1A illustrates change in CT26 tumor volume in Balb/cJ mice that receive rat IgG2a PD-1 antibody (control).

[0008] FIG. 1B illustrates change in CT26 tumor volume in Balb/cJ mice that receive 150 µg of rat IgG2a anti-PD-1 antibody on Days 0, 3, 6 and 10.

[0009] FIG. 1C illustrates change in CT26 tumor volume in Balb/cJ mice that receive 6 mg/kg of CFI-402257 daily for 21 days.

[0010] FIG. 1D illustrates change in CT26 tumor volume in Balb/cJ mice that receive the combination of 150 µg of rat IgG2a anti-PD-1 antibody on Days 0, 3, 6 and 10 and 6 mg/kg of CFI-402257 daily for 21 days.

[0011] FIG. 2A illustrates change in CT26 tumor volume in Balb/cJ mice that receive rat IgG2a PD-1 antibody (control) in a duplicate experiment.

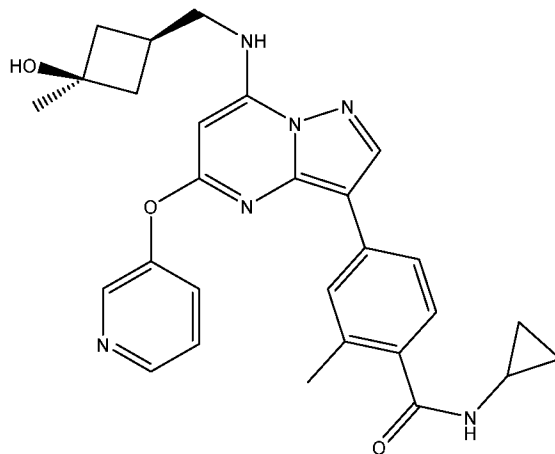
[0012] FIG. 2B illustrates change in CT26 tumor volume in Balb/cJ mice that receive 150 µg of rat IgG2a anti-PD-1 antibody on Days 0, 3, 6 and 10 in a duplicate experiment.

[0013] FIG. 2C illustrates change in CT26 tumor volume in Balb/cJ mice that receive 6 mg/kg of CFI-402257 daily for 21 days in a duplicate experiment.

[0014] FIG. 2D illustrates change in CT26 tumor volume in Balb/cJ mice that receive the combination of 150 µg of rat IgG2a anti-PD-1 antibody on Days 0, 3, 6 and 10 and 6 mg/kg of CFI-402257 daily for 21 days in a duplicate experiment.

DETAILED DESCRIPTION

[0015] In one aspect, the present disclosure provides a method of treating cancer in a subject, comprising the step of administering to the subject an effective amount of the compound CFI-402257 that is represented by the formula:



or a pharmaceutically acceptable salt thereof and an effective amount of an immune checkpoint inhibitor.

[0016] It will be understood that unless otherwise indicated, the administrations described herein include administering the described compound CFI-402257 or a pharmaceutically acceptable salt thereof prior to, concurrently with, or after administration of the immune checkpoint inhibitor described herein. Thus, simultaneous administration is not necessary for therapeutic purposes. In one embodiment, however, the compound CFI-402257 or a pharmaceutically acceptable salt thereof is administered concurrently with the immune checkpoint inhibitor.

[0017] The compound CFI-402257 described herein has basic amine groups and therefore can form pharmaceutically acceptable salts with pharmaceutically acceptable acid(s). Accordingly, the term “pharmaceutically acceptable salt” as used herein refers to any suitable

pharmaceutically acceptable acid addition salt of the compound CFI-402257 described herein, which includes but is not limited to salts of inorganic acids (e.g., hydrochloric acid, hydrobromic, phosphoric, nitric, and sulfuric acids) and of organic acids (such as, acetic acid, benzenesulfonic, benzoic, methanesulfonic, and p-toluenesulfonic acids). Other examples of such salts include hydrochlorides, hydrobromides, sulfates, methanesulfonates, nitrates, benzoates, fumarates, maleates, bisphosphate hemihydrates and salts with amino acids such as glutamic acid.

[0018] As used herein, an “immune check point inhibitor” or simply a “checkpoint inhibitor” refers to any compound that, either directly or indirectly, decreases the level of or inhibits the function of an immune checkpoint receptor protein found on the surface of an immune cell (e.g., T-cells, B-cells, etc.). Alternatively, the immune checkpoint inhibitor is a compound that, either directly or indirectly, decreases the level of or inhibits the function of a ligand on the surface of an immune cell-inhibitory cell (e.g., regulatory T-cells, tolerogenic antigen presenting cells (APC), myeloid-derived suppressor cells (MDSC), tumor-associated macrophages (TAM), cancer-associated fibroblasts (CAF), other cancer cells and APCs, etc.), or secreted by an immune cell-inhibitory cell. This ligand is typically capable of binding the immune checkpoint receptor protein of the immune cell. A non-limiting example of an immune checkpoint receptor protein-ligand pair is PD-1/PD-L1. PD-1 is an immune checkpoint receptor protein found on T-cells. PD-L1 that is often over-expressed by cancer cells binds to PD-1 and helps the cancer cells evade the host immune system attack. Accordingly, an immune checkpoint inhibitor prevents or reverses this PD-1/PD-L1 binding, by either blocking the PD-1 on the T-cells (i.e., a PD-1 inhibitor) or the PD-L1 on the cancer cells (i.e., a PD-L1 inhibitor), thereby maintaining or restoring anti-tumor T-cell activity or blocking T-cell-inhibitory cell activity. Additionally, an immune checkpoint inhibitor refers to a compound as described in US Patent Application Publication Nos. US 2017/0190675 and US 2016/0185870, and International Patent Application Publication Nos. WO 2015/112900, WO 2010/027828 and WO 2010/036959.

[0019] An immune checkpoint inhibitor in accordance with the present invention may be a small-molecule organic compound or a large molecule such as a peptide or a nucleic acid. In at least one embodiment, an immune checkpoint inhibitor is an antibody or an antigen binding fragment thereof. In at least one embodiment, an immune checkpoint inhibitor is a monoclonal antibody or an antigen binding fragment thereof.

[0020] As used herein, the term “antibody” means an immunoglobulin molecule that recognizes and specifically binds to a target, such as a protein, polypeptide, peptide, carbohydrate, polynucleotide, lipid, or combinations of the foregoing through at least one antigen recognition site within the variable region of the immunoglobulin molecule. As used herein, the term “antibody” encompasses intact polyclonal antibodies, intact monoclonal antibodies, antibody fragments (e.g., Fab, Fab', F(ab')₂, and Fv fragments), single chain Fv (scFv) mutants, multispecific antibodies such as bispecific antibodies, chimeric antibodies, humanized antibodies, human antibodies, fusion proteins comprising an antigen determination portion of an antibody, and any other modified immunoglobulin molecule comprising an antigen recognition site so long as the antibodies exhibit the desired biological activity. An antibody can be of any of the five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, or subclasses (isotypes) thereof (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2), based on the identity of their heavy-chain constant domains referred to as alpha, delta, epsilon, gamma, and mu, respectively. The different classes of immunoglobulins have different and well known subunit structures and three-dimensional configurations.

