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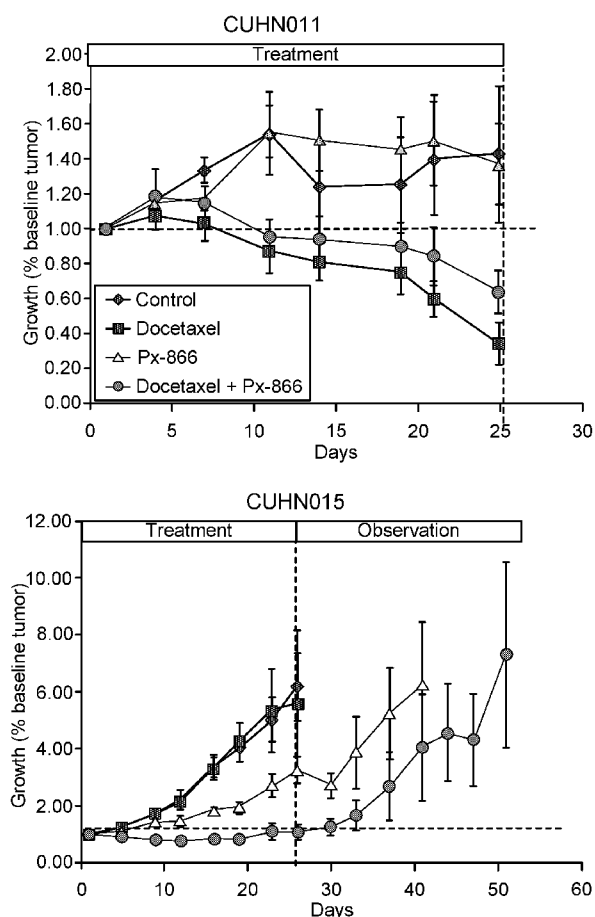
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[Continued on next page]

(54) Title: METHODS FOR TREATING ONCOVIRUS POSITIVE CANCERS

FIG. 17



(57) **Abstract:** Provided herein are methods for the treatment of certain cancers in a subject by administering a PI-3 kinase inhibitor, or a combination of a PI-3 kinase inhibitor and a second anticancer agent, to the subject.



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- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))

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METHODS FOR TREATING ONCOVIRUS POSITIVE CANCERS

CROSS REFERENCE

- [001] This application claims the benefit of U.S. Provisional Application No. 61/448,984, filed on March 3, 2011, which is incorporated herein by reference in its entirety.

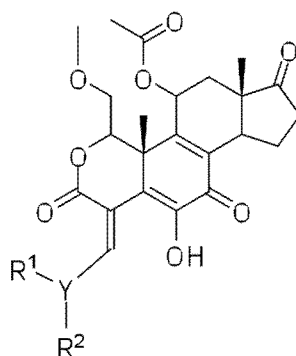
BACKGROUND OF THE INVENTION

- [002] Experimental and epidemiological data evidence a causative role for certain oncoviruses in the development of cancer in humans.

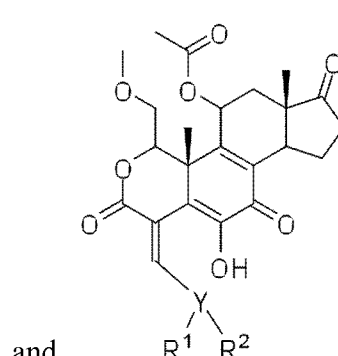
SUMMARY OF THE INVENTION

- [003] Provided herein are methods for treating cancer in a subject with PI-3 kinase monotherapy or a combination therapy of a PI-3 kinase inhibitor and a second anticancer agent. Provided herein are methods for treating cancer in a subject with a combination therapy of a wortmannin analog and a second anticancer agent. Cancers to be treated with a combination therapy described herein, include head and neck cancer, lung cancer, ovarian cancer, liver cancer, colon cancer, breast cancer, pancreatic cancer, kidney cancer, cervical cancer, uterine cancer, prostate cancer, esophageal cancer, nasopharyngeal cancer, oropharyngeal cancer, gastric cancer, skin cancer, vulvar cancer, vaginal cancer, anal cancer and penile cancer. Provided herein also are methods for reducing solid tumors in a subject suffering from or suspected to be suffering from cancer with PI-3 kinase monotherapy or a combination therapy of a PI-3 kinase inhibitor and a second cancer agent. Also provided herein are methods of improving or maintaining the quality of life in a subject with cancer with PI-3 kinase monotherapy or a combination therapy of a PI-3 kinase inhibitor and a second cancer agent. Also provided herein are methods for potentiation of existing treatment of cancer with a combination therapy of a PI-3 kinase inhibitor and a second cancer agent. In some of the aforementioned embodiments, the cancer is a cancer that tests positive for a virus. In some of the aforementioned embodiments, the cancer is an oncovirus-positive cancer. In some of the aforementioned embodiments, a PI-3 kinase inhibitor is a wortmannin analog as described herein.
- [004] In one aspect, provided herein is the use of a therapeutically effective amount of a PI-3 kinase inhibitor for the treatment of an oncovirus-positive cancer selected from human papilloma virus (HPV)-positive cancer, herpes virus-positive cancer, hepatitis virus-positive cancer, and Merkel cell polyoma virus (MCV)-positive cancer.

- [005] In some embodiments of the uses of PI-3 kinase inhibitors described above and herein, and the methods of treatment described herein, the PI-3 kinase inhibitor is selected from PX-866, XL 147, GDC-0941 (Genentech/Roche), CAL-101 (Calistoga), NVP-BKM120 (Novartis), ZSTK474 (Zenyaku Kogyo), NVP-BYL179 (Novartis), AMG319 (Amgen), GDC0032 (Genentech/Roche), A66, AS-252424, AS-604850, AS-605240, AZD6482, CAY10505, CH5132799, D-106669, GSK1059615, PIK-293, PIK-90, PIK-93, GNE-490, CNX-1351 (Celgene/Avila), INK117 (Intellikine), PIK-39, BAY806946 (Bayer), XL 765 (Exelixis), GDC-0980 (Genentech), GSK 2126458 (GlaxoSmithKline), NVP-BEZ235 (Novartis), NVP-BGT226 (Novartis), PF04691503 (Pfizer), PKI587 (Pfizer), SF1126 (Semaphore), D-87503, GSK2126458, IC-87114, PI-103, PIK-294, PIK-75, PKI-402, PKI-587 (PF-05212384), Quercetin (Sophoretin), TG100-115, TGX-221, A-769662, phenformin hydrochloride, and PP121.
- [006] In some embodiments of the uses of PI-3 kinase inhibitors described above and herein and the methods of treatment described herein, the PI-3 kinase inhibitor is a compound selected from



Formula IIA



and

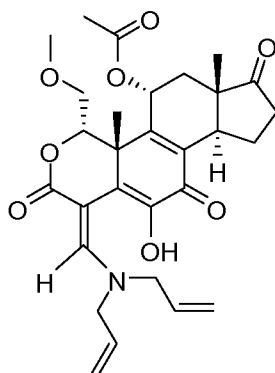
Formula IIB

wherein Y is a heteroatom selected from nitrogen and sulfur;

R¹ and R² are independently selected from an unsaturated alkyl, cyclic alkyl, or R¹ and R² together with Y form a heterocycle;

or pharmaceutically acceptable salt, solvate or polymorph thereof.

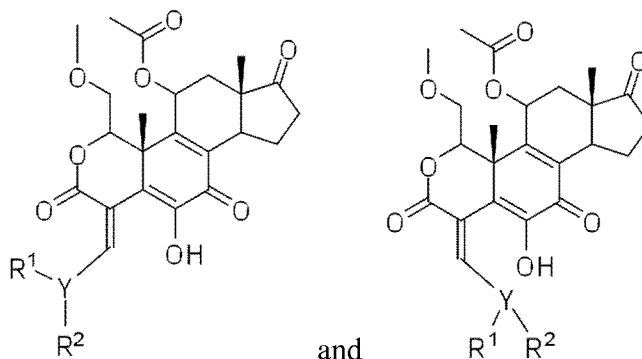
- [007] In some embodiments of the uses of PI-3 kinase inhibitors described above and herein, and the methods of treatment described herein, the PI-3 kinase inhibitor is an irreversible inhibitor of PI-3 kinase.
- [008] In some embodiments of the uses of PI-3 kinase inhibitors described above and herein, and the methods of treatment described herein, the PI-3 kinase inhibitor is



(PX-866),

or pharmaceutically acceptable salt, solvate, or polymorph thereof.

- [009] In a second aspect, provided herein is the use of a therapeutically effective amount of a PI-3 kinase inhibitor in combination with a second anticancer agent for the treatment of an oncovirus-positive cancer selected from HPV-positive cancer, MCV-positive cancer, herpes-virus positive cancer and hepatitis virus-positive cancer.
- [010] In some embodiments of the uses of PI-3 kinase inhibitors described above and herein, and the methods of treatment described herein, the PI-3 kinase inhibitor is selected from PX-866, XL 147, GDC-0941 (Genentech/Roche), CAL-101 (Calistoga), NVP-BKM120 (Novartis), ZSTK474 (Zenyaku Kogyo), NVP-BYL179 (Novartis), AMG319 (Amgen), GDC0032 (Genentech/Roche), A66, AS-252424, AS-604850, AS-605240, AZD6482, CAY10505, CH5132799, D-106669, GSK1059615, PIK-293, PIK-90, PIK-93, GNE-490, CNX-1351 (Celgene/Avila), INK117 (Intellikine), PIK-39, BAY806946 (Bayer), XL 765 (Exelixis), GDC-0980 (Genentech), GSK 2126458 (GlaxoSmithKline), NVP-BEZ235 (Novartis), NVP-BGT226 (Novartis), PF04691503 (Pfizer), PKI587 (Pfizer), SF1126 (Semaphore), D-87503, GSK2126458, IC-87114, PI-103, PIK-294, PIK-75, PKI-402, PKI-587 (PF-05212384), Quercetin (Sophoretin), TG100-115, TGX-221, A-769662, phenformin hydrochloride, and PP121.
- [011] In some embodiments of the uses of PI-3 kinase inhibitors described above and herein, and the methods of treatment described herein, the PI-3 kinase inhibitor is a compound selected from



Formula IIA

Formula IIB

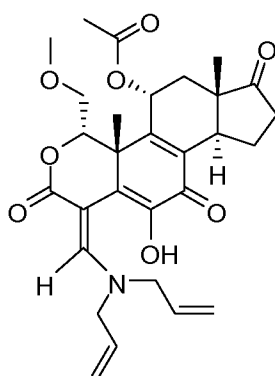
wherein Y is a heteroatom selected from nitrogen and sulfur;

R¹ and R² are independently selected from an unsaturated alkyl, cyclic alkyl, or R¹ and R² together with Y form a heterocycle;

or pharmaceutically acceptable salt, solvate, or polymorph thereof.

[012] In some embodiments of the uses of PI-3 kinase inhibitors described above and herein, and the methods of treatment described herein, the PI-3 kinase inhibitor is an irreversible inhibitor of PI-3 kinase.

[013] In some embodiments of the uses of PI-3 kinase inhibitors described above and herein, and the methods of treatment described herein, the PI-3 kinase inhibitor is

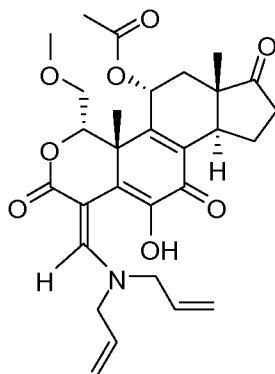


(PX-866),

or pharmaceutically acceptable salt, solvate or polymorph thereof.

[014] In a further aspect, provided herein is the use of a composition comprising a therapeutically effective amount of an irreversible PI-3 kinase inhibitor in combination with a second anticancer agent for the treatment of an Epstein-Barr virus (EBV)-positive cancer.

[015] In some embodiments of the uses of PI-3 kinase inhibitors described above and herein, and the methods of treatment described herein, the irreversible PI-3 kinase inhibitor is



(PX-866),

or pharmaceutically acceptable salt, solvate or polymorph thereof.

[016] In some embodiments of the uses of PI-3 kinase inhibitors described above and herein, and the methods of treatment described herein, the cancer is selected from the group

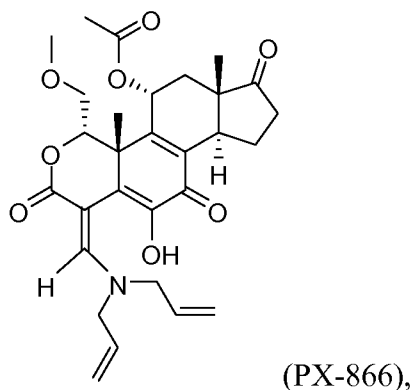
consisting of head and neck cancer, lung cancer, ovarian cancer, liver cancer, colon cancer, breast cancer, pancreatic cancer, kidney cancer, cervical cancer, uterine cancer, prostate cancer, esophageal cancer, nasopharyngeal cancer, oropharyngeal cancer, gastric cancer, skin cancer, vulvar cancer, vaginal cancer, anal cancer and penile cancer.

- [017] In some embodiments of the uses of PI-3 kinase inhibitors described above and herein, and the methods of treatment described herein, the cancer is a solid tumor.
- [018] In some embodiments of the uses of PI-3 kinase inhibitors described above and herein, and the methods of treatment described herein, the cancer is unresectable.
- [019] In some embodiments of the uses of PI-3 kinase inhibitors described above and herein, and the methods of treatment described herein, the cancer is a HPV positive squamous cell carcinoma, HPV positive head and neck cancer, HPV positive oropharyngeal cancer, HPV positive cervical cancer, HPV positive lung cancer, or HPV positive non-small cell lung cancer.
- [020] In some embodiments of the uses of PI-3 kinase inhibitors described above and herein, and the methods of treatment described herein, the cancer is Hepatitis B positive hepatocellular carcinoma, or Hepatitis C positive hepatocellular carcinoma.
- [021] In some embodiments of the uses of PI-3 kinase inhibitors described above and herein, and the methods of treatment described herein, the cancer is an EBV positive B-cell malignancy, EBV positive Burkitt's lymphoma, Hodgkin's lymphoma, or diffuse large B-cell lymphoma, EBV positive nasopharyngeal cancer, or EBV positive gastric carcinoma.
- [022] In some embodiments of the uses of PI-3 kinase inhibitors described above and herein, and the methods of treatment described herein, the second anticancer agent is selected from methotrexate (RHEUMATREX®, Amethopterin) cyclophosphamide (CYTOXAN®), thalidomide (THALIDOMID®), acridine carboxamide, actimid®, actinomycin, 17-N-allylamino-17-demethoxygeldanamycin, aminopterin, amsacrine, anthracycline, bevacuzimab (Avastin®), antineoplaston, 5-azacytidine, azathioprine, BL22, bendamustine, biricodar, bleomycin, bortezomib, bryostatin, busulfan, calyculin, camptothecin, capecitabine, carboplatin, cetuximab, chlorambucil, cisplatin, cladribine, clofarabine, cytarabine, dacarbazine, dasatinib, daunorubicin, decitabine, dichloroacetic acid, discodermolide, docetaxel, doxorubicin, epirubicin, epothilone, eribulin, estramustine, etoposide, exatecan, exisulind, ferruginol, floxuridine, fludarabine, fluorouracil, fosfestrol, fotemustine, ganciclovir, gefitinib (Iressa®), gemcitabine, hydroxyurea, IT-101, idarubicin, ifosfamide, imiquimod, irinotecan, irofulven,

ixabepilone, laniquidar, lapatinib, lenalidomide, lomustine, lurtotecan, mafosfamide, masoprocol, mechlorethamine, melphalan, mercaptopurine, mitomycin, mitotane, mitoxantrone, nelarabine, nilotinib, oblimersen, oxaliplatin, PAC-1, paclitaxel, pemetrexed, pentostatin, pipobroman, pixantrone, plicamycin, procarbazine, proteasome inhibitors (e.g., bortezomib), raltitrexed, rebeccamycin, revlimid®, rubitecan, SN-38, salinosporamide A, satraplatin, streptozotocin, swainsonine, tariquidar, taxane, tegafur-uracil, temozolomide, testolactone, thioTEPA, tioguanine, topotecan, trabectedin, tretinoin, triplatin tetranitrate, tris(2-chloroethyl)amine, troxacitabine, uracil mustard, valrubicin, vinblastine, vincristine, vinorelbine, vorinostat, and zosuquidar.

- [023] In some embodiments of the uses of PI-3 kinase inhibitors described above and herein, and the methods of treatment described herein, the second anticancer agent is selected from docetaxel, cetuximab, gefitinib, and a platinum based chemotherapeutic agent.
- [024] In some embodiments of the uses of PI-3 kinase inhibitors described above and herein, and the methods of treatment described herein, the second anticancer agent and the PI-3 kinase inhibitor are administered simultaneously.
- [025] In some embodiments of the uses of PI-3 kinase inhibitors described above and herein, and the methods of treatment described herein, the second anticancer agent and the PI-3 kinase inhibitor are administered sequentially.
- [026] In some embodiments of the uses of PI-3 kinase inhibitors described above and herein, and the methods of treatment described herein, the second anticancer agent and the PI-3 kinase inhibitor are administered in a single composition.
- [027] In some embodiments of the uses of PI-3 kinase inhibitors described above and herein, and the methods of treatment described herein, administration of the PI-3 kinase inhibitor is by intramuscular, intravenous, subcutaneous, intranodal, intratumoral, intracisternal, intraperitoneal, intradermal, transdermal, nasal, pulmonary, vaginal, rectal, buccal, ocular, otic, local, topical, or oral delivery.
- [028] In some embodiments of the uses of PI-3 kinase inhibitors described above and herein, and the methods of treatment described herein, the PI-3 kinase inhibitor is administered orally.
- [029] In some embodiments of the uses of PI-3 kinase inhibitors described above and herein, and the methods of treatment described herein, the PI-3 kinase inhibitor is administered in a capsule form.

[030] In some embodiments of the uses of PI-3 kinase inhibitors described above and herein, and the methods of treatment described herein, the PI-3 kinase inhibitor is a crystalline form of a compound having the structure



which is substantially free of wortmannin.

[031] In some embodiments of the uses of PI-3 kinase inhibitors described above and herein, and the methods of treatment described herein, the PI-3 kinase inhibitor is a crystalline form of PX-866 which is an anisole solvate form of PX-866, as described herein.

INCORPORATION BY REFERENCE

[032] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference for the purposes cited.

BRIEF DESCRIPTION OF THE DRAWINGS

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

[034] FIG. 1 shows the X-ray powder diffraction patterns of an anisole solvate of PX-866; redrawn by draftsman.

[035] FIG. 2 shows the DSC thermogram of an anisole solvate of PX-866; redrawn by draftsman.

[036] FIG. 3 shows the a-axis projection of the crystal packing in an anisole solvate of PX-866; redrawn by draftsman.

[037] FIG. 4 shows the X-ray powder diffraction patterns of a toluene solvate of PX-866; redrawn by draftsman.

[038] FIG. 5 shows the DSC thermogram of a toluene solvate of PX-866; redrawn by draftsman.

- [039] FIG. 6 shows the X-ray powder diffraction patterns of a propyl acetate solvate of PX-866; redrawn by draftsman.
- [040] FIG. 7 shows the DSC thermogram of a propyl acetate solvate of PX-866; redrawn by draftsman.
- [041] FIG. 8 shows the a-axis projection of the crystal packing in a propyl acetate solvate of PX-866; redrawn by draftsman.
- [042] FIG. 9 shows the X-ray powder diffraction patterns of the crystalline PX-866 prepared by from a solution comprising 4-methyl-2-pentanone; redrawn by draftsman.
- [043] FIG. 10 shows the X-ray powder diffraction patterns of the crystalline PX-866 prepared by from a solution comprising cumene; redrawn by draftsman.
- [044] FIG. 11 shows the X-ray powder diffraction patterns of the crystalline PX-866 prepared by from a solution comprising 1-pentanol; redrawn by draftsman.
- [045] FIG. 12 shows the X-ray powder diffraction patterns of the crystalline PX-866 prepared by from a solution comprising chlorobenzene; redrawn by draftsman.
- [046] FIG. 13 shows results of stability testing at 40 °C, 75% relative humidity between crystalline anisole solvate, toluene solvate, propyl acetate solvate and amorphous forms of PX-866; redrawn by draftsman.
- [047] FIG. 14 shows data from combination efficacy studies in two direct patient tumor xenograft models (DPTM) using PX-866 in combination with cetuximab, as described in Example 20.
- [048] FIG. 15 shows data from combination efficacy studies in two direct patient tumor xenograft models using PX-866 in combination with cetuximab, as described in Example 20.
- [049] FIG. 16 shows data from combination efficacy studies in two direct patient tumor xenograft models using PX-866 in combination with docetaxel, as described in Example 20.
- [050] FIG. 17 shows data from combination efficacy studies in two direct patient tumor xenograft models using PX-866 in combination with docetaxel, as described in Example 20.

DETAILED DESCRIPTION OF THE INVENTION

- [051] Provided herein, in certain embodiments, are methods for treating cancer in a subject with a combination therapy of a PI-3 kinase inhibitor and a second anticancer agent. Also provided herein are compounds, pharmaceutical compositions and medicaments comprising such compounds for a combination therapy with an anticancer agent and a

PI-3 kinase inhibitor (e.g., a wortmannin analog). In some embodiments, the methods provided herein are suitable for treatment of oncovirus positive cancers. In some of such embodiments, the methods of treatment provided herein reduce mortality in patient populations that suffer from or are suspected to be suffering from cancers that test positive for one or more oncoviruses.

Oncoviruses

- [052] As used herein, “a cancer that tests positive for a virus” is a cancer that is associated with any oncogenic viral mechanism. A virus that a cancer tests positive for, or an oncovirus, or a tumor virus, is a virus that, in some cases, induces development of cancer (e.g., after a chronic infection, in individuals with a compromised immune system, or the like).
- [053] Tumor viruses come in a variety of forms: adenoviruses with a DNA genome, viruses with a RNA genome (e.g., Hepatitis C virus (HCV)), retroviruses having both DNA and RNA genomes (e.g., Human T-lymphotropic virus, hepatitis B virus). Some viruses are tumorigenic when they infect a cell and persist as circular episomes or plasmids, replicating separately from a host cell DNA (e.g., Epstein-Barr virus, Kaposi's sarcoma-associated herpesvirus). Other viruses are carcinogenic when they integrate into the host cell genome as part of a biological accident (e.g., polyomaviruses such as MCV and papillomaviruses such as HPV).
- [054] Oncogenic viral mechanisms include direct insertion of viral oncogenic genes into a host cell, and indirect oncogenicity involving chronic nonspecific inflammation occurring over decades of infection which leads to a cancer that has accumulated sufficient mutations and growth conditions (hyperplasia) from the chronic inflammation of viral infection, (e.g., HCV-induced liver cancer). In certain instances, virus antigens are expressed in such cancerous tumors and such cancers test positive for the virus (e.g., any oncovirus described herein).
- [055] In some embodiments, a cancer that is treatable by methods described herein is an HPV positive cancer. HPV typically establishes infection in epithelia of skin or mucous membranes. In some instances, persistent infection leads to cancerous lesions. Examples of HPV positive cancers include cervical cancer, oral cancer, oropharyngeal cancer, laryngeal cancer and the like.
- [056] In some embodiments, a cancer that is treatable by methods described herein is an EBV positive cancer. EBV, together with HHV-8 (Kaposi sarcoma-associated virus), belongs to the genus Lymphocryptovirus, family Herpesviridae. The EBV virus is an enveloped DNA virus, which multiplies in the nucleus of a host cell. In some instances, EBV

infects resting human B-lymphocytes, establishing latent infection in memory B-lymphocytes. In some instances, EBV infects epithelial cells and multiplies. Examples of EBV positive cancers include and are not limited to nasopharyngeal cancers, oropharyngeal cancers and B-cell lymphomas.

- [057] In some embodiments, a cancer that is treatable by methods described herein is an HTLV positive cancer. HTLV viruses are sexually transmitted viruses. The HTLV-1 genome is diploid, composed of two copies of a single-stranded RNA virus whose genome is copied into a double-stranded DNA form that integrates into the host cell genome. Examples of HTLV-1 positive cancers include T-cell leukemia, including Adult T-cell leukemia/lymphoma (ATL).
- [058] In some embodiments, a cancer that is treatable by methods described herein is an MCV positive cancer. MCV is a non-enveloped, double-stranded DNA virus. In some instances, the virus is monoclonally integrated into a tumor, with additional host cell mutations acting in concert with the integrated virus to cause cancer. Examples of MCV positive cancers include Merkel cell carcinoma, an aggressive form of skin cancer.
- [059] In some embodiments, a cancer that is treatable by methods described herein is Hepatitis virus positive cancer. In some instances chronic infection due to Hepatitis C virus induces Hepatitis C positive liver cancer. In some instances chronic infection due to Hepatitis B or Hepatitis C viruses induces Hepatitis B positive or Hepatitis C positive hepatocellular carcinomas.
- [060] In some embodiments, a cancer that is treatable by methods described herein is a Herpes virus positive cancer. KSHV is a herpesvirus, and is a large double-stranded DNA virus that enters lymphocytes. Examples of KSHV (also known as HHV-8) positive cancers include Kaposi's sarcoma, effusion lymphoma and the like.

Treatment of Cancer

- [061] Cancers treatable by methods described herein include, but are not limited to, breast cancer, lung cancer, head and neck cancer, brain cancer, abdominal cancer, colon cancer, colorectal cancer, esophageal cancer, parapharyngeal cancer, gastrointestinal cancer, glioma, liver cancer, hepatocellular cancer, parotid gland cancer, tongue cancer, neuroblastoma, osteosarcoma, ovarian cancer, renal cancer, pancreatic cancer, retinoblastoma, cervical cancer, uterine cancer, Wilm's tumor, multiple myeloma, skin cancer, lymphoma, leukemia, blood cancer, anaplastic thyroid tumor, sarcoma of the skin, melanoma, adenocystic tumor, hepatoid tumor, non-small cell lung cancer, chondrosarcoma, pancreatic islet cell tumor, prostate cancer, ovarian cancer, gall bladder

cancer, urinary bladder cancer, renal cancer, urinary tract cancer, vulvar cancer, vaginal cancer, anal cancer, penile cancer, and/or carcinomas including but not limited to squamous cell carcinoma of the head and neck, colorectal carcinoma, glioblastoma, cervical carcinoma, endometrial carcinoma, gastric carcinoma, pancreatic carcinoma, leiomyosarcoma and breast carcinoma. In some embodiments, cancers treatable by methods provided herein are hematological cancers, including and not limited to multiple myeloma, acute myelogenous leukemia, acute/chronic lymphoblastic leukemia, hairy-cell leukemia, follicular lymphoma, multiple myeloma, plasmacytoma, diffuse large B-cell lymphoma, Hodgkin's lymphoma, Non-Hodgkin's lymphoma and the like. In some embodiments, cancers treated by the methods provided herein are carcinomas. In some embodiments, cancers treated by the methods provided herein are hematological cancers. In some embodiments, cancers treated by the methods provided herein are sarcomas. In some embodiments, cancers treated by the methods provided herein are solid tumors. In specific embodiments, cancers treated by the methods provided herein are oncovirus positive cancers. In specific embodiments, cancers treated by the methods provided herein are oncovirus positive solid tumors.

[062] In some embodiments, the methods described herein treat a lung cancer such as non-small cell lung cancer (NSCLC). In other embodiments, the methods described herein treat a head and neck cancer such as squamous cell carcinoma of the head and neck (SCCHN). Squamous-cell carcinoma (SCC) is a carcinomatous cancer occurring in many different organs, including and not limited to the skin, lips, gums, tongue, mouth, head and neck, esophagus, urinary bladder, prostate, lungs, vagina, and cervix. It is a malignant tumor of squamous epithelium (epithelium that shows squamous-cell differentiation). In certain specific embodiments, cancers treated by methods described herein are HPV positive squamous cell carcinomas. In certain specific embodiments, cancers treated by methods described herein are HPV positive cancers (e.g., HPV positive head and neck cancer).

[063] The methods described herein are suitable for treatment of various stages of cancer including stages which are locally advanced, metastatic and/or recurrent. In cancer staging, locally advanced is generally defined as cancer that has spread from a localized area to nearby tissues and/or lymph nodes. In the Roman numeral staging system, locally advanced usually is classified in Stage II or III. Cancer which is metastatic is a stage where the cancer spreads throughout the body to distant tissues and organs (stage IV). Cancer designated as recurrent generally is defined as the cancer has recurred,

usually after a period of time, after being in remission or after a tumor has visibly been eliminated. Recurrence can either be local, i.e., appearing in the same location as the original, or distant, i.e., appearing in a different part of the body. In certain instances, a cancer treatable by methods described herein is unresectable, or unable to be removed by surgery.

- [064] In some embodiments, the methods described herein are administered as a first-line or primary therapy. Other subjects suitable for treatment by the methods described herein include those that have completed first-line anti-cancer therapy. First-line anti-cancer therapies include chemotherapy, radiotherapy, immunotherapy, gene therapy, hormone therapy, surgery or other therapies that are capable of negatively affecting cancer in a patient, such as for example, by killing cancer cells, inducing apoptosis in cancer cells, reducing the growth rate of cancer cells, reducing the incidence or number of metastases, reducing tumor size, inhibiting tumor growth, reducing the blood supply to a tumor or cancer cells, promoting an immune response against cancer cells or a tumor, preventing or inhibiting the progression of cancer, or increasing the lifespan of a subject with cancer.

Definitions

- [065] As used herein and in the appended claims, the singular forms "a", "an", and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, reference to "a cell" is a reference to one or more cells and equivalents thereof known to those skilled in the art, and so forth.
- [066] The term "about" is used to indicate that a value includes the standard deviation of error for the device or method being employed to determine the value. The use of the term "or" in the claims is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and to "and/or." The terms "comprise," "have" and "include" are open-ended linking verbs. Any forms or tenses of one or more of these verbs, such as "comprises," "comprising," "has," "having," "includes" and "including," are also open-ended. For example, any method that "comprises," "has" or "includes" one or more steps is not limited to possessing only those one or more steps and also covers other unlisted steps.
- [067] As used herein, the terms "patient," "subject" and "individual" are intended to include living organisms in which certain conditions as described herein can occur. Examples include humans, monkeys, cows, sheep, goats, dogs, cats, mice, rats, and transgenic

species thereof. In a preferred embodiment, the patient is a primate. In certain embodiments, the primate or subject is a human. Other examples of subjects include experimental animals such as mice, rats, dogs, cats, goats, sheep, pigs, and cows. The experimental animal are an animal model for a disorder, e.g., a transgenic mouse with a cancerous pathology. A patient are a human suffering from cancer, such as lung cancer.

[068] The term “wortmannin analogs” or “analogs of wortmannin” as used herein refers to any compounds in which one or more atoms, functional groups, or substructures in wortmannin have been replaced with different atoms, groups, or substructures while retaining or improving upon the functional activity of wortmannin and/or improving pharmacokinetic profiles and/or reducing toxicity of wortmannin.

Phosphatidylinositol-3-kinases (PI-3Ks)

[069] Phosphatidylinositol-3-kinases (PI-3Ks) are a family of intracellular lipid kinases that play a critical role in transmitting signals from cell surface receptors on the plasma membrane to downstream signaling intermediates. PI-3Ks are linked to a diverse list of cellular functions, including cell growth, proliferation, differentiation, motility, survival and intracellular trafficking. There are 3 classes of PI-3K (Class I, II and III) which are classified based upon their structure and substrate specificity. Class I PI-3K are heterodimers formed by a regulatory subunit and a catalytic p110 subunit that phosphorylate membrane-associated phosphatidylinositol 4,5-bisphosphate (PIP2) to form phosphatidylinositol 3, 4, 5-trisphosphate (PIP3). PIP3 binds to the serine protein kinase AKT, which is reportedly the primary effector of PI-3K, triggering activation of downstream signaling intermediates, including mammalian target of rapamycin (mTOR), with subsequent effects on cell growth and metabolism, survival, and proliferation, as well as angiogenesis. The tumor suppressor gene phosphatase and tensin homolog (PTEN) reportedly counteracts the activity of Class I PI-3K by dephosphorylating PIP3 back to PIP2. PI-3K activation reportedly affects other AKT-independent pathways including Bruton tyrosine kinase and Tec family kinases, serum and glucocorticoid regulated kinases, and regulators of GTPases, although the role of these pathways is less well defined.

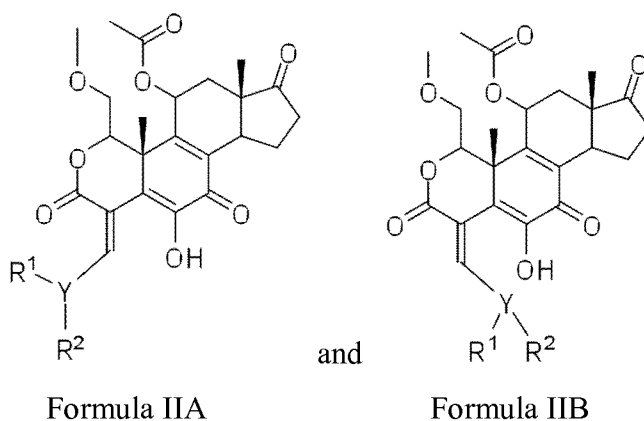
[070] Class I PI-3K is further divided into Class I_A and Class I_B subfamilies. Class I_A PI-3K are formed by a regulatory p85 subunit (PIK3R1) and a catalytic p110 subunit that are primarily activated by receptor tyrosine kinases such as epidermal growth factor receptor (EGFR), insulin-like growth factor (IGF), platelet-derived growth factor (PDGF) and Her2/neu. Several isoforms exist for each subunit, including α , β , γ and δ isoforms of

p110. The α and β isoforms are expressed ubiquitously, whereas expression of the δ isoform is restricted to leukocytes. Class I_B PI-3K are composed of a p110 subunit and a p101 regulatory subunit. Class I_B PI-3K are activated by G protein-coupled receptors. The best characterized Class I_B PI-3K contains the gamma isoform of p110, and is expressed primarily in leukocytes, as well as heart, pancreas, skeletal muscle, and liver.

