CRYSTALLINE FORM OF (*S*)-*N*-ETHYL-3-((9-ETHYL-2-(((2*R*,3*S*)-2-HYDROXYPENTAN-3-YL)AMINO)-9*H*-PURIN-6-YL)AMINO)-PYRROLIDINE-1-SULFONAMIDE

FIELD

[0001] The present disclosure relates generally to a solid-state form of *N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide, particularly a crystalline form of (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide. The present disclosure further relates to pharmaceutical compositions comprising a crystalline form of (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)-pyrrolidine-1-sulfonamide; use of a pharmaceutical composition comprising a crystalline form of (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide to treat or prevent cyclin-dependent kinase 2 (CDK2)-mediated conditions; kits comprising a pharmaceutical composition comprising a crystalline form of (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide; and methods for preparing crystalline forms of (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)-pyrrolidine-1-sulfonamide.

BACKGROUND

[0002] Cyclin-dependent kinases (CDKs), including CDK2, are serine/threonine protein kinases involved in cell cycle regulation. CDK2 drives the progression of cells into the S- and M-phases of the cell cycle. Overexpression of CDK2 is associated with abnormal regulation of the cell-cycle and tumor growth in multiple cancer types. The monomeric form of CDK2 is inactive, but is activated when it forms a heterodimeric complex with one of its two regulatory partners, Cyclin A or Cyclin E. Cyclin E binding to CDK2 in the late G1 phase of the cell cycle is required for the transition from the G1 to S phase of the cell cycle. Cyclin A binding to CDK2 is then required to progress through the S phase of the cell cycle. The activated CDK2-cyclin A/E complex governs the phosphorylation of a wide range of transcription factors that modulate a variety of oncogenic signaling pathways impacting cell cycle progression. CDK2 activation also leads to hyperphosphorylation and inactivation of the retinoblastoma protein (pRB), a tumor suppressor protein that helps maintain cells in a quiescent state (*i.e.*, the G0 phase of the cell cycle).

[0003] Overexpression of the CCNE1 gene, which produces Cyclin E, occurs in many tumor cells causing those cells to become dependent on CDK2 and Cyclin E. Abnormal Cyclin E activity has been observed, for example, in solid tumor cancers such as breast, ovarian, lung, colorectal, gastric, endometrial, and bone cancers, and in blood cancers such as leukemia and lymphoma. In addition, amplification and/or overexpression of Cyclin E has been reported as a potential mechanism of resistance to CDK4/6 therapies in ER-positive HER2-negative breast cancer. Likewise, abnormal expression of Cyclin A is associated with chromosomal instability and tumor proliferation while inhibition of Cyclin A leads to decreased tumor growth.

[0004] Inhibition of CDK2 activity is presently an unexploited therapeutic approach for treating cancer and other diseases associated with CDK2 activity. Despite significant efforts, no approved pharmacological agents that inhibit CDK2 activity generally, or that inhibit CDK2 activity specifically, are currently available. Efforts to identify such inhibitors have been challenging, in part, due to two major hurdles. First, the mechanism of CDK2 degradation remains poorly understood. Second, the CDK2 inhibitors developed to date generally lack the requisite specificity or otherwise exhibit off-target CDK-driven toxicities. Sequence and structure similarity among the various CDKs are generally high and the similarities among the CDK binding sites render most CDK2 inhibitors poorly specific and/or highly toxic. Accordingly, there is a need for CDK2 inhibitors, particularly CDK2 inhibitors that have pharmacologically appropriate properties and that possess physical properties suitable for manufacturing a drug substance and formulating a corresponding drug product.

[0005] The present disclosure addresses this large unmet need by providing a crystalline form of the CDK2 inhibitor, (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide, that is suitable for use in pharmaceutical compositions and methods for treating CDK2-mediated conditions such as breast and ovarian cancers.

SUMMARY

[0006] In one aspect, the present disclosure provides a crystalline form of *N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide.

[0007] In another aspect, the present disclosure provides a crystalline form of (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)-pyrrolidine-1-sulfonamide.

[0008] In another aspect, the present disclosure provides crystalline Form A of (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)-pyrrolidine-1-sulfonamide.

[0009] In another aspect, the present disclosure provides pharmaceutical compositions comprising crystalline Form A of (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide, and one or more pharmaceutically acceptable excipients.

[0010] In another aspect, the present disclosure provides methods of treating or preventing a cyclin-dependent kinase 2 (CDK2)-mediated condition by administering to a subject in need thereof a pharmaceutical composition comprising a therapeutically effective amount of crystalline Form A of (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide.

[0011] In another aspect, the present disclosure provides use of a pharmaceutical composition comprising crystalline Form A of (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide for treating or preventing a cyclin-dependent kinase 2 (CDK2)-mediated condition.

[0012] In another aspect, the present disclosure provides use of crystalline Form A of (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)-pyrrolidine-1-sulfonamide for the manufacture of medicaments for treating or preventing a cyclin-dependent kinase 2 (CDK2)-mediated condition.

[0013] In another aspect, the present disclosure provides kits comprising a pharmaceutical composition comprising crystalline Form A of (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide.

[0014] In another aspect, the present disclosure provides methods for preparing crystalline Form A of (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] FIG. 1 is a representative powder X-ray diffraction (PXRD) pattern for amorphous (S)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)-pyrrolidine-1-sulfonamide.

[0016] FIG. 2 is a representative differential scanning calorimetry (DSC) curve for amorphous (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)-pyrrolidine-1-sulfonamide.

[0017] FIG. 3 is a representative thermogravimetric analysis (TGA) thermogram for amorphous (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide.

[0018] FIG. 4 is a representative gravimetric vapor sorption (GVS) plot for amorphous (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)p-yrrolidine-1-sulfonamide.

[0019] FIG. 5-A is a thermal ellipsoid drawing of the crystal structure of crystalline Form A of (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide based on single crystal analysis and drawn at a 50% probability level.

[0020] FIG. 5-B is a representative powder X-ray diffraction (PXRD) pattern for crystalline Form A of (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide.

[0021] FIG. 6 is a representative differential scanning calorimetry (DSC) curve for crystalline Form A of (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide.

[0022] FIG. 7 is a representative thermogravimetric analysis (TGA) thermogram for crystalline Form A of (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide.

[0023] FIG. 8 is a representative gravimetric vapor sorption (GVS) plot for crystalline Form A of (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)-pyrrolidine-1-sulfonamide.

[0024] FIG. 9 is a representative ^{13}C CPMAS spectrum for crystalline Form A of (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)-pyrrolidine-1-sulfonamide.

DETAILED DESCRIPTION

[0025] Many embodiments are detailed throughout the specification and will be apparent to a reader skilled in the art. Such embodiments are provided by way of example only and are not intended to otherwise limit the scope of the invention. Various alternatives to the described embodiments may be employed in practicing the invention.

I. Definitions

[0026] With respect to the embodiments disclosed in this specification, the following terms have the meanings set forth below:

[0027] Reference to “a” or “an” means “one or more.” Throughout, the plural and singular should be treated as interchangeable, other than the indication of number.

[0028] When ranges are used herein to describe, for example, physical or chemical properties, all combinations and subcombinations of ranges and specific embodiments therein are intended to be included. Use of the term “about” or “approximately” when referring to a number or a numerical range means that the number or numerical range referred to is an approximation within experimental variability (or within statistical experimental error), and thus the number or numerical range may vary from, for example, between 1% and 15% of the stated number or numerical range.

[0029] Unless the context requires otherwise, the words "comprise" or "comprises" or “comprising" are used on the basis and clear understanding that they are to be interpreted inclusively, rather than exclusively, and that Applicant intends each of those words to be so interpreted in construing this patent, including the claims below.

[0030] The term “amorphous form” refers to a form of a compound that lacks long range crystalline order.

[0031] The terms “co-administration,” “co-administering,” “administered in combination with,” and “administering in combination with” as used herein, encompass administration of two or more agents to a subject so that both agents and/or their metabolites are present in the subject at the same time. Co-administration includes simultaneous administration in

separate compositions, administration at different times in separate compositions, or administration in a composition in which two or more agents are present.

[0032] The term "crystalline form" is intended to include all crystalline forms of the compound, including, for example, polymorphs, pseudopolymorphs, solvates, hydrates, unsolvated polymorphs (including anhydrates), and conformational polymorphs, as well as mixtures thereof, unless a particular crystalline form is referred to.

[0033] The term "therapeutically effective amount" of a pharmacological agent is an amount that is sufficient to effect beneficial or desired results, including clinical results, and, as such, will depend upon the situation in which it is being administered. Where the pharmacological agent is being administered to treat a cancer, for example, a therapeutically effective amount of the agent is an amount of the agent that is sufficient, either alone or in combination with additional therapies, to provide an anti-cancer effect in a subject as compared to the response obtained without administration of the agent.

[0034] The term "pharmaceutically acceptable" is used adjectivally in this specification to mean that the modified noun is appropriate for use as a pharmaceutical product or as a part of a pharmaceutical product. For example, the term "pharmaceutically acceptable carrier" or "pharmaceutically acceptable excipient" is intended to include any and all carriers or excipients that are suitable for use in mammals, particularly humans.

[0035] The terms "reflection" or "reflection mode," when used in conjunction with powder X-ray diffraction, refers to the reflection (also known as Bragg-Brentano) sampling mode.

[0036] The term "preventing" is readily understood by an ordinarily skilled physician and, with respect to treatment of a particular condition, can include is intended to have its normal meaning and includes primary prophylaxis to prevent the development of the condition and secondary prophylaxis whereby the condition has already developed and the patient is temporarily or permanently protected against exacerbation or worsening of the disease or the development of new symptoms associated with the condition.

[0037] The term "solvate" refers to a crystalline phase of a compound in physical association with one or more molecules of a solvent. The crystalline phase of a compound in physical association with one or more molecules of water is referred to as a "hydrate."

[0038] The terms “transmission” or “transmission mode,” when used in conjunction with powder X-ray diffraction, refers to the transmission (also known as Debye-Scherrer) sampling mode.

[0039] The term "treating” is readily understood by an ordinarily skilled physician and, with respect to treatment of a particular condition, can include (1) diminishing the extent or cause of the condition being treated, and/or (2) alleviating or ameliorating one or more symptoms associated with that condition. Treatment of a cancer, for example, can include stabilizing (*i.e.*, not worsening), delaying, or slowing the spread or progression of the cancer; prolonging survival as compared to expected survival if not receiving treatment; and/or otherwise ameliorating or palliating the severity of the cancer, in whole or in part.

[0040] “Enantiomeric purity” as used herein refers to the relative amounts, expressed as a percentage, of the presence of a specific enantiomer relative to the other enantiomer. For example, if a compound, which may potentially have an (*R*)- or an (*S*)-isomeric configuration, is present as a racemic mixture, the enantiomeric purity is about 50% with respect to either the (*R*)- or (*S*)-isomer. If that compound has one isomeric form predominant over the other, for example, 80% (*S*)-isomer and 20% (*R*)-isomer, the enantiomeric purity of the compound with respect to the (*S*)-isomeric form is 80%. The enantiomeric purity of a compound can be determined in a number of ways, including but not limited to chromatography using a chiral support, polarimetric measurement of the rotation of polarized light, nuclear magnetic resonance spectroscopy using chiral shift reagents which include but are not limited to lanthanide containing chiral complexes or Pirkle’s reagents, or derivatization of a compounds using a chiral compound such as Mosher’s acid followed by chromatography or nuclear magnetic resonance spectroscopy.

[0041] In some embodiments, the enantiomerically enriched composition has a higher potency with respect to therapeutic utility per unit mass than does the racemic mixture of that composition. Enantiomers can be isolated from mixtures by methods known to those skilled in the art, including chiral high pressure liquid chromatography (HPLC) and the formation and crystallization of chiral salts; or preferred enantiomers can be prepared by asymmetric syntheses. See, for example, Jacques, et al., Enantiomers, Racemates and Resolutions, Wiley Interscience, New York, 1981; Eliel, Stereochemistry of Carbon Compounds, McGraw-Hill, NY, 1962; and Eliel and Wilen, Stereochemistry of Organic

Compounds, Wiley-Interscience, New York, 1994.

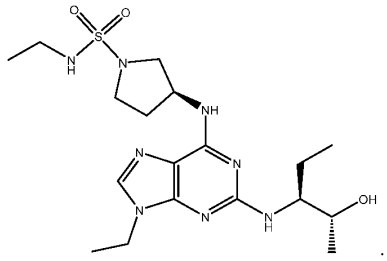
[0042] The terms "enantiomerically enriched" and "non-racemic," as used herein, refer to compositions in which the percent by weight of one enantiomer is greater than the amount of that one enantiomer in a control mixture of the racemic composition (*e.g.*, greater than 1:1 by weight). For example, an enantiomerically enriched preparation of the (*R*)-enantiomer, means a preparation of the compound having greater than 50% by weight of the (*R*)-enantiomer relative to the (*S*)-enantiomer, such as at least 75% by weight, or such as at least 80% by weight. In some embodiments, the enrichment can be significantly greater than 80% by weight, providing a "substantially enantiomerically enriched" or a "substantially non-racemic" preparation, which refers to preparations of compositions which have at least 85% by weight of one enantiomer relative to other enantiomer, such as at least 90% by weight, or such as at least 95% by weight. The terms "enantiomerically pure" or "substantially enantiomerically pure" refers to a composition that comprises at least 98% of a single enantiomer and less than 2% of the opposite enantiomer.

II. Crystalline Form A

[0043] A compound that is an active pharmaceutical ingredient in a drug product potentially can exist in different solid-state forms exhibiting different physical properties. These physical property differences can impact the manufacturing and formulation of the drug product. Such physical properties can include, but are not limited to: (1) packing properties such as molar volume, density, and hygroscopicity; (2) thermodynamic properties such as melting temperature, vapor pressure, and solubility; (3) kinetic properties such as dissolution rate and stability (including stability at ambient conditions, especially to moisture and under storage conditions); (4) surface properties such as surface area, wettability, interfacial tension, and shape; (5) mechanical properties such as hardness, tensile strength, compressibility, compactibility, handling, flow and blend; and (6) filtration properties. Accordingly, solid-state forms of a compound, particularly crystalline forms of the compound, that provide an improvement in one or more of these physical properties relative to other solid-state forms of the compound are desirable. The discovery of a new solid-state form of a pharmaceutically useful compound therefore provides a potential opportunity to improve the performance characteristics of the corresponding drug product and related manufacturing process.