Antibodies can be naked or conjugated to other molecules such as toxins, radioisotopes, etc

[0021] In some embodiments, an antibody is a non-naturally occurring antibody. In some embodiments, an antibody is purified from natural components. In some embodiments, an antibody is recombinantly produced. In some embodiments, an antibody is produced by a hybridoma.

[0022] The term “antibody fragment” refers to a portion of an intact antibody and refers to the antigenic determining variable regions of an intact antibody. Examples of antibody fragments include, but are not limited to, Fab, Fab', F(ab')₂, and Fv fragments, linear antibodies, single chain antibodies, and multispecific antibodies formed from antibody fragments. The term “antigen-binding fragment” of an antibody includes one or more fragments of an antibody that retain the ability to specifically bind to an antigen. It has been shown that the antigen-binding function of an antibody can be performed by certain fragments of a full-length antibody. Examples of binding fragments encompassed within the term “antigen-binding fragment” of an antibody include (without limitation): (i) an Fab fragment, a monovalent fragment consisting of the V_L, V_H, C_L, and C_{H1} domains (e.g., an antibody digested by papain yields three fragments: two antigen-binding Fab fragments, and one Fc fragment that does not bind antigen); (ii) a F(ab')₂ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region (e.g., an

antibody digested by pepsin yields two fragments: a bivalent antigen-binding $F(ab')_2$ fragment, and a pFc' fragment that does not bind antigen) and its related $F(ab')$ monovalent unit; (iii) a F_d fragment consisting of the V_H and C_{H1} domains (*i.e.*, that portion of the heavy chain which is included in the Fab); (iv) a F_v fragment consisting of the V_L and V_H domains of a single arm of an antibody, and the related disulfide linked F_v ; (v) a dAb (domain antibody) or sdAb (single domain antibody) fragment (Ward et al., *Nature* 341:544-546, 1989), which consists of a V_H domain; and (vi) an isolated complementarity determining region (CDR).

[0023] As used herein, a “monoclonal antibody” refers to a homogeneous antibody population involved in the highly specific recognition and binding of a single antigenic determinant, or epitope. This is in contrast to polyclonal antibodies that typically include different antibodies directed against different antigenic determinants. The term “monoclonal antibody” encompasses both intact and full-length monoclonal antibodies as well as antibody fragments (such as Fab, Fab', $F(ab')_2$, F_v), single chain (scFv) mutants, fusion proteins comprising an antibody portion, and any other modified immunoglobulin molecule comprising an antigen recognition site. Furthermore, “monoclonal antibody” refers to such antibodies made in any number of manners including but not limited to by hybridoma, phage selection, recombinant expression, and transgenic animals.

[0024] The term “humanized antibody” refers to forms of non-human (e.g., murine) antibodies that are specific immunoglobulin chains, chimeric immunoglobulins, or fragments thereof that contain minimal non-human (e.g., murine) sequences. Typically, humanized antibodies are human immunoglobulins in which residues from the complementary determining region (CDR) are replaced by residues from the CDR of a non-human species (e.g., mouse, rat, rabbit, hamster) that have the desired specificity, affinity, and capability (Jones et al., *Nature* 321:522-525, 1986; Riechmann et al., *Nature* 332:323-327, 1988; Verhoeyen et al., *Science* 239:1534-1536, 1988).

[0025] A non-exhaustive list of examples of an immune checkpoint inhibitor is provided as following: a CD40L inhibitor, a DR3 inhibitor, a TL1A inhibitor, a GITR inhibitor, a GITRL inhibitor, a 4-1BB inhibitor, a 4-1BBL inhibitor, an OX40 inhibitor, an OX40L inhibitor, a CD27 inhibitor, a CD70 inhibitor, a TMIGD2 inhibitor, an HHLA2 inhibitor, an ICOS inhibitor, an ICOSL inhibitor, a B7RP1 inhibitor, a CD28 inhibitor, a PD-1 inhibitor, a PD-L1 inhibitor, a PD-L2 inhibitor, a CTLA-4 inhibitor, a CD80 inhibitor, a CD86 inhibitor, a KIR inhibitor, a TCR inhibitor, a LAG3 inhibitor, an MHCI inhibitor, an MHCII inhibitor,

a CD80 inhibitor, a TIM-3 inhibitor, a GAL9 inhibitor, a BTLA inhibitor, an HVEM inhibitor, a CD160 inhibitor, a CD137 inhibitor, a CD137L inhibitor, a LIGHT inhibitor, a phosphatidylserine inhibitor, a VISTA inhibitor, a BTNL2 inhibitor, a B7-H3 inhibitor and a B7-H4 inhibitor. In certain embodiments, the immune checkpoint inhibitor applied in a cancer treatment method of the invention is one or more selected from the aforementioned examples.

[0026] In one embodiment, the immune checkpoint inhibitor is one or more selected from a PD-1 inhibitor, a PD-L1 inhibitor and a CTLA-4 inhibitor.

[0027] In one embodiment, the immune checkpoint inhibitor is a PD-1 inhibitor. In another embodiment, the immune checkpoint inhibitor is a PD-L1 inhibitor.

[0028] In some embodiments, the immune checkpoint inhibitor is one or more selected from pembrolizumab, ipilimumab, nivolumab, atezolizumab, avelumab and durvalumab.

[0029] In some embodiments, the immune checkpoint inhibitor is one or more selected from JS001, SHR-1210, BGB-A317, IBI-308, REGN2810, JS003, SHR-1316, KN035, BMS-936559, LAG525, BMS-986016, MBG453, MEDI-570, OREG-103/BY40 and lirilumab. In one embodiment, the immune checkpoint inhibitor is one or more selected from CJS001, SHR-1210, BGB-A317, IBI-308 and REGN2810. In an alternative embodiment, the immune checkpoint inhibitor is one or more selected from JS003, SHR-1316, KN035 and BMS-936559.