[071] Increased signaling through Class I_A PI-3Ks has been implicated in many different forms of cancer. Cancers in which PI-3K pathway abnormalities have been identified include non-small cell lung cancer (NSCLC), breast carcinoma, ovarian carcinoma, endometrial carcinoma, prostate carcinoma, squamous cell carcinoma of the head and neck (SCCHN), cervical cancer, glioblastoma, melanoma, and colorectal carcinoma. PI-3Ks are also contemplated in other cancers. Reported mechanisms which lead to increased signaling through the PI-3K pathway include increased receptor tyrosine kinase (RTK) activity, activating mutations in the p110 α isoform, mutations in the p85 subunit, and mutations and deletions in PTEN. Amplification of the PIK3CA gene has also been observed in a number of tumors, including squamous cell carcinomas of the lung and head and neck, although this observation has not yet been linked directly to increased PI-3K activity. Accordingly, in some embodiments, the methods of treatment provided herein treat cancers that test positive for a virus and are associated with increased PI-3K activity.

Methods of treatment

[072] In one aspect, provided herein are methods for treating an individual suffering from or suspected to be suffering from a cancer that tests positive for a virus comprising administering to the individual in need thereof a compound selected from



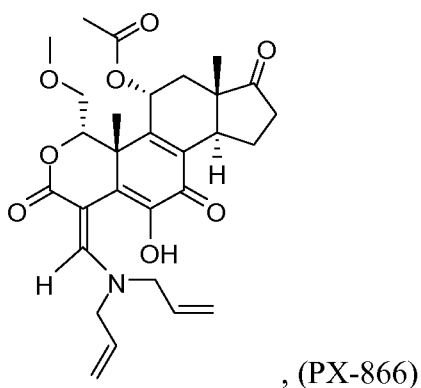
wherein Y is a heteroatom selected from nitrogen and sulfur;

R^1 and R^2 are independently selected from an unsaturated alkyl, cyclic alkyl, or R^1 and R^2 together with Y form a heterocycle;

or pharmaceutically acceptable salt, solvate, or polymorph thereof; in combination with a second anticancer agent.

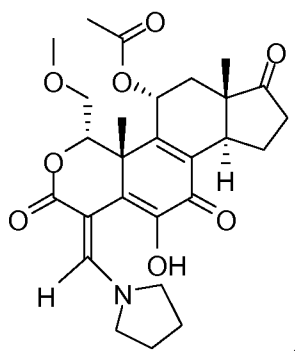
- [073] In some embodiments, the virus that a cancer tests positive for is an oncovirus.
- [074] In some of such embodiments, the virus that a cancer tests positive for is selected from Human T-lymphotropic virus (HTLV), Herpes virus, Human Papilloma Virus (HPV), Epstein Barr Virus (EBV), Hepatitis virus, and Merkel cell polyoma virus (MCV).
- [075] In some of such embodiments, the virus that the cancer tests positive for is HPV, EBV, Hepatitis A virus, Hepatitis B virus, Hepatitis C virus, MCV, HTLV1, HTLV2 or Kaposi's sarcoma-associated herpesvirus (KSHV).
- [076] In some of the embodiments described above, the cancer is selected from the group consisting of head and neck cancer, lung cancer, ovarian cancer, liver cancer, colon cancer, breast cancer, pancreatic cancer, kidney cancer, cervical cancer, uterine cancer, prostate cancer, esophageal cancer, nasopharyngeal cancer, oropharyngeal cancer, gastric cancer, skin cancer, vulvar cancer, vaginal cancer, anal cancer and penile cancer.
- [077] In some embodiments, the cancer is a solid tumor. In some embodiments, the cancer is unresectable. In some embodiments, the cancer is a locally advanced, recurrent or metastatic cancer.
- [078] In some embodiments, the cancer is a HPV positive squamous cell carcinoma. In some embodiments, the cancer is HPV positive head and neck cancer. In some embodiments, the cancer is HPV positive oropharyngeal cancer. In some embodiments, the cancer is HPV positive cervical cancer. In some embodiments, the cancer is HPV positive lung cancer. In some embodiments, the cancer is HPV positive non-small cell lung cancer.
- [079] In some embodiments, the cancer is an EBV positive B-cell malignancy. In some embodiments, the cancer is EBV positive Burkitt's lymphoma, Hodgkin's lymphoma, or diffuse large B-cell lymphoma. In some embodiments, the cancer is EBV positive nasopharyngeal cancer. In some embodiments, the cancer is EBV positive gastric carcinoma.
- [080] In some embodiments, the cancer is Hepatitis B positive hepatocellular carcinoma. In some embodiments, the cancer is Hepatitis C positive hepatocellular carcinoma.
- [081] In some embodiments, the cancer is HTLV positive leukemia. In some embodiments, the cancer is MCV positive skin cancer. In some embodiments, the cancer is a HHV-8 (Kaposi sarcoma-associated virus) positive Kaposi's sarcoma or primary effusion lymphoma.

[082] In some embodiments, the compound of Formula IIA or Formula IIB is:



or pharmaceutically acceptable salt, solvate or polymorph thereof.

[083] In some embodiments, the compound of Formula IIA or Formula IIB is

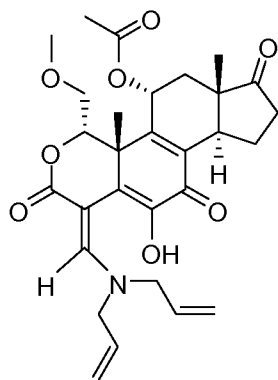


or pharmaceutically acceptable salt, solvate or polymorph thereof.

[084] In some embodiments, the second anticancer agent and the compound of Formula IIA or Formula IIB are administered simultaneously. In some embodiments, the second anticancer agent and the compound of Formula IIA or Formula IIB are administered sequentially. In some embodiments, the second anticancer agent and the compound of Formula IIA or Formula IIB are administered in a single composition.

[085] In some embodiments, administration of the compound of Formula IIA or Formula IIB is by intramuscular, intravenous, subcutaneous, intranodal, intratumoral, intracisternal, intraperitoneal, intradermal, transdermal, nasal, pulmonary, vaginal, rectal, buccal, ocular, otic, local, topical, or oral delivery. In some embodiments, the compound of Formula IIA or Formula IIB is administered orally. In some embodiments, the compound of Formula IIA or Formula IIB is administered in a capsule form.

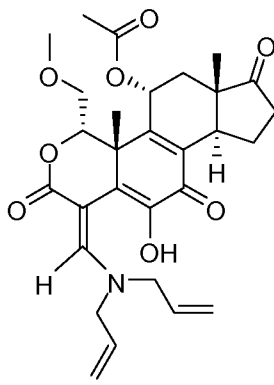
[086] In some embodiments, the compound of Formula IIA or Formula IIB is a crystalline form of a compound having the structure



(PX-866),

which is substantially free of wortmannin.

- [087] In some embodiments, the crystalline form described above has up to about 1% wortmannin. In some embodiments, the crystalline form described above has up to about 0.5 % wortmannin. In some embodiments, the crystalline form described above has up to about 0.25% wortmannin. In some embodiments, the crystalline form has undetectable levels of wortmannin (e.g., as measured on HPLC).
- [088] In some embodiments, the compound of Formula IIA or Formula IIB is a substantially pure crystalline form of a compound having a structural formula



wherein the crystalline form is a solvate.

- [089] In some embodiments, the solvate is a toluene, anisole, cumene, propyl acetate, 4-methyl-2-pentanone, chlorobenzene or 1-pentanol solvate.
- [090] In some embodiments, the crystalline form is crystalline anisole solvate. In some embodiments, the crystalline form is prepared from a solution comprising anisole.
- [091] In some embodiments, the crystalline form exhibits a predominant endotherm at about 146.0 °C as measured by Differential Scanning Calorimeter at a scan rate of 10 °C per minute.
- [092] In some embodiments, the crystalline form has an X-ray powder diffraction pattern having at least two 2-theta values selected from 7.9, 8.5, 10.2, 11.1, 14.0, 14.2, 17.9, 18.7, 21.0, 21.2, and 28.2 ±0.1.

- [093] In some embodiments, the crystalline form exhibits a single crystal X-ray crystallographic analysis at 120 K with crystal parameters as the following:

Space Group	P2 ₁ 2 ₁ 2 ₁
a, Å	13.7140(3)
b, Å	15.4272(4)
c, Å	15.6890(4)
α	90
β	90
γ	90
Z (molecules/unit cell)	4
Calculated Density (g/cm) 1.268.	

- [094] In some embodiments, the crystalline form is crystalline propyl acetate solvate. In some embodiments, the crystalline form is prepared from a solution comprising propyl acetate.
- [095] In some embodiments, the crystalline form exhibits a predominant endotherm at about 80.5 °C as measured by Differential Scanning Calorimeter at a scan rate of 10 °C per minute.
- [096] In some embodiments, the crystalline form has an X-ray powder diffraction pattern having at least two 2-theta values selected from 8.0, 8.4, 10.2, 11.0, 14.0 and 19.2, ± 0.1 .
- [097] In some embodiments, the crystalline form exhibits a single crystal x-ray crystallographic analysis at 100 K with crystal parameters as the following:

Space Group	P2 ₁ 2 ₁ 2 ₁
a, Å	13.4963(5)
b, Å	15.5158(5)
c, Å	15.6912(6)
α	90

β 90

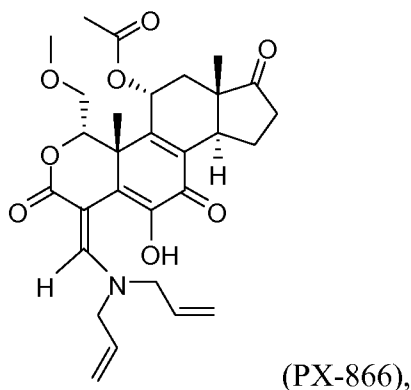
γ 90

Z (molecules/unit cell) 4

Calculated Density (g/cm) 1.269.

- [098] In some embodiments, the crystalline form is crystalline toluene solvate. In some embodiments, the crystalline form is prepared from a solution comprising toluene.
- [099] In some embodiments, the crystalline form exhibits a predominant endotherm at about 142.0 °C as measured by Differential Scanning Calorimeter at a scan rate of 10 °C per minute.
- [0100] In some embodiments, the crystalline form has an X-ray powder diffraction pattern having at least two 2-theta values selected from 12.5, 14.0, and 21.1, ± 0.1 .
- [0101] In some embodiments, the crystalline form is crystalline cumene solvate. In some embodiments, the crystalline form is prepared from a solution comprising cumene.
- [0102] In some embodiments, the crystalline form has an X-ray powder diffraction pattern expressed in degrees 2-theta at 7.8, 8.4, 10.1, 10.7, 13.7, 14.1, 18.1, 18.9, 20.6, and 20.8, ± 0.1 .
- [0103] In some embodiments, the crystalline form is crystalline 4-methyl-2-pentanone solvate. In some embodiments, the crystalline form is prepared from a solution comprising 4-methyl-2-pentanone.
- [0104] In some embodiments, the crystalline form has an X-ray powder diffraction pattern expressed in degrees 2-theta at 7.9, 8.4, 10.2, 10.9, 13.9, 14.2, 18.5, 19.2, and 20.7, ± 0.1 .
- [0105] In some embodiments, the crystalline form is crystalline PX-866-pentanol solvate. In some embodiments, the crystalline form is prepared from a solution comprising 1-pentanol.
- [0106] In some embodiments, the crystalline form has an X-ray powder diffraction pattern expressed in degrees 2-theta at 8.1, 8.5, 10.2, 11.1, 12.5, 14.0, 14.3, 17.9, 18.8, 20.7, and 21.3, ± 0.1 .
- [0107] In some embodiments, the crystalline form is crystalline chlorobenzene solvate. In some embodiments, the crystalline form is prepared from a solution comprising chlorobenzene.

- [0108] In some embodiments, the crystalline form has an x-ray powder diffraction pattern expressed in degrees 2-theta at 8.0, 8.5, 10.3, 11.1, 14.1, 17.9, 18.8, 19.1, 21.0 and 28.3, ± 0.1 .
- [0109] In some embodiments, the crystalline form exhibits higher stability than amorphous form.
- [0110] In any of the aforementioned embodiments, administration of PX-866 alone, or in combination with an anticancer described herein allows for treatment of an oncovirus positive cancer (e.g., HPV positive cancer, EBV positive cancer, Hepatitis B positive cancer, Hepatitis C positive cancer, and the like).
- [0111] Also contemplated within the scope of embodiments presented herein are methods of treatment of cancers that test positive for a virus comprising monotherapy with a PI-3 kinase inhibitor (e.g., a wortmannin analog (e.g. a compound of Formula IA, Formula IB, Formula IIA or Formula IIB, e.g., PX-866, or salt or polymorph thereof)) described herein. Also contemplated within the scope of methods described herein are single agent methods of treatment of cancers that test positive for a virus (e.g., an oncovirus described herein) comprising administration of any wortmanin analog described herein (e.g. a compound of Formula IA, Formula IB, Formula IIA or Formula IIB) to an individual in need thereof. In some of such embodiments, a cancer that tests positive for a virus is an HPV positive cancer as described herein, an EBV positive cancer as described herein, Hepatitis virus positive cancer as described herein (e.g., Hepatitis A, or B or C positive cancer), an MCV positive cancer as described herein, a KSHV positive cancer as described herein, or an HTLV positive cancer as described herein.
- [0112] In some embodiments of the uses of PI-3 kinase inhibitors described above and herein and herein, and the methods of treatment described herein, the PI-3 kinase inhibitor is a crystalline form of a compound having a structural formula



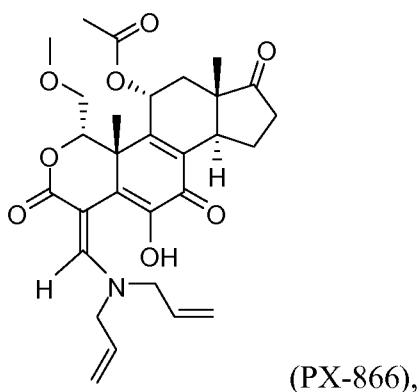
wherein the form is

(a) a crystalline anisole solvate; and

(b) has an X-ray powder diffraction pattern (XRPD) with characteristic peaks at 7.9 ± 0.1 degrees 2-Theta, 8.5 ± 0.1 degrees 2-Theta, 10.2 ± 0.1 degrees 2-Theta, 11.1 ± 0.1 degrees 2-Theta, 14.0 ± 0.1 degrees 2-Theta, 14.2 ± 0.1 degrees 2-Theta, 17.9 ± 0.1 degrees 2-Theta, 18.7 ± 0.1 degrees 2-Theta, 21.0 ± 0.1 degrees 2-Theta, 21.2 ± 0.1 degrees 2-Theta, and 28.2 ± 0.1 degrees 2-Theta.

[0113] Accordingly, provided herein is a crystalline anisole solvate form of PX-866 having an XRPD of FIG. 1.

[0114] In some embodiments of the uses of PI-3 kinase inhibitors described above and herein and herein, and the methods of treatment described herein, the PI-3 kinase inhibitor is a crystalline form of a compound having a structural formula



wherein the form is

(a) a crystalline anisole solvate; and

(b) has an X-ray powder diffraction pattern (XRPD) with characteristic peaks at 10.2 ± 0.1 degrees 2-Theta, 11.1 ± 0.1 degrees 2-Theta, 14.0 ± 0.1 degrees 2-Theta, 14.2 ± 0.1 degrees 2-Theta, and 21.0 ± 0.1 degrees 2-Theta.

[0115] In some embodiments, the crystalline form described above is a substantially pure crystalline form. In some embodiments, the crystalline form described above has a purity of at least 90%. In some embodiments, the crystalline form described above has a purity of at least 95%. In some embodiments, the crystalline form described above has a purity of at least 98%.

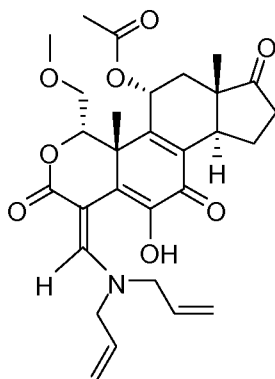
[0116] The crystalline form described above exhibits a predominant endotherm at about 146°C as measured by Differential Scanning Calorimeter. In some of such embodiments, the endotherm is observed when using a scan rate of 10°C per minute.

[0117] In one embodiment, provided herein are crystalline forms of PX-866, wherein the forms have a general space group $P2_12_12_1$.

[0118] The crystalline form described above exhibits a single crystal X-ray crystallographic analysis at 120 K with the following crystal parameters:

Space Group	P2 ₁ 2 ₁ 2 ₁
a, Å	13.7140(3) [°]
b, Å	15.4272(4)
c, Å	15.6890(4)
α	90
β	90
γ	90
Z (molecules/unit cell)	4 [°]
Calculated Density (g/cm)	1.268. [°]

[0119] In some embodiments of the uses of PI-3 kinase inhibitors described above and herein and herein, and the methods of treatment described herein, the PI-3 kinase inhibitor is a crystalline form of a compound having a structural formula



(PX-866),

wherein the crystalline form exhibits a predominant endotherm at about 146 °C as measured by Differential Scanning Calorimeter.

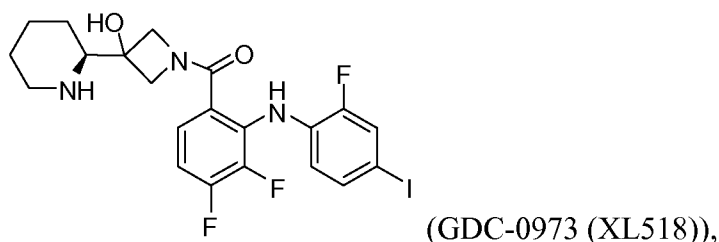
Anticancer agents for combination therapies

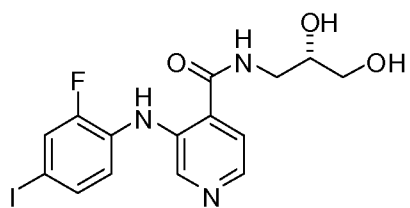
[0120] Contemplated for use within the scope of methods described herein are anticancer agents that are used in combination with a PI-3 kinase inhibitor (e.g., any PI-3 kinase inhibitor described herein, a wortmannin analog (e.g., a compound of Formula IA, IB, IIA or IIB as described herein, e.g., PX-866 and the like)) for treatment of cancers, including oncovirus positive cancers. Non-limiting examples of anticancer agents include methotrexate (RHEUMATREX®, Amethopterin) cyclophosphamide (CYTOXAN®), thalidomide (THALIDOMID®), acridine carboxamide, actimid®, actinomycin, 17-N-allylamino-17-demethoxygeldanamycin, aminopterin, amsacrine, anthracycline, bevacuzimab (Avastin®), antineoplaston, 5-azacytidine, azathioprine, BL22, bendamustine, biricodar, bleomycin, bortezomib, bryostatin, busulfan, calyculin, camptothecin, capecitabine, carboplatin, cetuximab, chlorambucil, cisplatin, cladribine,

clofarabine, cytarabine, dacarbazine, dasatinib, daunorubicin, decitabine, dichloroacetic acid, discodermolide, docetaxel, doxorubicin, epirubicin, epothilone, eribulin, estramustine, etoposide, exatecan, exisulind, ferruginol, floxuridine, fludarabine, fluorouracil, fosfestrol, fotemustine, ganciclovir, gefitinib (Iressa®), gemcitabine, hydroxyurea, IT-101, idarubicin, ifosfamide, imiquimod, irinotecan, irofulven, ixabepilone, laniquidar, lapatinib, lenalidomide, lomustine, lurtotecan, mafosfamide, masoprocol, mechlorethamine, melphalan, mercaptopurine, mitomycin, mitotane, mitoxantrone, nelarabine, nilotinib, oblimersen, oxaliplatin, PAC-1, paclitaxel, pemetrexed, pentostatin, pipobroman, pixantrone, plicamycin, procarbazine, proteasome inhibitors (e.g., bortezomib), raltitrexed, rebeccamycin, revlimid®, rubitecan, SN-38, salinosporamide A, satraplatin, streptozotocin, swainsonine, tariquidar, taxane, tegafur-uracil, temozolomide, testolactone, thioTEPA, tioguanine, topotecan, trabectedin, tretinoin, triplatin tetranitrate, tris(2-chloroethyl)amine, troxacitabine, uracil mustard, valrubicin, vinblastine, vincristine, vinorelbine, vorinostat, and zosuquidar. In some embodiments, anticancer agents include farnesyl protein transferase inhibitors, topoisomerase inhibitors, microtubule stabilizers, anti-metabolites and the like.

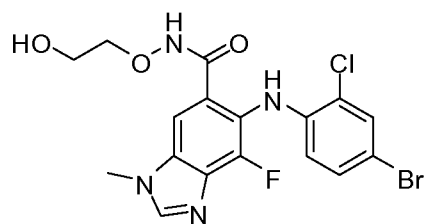
[0121] In some embodiments, the second anticancer agent is selected from docetaxel, cetuximab, gefitinib, and/or a platinum based chemotherapeutic agent. In some of such embodiments, a platinum based chemotherapeutic agent is cisplatin, carboplatin, oxiplatin, bisplatin agents and the like. In some embodiments, the second anticancer agent is docetaxel. In some embodiments, the second anticancer agent is cetuximab. In some embodiments, the second anticancer agent is gefitinib. Example 20, FIG. 14, FIG. 15, FIG. 16, FIG. 17 describe experiments with certain direct patient tumor models (DPTM) involving the use of PX-866 in combination with docetaxel or cetuximab for treatment of oncovirus positive cancers.

[0122] Also contemplated within the scope of the uses of PI-3 kinase inhibitors described above and herein, and methods of treatment described herein is the use of MEK inhibitors as a second anticancer agent. Examples of such MEK inhibitors include, and are not limited, to

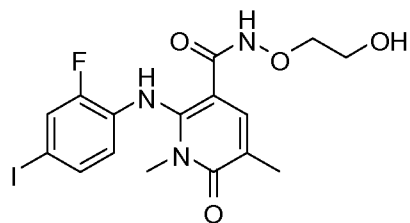




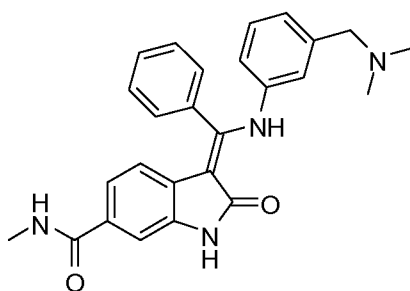
(AS703026),



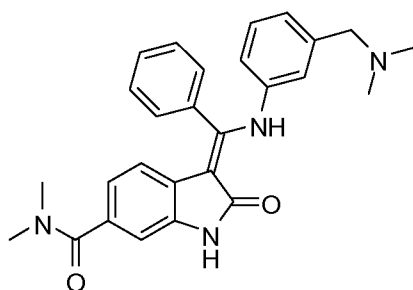
(AZD6244 (Selumetinib) (ARRY-142886)),



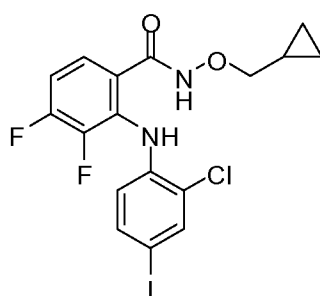
(AZD8330 (ARRY-424704, ARRY-704)),



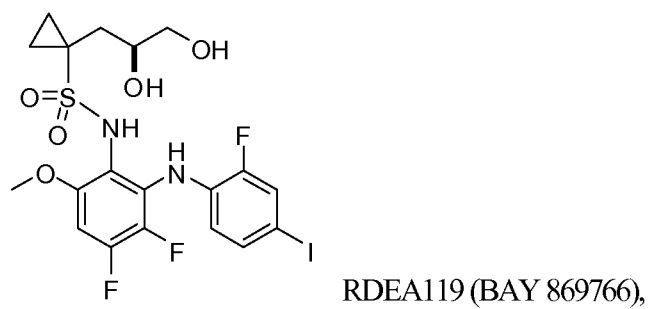
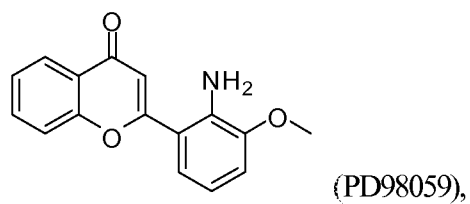
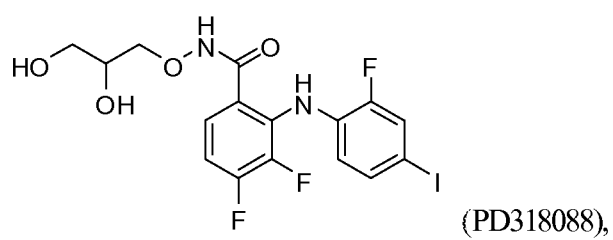
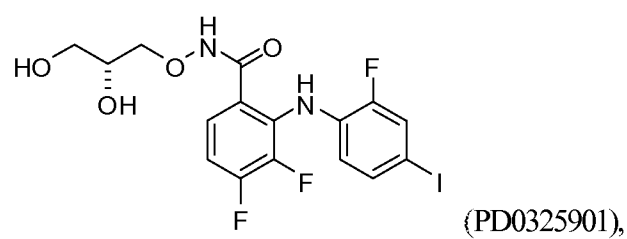
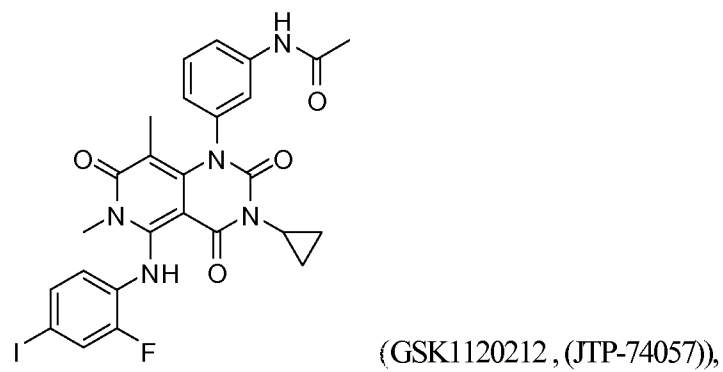
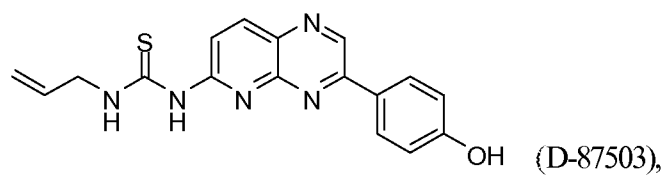
(BIX 02188),

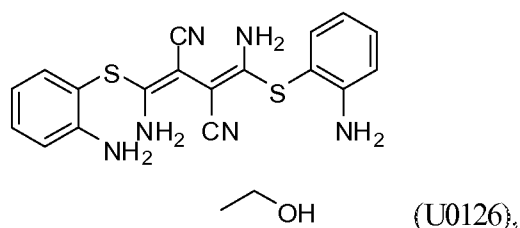
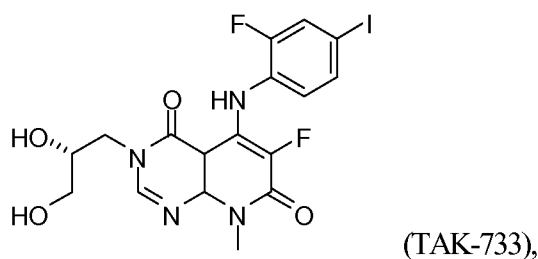


(BIX 02189),



(CI-1040 (PD184352)),





ARRY-162/ OP-0124 (Array/Novartis) and the like.

Further Combinations

[0123] In some embodiments, the uses of PI-3 kinase inhibitors described above and herein, and methods of treatment described herein further comprise the use of additional therapeutic agents.

[0124] Additional anti-cancer therapies include radiotherapy, immunotherapy, gene therapy, surgery or other therapies that are capable of negatively affecting cancer in a patient, such as for example, by killing cancer cells, inducing apoptosis in cancer cells, reducing the growth rate of cancer cells, reducing the incidence or number of metastases, reducing tumor size, inhibiting tumor growth, reducing the blood supply to a tumor or cancer cells, promoting an immune response against cancer cells or a tumor, preventing or inhibiting the progression of cancer, or increasing the lifespan of a subject with cancer.

[0125] Radiotherapies include factors that cause DNA damage and have been used extensively include what are commonly known as γ -rays, X-rays, and/or the directed delivery of radioisotopes to tumor cells. Other forms of DNA damaging factors are also contemplated such as microwaves and UV-irradiation. It is likely that all of these factors affect a broad range of damage on DNA, on the precursors of DNA, on the replication and repair of DNA, and on the assembly and maintenance of chromosomes. Dosage ranges for X-rays may range from daily doses of 50 to 200 roentgens for prolonged periods of time (e.g., 3 to 4 weeks), to single doses of 2000 to 6000 roentgens. Dosage ranges for radioisotopes vary widely, and depend on the half-life of the isotope, the strength and type of radiation emitted, and the uptake by the neoplastic cells. In some embodiments, the combination therapies described herein are administered with a radiotherapy.

- [0126] Immunotherapies, generally, rely on the use of immune effector cells and molecules to target and destroy cancer cells. The immune effector may be, for example, a tumor antigen or an antibody specific for some marker on the surface of a tumor cell. The tumor antigen or antibody alone may serve as an effector of therapy or it may recruit other cells to actually effect cell killing. An antibody also may be conjugated to a drug or toxin (chemotherapeutic, radionuclide, ricin A chain, cholera toxin, pertussis toxin, etc.) and serve merely as a targeting agent. Alternatively, the effector may be a lymphocyte carrying a surface molecule that interacts, either directly or indirectly, with a tumor cell target. Various effector cells include cytotoxic T cells and NK cells. Alternatively, a tumor antigen may stimulate a subject's immune system to target the specific tumor cells using cytotoxic T cells and NK cells. In some embodiments, the combination therapies described herein are administered with an immunotherapy.
- [0127] In other embodiments, an additional anti-cancer therapy is a gene therapy in which a therapeutic polynucleotide is administered before, after, or at the same time as a combination therapy. Therapeutic genes may include an antisense version of an inducer of cellular proliferation (oncogene), an inhibitor of cellular proliferation (tumor suppressor), or an inducer of programmed cell death (pro-apoptotic gene). In some embodiments, the combination therapies described herein are administered with a gene therapy.
- [0128] In further embodiments, surgery of some type is performed in conjunction with the combination therapies described herein. Surgery types include preventative, diagnostic or staging, curative and palliative surgery and are performed prior to, during, or subsequent to the combination therapy.
- [0129] In some embodiments, an additional agent used to treat adjunct conditions associated with the cancer or a side effect from either the PI-3 kinase inhibitor or the second anticancer agent in the combination therapy. Additional agents include, but are not limited to, anti-inflammatories, anti-emetics, anti-diarrheals, analgesics, anti-hyperglycemics, antivirals and the like. In certain instances, the additional agents are administered prophylactically or as a pre-treatment prior to the anticancer agent or the PI-3 kinase inhibitor. In other instances, the additional agents are administered on a needed basis, i.e., when a condition or side effect arises.
- [0130] Anti-inflammatories are used to treat or reduce the incidence and severity of, for example, inflammatory conditions, fluid retention or hypersensitivity reactions that result from the one or both of the agents in the combination therapy and/or conditions from the

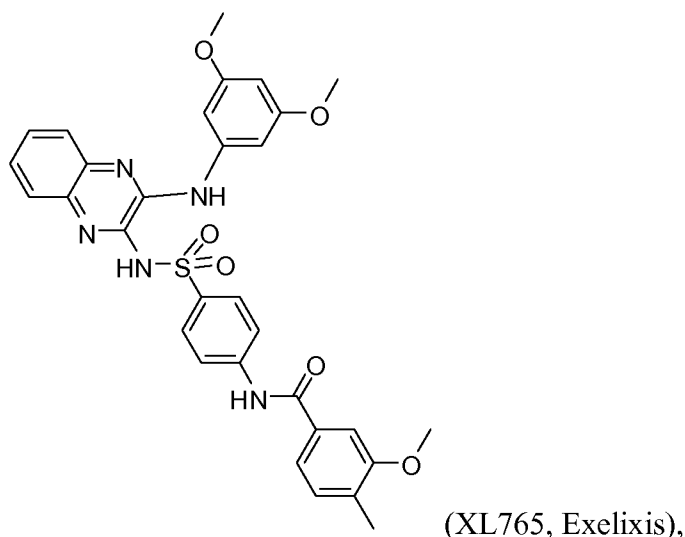
cancer. Anti-inflammatories include, but are not limited to corticosteroids (e.g., dexamethasone, prednisone, hydrocortisone, betamethasone, and the like); NSAIDS such as arylcarboxylic acids (salicylic acid, acetylsalicylic acid, diflunisal, choline magnesium trisalicylate, salicylate, benorylate, flufenamic acid, mefenamic acid, meclofenamic acid and triflumic acid), arylalkanoic acids (diclofenac, fenclofenac, alclofenac, fentiazac, ibuprofen, flurbiprofen, ketoprofen, naproxen, fenoprofen, fenbufen, suprofen, indoprofen, tiaprofenic acid, benoxaprofen, piroprofen, tolmetin, zomepirac, clopinac, indomethacin and sulindac) and enolic acids (phenylbutazone, oxyphenbutazone, azapropazone, feprazone, piroxicam, and isoxicam); and anti-histamines such as cimetidine, ranitidine, famotidine and nizatidine.