[0044] The present disclosure provides a crystalline form of *N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide. In one aspect, the crystalline form possesses one or more of the above-described advantageous properties relative to one or more of the other solid-state forms of the compound. In another aspect, the crystalline form is a crystalline anhydrate. In further aspects, the crystalline form is substantially pure. As used in the present specification, the term "substantially pure" means that the crystalline form of the compound comprises at least about 90 weight % of the desired crystalline form relative to any other solid-state form of the compound. In one aspect, the crystalline form of the compound comprises at least about 95 weight % of the desired crystalline form relative to any other solid-state form of the compound. In another aspect, the crystalline form of the compound comprises at least about 96 weight % of the desired crystalline form relative to any other solid-state form of the compound. In another aspect, the crystalline form of the compound comprises at least about 97 weight % of the desired crystalline form relative to any other solid-state form of the compound relative to any other solid-state form of the compound. In another aspect, the crystalline form of the compound comprises at least about 98 weight % of the desired crystalline form relative to any other solid-state form of the compound. In another aspect, the crystalline form of the compound comprises at least about 99 weight % of the desired crystalline form relative to any other solid-state form of the compound.

[0045] In some embodiments, the present disclosure provides a crystalline form of (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)-pyrrolidine-1-sulfonamide which has the following chemical structure:



[0046] In some embodiments, the present disclosure provides crystalline Form A of (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)-pyrrolidine-1-sulfonamide.

[0047] In some embodiments, crystalline Form A is characterized by a powder X-ray diffraction (PXRD) pattern that comprises at least one peak selected from the group consisting of 8.1 ± 0.2° 2θ, 13.9 ± 0.2° 2θ, 16.1 ± 0.2° 2θ, 16.7 ± 0.2° 2θ, and 22.6 ± 0.2 °2θ. In one aspect, the powder X-ray diffraction pattern comprises at least two, three, or four peaks selected from the group consisting of 8.1 ± 0.2° 2θ, 13.9 ± 0.2° 2θ, 16.1 ± 0.2° 2θ, 16.7 ± 0.2° 2θ, and 22.6 ± 0.2 °2θ. In another aspect, the powder X-ray diffraction pattern comprises peaks at 8.1 ± 0.2° 2θ, 13.9 ± 0.2° 2θ, 16.1 ± 0.2° 2θ, 16.7 ± 0.2° 2θ, and 22.6 ± 0.2 °2θ. In another aspect, the powder X-ray diffraction pattern further comprises at least one, two, three, or four peaks selected from the group consisting of 17.7 ± 0.2° 2θ, 20.2 ± 0.2° 2θ, 21.3 ± 0.2° 2θ, 25.9 ± 0.2° 2θ, and 29.2 ± 0.2 °2θ. In another aspect, the powder X-ray diffraction pattern further comprises peaks at 17.7 ± 0.2° 2θ, 20.2 ± 0.2° 2θ, 21.3 ± 0.2° 2θ, 25.9 ± 0.2° 2θ, and 29.2 ± 0.2 °2θ. In another aspect, the powder X-ray diffraction pattern comprises peaks at 8.1 ± 0.2° 2θ, 13.9 ± 0.2° 2θ, 16.1 ± 0.2° 2θ, 16.7 ± 0.2° 2θ, 17.7 ± 0.2 °2θ, 20.2 ± 0.2° 2θ, 21.3 ± 0.2° 2θ, 22.6 ± 0.2° 2θ, 25.9 ± 0.2 °2θ, and 29.2 ± 0.2 °2θ. In another aspect, the powder X-ray diffraction is reflection powder X-ray diffraction. In another aspect, the powder X-ray diffraction is transmission powder X-ray diffraction. In another aspect, the powder X-ray diffraction pattern is substantially the same as the powder X-ray diffraction pattern of FIG. 5-B.

[0048] In some embodiments, Form A is characterized by a differential scanning calorimetry (DSC) curve comprising a melting endotherm having an onset temperature of about 122 °C ± 5 °C. In another aspect, the endotherm has a peak at about 128 °C ± 5 °C. In another aspect, the endotherm comprises a melting endotherm having an onset temperature of about 122 °C ± 5 °C and a peak at about 128 °C ± 5 °C. In another aspect, the endotherm has an onset temperature of about 122 °C ± 2 °C. In another aspect, the endotherm has a peak at about 128 °C ± 2 °C. In another aspect, the endotherm comprises a melting endotherm having an onset temperature of about 122 °C ± 2 °C and a peak at about 128 °C ± 2 °C. In another aspect, the endotherm has an onset temperature of about 122 °C. In another aspect, the endotherm has a peak at about 128 °C. In another aspect, the endotherm comprises a melting endotherm having an onset temperature of about 122 °C and a peak at about 128 °C. In another aspect, the differential scanning calorimetry curve is substantially the same as the differential scanning calorimetry curve of FIG. 6.

[0049] In some embodiments, Form A is characterized by a thermogravimetric analysis (TGA) thermogram wherein the crystalline form exhibits a weight loss of less than about 1.0 weight % from about 25 ºC to about 100 ºC. In one aspect, the weight loss is less than about 0.5 weight %. In another aspect, the weight loss is less than about 0.1 weight %. In another aspect, the thermogravimetric analysis thermogram is substantially the same as the thermogravimetric analysis thermogram of FIG. 7.

[0050] In some embodiments, Form A is characterized by a gravimetric vapor sorption (GVS) plot wherein the crystalline form exhibits a reversible moisture uptake of less than about 1.0 weight % from about 0% relative humidity to about 80% relative humidity at 25 ºC ± 0.1 ºC. In one aspect, the reversible moisture uptake is less than about 0.7 weight %. In another aspect, the reversible moisture uptake is less than about 0.4 weight %. In another aspect, the gravimetric vapor sorption plot is substantially the same as the gravimetric vapor sorption plot of FIG. 8.

[0051] In some embodiments, Form A is characterized by a solid-state ^{13}C NMR spectrum comprising at least one peak selected from the group consisting of 161.2 ±0.2 ppm, 137.6 ±0.2 ppm, 113.1 ±0.2 ppm, 72.3 ±0.2 ppm, and 60.0 ±0.2 ppm. In one aspect, the solid-state ^{13}C NMR spectrum comprises at least two, three, or four peaks selected from the group consisting of 161.2 ±0.2 ppm, 137.6 ±0.2 ppm, 113.1 ±0.2 ppm, 72.3 ±0.2 ppm, and 60.0 ±0.2 ppm. In another aspect, the solid-state ^{13}C NMR spectrum comprises peaks at 161.2 ±0.2 ppm, 137.6 ±0.2 ppm, 113.1 ±0.2 ppm, 72.3 ±0.2 ppm, and 60.0 ±0.2 ppm. In another aspect, the solid-state ^{13}C NMR spectrum comprises peaks at 161.2 ±0.2 ppm, 153.9 ±0.2 ppm, 152.8 ±0.2 ppm, 137.6 ±0.2 ppm, 113.1 ±0.2 ppm, 72.3 ±0.2 ppm, 60.0 ±0.2 ppm, 54.9 ±0.2 ppm, 51.0 ±0.2 ppm, 47.2 ±0.2 ppm, 40.1 ±0.2 ppm, 39.1 ±0.2 ppm, 29.3 ±0.2 ppm, 23.1 ±0.2 ppm, 17.1 ±0.2 ppm, 16.5 ±0.2 ppm, 13.3 ±0.2 ppm, and 10.7 ±0.2 ppm. In another aspect, the solid-state ^{13}C NMR spectrum is substantially the same as the solid-state ^{13}C NMR spectrum of FIG. 10.

[0052] In some embodiments, Form A is characterized by at least two of the above-described physical characterization embodiments selected from powder X-ray diffraction, differential scanning calorimetry, thermogravimetric analysis, and/or gravimetric vapor sorption.

[0053] In some embodiments, Form A is characterized by at least three of the above-

described physical characterization embodiments selected from powder X-ray diffraction, differential scanning calorimetry, thermogravimetric analysis, and/or gravimetric vapor sorption.

[0054] In some embodiments, Form A is characterized by at least four of the above-described physical characterization embodiments selected from powder X-ray diffraction, differential scanning calorimetry, thermogravimetric analysis, and gravimetric vapor sorption.

[0055] In some embodiments, Form A is characterized by at least two of the above-described physical characterization embodiments selected from powder X-ray diffraction, solid-state ^{13}C NMR, differential scanning calorimetry, thermogravimetric analysis, and/or gravimetric vapor sorption.

[0056] In some embodiments, Form A is characterized by at least three of the above-described physical characterization embodiments selected from powder X-ray diffraction, solid-state ^{13}C NMR, differential scanning calorimetry, thermogravimetric analysis, and/or gravimetric vapor sorption.

[0057] In some embodiments, Form A is characterized by at least four of the above-described physical characterization embodiments selected from powder X-ray diffraction, solid-state ^{13}C NMR, differential scanning calorimetry, thermogravimetric analysis, and gravimetric vapor sorption.

[0058] In some embodiments, Form A is characterized by at least five of the above-described physical characterization embodiments selected from powder X-ray diffraction, solid-state ^{13}C NMR, differential scanning calorimetry, thermogravimetric analysis, and gravimetric vapor sorption.

[0059] In some embodiments, Form A is characterized by the following:

a powder X-ray diffraction pattern that comprises peaks at 8.1 ± 0.2° 2θ, 13.9 ± 0.2° 2θ, 16.1 ± 0.2° 2θ, 16.7 ± 0.2° 2θ, and 22.6 ± 0.2 °2θ; and

a solid-state ^{13}C NMR spectrum that comprises peaks at 161.2 ±0.2 ppm, 137.6 ±0.2 ppm, 113.1 ±0.2 ppm, 72.3 ±0.2 ppm, and 60.0 ±0.2 ppm.

[0060] In some embodiments, Form A is characterized by the following:

a powder X-ray diffraction pattern that comprises peaks at 8.1 ± 0.2° 2θ, 13.9 ± 0.2° 2θ, 16.1 ± 0.2° 2θ, 16.7 ± 0.2° 2θ, and 22.6 ± 0.2 °2θ; and

a differential scanning calorimetry curve comprising a melting endotherm having an onset temperature of about 122 ºC ± 5 ºC.

In another aspect, Form A is further characterized by a solid-state ^{13}C NMR spectrum that comprises peaks at 161.2 ±0.2 ppm, 137.6 ±0.2 ppm, 113.1 ±0.2 ppm, 72.3 ±0.2 ppm, and 60.0 ±0.2 ppm.

[0061] In some embodiments, Form A is characterized by the following:

a powder X-ray diffraction pattern that comprises peaks at 8.1 ± 0.2° 2θ, 13.9 ± 0.2° 2θ, 16.1 ± 0.2° 2θ, 16.7 ± 0.2° 2θ, and 22.6 ± 0.2 °2θ; and

a thermogravimetric analysis thermogram wherein the crystalline form exhibits a weight loss of less than about 1.0 weight % from about 25 ºC to about 100 ºC.

In another aspect, Form A is further characterized by a solid-state ^{13}C NMR spectrum that comprises peaks at 161.2 ±0.2 ppm, 137.6 ±0.2 ppm, 113.1 ±0.2 ppm, 72.3 ±0.2 ppm, and 60.0 ±0.2 ppm.

[0062] In some embodiments, Form A is characterized by the following:

a powder X-ray diffraction pattern that comprises peaks at 8.1 ± 0.2° 2θ, 13.9 ± 0.2° 2θ, 16.1 ± 0.2° 2θ, 16.7 ± 0.2° 2θ, and 22.6 ± 0.2 °2θ; and

a gravimetric vapor sorption plot wherein the crystalline form exhibits a reversible moisture uptake of less than about 1.0 weight % from about 0% relative humidity to about 80% relative humidity at 25 ºC ± 0.1 ºC.

In another aspect, Form A is further characterized by a solid-state ^{13}C NMR spectrum that comprises peaks at 161.2 ±0.2 ppm, 137.6 ±0.2 ppm, 113.1 ±0.2 ppm, 72.3 ±0.2 ppm, and 60.0 ±0.2 ppm.

[0063] In some embodiments, Form A is characterized by the following:

a powder X-ray diffraction pattern that comprises peaks at 8.1 ± 0.2° 2θ, 13.9 ± 0.2° 2θ, 16.1 ± 0.2° 2θ, 16.7 ± 0.2° 2θ, and 22.6 ± 0.2 °2θ;

a differential scanning calorimetry curve comprising a melting endotherm having an onset temperature of about 122 ºC ± 5 ºC; and

a thermogravimetric analysis thermogram wherein the crystalline form exhibits a weight loss of less than about 1.0 weight % from about 25 ºC to about 100 ºC.

In another aspect, Form A is further characterized by a solid-state ^{13}C NMR spectrum that comprises peaks at 161.2 ±0.2 ppm, 137.6 ±0.2 ppm, 113.1 ±0.2 ppm, 72.3 ±0.2 ppm, and 60.0 ±0.2 ppm.

[0064] In some embodiments, Form A is characterized by the following:

a powder X-ray diffraction pattern that comprises peaks at 8.1 ± 0.2° 2θ, 13.9 ± 0.2° 2θ, 16.1 ± 0.2° 2θ, 16.7 ± 0.2° 2θ, and 22.6 ± 0.2 °2θ;

a differential scanning calorimetry curve comprising a melting endotherm having an onset temperature of about 122 ºC ± 5 ºC; and

a gravimetric vapor sorption plot wherein the crystalline form exhibits a reversible moisture uptake of less than about 1.0 weight % from about 0% relative humidity to about 80% relative humidity at 25 ºC ± 0.1 ºC.

In another aspect, Form A is further characterized by a solid-state ^{13}C NMR spectrum that comprises peaks at 161.2 ±0.2 ppm, 137.6 ±0.2 ppm, 113.1 ±0.2 ppm, 72.3 ±0.2 ppm, and 60.0 ±0.2 ppm.

[0065] In some embodiments, Form A is characterized by the following:

a powder X-ray diffraction pattern that comprises peaks at 8.1 ± 0.2° 2θ, 13.9 ± 0.2° 2θ, 16.1 ± 0.2° 2θ, 16.7 ± 0.2° 2θ, and 22.6 ± 0.2 °2θ;

a thermogravimetric analysis thermogram wherein the crystalline form exhibits a weight loss of less than about 1.0 weight % from about 25 ºC to about 100 ºC; and

a gravimetric vapor sorption plot wherein the crystalline form exhibits a reversible moisture uptake of less than about 1.0 weight % from about 0% relative humidity to about 80% relative humidity at 25 ºC ± 0.1 ºC.