[0030] As used herein, the terms “treatment,” “treat,” and “treating” refer to reversing, alleviating, ameliorating, inhibiting or slowing the progression of a cancer, reducing the likelihood of recurrence of a cancer, or one or more symptoms thereof, as described herein. Exemplary types of cancer treated by the methods and compositions of the invention include but are not limited to breast cancer (including metastatic breast cancer); colon cancer; rectal cancer; colorectal cancer; lung cancer (including small-cell lung cancer (SCLC), non-small cell lung cancer (NSCLC), adenocarcinoma of the lung, and squamous carcinoma of the lung); cancer of the peritoneum; gastric or stomach cancer; gastrointestinal cancer; cervical cancer; liver cancer; bladder cancer; hepatoma; ovarian cancer; endometrial or uterine cancer; prostate cancer; testicular cancer; leukemias; lymphomas; hematological malignancies; brain cancer (including glioma, glioblastoma multiforme, medulloblastoma, and neuroblastoma); head and neck cancer; pancreatic cancer; melanoma; hepatocellular cancer; kidney or renal cancer; vulval cancer; thyroid cancer; hepatic carcinoma; anal carcinoma; penile carcinoma; Merkel cell cancer; mycoses fungoids; esophageal cancer; tumors of the biliary tract; salivary

gland cancer; sarcomas; retinoblastoma; liposarcoma, synovial cell sarcoma; neuroendocrine tumors; gastrinoma; islet cell cancer; mesothelioma; schwannoma; acoustic neuroma; meningioma; adenocarcinoma; squamous cell cancer and epithelial squamous cell cancer. In another embodiment, the cancer treated by the methods and compositions of the invention is pancreatic cancer, lung cancer, breast cancer, colon cancer, brain cancer, neuroblastoma, prostate cancer, melanoma, glioblastoma multiforme, ovarian cancer, lymphoma, leukemia, melanoma, sarcoma, paraneoplasia, osteosarcoma, germinoma, glioma or mesothelioma. In yet another embodiment, the cancer is renal cell carcinoma, non-small cell lung cancer, urothelial cancer, head and neck cancer, ovarian cancer, lymphoma, melanoma, pancreatic cancer, myeloma, acute myeloid leukemia, bladder cancer and Hodgkin's lymphoma.

[0031] The term "an effective amount" means an amount when administered to a subject which results in beneficial or desired results, including clinical results, i.e., reversing, alleviating, inhibiting or slowing the progression of a cancer, reducing the likelihood of recurrence of a cancer, or one or more symptoms thereof, e.g., as determined by clinical symptoms, the amount or volume of cancer cells or tumors in a subject compared to a control.

[0032] In an embodiment, an effective amount of the compound CFI-402257 or a pharmaceutically acceptable salt thereof taught herein ranges from about 0.1 to about 1000 mg/kg body weight, alternatively about 1 to about 500 mg/kg body weight, and in another alternative, from about 1 to about 100 mg/kg body weight, and in yet another alternative, from about 1 to about 50 mg/kg. In an embodiment, an effective amount of an immune checkpoint inhibitor taught herein ranges from about 0.01 to about 1000 µg per subject, alternatively from about 0.05 to about 500 µg per subject. The skilled artisan will appreciate that certain factors may influence the dosage required to effectively treat a subject suffering from cancer or reduce the likelihood of recurrence of a cancer. These factors include, but are not limited to, the classification and/or severity of the disease or disorder, previous treatments, the general health and/or age of the subject and other diseases present.

[0033] In another aspect, pharmaceutical compositions comprising the compound CFI-402257 or a pharmaceutically acceptable salt thereof and an immune checkpoint inhibitor are also included in the present disclosure.

[0034] Also included are the use of the compound CFI-402257 or a pharmaceutically acceptable salt thereof in the manufacture of a medicament to be used in combination with an immune checkpoint inhibitor as described herein for the treatment of one or more cancers described herein. Also included herein are pharmaceutical compositions comprising the

compound CFI-402257 or a pharmaceutically acceptable salt thereof and an immune checkpoint inhibitor as described herein optionally together with a pharmaceutically acceptable carrier, in the manufacture of a medicament for the treatment of one or more cancers described herein. Also included is the compound CFI-402257 for use in combination with an immune checkpoint inhibitor as described herein for the treatment of a subject with cancer. Further included are pharmaceutical compositions comprising the compound CFI-402257 or a pharmaceutically acceptable salt thereof and an immune checkpoint inhibitor as described herein, optionally together with a pharmaceutically acceptable carrier, for use in the treatment of one or more cancers described herein. Further included are pharmaceutical compositions comprising the compound CFI-402257 or a pharmaceutically acceptable salt thereof and an immune checkpoint inhibitor as described herein optionally together with a pharmaceutically acceptable carrier for use in the treatment of one or more cancers described herein.

[0035] The term “pharmaceutically acceptable carrier” refers to a non-toxic carrier, diluent, adjuvant, vehicle or excipient that does not adversely affect the pharmacological activity of the compound with which it is formulated, and which is also safe for human use. Pharmaceutically acceptable carriers that may be used in the compositions of this disclosure include, but are not limited to, ion exchangers, alumina, aluminum stearate, magnesium 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 (e.g., microcrystalline cellulose, hydroxypropyl methylcellulose, lactose monohydrate, sodium lauryl sulfate, and crosscarmellose sodium), polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat.

[0036] Other excipients, such as flavoring agents; sweeteners; and preservatives, such as methyl, ethyl, propyl and butyl parabens, can also be included. More complete listings of suitable excipients can be found in the Handbook of Pharmaceutical Excipients (5th Ed., a Pharmaceutical Press (2005)). A person skilled in the art would know how to prepare formulations suitable for various types of administration routes. Conventional procedures and ingredients for the selection and preparation of suitable formulations are described, for

example, in Remington's Pharmaceutical Sciences (2003, 20th edition) and in The United States Pharmacopeia: The National Formulary (USP 24 NF19) published in 1999.

[0037] The compound CFI-402257 or a pharmaceutically acceptable salt thereof and the immune checkpoint inhibitor, or the compositions of the present teachings may be administered, for example, by oral, parenteral, sublingual, topical, rectal, nasal, buccal, vaginal, transdermal, patch, pump administration or via an implanted reservoir, and the pharmaceutical compositions would be formulated accordingly. Parenteral administration includes intravenous, intraperitoneal, subcutaneous, intramuscular, transepithelial, nasal, intrapulmonary, intrathecal, rectal and topical modes of administration. Parenteral administration can be by continuous infusion over a selected period of time.

[0038] Other forms of administration included in this disclosure are as described in WO 2013/075083, WO 2013/075084, WO 2013/078320, WO 2013/120104, WO 2014/124418, WO 2014/151142, and WO 2015/023915, the contents of which are incorporated herein by reference.