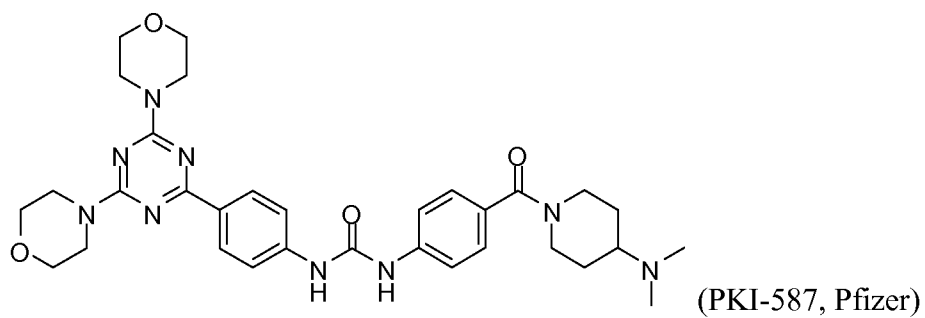
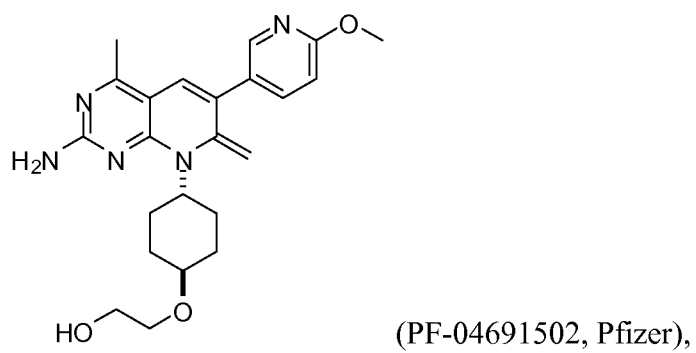
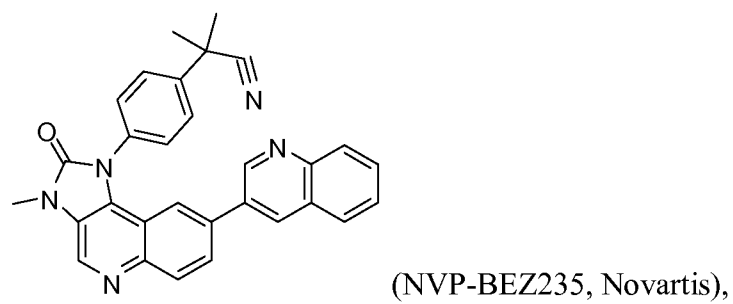
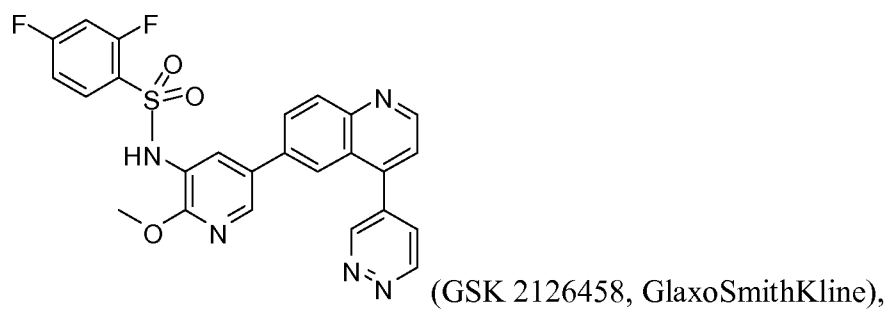
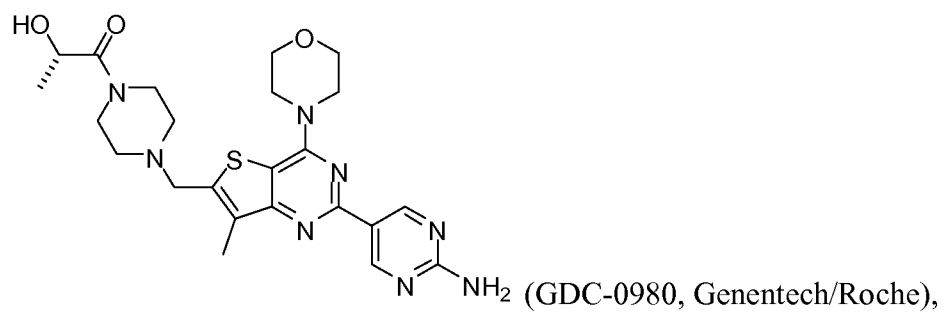
- [0131] Anti-emetics are used to treat or prevent nausea or vomiting associated with the cancer or one or both of the agents of the combination therapy. Anti-emetics include 5-HT receptor antagonists (ondansetron, granisetron, dolasetron, tropisetron, palonosetron, mirtazapine, etc.), dopamine antagonists (haloperidol, droperidol, prochlorperazine, etc.), antihistamines such as H₁ antagonists (promethazine, diphenhydramine, meclizine, etc.), benzodiazepines (lorazepam, midazolam), cannabinoids, and dexamethasone. Other known anti-emetics are used as in conjunction with the combination therapy in some embodiments.
- [0132] Anti-diarrheals are used to treat or prevent diarrhea associated with the cancer or one or both of the agents of the combination therapy. Anti-diarrheals include bismuth subsalicylate, loperamide, diphenoxylate, difenoxin, as well as other opioids.
- [0133] Analgesics are used to treat or prevent acute or chronic pain associated with the cancer or one or both of the agents of the combination therapy. Analgesics include acetaminophen, NSAIDS and opioid drugs (morphine, hydromorphone, fentanyl, tramadol, oxymorphone, oxycodone, hydrocodone, etc.) and COX-2 inhibitors.
- [0134] Anti-hyperglycemic agents are used to treat or prevent side effects such as hyperglycemia. Anti-hyperglycemic agents include biguanides such as metformin; sulfonyl ureas such as chlorpropamide tolbutamide, glyburide (DiaBeta®), glipizide (Glucotrol®), glimepiride (Amaryl®); meglitinides such as repaglinide (Prandin®), nateglinide (Starlix®); thiazolidinediones such as pioglitazone and rosiglitazone; pramlintide (Symlin®), exenatide (Byetta®), liraglutide (Victoza®); DPP-IV inhibitors such as sitagliptin (Januvia®), saxagliptin (Onglyza®), linagliptin (Tradjenta®); insulin, and the like.

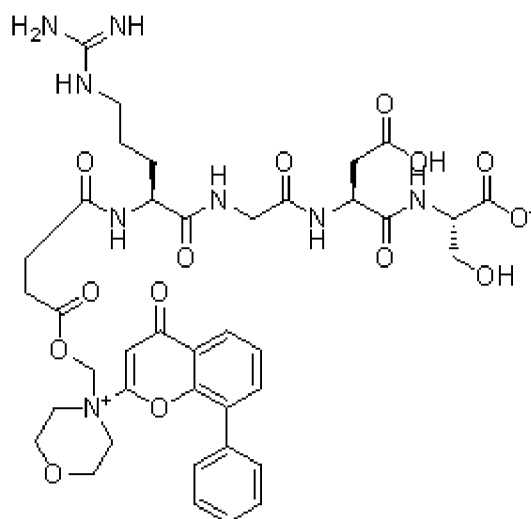
[0135] Anti-virals are used to treat or prevent viral infections that a oncovirus cancer is associated with. For example, in one embodiment, the uses of PI-3 kinase inhibitors and the methods of treatment described above and herein further comprise the use of antivirals such as acyclovir, famciclovir, valacyclovir, abacavir, aciclovir, adfovir, amantadine, amprenavir, arbidol, atazanavir, artipia, brivudine, cidofovir, combivir, edoxudine, efavirenz, emtricitabine, enfuvirtide, entecavir, fomvirsen, fosamprenavir, foscarnet, fosfonet, gancyclovir, gardasil, ibacitabine, imunovir, idoxuridine, imiquimod, indinavir, inosine, integrase inhibitors, interferons, including interferon type III, interferon type II, interferon type I, lamivudine, lopinavir, loviride, MK-0518, maraviroc, moroxydine, nelfinavir, nevirapine, nexavir, nucleoside analogues, oseltamivir, penciclovir, peramivir, pleconaril, podophyllotoxin, protease inhibitors, reverse transcriptase inhibitors, ribavirin, rimantadine, ritonavir, saquinavir, stavudine, tenofovir, tenofovir disoproxil, tipranavir, trifluridine, trizivir, tromantadine, truvada, valganciclovir, vicriviroc, vidarabine, viramidine, zalcitabine, zanamivir, zidovudine, and/or combinations thereof.

PI-3 kinase inhibitors

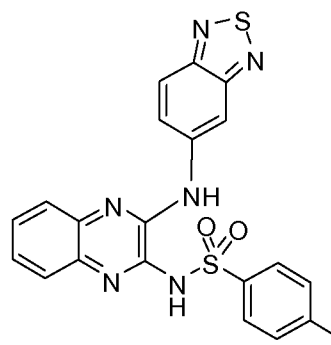
[0136] In some embodiments, PI-3 kinase inhibitors suitable for treatment of oncovirus-positive cancers as described above and herein include, and are not limited to, small molecule PI-3 kinase inhibitors and antibodies. In some embodiments, PI-3 kinase inhibitors suitable for treatment of oncovirus-positive cancers as described above and herein include one or more of the following:



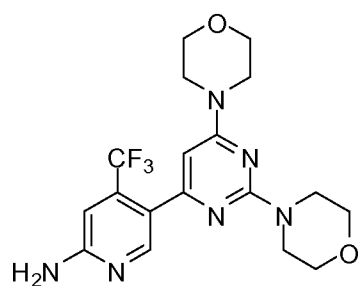




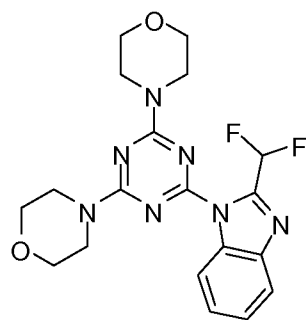
(SF 1126, Semafore),



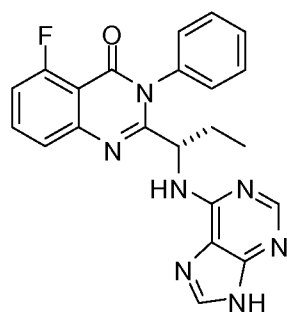
(XL147, Exelixis),



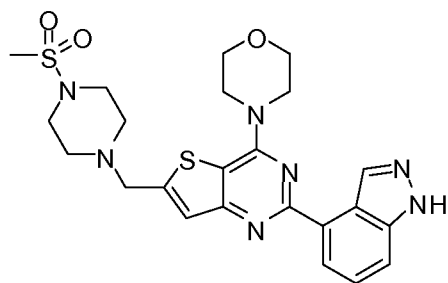
(NVP-BKM120, Novartis),



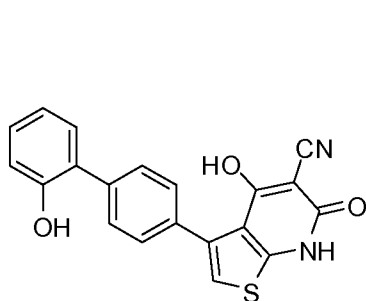
(ZSTK474, Zenyaku Kogyo),



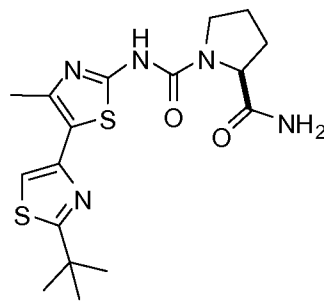
(CAL 101, Calistoga),



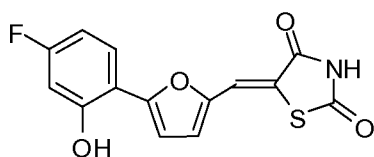
(GDC0941, Genentech/Roche),



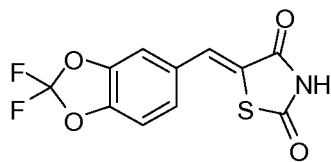
(A-769662),



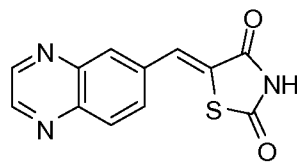
(A66),



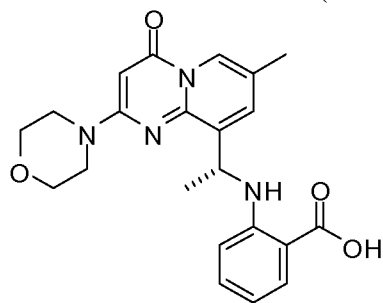
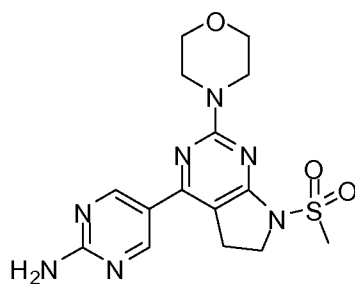
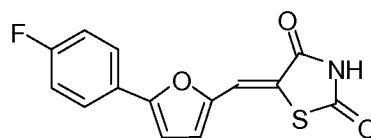
(AS-252424),



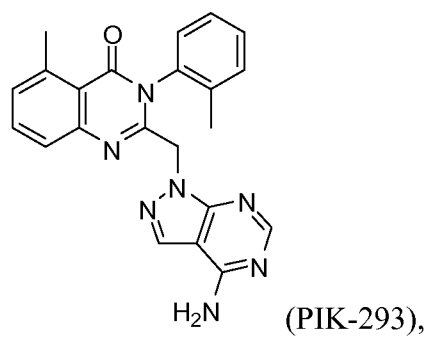
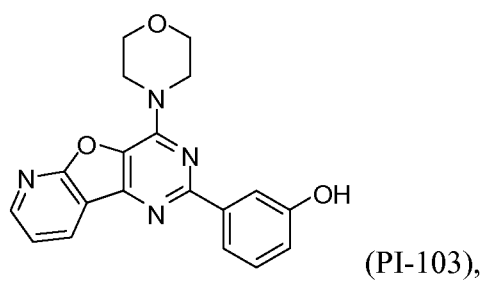
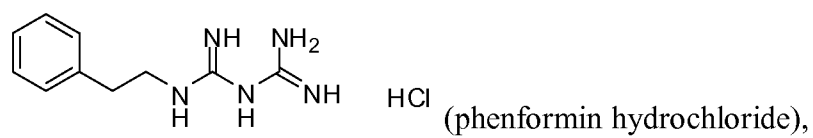
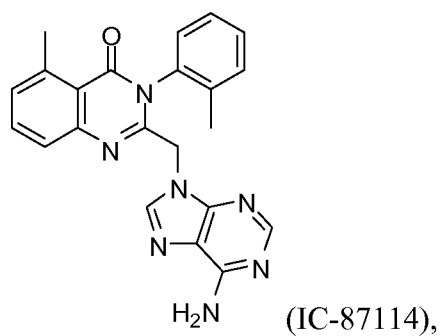
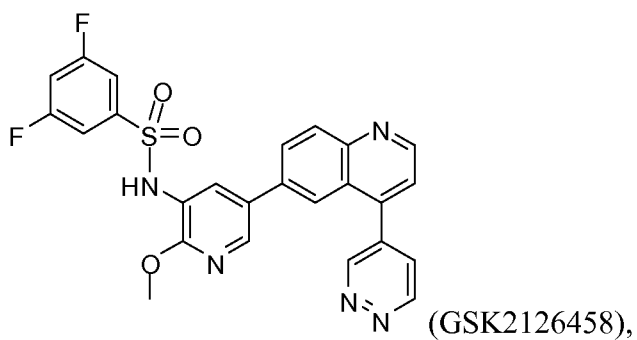
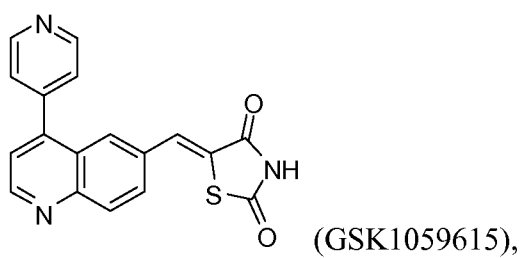
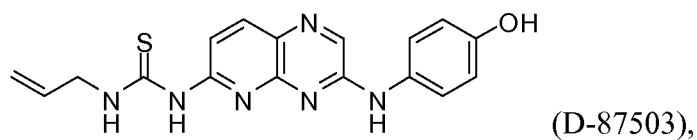
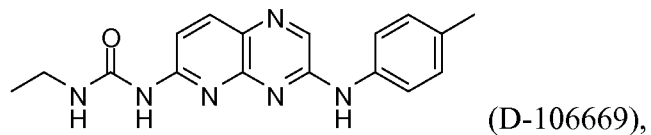
(AS-604850),

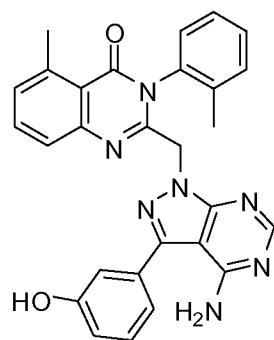


(AS605240),

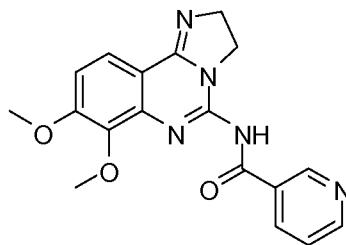
(AZD6482),
(CAY10505),

(CH5132799),

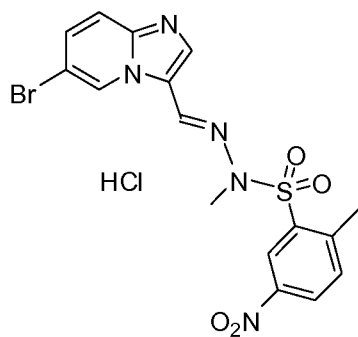




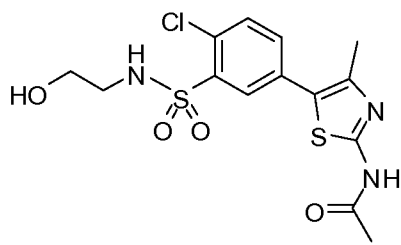
(PIK-294),



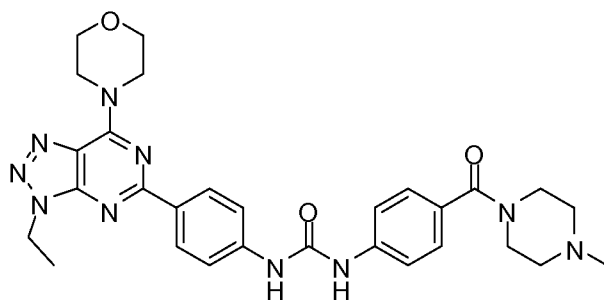
(PIK-90),



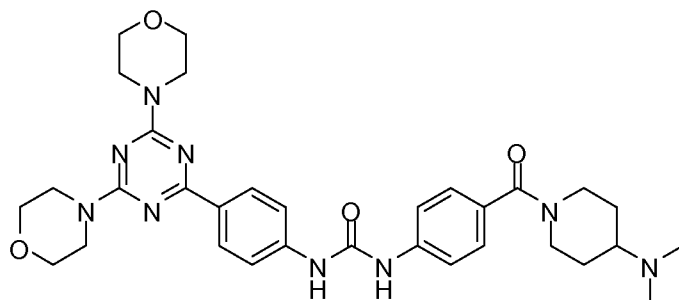
(PIK-75),



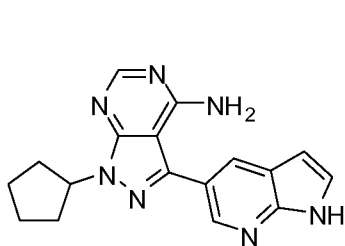
(PIK-93),



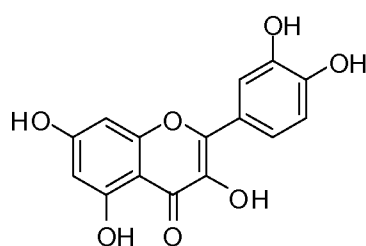
(PKI-402),



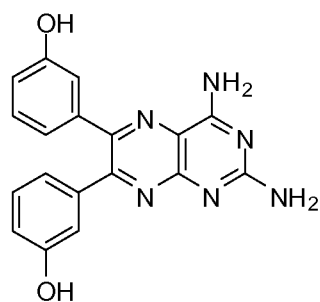
(PKI-587 (PF-05212384)),



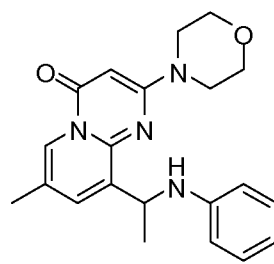
(PP121),



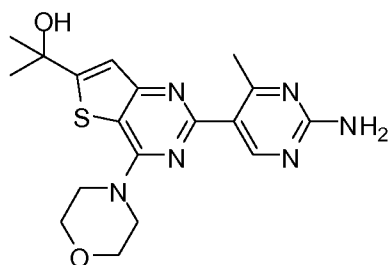
(Quercetin (Sophoretin))



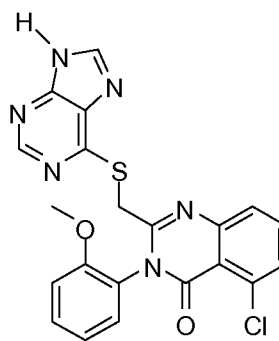
(TG100-115),



(TGX-221),



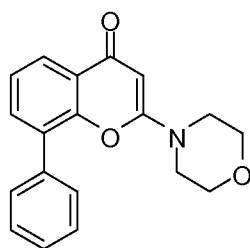
(GNE-490),



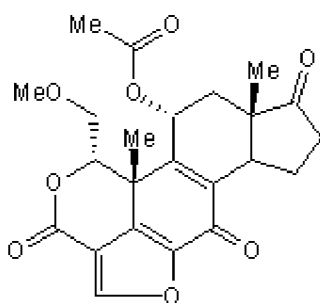
(PIK-39),

BAY806946 (Bayer), GDC0032 (Genentech/Roche), BYL719 (Novartis), AMG319 (Amgen), CNX-1351 (Celgene/Avila), INK117 (Intellikine) and the like.

[0137] In some further embodiments, PI-3 kinase inhibitors suitable for uses and methods described herein are



(LY294002, Lilly), and



(wortmannin).

[0138] In further embodiments, PI-3 kinase inhibitors suitable for uses and methods described herein are wortmannin analogs as described herein.

- [0139] In some embodiments of the uses of PI-3 kinase inhibitors described above and herein, and the methods of treatment described herein, the PI-3 kinase inhibitor is selected from PX-866, XL 147, GDC-0941 (Genentech/Roche), CAL-101 (Calistoga), NVP-BKM120 (Novartis), ZSTK474 (Zenyaku Kogyo), NVP-BYL179 (Novartis), AMG319 (Amgen), GDC0032 (Genentech/Roche), A66, AS-252424, AS-604850, AS-605240, AZD6482, CAY10505, CH5132799, D-106669, GSK1059615, PIK-293, PIK-90, PIK-93, GNE-490, CNX-1351 (Celgene/Avila), INK117 (Intellikine) and PIK-39.
- [0140] In some embodiments of the uses of PI-3 kinase inhibitors described above and herein, and the methods of treatment described herein, the PI-3 kinase inhibitor is selected from BAY806946 (Bayer), XL 765 (Exelixis), GDC-0980 (Genentech), GSK 2126458 (GlaxoSmithKline), NVP-BEZ235 (Novartis), NVP-BGT226 (Novartis), PF04691503 (Pfizer), PKI587 (Pfizer), SF1126 (Semaphore), D-87503, GSK2126458, IC-87114, PI-103, PIK-294, PIK-75, PKI-402, PKI-587 (PF-05212384), Quercetin (Sophoretin), TG100-115, and TGX-221.
- [0141] In some embodiments of the uses of PI-3 kinase inhibitors described above and herein, and the methods of treatment described herein, the PI-3 kinase inhibitor is selected from A-769662, phenformin hydrochloride, and PP121.
- [0142] In some embodiments of the uses of PI-3 kinase inhibitors described above and herein, and the methods of treatment described herein, the PI-3 kinase inhibitor is a wortmannin analog.
- [0143] In some embodiments of the uses of PI-3 kinase inhibitors described above and herein, and the methods of treatment described herein, the PI-3 kinase inhibitor is selected from LY294002 (Lilly), and wortmannin.
- [0144] In some embodiments of the uses of PI-3 kinase inhibitors described above and herein, and the methods of treatment described herein, the PI-3 kinase inhibitor is a selective PI-3 kinase inhibitor (e.g., selective for PI-3 kinase over mTOR, PX-866, XL 147, GDC-0941 (Genentech/Roche), CAL-101 (Calistoga), NVP-BKM120 (Novartis), ZSTK474 (Zenyaku Kogyo), NVP-BYL179 (Novartis), AMG319 (Amgen), GDC0032 (Genentech/Roche), A66, AS-252424, AS-604850, AS-605240, AZD6482, CAY10505, CH5132799, D-106669, GSK1059615, PIK-293, PIK-90, PIK-93, PIK-39, GNE-490 and the like). In some embodiments of the uses of PI-3 kinase inhibitors described above and herein, and the methods of treatment described herein, the PI-3 kinase inhibitor is an isoform selective PI-3 kinase inhibitor (e.g., selectively inhibits one isoform of PI-3 kinase over other isoforms of PI-3 kinase, e.g., CNX-1351 (Celgene/Avila), INK117

(Intellikine)). In some embodiments of the uses of PI-3 kinase inhibitors described above and herein, and the methods of treatment described herein, the PI-3 kinase inhibitor is a pan-PI-3 kinase inhibitor (e.g., inhibits more than one PI-3 kinase isoforms). By way of example, Berndt et al. describe certain isoform selective PI-3 kinase inhibitors (*Nat Chem Biol.* 2010 February; 6(2): 117–124) which disclosure is incorporated herein by reference.

[0145] In some embodiments of the uses of PI-3 kinase inhibitors described above and herein, and the methods of treatment described herein, the PI-3 kinase inhibitor is a mixed kinase inhibitor inhibits multiple kinases including at least one isoform of a PI-3 kinase (e.g., inhibits one or more PI-3 kinase isoforms, mTOR, and/or AKT, or a combination thereof, and the like, e.g., BAY806946 (Bayer), XL 765 (Exelixis), GDC-0980 (Genentech), GSK 2126458 (GlaxoSmithKline), NVP-BEZ235 (Novartis), NVP-BGT226 (Novartis), PF04691503 (Pfizer), PKI587 (Pfizer), SF1126 (Semaphore), D-87503, GSK2126458, IC-87114, PI-103, PIK-294, PIK-75, PKI-402, PKI-587 (PF-05212384), Quercetin (Sophoretin), TG100-115, TGX-221 and the like). In some embodiments of the uses of PI-3 kinase inhibitors described above and herein, and the methods of treatment described herein, the PI-3 kinase inhibitor is an AMPK modulator (e.g., A-769662) and indirectly inhibits PI-3 kinase.

Wortmannin Analogs

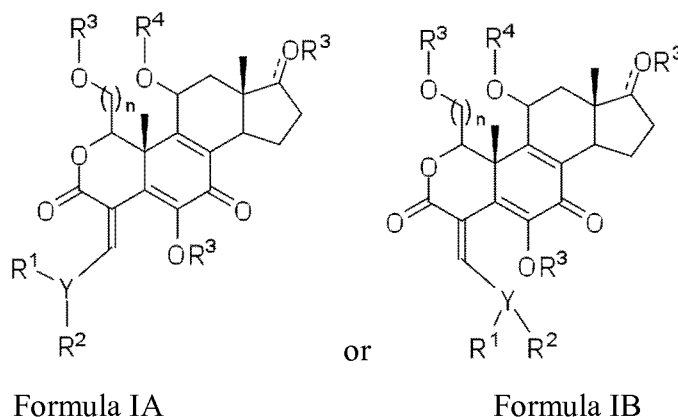
[0146] In further embodiments, PI-3 kinase inhibitors suitable for treatment of oncovirus-positive cancers as described above and herein include, and are not limited to, wortmannin analogs as described herein.

[0147] Wortmannin is a naturally occurring compound isolated from culture broths of fungal strains, *Penicillium wortmannin*, *Talaromyces wortmannin*, *Penicillium Funiculosum* and related micro-organisms. Wortmannin irreversibly inhibits PI-3K through covalent interaction with a specific lysine on the kinase: Lys⁸⁰² of the ATP binding pocket of the catalytic site of the p110 α isoform or Lys⁸⁸³ of the p110 γ isoform. Most isoforms of PI-3K, such as p110 α , p110 β , p110 δ and p110 γ for example, are inhibited equally by wortmannin. Wortmannin demonstrates liver and hematologic toxicity, however, and is a biologically unstable molecule. Samples stored as aqueous solutions at either 37°C or 0°C at neutral pH are subject to decomposition by hydrolytic opening of the furan ring. It has been shown that the electrophilicity of the furan ring is central to the inhibitory activity of wortmannin. The irreversible inhibition of PI-3K occurs by formation of an enamine following the attack of the active lysine of the kinase on the furan ring at

position C(20) of wortmannin. Decomposition of wortmannin interferes with its inhibitory activity on PI-3Ks. Although wortmannin is a nanomolar inhibitor of PI-3K, its instability and toxicity to the liver results in variable activity in animal models.

Wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB) described herein have reduced toxicity and better stability compared to the base wortmannin compound.

[0148] In some embodiments, wortmannin analogs suitable for methods/uses described herein and/or for preparation of medicaments described herein include compounds of Formula IA or IB:



wherein:

--- is an optional bond;

n is 1-6;

Y is a heteroatom;

R¹ and R² are independently selected from an unsaturated alkyl, non-linear alkyl, cyclic alkyl, and substituted alkyl or R¹ and R² together with the atom to which they are attached form a heterocycloalkyl group;

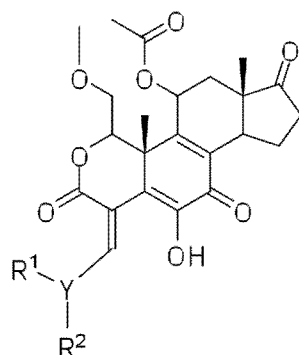
R³ is absent, H, or C₁-C₆ substituted or unsubstituted alkyl;

R⁴ is (C=O)R⁵, (C=O)OR⁵, (S=O)R⁵, (SO₂)R⁵, (PO₃)R⁵, (C=O)NR⁵R⁶;

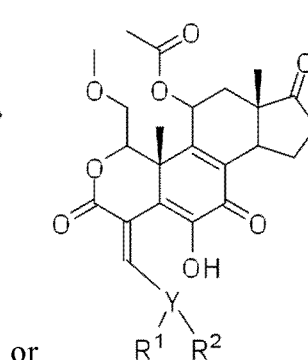
R⁵ is substituted or unsubstituted C₁-C₆ alkyl; and

R⁶ is substituted or unsubstituted C₁-C₆ alkyl.

[0149] In some embodiments, wortmannin analogs suitable for methods/uses described herein include compounds of Formula IIA or IIB:



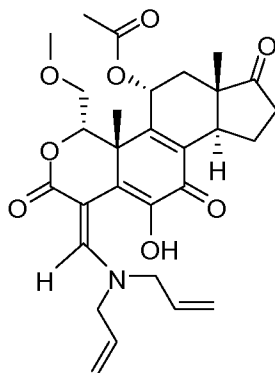
Formula IIA

or
Formula IIB

wherein Y is a heteroatom and R^1 and R^2 are independently selected from an unsaturated alkyl, cyclic alkyl, or R^1 and R^2 together with Y form a heterocycle.

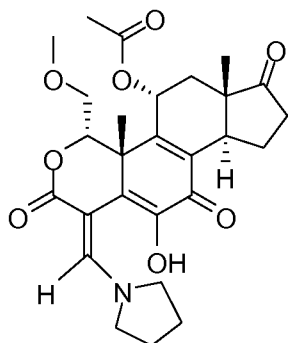
[0150] In certain embodiments of compounds of formula IIA or IIB, Y is a heteroatom selected from nitrogen and sulfur and R^1 and R^2 are independently selected from an unsaturated alkyl, cyclic alkyl, or R^1 and R^2 together with Y form a heterocycle.

[0151] In further embodiments, a wortmannin analog is Acetic acid 4-diallylaminomethylene-6-hydroxy-1- α -methoxymethyl-10 β ,13 β -dimethyl-3,7,17-trioxo-1,3,4,7,10,11 β ,12,13,14 α ,15,16,17-dodecahydro-2-oxa-cyclopenta[*a*]phenanthren-11-yl ester (PX-866) having the structure,



(PX-866).

[0152] In yet further embodiments, a wortmannin analog is Acetic acid 6-hydroxy-1 α -methoxymethyl-10 β ,13 β -dimethyl-3,7,17-trioxo-4-pyrrolidin-1-methylene-1,3,4,7,10,11 β ,12,13,14 α ,15,16,17-dodecahydro-2-oxa-cyclopenta[*a*]phenanthren-11-yl (PX-867) having the structure,



(PX-867).

[0153] In additional embodiments, wortmannin analogs suitable for methods described herein include compounds selected from, but not limited to, PX-868, PX-870, PX-871, PX-880, PX-881, PX-882, PX-889, PX-890, DJM2-170, DJM2-171, DJM2-177, DJM2-181 and combinations thereof. In some embodiments, wortmannin analogs suitable for methods described herein include compounds described in GB Pat. No. 2302021, which compounds are incorporated herein by reference.

Further forms of Wortmannin analogs

[0154] In the scope of the embodiments, wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) include further forms of the compounds described herein such as pharmaceutically acceptable salts, solvates (including hydrates), amorphous phases, partially crystalline and crystalline forms (including all polymorphs), prodrugs, metabolites, N-oxides, isotopically-labeled and stereo-isomers. Wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) are prepared as a pharmaceutically acceptable salts formed when an acidic proton present in the parent compound either is replaced by a metal ion, for example an alkali metal ion, an alkaline earth ion, or an aluminum ion; or coordinates with an organic base. In addition, the salt forms of the disclosed compounds are prepared using salts of the starting materials or intermediates.

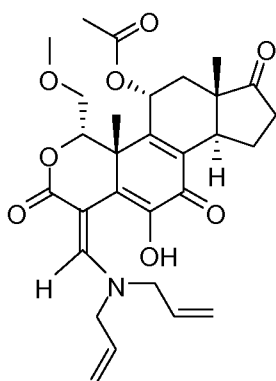
[0155] In some of the embodiments described herein, wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) are prepared as a pharmaceutically acceptable acid addition salt (which is a type of a pharmaceutically acceptable salt) by reacting the free base form of the compound with a pharmaceutically acceptable inorganic or organic acid, including, but not limited to, inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid metaphosphoric acid, and the like; and organic acids such as acetic acid, propionic acid, hexanoic acid, cyclopentanepropionic acid, glycolic acid, pyruvic acid, lactic acid, malonic acid, succinic acid, malic acid, maleic acid, fumaric acid, Q-toluenesulfonic

acid, tartaric acid, trifluoroacetic acid, citric acid, benzoic acid, 3-(4-hydroxybenzoyl)benzoic acid, cinnamic acid, mandelic acid, arylsulfonic acid, methanesulfonic acid, ethanesulfonic acid, 1,2-ethanedisulfonic acid, 2-hydroxyethanesulfonic acid, benzenesulfonic acid, 2-naphthalenesulfonic acid, 4-methylbicyclo-[2.2.2]oct-2-ene-1-carboxylic acid, glucoheptonic acid, 4,4'-methylenebis-(3-hydroxy-2-ene-1-carboxylic acid), 3-phenylpropionic acid, trimethylacetic acid, tertiary butylacetic acid, lauryl sulfuric acid, gluconic acid, glutamic acid, hydroxynaphthoic acid, salicylic acid, stearic acid, and muconic acid.