In another aspect, Form A is further characterized by a solid-state ^{13}C NMR spectrum that comprises peaks at 161.2 ±0.2 ppm, 137.6 ±0.2 ppm, 113.1 ±0.2 ppm, 72.3 ±0.2 ppm, and 60.0 ±0.2 ppm.

[0066] In some embodiments, Form A is characterized by the following:

a powder X-ray diffraction pattern that comprises peaks at 8.1 ± 0.2° 2θ, 13.9 ± 0.2° 2θ, 16.1 ± 0.2° 2θ, 16.7 ± 0.2° 2θ, and 22.6 ± 0.2 °2θ;

a differential scanning calorimetry curve comprising a melting endotherm having an onset temperature of about 122 °C ± 5 °C;

a thermogravimetric analysis thermogram wherein the crystalline form exhibits a weight loss of less than about 1.0 weight % from about 25 °C to about 100 °C; and

a gravimetric vapor sorption plot wherein the crystalline form exhibits a reversible moisture uptake of less than about 1.0 weight % from about 0% relative humidity to about 80% relative humidity at 25 °C ± 0.1 °C.

In another aspect, Form A is further characterized by a solid-state ^{13}C NMR spectrum that comprises peaks at 161.2 ±0.2 ppm, 137.6 ±0.2 ppm, 113.1 ±0.2 ppm, 72.3 ±0.2 ppm, and 60.0 ±0.2 ppm.

[0067] In some embodiments, Form A is a crystalline anhydrate.

[0068] In some embodiments, Form A is substantially pure. In one aspect, Form A comprises less than 10 weight % of any other crystalline form of (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3S)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide. In another aspect, Form A comprises less than 5 weight % of any other crystalline form of (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide. In another aspect, Form A comprises less than 4 weight % of any other crystalline form of (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide. In another aspect, Form A comprises less than 3 weight % of any other crystalline form of (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide. In another aspect, Form A comprises less than 2 weight % of any other crystalline form of (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide. In another aspect, Form A comprises less than 1 weight % of any other crystalline form of (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide. In another aspect, Form A is substantially free of any other crystalline form of (*S*)-*N*-ethyl-3-((9-ethyl-

2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide.

III. Methods of Use

[0069] (*S*)-*N*-Ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide is an inhibitor of cyclin-dependent kinase 2 (CDK2) activity. Overexpression of CDK2 is associated with abnormal regulation of the cell-cycle and tumor growth in multiple cancer types.

[0070] In some embodiments, therefore, the present disclosure provides a method for treating or preventing a CDK2-mediated condition in a subject in need thereof by administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of crystalline Form A.

[0071] In some embodiments, the present disclosure provides a method for inhibiting CDK2 activity in a subject suffering from or susceptible to the CDK2-mediated condition by administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of crystalline Form A.

[0072] In some embodiments, the present disclosure provides a method for treating a cancer in a subject suffering from or susceptible to the cancer by administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of crystalline Form A. In one aspect, the cancer is a solid tumor cancer. In another aspect the cancer is a hematological cancer. In another aspect, the cancer is mediated, in whole or in part, by CDK2.

[0073] In some embodiments, the present disclosure provides a method for treating a cancer characterized by amplification or overexpression of the cyclin E (CCNE) gene in a subject suffering from or susceptible to the cancer by administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of crystalline Form A. In one aspect, the cancer is characterized by amplification or overexpression of CCNE1. In another aspect, the cancer is characterized by amplification or overexpression of CCNE2. In another aspect, the cancer is characterized by amplification or overexpression of CCNE1 and CCNE2. In another aspect, the cancer is a solid tumor cancer characterized by amplification or overexpression of CCNE1 and/or CCNE2. In another aspect, the solid tumor cancer is selected from the group consisting of breast cancer, ovarian cancer, endometrial cancer, and lung cancer. In another aspect, the solid tumor cancer is breast

cancer or ovarian cancer. In another aspect the cancer is a hematological cancer characterized by amplification or overexpression of CCNE1 and/or CCNE2.

[0074] In some embodiments, the present disclosure provides a method for treating or preventing a solid tumor cancer in a subject suffering from or susceptible to the cancer by administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of crystalline Form A, wherein the solid tumor cancer is selected from the group consisting of breast cancer, ovarian cancer, endometrial cancer, cervical cancer, uterine cancer, gastric cancer, prostate cancer, bladder cancer, lung cancer, esophageal cancer, head and neck cancer, kidney cancer, liver cancer, pancreatic cancer, thyroid cancer, colorectal cancer, and skin cancer. In one aspect, the solid tumor cancer is selected from the group consisting of breast cancer, ovarian cancer, endometrial cancer, cervical cancer, lung cancer, colorectal cancer, and skin cancer. In another aspect, the solid tumor cancer is selected from the group consisting of breast cancer, ovarian cancer, endometrial cancer, and lung cancer. In another aspect, the solid tumor cancer is breast cancer or ovarian cancer.

[0075] In some embodiments, the cancer is breast cancer. In one aspect, the breast cancer is selected from the group consisting of hormone receptor positive (HR+) breast cancer, hormone receptor negative (HR-) breast cancer, and triple negative breast cancer. In another aspect, the breast cancer is HR+ HER2- breast cancer. In another aspect, the breast cancer is a chemotherapy-resistant breast cancer. In another aspect, the breast cancer is a radiotherapy-resistant breast cancer. In another aspect, the breast cancer is characterized by amplification or overexpression of CCNE1 and/or CCNE2. In another aspect, the breast cancer is an advanced or metastatic breast cancer. In another aspect, the subject suffering from breast cancer was previously treated with a CDK4/6 inhibitor.

[0076] In some embodiments, the cancer is ovarian cancer. In one aspect, the ovarian cancer is platinum-sensitive or platinum-resistant ovarian cancer. In another aspect, the ovarian cancer is characterized by amplification or overexpression of CCNE1 and/or CCNE2. In another aspect, the cancer is epithelial ovarian cancer. In another aspect, the ovarian cancer is serous ovarian cancer. In another aspect, the ovarian cancer is high-grade serous ovarian cancer (HGSOC). In another aspect, the ovarian cancer is an advanced or metastatic ovarian cancer. In another aspect, the ovarian cancer is metastatic high-grade serous ovarian cancer (HGSOC). In another aspect, the subject suffering from ovarian

cancer was previously treated with a platinum-based chemotherapy.

[0077] In some embodiments, the cancer is lung cancer. In one aspect, the lung cancer is small cell lung cancer (SCLC). In another aspect, the lung cancer is non-small cell lung cancer (NSCLC). In another aspect, the non-small cell lung cancer (NSCLC) is squamous cell carcimoma. In another aspect, the non-small cell lung cancer (NSCLC) is adenocarcinoma. In another aspect, the non-small cell lung cancer (NSCLC) is large-cell carcinoma.

[0078] In some embodiments, the present disclosure provides a method for treating or preventing a hematological cancer in a subject suffering from or susceptible to the cancer by administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of crystalline Form A, wherein the hematological cancer is selected from the group consisting of non-Hodgkin's lymphoma, leukemia, multiple myeloma (MM), and myelodysplastic syndrome (MDS). In one aspect, the hematological cancer is non-Hodgkin's lymphoma (NHL). In another aspect, the non-Hodgkin's lymphoma (NHL) is selected from diffuse large B-cell lymphoma (DLBCL), follicular lymphoma, mantle cell lymphoma (MCL), and marginal zone lymphoma. In another aspect, the hematological cancer is leukemia. In another aspect, the leukemia is selected from the group consisting of acute lymphoblastic leukemia (ALL), acute myelogenous leukemia (AML), chronic lymphocytic leukemia (CLL), and chronic myeloid leukemia (CML). In another aspect, the hematological cancer is multiple myeloma (MM). In another aspect, the hematological cancer is myelodysplastic syndrome (MDS).

[0079] In some embodiments, a pharmaceutical composition comprising a therapeutically effective amount of crystalline Form A is administered as first line therapy.

[0080] In some embodiments, a pharmaceutical composition comprising a therapeutically effective amount of crystalline Form A is administered as second line (or later) therapy.

[0081] In some embodiments, the subject to whom a pharmaceutical composition comprising a therapeutically effective amount of crystalline Form A is administered exhibits a partial response (PR) in response to such treatment.

[0082] In some embodiments, the subject to whom a pharmaceutical composition comprising a therapeutically effective amount of crystalline Form A is administered exhibits

a complete response (CR) in response to such treatment.

[0083] In some embodiments, the subject to whom a pharmaceutical composition comprising a therapeutically effective amount of crystalline Form A is administered exhibits an improved progression free survival (PFS) in response to such treatment.

[0084] In some embodiments, the subject to whom a pharmaceutical composition comprising a therapeutically effective amount of crystalline Form A is administered exhibits an improved overall survival (OR) in response to such treatment.

[0085] PR, CR, PFS, and OR can be assessed, for example, in accordance with RECIST (Response Evaluation Criteria in Solid Tumours) guidelines (version 1.1).

[0086] The subject treated typically will be a human or non-human mammal, particularly a human. Suitable subjects can also include domestic or wild animals; companion animals (including dogs, cats, and the like); livestock (including horses, cows and other ruminants, pigs, poultry, rabbits, and the like); primates (including monkeys such as rhesus monkeys, cynomolgus (also known as crab-eating or long-tailed) monkeys, marmosets, tamarins, chimpanzees, macaques, and the like); and rodents (including rats, mice, gerbils, guinea pigs, and the like).

[0087] In some embodiments, the present disclosure provides crystalline Form A for use as a medicament for treating a cancer mediated, in whole or in part, by CDK2.

[0088] In some embodiments, the present disclosure provides for the use of crystalline Form A for treating a cancer mediated, in whole or in part, by CDK2.

[0089] In some embodiments, the present disclosure provides for the use of crystalline Form A for the manufacture of medicaments for treating a cancer mediated, in whole or in part, by CDK2.

IV. Combination Therapies and Fixed-Dose Combinations

[0090] Crystalline Form A of the present disclosure may be used in the methods described above as either as a single pharmacological agent or in combination with other pharmacological agents or techniques. Such combination therapies may be achieved by way of the simultaneous, sequential, or separate dosing of the individual components of the treatment. These combination therapies (and corresponding combination products) employ crystalline Form A within the dosage ranges described in this specification and the other

pharmacological agent(s), typically within its approved dosage range(s).

[0091] In some embodiments, the present disclosure provides a combination suitable for use in the treatment of a cancer mediated, in whole or in part, by CDK2, wherein the combination comprises crystalline Form A and a cyclin-dependent kinase 4/6 (CDK4/6) inhibitor. In one aspect, the CDK4/6 inhibitor is selected from the group consisting of palbociclib, abemaciclib, ribociclib, lerociclib (G1T38), trilaciclib (G1T28), dalpiciclib (SHR-6390), and BPI-16350. In another aspect, the CDK4/6 inhibitor is selected from the group consisting of palbociclib, abemaciclib, ribociclib, and dalpiciclib. In another aspect, the CDK4/6 inhibitor is palbociclib. In another aspect, the CDK4/6 inhibitor is abemaciclib. In another aspect, the CDK4/6 inhibitor is ribociclib. In another aspect, the CDK4/6 inhibitor is dalpiciclib.

[0092] In some embodiments, the present disclosure provides a combination suitable for use in the treatment of a cancer mediated, in whole or in part, by CDK2, wherein the combination comprises crystalline Form A and endocrine therapy. In one aspect, the cancer is breast cancer.

[0093] In some embodiments, the present disclosure provides a combination suitable for use in the treatment of a cancer mediated, in whole or in part, by CDK2, wherein the combination comprises crystalline Form A and an aromatase inhibitor. In one aspect, the aromatase inhibitor is selected from the group consisting of anastrozole, letrozole, exemestane, vorozole, formestane, and fadrozole. In another aspect, the combination further comprises everolimus. In another aspect, the cancer is breast cancer.

[0094] In some embodiments, the present disclosure provides a combination suitable for use in the treatment of a cancer mediated, in whole or in part, by CDK2, wherein the combination comprises crystalline Form A and a selective estrogen receptor degrader (SERD). In one aspect, the SERD is selected from the group consisting of fulvestrant, giredestrant (GDC-9545), amcenestrant (SAR439859), camizestrant (AZD9833), rintodestrant (G1T48), imlunestrant (LY3484356), elacestrant (RAD-1901), taragarestrant (D-0502), OP1250 (Olema), LSZ102 (Novartis), ZN-c5 (Zentalis), and SHR9549 (Jiangsu Hengrui Medicine). In another aspect, the SERD is selected from the group consisting of fulvestrant, giredestrant, camizestrant, imlunestrant, and elacestrant. In another aspect, the SERD is fulvestrant. In another aspect, the SERD is fulvestrant and the combination

administered further comprises alpelisib. In another aspect, the SERD is camizestrant (AZD9833). In another aspect, the SERD is camizestrant and the combination administered further comprises alpelisib. In another aspect, the cancer is breast cancer.

[0095] In some embodiments, the present disclosure provides a combination suitable for use in the treatment of a cancer mediated, in whole or in part, by CDK2, wherein the combination comprises crystalline Form A, a selective estrogen receptor degrader (SERD), and a cyclin-dependent kinase 4/6 (CDK4/6) inhibitor. In one aspect, the SERD is selected from the group consisting of fulvestrant, giredestrant (GDC-9545), amcenestrant (SAR439859), camizestrant (AZD9833), rintodestrant (G1T48), imlunestrant (LY3484356), elacestrant (RAD-1901), taragarestrant (D-0502), OP1250 (Olema), LSZ102 (Novartis), ZN-c5 (Zentalis), and SHR9549 (Jiangsu Hengrui Medicine); and the CDK4/6 inhibitor is selected from the group consisting of palbociclib, abemaciclib, ribociclib, lerociclib (G1T38), trilaciclib (G1T28), dalpiciclib (SHR-6390), and BPI-16350. In another aspect, the SERD is selected from the group consisting of fulvestrant, giredestrant, camizestrant, imlunestrant, and elacestrant; and the CDK4/6 inhibitor is selected from the group consisting of palbociclib, abemaciclib, ribociclib, and dalpiciclib. In another aspect, the SERD is selected from the group consisting of fulvestrant and camizestrant; and the CDK4/6 inhibitor is selected from the group consisting of palbociclib, abemaciclib, and ribociclib. In another aspect, the SERD is camizestrant; and the CDK4/6 inhibitor is selected from the group consisting of palbociclib, abemaciclib, and ribociclib. In another aspect, the cancer is breast cancer.