EXEMPLIFICATION

[0039] While have described a number of embodiments of this, it is apparent that our basic examples may be altered to provide other embodiments that utilize the compounds and methods of this disclosure. Therefore, it will be appreciated that the scope of this disclosure is to be defined by the appended claims rather than by the specific embodiments that have been represented by way of example.

[0040] The contents of all references (including literature references, issued patents, published patent applications, and co-pending patent applications) cited throughout this application are hereby expressly incorporated herein in their entireties by reference. Unless otherwise defined, all technical and scientific terms used herein are accorded the meaning commonly known to one with ordinary skill in the art.

Example 1

Materials

[0041] Salt forms of the compound CFI-402257 were prepared using the one or more of the procedures described in US Patent No. 9,657,025, International Publication Application Nos. WO 2014/075168 and WO 2015/070349, and Liu et al. (2016) *ACS Med Chem Lett* 7(7):671-675. The hydrochloride or bisphosphate hemihydrate salt forms of the compound CFI-402257 were used in all of the studies described herein.

[0042] Rat IgG2a anti-PD-1 antibody (clone RMP1-14; cat. no. BE0146) and rat IgG2a isotype control (clone 2A3; cat. no. BE0089) were obtained from Bio X Cell.

[0043] BALB/cJ mice were obtained from The Jackson Laboratory. Six- to eight-week-old female animals were used for all of the studies described herein and were allowed unrestricted access to food and water. All animal procedures were approved by the institutional animal care and use committee of the University Health Network (Toronto).

[0044] The CT26 mouse colon carcinoma cell line was obtained from American Type Culture Collection (ATCC) and maintained according to the supplier's instructions. Short tandem repeat (STR) profiling was used to verify authenticity of the cell lines. Sixteen STR loci were simultaneously amplified in multiplex PCR at The Centre for Applied Genomics (Toronto), and the ATCC database was used for comparison when possible. Cell lines were routinely tested for mycoplasma and used at low passage numbers (<15).

Example 2

Methods

[0045] BALB/cJ mice were inoculated subcutaneously with 1×10^6 CT26 cells. The mice were then randomized. Animal weights were monitored daily, and tumor volume was measured three times per week.

[0046] Tumor volume (in cubic millimeters or mm^3) was defined as $100 \times [1 - (TV_{f, \text{treated}} - TV_{i, \text{treated}}) / (TV_{f, \text{control}} - TV_{i, \text{control}})]$, where TV_f is the average tumor volume at the end of study and TV_i is the average tumor volume at the end initiation of treatment. In cases in which tumor regression occurred, percentage of tumor regression was defined as $100 \times [1 - (TV_{f, \text{treated}} / TV_{i, \text{treated}})]$. At the completion of each study, the mice were killed by an anesthetic overdose, and tumor tissue was removed for further analysis.

[0047] Treatments were initiated when tumor volumes reached an average size of $\sim 60 \text{ mm}^3$. To treat an established tumor, the animals were first assigned into groups, i.e., the control group receiving rat IgG2a PD-1 antibody, the group receiving the compound CFI-402257 monotherapy, the group receiving the rat IgG2a anti-PD-1 antibody monotherapy and the group receiving the combination therapy. The compound CFI-402257 and the vehicle [10% (v/v) NMP/40% (v/v) PEG-300/50% (v/v) water for the hydrochloride form; or 80-90% (v/v) PEG-300 or PEG-400 20-10% (v/v) water for the bisphosphate hemihydrate form] were administered by oral gavage (PO), at 6 mg/kg daily (QD) for 21 days. The anti-PD-1 antibody or the isotype control were administered by intraperitoneal (IP) injection. 150 μg

anti-PD-1 antibody was administered in four doses, i.e., on Days 0, 3, 6 and 10. These treatment studies were carried out in duplicates.

Example 3

Complete regression in combination CFI-402257 and anti-PD-1-antibody-treated tumors

[0048] The size of each individual tumor within each treatment arm is plotted (*See* FIGS. 1A-1D and 2A-2D). As can be seen in FIGS. 1A and 2A, tumors in the vehicle-treated control arm grew rapidly, and the average tumor was $>1500 \text{ mm}^3$ by Day 11 of treatment.

[0049] As shown in FIGS. 1B and 2B, there was tumor growth delay in the anti-PD-1-antibody-treated single agent arms. Similarly, the CFI-402257- treated single agent arm in FIGS. 1C and 2C indicate tumor growth delay and that the administered dosage was well-tolerated. Although there was tumor growth inhibition in both monotherapeutic arms, there were no instances in which complete regression was observed.

[0050] Surprisingly and notably, in the combination anti-PD-1antibody and CFI-402257-treated arm of FIG. 1D, two of the eight tumors completely regressed. Very similar results were achieved in the duplicate experiment of FIG. 2D, where complete regression was again observed in two of eight tumors.

Example 4

CFI-402257 and anti-PD-1-antibody generates tumor immunity

[0051] Furthermore, in the first experiment, the two animals in which complete regression had occurred were re-challenged by inoculation with CT26 cells on Day 31. Tumors did not grow in either mouse, indicating that immunity to the CT26 cells had been generated.

Example 5

Animal body weight monitoring

[0052] In the first experiment, none of the animals receiving the CFI-402257 or anti-PD-1-antibody single agent treatment or the combination treatment recorded any significant body weight loss. Limited body weight loss ($\leq 7.5\%$) was observed only in animals treated with only CFI-402257 in the second experiment.

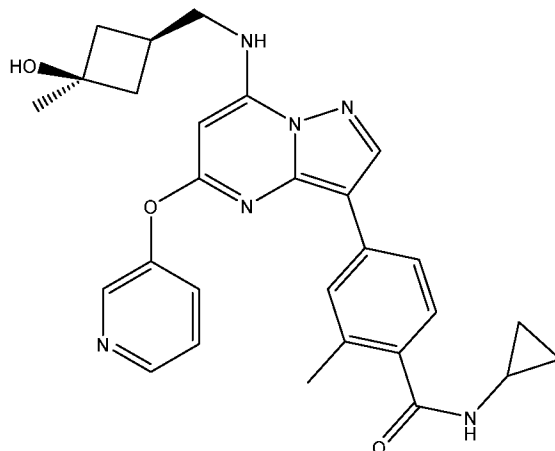
[0053] While the applicants have described a number of embodiments of this invention, it is apparent that these basic examples may be altered to provide other embodiments that utilize the compounds and methods of this invention. Therefore, it will be appreciated that the scope of this invention is to be defined by the appended claims rather than by the specific embodiments that have been represented by way of example.

CLAIMS

What is claimed is:

1. A method for treating cancer, comprising:

administering to a subject an effective amount of a compound represented by the formula:



or a pharmaceutically acceptable salt thereof and an effective amount of an immune checkpoint inhibitor.