- [0156] Alternatively, in some of the embodiments described herein, wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) are prepared as a pharmaceutically acceptable base addition salts (which is a type of a pharmaceutically acceptable salt) by reacting the free acid form of the compound with a pharmaceutically acceptable inorganic or organic base, including, but not limited to organic bases such as ethanolamine, diethanolamine, triethanolamine, tromethamine, N-methylglucamine, and the like and inorganic bases such as aluminum hydroxide, calcium hydroxide, potassium hydroxide, sodium carbonate, sodium hydroxide, and the like.
- [0157] It should be understood that a reference to a pharmaceutically acceptable salt includes the solvent addition forms or crystal forms thereof, particularly solvates or polymorphs. Solvates contain either stoichiometric or non-stoichiometric amounts of a solvent, and are formed during the process of crystallization with pharmaceutically acceptable solvents such as water, ethanol, and the like. Hydrates are formed when the solvent is water, or alcoholates are formed when the solvent is alcohol. Solvates of wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) are prepared or formed during the processes described herein. By way of example only, hydrates of wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) are conveniently prepared by recrystallization from an aqueous/organic solvent mixture, using organic solvents including, but not limited to, dioxane, toluene, alkyl acetate, anisole, tetrahydrofuran or methanol. In addition, the compounds provided herein can exist in unsolvated as well as solvated forms. In general, the solvated forms are considered equivalent to the unsolvated forms for the purposes of the compounds and methods provided herein.
- [0158] In some of the embodiments described herein, wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) include crystalline forms, also known as polymorphs. Polymorphs include the different crystal packing arrangements of the same

elemental composition of a compound. Polymorphs usually have different X-ray diffraction patterns, infrared spectra, melting points, density, hardness, crystal shape, optical and electrical properties, stability, and solubility. Various factors such as the recrystallization solvent, rate of crystallization, and storage temperature cause a single crystal form to dominate.

[0159] Accordingly, contemplated within the scope of methods of treatment presented herein for treatment of cancers that test positive for a virus (e.g., an oncovirus described herein) is administration of compositions comprising compounds of Formula IA, Formula IB, Formula IIA or Formula IIB, or crystalline forms thereof or polymorphs thereof. By way of example, PX-866 has the following structural formula:



(PX-866).

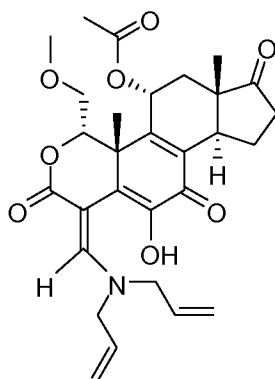
[0160] Typically, PX-866 is prepared from wortmannin in a number of synthetic steps. PX-866 is prepared as an orange oil after chromatography. *See*, for example US 7,081,475. The polymorphs of the present embodiments described herein encompass racemates, racemic mixtures, and diastereomeric mixtures with all possible isomers and mixtures thereof of PX-866.

[0161] Because of the structural similarity between the starting material, wortmannin, and PX-866, purification of PX-866 (e.g., chromatography) presents a challenge. Accordingly, provided herein are certain conditions for purification and/or crystallization of PX-866 and/or analog thereof.

[0162] In some embodiments, the crystalline form described herein increases purity of PX-866, especially decreasing the content of wortmannin. In some embodiments, the crystalline form has up to about 2% wortmannin. In certain embodiments, the crystalline form has up to about 1% wortmannin, up to about 0.5% wortmannin, up to about 0.3% wortmannin, or up to about 0.1% wortmannin. In certain embodiments, the crystalline form is free of wortmannin (e.g., undetectable levels of wortmannin by HPLC, or mass spec or the like). In certain embodiments, the crystalline form of PX-866 and/or analog

thereof is a toluene, anisole, cumene, propyl acetate, 4-methyl-2-pentanone, chlorobenzene, or 1-pentanol solvate crystalline form.

[0163] In some embodiments of the uses of PI-3 kinase inhibitors and methods of treatment described herein, the PI-3 kinase inhibitors are substantially pure crystalline forms of a compound having a structural formula



(PX-866),

and/or analog thereof wherein the crystalline form is a solvate of PX-866 and/or analog thereof. In certain embodiments, the substantially pure crystalline solvate form of PX-866 and/or analog thereof is a toluene, anisole, cumene, propyl acetate, 4-methyl-2-pentanone, chlorobenzene, 1-pentanol solvate, or the like. Such solvate forms include anisole solvate, cumene solvate, propyl acetate solvate, 4-methyl-2-pentanone solvate, 1-pentanol solvate, or the like.

[0164] In some embodiments, the crystalline form of PX-866 and/or analog thereof is an anisole solvate. In certain embodiments, the crystalline form of PX-866 and/or analog thereof is a crystalline form prepared from a solution comprising anisole. For instance, the anisole solvate of PX-866 and/or analog thereof is prepared from an anisole supernatant. In other embodiments, the anisole solvate is prepared from addition of anisole as antisolvent to a solution comprising PX-866 and/or analog thereof. In some embodiments, the process of preparing the anisole solvate utilizes seeding (e.g., addition of crystals of the anisole solvate or glass powder) or via any other known processes. In other embodiments, the process of preparing the anisole solvate does not use seeding. Typically, the crystalline form is dried over a flow of nitrogen or under vacuum at room temperature or raised temperature (e.g. 40 °C). The crystalline form is determined by XRPD, DSC, single crystal X-ray crystallography and/or other suitable instrumental analysis.

[0165] In certain embodiments, the crystalline form of the anisole solvate of PX-866 and/or analog thereof exhibits a predominant endotherm at about 146.0 °C as measured by Differential Scanning Calorimeter at a scan rate of 10 °C per minute in a crimped pan. In

certain embodiments, the crystalline form has an X-ray powder diffraction pattern having at least two 2-theta values selected from 7.9, 8.5, 10.2, 11.1, 14.0, 14.2, 17.9, 18.7, 21.0, 21.2, and 28.2 ± 0.1 . In certain embodiments, the crystalline form exhibits a single crystal X-ray crystallographic analysis at 120 K with crystal parameters as the following:

Space Group	P2 ₁ 2 ₁ 2 ₁
a, Å	13.7140(3)
b, Å	15.4272(4)
c, Å	15.6890(4)
α	90
β	90
γ	90
Z (molecules/unit cell)	4

Calculated Density (g/cm) 1.268.

[0166] In some instances, similar solvents such as toluene, cumene, chlorobenzene, *o*-xylene, *m*-xylene, *p*-xylene and the like are used to prepare crystalline solvates of PX-866.

[0167] In some embodiments, the crystalline form of PX-866 and/or analog thereof is a propyl acetate solvate. In certain embodiments, the crystalline form of PX-866 and/or analog thereof is a crystalline form prepared from a solution comprising propyl acetate. For instance, the propyl acetate solvate of PX-866 and/or analog thereof is prepared from a propyl acetate supernatant. In other embodiments, the propyl acetate solvate is prepared from addition of propyl acetate as antisolvent to a solution comprising PX-866 and/or analog thereof. In some embodiments, the process of preparing the propyl acetate solvate utilizes seeding (e.g., addition of crystals of the propyl acetate solvate or glass powder) or via any other known processes. In other embodiments, the process of preparing the propyl acetate solvate does not use seeding. Typically, the crystalline form is dried over a flow of nitrogen or under vacuum at room temperature or raised temperature (e.g. 40 °C). The crystalline form is determined by XRPD, DSC, single crystal X-ray crystallography and/or other suitable instrumental analysis.

[0168] In certain embodiments, the crystalline propyl acetate solvate of PX-866 and/or analog thereof exhibits a predominant endotherm at about 80.5 °C as measured by Differential

Scanning Calorimeter at a scan rate of 10 °C per minute in a crimped pan. In certain embodiments, the crystalline propyl acetate solvate has an X-ray powder diffraction pattern having at least two 2-theta values selected from 8.0, 8.4, 10.2, 11.0, 14.0 and 19.2, ± 0.1 . In certain embodiments, the crystalline propyl acetate solvate exhibits a single crystal X-ray crystallographic analysis at 100 K with crystal parameters as the following:

Space Group	P2 ₁ 2 ₁ 2 ₁
a, Å	13.4963(5)
b, Å	15.5158(5)
c, Å	15.6912(6)
α	90
β	90
γ	90
Z (molecules/unit cell)	4

Calculated Density (g/cm) 1.269.

[0169] In some embodiments, other similar solvents such as methyl acetate, ethyl acetate, isopropyl acetate, butyl acetate, and the like are used to prepare crystalline solvates of PX-866.

[0170] In some embodiments, the crystalline form of PX-866 and/or analog thereof is a toluene solvate. In certain embodiments, the crystalline form of PX-866 and/or analog thereof is a crystalline form prepared from a solution comprising toluene. For instance, the toluene solvate of PX-866 and/or analog thereof is prepared from a toluene supernatant. In other embodiments, the toluene solvate is prepared from addition of toluene as antisolvent to a solution comprising PX-866 and/or analog thereof. In some embodiments, the process of preparing the toluene solvate utilizes seeding (e.g., addition of crystals of the toluene solvate or glass powder) or via any other known processes. In other embodiments, the process of preparing the toluene solvate does not use seeding. Typically, the crystalline form is dried over a flow of nitrogen or under vacuum at room temperature or raised temperature (e.g. 40 °C). The crystalline form is determined by XRPD, DSC, single crystal X-ray crystallography and/or other suitable instrumental analysis.

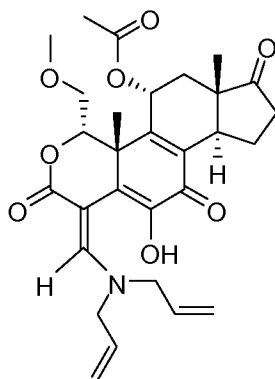
- [0171] In certain embodiments, the crystalline toluene solvate exhibits a predominant endotherm at about 142.0 °C as measured by Differential Scanning Calorimeter at a scan rate of 10 °C per minute in a crimped pan. In certain embodiments, the crystalline toluene solvate has an X-ray powder diffraction pattern having at least two 2-theta values selected from 12.5, 14.0, and 21.1, ± 0.1 .
- [0172] In some embodiments, the crystalline form of PX-866 and/or analog thereof is a cumene solvate. In certain embodiments, the crystalline form is a crystalline form of PX-866 and/or analog thereof prepared from a solution comprising cumene. For instance, the cumene solvate of PX-866 and/or analog thereof is prepared from a cumene supernatant. In other embodiments, the cumene solvate is prepared from addition of cumene as antisolvent to a solution comprising PX-866 and/or analog thereof. In some embodiments, the process of preparing the cumene solvate utilizes seeding (e.g., addition of crystals of the cumene solvate or glass powder) or via any other known processes. In other embodiments, the process of preparing the cumene solvate does not use seeding. Typically, the crystalline form is dried over a flow of nitrogen or under vacuum at room temperature or raised temperature (e.g. 40 °C). The crystalline form is determined by XRPD, DSC, single crystal X-ray crystallography and/or other suitable instrumental analysis.
- [0173] In certain embodiments, the crystalline cumene solvate has an X-ray powder diffraction pattern expressed in degrees 2-theta at 7.8, 8.4, 10.1, 10.7, 13.7, 14.1, 18.1, 18.9, 20.6, and 20.8, ± 0.1 .
- [0174] In some embodiments, the crystalline form of PX-866 and/or analog thereof is a chlorobenzene solvate. In certain embodiments, the crystalline form of PX-866 and/or analog thereof is a crystalline form prepared from a solution comprising chlorobenzene. In certain embodiments, the crystalline chlorobenzene solvate has an X-ray powder diffraction pattern expressed in degrees 2-theta at 8.0, 8.5, 10.3, 11.1, 14.1, 17.9, 18.8, 19.1, 21.0 and 28.3, ± 0.1 .
- [0175] In some embodiments, the crystalline form of PX-866 and/or analog thereof is a 4-methyl-2-pentanone solvate. In certain embodiments, the crystalline form is a crystalline form of PX-866 and/or analog thereof prepared from a solution comprising 4-methyl-2-pentanone. For instance, the 4-methyl-2-pentanone solvate of PX-866 and/or analog thereof is prepared from a 4-methyl-2-pentanone supernatant. In other embodiments, the 4-methyl-2-pentanone solvate is prepared from addition of 4-methyl-2-pentanone as antisolvent to a solution comprising PX-866 and/or analog thereof. In some

embodiments, the process of preparing the 4-methyl-2-pentanone solvate utilizes seeding (e.g., addition of crystals of the 4-methyl-2-pentanone solvate or glass powder) or via any other known processes. In other embodiments, the process of preparing the 4-methyl-2-pentanone solvate does not use seeding. Typically, the crystalline form is dried over a flow of nitrogen or under vacuum at room temperature or raised temperature (e.g. 40 °C). The crystalline form is determined by XRPD, DSC, single crystal X-ray crystallography and/or other suitable instrumental analysis.

- [0176] In certain embodiments, the crystalline 4-methyl-2-pentanone solvate has an X-ray powder diffraction pattern expressed in degrees 2-theta at 7.9, 8.4, 10.2, 10.9, 13.9, 14.2, 18.5, 19.2, and 20.7, ± 0.1 .
- [0177] Similarly, in some instances, other ketone solvents such as acetone, 2-butanone, and the like are used to prepare a similar crystalline solvate.
- [0178] In other embodiments, the crystalline form of PX-866 and/or analog thereof is a 1-pentanol solvate. In certain embodiments, the crystalline form of PX-866 and/or analog thereof is a crystalline form prepared from a solution comprising 1-pentanol. For instance, the 1-pentanol solvate of PX-866 and/or analog thereof is prepared from a 1-pentanol supernatant. In other embodiments, the 1-pentanol solvate is prepared from addition of 1-pentanol as antisolvent to a solution comprising PX-866 and/or analog thereof. In some embodiments, the process of preparing the 1-pentanol solvate utilizes seeding (e.g., addition of crystals of the 1-pentanol solvate or glass powder) or via any other known processes. In other embodiments, the process of preparing the 1-pentanol solvate does not use seeding. Typically, the crystalline form is dried over a flow of nitrogen or under vacuum at room temperature or raised temperature (e.g. 40 °C). The crystalline form is determined by XRPD, DSC, single crystal X-ray crystallography and/or other suitable instrumental analysis.
- [0179] In certain embodiments, the crystalline 1-pentanol solvate has an X-ray powder diffraction pattern expressed in degrees 2-theta at 8.1, 8.5, 10.2, 11.1, 12.5, 14.0, 14.3, 17.9, 18.8, 20.7, and 21.3, ± 0.1 .
- [0180] Similarly, in some embodiments, other alcohol solvents such as ethanol, isopropyl alcohol, 1-butanol, *t*-butanol, methanol, isopentanol, glycerol, 1-octanol, 2,2,2-trifluoroethanol, and the like are used to prepare a crystalline solvate.
- [0181] In some embodiments, the crystalline forms of PX-866 and/or analogs thereof exhibit higher stability than amorphous form. In exemplary formulation studies, it was observed that amorphous forms of PX-866 exhibited undesirable properties for formulation

including rapid degradation from heat and humidity, low flowability as well as unwanted hygroscopicity which required low-moisture conditions to produce sample formulations. In some instances, the synthetic routes for the amorphous forms led to variable purity and it was observed in some cases that heptane was trapped in the last step to widely varying degrees. Dissolution studies also have shown capsules filled with amorphous PX-866 to exhibit plugging (i.e., a sticky mass) in the capsule. In some embodiments, the crystalline forms and solvates thereof of PX-866 and/or analogs thereof described herein have improved properties with respect to the amorphous form.

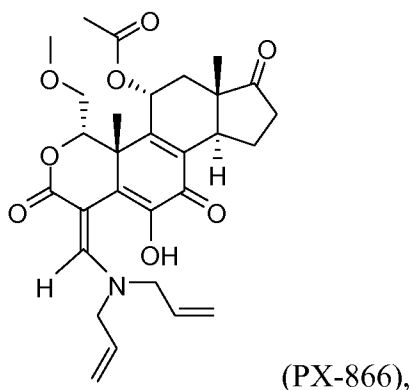
- [0182] By way of example, a stability study established improved stability of the crystalline solvates against heat and humidity compared to the stability of the amorphous material. The study was conducted under 40°C and 75% relative humidity to be pulled at 1, 2, 3, 4 and 8 week time points for HPLC and XRPD analysis. (See Example 17 and FIG. 13). Further, it is observed that exemplary anisole and toluene solvates have a better stability over amorphous PX-866 after only one week. The propyl acetate solvate is also more stable than the amorphous material. XRPD analysis also showed that crystalline forms did not convert or undergo a physical change under the accelerated stability conditions.
- [0183] In other embodiments, crystalline forms of PX-866 and/or analogs thereof allow for ease of formulation. Exemplary studies with crystalline forms of PX-866 and/or analogs thereof show that these forms have better flowability than the amorphous form, do not exhibit plugging when filled in capsules, have good aqueous solubility and have a higher melting point, and better stability as described above. In some embodiments, crystalline forms of PX-866 and/or analogs thereof have better *in vitro* dissolution profile as compared to the amorphous form. In some instances, crystalline forms of PX-866 and/or analogs thereof have better flowability as compared to the amorphous form.
- [0184] In some embodiments provide herein methods of making a crystalline solvate form of a compound having a structural formula



(PX-866),

and/or analog thereof comprising adding antisolvent to a solution of the compound in THF. In some embodiments, the antisolvent is a benzene like solvent such as toluene, anisole, cumene, xylene, chlorobenzene, or the like. In some embodiments, the antisolvent is an ester type solvent such as methyl acetate, ethyl acetate, propyl acetate, butyl acetate, or the like. In some embodiments, the antisolvent is a ketone type solvent such as acetone, 2-butanone, 2-pentanone, 3-pentanone, 3-methyl-2-pentanone, 4-methyl-2-pentanone, or the like. In some embodiments, the antisolvent is an alcohol type solvent such as methanol, ethanol, propanol, butanol, pentanol, or the like. In certain embodiments, the antisolvent is toluene, anisole, cumene, propyl acetate, 4-methyl-2-pentanone, chlorobenzene, or 1-pentanol.

[0185] In some embodiments, provided herein are methods of making a crystalline form of a compound having a structural formula



and/or analog thereof that is substantially free of wortmannin, comprising cooling down a supernatant of the compound to 4 °C to -20 °C. In some embodiments, the supernatant is prepared from an ester type solvent such as methyl acetate, ethyl acetate, propyl acetate, butyl acetate, or the like. In some embodiments, the supernatant is prepared from a ketone type solvent such as acetone, 2-butanone, 2-pentanone, 3-pentanone, 3-methyl-2-pentanone, 4-methyl-2-pentanone, or the like. In some embodiments, the supernatant is prepared from an alcohol type solvent such as methanol, ethanol, propanol, butanol, pentanol, or the like. In certain embodiments, the supernatant is toluene, anisole, cumene, propyl acetate, 4-methyl-2-pentanone, chlorobenzene, or 1-pentanol supernatant.

[0186] In some of the embodiments described herein, wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) in unoxidized form are prepared from N-oxides of compounds of Formula (1) by treating with a reducing agent, such as, but not limited to, sulfur, sulfur dioxide, triphenyl phosphine, lithium borohydride, sodium borohydride, phosphorus trichloride, tribromide, or the like in a suitable inert organic

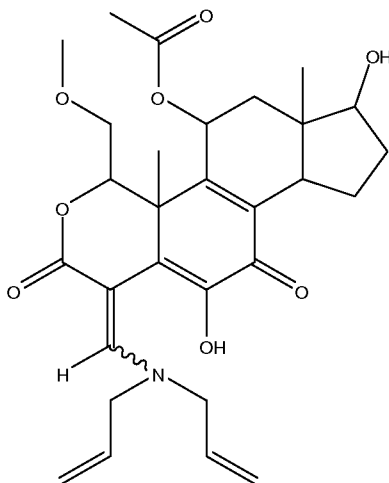
solvent, such as, but not limited to, acetonitrile, ethanol, aqueous dioxane, or the like at 0 to 80°C.

- [0187] In some embodiments, wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) are isotopically-labeled, which are identical to those recited in the various formulae and structures presented herein, but for the fact that one or more atoms are replaced by an atom having an atomic mass or mass number different from the atomic mass or mass number usually found in nature. In some embodiments, one or more hydrogen atoms are replaced with deuterium. In some embodiments, metabolic sites on the compounds described herein are deuterated. In some embodiments, substitution with deuterium affords certain therapeutic advantages resulting from greater metabolic stability, such as, for example, increased *in vivo* half-life or reduced dosage requirements.
- [0188] In some of the embodiments described herein, wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) are prepared as prodrugs. Prodrugs are generally drug precursors that, following administration to a subject and subsequent absorption, are converted to an active, or a more active species via some process, such as conversion by a metabolic pathway. Some prodrugs have a chemical group present on the prodrug that renders it less active and/or confers solubility or some other property to the drug. Once the chemical group has been cleaved and/or modified from the prodrug the active drug is generated. Prodrugs are often useful because, in some situations, they are easier to administer than the parent drug. They may, for instance, be bioavailable by oral administration whereas the parent is not. The prodrug may also have improved solubility in pharmaceutical compositions over the parent drug. An example, without limitation, of a prodrug would be a wortmannin analog which is administered as an ester (the “prodrug”) to facilitate transmittal across a cell membrane where water solubility is detrimental to mobility but which then is metabolically hydrolyzed to the carboxylic acid, the active entity, once inside the cell where water-solubility is beneficial. A further example of a prodrug might be a short peptide (polyaminoacid) bonded to an acid group where the peptide is metabolized to reveal the active moiety.
- [0189] In some of the embodiments described herein, wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) are metabolites. A “metabolite” of a wortmannin analog disclosed herein is a derivative of that wortmannin analog that is formed when the wortmannin analog is metabolized. The term “active metabolite” refers to a biologically active derivative of a wortmannin analog that is formed when the

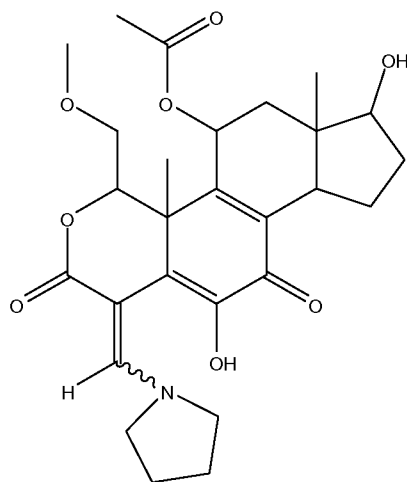
wortmannin analog is metabolized (biotransformed). The term “metabolized,” as used herein, refers to the sum of the processes (including, but not limited to, hydrolysis reactions and reactions catalyzed by enzymes) by which a particular substance is changed by an organism. Thus, in some instances, enzymes produce specific structural alterations to a wortmannin analog. For example, cytochrome P450 catalyzes a variety of oxidative and reductive reactions while uridine diphosphate glucuronyltransferases (UGT) catalyze the transfer of an activated glucuronic-acid molecule to aromatic alcohols, aliphatic alcohols, carboxylic acids, amines and free sulphydryl groups (e.g. conjugation reactions). Further information on metabolism is available in *The Pharmacological Basis of Therapeutics*, 9th Edition, McGraw-Hill (1996). In one embodiment, metabolites of the compounds disclosed herein are identified either by administration of compounds to a host and analysis of tissue samples from the host, or by incubation of compounds with hepatic cells in vitro and analysis of the resulting compounds.

[0190] Metabolites of wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein), in some embodiments described herein, include, but are not limited to, metabolites resulting from first pass metabolism. In some embodiments, the metabolite is a 17-hydroxy (17-OH) derivative of a wortmannin analog. In some embodiments, the metabolite is a derivative of PX-866. In other embodiments, the metabolite is a derivative of PX-867.

[0191] In some instances the metabolite has the following structural formula:



[0192] In other instances the metabolite has the following structural formula:

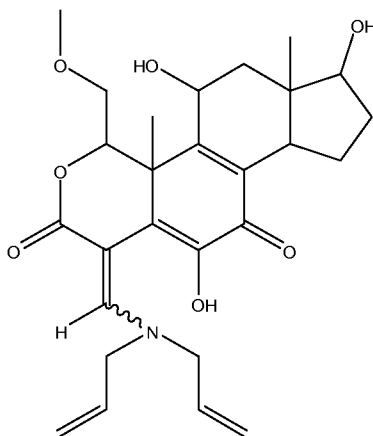


[0193] Table 1 illustrates the potency of 17-hydroxy PX-866 metabolite in *in vitro* kinase assays:

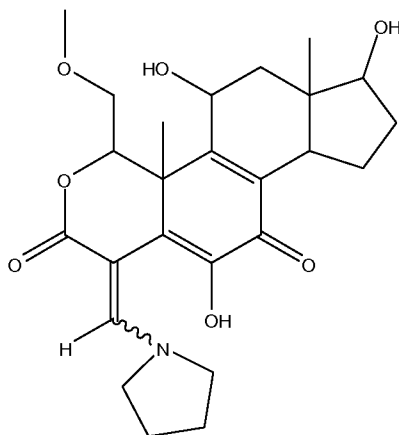
Target	IC ₅₀ nM	
	PX-866	17-OH PX-866
PIK3CA	39	14
PIK3CB	88	57
PIK3CD	124	131
PIK3CG	198	148

[0194] In further embodiments, the metabolite is a 11,17-hydroxy (11,17-OH) derivative of a wortmannin analog.

[0195] In some instances the metabolite has the following structural formula:



[0196] In other instances the metabolite has the following structural formula:



Synthesis of Wortmannin Analogs

[0197] Wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) described herein are synthesized using standard synthetic techniques known to those of skill in the art or using methods known in the art in combination with methods described herein. In additions, solvents, temperatures and other reaction conditions presented herein vary according to the practice and knowledge of those of skill in the art.

[0198] The starting material used for the synthesis of wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) described herein are obtained from commercial sources, such as Aldrich Chemical Co. (Milwaukee, Wis.), Sigma Chemical Co. (St. Louis, Mo.), or the starting materials are synthesized. The wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) described herein, and other related compounds having different substituents are synthesized using techniques and materials known to those of skill in the art, such as described, for example, in March, *ADVANCED ORGANIC CHEMISTRY* 4th Ed., (Wiley 1992); Carey and Sundberg, *ADVANCED ORGANIC CHEMISTRY* 4th Ed., Vols. A and B (Plenum 2000, 2001), and Green and Wuts, *PROTECTIVE GROUPS IN ORGANIC SYNTHESIS* 3rd Ed., (Wiley 1999) (all of which are incorporated by reference in their entirety). General methods for the preparation of wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) as disclosed herein are derived from known reactions in the field, and the reactions are modified by the use of appropriate reagents and conditions, as would be recognized by the skilled person, for the introduction of the various moieties found in the formulae as provided herein.

[0199] Additional synthesis methods and schemes for the wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) described herein are found in, for example, U.S. Patent No. 5,480,906, U.S. Patent No. 7,335,679, and U.S. Patent Appl. Pub. No. 2007/0191466, each of which is incorporated herein by reference for the

synthesis methods and schemes of wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein).

Pharmaceutical Compositions of PI-3 kinase inhibitors

[0200] Pharmaceutical compositions containing PI-3 kinase inhibitors (e.g., any PI-3 kinase inhibitor described herein, e.g., wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein)) are administered in therapeutically effective amounts as pharmaceutical compositions by any conventional form and route known in the art including, but not limited to: injection, transdermal, nasal, pulmonary, vaginal, rectal, buccal, ocular, otic, local, topical, or oral administration. In certain embodiments, an injectable pharmaceutical composition of a wortmannin analog is an intramuscular, intravenous, subcutaneous, intranodal, intratumoral, intracisternal, intraperitoneal, or intradermal injection. In some embodiments, the pharmaceutical composition containing wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) is provided in the form of a rapid release formulation, in the form of an extended release formulation, or in the form of an intermediate release formulation.

[0201] In some embodiments, for oral administration, wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) are formulated by combining the active compounds with pharmaceutically acceptable carriers or excipients. Such carriers enable the compounds described herein to be formulated as tablets, powders, pills, dragees, capsules, liquids, gels, syrups, elixirs, slurries, suspensions and the like, for oral ingestion by a patient to be treated.

[0202] Pharmaceutical preparations for oral use are obtained by mixing one or more solid excipient with one or more of the compounds described herein, optionally grinding the resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as: for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methylcellulose, microcrystalline cellulose, hydroxypropylmethylcellulose, sodium carboxymethylcellulose; or others such as: polyvinylpyrrolidone (PVP or povidone) or calcium phosphate. If desired, disintegrating agents are added, such as the cross linked croscarmellose sodium, polyvinylpyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

[0203] Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions are used, which optionally contain gum arabic, talc, polyvinylpyrrolidone,

carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments are added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

- [0204]** Pharmaceutical preparations which are used orally include push fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds are dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers are added. All formulations for oral administration should be in dosages suitable for such administration. In some embodiments, a wortmannin analog is in powder form and is directly filled into hard gelatin capsules.
- [0205]** For buccal or sublingual administration, the compositions optionally take the form of tablets, lozenges, or gels formulated in conventional manner.
- [0206]** In some embodiments, injectable compositions are for bolus injection or continuous infusion. An injectable composition of wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) is in a form suitable for parenteral or any other type of injection as a sterile suspensions, solutions or emulsions in oily or aqueous vehicles, and optionally contains formulatory agents such as suspending, stabilizing and/or dispersing agents. In some embodiments, the composition is formulated for intramuscular, intravenous, subcutaneous, intranodal, intratumoral, intracisternal, intraperitoneal, and/or intradermal injection. Pharmaceutical formulations for injection administration include aqueous solutions of the active compounds in water soluble form. Additionally, suspensions of the active compounds are prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions optionally contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension contains suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions. Alternatively, the active ingredient is in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

- [0207] In various embodiments, wortmannin analog compositions (e.g., compositions comprising compounds of Formula IA, IB, IIA or IIB described herein) are in liquid form for ocular or otic delivery. Liquid forms include, by way of non-limiting example, neat liquids, solutions, suspensions, dispersions, colloids, foams and the like and are formulated by known methods.
- [0208] In some embodiments, wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) are administered topically and are formulated into a variety of topically administrable compositions, such as solutions, suspensions, lotions, gels, pastes, medicated sticks, balms, creams or ointments. Such pharmaceutical compounds contain solubilizers, stabilizers, tonicity enhancing agents, buffers and preservatives.
- [0209] Formulations suitable for transdermal administration of wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) employ transdermal delivery devices and transdermal delivery patches and are lipophilic emulsions or buffered, aqueous solutions, dissolved and/or dispersed in a polymer or an adhesive. Such patches are constructed for continuous, pulsatile, or on demand delivery of pharmaceutical agents. Still further, transdermal delivery of the wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) are accomplished by means of iontophoretic patches and the like. Additionally, transdermal patches provide controlled delivery of the wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein). The rate of absorption is slowed by using rate-controlling membranes or by trapping the compound within a polymer matrix or gel. Conversely, absorption enhancers are used to increase absorption. An absorption enhancer or carrier optionally includes absorbable pharmaceutically acceptable solvents to assist passage through the skin. For example, transdermal devices are in the form of a bandage comprising a backing member, a reservoir containing the compound optionally with carriers, optionally a rate controlling barrier to deliver the compound to the skin of the host at a controlled and predetermined rate over a prolonged period of time, and means to secure the device to the skin.
- [0210] In some embodiments, for administration by inhalation for pulmonary or nasal delivery, wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) are formulated in a form such as an aerosol, a mist or a powder. Pharmaceutical compositions of wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g.,

dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit is determined by providing a valve to deliver a metered amount. Capsules and cartridges of, such as, by way of example only, gelatin for use in an inhaler or insufflator are formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

[0211] In some embodiments, wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) are formulated in rectal or vaginal compositions such as enemas, douches, gels, foams, aerosols, suppositories, jelly suppositories, or retention enemas, containing conventional suppository bases such as cocoa butter or other glycerides, as well as synthetic polymers such as polyvinylpyrrolidone, PEG, and the like. In suppository forms of the compositions, a low-melting wax such as, but not limited to, a mixture of fatty acid glycerides, optionally in combination with cocoa butter is first melted.

[0212] In some embodiments, wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) are administered in a local rather than systemic manner, for example, via injection of the compound directly into an organ, often in a depot or sustained release formulation. Furthermore, pharmaceutical composition comprising wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) are optionally administered in a targeted drug delivery system, for example, in a liposome coated with organ-specific antibody. The liposomes are targeted to and taken up selectively by the organ. Pharmaceutical compositions of wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) are formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which are used pharmaceutically. Proper formulation is dependent upon the route of administration chosen. Pharmaceutical compositions comprising wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) are manufactured by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or compression processes, and the like.

[0213] The pharmaceutical compositions include at least one pharmaceutically acceptable carrier, diluent or excipient and a wortmannin analog described herein as an active ingredient in free-acid or free-base form, or in a pharmaceutically acceptable salt form.

In addition, the methods and pharmaceutical compositions described herein include the use of N-oxides, crystalline forms (also known as polymorphs), as well as active metabolites of these compounds having the same type of activity. In some situations, wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) exist as tautomers. All tautomers are included within the scope of embodiments presented herein. Additionally, in some embodiments, wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) described herein exist in unsolvated as well as solvated forms with pharmaceutically acceptable solvents such as water, ethanol, and the like. The solvated forms of wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) presented herein are also considered to be disclosed herein. In other embodiments, the pharmaceutical compositions include other medicinal or pharmaceutical agents, carriers, adjuvants, such as preserving, stabilizing, wetting or emulsifying agents, solution promoters, salts for regulating the osmotic pressure, and/or buffers.

[0214] Methods for the preparation of compositions comprising wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) described herein include formulating the wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) with one or more inert, pharmaceutically acceptable excipients or carriers to form a solid, semi-solid or liquid. Solid compositions include, but are not limited to, powders, tablets, dispersible granules, capsules, cachets, and suppositories. Liquid compositions include solutions in which a compound is dissolved, emulsions comprising a compound, or a solution containing liposomes, micelles, or nanoparticles comprising a compound as disclosed herein. Semi-solid compositions include, but are not limited to, gels, suspensions and creams. The compositions are formulated as liquid solutions or suspensions, solid forms suitable for solution or suspension in a liquid prior to use, or as emulsions. In some embodiments, these compositions contain minor amounts of nontoxic, auxiliary substances, such as wetting or emulsifying agents, pH buffering agents, and so forth.

[0215] Further forms of pharmaceutical compositions of wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) are integrated with other active agents, e.g., any anticancer agent described herein, e.g., docetaxel, in a unitary dosage form for methods. The unitary dosage forms are formulated to release such that both agents are released simultaneously or where there is sequential release of each agent via known

modified release mechanisms including but not limited to timed release, delayed release, pH release, pulsatile release and the like.