[0096] In some embodiments, the present disclosure provides a combination suitable for use in the treatment of a cancer mediated, in whole or in part, by CDK2, wherein the combination comprises crystalline Form A and a PROTAC estrogen receptor degrader (PROTAC ER Degrader). In one aspect, the PROTAC ER Degrader is vepdegestrant. In another aspect, the cancer is breast cancer.

[0097] In some embodiments, the present disclosure provides a combination suitable for use in the treatment of a cancer mediated, in whole or in part, by CDK2, wherein the combination comprises crystalline Form A and a selective estrogen receptor modulator (SERM). In one aspect, the SERM is selected from the group consisting of anordrin, bazedoxifene, broparestrol, clomifene, cyclofenil, lasofoxifene, ormeloxifene, ospemifene,

raloxifene, tamoxifen, and toremifene. In another aspect, the SERM is tamoxifen. In another aspect, the SERM is toremifene. In another aspect, the SERM is selected from the group consisting of acolbifene, afimoxifene, enclomifene, endoxifen, and zuclomifene. In another aspect, the SERM is selected from the group consisting of arzoxifene, brilanestrant, clomifenoxide, droloxifene, etacstil, fispemifene, idoxifene, levormeloxifene, miproxifene, nafoxidine, nitromifene, panomifene, pipendoxifene, trioxifene, zindoxifene, GW-7604 (Glaxo Wellcome), and NNC 45-0095 (Novo Nordisk). In another aspect, the cancer is breast cancer.

[0098] In some embodiments, the present disclosure provides a combination suitable for use in the treatment of a cancer mediated, in whole or in part, by CDK2, wherein the combination comprises crystalline Form A and an anti-HER2 agent. In one aspect, the anti-HER2 agent is an anti-HER2 monoclonal antibody. In another aspect, the anti-HER2 monoclonal antibody is trastuzumab or pertuzumab. In another aspect, the cancer is breast cancer.

[0099] In some embodiments, the present disclosure provides a combination suitable for use in the treatment of a cancer mediated, in whole or in part, by CDK2, wherein the combination comprises crystalline Form A and a poly ADP ribose polymerase (PARP) inhibitor. In one aspect, the PARP inhibitor is selected from the group consisting of olaparib, rucaparib, niraparib, talazoparib, and saruparib (AZD5305; CAS No. 2589531-76-8). In another aspect, the PARP inhibitor is olaparib. In another aspect, the PARP inhibitor is saruparib (AZD5305). In another aspect, the cancer is breast cancer. In another aspect, the cancer is ovarian cancer.

[00100] In some embodiments, the present disclosure provides a combination suitable for use in the treatment of a cancer mediated, in whole or in part, by CDK2, wherein the combination comprises crystalline Form A and a Protein kinase B (Akt) inhibitor. In one aspect, the Akt inhibitor is selected from the group consisting of capivasertib (AZD5363) and ipatasertib (RG7440). In another aspect, the Akt inhibitor is capivasertib. In another aspect, the Akt inhibitor is ipatasertib. In another aspect, the cancer is breast cancer.

[00101] In some embodiments, the present disclosure provides a combination suitable for use in the treatment of a cancer mediated, in whole or in part, by CDK2, wherein the combination comprises crystalline Form A and radiotherapy.

[00102] In some embodiments, the present disclosure provides a combination suitable for use in the treatment of a cancer mediated, in whole or in part, by CDK2, wherein the combination comprises crystalline Form A and chemotherapy.

[00103] In some embodiments, the present disclosure provides a combination suitable for use in the treatment of breast cancer, wherein the combination comprises crystalline Form A and chemotherapy. In one aspect, chemotherapy comprises administration of a combination of cyclophosphamide and doxorubicin ("AC"). In another aspect, chemotherapy comprises administration of a combination of cyclophosphamide, doxorubicin, and a taxane such as paclitaxel or docetaxel ("CAT"). In another aspect, chemotherapy comprises administration of a combination of cyclophosphamide, methotrexate, and fluorouracil (or "CMF").

[00104] In some embodiments, the present disclosure provides a combination suitable for use in the treatment of ovarian cancer, wherein the combination comprises crystalline Form A and chemotherapy. In one aspect, chemotherapy comprises administration of one or more chemotherapeutics selected from the group consisting of cisplatin, carboplatin, paclitaxel, docetaxel, topotecan, doxorubicin, epirubicin, and gemcitabine. In another aspect, chemotherapy comprises administration of a combination of carboplatin and either doxorubicin, gemcitabine, paclitaxel, or docetaxel.

[00105] In another aspect, chemotherapy comprises administration of a combination of carboplatin and either paclitaxel or docetaxel. In another aspect, chemotherapy comprises administration of topotecan. In another aspect, chemotherapy comprises administration a combination of bleomycin, etoposide, and cisplatin (BEP). In another aspect, chemotherapy comprises administration of vincristine, dactinomycin, and cyclophosphamide (VAC). In another aspect, chemotherapy comprises administration of combination of paclitaxel, gemcitabine, and oxaliplatin.

V. Pharmaceutical Compositions

[00106] Crystalline Form A of the present disclosure may be administered as pharmaceutical compositions, comprising one or more pharmaceutically acceptable excipients. Therefore, in some embodiments the present disclosure provides pharmaceutical compositions comprising crystalline Form A, and at least one pharmaceutically acceptable excipient.

[00107] The excipient(s) selected for inclusion in a particular composition will depend on

factors such as the mode of administration and the form of the composition provided. Suitable pharmaceutically acceptable excipients are well known to persons skilled in the art and are described, for example, in the Handbook of Pharmaceutical Excipients, Sixth Edition, Pharmaceutical Press, edited by Rowe, Ray C; Sheskey, Paul J; Quinn, Marian. Pharmaceutically acceptable excipients may function as, for example, adjuvants, diluents, carriers, stabilisers, flavourings, colorants, fillers, binders, disintegrants, lubricants, glidants, thickening agents and coating agents. As persons skilled in the art will appreciate, certain pharmaceutically acceptable excipients may serve more than one function and may serve alternative functions depending on how much of the excipient is present in the composition and what other excipients are present in the composition.

[00108] The compositions may be in a form suitable for oral use (for example as tablets, lozenges, hard or soft capsules, aqueous or oily suspensions, emulsions, dispersible powders or granules, syrups or elixirs), for topical use (for example as creams, ointments, gels, or aqueous or oily solutions or suspensions), for administration by inhalation (for example as a finely divided powder or a liquid aerosol), for administration by insufflation (for example as a finely divided powder) or for parenteral administration (for example as a sterile aqueous or oily solution for intravenous, subcutaneous or intramuscular dosing), or as a suppository for rectal dosing. The compositions may be obtained by conventional procedures using conventional pharmaceutical excipients, well known in the art. Thus, compositions intended for oral use may contain, for example, one or more coloring, sweetening, flavoring and/or preservative agents.

[00109] The total daily dose will necessarily be varied depending upon the subject treated, the route of administration, any therapies being co-administered, and the severity of the illness being treated, and may include single or multiple doses. Specific dosages can be adjusted, for example, depending upon the condition being treated; the age, body weight, general health condition, sex, and diet of the subject; administration routes; dose intervals; excretion rate; and other drugs being co-administered to the subject. An ordinarily skilled physician provided with the disclosure of the present specification will be able to determine appropriate dosages and regimens for administration of the therapeutic agent to the subject, and to adjust such dosages and regimens as necessary during the course of treatment, in accordance with methods well-known in the therapeutic arts. The crystalline form of the

present disclosure typically will be administered to a warm-blooded animal at a unit dose within the range 2.5 to 5000 mg/m^2 body area of the animal, or approximately 0.05 mg/kg to 100 mg/kg, and this normally provides a therapeutically effective dose.

[00110] In some embodiments, the present disclosure provides pharmaceutical compositions for use in therapy, comprising crystalline Form A and at least one pharmaceutically acceptable excipient.

[00111] In some embodiments, the present disclosure provides pharmaceutical compositions for use in the treatment of an CDK2-mediated condition, comprising crystalline Form A and at least one pharmaceutically acceptable excipient. In one aspect, the CDK2-mediated condition is a solid tumor cancer. In another aspect, the solid tumor cancer is selected from the group consisting of breast cancer, ovarian cancer, endometrial cancer, cervical cancer, lung cancer, colorectal cancer, and skin cancer. In another aspect, the solid tumor cancer is selected from the group consisting of breast cancer, ovarian cancer, endometrial cancer, and lung cancer. In another aspect, the solid tumor cancer is breast cancer or ovarian cancer. In another aspect, the CDK2-mediated condition is breast cancer. In another aspect, the CDK2-mediated condition is ovarian cancer. In another aspect, the CDK2-mediated condition is endometrial cancer. In another aspect, the CDK2-mediated condition is lung cancer.

[00112] In some embodiments, the present disclosure provides a pharmaceutical composition comprising crystalline Form A, and one or more pharmaceutically acceptable excipients. In one aspect, the composition comprises at least 90 weight % of Form A relative to any other crystalline forms of the compound. In another aspect, the composition comprises at least 95 weight % of Form A relative to any other crystalline forms of the compound. In another aspect, the composition comprises at least 96 weight % of Form A relative to any other crystalline forms of the compound. In another aspect, the composition comprises at least 97 weight % of Form A relative to any other crystalline forms of the compound. In another aspect, the composition comprises at least 98 weight % of Form A relative to any other crystalline forms of the compound. In another aspect, the composition comprises at least 99 weight % of Form A relative to any other crystalline forms of the compound. In another aspect, the composition comprises Form A substantially free of any other crystalline forms of the compound.

VI. Kits

[00113] The present disclosure further provides kits comprising a unit dosage form comprising crystalline Form A contained within a packaging material and a label or package insert which indicates that the unit dosage form can be used for treating one or more of the previously described conditions.

[00114] In some embodiments, the kit comprises a unit dosage form comprising crystalline Form A contained within a packaging material and a label or package insert which indicates that the pharmaceutical composition can be used for treating a CDK2-mediated condition. In one aspect, the CDK2-mediated condition is a solid tumor cancer. In another aspect, the solid tumor cancer is selected from the group consisting of breast cancer, ovarian cancer, endometrial cancer, cervical cancer, lung cancer, colorectal cancer, and skin cancer. In another aspect, the solid tumor cancer is selected from the group consisting of breast cancer, ovarian cancer, endometrial cancer, and lung cancer. In another aspect, the solid tumor cancer is breast cancer or ovarian cancer. In another aspect, the CDK2-mediated condition is breast cancer. In another aspect, the CDK2-mediated condition is ovarian cancer. In another aspect, the CDK2-mediated condition is endometrial cancer. In another aspect, the CDK2-mediated condition is lung cancer.

[00115] In some embodiments, the kit comprises: (a) a first unit dosage form comprising a crystalline form of the present disclosure; (b) a second unit dosage form comprising a pharmacological agent selected from the group consisting of CDK4/6 inhibitors, aromatase inhibitors, selective estrogen receptor degraders, PROTAC estrogen receptor degraders, selective estrogen receptor modulators, anti-HER2 agents, poly ADP ribose polymerase inhibitors, and Protein kinase B inhibitors; (c) a container means for containing said first and second dosage forms; and (d) a label or package insert which indicates that the first unit dosage form and second unit dosage form can be used for treating a CDK2-mediated condition.

[00116] In some embodiments, the kit comprises: (a) a first unit dosage form comprising crystalline Form A; (b) a second unit dosage form comprising a CDK4/6 inhibitor; (c) a container means for containing said first and second dosage forms; and (d) a label or package insert which indicates that the first unit dosage form and second unit dosage form can be used for treating a CDK2-mediated condition. In one aspect, the second unit dosage

form comprises a CDK4/6 inhibitor selected from the group consisting of palbociclib, abemaciclib, ribociclib, and dalpiciclib. In another aspect, the CDK2-mediated condition is selected from the group consisting of breast cancer, ovarian cancer, endometrial cancer, and lung cancer. In another aspect, the CDK2-mediated condition is breast cancer or ovarian cancer.

[00117] In some embodiments, the kit comprises: (a) a first unit dosage form comprising crystalline Form A; (b) a second unit dosage form comprising a CDK4/6 inhibitor; (c) a third unit dosage form comprising a selective estrogen receptor degrader (SERD); (d) a container means for containing said first and second dosage forms; and (e) a label or package insert which indicates that the first unit dosage form and second unit dosage form can be used for treating a CDK2-mediated condition. In one aspect, the second unit dosage form comprises a CDK4/6 inhibitor selected from the group consisting of palbociclib, abemaciclib, ribociclib, and dalpiciclib; and the third unit dosage form comprises camizestrant (AZD9833). In another aspect, the CDK2-mediated condition is selected from the group consisting of breast cancer, ovarian cancer, endometrial cancer, and lung cancer. In another aspect, the CDK2-mediated condition is breast cancer or ovarian cancer.

Representative Embodiments:

[00118] **Embodiment 1:** A crystalline form of *N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide.

[00119] **Embodiment 2:** A crystalline form of (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide.

[00120] **Embodiment 3:** The crystalline form of Embodiment 2 characterized by a powder X-ray diffraction pattern comprising at least one peak selected from the group consisting of 8.1 ± 0.2° 2θ, 13.9 ± 0.2° 2θ, 16.1 ± 0.2° 2θ, 16.7 ± 0.2° 2θ, and 22.6 ± 0.2 °2θ.

[00121] **Embodiment 4:** The crystalline form of Embodiment 2 characterized by a powder X-ray diffraction pattern comprising at least three peaks selected from the group consisting of 8.1 ± 0.2° 2θ, 13.9 ± 0.2° 2θ, 16.1 ± 0.2° 2θ, 16.7 ± 0.2° 2θ, and 22.6 ± 0.2 °2θ.

[00122] **Embodiment 5:** The crystalline form of Embodiment 2 characterized by a powder X-ray diffraction pattern comprising peaks at 8.1 ± 0.2° 2θ, 13.9 ± 0.2° 2θ, 16.1 ± 0.2° 2θ, 16.7 ± 0.2° 2θ, and 22.6 ± 0.2 °2θ.