2. The method according to claim 1, wherein the immune checkpoint inhibitor is an antibody or an antigen binding fragment thereof.

3. The method according to any one of claims 1 and 2, wherein the immune checkpoint inhibitor is a monoclonal antibody or an antigen binding fragment thereof.

4. The method according to any one of claims 1-3, wherein the immune checkpoint inhibitor is one or more selected from a CD40L inhibitor, a DR3 inhibitor, a TL1A inhibitor, a GITR inhibitor, a GITRL inhibitor, a 4-1BB inhibitor, a 4-1BBL inhibitor, an OX40 inhibitor, an OX40L inhibitor, a CD27 inhibitor, a CD70 inhibitor, a TMIGD2 inhibitor, an HHLA2 inhibitor, an ICOS inhibitor, an ICOSL inhibitor, a B7RP1 inhibitor, a CD28 inhibitor, a PD-1 inhibitor, a PD-L1 inhibitor, a PD-L2 inhibitor, a CTLA-4 inhibitor, a CD80 inhibitor, a CD86 inhibitor, a KIR inhibitor, a TCR inhibitor, a LAG3 inhibitor, an MHCI inhibitor, an MHCII inhibitor, a CD80 inhibitor, a TIM-3 inhibitor, a GAL9 inhibitor, a BTLA inhibitor, an HVEM inhibitor, a CD160 inhibitor, a CD137 inhibitor, a CD137L inhibitor, a LIGHT inhibitor, a

phosphatidylserine inhibitor, a VISTA inhibitor, a BTNL2 inhibitor, a B7-H3 inhibitor and a B7-H4 inhibitor.

5. The method according to any one of claims 1-4, wherein the checkpoint inhibitor is one or more selected from a PD-1 inhibitor, a PD-L1 inhibitor and a CTLA-4 inhibitor.

6. The method according to any one of claims 1-5, wherein the immune checkpoint inhibitor is a PD-1 inhibitor.

7. The method according to any one of claims 1-5, wherein the immune checkpoint inhibitor is a PD-L1 inhibitor.

8. The method according to any one of claims 1-6, wherein the immune checkpoint inhibitor restores anti-tumor T-cell activity.

9. The method according to any one of claims 1-5 and 7, wherein the immune checkpoint inhibitor blocks T-cell-inhibitory cell activity.

10. The method according to any one of claims 1-5, wherein the immune checkpoint inhibitor is one or more selected from pembrolizumab, ipilimumab, nivolumab, atezolizumab, avelumab and durvalumab.

11. The method according to any one of claims 1-4, wherein the immune checkpoint inhibitor is one or more selected from JS001, SHR-1210, BGB-A317, IBI-308, REGN2810, JS003, SHR-1316, KN035, BMS-936559, LAG525, BMS-986016, MBG453, MEDI-570, OREG-103/BY40 and lirilumab.

12. The method according to claims 1-6, 8 and 11, wherein the immune checkpoint inhibitor is one or more selected from CJS001, SHR-1210, BGB-A317, IBI-308 and REGN2810.

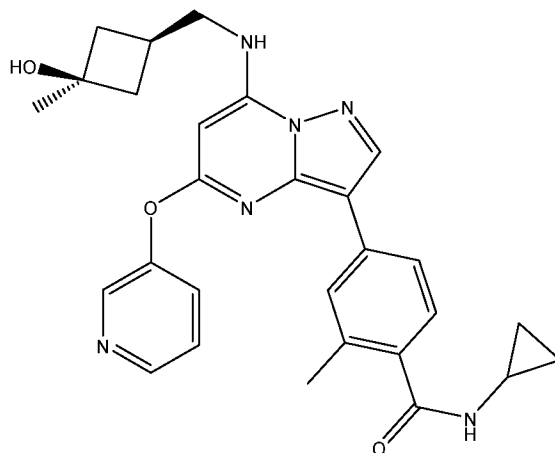
13. The method according to claims 1-5, 7, 9 and 11, wherein the immune checkpoint inhibitor is one or more selected from JS003, SHR-1316, KN035 and BMS-936559.

14. The method according to any one of claims 1-13, wherein the cancer is selected from the group consisting of breast cancer, colon cancer, rectal cancer, colorectal cancer, lung cancer, cancer of the peritoneum, gastric or stomach cancer, gastrointestinal cancer, cervical cancer, liver cancer, bladder cancer, hepatoma, ovarian cancer, endometrial or uterine cancer, prostate cancer, testicular cancer, leukemias, lymphomas, hematological malignancies, brain cancer, head and neck cancer, pancreatic cancer, melanoma, hepatocellular cancer, kidney or renal cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, Merkel cell cancer, mycoses fungoids, esophageal cancer, tumors of the biliary tract, salivary gland cancer, sarcomas, retinoblastoma, liposarcoma, synovial cell sarcoma, neuroendocrine tumors, gastrinoma, islet cell cancer, mesothelioma, schwannoma, acoustic neuroma, meningioma, adenocarcinoma, squamous cell cancer and epithelial squamous cell cancer.

15. The method according to any one of claims 1-13, wherein the cancer is selected from the group consisting of pancreatic cancer, lung cancer, breast cancer, colon cancer, brain cancer, neuroblastoma, prostate cancer, melanoma, glioblastoma multiforme, ovarian cancer, lymphoma, leukemia, melanoma, sarcoma, paraneoplasia, osteosarcoma, germinoma, glioma and mesothelioma.

16. The method according to any one of claims 1-13, wherein the cancer is selected from renal cell carcinoma, non-small cell lung cancer, urothelial cancer, head and neck cancer, ovarian cancer, lymphoma, melanoma, pancreatic cancer, myeloma, acute myeloid leukemia, bladder cancer and Hodgkin's lymphoma.

17. A pharmaceutical composition comprising a compound represented by the formula:



or a pharmaceutically acceptable salt thereof and an immune checkpoint inhibitor.

18. The pharmaceutical composition according to claim 17, wherein the immune checkpoint inhibitor is an antibody or an antigen binding fragment thereof.

19. The pharmaceutical composition according to any one of claims 17 and 18, wherein the immune checkpoint inhibitor is a monoclonal antibody or an antigen binding fragment thereof.