- [0216] A summary of pharmaceutical compositions described herein is found, for example, in *Remington: The Science and Practice of Pharmacy*, Nineteenth Ed (Easton, Pa.: Mack Publishing Company, 1995); Hoover, John E., *Remington's Pharmaceutical Sciences*, Mack Publishing Co., Easton, Pennsylvania 1975; Liberman, H.A. and Lachman, L., Eds., *Pharmaceutical Dosage Forms*, Marcel Decker, New York, N.Y., 1980; and *Pharmaceutical Dosage Forms and Drug Delivery Systems*, Seventh Ed. (Lippincott Williams & Wilkins 1999), herein incorporated by reference in their entirety.

PI-3 kinase inhibitors dosages

- [0217] Dosages of PI-3 kinase inhibitors (e.g., any PI-3 kinase inhibitor described herein, e.g., wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein)) described herein are determined by known methods as well as in the examples. Maximum tolerated doses (MTD) and maximum response doses (MRD) are determined via established animal and human experimental protocols as well as in the examples described herein. For example, toxicity and therapeutic efficacy of wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) are determined by standard pharmaceutical procedures in cell cultures or experimental animals, including, but not limited to, for determining the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between the toxic and therapeutic effects is the therapeutic index and it are expressed as the ratio between LD₅₀ and ED₅₀. Wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) exhibiting high therapeutic indices are of interest. The data obtained from cell culture assays and animal studies are used in formulating a range of dosage for use in human. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED₅₀ with minimal toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. Additional relative dosages, represented as a percent of maximal response or of maximum tolerated dose, are obtained via suitable protocols.
- [0218] In some embodiments, wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) are provided in a dose per day from about 0.01 mg to 1000 mg, from about 0.1 mg to about 100 mg, from about 1 to about 20, from about 2 mg to about 12 mg. In certain embodiments, wortmannin analogs (e.g., compounds of Formula IA,

IB, IIA or IIB described herein) are provided in a daily dose of about 0.01 mg, about 0.05 mg, about 0.1 mg, about 0.2 mg, about 0.4 mg, about 0.6 mg, about 0.8 mg, about 1 mg, about 1.5 mg, about 2 mg, about 2.5 mg, about 3 mg, about 4 mg, about 5 mg, about 6 mg, about 7 mg, about 8 mg, about 9 mg, about 10 mg, about 11 mg, about 12 mg, about 15 mg, about 20 mg, about 25 mg, about 30 mg, about 40 mg, about 50 mg, about 75 mg, about 100 mg, about 150 mg, about 200 mg, about 250 mg, about 300 mg, about 500, mg, about 750 mg, about 1000 mg, or more, or any range derivable therein. In certain instances, wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) are provided in a dose per day of about 1 mg. In certain instances, wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) are provided in a dose per day of about 2 mg. In certain instances, wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) are provided in a dose per day of about 3 mg. In certain instances, wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) are provided in a dose per day of about 4 mg. In certain instances, wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) are provided in a dose per day of about 5 mg. In certain instances, wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) are provided in a dose per day of about 6 mg. In certain instances, wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) are provided in a dose per day of about 7 mg. In certain instances, wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) are provided in a dose per day of about 8 mg. In certain instances, wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) are provided in a dose per day of about 9 mg. In certain instances, wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) are provided in a dose per day of about 10 mg. In certain instances, wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) are provided in a dose per day of about 11 mg. In certain instances, wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) are provided in a dose per day of about 12 mg. The dose per day described herein are given once per day or multiple times per day in the form of sub-doses given b.i.d., t.i.d., q.i.d., or the like where the number of sub-doses equal the dose per day.

[0219] In other embodiments wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) are provided at the maximum tolerated dose (MTD). In other embodiments, the amount of wortmannin analogs (e.g., compounds of Formula IA, IB,

IIA or IIB described herein) administered is from about 10% to about 90% of the maximum tolerated dose (MTD), from about 25% to about 75% of the MTD, or about 50% of the MTD. In particular embodiments, the amount of wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) administered is from about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, or higher, or any range derivable therein, of the MTD.

Administration of anticancer agent and Wortmannin Analog Combination Therapy

[0220] Administration of a PI-3 kinase inhibitor (e.g., any PI-3 kinase inhibitor described herein, e.g., a wortmannin analog (e.g., a compound of Formula IA, Formula IB, Formula IIA or Formula IIB as described herein)) and a second anticancer agent (e.g., docetaxel) are at dosages and compositions described herein or at other dose levels and compositions determined and contemplated by a medical practitioner. When administered as an intravenous infusion or by other known methods, an anticancer agent (e.g., docetaxel) is given to a subject periodically, where each period is referred to as a treatment cycle. Administration periods include, but are not limited to, once every 3 days, once every 7 days, once every 10 days, once every 2 weeks, once every 3 weeks, once every 4 weeks, once every 5 weeks, once every 6 weeks or more. In some embodiments, docetaxel is administered once every 3 weeks. Treatment cycles also include, but are not limited to 1 cycle, 2 cycles, 3 cycles, 4 cycles, 5 cycles, 6 cycles, 7 cycles, 8 cycles, 9 cycles, 10 cycles, 11 cycles, 12 cycles, 13 cycles, 14 cycles, 15 cycles, 16 cycles, 17 cycles, 18 cycles, 19 cycles, 20 cycles, 25 cycles, 30 cycles, 40 cycles, or more. For an administration period of once every three weeks, the above representative cycles would last 3 weeks, 6 weeks, 9 weeks, 12 weeks, 15 weeks, 18 weeks, 21 weeks, 24 weeks, 27 weeks, 30 weeks, 33 weeks, 36 weeks, 39 weeks, 42 weeks, 45 weeks, 48 weeks, 51 weeks, 54 weeks, 57 weeks, 60 weeks, 75 weeks, 90 weeks, and 120 weeks respectively.

[0221] Dosages for the second anticancer agent (e.g., docetaxel) are, in some embodiments, the same for each treatment cycle. In other embodiments, the dosage of the second anticancer agent (e.g., docetaxel) dosages varies per cycle. In some embodiments, a higher initial dose of an anticancer agent (e.g., docetaxel) is administered for the first cycle and a lower dose is administered for all subsequent cycles. In other embodiments, the dosage of an anticancer agent (e.g., docetaxel) is decreased gradually per administration for each cycle. In yet other embodiments, the dosages are increased gradually per administration for each cycle.

- [0222] In some embodiments, a PI-3 kinase inhibitor (e.g., any PI-3 kinase inhibitor described herein, e.g., a wortmannin analog (e.g., a compound of Formula IA, Formula IB, Formula IIA or Formula IIB as described herein)), is administered daily to a subject when receiving one of the described anticancer agent (e.g., docetaxel) regimens. In other embodiments, wortmannin analogs (e.g., compounds of Formula IA, IB, IIA or IIB described herein) are administered every other day, every 2 days, every 3 days, every 4 days, every 5 days, every 6 days or every 7 days to a subject.
- [0223] In some embodiments, a PI-3 kinase inhibitor (e.g., any PI-3 kinase inhibitor described herein, e.g., a wortmannin analog (e.g., a compound of Formula IA, Formula IB, Formula IIA or Formula IIB as described herein)), is administered in reference to the second anticancer agent treatment cycles. In some embodiments, a PI-3 kinase inhibitor (e.g., any PI-3 kinase inhibitor described herein, e.g., a wortmannin analog (e.g., a compound of Formula IA, Formula IB, Formula IIA or Formula IIB as described herein)) is administered daily or every other day, every 2 days and the like for one treatment cycle for the anticancer agent and subsequently for the next treatment cycle, administration of a PI-3 kinase inhibitor (e.g., any PI-3 kinase inhibitor described herein, e.g., a wortmannin analog (e.g., a compound of Formula IA, Formula IB, Formula IIA or Formula IIB as described herein)) is withheld or given a “drug holiday”. In other embodiments, a PI-3 kinase inhibitor (e.g., any PI-3 kinase inhibitor described herein, e.g., a wortmannin analog (e.g., a compound of Formula IA, Formula IB, Formula IIA or Formula IIB as described herein)) is given to a subject every other anticancer treatment cycle, every two treatment cycles, every three treatment cycles, every four treatment cycles, or every five treatment cycles.
- [0224] In alternative embodiments, a PI-3 kinase inhibitor (e.g., any PI-3 kinase inhibitor described herein, e.g., a wortmannin analog (e.g., a compound of Formula IA, Formula IB, Formula IIA or Formula IIB as described herein)), is administered to an individual in need thereof on an intermittent dosing schedule. Intermittent dosing schedules include administering a wortmannin analog for a number of days, withholding administration for a certain period of time, subsequently administering a PI-3 kinase inhibitor (e.g., any PI-3 kinase inhibitor described herein, e.g., a wortmannin analog (e.g., a compound of Formula IA, Formula IB, Formula IIA or Formula IIB as described herein)) again with another subsequent withholding. In a non-limiting example, for a 28-day treatment cycle, a PI-3 kinase inhibitor (e.g., any PI-3 kinase inhibitor described herein, e.g., a wortmannin analog (e.g., a compound of Formula IA, Formula IB, Formula IIA or

Formula IIB as described herein)) is administered for days 1-5 and 8-12. Other intermittent dosing schedules are contemplated that include administration of a PI-3 kinase inhibitor (e.g., any PI-3 kinase inhibitor described herein, e.g., a wortmannin analog (e.g., a compound of Formula IA, Formula IB, Formula IIA or Formula IIB as described herein)) daily for one, two, three, four, five, six, seven, eight, nine or ten days, a withholding period of one, two, three, four, five, six, seven, eight, nine or ten days and an optional daily and withholding period similar or different from the previous administration within a treatment cycle.

- [0225] In certain embodiments, a PI-3 kinase inhibitor (e.g., any PI-3 kinase inhibitor described herein, e.g., a wortmannin analog (e.g., a compound of Formula IA, Formula IB, Formula IIA or Formula IIB as described herein)) is provided the same day as an anticancer agent administration. In yet other embodiments, a PI-3 kinase inhibitor (e.g., any PI-3 kinase inhibitor described herein, e.g., a wortmannin analog (e.g., a compound of Formula IA, Formula IB, Formula IIA or Formula IIB as described herein)) is provided on a previous day to the second anticancer agent (e.g., docetaxel) administration. In yet other embodiments, a PI-3 kinase inhibitor (e.g., any PI-3 kinase inhibitor described herein, e.g., a wortmannin analog (e.g., a compound of Formula IA, Formula IB, Formula IIA or Formula IIB as described herein)) is provided the subsequent day of the second anticancer agent administration. In certain instances where a PI-3 kinase inhibitor (e.g., any PI-3 kinase inhibitor described herein, e.g., a wortmannin analog (e.g., a compound of Formula IA, Formula IB, Formula IIA or Formula IIB as described herein)) is provided prior to anticancer agent administration, a PI-3 kinase inhibitor (e.g., any PI-3 kinase inhibitor described herein, e.g., a wortmannin analog (e.g., a compound of Formula IA, Formula IB, Formula IIA or Formula IIB as described herein)) is administered multiple days prior to administration of the anticancer agent, including, administration of a PI-3 kinase inhibitor (e.g., any PI-3 kinase inhibitor described herein, e.g., a wortmannin analog (e.g., a compound of Formula IA, Formula IB, Formula IIA or Formula IIB as described herein)) for two days prior, three days prior, four days prior, five days prior, six days prior, or seven days prior to administration of the anticancer agent.
- [0226] When a PI-3 kinase inhibitor (e.g., any PI-3 kinase inhibitor described herein, e.g., a wortmannin analog (e.g., a compound of Formula IA, Formula IB, Formula IIA or Formula IIB as described herein)) is provided the same day as administration of an anticancer agent, the PI-3 kinase inhibitor is administered at a set time in reference to the

anticancer agent administration. In some embodiments, a PI-3 kinase inhibitor (e.g., any PI-3 kinase inhibitor described herein, e.g., a wortmannin analog (e.g., a compound of Formula IA, Formula IB, Formula IIA or Formula IIB as described herein)) is administered about 1 hour, about 2 hours, about 4 hours, about 6 hours, about 8 hours, about 12 hours, about 18 hours, about 24 hours prior to anticancer agent administration. In some embodiments, a PI-3 kinase inhibitor (e.g., any PI-3 kinase inhibitor described herein, e.g., a wortmannin analog (e.g., a compound of Formula IA, Formula IB, Formula IIA or Formula IIB as described herein)) is administered about 1 hour, about 2 hours, about 4 hours, about 6 hours, about 8 hours, about 12 hours, about 18 hours, about 24 hours subsequent to anticancer agent administration.

[0227] In some embodiments when a PI-3 kinase inhibitor (e.g., any PI-3 kinase inhibitor described herein, e.g., a wortmannin analog (e.g., a compound of Formula IA, Formula IB, Formula IIA or Formula IIB as described herein)) is administered orally, the oral administration is given to a subject who is in a fasted state. A fasted state refers to a subject who has gone without food or fasted for a certain period of time. General fasting periods include at least 4 hours, at least 6 hours, at least 8 hours, at least 10 hours, at least 12 hours, at least 14 hours and at least 16 hours without food. In some embodiments, a PI-3 kinase inhibitor (e.g., any PI-3 kinase inhibitor described herein, e.g., a wortmannin analog (e.g., a compound of Formula IA, Formula IB, Formula IIA or Formula IIB as described herein)) is administered orally to a subject who is in a fasted state for at least 8 hours. In other embodiments, a PI-3 kinase inhibitor (e.g., any PI-3 kinase inhibitor described herein, e.g., a wortmannin analog (e.g., a compound of Formula IA, Formula IB, Formula IIA or Formula IIB as described herein)) is administered orally to a subject who is in a fasted state for at least 10 hours. In yet other embodiments, a PI-3 kinase inhibitor (e.g., any PI-3 kinase inhibitor described herein, e.g., a wortmannin analog (e.g., a compound of Formula IA, Formula IB, Formula IIA or Formula IIB as described herein)) is administered orally to a subject who is in a fasted state for at least 12 hours. In other embodiments, a PI-3 kinase inhibitor (e.g., any PI-3 kinase inhibitor described herein, e.g., a wortmannin analog (e.g., a compound of Formula IA, Formula IB, Formula IIA or Formula IIB as described herein)) is administered orally to a subject who has fasted overnight.

[0228] In other embodiments when a PI-3 kinase inhibitor (e.g., any PI-3 kinase inhibitor described herein, e.g., a wortmannin analog (e.g., a compound of Formula IA, Formula IB, Formula IIA or Formula IIB as described herein)) is administered orally, the oral

administration is given to a subject who is in a fed state. A fed state refers to a subject who has taken food or has had a meal. In certain embodiments, a PI-3 kinase inhibitor (e.g., any PI-3 kinase inhibitor described herein, e.g., a wortmannin analog (e.g., a compound of Formula IA, Formula IB, Formula IIA or Formula IIB as described herein)) is administered orally to a subject in a fed state 5 minutes post-meal, 10 minutes post-meal, 15 minutes post-meal, 20 minutes post-meal, 30 minutes post-meal, 40 minutes post-meal, 50 minutes post-meal, 1 hour post-meal, or 2 hours post-meal. In certain instances, a PI-3 kinase inhibitor (e.g., any PI-3 kinase inhibitor described herein, e.g., a wortmannin analog (e.g., a compound of Formula IA, Formula IB, Formula IIA or Formula IIB as described herein)) is administered orally to a subject in a fed state 30 minutes post-meal. In other instances, a PI-3 kinase inhibitor (e.g., any PI-3 kinase inhibitor described herein, e.g., a wortmannin analog (e.g., a compound of Formula IA, Formula IB, Formula IIA or Formula IIB as described herein)) is administered orally to a subject in a fed state 1 hour post-meal. In yet further embodiments, a PI-3 kinase inhibitor (e.g., any PI-3 kinase inhibitor described herein, e.g., a wortmannin analog (e.g., a compound of Formula IA, Formula IB, Formula IIA or Formula IIB as described herein)) is administered orally to a subject with food.

[0229] In further embodiments of the second anticancer agent and a PI-3 kinase inhibitor (e.g., any PI-3 kinase inhibitor described herein, e.g., a wortmannin analog (e.g., a compound of Formula IA, Formula IB, Formula IIA or Formula IIB as described herein)) combinations described herein, the a PI-3 kinase inhibitor (e.g., any PI-3 kinase inhibitor described herein, e.g., a wortmannin analog (e.g., a compound of Formula IA, Formula IB, Formula IIA or Formula IIB as described herein)) is administered at a certain time of day for the entire administration period. For example, a wortmannin analog (e.g., a compound of Formula IA, Formula IB, Formula IIA or Formula IIB as described herein) is administered at a certain time in the morning, in the evening, or prior to bed. In certain instances, a wortmannin analog is administered in the morning. In other embodiments, a wortmannin analog is administered at different times of the day for the entire administration period. For example, a wortmannin analog are administered in 8:00 am in the morning for the first day, 12 pm noon for the next day or administration, 4 pm in the afternoon for the third day or administration, and so on.

[0230] Any administration of the anticancer agent and a PI-3 kinase inhibitor (e.g., any PI-3 kinase inhibitor described herein, e.g., a wortmannin analog (e.g., a compound of Formula IA, Formula IB, Formula IIA or Formula IIB as described herein))

combinations described herein is adjusted and modified via factoring conditions as a subject's response, age, sex, disease, etc. at the beginning of treatment and throughout the course of the administration at the discretion of a physician. Administration of the second anticancer agent a PI-3 kinase inhibitor (e.g., any PI-3 kinase inhibitor described herein, e.g., a wortmannin analog (e.g., a compound of Formula IA, Formula IB, Formula IIA or Formula IIB as described herein)) is also adjusted and modified according to desired bioavailability of the two agents. In some embodiments, bioavailability of a wortmannin analog (e.g., a compound of Formula IA, Formula IB, Formula IIA or Formula IIB as described herein) is measured by a wortmannin analog metabolite described herein in the subject such as the 17-hydroxy or 11,17-hydroxy metabolite.

Effects of Treatment

[0231] In some embodiments, treatment with methods described herein results in various effects that provide benefit to a patient. In one embodiment, the effect of combination therapy is an additive effect; in another embodiment, the effect of the combination therapy is a synergistic effect; and in another embodiment, the effect of the combination therapy is a potentiating effect. One effect of treating a subject, with a combination therapy described herein is an increase in the length of survival. Another effect of treating a subject, with a combination therapy described herein is progression-free survival. Yet another effect is potentiation of the effect of either single agent, i.e., PI-3 kinase inhibitor or the second anticancer agent. For example, a potentiating effect is associated with a delay in tumor growth, progression free survival, disease stabilization, improved overall survival, or the like, or a combination thereof. Similarly, administering a described combination therapy to a subject impacts that subject's "quality of life" or "health-related quality of life." Moreover, in certain subjects, treatment with a combination therapy described herein results in modulating assessed biomarkers including, but not limited to, decreases in phosphatase and tensin homolog (PTEN) mutational status, PI3K gene amplification, PI3K catalytic subunit alpha (PIK3CA) mutational status, K-ras mutational status, and/or B-raf mutational status as well as biomarkers specific in various cancers. For example, a treatment with a described combination therapy to an individual with prostate cancer can lower prostate-specific antigen (PSA), stabilize PSA, or decrease PSA doubling rates.

[0232] Comparisons of the effects of treatment with a combination therapy described herein are made between treated subjects and subjects who are either undergoing no care, subjects

who are undergoing a standard of care (SOC) or subjects who are receiving only one of the active agents in a combination therapy described herein. SOC comprises many alternative types of care that do not include treatment with a combination therapy described herein. For example, SOC, although usually discretionary depending on the circumstances, may include psychosocial support, analgesics, and nutritional support. In some embodiments, comparison of the effects of treatment will be made between subjects receiving differing amounts of active agents a combination therapy described herein. In yet further embodiments, individuals will undergo SOC in conjunction with treatment with a combination therapy described herein.

- [0233] In some embodiments, before treatment of a subject with a combination therapy described herein, the subject may undergo pre-treatment evaluation. A non-limiting example of a pre-treatment evaluation includes a complete history and physical examination. The physical examination may include such things as a CT scan or X-ray. Subjects may also undergo treatment evaluations during the course of treatment. A treatment evaluation may include monitoring a subject's vital signs, inspecting injection sites, and analyzing blood samples.
- [0234] In some embodiments, an individual treated with a combination therapy regimen described herein has treatment effects evaluated by determining: (a) tumor size, (b) tumor location, (c) nodal stage, (d) growth rate of the cancer, (e) survival rate of the subject, (f) changes in the subject's cancer symptoms, (g) changes in the subject's Prostate Specific Antigen (PSA) concentration, (h) changes in the subject's PSA concentration doubling rate, (i) changes in the subject's biomarkers, or (i) changes in the subject's quality of life. Tumor evaluations are determined by any standardized criteria including Response Evaluation Criteria In Solid Tumors (RECIST) criteria.
- [0235] Survival rates are determined by comparing the current number of survivors with the number of individuals who started treatment a described combination therapy. In other embodiments, survival rates are compared to published survival rates for a particular type of cancer. In yet other embodiments, survival rates are compared to survival rates of individuals treated with one of the active agents in a combination therapy. In general, the survival rate is measured at any time following the start of treatment.
- [0236] For example, the survival rate is measured at less than 6 months following the start of treatment, greater than 6 months but less than a year, a year or greater but less than 2 years, 2 years or greater but less than 5 years, or 5 or greater years. In some

embodiments, an increased survival rate will be evidence that a described combination therapy has effects on a particular subject.

- [0237] In some embodiments, an effect of treating a subject having cancer a combination therapy described herein is maintenance or an increase in a subject's quality of life. Clinicians and regulatory agencies recognize that a subject's "quality of life" ("QoL") is an important endpoint in cancer clinical trials. See, for instance, Plunkett et al., *Clin. Lung Cancer*, 5(1):28-32 (2003), and Cella et al., *J. Clin. Epidemiol.*, 55(3):285-95 (2002), which are each incorporated herein by reference.
- [0238] Four important quality of life indicators are physical and occupational function, psychologic state, social interaction, and somatic sensations. For example, in individuals with NSCLC, two lung cancer questionnaires, the European Organization for Research and Treatment of Cancer ("EORTC") and the Functional Assessment of Cancer Therapy ("FACT-L"), are used to assess an individual's, specifically an individual's, health-related quality of life before, during, and after treatment with a combination therapy described herein.
- [0239] In various embodiments, the above evaluations are used in conjunction with assessments according to various subscales that monitor a subject's Physical Well-being (PWB), Social/Family Well-being (SWB), Emotional Well-being (EWB), Functional Well-being (FWB), and Lung Cancer Symptom subscale (LCS). Although the Lung Cancer Symptom subscale is obviously tailored to individuals with lung cancer, different subscales may be used with different types of cancer. Thus, a different subscale may be used with individuals with other cancers. Depending on which "Well-being" scores are combined, one may obtain a "FACT-L score" (the sum of all of the subscales) or a "Trial Outcome Score (TOI)" (the sum of the PWB, FWB, and LCS subscales). The TOI is a reliable indicator of meaningful change in quality of life. See, Cella et al., *supra*.
- [0240] A subject may be assessed for their FACT-L and TOI scores before, during, and after treatment with a combination therapy described herein. For instance, the TOI score may be taken at baseline, i.e., pre-treatment, and then at various intervals after treatment has started, i.e., at 4 weeks, 8 weeks, 19 weeks, 31 weeks, or 43 weeks, or longer. These various intervals are examples only and the quality of life indicators are taken at any appropriate time. For example, the first TOI score is taken after the first treatment, instead of at a baseline. Then, the change in scores between various time points is calculated to determine trends relating to improving, worsening, or maintaining of quality of life.

- [0241] It has been calculated that a decrease of 3 points or more from baseline for LCS is a clinically meaningful worsening in lung cancer symptoms and an increase in 3 or more points is a clinically meaningful improvement in lung cancer symptoms. Likewise for TOI scores, a decrease of 7 or more points indicates a worsening in quality of life, while an increase of 7 or more points indicates an improvement in quality of life.
- [0242] In some embodiments, a clinical improvement in cancer symptoms or quality of life demonstrates that a described combination therapy has effects on a particular subject.
- [0243] Administering a combination therapy described herein is useful in improving or maintaining the quality of life of treated subjects that have cancer. In measuring the effect on the quality of life, an effect size is determined from baseline or from any treatment point. In some embodiments, an effect size of between 0.2 to <0.49 indicates a small effect, 0.5 to 0.79 indicates a moderate effect, and 0.8 or greater indicates a large effect. These numbers are examples only and the observed effect size changes based on treatment and individual.
- [0244] Administration of a combination therapy described herein is useful in preventing the worsening in quality of life seen over time in many cancer patients. For example, in some embodiments, administration of a combination therapy described herein results in quality of life indexes that essentially remain unchanged or do not reach the level of worsening or improving quality of life.
- [0245] In other embodiments, the present treatments described herein encompasses improving or maintaining the quality of life or improving or stabilizing lung cancer symptoms in an individual diagnosed with NSCLC by determining the individual's TOI or LCS scores before, during, and after treatment with a combination therapy described herein.
- [0246] In other embodiments, the response of subjects to a combination therapy described herein is measured by changes in certain biomarkers including, but not limited, decreases in phosphatase and tensin homolog (PTEN) mutational status, PI3K gene amplification, PI3K catalytic subunit alpha (PIK3CA) mutational status, K-ras mutational status, and/or B-raf mutational status. Biomarkers include other changes in copy number, nucleotide and protein concentrations, and/or mutational status in other genes involved in one of the PI-3K signal transduction pathways. The effects of a combination therapy on biomarkers are measured at any time. For example, although a PTEN copy number are compared to a baseline value, PTEN copy number is also be compared between treatment points or between a specific treatment point and the end of treatment.

[0247] In further embodiments, the response of subjects to a combination therapy described herein is measured using tests of immune function, such as a T-cell proliferation response assay. In some embodiments, the results from T-cell proliferation response assays will be used to determine whether a combination therapy described herein has an effect on a subject. Results from these assays are also used to determine individual response to the formulations during different time points during the course of the treatment. Comparison of the T-cell proliferation response is undertaken to compare pre-treatment versus post-treatment response as well as to compare immune responses within treatment.

Kits/Articles of Manufacture

[0248] Provided herein are kits and articles of manufacture for use in the methods described herein. Such kits comprise a carrier, package, or container that is compartmentalized to receive one or more containers such as vials, tubes, and the like, each of the container(s) comprising one of the separate elements to be used in a method described herein including an anticancer agent and a PI-3 kinase inhibitor (e.g., a wortmannin analog). Suitable containers include, for example, bottles, vials, syringes, and test tubes. The containers are formed from a variety of materials such as glass or plastic.

[0249] A kit typically comprises one or more additional containers, each with one or more of various materials (such as reagents, optionally in concentrated form, and/or devices) desirable from a commercial and user standpoint for a combination therapy described herein. Non-limiting examples of such materials include, but not limited to, buffers, diluents, filters, needles, syringes; carrier, package, container, vial and/or tube labels listing contents and/or instructions for use, and package inserts with instructions for use associated with docetaxel and/or a wortmannin analog. A set of instructions will also typically be included.

[0250] A label is on or associated with the container. A label is on a container when letters, numbers or other characters forming the label are attached, molded or etched into the container itself; a label is associated with a container when it is present within a receptacle or carrier that also holds the container, e.g., as a package insert. A label is used to indicate that the contents are to be used for a specific therapeutic application. The label can also indicate directions for use of the contents, such as in the methods described herein.

[0251] Kits are supplied and manufactured according to dosages or administration methods described herein.

EXAMPLES

General procedures

[0252] HPLC method:

[0253] Instrument: Agilent HP1100

[0254] Detector: UV 254 nm

[0255] Column: Phenomenex Luna C18 (2), 3 μ m, 4.6 x 150 mm

[0256] Temperature: 25°C

[0257] Mobile Phase A: 10mM ammonium formate in 80% water 20% ACN, adjusted to pH 4.2 with formic acid

[0258] Mobile Phase B: acetonitrile

[0259] Flow Rate: 1.0 mL/min

[0260] Injection volume: 5 μ L (with ACN needle wash)

[0261] Detection Time: 30 min

[0262] Run Time: 38 min

[0263] Sample Preparation: dilution in acetonitrile

[0264] Gradient:

time (min)	% A	% B
0	100	0
6	79	21
16	79	21
25	6	94
30	6	94
31	100	0
38	100	0

[0265] XRPD analyses were performed using an Inel XRG-3000 diffractometer equipped with a CPS (Curved Position Sensitive) detector with a 2θ range of 120° . Real time data were collected using Cu-K α radiation at a resolution of $0.03^\circ 2\theta$. The tube voltage and amperage were set to 40 kV and 30 mA, respectively. The monochromator slit was set at 5 mm by 160 μ m. The pattern is displayed from 2.5 - $40^\circ 2\theta$. Each sample was prepared for analysis by packing it into a thin-walled glass capillary. The capillary was mounted onto a goniometer head that is motorized to permit spinning of the capillary during data acquisition. The samples were analyzed for 5 min. Instrument calibration was performed using a silicon reference standard.

[0266] Differential scanning calorimetry (DSC) was performed using a TA Instruments Q2000 differential scanning calorimeter. Temperature calibration was performed using NIST

traceable indium metal. The sample use placed into an aluminum DSC pan, and the weight was accurately recorded. The pan was covered with a lid, and the lid was crimped. A weighed, crimped aluminum pan was placed on the reference side of the cell. The sample cell was equilibrated at -30 °C and heated under a nitrogen purge at a rate of 10 °C/minute, up to a final temperature of 250 °C.

Example 1 Solubility Screening Test I

- [0267] For each solvent, a 100 µL glass crimp-top vial was charged with ~10 mg of PX-866 and sealed. By syringe, 2 parts of the appropriate solvent was added. If PX-866 dissolved completely, no further work was done. If necessary, more solvent was added to obtain a supernatant. The crimped vial was sonicated for 15 minutes and then centrifuged for 30 seconds. 1.0 µL of the supernatant was removed by syringe and added to a sealed HPLC vial containing 500 µL of acetonitrile. The same saturated solution was then heated for 30 minutes to a set point of 50°C, centrifuged again, and a second HPLC vial was prepared with 1.0 µL of the supernatant at 50°C. In the case of toluene, anisole, *o*-xylene, propyl acetate, 4-methyl-2-pentanone, and 1-pentanol the room temperature supernatant was transferred to a clean vial and cooled down to 0°C, and then -20°C. For each temperature, an HPLC vial was prepared with 1.0 µL of the supernatant. HPLC samples were run using the method described below and the 254 nm AUC was plotted on a calibration curve to obtain solubility data.
- [0268] Screening on the first group of solvents showed that PX-866 was very soluble in most of the common solvents. Furthermore, for the solvents with intermediate solubility, increasing the temperature from room temperature to 50°C had little to no effect on solubility. The alkanes were all good antisolvents, but appeared to be absorbed and retained by the amorphous solid in the same way as the heptane antisolvent in the supplied process. Upon storing the solubility samples at -20°C, the compound oiled out in the case of toluene and isopropyl alcohol (IPA), which suggested that these solutions might have been supersaturated.
- [0269] The solubility of wortmannin in toluene, anisole, and *o*-xylene was also investigated to ensure that wortmannin could be excluded in a crystallization procedure. In each case, the solubility was low; however, the conversion of wortmannin to PX-866 was always close enough to quantitative that it was not a concern.

[0270] Table 1. PX-866 solubility Screening I

Solvent	b.p. (°C)	Solubility (mg/mL)		Comments
ethyl acetate	77.1	>500 @ RT		Highly soluble
isopropyl acetate	89	>500 @ RT		Highly soluble
methyl <i>t</i> -butyl ether	55.2	RT	41.0	Moderately soluble
2-butanone	79.6	>500 @ RT		Highly soluble
acetone	56.5	>500 @ RT		Highly soluble
ethanol	78.4	>500 @ RT		Highly soluble, product oiled out after cooling
isopropyl alcohol	82.3	>500 @ RT		Highly soluble, but tends to oil out at RT
1-butanol	117.8	>500 @ RT		Highly soluble, product oiled out after cooling
<i>t</i> -Butanol	82.4	30°C	340.0	Partially soluble, unfavorable due to its melting
heptanes	98.5	RT	0.88	Effectively insoluble
2,2,4-	99.3	RT	0.37	Effectively insoluble
pentane	36.1	RT	0.79	Effectively insoluble
dimethylsulfoxide	189	RT	252.5	Partially soluble
methyltetrahydrofura	80	>500 @ RT		Highly soluble
water	100	RT	3.5	Slightly soluble, significant decomposition
anisole	152	RT	9.9	Supersaturated solution in 2 parts
		0°C	7.0	
		-20°C	6.2	Slightly soluble, data obtained from supernatant concentration after crystallization
diethyl ether	34.6	RT	9.5	Slightly soluble
toluene	110.6	RT	5.2	Supersaturated solution in 2 parts
		0°C	2.7	
		-20°C	2.6	Slightly soluble, data obtained from supernatant concentration after crystallization
methanol	64.7	>500 @ RT		Highly soluble
1,4-dioxane	101.1	>500 @ RT		Highly soluble
hexanes	69	RT	0.31	Effectively insoluble
N-methyl-2-	203	>500 @ RT		Highly soluble
dimethylformamide	153	>500 @ RT		Highly soluble
tetrahydrofuran	66	>500 @ RT		Highly soluble
dichloromethane	40	>500 @ RT		Highly soluble
<i>m</i> -xylene	137	RT	4.5	Slightly soluble
<i>o</i> -xylene	143	RT	5.5	Supersaturated solution in 2 parts
		0°C	7.3	
		-20°C	5.7	Slightly soluble, data obtained from supernatant concentration after crystallization
<i>p</i> -xylene	137	RT	1.0	Effectively insoluble
<i>sec</i> -butyl ether	121	RT	1.3	Effectively insoluble
diisopropyl ether	65	RT	4.2	Effectively insoluble

dibutyl ether	142	RT	3.1	Effectively insoluble
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Example 2. Crystallization Test I

- [0271] The first single solvent systems targeted were those with intermediate solubility from table 1: MTBE and DMSO. PX-866 was dissolved in a minimal amount of solvent at RT and cooled to -20°C for several days. Glass powder was introduced for nucleation. These attempts were unsuccessful.
- [0272] Some of the solvents which caused the compound to oil out at -20°C (toluene, IPA) were also investigated as single solvent systems. PX-866 was dissolved in a minimal amount of solvent and the solutions were added to new vials containing glass powder. In the case of toluene, the compound crystallized on the syringe tip and on the walls of the vial. This solid appeared crystalline by microscope. With IPA, the compound crystallized upon cooling but oiled out upon warming back to RT.
- [0273] Given the positive result with toluene, anisole and the three xylene isomers were screened because they are analogous to toluene. PX-866 crystallized from anisole and *o*-xylene in a similar way to toluene. The crystallizations from toluene, anisole, and *o*-xylene were scaled up to 50 mg (from 10 mg) using larger solvent volumes (20 vol. equivalents) and seeding with crystals from the previous experiment instead of glass powder. The resulting solids were all crystalline by XRPD.
- [0274] Residual solvents in the three solids were determined by ¹H NMR integration. In each case no heptane was present but the aromatic solvent remained in roughly a 1:1 molar ratio. The material (from anisole) was dried at 90°C under vacuum for 18 hours, which failed to remove any of the anisole. Drying under the same conditions for 88 hours removed a significant amount of the anisole (qualitatively, by HPLC), but also caused the material to turn into a glass and decompose to 95% purity (from 99%). These data (along with early stability results) led to the consensus that the crystalline forms were solvates, and PX-866 was stabilized against decomposition by interactions with solvent molecules within the crystal.
- [0275] A 10 g synthesis of PX-866 was started to investigate how to incorporate the crystallization into the supplied process. Once conversion was complete by HPLC, several aliquots of the reaction mixture were removed to test various workup conditions (see experiment 16 in Table 4). In the best procedure (highest yield and purity), the mixture was dried to a thick oil, dissolved in 3.5 parts of THF, and the antisolvent (anisole/toluene) was slowly added up to 8 parts total. Due to the toxicity concern, it was

opted to work up the batch with anisole and 7.9 g of PX-866 anisole solvate was isolated (78% yield). This material was 99.2% pure by HPLC (254 nm AUC), crystalline by XRPD, and contained 1.18 mol equivalents of anisole but no heptane or diallylamine.