[00123] Embodiment 6: The crystalline form of Embodiment 5 characterized by a powder X-ray diffraction pattern further comprising at least one peak selected from the group consisting of 17.7 ± 0.2° 2θ, 20.2 ± 0.2° 2θ, 21.3 ± 0.2° 2θ, 25.9 ± 0.2° 2θ, and 29.2 ± 0.2 °2θ.

[00124] Embodiment 7: The crystalline form of Embodiment 5, wherein the powder X-ray diffraction pattern further comprises peaks at 17.7 ± 0.2° 2θ, 20.2 ± 0.2° 2θ, 21.3 ± 0.2° 2θ, 25.9 ± 0.2° 2θ, and 29.2 ± 0.2 °2θ.

[00125] Embodiment 8: The crystalline form of any of Embodiments 3 to 7, wherein the powder X-ray diffraction is carried out using Cu radiation.

[00126] Embodiment 9: The crystalline form of any of Embodiments 3 to 8, wherein the powder X-ray diffraction is carried out using a Bruker D8 Advantage diffractometer operating in reflection geometry, a tube voltage of 45 kV, and filament emission of 40 mA.

[00127] Embodiment 10: The crystalline form of Embodiment 3, wherein the powder X-ray diffraction pattern is substantially the same as the powder X-ray diffraction pattern of FIG. 5-B.

[00128] Embodiment 11: The crystalline form of any of Embodiments 3 to 10 further characterized by a differential scanning calorimetry curve comprising a melting endotherm having an onset temperature of about 122 °C ± 5 °C.

[00129] Embodiment 12: The crystalline form of any of Embodiments 3 to 10 further characterized by a differential scanning calorimetry curve comprising a melting endotherm having an onset temperature of about 122 °C ± 2 °C.

[00130] Embodiment 13: The crystalline form of any of Embodiments 3 to 10 further characterized by a differential scanning calorimetry curve comprising a melting endotherm having a peak at about 128 °C ± 5 °C.

[00131] Embodiment 14: The crystalline form of any of Embodiments 3 to 10 further characterized by a differential scanning calorimetry curve comprising a melting endotherm having a peak at about 128 °C ± 2 °C.

[00132] Embodiment 15: The crystalline form of any of Embodiments 3 to 10 further characterized by a differential scanning calorimetry curve comprising a melting endotherm

having an onset temperature of about 122 °C ± 5 °C and a peak at about 128 °C ± 5 °C.

[00133] Embodiment 16: The crystalline form of any of Embodiments 3 to 10 further characterized by a differential scanning calorimetry curve comprising a melting endotherm having an onset temperature of about 122 °C ± 2 °C and a peak at about 128 °C ± 2 °C.

[00134] Embodiment 17: The crystalline form of any of Embodiments 11 to 16, wherein the differential scanning calorimetry is conducted on a TA Instruments Differential Scanning Calorimeter, model Q2000, with a sample placed in an aluminum pan and heated under nitrogen at a rate of 10 °C/minute to a temperature of 300 °C.

[00135] Embodiment 18: The crystalline form of Embodiment 11, wherein the differential scanning calorimetry curve is substantially the same as the differential scanning calorimetry curve of FIG. 6.

[00136] Embodiment 19: The crystalline form of any of Embodiments 3 to 18 further characterized by a thermogravimetric analysis thermogram wherein the crystalline form exhibits a weight loss of less than about 1.0 weight % from about 25 °C to about 100 °C.

[00137] Embodiment 20: The crystalline form of Embodiment 19, wherein the weight loss is less than about 0.5 weight %.

[00138] Embodiment 21: The crystalline form of Embodiment 19, wherein the weight loss is less than about 0.1 weight %.

[00139] Embodiment 22: The crystalline form of Embodiment 19, wherein the thermogravimetric analysis thermogram is substantially the same as the thermogravimetric analysis thermogram of FIG. 7.

[00140] Embodiment 23: The crystalline form of any of Embodiments 3 to 22 further characterized by a gravimetric vapor sorption plot wherein the crystalline form exhibits a reversible moisture uptake of less than about 1.0 weight % from about 0% relative humidity to about 80% relative humidity at 25 °C ± 0.1 °C.

[00141] Embodiment 24: The crystalline form of Embodiment 23, wherein the reversible moisture uptake is less than about 0.7 weight %.

[00142] Embodiment 25: The crystalline form of Embodiment 23, wherein the reversible moisture uptake is less than about 0.4 weight %.

[00143] Embodiment 26: The crystalline form of Embodiment 23, wherein the gravimetric vapor sorption plot is substantially the same as the gravimetric vapor sorption plot of FIG. 8.

[00144] Embodiment 27: The crystalline form of any of Embodiments 2 to 26, wherein the crystalline form is a crystalline anhydrate.

[00145] Embodiment 28: The crystalline form of any of Embodiments 3 to 10, wherein the crystalline form is further characterized by the following: a differential scanning calorimetry curve comprising a melting endotherm having an onset temperature of about 122 °C ± 5 °C; and a gravimetric vapor sorption plot wherein the crystalline form exhibits a reversible moisture uptake of less than about 1.0 weight % from about 0% relative humidity to about 80% relative humidity at 25 °C ± 0.1 °C.

[00146] Embodiment 29: The crystalline form of any of Embodiments 3 to 10, wherein the crystalline form is further characterized by the following: a thermogravimetric analysis thermogram wherein the crystalline form exhibits a weight loss of less than about 1.0 weight % from about 25 °C to about 100 °C; and a gravimetric vapor sorption plot wherein the crystalline form exhibits a reversible moisture uptake of less than about 1.0 weight % from about 0% relative humidity to about 80% relative humidity at 25 °C ± 0.1 °C.

[00147] Embodiment 30: The crystalline form of any of Embodiments 3 to 10, wherein the crystalline form is further characterized by the following: a differential scanning calorimetry curve comprising a melting endotherm having an onset temperature of about 122 °C ± 5 °C; a thermogravimetric analysis thermogram wherein the crystalline form exhibits a weight loss of less than about 1.0 weight % from about 25 °C to about 100 °C; and a gravimetric vapor sorption plot wherein the crystalline form exhibits a reversible moisture uptake of less than about 1.0 weight % from about 0% relative humidity to about 80% relative humidity at 25 °C ± 0.1 °C.

[00148] Embodiment 31: The crystalline form of any of Embodiments 3 to 30 further characterized by a solid-state ^{13}C NMR spectrum comprising one, two, three, or four peaks selected from the group consisting of 161.2 ±0.2 ppm, 137.6 ±0.2 ppm, 113.1 ±0.2 ppm, 72.3 ±0.2 ppm, and 60.0 ±0.2 ppm.

[00149] Embodiment 32: The crystalline form of any of Embodiments 3 to 30 further

characterized by a solid-state ^{13}C NMR spectrum comprising peaks at 161.2 ±0.2 ppm, 137.6 ±0.2 ppm, 113.1 ±0.2 ppm, 72.3 ±0.2 ppm, and 60.0 ±0.2 ppm.

[00150] Embodiment 33: The crystalline form of any of Embodiments 3 to 30 further characterized by a solid-state ^{13}C NMR spectrum comprising peaks at 161.2 ±0.2 ppm, 153.9 ±0.2 ppm, 152.8 ±0.2 ppm, 137.6 ±0.2 ppm, 113.1 ±0.2 ppm, 72.3 ±0.2 ppm, 60.0 ±0.2 ppm, 54.9 ±0.2 ppm, 51.0 ±0.2 ppm, 47.2 ±0.2 ppm, 40.1 ±0.2 ppm, 39.1 ±0.2 ppm, 29.3 ±0.2 ppm, 23.1 ±0.2 ppm, 17.1 ±0.2 ppm, 16.5 ±0.2 ppm, 13.3 ±0.2 ppm, and 10.7 ±0.2 ppm.

[00151] Embodiment 34: The crystalline form of Embodiment 31, wherein the solid-state ^{13}C NMR spectrum is substantially the same as the solid-state ^{13}C NMR spectrum of FIG. 10.

[00152] Embodiment 35: The crystalline form of any of Embodiments 28 to 34, wherein the crystalline form is a crystalline anhydrate.

[00153] Embodiment 36: The crystalline form of any of Embodiments 3 to 35, wherein the crystalline form comprises less than 5 weight % of any other crystalline form of (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)-pyrrolidine-1-sulfonamide.

[00154] Embodiment 37: The crystalline form of any of Embodiments 3 to 35, wherein the crystalline form is substantially free of any other crystalline form of (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)-pyrrolidine-1-sulfonamide.

[00155] Embodiment 38: A pharmaceutical composition comprising a crystalline form of any of Embodiments 1 to 37, and one or more pharmaceutically acceptable excipients.

[00156] Embodiment 39: A method of treating or preventing a CDK2-mediated condition in a subject suffering from or susceptible to the CDK2-mediated condition, the method comprising administering to the subject a therapeutically effective amount of a crystalline form of any of Embodiments 1 to 37.

[00157] Embodiment 40: The method of Embodiment 39, wherein the CDK2-mediated condition is selected from the group consisting of breast cancer, ovarian cancer, endometrial cancer, cervical cancer, uterine cancer, gastric cancer, prostate cancer, bladder cancer, lung cancer, esophageal cancer, head and neck cancer, kidney cancer, liver cancer, pancreatic

cancer, thyroid cancer, colorectal cancer, and skin cancer.

[00158] Embodiment 41: The method of Embodiment 39, wherein the CDK2-mediated condition is breast cancer.

[00159] Embodiment 42: The method of Embodiment 39, wherein the CDK2-mediated condition is ovarian cancer.

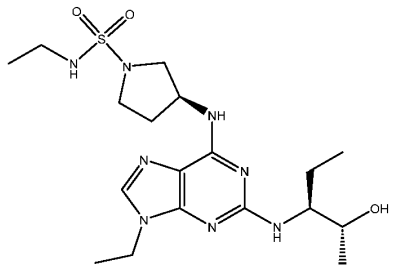
[00160] Embodiment 43: The use of a crystalline form of any of Embodiments 1 to 37 for the manufacture of a medicament for treating or preventing a CDK2-mediated condition.

[00161] Embodiment 44: A kit comprising a unit dosage form comprising a crystalline form of any of Embodiments 1 to 37 contained within a packaging material, and a label or package insert which indicates that the unit dosage form can be used for treating a CDK2-mediated condition.

VII. Examples

[00162] The following descriptions of experiments, procedures, examples, and intermediates are intended to exemplify embodiments of the disclosure. They are in no way intended to be limiting. Other embodiments of this disclosure may be prepared using the methods illustrated in these examples, either alone or in combination with techniques generally known in the art.

Example 1: Preparation of (S)-N-ethyl-3-((9-ethyl-2-(((2R,3S)-2-hydroxypentan-3-yl)amino)-9H-purin-6-yl)amino)pyrrolidine-1-sulfonamide



[00163] (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide can be prepared as described below:

A. Preparation of 6-chloro-9-ethyl-2-fluoro-9H-purine (Intermediate 2)

[00164] Iodoethane (5.15 ml, 63.75 mmol) was added to a stirred suspension of 6-chloro-

2-fluoro-9*H*-purine (**Intermediate 1**, 10 g, 57.96 mmol) and potassium carbonate (10.01 g, 72.44 mmol) in dimethyl sulfoxide (DMSO, 52.8 mL) at 23°C. The resulting suspension was stirred at 23 °C for 20 hours (h). The reaction was diluted with water, adjusted to pH 7-8 with glacial acetic acid and then extracted with ethyl acetate. The combined organic layer was washed with brine, dried over sodium sulfate, filtered, and concentrated. The resulting residue was purified by flash silica chromatography, elution gradient 0 to 100% ethyl acetate (EtOAc) in hexanes. Product fractions were concentrated to afford 6-chloro-9-ethyl-2-fluoro-9*H*-purine (**Intermediate 2**, 7.00 g, 60.2 %) ^{1}H NMR (Chloroform-d, 300 MHz) δ 1.58 (3H, t), 4.31 (2H, q), 8.11 (1H, s); m/z (ES$^+$) $[M+H]^+$ = 201; and 6-chloro-7-ethyl-2-fluoro-7*H*-purine, both as off-white solids.

B. Preparation of tert-butyl (S)-3-((9-ethyl-2-fluoro-9H-purin-6-yl)amino)-pyrrolidine-1-carboxylate (Intermediate 3)

[00165] 6-Chloro-9-ethyl-2-fluoro-9*H*-purine (**Intermediate 2**, 2.30 g, 11.47 mmol) and *tert*-butyl (*S*)-3-aminopyrrolidine-1-carboxylate (2.093 g, 11.24 mmol) was dissolved in dimethylformamide (DMF, 15 mL) under nitrogen, cooled to 0 °C, then added *N,N*-diisopropylethylamine (4.01 ml, 22.93 mmol) dropwise. The reaction was warmed to 80 °C, stirred for 1 h. The solvent was removed under reduced pressure and the white residue was purified by flash silica chromatography, elution gradient 50 to 100% EtOAc in hexanes, then 15% methanol (MeOH) in dichloromethane (DCM). Product fractions were concentrated under reduced pressure to afford *tert*-butyl (*S*)-3-((9-ethyl-2-fluoro-9*H*-purin-6-yl)amino)pyrrolidine-1-carboxylate (**Intermediate 3**, 2.37 g, 59.1 %) as a white solid. ^{1}H NMR (300 MHz, DMSO-d$_6$) δ 1.37 – 1.45 (12H, m), 2.00 (1H, s), 2.14 (1H, s), 3.16 – 3.31 (2H, m), 3.38 – 3.66 (2H, m), 4.12 (2H, q), 4.58 (1H, s), 8.18 (1H, s), 8.56 (1H, s). m/z (ES$^+$) $[M+H]^+$ = 351.