20. The pharmaceutical composition according to any one of claims 17-19, wherein the immune checkpoint inhibitor is one or more selected from a CD40L inhibitor, a DR3 inhibitor, a TL1A inhibitor, a GITR inhibitor, a GITRL inhibitor, a 4-1BB inhibitor, a 4-1BBL inhibitor, an OX40 inhibitor, an OX40L inhibitor, a CD27 inhibitor, a CD70 inhibitor, a TMIGD2 inhibitor, an HHLA2 inhibitor, an ICOS inhibitor, an ICOSL inhibitor, a B7RP1 inhibitor, a CD28 inhibitor, a PD-1 inhibitor, a PD-L1 inhibitor, a PD-L2 inhibitor, a CTLA-4 inhibitor, a CD80 inhibitor, a CD86 inhibitor, a KIR inhibitor, a TCR inhibitor, a LAG3 inhibitor, an MHC I inhibitor, an MHC II inhibitor, a CD80 inhibitor, a TIM-3 inhibitor, a GAL9 inhibitor, a BTLA inhibitor, an HVEM inhibitor, a CD160 inhibitor, a CD137 inhibitor, a CD137L inhibitor, a LIGHT inhibitor, a phosphatidylserine inhibitor, a VISTA inhibitor, a BTNL2 inhibitor, a B7-H3 inhibitor and a B7-H4 inhibitor.

21. The pharmaceutical composition according to any one of claims 17-20, wherein the immune checkpoint inhibitor is one or more selected from a PD-1 inhibitor, a PD-L1 inhibitor and a CTLA-4 inhibitor.
22. The pharmaceutical composition according to any one of claims 17-21, wherein the immune checkpoint inhibitor is a PD-1 inhibitor.
23. The pharmaceutical composition according to any one of claims 17-21, wherein the immune checkpoint inhibitor is a PD-L1 inhibitor.
24. The pharmaceutical composition according to any one of claims 17-22, wherein the immune checkpoint inhibitor restores anti-tumor T cell activity.
25. The pharmaceutical composition according to any one of claims 17-21 and 23, wherein the immune checkpoint inhibitor blocks T-cell-inhibitory cell activity.
26. The pharmaceutical composition according to any one of claims 17-21, wherein the immune checkpoint inhibitor is one or more selected from pembrolizumab, ipilimumab, nivolumab, atezolizumab, avelumab and durvalumab.
27. The pharmaceutical composition according to any one of claims 17-20, wherein the immune checkpoint inhibitor is one or more selected from JS001, SHR-1210, BGB-A317, IBI-308, REGN2810, JS003, SHR-1316, KN035, BMS-936559, LAG525, BMS-986016, MBG453, MEDI-570, OREG-103/BY40 and lirilumab.
28. The pharmaceutical composition according to claims 17-22, 24 and 27, wherein the immune checkpoint inhibitor is one or more selected from CJS001, SHR-1210, BGB-A317, IBI-308 and REGN2810.
29. The pharmaceutical composition according to claims 17-21, 23, 25 and 27, wherein the immune checkpoint inhibitor is one or more selected from JS003, SHR-1316, KN035 and BMS-936559.