[0276] A serial recrystallization of the anisole solvate was attempted to determine if subsequent recrystallizations offered any improvement in purity (experiment 18 in Table 4). The experiment failed to yield material of > 99.2% purity, and nearly 50% of the material was lost in each recrystallization. Vapor diffusion with several solvents was also attempted (experiment 19 in Table 4) in order to obtain an anhydrous, crystalline form of PX-866, but this was abandoned after one month with no positive results observed.

Example 3. Solubility Screening Test II

[0277] A new set of solvents (Table 2) were screened in an attempt to find a crystallization solvent that did not produce a solvate. Several of the new solvents caused the compound to spontaneously crystallize immediately after dissolution. The samples that crystallized were cooled to 0°C and subsequently -20°C to acquire two additional temperature points. Solubility in the aliphatic solvents (propyl acetate, 4-methyl-2-pentanone, and 1-pentanol) decreased drastically with temperature from RT to -20°C.

[0278] Table 2. PX-866 Solubility Screening II

Solvent	b.p. (°C)	Solubility (mg/mL)		Comments
butyl acetate	126	>500 @ RT		Highly soluble
ethyl formate	54	>500 @ RT		Highly soluble
isopentanol	131	RT	228.2	Oiled out of 2 parts
cumene	152	RT	4.9	Slightly soluble, crystallized from saturated solution
methyl acetate	57	>500 @ RT		Highly soluble
4-methyl-2-pentanone	117	RT	38.4	Partially soluble, crystallized from saturated solution
		0°C	33.4	
		-20°C	8.5	
1-pentanol	138	RT	84.4	Partially soluble, crystallized from saturated solution
		0°C	22.8	
		-20°C	2.4	
propyl acetate	102	RT	84.6	Partially soluble, crystallized from saturated solution

		0°C	54.8	
		- 20°C	18.6	
chlorobenzene	132	RT	15.6	Slightly soluble, crystallized from saturated solution
ethylene glycol	197	RT	135.9	Partially soluble
2-ethoxyethanol	135	>500 @ RT		Highly soluble
nitromethane	101	>500 @ RT		Highly soluble
benzyl alcohol	205	RT	498.0	Highly soluble, crystallized from saturated solution
di(ethylene glycol)	197	RT	374.8	Partially soluble
diethylamine	56	>500 @ RT		Highly soluble
ethanolamine	171	>500 @ RT		Solution turned dark orange, no PX-866 detected in supernatant
glycerol	182	RT	12.6	Slightly soluble
1,1,1,3,3,3,hexafluoro-2-propanol	59	>500 @ RT		Highly soluble
1-octanol	195	RT	90.6	Partially soluble
perfluorohexane	59	RT	0.28	Effectively insoluble
1,1,2,2-tetrachloroethane	131	>500 @ RT		Highly soluble
1,1,1-trichloroethane	74	>500 @ RT		Highly soluble
2,2,2-trifluoroethanol	74	>500 @ RT		Highly soluble

Example 4. Crystallization Test II

[0279] The anisole solvated PX-866 was recrystallized from propyl acetate, 4-methyl-2-pentanone, 1-pentanol, cumene, and chlorobenzene by dissolving the solid in a minimal amount of solvent at RT and cooling to -20°C overnight. Much slower crystallization and larger crystal size resulted in the case of the aliphatic solvents. NMR residual solvent analysis was performed on each of the solids; each contained both the crystallization solvent and anisole which added to roughly 1 mol equivalent. The XRPD patterns of the solids were all remarkably similar to each other and to previous lots.

- [0280] The crystallizations were repeated starting with amorphous PX-866 to see if completely excluding anisole would yield an anhydrous crystal form. However, the XRPD pattern of these crystals was indicative of the same crystal form.
- [0281] The new aliphatic solvents were incorporated into the manufacturing procedure on a 10 g batch. Three aliquots of the completed reaction mixture were removed to test propyl acetate, 4-methyl-2-pentanone, and 1-pentanol in the workup (see experiment 26 in table 4). All three options resulted in acceptable yields of similarly high purity material with ~0.8 mol equivalents of solvent. It was elected to work up the batch with propyl acetate and 8.79 g of PX-866 propyl acetate solvate was obtained (80.7% yield). The material was 99.3% pure by HPLC (254 nm AUC) and contained 0.85 mol equivalents of propyl acetate by NMR.
- [0282] The DSC and TGA profiles of the anisole and propyl acetate solvates were acquired for the purpose of determining the temperature of solvent volatilization. In both cases only one DSC endotherm was observed due to melting, but the melting points of the two solvates were drastically different (146.0°C for anisole, 80.5°C for PrOAc). The TGA results were inconclusive, as weight losses occurred over a broad temperature range and did not correspond to the solvent content. TG-IR would be a useful technique to gain insight into the solvent loss as well as decomposition pathway.

Example 5. Crystallization Study in Single Solvent: Anisole (18 volumes, no glass powder)

- [0283] 53 mg of PX-866 was placed in a 2 mL flat bottom vial and then added ~940 μ L (18 vol.) of anisole at RT. The supernatant was transferred to another vial and cooled down first to 4°C, and then to -20°C (PX-866 quantified in aliquots of the supernatant at both temperatures). The resulted solids were dried under a stream of nitrogen and further dried under vacuum at RT for 16 hours, then dried under vacuum at 40°C for 16 hours. PX-866 concentration in supernatant: RT: 9.9 mg/mL; 4°C: 7.0 mg/mL ; -20°C: 6.2 mg/mL.
- [0284] Isolated solids appear to be crystalline under microscope. The crystalline contained 15.8% (w/w) anisole, 0.8% heptane (by ^1H NMR integration) after nitrogen drying. After RT vacuum drying, no more heptane was detected and anisole amount was unchanged. After 40°C vacuum drying the crystalline was 98.5% pure by HPLC (254 nm AUC), and anisole amount appeared unchanged by NMR. XRPD pattern of the isolated crystalline shows a crystalline material (see Figure 1).
- [0285] XRPD data: (2-theta) 7.9, 8.5, 10.2, 11.1, 14.0, 14.2, 17.9, 18.7, 21.0, 21.2, and 28.2 ± 0.1 (See FIG. 1).

[0286] DSC data: a predominant endotherm at 146.0 °C (*See* FIG. 2).

[0287] The a-axis projection of the crystal packing in an anisole solvate of PX-866 is shown in FIG. 3. The crystalline form exhibits a single crystal X-ray crystallographic analysis at 120 K with crystal parameters as the following:

Space Group	P2 ₁ 2 ₁ 2 ₁
a, Å	13.7140(3)
b, Å	15.4272(4)
c, Å	15.6890(4)
α	90
β	90
γ	90
Z (molecules/unit cell)	4
Calculated Density (g/cm) 1.268.	

Example 6. Anisole crystallization study with PX-866 in THF solution

[0288] To a 100 μ L V-shaped glass vial containing 10 mg PX-866 was added 20 μ L (2 vol.) THF for complete solubilization. Anisole (1 to 3 volumes) was slowly added to the resulting solution; solids were visible after 2 volumes of anisole. Solids formed and dried under nitrogen. Isolated solids appeared crystalline under the microscope.

[0289] A larger scale was also conducted (51 mg in 102 μ L of THF and 408 μ L of anisole). XRPD pattern suggests same crystal as Example 5. NMR integration shows 1.74 equivalent anisole in the crystal.

Example 7. Crystallization Study in Single Solvent: Toluene (2 to 5 volumes)

[0290] About 10 mg of PX-866 was dissolved in toluene (20 μ L; 2 volumes) at RT with or without glass powder added. Upon gentle agitation to dissolve solid, product crystallized on walls of vial. A larger scale experiment was also conducted. About 50 mg of PX-866 was placed in 2 mL flat bottom HPLC vial. About 250 μ L (5 volumes) toluene was added to the vial at RT. The supernatant was transferred to another vial containing seeds from the 10 mg scale and cooled down first to 4 °C, and then -20 °C (PX-866 quantified in aliquots of the supernatant at both temperatures). The crystalline was formed and dried

under a stream of nitrogen. XRPD pattern of the isolated crystalline typical of a crystalline material.

Example 8. Crystallization Study in Single Solvent: Toluene (20 volumes, no glass powder)

[0291] About 47 mg of PX-866 was placed in 2 mL flat bottom HPLC vial. About 1000 μ L (20 volumes) toluene was added to the vial at RT. The supernatant was transferred to another vial and cooled down first to 4 °C, and then -20 °C (PX-866 quantified in aliquots of the supernatant at both temperatures). The crystalline was formed and dried under a stream of nitrogen.

[0292] The crystalline contained 12.5% (w/w) toluene, 0.98% heptane (by ^1H NMR integration) after nitrogen drying. After RT vacuum drying, no more heptane was detected and toluene amount was unchanged. After 40°C vacuum drying the crystalline was 97.7% pure by HPLC (254 nm AUC), and toluene amount appeared unchanged by NMR.

[0293] XRPD data: (2-theta) 7.9, 8.5, 10.2, 11.1, 12.5, 14.0, 18.7, and 21.1, ± 0.1 (FIG. 4).

[0294] DSC data: a predominant endotherm at 142.0 °C (FIG. 5).

Example 9. Toluene crystallization study with PX-866 in THF solution

[0295] To a 100 μ L V-shaped glass vial containing 10 mg PX-866 was added 20 μ L (2 vol.) THF for complete solubilization. Toluene (1 to 3 volumes) was slowly added to the resulting solution; solids were visible after 2 volumes of toluene. Evaporation of ~ 50% of the initial volume under a stream of nitrogen gave a supernatant that was transferred to another vial. Solids formed and dried under nitrogen. Isolated solids appeared crystalline under the microscope.

[0296] A larger scale was also conducted (64.5 mg in 129 μ L of THF and 516 μ L of toluene). XRPD pattern suggests same crystal as Example 8. NMR integration shows 1.54 equivalent toluene in the crystal.

Example 10. Propyl acetate crystallization

[0297] To a 100 mg of anisole solvate in scintillation vial was added 12 volumes of propyl acetate for complete dissolution. The resulting solution was held at room temperature for 2 hours and then cooled to -20 °C overnight. The produced solids were filtered and washed with minimal amount of cold propyl acetate and dried at room temperature under vacuum overnight. The crystal was analyzed by NMR for solvent content and by XRPD for crystallinity (see FIG. 6). It was found that the crystal contains 0.76 mol equiv PrOAc and 0.34 mol equiv anisole.

[0298] XRPD data: (2-theta) 8.0, 8.4, 10.2, 11.0, 14.0 and 19.2, ± 0.1 . (FIG. 6)

[0299] DSC data: a predominant endotherm at 80.5 °C. (FIG. 7)

[0300] The a-axis projection of the crystal packing in a propyl acetate solvate of PX-866 is illustrated in FIG. 8. The crystalline propyl acetate solvate exhibits a single crystal X-ray crystallographic analysis at 100 K with crystal parameters as the following:

Space Group	P2 ₁ 2 ₁ 2 ₁
a, Å	13.4963(5)
b, Å	15.5158(5)
c, Å	15.6912(6)
α	90
β	90
γ	90
Z (molecules/unit cell)	4

Calculated Density (g/cm) 1.269.

Example 11. 4-Methyl-2-pentanone crystallization

[0301] To a 100 mg of anisole solvate in scintillation vial was added 25 volumes of 4-methyl-2-pentanone for complete dissolution. The resulting solution was held at room temperature for 2 hours and then cooled to -20 °C overnight. The produced solids were filtered and washed with minimal amount of cold 4-methyl-2-pentanone and dried at room temperature under vacuum overnight. The crystal was analyzed by NMR for solvent content and by XRPD for crystallinity. It was found that the crystal contains 0.90 mol equiv 4-methyl-2-pentanone and 0.15 mol equiv anisole.

[0302] XRPD data: (2-theta) 7.9, 8.4, 10.2, 10.9, 13.9, 14.2, 18.5, 19.2, and 20.7, ± 0.1 . (FIG. 9)

Example 12. Cumene crystallization

[0303] To a 100 mg of anisole solvate in scintillation vial was added 221 volumes of cumene for complete dissolution. The resulting solution was held at room temperature for 2 hours and then cooled to -20 °C overnight. The produced solids were filtered and washed with minimal amount of cold cumene and dried at room temperature under vacuum overnight.

The crystal was analyzed by NMR for solvent content and by XRPD for crystallinity. It was found that the crystal contains 0.96 mol equiv cumene and 0.06 mol equiv anisole.

[0304] XRPD data: (2-theta) 7.8, 8.4, 10.1, 10.7, 13.7, 14.1, 18.1, 18.9, 20.6, and 20.8, ± 0.1 . (See FIG. 10).

Example 13. 1-Pentanol crystallization

[0305] To a 100 mg of anisole solvate in scintillation vial was added 35 volumes of 1-pentanol for complete dissolution. The resulting solution was held at room temperature for 2 hours and then cooled to -20°C overnight. The produced solids were filtered and washed with minimal amount of cold 1-pentanol and dried at room temperature under vacuum overnight. The crystal was analyzed by NMR for solvent content and by XRPD for crystallinity. It was found that the crystal contains 0.46 mol equiv 1-pentanol and 0.77 mol equiv anisole.

[0306] XRPD data: (2-theta) 8.1, 8.5, 10.2, 11.1, 12.5, 14.0, 14.3, 17.9, 18.8, 20.7, and 21.3, ± 0.1 . (FIG. 11)

Example 14. Chlorobenzene crystallization

[0307] To a 100 mg of anisole solvate in scintillation vial was added 52 volumes of chlorobenzene for complete dissolution. The resulting solution was held at room temperature for 2 hours and then cooled to -20°C overnight. The produced solids were filtered and washed with minimal amount of cold chlorobenzene and dried at room temperature under vacuum overnight. The crystal was analyzed by NMR for solvent content and by XRPD for crystallinity. It was found that the crystal contains 0.86 mol equiv chlorobenzene and 0.15 mol equiv anisole.

[0308] XRPD data: (2-theta) 8.0, 8.5, 10.3, 11.1, 14.1, 17.9, 18.8, 19.1, 21.0 and 28.3, ± 0.1 (FIG. 12).

Example 15. Synthesis of PX-866 anisole solvate from Wortmannin

[0309] In a 250 mL RBF equipped with a nitrogen bubbler, thermocouple, magnetic stirring, and cooling water bath, wortmannin (10 g, 23.34 mmol) was suspended in 50 mL of anhydrous THF resulting in a thin yellow slurry. To the slurry, diallylamine (34.5 mL, 280.1 mmol) was slowly added maintaining the temperature below 30°C . A 10 minute COR sample showed $\sim 2\%$ wortmannin remaining. After 90 minutes no wortmannin was detected. Six 2.5 mL aliquots of the reaction mixture were removed to test workup conditions; meanwhile the remaining solution was stored at -20°C . The solvent was

removed to afford a thick orange oil and THF (29 mL, 3.5 vol) was added and stirred until homogeneous. Anisole (25 mL, 3 vol) was added and the solution was seeded with anisole solvate crystals. Slowly, additional anisole was added up to a total of 8 vol. The suspension was agitated overnight. The solid was isolated on a Buchner funnel and washed with 2 vol of 3.5:8 THF/anisole. The isolated solid was dried overnight at RT in a vacuum oven to afford 7.9 g of PX-866 anisole solvate (78% yield).

Example 16. Synthesis of PX-866 propyl acetate solvate from wortmannin

[0310] In a 250 mL RBF equipped with a nitrogen bubbler, thermocouple, magnetic stirring, and cooling water bath, wortmannin (10 g, 23.34 mmol) was suspended in 50 mL of anhydrous THF resulting in a thin yellow slurry. To the slurry, diallylamine (3.5 mL, 28.0 mmol) was slowly added maintaining the temperature below 30°C. A 60 minute COR sample showed 0.2% wortmannin remaining. After 90 minutes, three 2 mL aliquots of the reaction mixture were removed to investigate workup conditions; meanwhile the remaining solution was stored at -20°C. The solvent was removed to afford a thick orange oil and propyl acetate (45 mL, 5 vol) was added and stirred until homogeneous. After ~1 minute the solution became cloudy. Agitation was continued at RT for 2 hours, and then the solution was cooled to -20°C overnight. The solid was isolated on a Buchner funnel and washed with 1 vol. of cold propyl acetate. The isolated solid was dried on a rotovap overnight (30°C bath temp, 8 mm Hg) to afford 8.79 g of PX-866 propyl acetate solvate (80.7% yield).

Example 17. Stability Test

[0311] A non-GMP stability study was initiated to establish the stability of the PX-866 solvates against heat and humidity compared to that of the amorphous material. Samples of the anisole solvate, toluene solvate, and amorphous PX-866 were placed in a stability chamber set to 40°C and 75% relative humidity to be pulled at 1,2,3,4, and 8 week time points for HPLC analysis. As soon as it was available, the propyl acetate solvate was added to the chamber to be pulled at a single 2.5 week point. (See Figure 13).

[0312] The anisole and toluene solvates offered a clear upgrade in stability over amorphous PX-866, which was evident after only one week. Based on limited data (only a single time point), the propyl acetate solvate was more stable than the amorphous material but less stable than the anisole solvate.

[0313] After the 8 week period in the stability chamber, the materials were analyzed by NMR for solvent content and XRPD for crystallinity. The anisole and toluene solvates

contained 1.34 and 1.25 mol equivalents of solvent respectively, which is roughly comparable to the original amount. The propyl acetate solvate contained 0.36 mol equivalents of solvent (roughly half the original amount) as well as a significant amount of water. All three solids were crystalline by XRPD.

Example 18. Amorphous and Crystalline PX-866 API-in-capsule in Rat Oral Pharmacokinetic Study

[0314] Absorption of orally administered amorphous and crystalline anisole solvate of PX-866 in rats (fed or fast) has been studied to investigate their pharmacokinetics *in vivo*. Rats were given 0.3 mg of amorphous and crystalline anisole solvate of PX-866, and blood was collected after administration.

Test Article	Dose (mg)	Food	Species	Formulation
Amorphous PX-866	0.3	Fast	SD rat	API-in-capsule
Crystalline PX-866	0.3	Fast	SD rat	API-in-capsule
Amorphous PX-866	0.3	Fed	SD rat	API-in-capsule
Crystalline PX-866	0.3	Fed	SD rat	API-in-capsule

Results

[0315] There is no statistically significant difference between plasma concentrations of PX-866 or the metabolite 17-OH-PX-866 in fed or fasted rats after administration of amorphous powder in capsules or crystalline anisole solvate PX-866 powder in capsules.

Example 19. Crystalline/Solvate PX-866 Formulations

[0316] *Example 19a: Hard Gelatin Capsule for Oral Administration*

[0317] To prepare a pharmaceutical formulation for oral administration, 10 mg of PX-866 in powder form was directly filled into hard gelatin capsules with no processing or blending with any excipients. The capsules were individually opened, tared, filled and closed with a capsule filling machine (Xcelodose, Capsugel).

[0318] *Example 19b: Direct-Compression Tablet for Oral Administration*

[0319] To prepare a pharmaceutical formulation for oral administration, crystalline anisole solvate of PX-866, Mg-Stearate, and Mannitol (Pearlitol 100SD, Roquette) were weighed, sieved and subsequently dry-blended (Turbula mixer). The API and mannitol were blended first and the dry blend is tested for uniformity. After passing that test, the Mg-Stearate was added for a short additional blend and the material was then tableted by a direct compression tableting machine with appropriate weight and hardness checks. 10 mg tablets of crystalline PX-866 were produced.

[0320] Tablets were then spray coated with a yellow moisture barrier (Opadry AMB 80W120002 Yellow) to both protect patients and pharmacists from directly contacting the API as well as to protect the API from possible moisture ingress.

[0321] *Example 19c: Hard Lozenge for Buccal Administration*

[0322] To prepare a sublingual pharmaceutical formulation for buccal delivery, e.g., hard lozenge, 10 mg of crystalline anisole solvate of PX-866 is mixed with 490 mg of powdered sugar, 1.6 ml of light corn syrup, 2.4 mL distilled water and 0.42 mL mint extract. The mixture is gently blended and poured into mold to form a lozenge suitable for buccal administration.

[0323] *Example 19d: Injectable Solution for Parenteral Administration*

[0324] To prepare a parenteral pharmaceutical formulation suitable for administration by injection, 10 mg of PX-866 is mixed with 10 mL of 0.9% sterile saline. The mixture is incorporated into a dosage unit form suitable for administration by injection.

Example 20. PX-866 and Docetaxel or cetuximab in a Direct Patient Tumor Xenograft Model of HPV positive and HPV negative head and neck cancer

[0325] A direct patient tumor model (DPTM) for Head and Neck Squamous Cell Carcinoma (HNSCC) was developed to preserve key features of human disease, including better replication of tumor-stroma interactions and preservation of human cancer stem cells. The model employed direct implantation of patients' tumors into nude mice according to Keysar et al., J Clin Oncol 28:15s, 2010 (suppl. abstract 5558). In vivo efficacy of PX-866, or a combination of PX-866 and docetaxel was tested.

[0326] Methods: DPTM were left untreated or exposed to PX-866, docetaxel, cetuximab, or a combination of PX-866 plus docetaxel, or PX-866 plus cetuximab, for 25-29 days. Tumor measurements were taken twice weekly during therapy and thereafter until study conclusion. Tumor initiating cell (TIC) population was determined by flow cytometry.

[0327] Results: Four DPTM (CUHN002, CUHN015, CUHN022, CUHN027) were treated with PX-866, or PX-866 plus cetuximab, and four DPTM (CUHN002, CUHN004, CUHN011, CUHN015) with PX-866, docetaxel, or the combination of PX-866 plus docetaxel. Neither combination had increased toxicity compared with the single agents. All untreated DPTM had rapid tumor growth. In two DPTM (CUHN015 and CUHN022) cetuximab was similar to no treatment, whereas single agent PX-866 was slightly more effective than no treatment in CUHN022 and significantly more effective in CUHN015. In CUHN027 both PX-866 and cetuximab arrested growth but the combination caused greater than 50% tumor reduction. In CUHN002 both cetuximab and PX-866 were effective at delaying tumor growth and the combination of PX-866 and cetuximab showed similar activity to cetuximab alone. Two xenografts treated with PX-866, docetaxel, or the combination showed decreased tumor growth with PX-866 compared to either docetaxel or no treatment (CUHN004 and CUHN015). In one DPTM (CUHN002) PX-866 had no effect alone, but in combination with docetaxel showed greater activity than docetaxel alone. FIG. 14, FIG. 15, FIG. 16 and FIG. 17 show data from the experiment.

[0328] PX-866 was equal or superior to cetuximab or docetaxel at slowing tumor growth in 6 of 8 DPTM of HNSCC. The combination of PX-866 plus another agent was superior to single agent therapy in 4 of 8 DPTM.

Example 21.

[0329] Following the procedure in Example 20, anisole solvate form of PX-866 is tested in combination with docetaxel in Hepatitis B positive and Hepatitis B negative hepatocellular DPTM.

Examples 22-24.

[0330] Following the procedure in Example 20, a combination of PX-866 and cisplatin is tested in a DPTM for HPV positive cervical cancer, a combination of PX-866 and bevacizumab is tested in a DPTM for EBV nasopharyngeal cancer, and a combination of PX-866 and dasatinib is tested in a DPTM for MCV positive skin cancer.

Example 25. Phase 1 Clinical trial PX-866 and Docetaxel Given to Patients with Newly Diagnosed HPV Positive, Locally Advanced Squamous Cell Carcinoma of the Oropharynx

[0331] This will be a Phase 1 open-label study of PX-866 given in combination with docetaxel to patients with HPV positive, locally advanced squamous cell carcinoma of the oropharynx.

Study Objectives

[0332] *Primary:* To determine the maximum tolerated dose (MTD) or recommended dose (RD) of PX-866 to be administered orally once per day in combination with docetaxel administered IV at a dose of 75 mg/m² once every 21 days in patients with HPV Positive, locally advanced squamous cell carcinoma of the oropharynx.

[0333] *Secondary:* To evaluate the preliminary antitumor activity of PX-866 administered in combination with docetaxel as assessed by objective response rate (ORR) and early progression rate (% of patients with progressive disease at 6 weeks).

Endpoints

[0334] *Primary Endpoint:* Incidence and severity of adverse events

[0335] *Secondary Endpoint:* Objective response rate (ORR) and early progression rate (% of patients with progressive disease at 6 weeks)

[0336] *Exploratory Endpoints*

[0337] Tumor mutational profile, including but not limited to phosphatase and tensin homolog (PTEN) mutational status, PI3K gene amplification, PI3K catalytic subunit alpha (PIK3CA) mutational status, K-ras mutational status, and B-raf mutational status

Example 26. Phase 1 Clinical trial PX-866 and Cetuximab Given to Patients with With Newly Diagnosed HPV Positive, Locally Advanced Squamous Cell Carcinoma of the Oropharynx

[0338] A Phase 1 open-label study of PX-866 given in combination with cetuximab to patients with HPV positive, locally advanced squamous cell carcinoma of the oropharynx is conducted following the protocol described in Example 25.

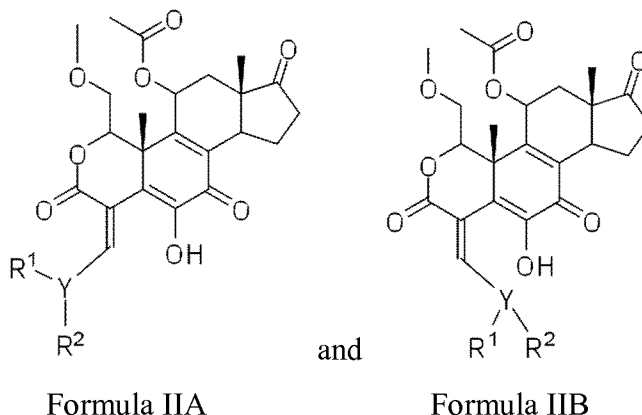
[0339] While embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

WHAT IS CLAIMED IS:

1. Use of a therapeutically effective amount of a PI-3 kinase inhibitor for the treatment of an oncovirus-positive cancer selected from human papilloma virus (HPV)-positive cancer, herpes virus-positive cancer, hepatitis virus-positive cancer, and Merkel cell polyoma virus (MCV)-positive cancer.

2. The use of claim 1, wherein the PI-3 kinase inhibitor is selected from PX-866, XL 147, GDC-0941 (Genentech/Roche), CAL-101 (Calistoga), NVP-BKM120 (Novartis), ZSTK474 (Zenyaku Kogyo), NVP-BYL179 (Novartis), AMG319 (Amgen), GDC0032 (Genentech/Roche), A66, AS-252424, AS-604850, AS-605240, AZD6482, CAY10505, CH5132799, D-106669, GSK1059615, PIK-293, PIK-90, PIK-93, GNE-490, CNX-1351 (Celgene/Avila), INK117 (Intellikine), PIK-39, BAY806946 (Bayer), XL 765 (Exelixis), GDC-0980 (Genentech), GSK 2126458 (GlaxoSmithKline), NVP-BEZ235 (Novartis), NVP-BGT226 (Novartis), PF04691503 (Pfizer), PKI587 (Pfizer), SF1126 (Semaphore), D-87503, GSK2126458, IC-87114, PI-103, PIK-294, PIK-75, PKI-402, PKI-587 (PF-05212384), Quercetin (Sophoretin), TG100-115, TGX-221, A-769662, phenformin hydrochloride, and PP121.

3. The use of claim 1, wherein the PI-3 kinase inhibitor is a compound selected from



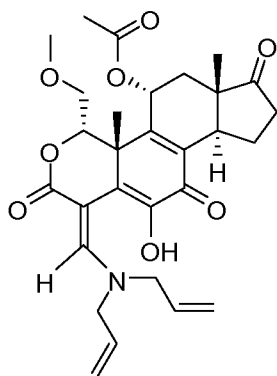
wherein Y is a heteroatom selected from nitrogen and sulfur;

R¹ and R² are independently selected from an unsaturated alkyl, cyclic alkyl, or R¹ and R² together with Y form a heterocycle;

or pharmaceutically acceptable salt, solvate, or polymorph thereof.

4. The use of claim 1, wherein the PI-3 kinase inhibitor is an irreversible inhibitor of PI-3 kinase.

5. The use of claim 1, wherein the PI-3 kinase inhibitor is



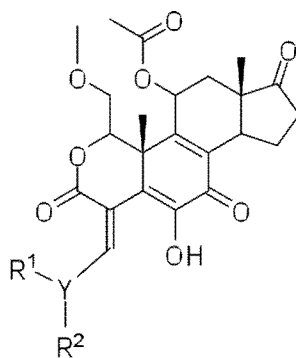
(PX-866),

or pharmaceutically acceptable salt, solvate, or polymorph thereof.

6. Use of a therapeutically effective amount of a PI-3 kinase inhibitor in combination with a second anticancer agent for the treatment of an oncovirus-positive cancer selected from HPV-positive cancer, MCV-positive cancer, herpes-virus positive cancer and hepatitis virus-positive cancer.

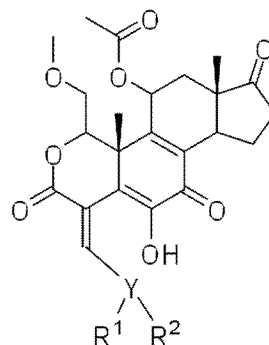
7. The use of claim 6, wherein the PI-3 kinase inhibitor is selected from PX-866, XL 147, GDC-0941 (Genentech/Roche), CAL-101 (Calistoga), NVP-BKM120 (Novartis), ZSTK474 (Zenyaku Kogyo), NVP-BYL179 (Novartis), AMG319 (Amgen), GDC0032 (Genentech/Roche), A66, AS-252424, AS-604850, AS-605240, AZD6482, CAY10505, CH5132799, D-106669, GSK1059615, PIK-293, PIK-90, PIK-93, GNE-490, BAY806946 (Bayer), XL 765 (Exelixis), GDC-0980 (Genentech), GSK 2126458 (GlaxoSmithKline), NVP-BEZ235 (Novartis), NVP-BGT226 (Novartis), PF04691503 (Pfizer), PKI587 (Pfizer), SF1126 (Semaphore), D-87503, GSK2126458, IC-87114, PI-103, PIK-294, PIK-75, PKI-402, PKI-587 (PF-05212384), Quercetin (Sophoretin), TG100-115, TGX-221, A-769662, phenformin hydrochloride, and PP121.

8. The use of claim 6, wherein the PI-3 kinase inhibitor is a compound selected from



Formula IIA

and



Formula IIB

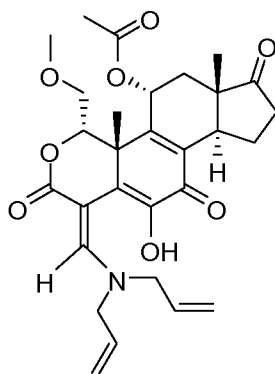
wherein Y is a heteroatom selected from nitrogen and sulfur;

R^1 and R^2 are independently selected from an unsaturated alkyl, cyclic alkyl, or R^1 and R^2 together with Y form a heterocycle;

or pharmaceutically acceptable salt, solvate, or polymorph thereof.

9. The use of claim 6, wherein the PI-3 kinase inhibitor is an irreversible inhibitor of PI-3 kinase.

10. The use of claim 6, wherein the PI-3 kinase inhibitor is

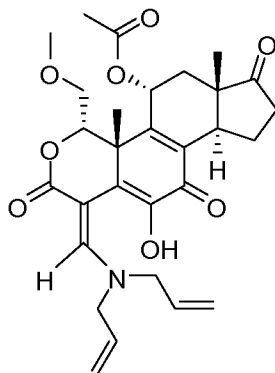


(PX-866),

or pharmaceutically acceptable salt, solvate or polymorph thereof.

11. Use of a composition comprising a therapeutically effective amount of an irreversible PI-3 kinase inhibitor in combination with a second anticancer agent for the treatment of an Epstein-Barr virus (EBV)-positive cancer.

12. The use of the composition of claim 11, wherein the irreversible PI-3 kinase inhibitor is



(PX-866),

or pharmaceutically acceptable salt, solvate or polymorph thereof.

13. The use of claim 1, claim 6 or composition of claim 11, wherein the cancer is selected from the group consisting of head and neck cancer, lung cancer, ovarian cancer, liver cancer, colon cancer, breast cancer, pancreatic cancer, kidney cancer, cervical cancer, uterine cancer, prostate cancer, esophageal cancer, nasopharyngeal cancer, oropharyngeal cancer, gastric cancer, skin cancer, vulvar cancer, vaginal cancer, anal cancer and penile cancer.

14. The use of claim 1, claim 6 or composition of claim 11, wherein the cancer is a solid tumor.

15. The use of claim 1, claim 6 or composition of claim 11, wherein the cancer is unresectable.

16. The use of any one of claims 1-10, wherein the cancer is a HPV positive squamous cell carcinoma, HPV positive head and neck cancer, HPV positive oropharyngeal cancer, HPV positive cervical cancer, HPV positive lung cancer, or HPV positive non-small cell lung cancer.