C. Preparation of tert-butyl (S)-3-((9-ethyl-2-(((2R,3S)-2-hydroxypentan-3-yl)-amino)-9H-purin-6-yl)amino)pyrrolidine-1-carboxylate (Intermediate 4)

[00166] *tert*-Butyl (*S*)-3-((9-ethyl-2-fluoro-9*H*-purin-6-yl)amino)pyrrolidine-1-carboxylate (**Intermediate 3**, 0.903 g, 2.58 mmol), (2*R*,3*S*)-3-aminopentan-2-ol (0.857 ml, 7.73 mmol), and *N,N*-diisopropylethylamine (DIEA, 1.350 ml, 7.73 mmol) were dissolved in n-butanol (6.13 mL)/dimethylsulfoxide (0.613 mL) and the reaction was heated at 100 °C. The reaction was stirred at 100 °C for 65 h, then continued at 120 °C for an additional 16 h. The reaction

was concentrated and purified by flash C18 chromatography, elution gradient 0 to 100% acetonitrile in water (containing 0.1% formic acid additive) to yield *tert*-butyl (*S*)-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-carboxylate (**Intermediate 4**, 0.700 g, 62.7 %) as a white solid. m/z (ES^+) $[M+H]^+ = 434$.

D. Preparation of (2R,3S)-3-((9-ethyl-6-(((S)-pyrrolidin-3-yl)amino)-9H-purin-2-yl)amino)pentan-2-ol hydrochloride (Intermediate 5)

[00167] In a scintillation vial was added *tert*-butyl (*S*)-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-carboxylate (**Intermediate 4**, 0.240 g, 0.55 mmol), dichloromethane (3 mL) and hydrogen chloride (0.692 ml, 2.77 mmol) (4 M solution in 1,4-dioxane). The vial was sealed and stirred at room temperature (rt) for 16 h. The reaction mixture was concentrated under vacuum to obtain (2*R*,3*S*)-3-((9-ethyl-6-(((*S*)-pyrrolidin-3-yl)amino)-9*H*-purin-2-yl)amino)pentan-2-ol.HCl (**Intermediate 5**, 0.205 g, 100 %) as a white solid. m/z (ES^+) $[M+H]^+ = 334$.

E. Preparation of (S)-N-ethyl-3-((9-ethyl-2-(((2R,3S)-2-hydroxypentan-3-yl)amino)-9H-purin-6-yl)amino)pyrrolidine-1-sulfonamide

[00168] (2*R*,3*S*)-3-((9-ethyl-6-(((*S*)-pyrrolidin-3-yl)amino)-9*H*-purin-2-yl)amino)pentan-2-ol. HCl (**Intermediate 5**, 0.482 g, 1.30 mmol) was weighed in a 40 mL scintillation vial, added dichloromethane (20 mL) and triethylamine (0.903 ml, 6.52 mmol), reaction was cooled to -78 °C. Ethylsulfamoyl chloride (0.177 g, 1.17 mmol) was added to the reaction mixture and stirred for 2 h. Additional ethylsulfamoyl chloride (0.030 g, 0.20 mmol) was added and stirred for 1 h. The reaction was quenched with aq. $NaHCO_3$ solution, warmed to 25 °C, stirred for 15 minutes, extracted with DCM, organic layer separated, dried over $MgSO_4$ and concentrated to yield a white foam. The solid was purified by flash silica chromatography using 0-20% MeOH in DCM to yield (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide **(Example 1,** 0.302 g, 52.6 %). ^1H NMR (400 MHz, DMSO-d_6) 0.86 (3H, t), 0.97 – 1.1 (6H, m), 1.27 – 1.49 (4H, m), 1.69 (1H, ddd), 2.05 (1H, dt), 2.20 (1H, td), 2.95 (2H, qd), 3.09 (1H, dd), 3.21 (1H, dt), 3.33 – 3.42 (1H, m), 3.54 (1H, dd), 3.58 – 3.66 (1H, m), 3.71 – 3.82 (1H, m), 3.98 (2H, q), 4.64 (2H, s), 5.96 (1H, s), 6.93 – 7.62 (2H, m), 7.73 (1H, s); m/z (ES^+) $[M+H]^+ = 441$.

Example 2: Analytical Methods

[00169] Unless otherwise stated, the following analytical methods were used to characterize the amorphous and crystalline forms described in the Examples:

A. Reflection Powder X-Ray Diffraction

[00170] The X-ray diffraction analysis is performed according to standard methods, which can be found, for example, in Kitaigorodsky, A.I. (1973), Molecular Crystals and Molecules, Academic Press, New York; Bunn, C.W. (1948), Chemical Crystallography, Clarendon Press, London; or Klug, H.P. & Alexander, L.E. (1974), X-ray Diffraction Procedures, John Wiley & Sons, New York.

[00171] The powder X-ray diffraction (PXRD) pattern is determined by mounting a sample on a zero-background holder, single silicon crystal, and spreading out the sample into a thin layer. The PXRD is recorded with a Theta-Theta Bruker D8 Advantage (wavelength of X-rays 1.5418 Å nickel-filtered Cu radiation, Voltage 45 kV, filament emission 40 mA). Variable divergence and anti-scatter slits and incident and diffracted soller slit 0.04° are used. The samples are rotated during measurement. Samples are scanned from 5 to 40°2Θ using a 0.013° step width and a 115.770 s count time together with a PIXcel1D detector (active length 3.35°2Θ). The PXRD patterns are obtained in Bragg-Brentano geometry.

[00172] One of skill in the art will recognize that a PXRD pattern may be obtained which has one or more measurement errors depending on measurement conditions, such as equipment or machine used (Jenkins, R & Snyder, R.L. 'Introduction to X-Ray Powder Diffractometry' John Wiley & Sons 1996; Bunn, C.W. (1948), Chemical Crystallography, Clarendon Press, London; Klug, H. P. & Alexander, L. E. (1974), X-Ray Diffraction Procedures). Those skilled in the art of powder X-ray diffraction will further recognize that the relative intensity of peaks can be affected by, for example, grains above 30 microns in size and non-unitary aspect ratios that may affect analysis of samples. Those skilled in the art would further understand that intensities might fluctuate depending on experimental conditions and sample preparation (*e.g.*, preferred orientation). The following definitions have been used for the relative intensity (%): 25% – 100%, vs (very strong); 10% – 25%, s (strong); 3% – 10%, m (medium); 1% – 3%, w (weak).

[00173] One of skill in the art will also recognize that the position of reflections can be

affected by the precise height at which the sample sits in the diffractometer and the zero calibration of the diffractometer. The surface planarity of the sample may also have a small effect. Hence the diffraction pattern data presented are not to be taken as absolute values. Generally, a measurement error of a diffraction angle in a powder X-ray diffractogram may be approximately plus or minus $0.2^{\circ}2\Theta$, and such a degree of a measurement error should be taken into account when considering the PXRD data.

[00174] The reflection mode PXRD pattern may be compared to the transmission mode PXRD pattern, although those skilled in the art will realize that the diffraction patterns may vary, particularly with respect to peak intensities.

B. Differential Scanning Calorimetry (DSC)

[00175] The melting point temperature onset (T_m) is determined by Differential Scanning Calorimetry using a TA Instruments DSC, model Q2000. A sample (approximately 1-3 mg) is weighed into an aluminum sample pan. The sample is packed to the bottom of the sample pan and a lid with a pin hole is used. The instrument is purged with nitrogen at 50 mL/min and data collected between 25 °C and 210-250 °C, using a heating rate of 10 °C/minute.

C. Thermogravimetric Analysis (TGA)

[00176] Thermal gravimetric analysis is performed using a TA Instruments TGA, model Q500. A sample (approximately 10 mg) is transferred to a tared sample holder. The instrument is purged with nitrogen, oven 60 mL/min and balance 40 mL/min, and data are collected between room temperature and 300 °C, using a heating rate of 10 °C/min. During heating, the buoyancy effect will result in an observed weight increase. This effect can be reduced by using more than 15 mg of material or performing a baseline subtraction on the sample curve.

D. Gravimetric Vapor Sorption (GVS)

[00177] Gravimetric vapor sorption analysis is performed using a TA Instruments TGA, model Q5000SA. A sample (approximately 5-10 mg) is transferred to a tared sample holder. The instrument is purged with nitrogen, chamber 200 mL/min and balance 10 mL/min at 25 °C and data are collected at different relative humidity (%RH). Starting at 40% relative humidity (RH) and going stepwise up to 90%RH, down stepwise to 0%RH, and eventually a second cycle going up to 90%RH and back to 0%RH. The equilibrium criteria for moving to

next %RH is reached when the drift criteria (dm/dt) is below 0.002 for 10 min.

[00178] Hygroscopicity can be assessed, for example, according to the European Pharmacopoeia (EP) classification: non-hygroscopic: < 0.2%; slightly hygroscopic: ≥ 0.2% and < 2%; hygroscopic: ≥ 2% and < 15%; very hygroscopic: ≥ 15%; deliquescent: sufficient water is absorbed to form a liquid; all values measured as weight increase at 80% RH and 25 °C).

Example 3: Amorphous (S)-N-ethyl-3-((9-ethyl-2-(((2R,3S)-2-hydroxypentan-3-yl)amino)-9H-purin-6-yl)amino)pyrrolidine-1-sulfonamide

A. Preparation of Amorphous (S)-N-ethyl-3-((9-ethyl-2-(((2R,3S)-2-hydroxypentan-3-yl)amino)-9H-purin-6-yl)amino)pyrrolidine-1-sulfonamide

(i) Evaporation from Acetone

[00179] Crystalline Form A of (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide (50 mg) was dissolved in acetone (1.0 mL). The resulting clear colorless solution was evaporated to dryness in vacuum by a GeneVac. A white powder was obtained. The isolated material was analyzed by PXRD analysis which showed that the material was amorphous.

(ii) Evaporation from Methanol

[00180] Crystalline Form A of (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide (50 mg) was dissolved in methanol (1.0 mL). The resulting clear colorless solution was evaporated to dryness in vacuum by a GeneVac. A gel with needle crystals was obtained.

(iii) Evaporation from Tetrahydrofuran

[00181] The material obtained from the methanol evaporation step was dissolved in tetrahydrofuran (1.0 mL). The resulting clear colorless solution was evaporated to dryness in vacuum by a GeneVac. A gel was obtained.

B. Physical Characterization of Amorphous (S)-N-ethyl-3-((9-ethyl-2-(((2R,3S)-2-hydroxypentan-3-yl)amino)-9H-purin-6-yl)amino)pyrrolidine-1-sulfonamide

[00182] Amorphous (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-

9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide was characterized by PXRD (FIG. 1), differential scanning calorimetry (DSC) (FIG. 2), thermogravimetric analysis (TGA) (FIG. 3), and gravimetric vapor sorption (GVS) (FIG. 4). The PXRD pattern of FIG. 1 shows that the amorphous compound has no regular crystalline order. The GVS plot of FIG. 4 shows that the amorphous compound is hygroscopic, absorbing over 2.0% water by weight at the 80% relative humidity level.

Example 4: Crystallization Study

[00183] A crystallization study was conducted as described below.

A. Acetone/Water Solvent System

[00184] Amorphous (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide (10 mg) was dissolved in acetone (0.2 ml) at room temperature. Water (0.8 mL) was added. The resulting clear solution was evaporated in air under the room conditions to provide needle-like crystals. PXRD analysis showed that the isolated material was crystalline Form A.

B. Ethyl Acetate/Heptane Solvent System

[00185] Amorphous (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide (10 mg) was dissolved in ethyl acetate (0.2 ml) at room temperature. Heptane (0.8 mL) was slowly added to the clear solution and a gel formed in the solution. After approximately 30 minutes the gel solidified providing a suspension of white solids. The suspension was stirred at room temperature for two days resulting in a slurry. The solid material isolated from the slurry was analyzed by PXRD analysis which showed that the material was crystalline Form A.

C. Methyl Tert-Butyl Ether Solvent System

[00186] Amorphous (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide (10 mg) was suspended in methyl *tert*-butyl ether (0.2 ml) at room temperature. After approximately five minutes a gel formed. More methyl *tert*-butyl ether (0.3 ml) was added to the suspension. The gel solidified and a slurry was obtained. The slurry was stirred at room temperature for two days resulting in a slurry. The solid material isolated from the slurry was analyzed by PXRD analysis which showed that the material was crystalline Form A.

D. Water Solvent System

[00187] Amorphous (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide (10 mg) was dissolved in water (0.2 ml) at room temperature. A gel formed and more water (0.3 mL) was added. The suspension obtained was stirred at room temperature for two days resulting in a slurry. The solid material isolated from the slurry was analyzed by PXRD analysis which showed that the material was crystalline Form A.

E. Methyl Tert-Butyl Ether Solvent System (With Form A Seeding)

[00188] Amorphous (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide (100 mg) was suspended in methyl *tert*-butyl ether (2.0 mL) at room temperature. Form A seeds (2 mg to 3 mg) were added. After five minutes, the amorphous material became a gel while the Form A seeds remained in solid form. After 15 minutes, the gel started to solidify and formed a solid cluster after one hour. The solid cluster was broken up by spatula and the resulting slurry was stirred at room temperature for one day. The methyl *tert*-butyl ether was evaporated from the slurry and a white solid was obtained. PXRD analysis showed that the isolated material was crystalline Form A. Thermal analysis showed that the isolated material was anhydrous and had a melting point of 119°C.

F. Ethyl Acetate Solvent System (With Form A Seeding)

[00189] Amorphous (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide (10 mg) was suspended in ethyl acetate (0.2 mL) at room temperature. Form A seeds (approximately 1 mg) were added. White solid started to precipitate (without forming gel) after a few minutes. The resulting slurry was stirred at room temperature for one day. Heptane (0.20 mL) was added to increase the precipitate. A white solid (5-6 mg) was collected by filtration and dried in air. PXRD analysis showed that the isolated material was crystalline Form A. Alternatively, Form A can be obtained by evaporating the ethyl acetate from the slurry without the need to add the heptane to the slurry first.

Example 5: Crystallization Study

[00190] A crystallization study was conducted as described below:

A. Water Slurry

[00191] Crystalline Form A of (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide (10 mg) was suspended in water (0.5 ml) and maintained at room temperature for one day. Acetone (1.0 ml) was then added to the suspension and the resulting solution was evaporated in air under the room conditions to provide a white solid. The test was repeated for a suspension maintained at room temperature for 5 days and again provided a white solid. The test was repeated a third time for a suspension maintained at 60°C for two hours and once again provided a white solid. PXRD analysis of the white solids isolated after evaporation indicated that they were all Form A.