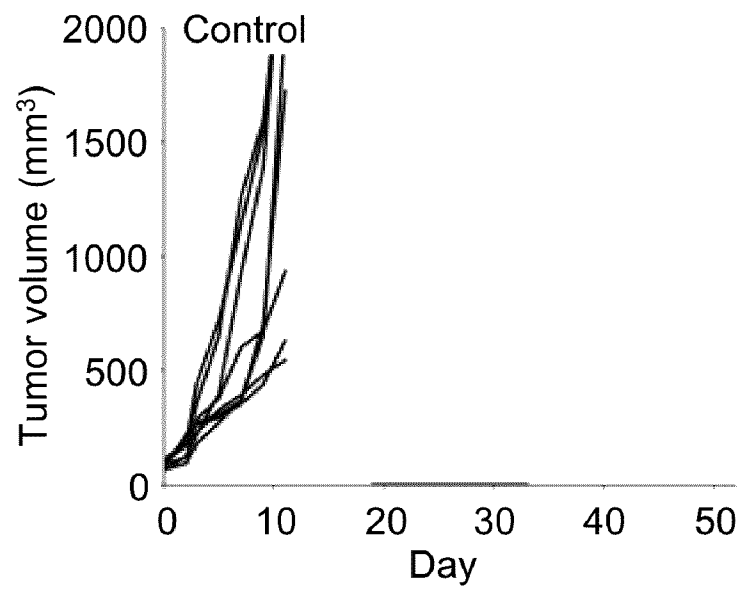


FIG. 1A

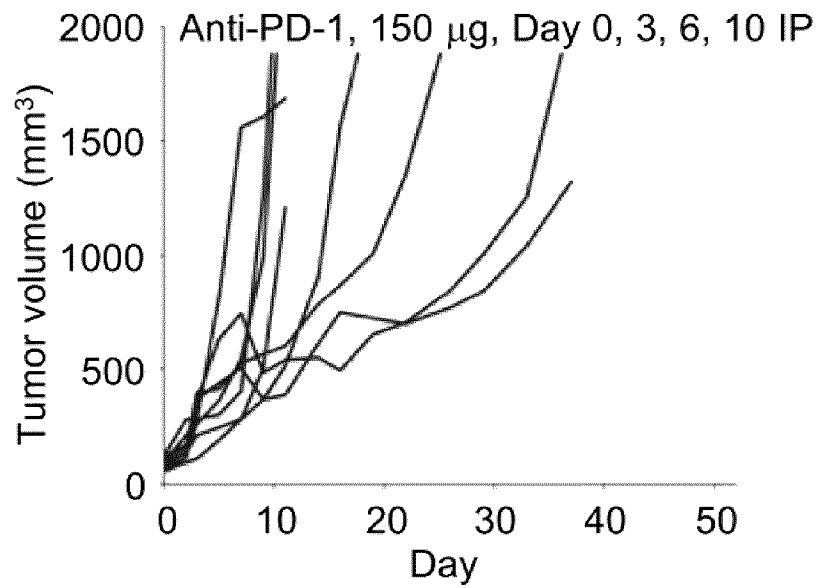


FIG. 1B

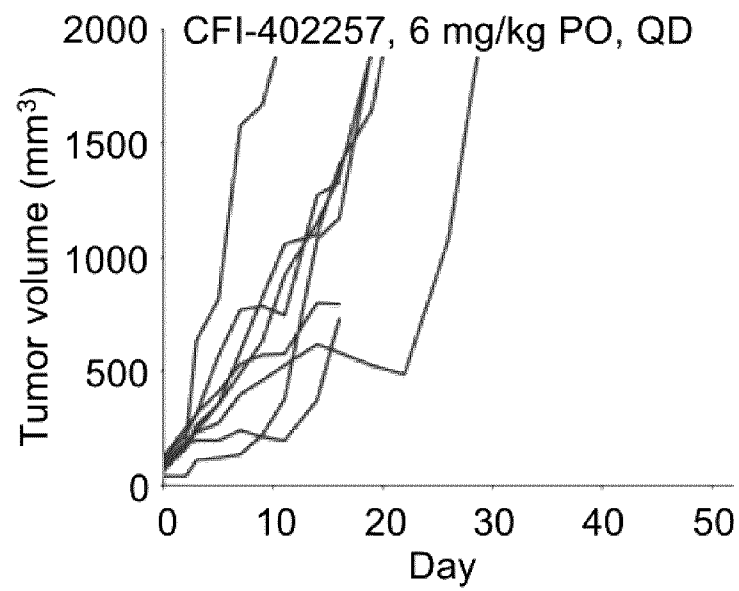


FIG. 1C

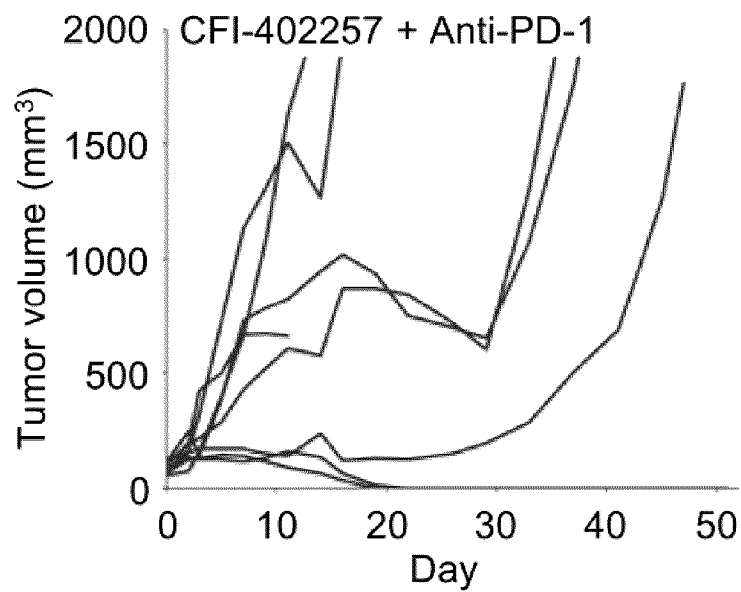


FIG. 1D

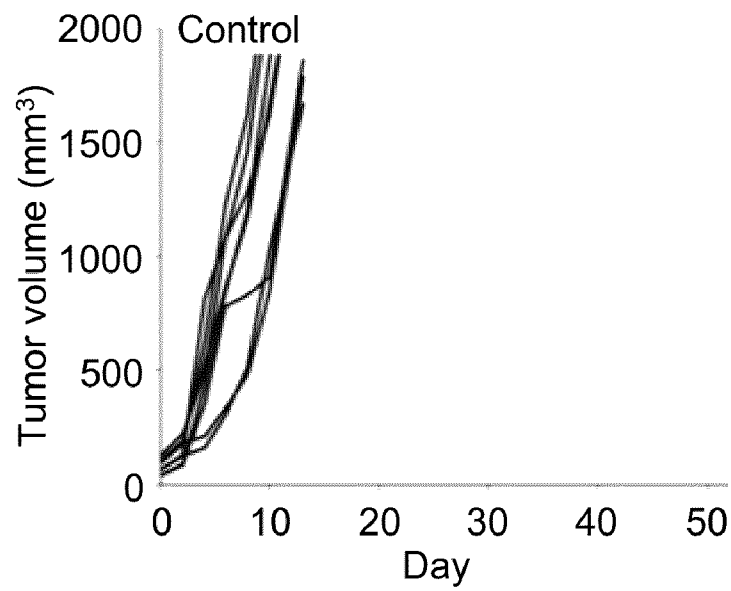


FIG. 2A

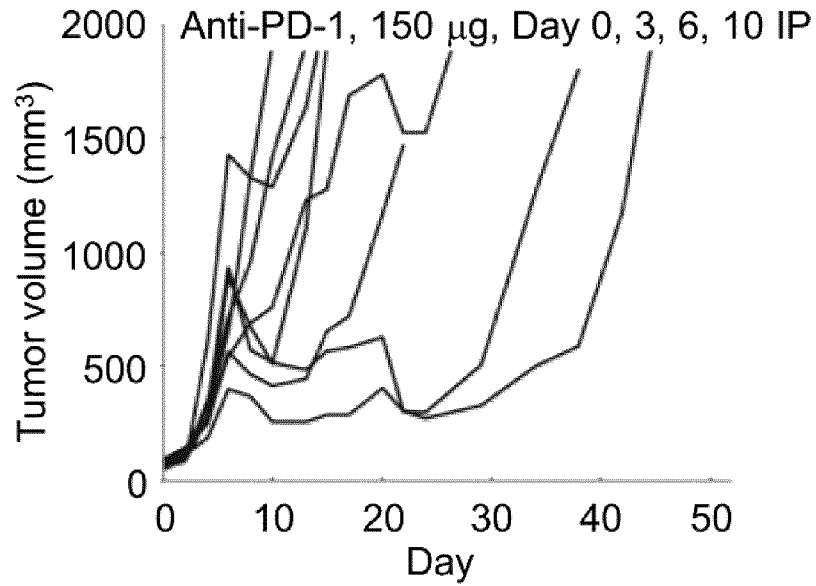


FIG. 2B

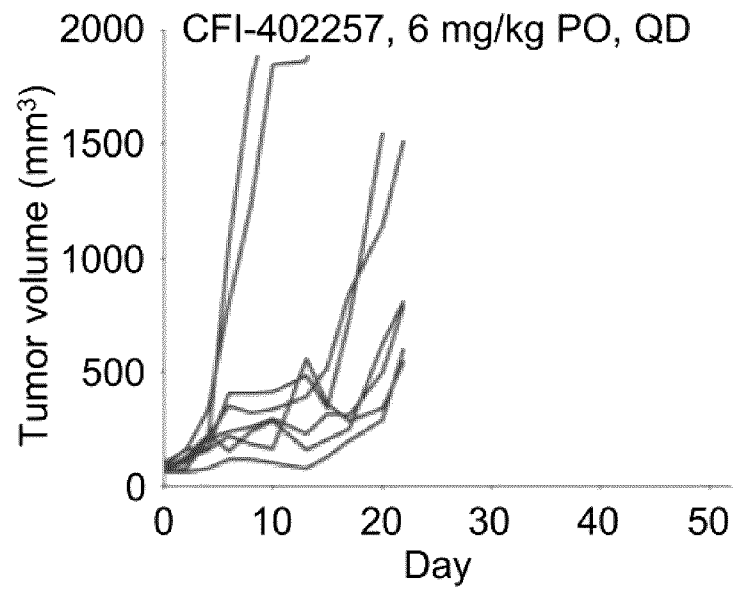


FIG. 2C

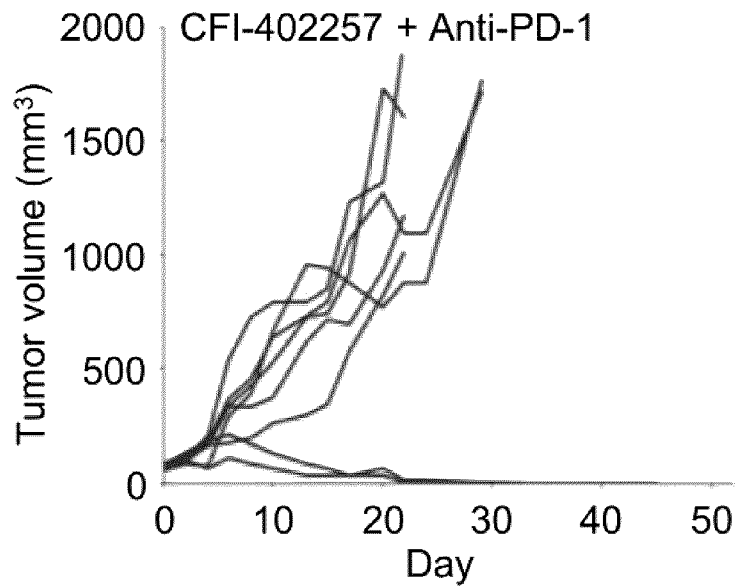


FIG. 2D

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA2018/050936

A. CLASSIFICATION OF SUBJECT MATTER

IPC: **A61K 31/519** (2006.01), **A61K 39/395** (2006.01), **A61P 35/00** (2006.01), **C07D 487/04** (2006.01), **C07K 16/18** (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC: **A61K** (2006.01), **C07K** (2006.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)

STN (compound search in Registry; CAPlus: anti-PD-1 monoclonal antibody, anti-Programmed cell death protein 1 monoclonal antibody, pembrolizumab, nivolumab, avelumab, durvalumab, atezolizumab)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	J. M. MASON et al: "Functional Characterization of CFI-402257, a Potent and Selective Mps1/TTK Kinase Inhibitor, for the Treatment of Cancer"; <i>Proceedings of the National Academy of Sciences of the United States of America</i> (March 2017), 114 (12), 3127-3132. See the whole document.	1-29
A	Y. LIU et al: "Discovery of Pyrazolo[1,5-a]pyrimidine TTK Inhibitors: CFI-402257 is a Potent, Selective, Bioavailable Anticancer Agent"; <i>ACS Medicinal Chemistry Letters</i> (2016), 7 (7), 671-675. See the whole document.	1-29
A	CA 2,963,281 A1 (CAO et al) 07 April 2016 (07-04-2016) See the whole document.	1-29
A	CA 2,966,042 A1 (MURRIEL et al) 06 May 2016 (06-05-2016) See the whole document.	1-29

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

* "A" "E" "L" "O" "P"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance earlier application or patent but published on or after the international filing date document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) document referring to an oral disclosure, use, exhibition or other means document published prior to the international filing date but later than the priority date claimed	"T" "X" "Y" "&"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art document member of the same patent family
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Date of the actual completion of the international search
11 October 2018 (11-10-2018)Date of mailing of the international search report
30 October 2018 (30-10-2018)Name and mailing address of the ISA/CA
Canadian Intellectual Property Office
Place du Portage I, C114 - 1st Floor, Box PCT
50 Victoria Street
Gatineau, Quebec K1A 0C9
Facsimile No.: 819-953-2476

Authorized officer

Lu Jiang (819) 639-4540