17. The use of any one of claims 1-10, wherein the cancer is Hepatitis B positive hepatocellular carcinoma, or Hepatitis C positive hepatocellular carcinoma.

18. The use of claim 11, wherein the cancer is an EBV positive B-cell malignancy, EBV positive Burkitt's lymphoma, Hodgkin's lymphoma, or diffuse large B-cell lymphoma, EBV positive nasopharyngeal cancer, or EBV positive gastric carcinoma.

19. The use of claim 6 or composition of claim 11, wherein the second anticancer agent is selected from methotrexate (RHEUMATREX®, Amethopterin) cyclophosphamide (CYTOXAN®), thalidomide (THALIDOMID®), acridine carboxamide, actimid®, actinomycin, 17-N-allylamino-17-demethoxygeldanamycin, aminopterin, amsacrine, anthracycline, bevacuzimab (Avastin®), antineoplaston, 5-azacytidine, azathioprine, BL22, bendamustine, biricodar, bleomycin, bortezomib, bryostatin, busulfan, calyculin, camptothecin, capecitabine, carboplatin, cetuximab, chlorambucil, cisplatin, cladribine, clofarabine, cytarabine, dacarbazine, dasatinib, daunorubicin, decitabine, dichloroacetic acid, discodermolide, docetaxel, doxorubicin, epirubicin, epothilone, eribulin, estramustine, etoposide, exatecan, exisulind, ferruginol, floxuridine, fludarabine, fluorouracil, fosfestrol, fotemustine, ganciclovir, gefitinib (Iressa®), gemcitabine, hydroxyurea, IT-101, idarubicin, ifosfamide, imiquimod, irinotecan, irofulven, ixabepilone, laniquidar, lapatinib, lenalidomide, lomustine, lurtotecan, mafosfamide, masoproc, mechlorethamine, melphalan, mercaptopurine, mitomycin, mitotane, mitoxantrone, nelarabine, nilotinib, oblimersen, oxaliplatin, PAC-1, paclitaxel, pemetrexed, pentostatin, pipobroman, pixantrone, plicamycin, procarbazine, proteasome inhibitors (e.g., bortezomib), raltitrexed, rebeccamycin, revlimid®, rubitecan, SN-38, salinosporamide A, satraplatin, streptozotocin, swainsonine, tariquidar, taxane, tegafur-uracil, temozolomide, testolactone, thioTEPA, tioguanine, topotecan, trabectedin, tretinoin, triplatin tetranitrate, tris(2-chloroethyl)amine, troxacitabine, uracil mustard, valrubicin, vinblastine, vincristine, vinorelbine, vorinostat, and zosuquidar.

20. The use of claim 6 or composition of claim 11, wherein the second anticancer agent is selected from docetaxel, cetuximab, gefitinib, and a platinum based chemotherapeutic agent.

21. The use of claim 6 or composition of claim 11, wherein the second anticancer agent and the PI-3 kinase inhibitor are administered simultaneously.

22. The use of claim 6 or composition of claim 11, wherein the second anticancer agent and the PI-3 kinase inhibitor are administered sequentially.

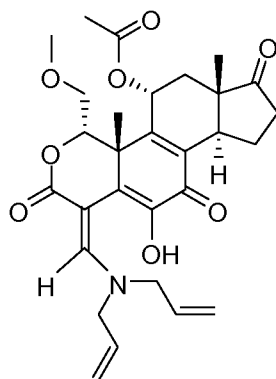
23. The use of claim 6 or composition of claim 11, wherein the second anticancer agent and the PI-3 kinase inhibitor are administered in a single composition.

24. The use of claim 1, claim 6 or composition of claim 11, wherein administration of the PI-3 kinase inhibitor is by intramuscular, intravenous, subcutaneous, intranodal, intratumoral, intracisternal, intraperitoneal, intradermal, transdermal, nasal, pulmonary, vaginal, rectal, buccal, ocular, otic, local, topical, or oral delivery.

25. The use of claim 1, claim 6 or composition of claim 11, wherein the PI-3 kinase inhibitor is administered orally.

26. The use of claim 1, claim 6 or composition of claim 11, wherein the PI-3 kinase inhibitor is administered in a capsule form.

27. The use of claim 1, claim 6 or composition of claim 11, wherein the PI-3 kinase inhibitor is a crystalline form of a compound having the structure



(PX-866),

which is substantially free of wortmannin.

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FIG. 1

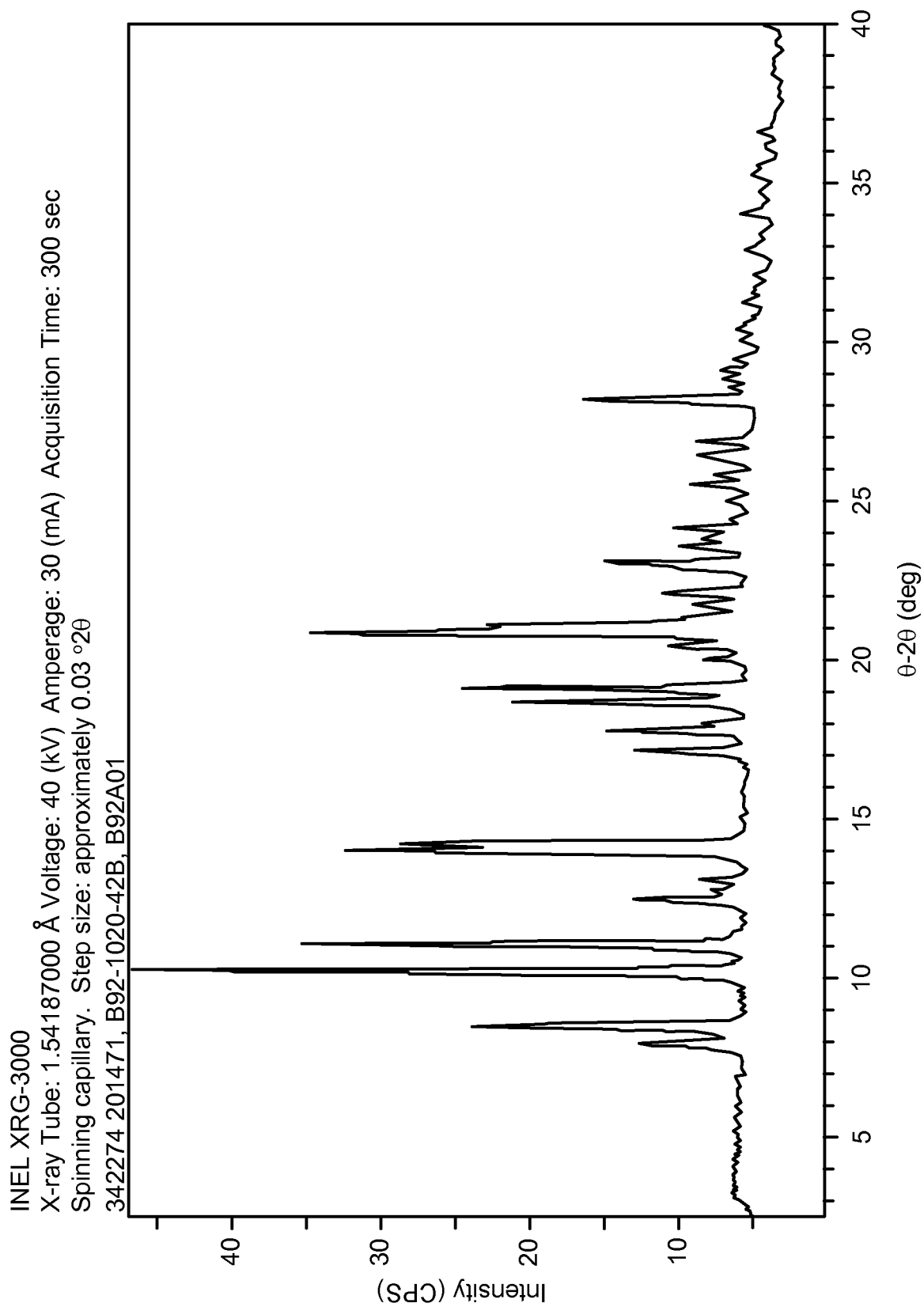
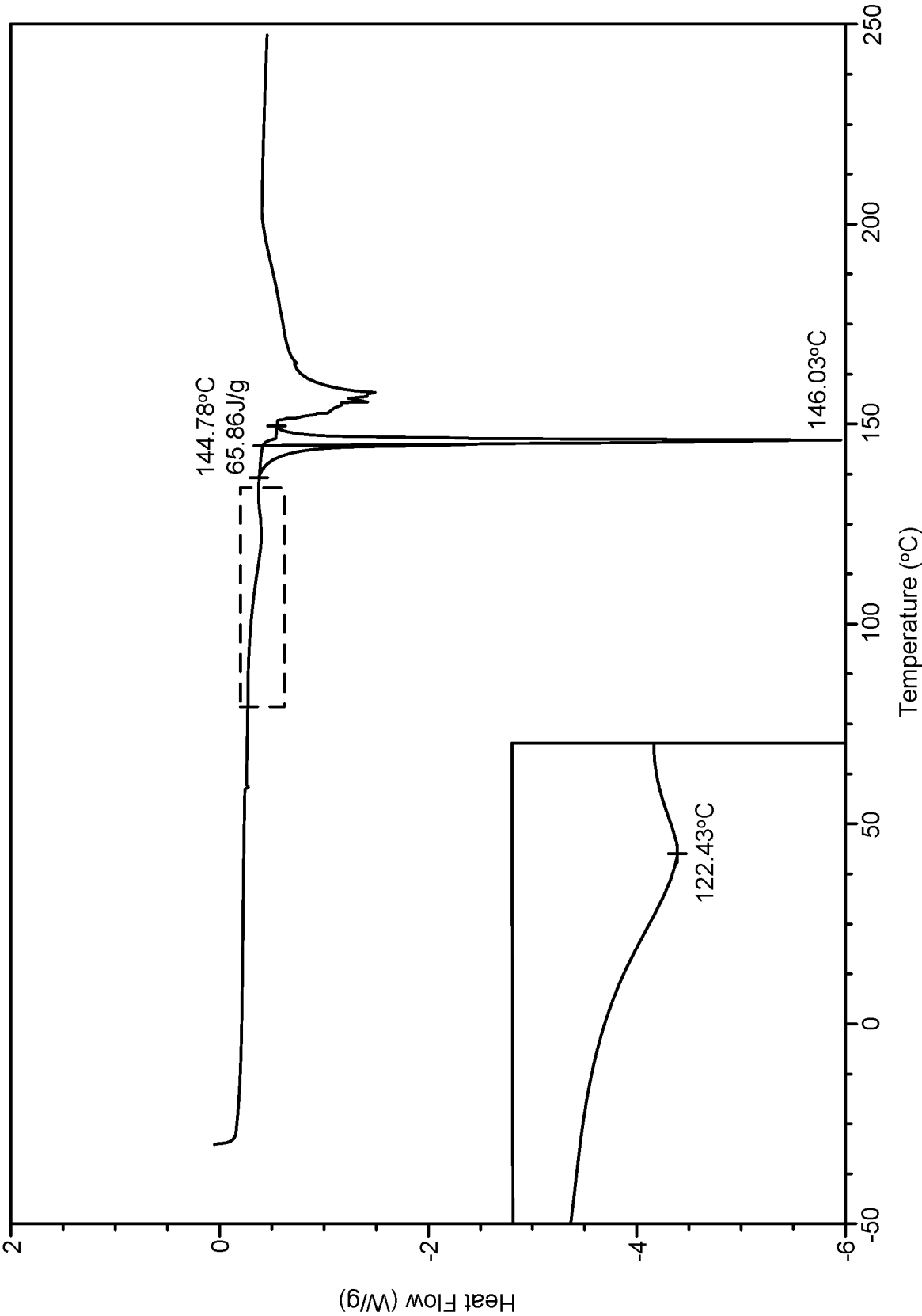
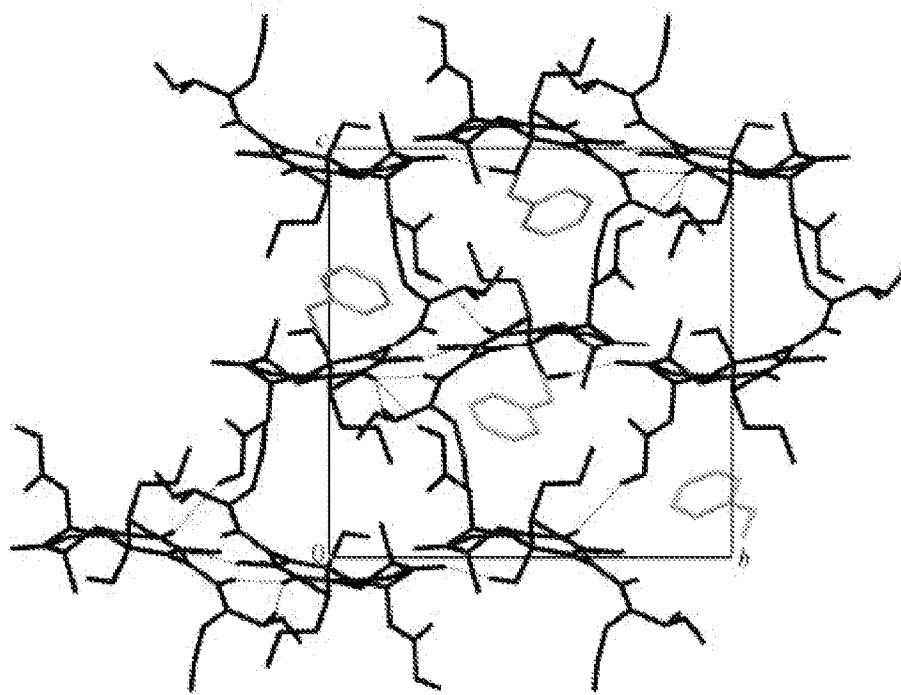


FIG. 2



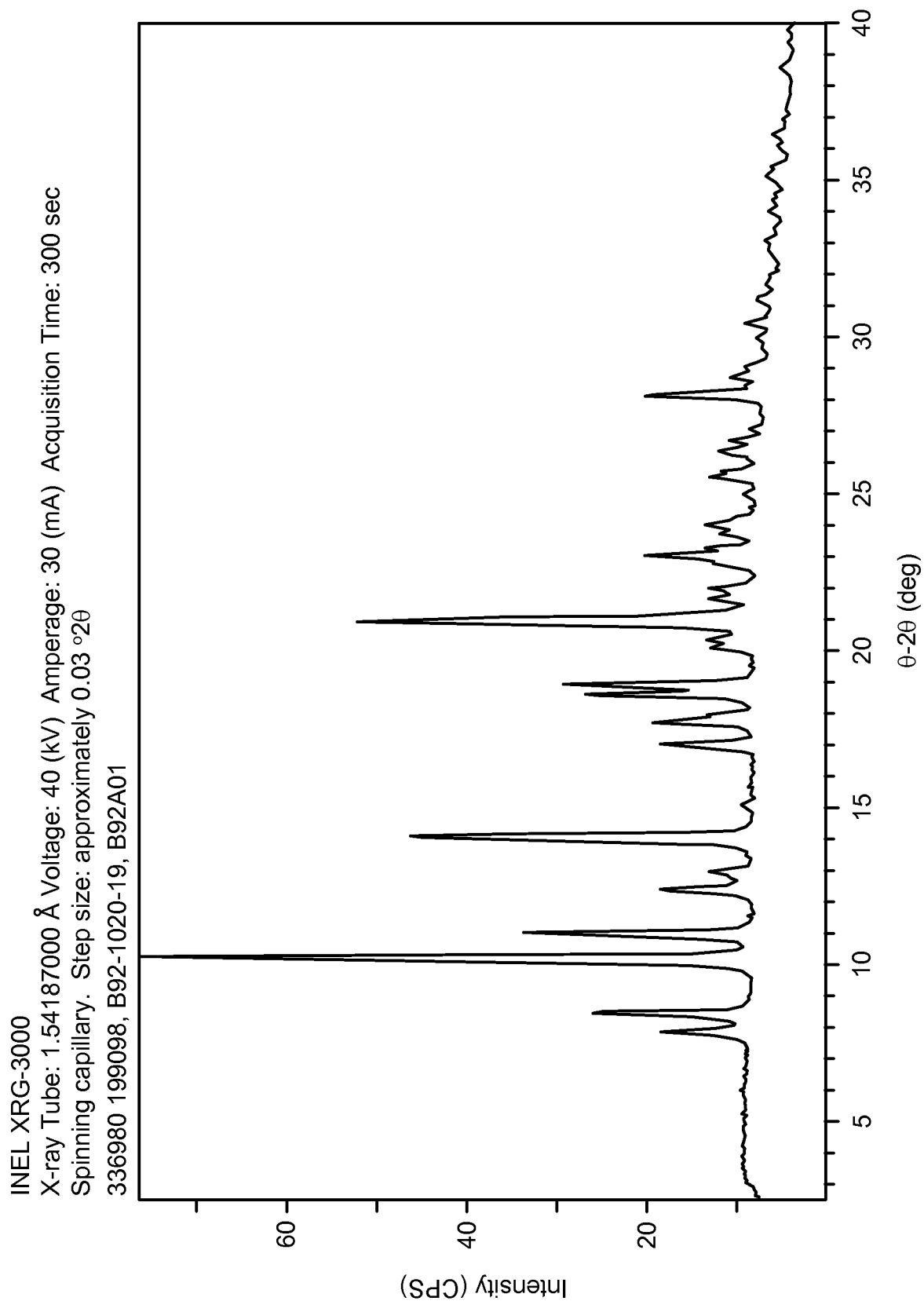
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FIG. 3



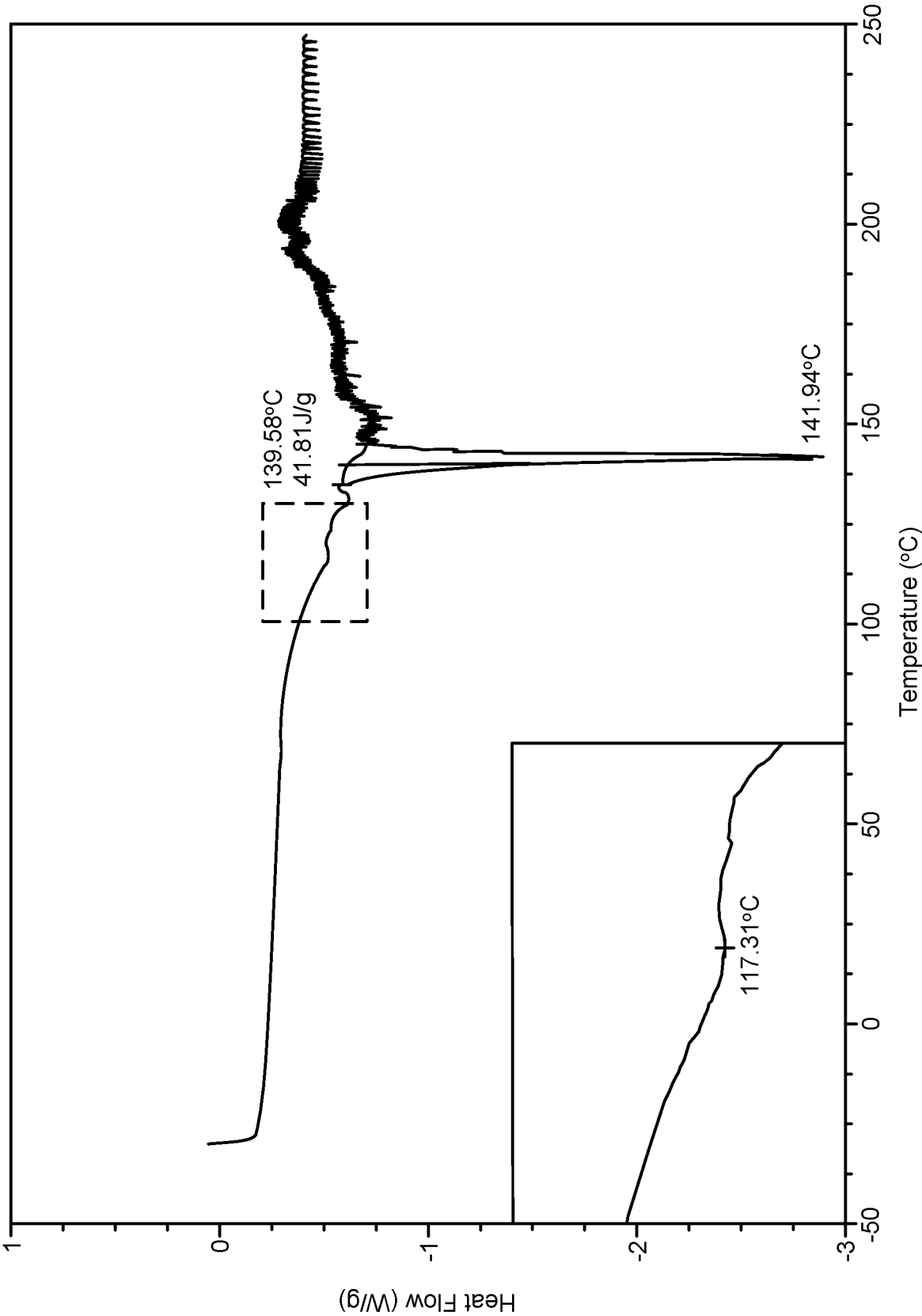
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FIG. 4



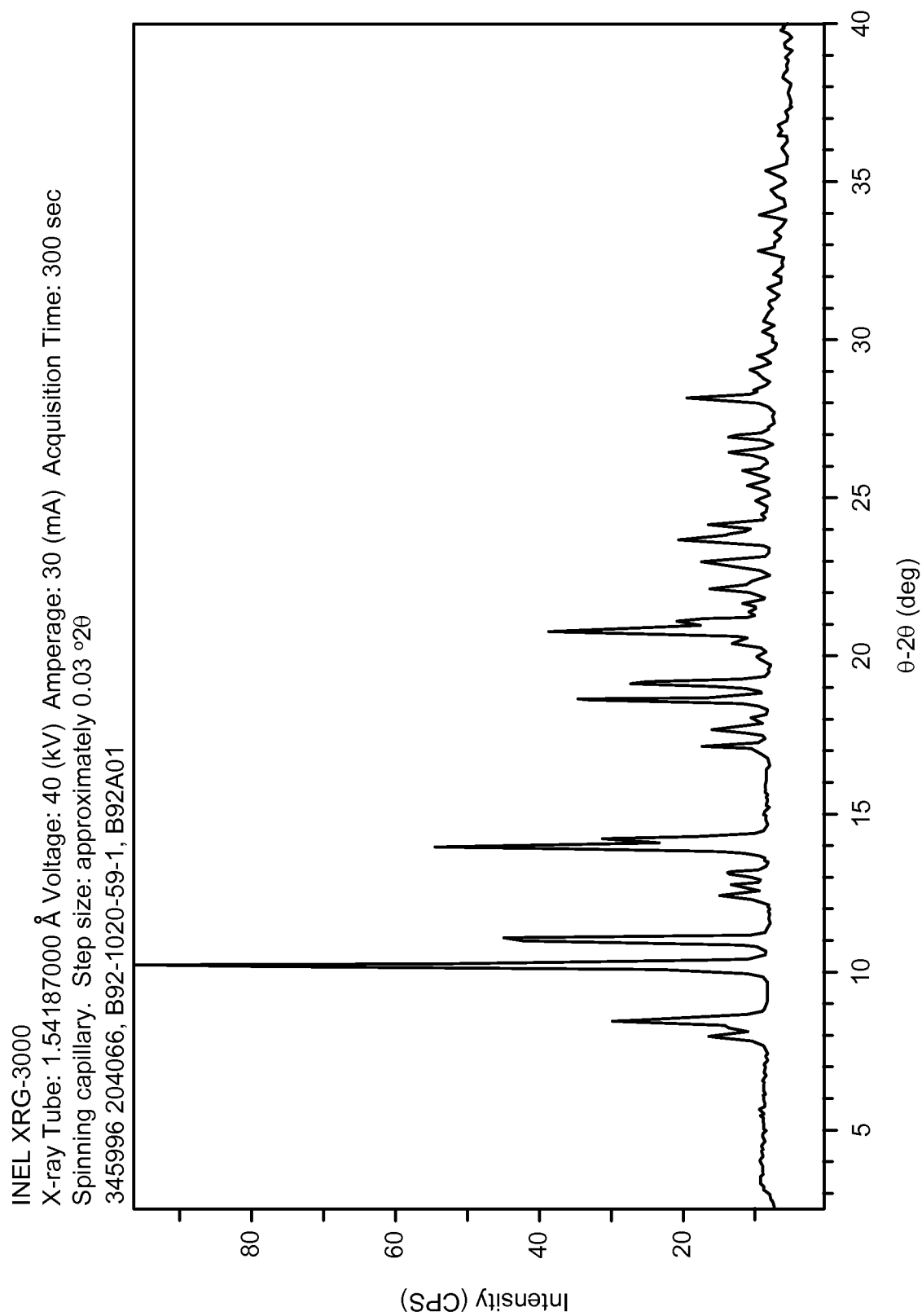
5/17

FIG. 5



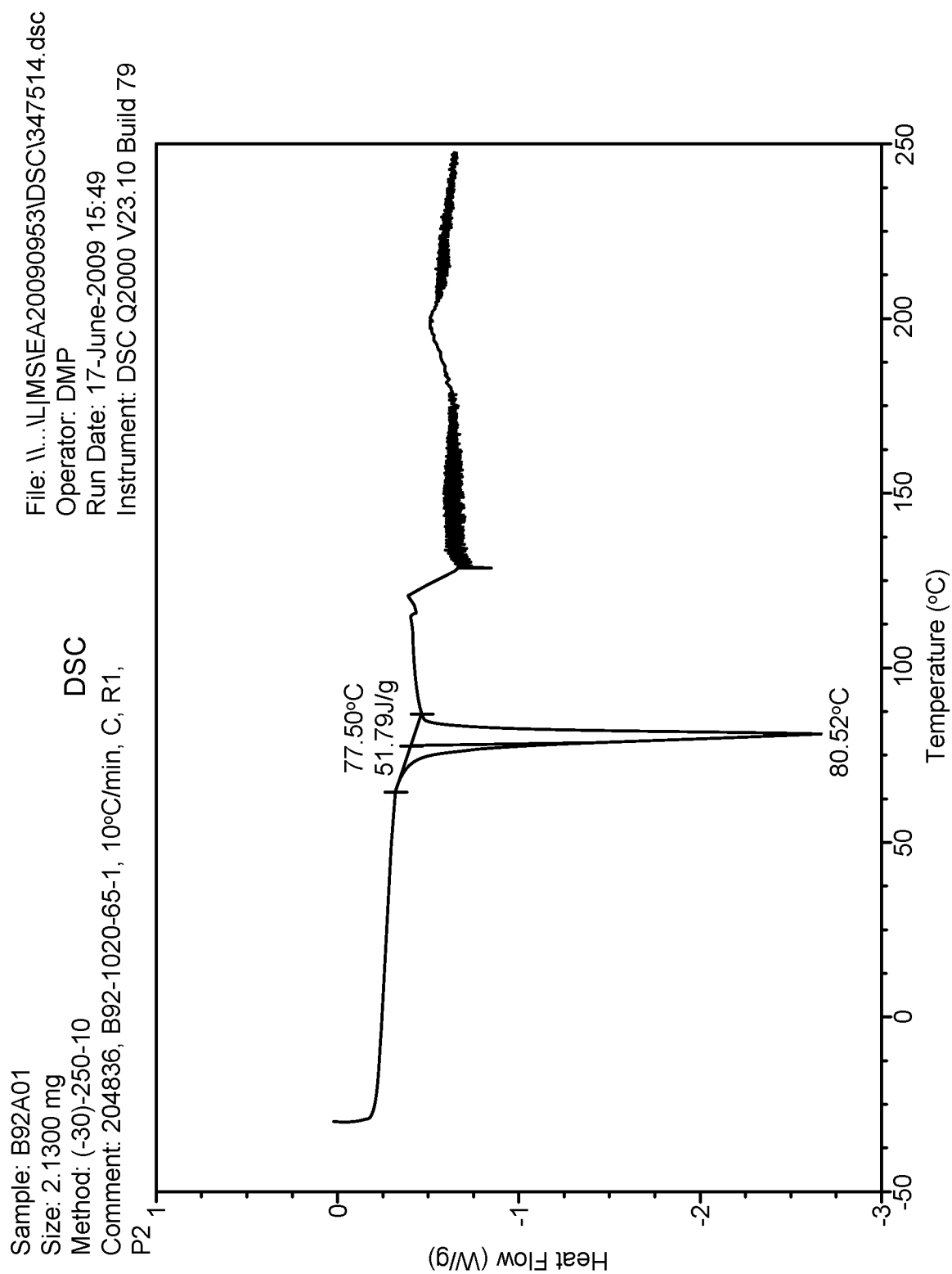
6/17

FIG. 6



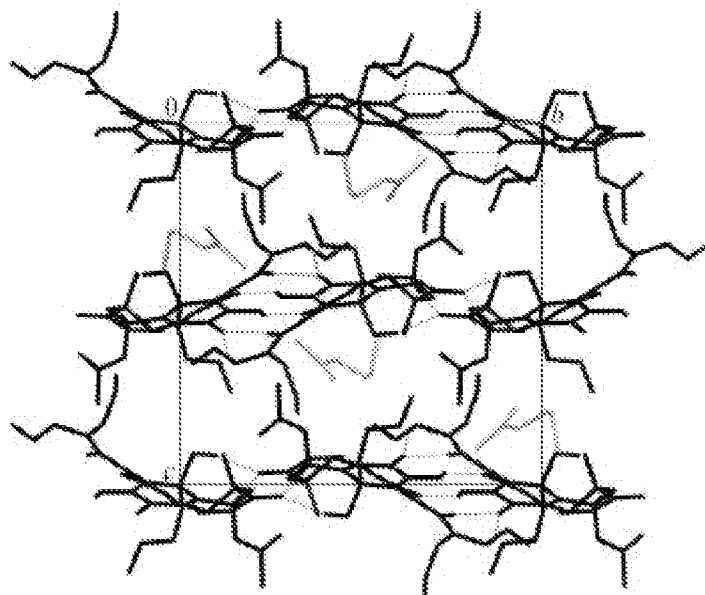
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FIG. 7



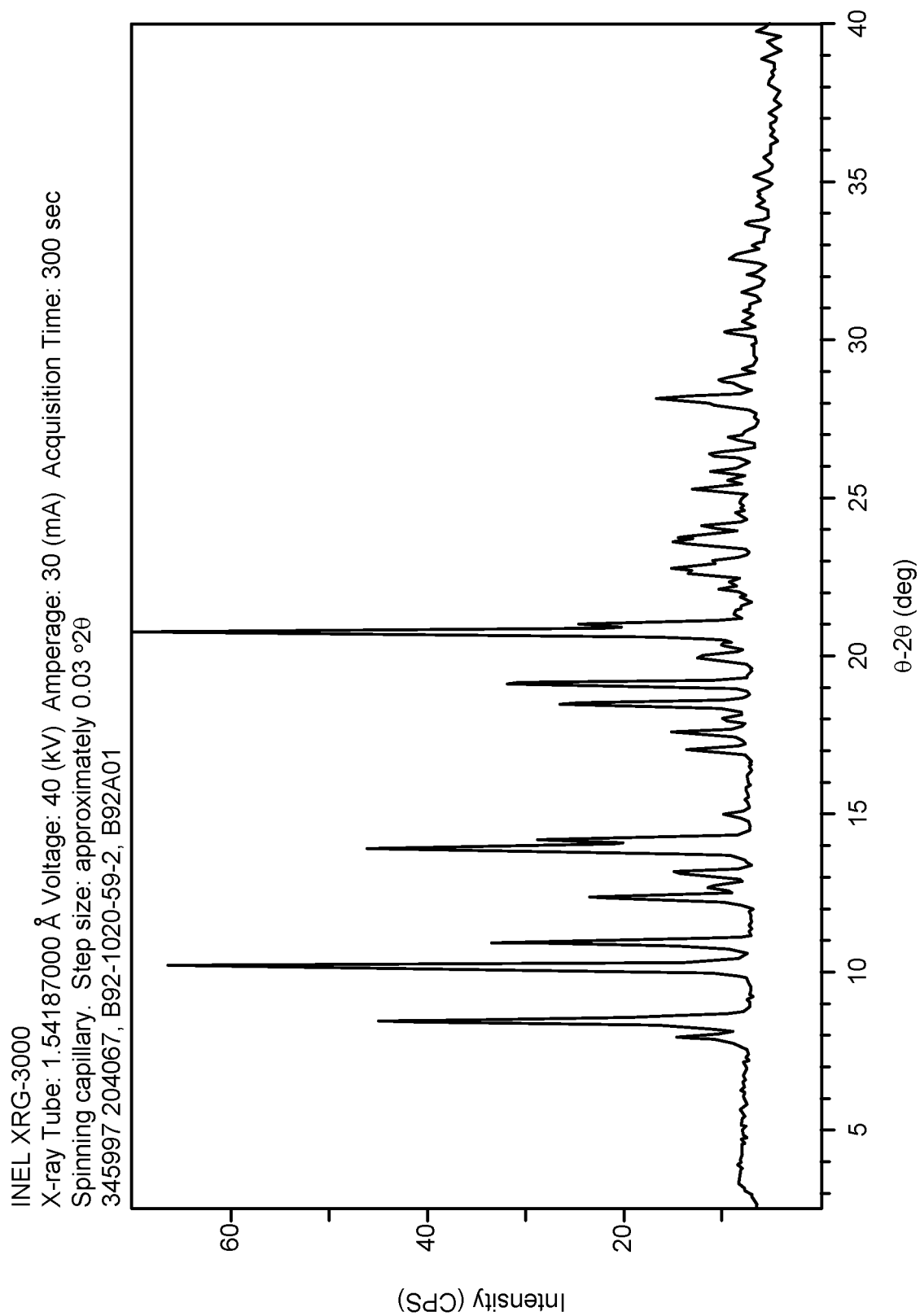
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FIG. 8