B. Ethyl Acetate Slurry

[00192] Crystalline Form A of (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide (10 mg) was suspended in ethyl acetate (0.2 ml) and maintained at room temperature for one day. Additional ethyl acetate (1.0 ml) was then added to the suspension and the resulting solution was evaporated in air under the room conditions to provide a white solid. The test was repeated for a suspension maintained at room temperature for 5 days and again provided a white solid. The test was repeated a third time for a suspension maintained at 60°C for two hours and once again provided a white solid. PXRD analysis of the white solids isolated after evaporation indicated that they were all Form A.

C. Methyl Tert-Butyl Ether Slurry

[00193] Crystalline Form A of (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide (10 mg) was suspended in methyl *tert*-butyl ether (0.2 ml) and maintained at room temperature for one day. Additional methyl *tert*-butyl ether (2.0 ml) was then added to the suspension and the resulting solution was evaporated in air under the room conditions to provide a white solid. The test was repeated for a suspension maintained at room temperature for 5 days and again provided a white solid. The test was repeated a third time for a suspension maintained at 60°C for two hours and once again provided a white solid. PXRD analysis of the white solids isolated after evaporation indicated that they were all Form A.

D. Isopropanol Slurry

[00194] Crystalline Form A of (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide (20 mg) was suspended in isopropanol (0.2 ml) and maintained at room temperature for one day. Additional isopropanol (1.0 ml) was then added to the suspension and the resulting solution was evaporated in air under the room conditions to provide a white solid. The test was repeated for a suspension maintained at room temperature for 5 days and again provided a white solid. The test was repeated a third time for a suspension maintained at 60°C for two hours and once again provided a white solid. PXRD analysis of the white solids isolated after evaporation indicated that they were all Form A.

E. Evaporation From Methanol

[00195] Crystalline Form A of (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide (10 mg) was dissolved in methanol (0.5 ml). The resulting solution was evaporated in air under the room conditions to provide a white solid. PXRD analysis of the white solid isolated after evaporation indicated that it was Form A. Thermal analysis showed that the isolated material was anhydrous and had a melting point of 122°C.

F. Evaporation From Acetone

[00196] Crystalline Form A of (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide (10 mg) was dissolved in acetone (0.5 ml). The resulting solution was evaporated in air under the room conditions to provide a white solid. PXRD analysis of the white solid isolated after evaporation indicated that it was Form A.

G. Evaporation From Acetonitrile

[00197] Crystalline Form A of (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide (10 mg) was dissolved in acetonitrile (0.5 ml). The resulting solution was evaporated in air under the room conditions to provide a white solid. PXRD analysis of the white solid isolated after evaporation indicated that it was Form A.

H. Evaporation From Tetrahydrofuran

[00198] Crystalline Form A of (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide (10 mg) was dissolved in tetrahydrofuran (0.5 ml). The resulting solution was evaporated in air under the room conditions to provide a white solid. PXRD analysis of the white solid isolated after evaporation indicated that it was Form A.

I. Evaporation From Dichloromethane

[00199] Crystalline Form A of (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide (10 mg) was dissolved in dichloromethane (0.5 ml). The resulting solution was evaporated in air under the room conditions to provide a white solid. PXRD analysis of the white solid isolated after evaporation indicated that it was Form A.

Example 6: Physical Characterization of Crystalline Form A

[00200] Characterization of Form A was carried out using various techniques including single crystal analysis, PXRD (FIG. 5-B), differential scanning calorimetry (DSC) (FIG. 6), thermogravimetic analysis (TGA) (FIG. 7), gravimetric vapor sorption (GVS) (FIG. 8), and ^{13}C solid-state NMR (FIG. 9).

A. Single Crystal Analysis

[00201] A single crystal analysis was conducted for Form A. Single clear colorless needle-shaped crystals of Form A were obtained from slow evaporation of an ethyl acetate solution of (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)-pyrrolidine-1-sulfonamide. A suitable crystal was selected and mounted on a Hampton loop in perfluoroether oil. X-ray diffraction data was collected at 100 K using Cryostream 800 (Oxford Cryosystem, UK) in ω-scan mode with XtaLab Synergy-S (Rigaku, Japan) equipped with Cu Kα micro focus source (50 kV, 0.01 mA) and Hypix-Arc 100 detector. The diffraction pattern was initially indexed and the total number of runs and images was based on the strategy calculation from the program CrysAlisPro 1.171.42.46a (Rigaku, Japan). Data reduction, scaling and absorption corrections were performed using CrysAlisPro 1.171.42.46a (Rigaku, Japan). The integrated and scaled data were corrected using numerical absorption correction based on gaussian integration over a multifaceted

crystal model and empirical absorption correction using spherical harmonics, implemented in SCALE3 ABSPACK scaling algorithm.

[00202] The structure was solved by the ShelXT (Sheldrick, 2015) structure solution program using dual methods and refined by full matrix least squares minimisation on F^2 using version 2018/3 of ShelXL 2018/3 (Sheldrick, 2015) within Olex2 (Dolomanov, 2009). All non-hydrogen atoms were refined anisotropically. All hydrogen atoms attached to oxygen and nitrogen atoms were located from the differential Fourier map and were refined isotropically. All other hydrogen atoms were determined geometrically and refined isotropically.

[00203] The crystal structure analysis showed that Form A crystallizes in a trigonal P3(1) space group with Z'=1. The asymmetric unit contains one molecule of (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)-pyrrolidine-1-sulfonamide, therefore the crystal structure is anhydrous Form A. Flack (Flack, 1999) and Hooft (Hooft, 2010) parameters were found to be 0.01(3) and -0.016(4), respectively. Crystallographic data is listed in Table 1-A. A thermal ellipsoid drawing of the crystal structure drawn at a 50% probability level is shown in Figure 5-A.

TABLE 1-A

Formula	$C_{18}H_{32}N_8O_3S$
Dcalc	1.322
m/mm^{-1}	1.609
Formula Weight	440.57
Colour	clear colourless
Shape	needle-shaped
Size/mm^3	0.30×0.04×0.02
T/K	100.01(10)
Crystal System	trigonal
Flack Parameter	0.01(3)
Hooft Parameter	-0.016(4)
Space Group	$P3_1$
a/Å	12.58380(10)
b/Å	12.58380(10)
c/Å	12.1104(2)
V/Å^3	1660.78(4)
Z	3
Z'	1
θ_{min}/°	4.057
θ_{max}/°	79.344

Measured Reflections	83452
Independent Reflections	4408
Reflections I≥2 *s*(I)	4303
R_{int}	0.0581
Parameters	291
Restraints	1
Largest Peak	0.290
Deepest Hole	-0.424
GooF	1.091
wR_2 (all data)	0.1343
wR_2	0.1337
R_1 (all data)	0.0495
R_1	0.0486

Reference:

- Dolomanov, O.V., Bourhis, J., Gildea. R.J., Howard, J.A.K., Puschmann, H. J. Appl. Cryst. 42 (2009) 339-431.
- Hooft, R.W.W., Straver, L.H., Spek, A.L., J. Appl. Cryst. 43 (2010) 665-668.
- Flack, H.D., Bernardinelli, G. Acta Cryst. A55 (1999) 908-915.
- Sheldrick, G.M., Acta Cryst. C71 (2015) 3-8.

B. PXRD

[00204] FIG. 5-B shows a representative PXRD pattern for Form A measured using reflection geometry. The PXRD pattern of FIG. 5-B confirms that Form A is crystalline. Table 1-B below lists selected peaks identified in the PXRD pattern of FIG. 5-B.

TABLE 1-B

PEAK POSITION (°2Θ)	RELATIVE INTENSITY (%)	DEFINITION
16.7	100.0	vs
16.1	65.8	vs
13.9	62.6	vs
8.1	57.8	vs
22.6	49.4	vs
20.2	38.6	vs
17.7	30.8	vs
25.9	24.3	s
21.3	23.7	s
29.2	15.3	s
10.9	14.3	s
15.8	14.0	s
27.3	9.9	m
29.0	9.5	m
28.0	9.2	m
22.0	6.8	m
30.1	6.4	m

The following definitions have been used for the relative intensity (%): 25% – 100%, vs (very strong); 10% – 25%, s (strong); 3% – 10%, m (medium); 1% – 3%, w (weak).

Form A shows characteristic peaks at 8.1 ± 0.2° 2θ, 13.9 ± 0.2° 2θ, 16.1 ± 0.2° 2θ, 16.7 ± 0.2° 2θ, and 22.6 ± 0.2 °2θ. Form A shows further characteristic peaks at 17.7 ± 0.2° 2θ, 20.2 ± 0.2° 2θ, 21.3 ± 0.2° 2θ, 25.9 ± 0.2° 2θ, and 29.2 ± 0.2 °2θ.

C. DSC

[00205] FIG. 6 shows a representative differential scanning calorimetry (DSC) thermogram for Form A. Exothermic events are plotted in the upward direction. The melting endotherm shown in FIG. 6 has an onset temperature of about 122°C and a heat enthalpy of approximately 105 J/g for the melting endotherm. The DSC values obtained can vary by as much as ± 5°C depending upon the instrument used, how samples are prepared, and differences between batches.

D. TGA

[00206] FIG. 7 shows a representative thermogravimetic analysis (TGA) thermogram for Form A. Form A exhibited a weight loss of less than about 1.0 weight % upon heating from about 25 °C to 100 °C, which confirms that Form A is an anhydrate.

E. DVS

[00207] FIG. 8 shows a representative gravimetric vapor sorption (GVS) plot for Form A. Form A exhibited a reversible moisture uptake of about 0.3 weight % between 0% relative humidity and 80% relative humidity at 25 °C ±0.1 °C. The desorption curve indicates that Form A lost moisture at a similar rate to the moisture gained during sorption, with limited hysteresis. No form change was observed by PXRD after the GVS experiment.

[00208] Section 5.11 of European Pharmacopoeia 6.0 provides the following hygroscopicity classification according to specified analytical method:

HYGROSCOPIC CLASS	CRITERIA[1]
Non-Hygroscopic	<0.2% (w/w)
Slightly Hygroscopic	0.2-2% (w/w)
Hygroscopic	2-15% (w/w)
Very Hygroscopic	>15% (w/w)

[1] Percent water uptake at 25°C/80% RH in first adsorption cycle of sorption isotherm.

F. ^{13}C Solid-State NMR

[00209] Form A was analyzed by ^{13}C solid-state NMR. The ^{13}C solid-state NMR spectrum obtained was consistent with the Form A sample being highly crystalline with a Z'=1.

[00210] Approximately 100 mg of Form A was packed into a 4 mm Magic Angle Spinning (MAS) rotor which was placed into the 4 mm HX probe (H12138_0092) fitted to the Bruker Avance IIIHD solid-state NMR spectrometer (Dopey). The rotor was spun about the magic angle at a frequency of 12 kHz. A series of ^{1}H Direct Polarisation (DP)MAS spectra were recorded with increasing values of recycle delay. When there was no further increase in spectral intensity, this value of recycle delay was recorded and used in the subsequent experiments. A ^{1}H-^{13}C cross-polarisation (CP) MAS spectrum was recorded using the recycle delay as determined as above. A contact pulse length of 2 ms was used. The ^{1}H spectra were externally referenced to the isotropic peak of Adamantane at 1.85 ppm and the ^{13}C spectrum was externally referenced to the peak assigned to the carbonyl carbon of Glycine at 176.03 ppm. All spectra were stored on the spectrometer.

[00211] The ^{1}H DPMAS spectra of Form A were recorded with increasing values of recycle delay (1, 5, 10, 20, 30, 45, 60 seconds). The ^{1}H DPMAS spectrum that first showed no further increase in spectral intensity was recorded with a recycle delay of 20 seconds. Figure 9 shows the ^{13}C CPMAS spectrum of Form A recorded with a recycle delay of 20 seconds based on the ^{1}H DPMAS experimental results. The peaks in the spectrum were very sharp which is consistent with the sample being highly crystalline. Comparing the number of resolved peaks (18) with the number of carbon atoms in the molecular formula of the compound (18), it was determined that the number of molecules in the asymmetric unit cell (Z') is 1.

[00212] Form A shows characteristic peaks at 161.2 ±0.2 ppm, 137.6 ±0.2 ppm, 113.1 ±0.2 ppm, 72.3 ±0.2 ppm, and 60.0 ±0.2 ppm. Form A shows further characteristic peaks at 161.2 ±0.2 ppm, 153.9 ±0.2 ppm, 152.8 ±0.2 ppm, 137.6 ±0.2 ppm, 113.1 ±0.2 ppm, 72.3 ±0.2 ppm, 60.0 ±0.2 ppm, 54.9 ±0.2 ppm, 51.0 ±0.2 ppm, 47.2 ±0.2 ppm, 40.1 ±0.2 ppm, 39.1 ±0.2 ppm, 29.3 ±0.2 ppm, 23.1 ±0.2 ppm, 17.1 ±0.2 ppm, 16.5 ±0.2 ppm, 13.3 ±0.2 ppm, and 10.7 ±0.2 ppm.

G. Cell Parameters

[00213] High resolution X-ray powder diffraction ("HR-XRPD") data for a sample of crystalline Form A of (S)-N-ethyl-3-((9-ethyl-2-(((2R,3S)-2-hydroxypentan-3-yl)amino)-9H-purin-6-yl)amino)pyrrolidine-1-sulfonamide were collected on a D8 Advance diffractometer using Cu Kα1 radiation (1.54056 Å) with germanium monochromator at RT. The diffraction data were collected in the 2θ range 2.15 - 41.5°. The detector scan on a solid state LynxEye detector was performed using 0.0157° per step with 15 sec/step scan speed. The samples were measured in 8 mm long glass capillary with 0.5 mm outer diameter. The analyzed material was polymorphic pure. No crystalline impurities were detected.

[00214] Cell parameters and crystal system were obtained using an LSI-Index (Coelho, 2003; Coelho & Kern, 2005) indexing program. The space group was selected on reflections condition and density of the crystal. The cell parameters, purity as well as instrument parameters were refined using **W**hole **P**owder **P**attern **D**ecomposition method (Pawley, 1981). The following criteria of fit were used:

- $Y_{o,m}$ and $Y_{c,m}$ are the observed and calculated data, respectively at data point m,
- M the number of data points,
- P the number of parameters,
- w_m the weighting given to data point m which for counting statistics is given by $w_m=1/\sigma(Y_{o,m})^2$ where $\sigma(Y_{o,m})$ is the error in $Y_{o,m}$,

$$R_{\exp}=\sqrt{\frac{M-P}{\sum w_m Y_{o,m}^2}};\quad R_{wp}=\sqrt{\frac{\sum w_m\left(Y_{o,m}-Y_{c,m}\right)^2}{\sum w_m Y_{o,m}^2}};\quad R_p=\sqrt{\frac{\sum\left|Y_{o,m}-Y_{c,m}\right|}{\sum Y_{o,m}}}$$

$$GOF=chi^2=\frac{R_{wp}}{R_{\exp}}=\sqrt{\frac{\sum w_m\left(Y_{o,m}-Y_{c,m}\right)^2}{M-P}}$$

[00215] Calculated cell parameters and related information are summarized in Table 1-C below.