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA2018/050936

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CA 2,949,121 A1 (FELTQATE et al) 19 November 2015 (19-11-2015) See the whole document.	1-29
A	CA 2,954,508 A1 (JUNTILA) 21 January 2016 (21-01-2016) See the whole document.	1-29

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claim Nos.: 1-9, 11-25, 27-29
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

See extra sheet

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

“Immune checkpoint inhibitor” encompasses all compounds having this common function or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). The expression does not define any particular kind of compound(s) but rather the effect that the desired compound(s) should possess. Again, this lack of clarity in the present application renders a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to an anti-PD-1 monoclonal antibody, the only “immune checkpoint inhibitor” disclosed in examples 1-5.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2018/050936

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
CA2963281A1	07 April 2016 (07-04-2016)	AU2015327868A1 CN107106687A EA201790737A1 EP3200775A2 JP2017535528A KR20170066546A MX2017004360A US2017209574A1 WO2016054555A2 WO2016054555A3	20 April 2017 (20-04-2017) 29 August 2017 (29-08-2017) 31 August 2017 (31-08-2017) 09 August 2017 (09-08-2017) 30 November 2017 (30-11-2017) 14 June 2017 (14-06-2017) 26 June 2017 (26-06-2017) 27 July 2017 (27-07-2017) 07 April 2016 (07-04-2016) 30 June 2016 (30-06-2016)
CA2966042A1	06 May 2016 (06-05-2016)	AU2015338974A1 CN107530419A EP3212233A2 JP2017537892A MX2017005258A US2016176962A1 WO2016070051A2 WO2016070051A3 WO2016070051A4	11 May 2017 (11-05-2017) 02 January 2018 (02-01-2018) 06 September 2017 (06-09-2017) 21 December 2017 (21-12-2017) 26 July 2017 (26-07-2017) 23 June 2016 (23-06-2016) 06 May 2016 (06-05-2016) 14 July 2016 (14-07-2016) 29 September 2016 (29-09-2016)
CA2949121A1	19 November 2015 (19-11-2015)	CN106714839A EP3142697A1 JP2017515859A KR20170003692A MX2016014753A US2017158776A1 WO2015176033A1	24 May 2017 (24-05-2017) 22 March 2017 (22-03-2017) 15 June 2017 (15-06-2017) 09 January 2017 (09-01-2017) 06 March 2017 (06-03-2017) 08 June 2017 (08-06-2017) 19 November 2015 (19-11-2015)
CA2954508A1	21 January 2016 (21-01-2016)	AR101210A1 AU2015289672A1 CN106573060A EP3169361A1 JP2017522307A KR20170026630A MX2017000546A SG11201700074YA US2017112925A1 WO2016011160A1	30 November 2016 (30-11-2016) 02 March 2017 (02-03-2017) 19 April 2017 (19-04-2017) 24 May 2017 (24-05-2017) 10 August 2017 (10-08-2017) 08 March 2017 (08-03-2017) 08 March 2017 (08-03-2017) 27 February 2017 (27-02-2017) 27 April 2017 (27-04-2017) 21 January 2016 (21-01-2016)