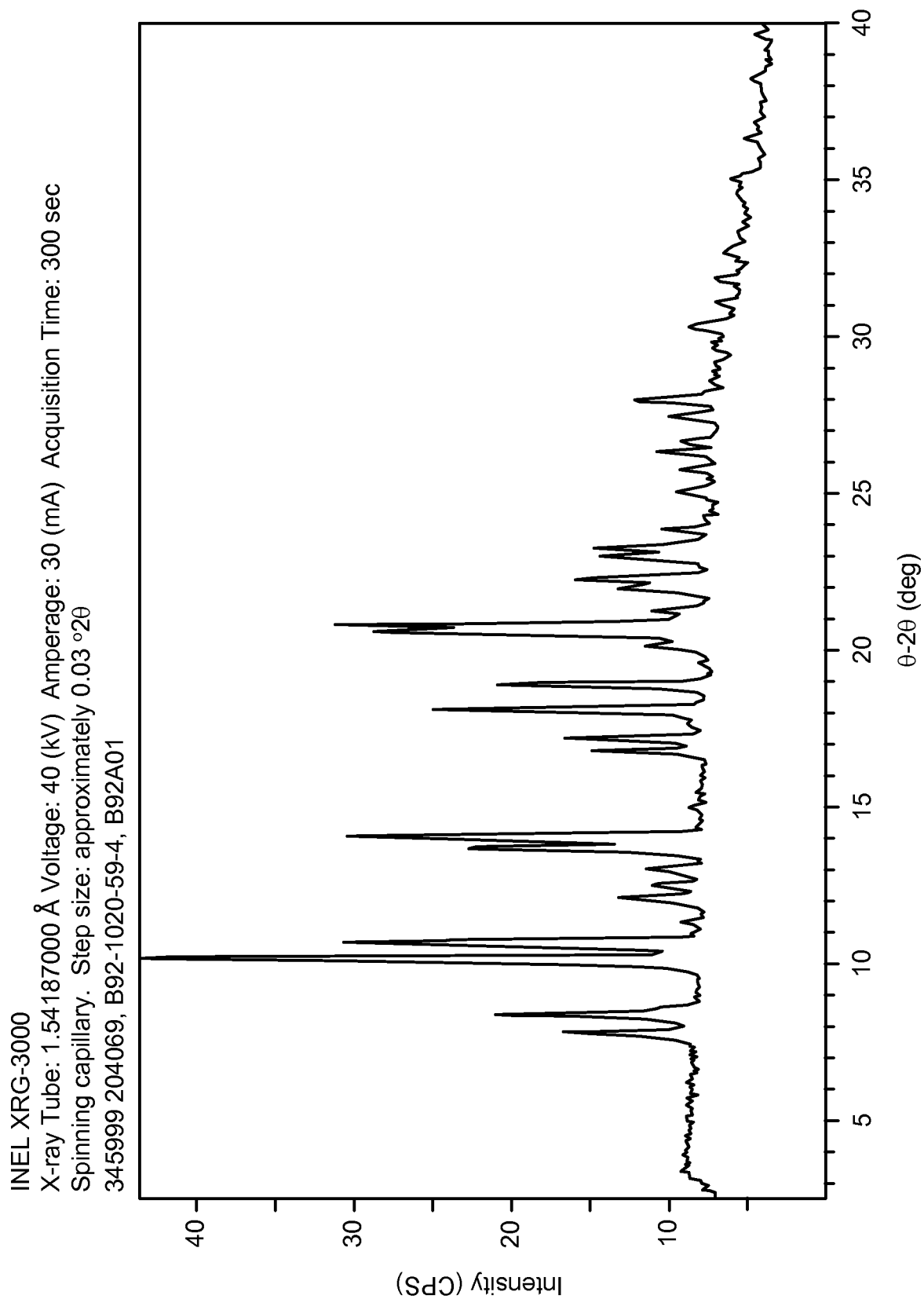
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FIG. 9



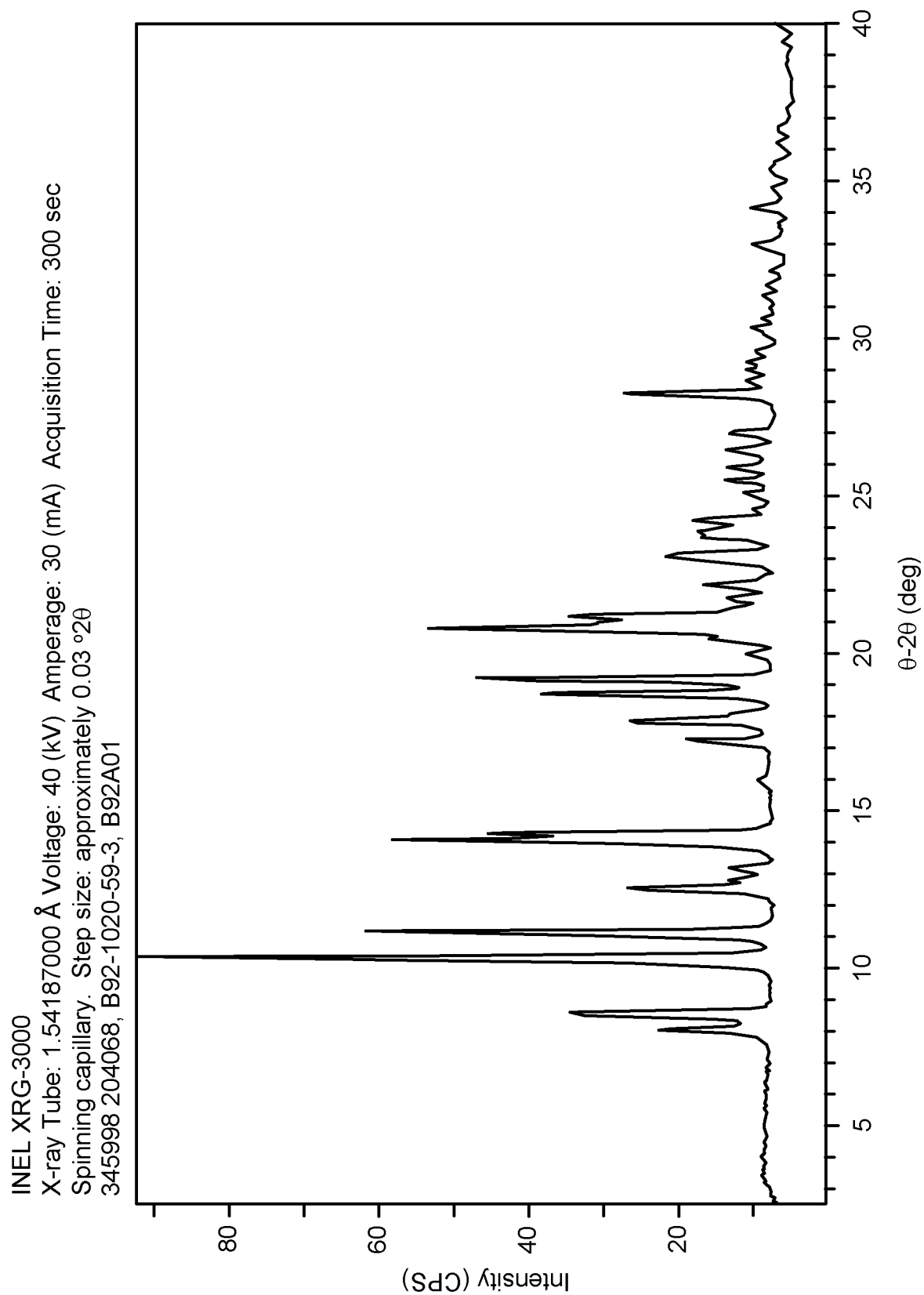
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FIG. 10



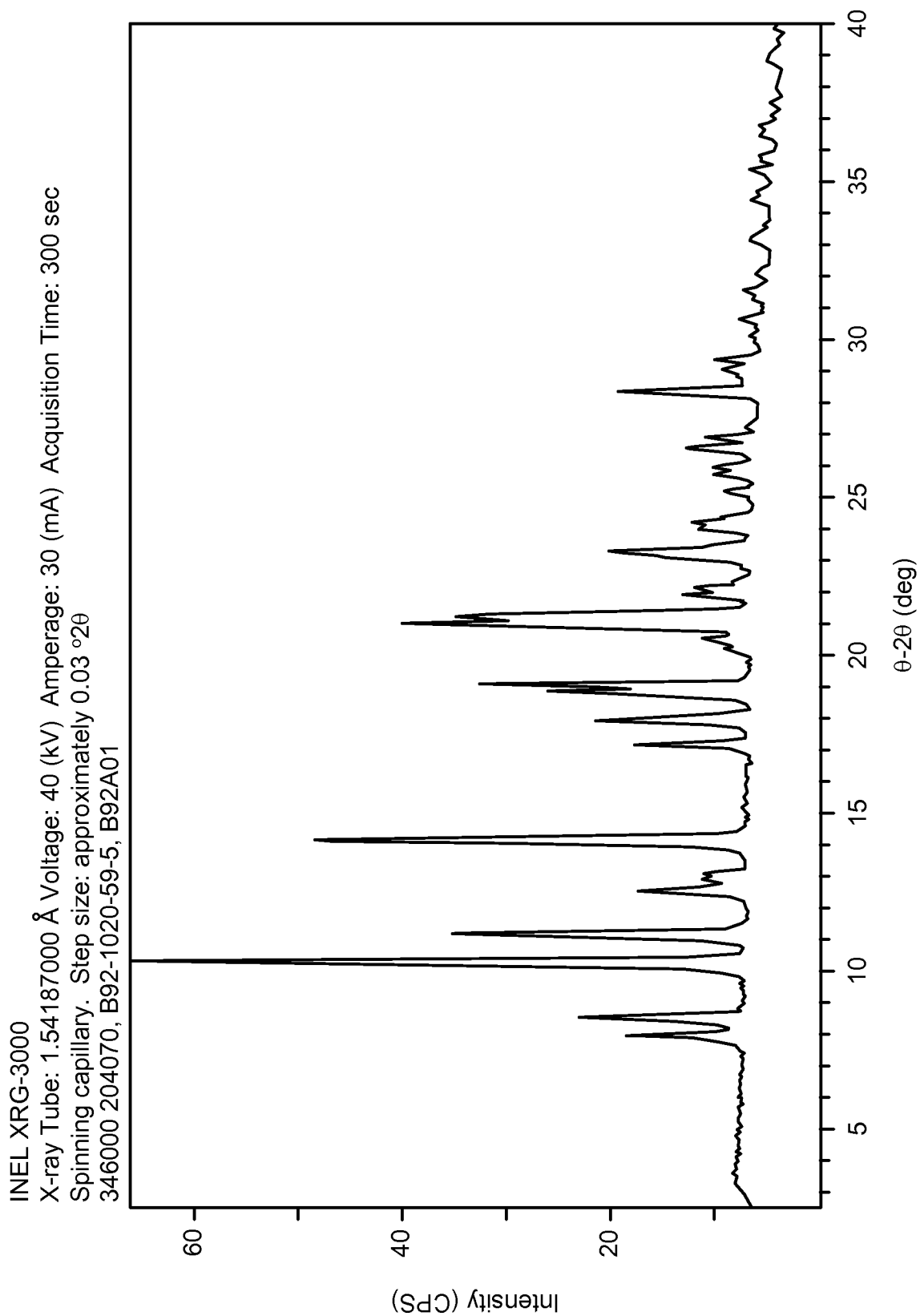
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FIG. 11



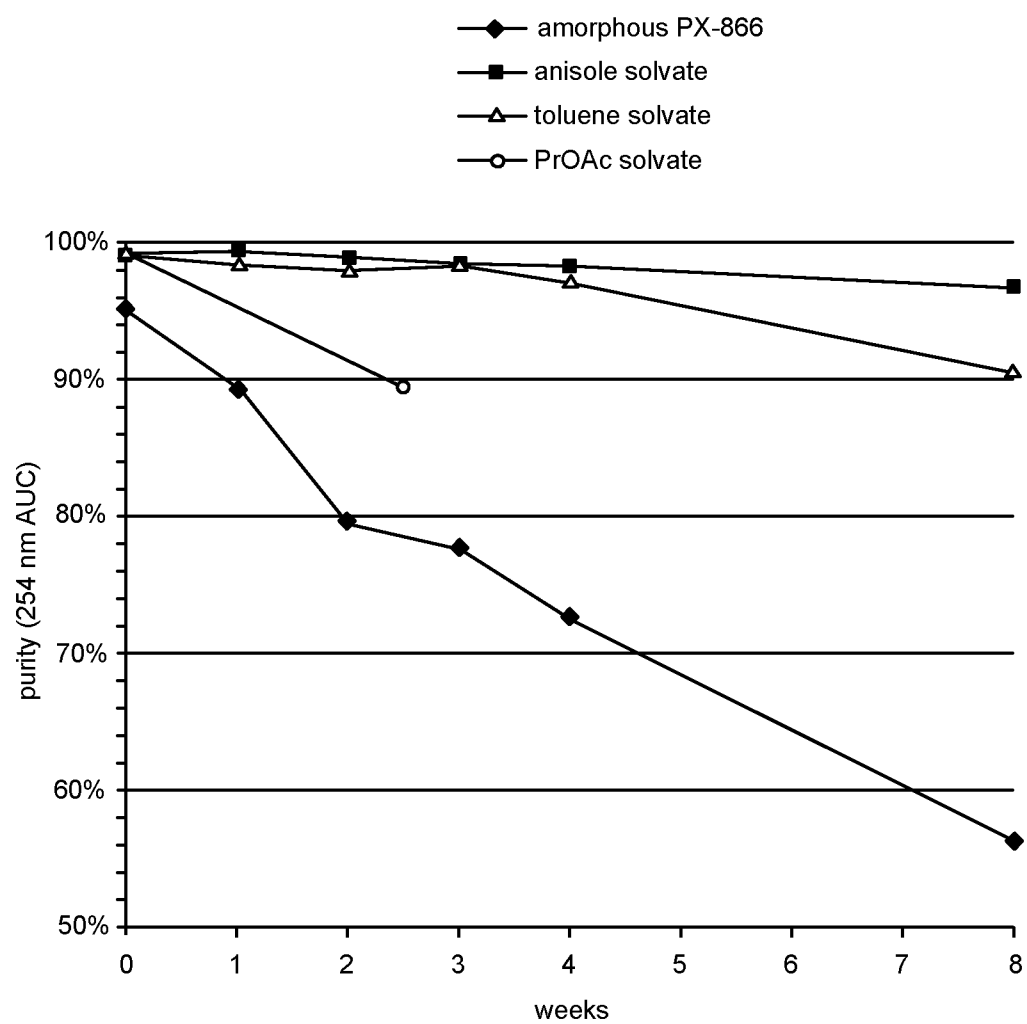
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FIG. 12



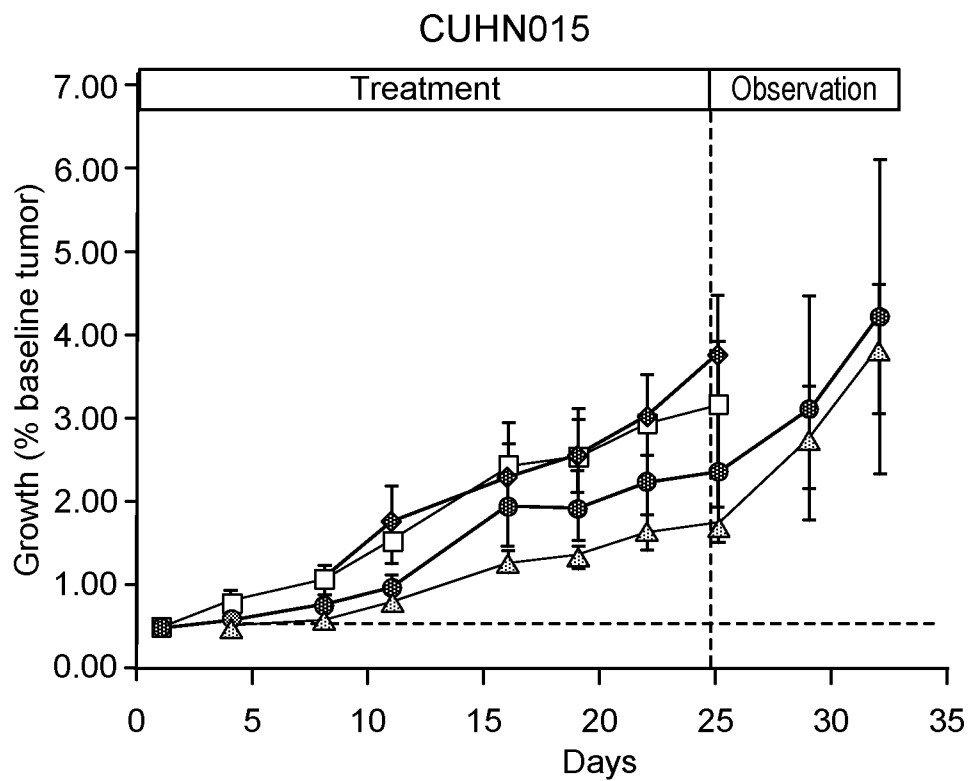
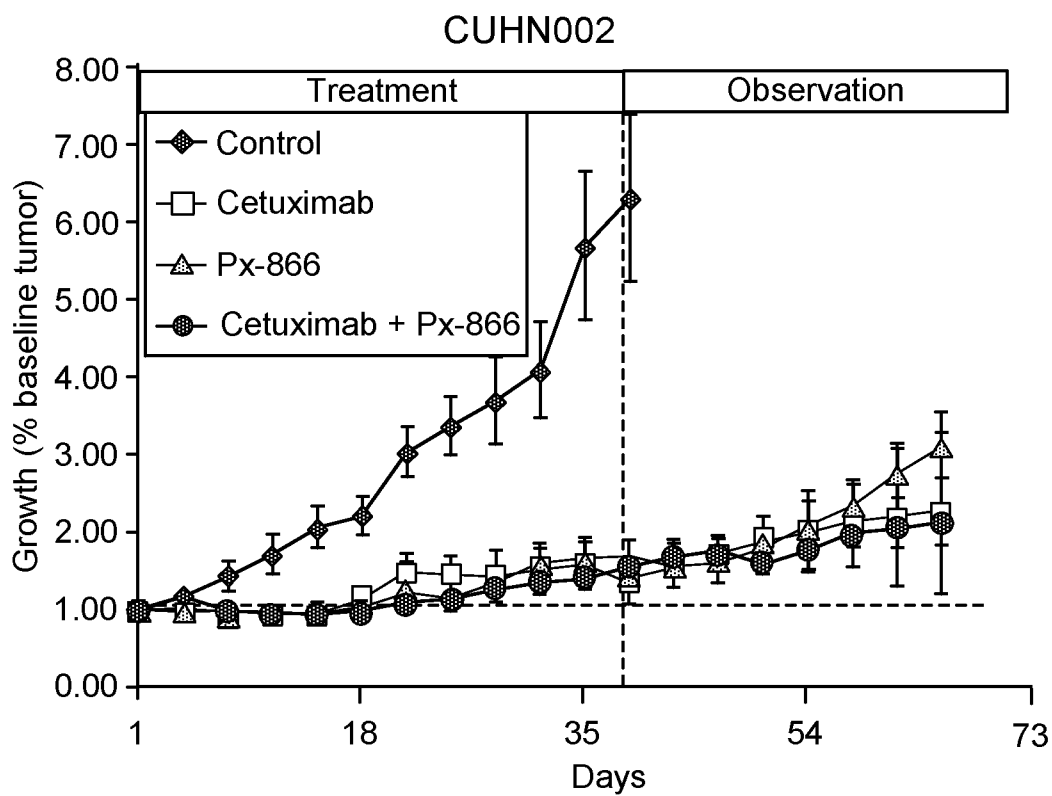
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FIG. 13



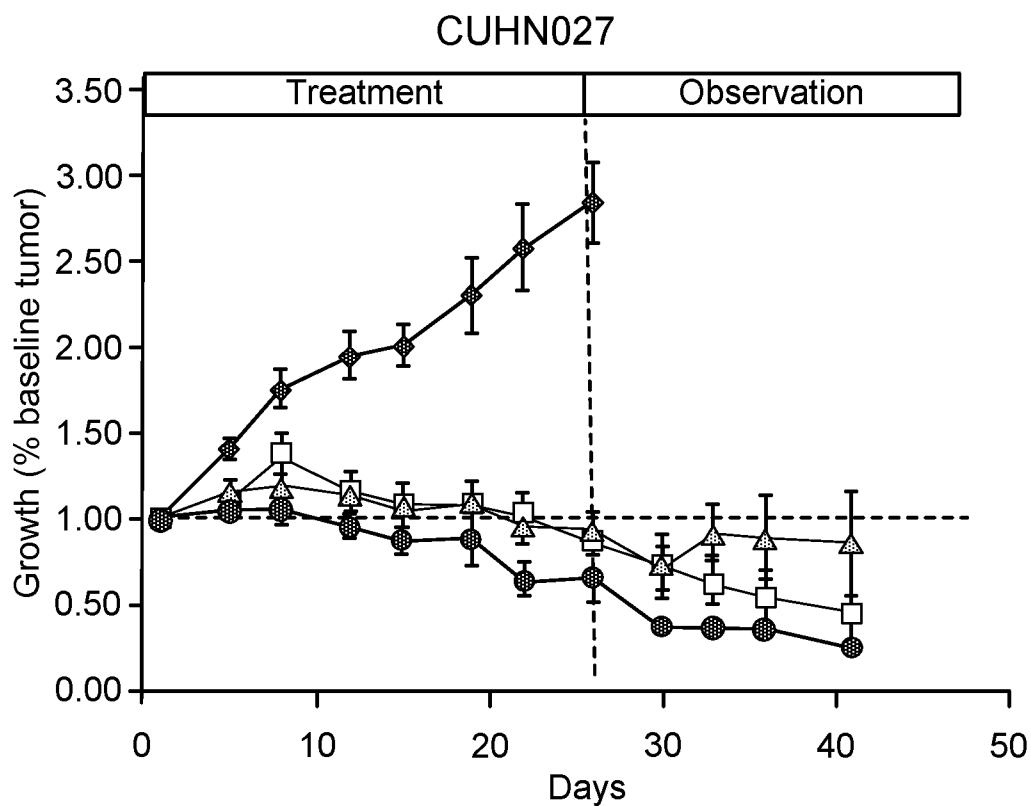
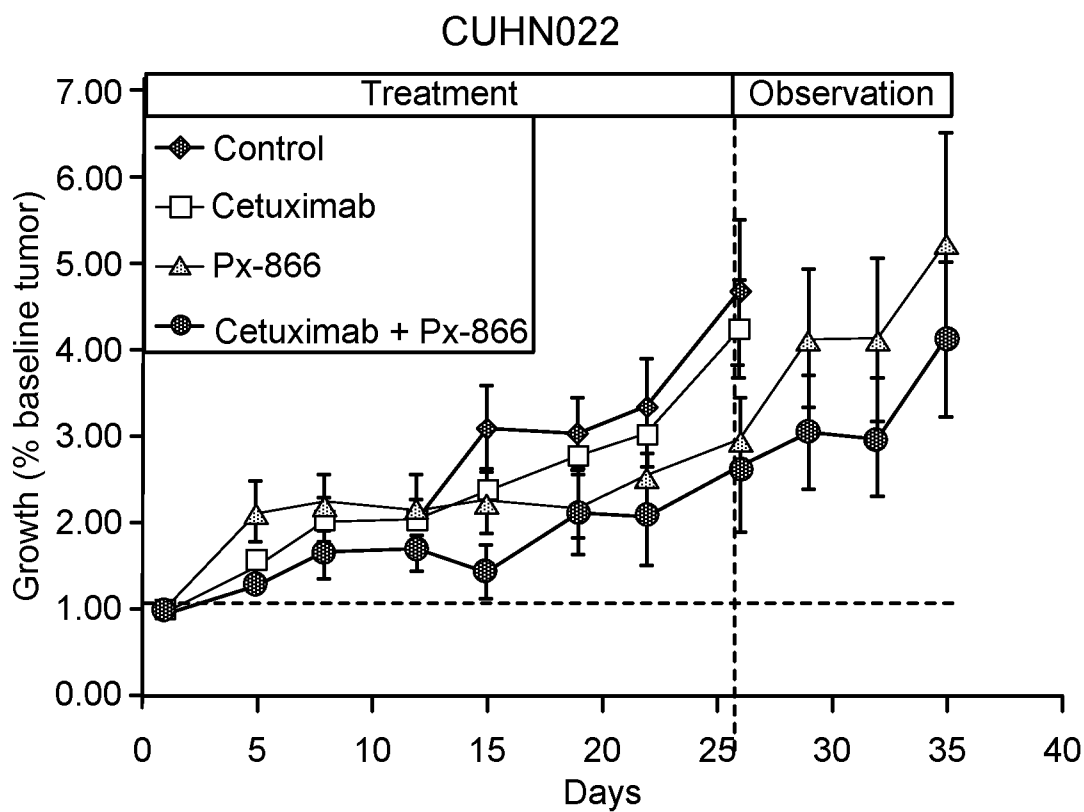
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FIG. 14



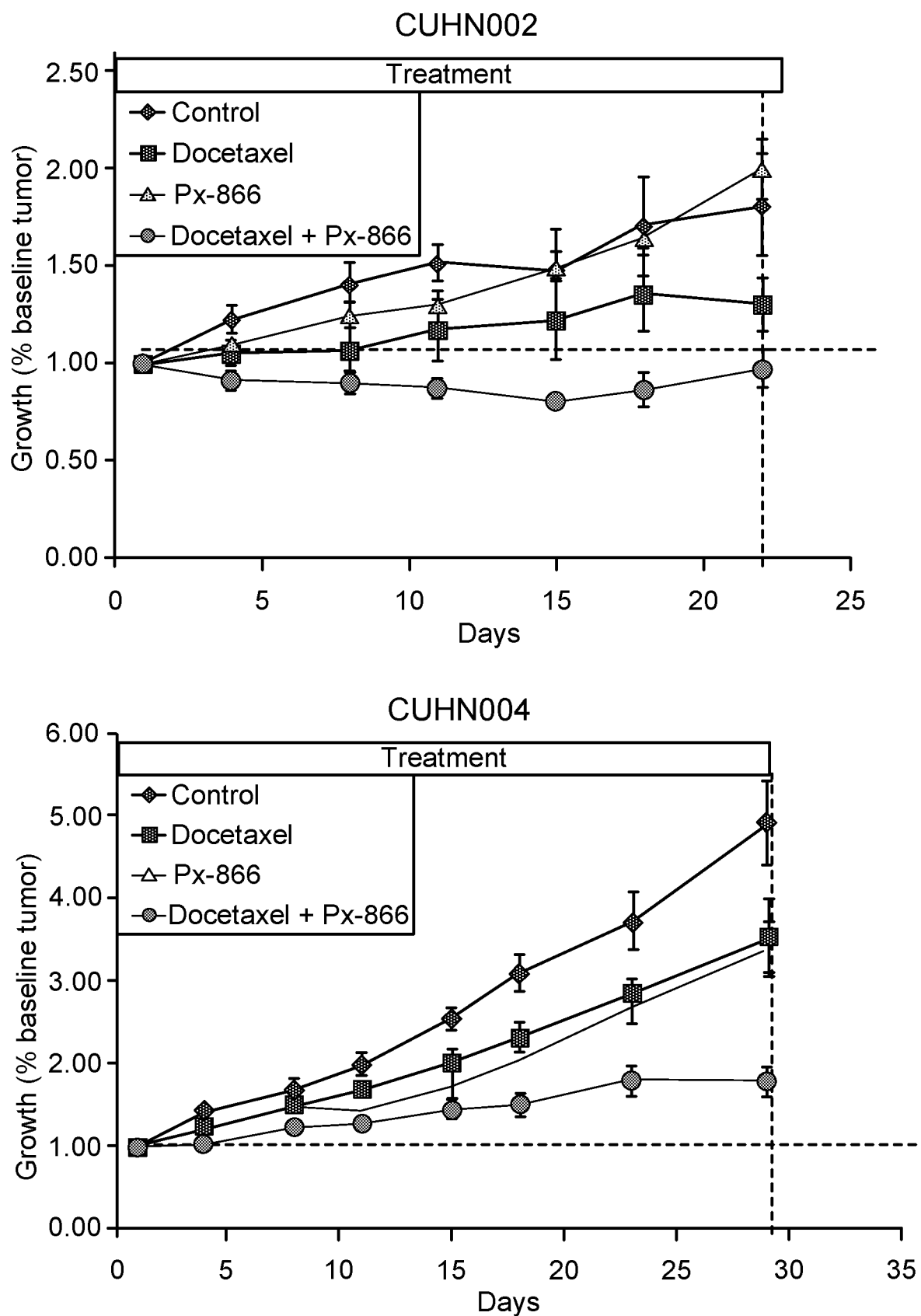
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FIG. 15



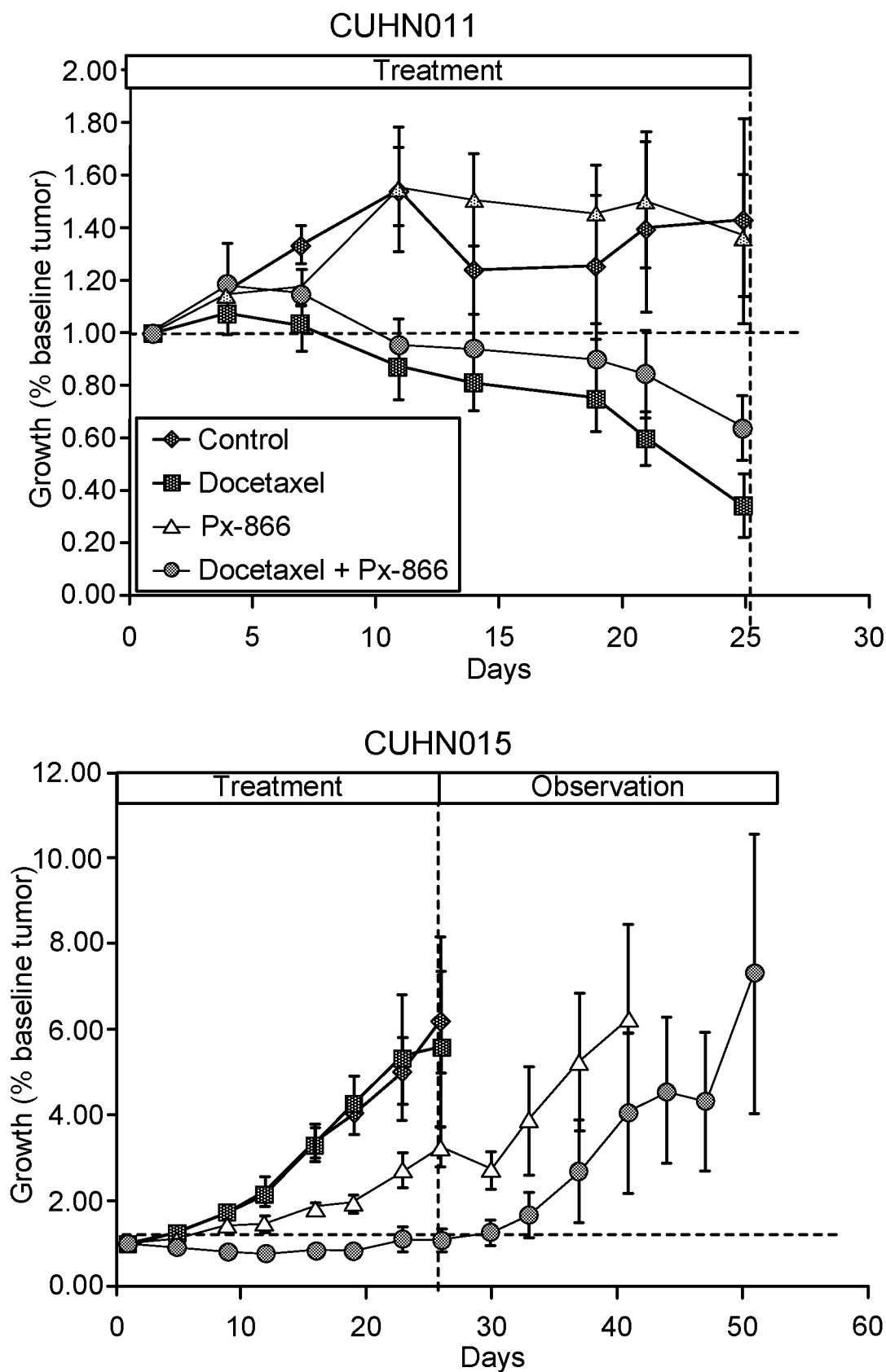
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FIG. 16



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FIG. 17



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/27294

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A01N 43/36; A61K 31/40; C07D 311/78; C07D 405/00 (2012.01)

USPC - 514/422

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(8) - A01N 43/36; A61K 31/40; C07D 311/78; C07D 405/00 (2012.01)

USPC - 514/422

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 549/276, 548/525, 514/453 (see search terms below)

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

PubWest, Google Patent, Google Scholar (wortmannin, kinase, inhibitor, irreversible, PI-3, Pi3, PI-3K, hepatitis, cancer, oncovirus-positive, HPV, merkel, PX-866, PX 866, anticancer, anti-cancer, Epstein, HPV, crystalline, free)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2009/0192127 A1 (SCHEURING, et al.) 30 July 2009 (30.07.2009) entire document, especially para[0001]-[0002], [0019], [0044], [0069], [0079], [0081], [0085], [0090]-[0091], [0094]-[0095].	1-2, 5-7, 10, 16/1-2,5-7,10, 21-23/6, 24-25/1,6
----- Y		3-4, 8-9, 11-15, 16/3-4,8-9, 17-20, 21-23/11, 24-25/11, 26-27
		3, 8, 16-17/3,8
Y	US 7,446,124 B2 (KIRKPATRICK, et al.) 04 November 2008 (04.11.2008) entire document, especially col 3, ln 50-col 4, ln 20 and claim 1.	4, 9, 11-14, 15/11, 16-17/4,9, 18, 19-26/11, 27/11
Y	US 2008/0026034 A1 (COOK, et al.) 31 January 2008 (31.01.2008) entire document, especially para[0333], [0090].	11-14, 15/11, 18, 19, 20, 21-25/11, 26, 27/11
Y	US 7,645,762 B2 (PARUCH, et al.) 12 January 2010 (12.01.2010) entire document, especially col 4, ln 6-9; col 35, ln 3-10, 43-46; col 26, ln 9-39; col 28, ln 58-67	15/1,6,11
Y	US 7,329,469 B2 (FISHER, et al.) 12 February 2008(12.02.2008) entire document, especially col 1, ln 22-34; col 2, ln 1-17; col 5, ln 38-53.	

☒ Further documents are listed in the continuation of Box C.

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"X" 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

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Date of the actual completion of the international search

29 May 2012 (29.05.2012)

Date of mailing of the international search report

14 JUN 2012

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/27294

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2010/0222343 A1 (CAI, et al.) 02 September 2010 (02.09.2010) entire document, especially para[0072], [0090]-[0091].	17/1-10
Y	US 2010/0249205 A1 (POWIS) 30 September 2010 (30.09.2010) entire document, especially para[0005], [0047], [0066]-[0067], [0111]-[0112].	27/1,6,11