TABLE 1-C

Form	Crystalline Form A
Empirical formula	$C_{18}H_{32}N_8O_3S$
Formula weight	440.56
T [K]	296(2)

λ [Å]	1.54056
Crystal system	Trigonal (hexagonal setting)
Proposed space group	P3(1) (No. 144)
Unit cell dimensions	
a [Å]	12.7888(5)
c [Å]	12.1924(6)
V [$Å^3$]	1726.96(18)
Z (Z')	3(1)
D_c [g/cm^3]	1.271
Cap. size [mm^2]	0.5 x 8
2θ Step size [°]	0.016
No of steps	2503
Time per step [s]	15
2θ range [°]	1.5 → 41.5
Rexp	3.52
R_{wp}	5.88
R_p	4.43
GOF	1.67
R_{Brag}	0.71
Impurities [%]	Below Detection Limit

Example 7: CDK1, CDK2, and CDK4 Activity (NanoBRET Assay in MCF-7 Cells)

[00216] (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide was tested in breast cancer cell line MCF-7 to assess inhibition of CDK1, CDK2, and CDK4 activity. MCF-7 cells transiently transfected with CDK1:CCNB1, CDK2:CCNE1, or CDK4:CCND1 were harvested at a density of 1E5 cells/mL in complete media, seeded 20 µL/well into 384-well Greiner 784080 plates using a Multidrop Combi, and incubated overnight at 37°C and 5% CO_2. The next day media were evacuated from the wells using the Bluewasher centrifugal plate washer (Bluecatbio) and 10 µL/well phenol red free OptiMEM was then added using a Multidrop Combi. Test compounds were then dispensed into the wells using an Echo instrument (555/655, Beckman Coulter). Immediately after compound addition, a Tecan HP300 dispensor was used to dispense the relevant NanoBRET™ tracer to the CDK1 wells (12.5nl, 400 µM, NanoBRET™ TE Tracer K-9), the CDK2 wells (12.5nl, 200 µM, NanoBRET™ TE Tracer K-9), and the CDK4 wells (8nl, 100 µM, NanoBRET™ TE Tracer K-7) and the plates were incubated for 2 hours at 37 °C and 5% CO_2. The plates were allowed to cool for 10 minutes at room temperature and 5 µL/well of TE Nano-Glo®Substrate/Inhibitor at 2.4 µM and 1:500 respectively (N2162 Promega) was added. The plates were incubated in subdued

lighting for 10 minutes and then read on a Pherastar FS plate reader (BMG Technologies) using a NanoBRET™ filter module (460 ± 80nm/610nm-LP). The ratio values were normalized to controls and the IC_{50} values of test compound determined using Genedata Screener software.

[00217] The IC_{50} values for the compound of Example 1 are reported in Table 2 below. The data confirm that the compound has nanomolar potency against CDK2 and is selective for CDK2 relative to CDK1 and CDK4.

TABLE 2

CDK2 IC_{50}[1] (μM)	CDK1 IC_{50}[1] (μM)	CDK4 IC_{50}[1] (μM)
0.009	0.528	9.4

[1] IC_{50} is reported after a single measurement (n=1) or as an average for multiple measurements (n>1).

Example 8: NPM Phosphorylation (Imaging Assay in MCF-7 Cells)

[00218] (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide was tested to assess the effects of CDK2 inhibition in a cellular context. Specifically, phosphorylation of nucleophosmin (NPM) was measured to determine whether the compound downregulated NPM phosphorylation in MCF-7 cells. During the cell cycle, the CDK2-cyclin E complex phosphorylates NPM at Thr199 which is a prerequisite step for initiation of centrosome duplication.

[00219] MCF-7 cells at a density of 2.5E5 cells/mL in complete media were seeded 40 μL/well into 384-well Greiner 781090 plates using a Multidrop Combi and incubated overnight at 37 °C and 5% CO_2. Test compounds were then dispensed into the wells using an Echo instrument (555/655, Beckman Coulter) and the plates were incubated for 2 hours at 37 °C and 5% CO_2. The cells were fixed by addition of 40 μL/well of 8% paraformaldehyde and incubated at room temperature for 10 minutes. The plates were washed three times with 50 μL/well PBS using a Biotek EL406 plate washer and then permeabilized for 10 minutes at room temperature in 0.3% Triton X100 in PBS. After washing as before, the plates were blocked using 30 μL/well of 2% BSA (w/v) in PBS-T for at least 30 minutes at room temperature. Following aspiration of the blocking solution, the plates were sealed and incubated overnight at 4°C in 20 μL/well primary antibody (CST#3541, 1/400 in PBS-T

with 0.05% BSA). The plates were washed three times with 50 μL/well PBS-T, and then incubated in 20 μL/well of secondary antibody solution (1/500 AlexaFluor488 goat anti-rabbit IgG, (Invitrogen A11008) and 1/10000 Hoechst 33342 (Invitrogen H21492) in 0.05% BSA in PBS-T) for one hour at room temperature, shielded from light. After washing in PBS as before, leaving each well in 40 μL PBS, the plates were sealed and imaged on a Cell Insight imaging system (Thermo) with a 10x objective and 6 fields of view per well. Cells containing 4N DNA were selected using the Hoechst staining. Phospho-NPM levels were normalized to controls and the IC_{50} values of test compounds determined using Genedata Screener software.

[00220] The IC_{50} value for the compound of Example 1 was 0.061 μM (average of multiple measurements).

Example 9: POLR2A Ser2 Phosphorylation (Imaging Assay in MCF-7 Cells)

[00221] (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide was tested to assess its effect on CDK9 activity in a cellular context. Specifically, phosphorylation of POLR2A Ser2 was measured to determine whether the compound downregulated POLR2A Ser2 phosphorylation in MCF-7 cells. During the cell cycle, CDK9 is a component of a multiprotein complex that phosphorylates POLR2A Ser2 which results in transcription elongation.

[00222] MCF-7 cells at a density of 1.25E5 cells/mL in complete media were seeded, 40 μL/well, into 384-well Greiner 781090 plates using a Multidrop Combi and incubated overnight at 37 °C and 5% CO_2. Test compounds were then dispensed using an Echo instrument (555/655, Beckman Coulter) and the plates were incubated for 2 hours at 37 °C and 5% CO_2. T cells were fixed by addition of 40 μL/well of 8% paraformaldehyde and incubated for 10 minutes at room temperature. The plates were washed three times with 50 μL/well PBS using a Biotek EL406 plate washer and then permeabilized for 10 minutes at room temperature in 0.3% Triton X100 in PBS. After washing as before, the plates were blocked using 30 μL/well of 2% BSA (w/v) in PBS-T for over 30 minutes at room temperature. Following aspiration of the blocking solution, the plates were sealed and incubated overnight at 4°C in 20 μL/well of primary antibody (CST#13499, 1/1000 in PBS-T with 0.05% BSA). The plates were washed three times with 50 μL/well of PBS-T, and then incubated in 20 μL/well of secondary antibody solution (1/500 AlexaFluor488 goat

anti-rabbit IgG, (Invitrogen A11008) and 1/10000 Hoechst 33342 (Invitrogen H21492) in 0.05% BSA in PBS-T) for one hour at room temperature, shielded from light. After washing in PBS as before, leaving each well in 40 µL of PBS, the plates were sealed and imaged on a Cell Insight imaging system (Thermo) with a 10x objective and 2 fields of view per well. POLR2A Ser2levels were normalized to controls and the IC_{50} values of test compounds determined using Genedata Screener software.

[00223] The IC_{50} value for the compound of Example 1 was >19.3 µM (average of multiple measurements).

Example 10: Cellular Proliferation in MCF-7 and OVCAR3 Cell Lines (EdU Assay)

[00224] (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide was tested to assess inhibition of cellular proliferation in the breast cancer cell line MCF-7 and the CCNE1-amplified ovarian cell line OVCAR3.

[00225] MCF-7 and OVCAR3 cells in RPMI supplemented with 10% Fetal bovine serum were seeded into 384-well plates (Greiner, Kremsmunster, Austria; 781091), 30 µL/well, using a WellMate. The MCF7 and OVCAR3 cells were seeded at 800 and 1200 cells/well, respectively. Test compounds were added to the wells using an Echo 555 liquid handler and the plates were placed in incubator maintained at 37°C and 5% CO_2 and incubated for two days. On Day 2, an EdU assay was performed following the manufacturer's protocol (Thermo Fisher, C10351). The cells were read on an Acumen eX3 instrument. The IC_{50} values of test compounds were determined in Genedata by assessing the ratio of signal intensity at 488nm to the area of signal at 405 nm.

[00226] IC_{50} values for the compound of Example 1 are reported in Table 3.

TABLE 3

MCF-7 IC_{50}[1] (µM)	OVCAR3 IC_{50}[1] (µM)
0.278	0.066

[1] IC_{50} is reported after a single measurement (n=1) or as an average for multiple measurements (n>1).

Example 11: Effect on Cell Cycle in OVCAR3 Cell Line (24 Hours Compound Dosing)

[00227] (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-

yl)amino)pyrrolidine-1-sulfonamide was tested in the CCNE1-amplified ovarian cell line OVCAR3 to assess the effect on cell cycle phases G1 and S and potential off-target effects on cell cycle phases G2 and M.

[00228] OVCAR3 cells were seeded at 60,000 cells per well in 24 well plates, 4 wells per sample, in RPMI with 10% Fetal bovine serum. Test compounds were diluted in 96 well plates in dimethyl sulfoxide (DMSO) and then added to the wells containing the cells. The 24 well plates were placed in an incubator maintained at 37°C and 5% CO_2 and incubated for 20 to 24 hours. The next day an EdU assay was performed following the manufacturer's protocol (Thermo Fisher, C10425 EdU Alexa Fluor 488). Antibody staining was carried out using PE Mouse Anti-Cleaved PARP (BD Biosciences Catalog No. 552933) and 4',6-diamidino-2-phenylindole (DAPI). Cells were then analyzed by flow cytometry.

[00229] The compound of Example 1 induced G1/S cell cycle arrest, inhibited pRB phosphorylation, and inhibited cell proliferation. Treatment of the OVCAR3 cells with the compound of Example 1 at concentrations of 0.03, 0.1, 0.3, 1, and 3 μM increased the population of cells in the G0 and G1 phases.

Example 12: pRB Phosphorylation in OVCAR3 Cell Line (Western Blot Analysis)

[00230] (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide was analyzed by Western blot to assess inhibition of pRB phosphorylation in the CCNE1-amplified ovarian cell line OVCAR3. pRB is a tumor suppressor protein that inhibits cell cycle progression. Once hyperphosphorylated, however, pRB is inactivated and the cell cycle can proceed.

[00231] On Day 1, OVCAR3 cells were seeded at 60K cells per well in 24 well plates in RPMI with 10% Fetal bovine serum. Test compounds were diluted in 96 well plates in DMSO and then added to the cells. The treated cells were placed in an incubator maintained at 37°C and 5% CO_2 and incubated for 20 to 24 hours. On Day 2, the cells were rinsed once with phosphate buffer saline (PBS) and scraped to 1% SDS lysing buffer. The protein lysate was quantified using a Pierce™ BCA Protein Assay Kit (Thermo Fisher 23225). An equal microgram of proteins was diluted in 1x Novex LDS Sample Buffer (Thermo Fisher NP0008) supplemented with reducing agent (Thermo Fisher NP0009). The gel samples were loaded into NuPAGE 4 to 12% Bis-Tris buffer and electrophoresis run at 200 volts. The samples were then transferred to Nitrocellulose by standard Wet Transfer method in 1x

NuPAGE transfer buffer 10% MeOH. The membranes were blocked for one hour in blocking buffer containing 5% milk in PBST (0.2% Tween in PBS). The blots were incubated with primary antibody Anti-Rb (phospho S780) antibody (Abcam ab173289) in blocking buffer for overnight at 4°C. After washing three times in PBST, the membranes were incubated into secondary antibody (CST Anti-rabbit IgG, HRP-linked Antibody #7074) in blocking buffer at room temperature for one hour. After washing three times in PBST, the signals were developed using SuperSignal™ West Dura Extended Duration Substrate (Thermo Fisher 3707) and imaged in chemiluminescence imager.

[00232] The Western blots showed that the compound of Example 1 downregulated pRB phosphorylation in the OVCAR3 cell line and provided further confirmation of the cell cycle effects reported in Example 11.

[00233] The compound of Example 1 was similarly analyzed by Western blot to assess modulation of pSer2 of RNAPII, a CDK9 phosphosubstrate. No modulation of pSer2 of RNAPII was observed for the compound indicating that it was not impacting CDK9 activity.

Example 13: Cell Proliferation in OVCAR3 and SKOV3 Cell Lines (CyQUANT™ Assay)

[00234] An imaging-based assay was used to investigate changes in cell number in OVCAR3 cells (CCNE1-amplified ovarian cancer cell line) and SKOV3 cells (non-amplified ovarian cancer cell line) treated with (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide.

[00235] Cells were plated in 384 well plates (Perkin-Elmer 6057300) in RPMI-1640 / 10% FBS / 1x L-Glu at 500 cells per well in 30uL and incubated with test compound at 37°C and 5% CO_2 for 7 days. Cells were then stained using the CyQuant Direct Cell Proliferation Assay Kit (Thermo Cat#C7026) in accordance with the kit protocol. The stained cells were imaged using an Operetta high content imager to generate cell counts and IC_{50} values were calculated relative to DMSO controls.

[00236] IC_{50} values for the compound of Example 1 are reported in Table 4. The compound inhibited cellular proliferation in the CCNE-1 amplified OVCAR3 cells more potently than in the non-amplified SKOV3 cell line.

TABLE 4

OVCAR3 IC_{50}[1] (μM)	SKOV3 IC_{50}[1] (μM)
0.077	1.965

[1] IC_{50} is reported after a single measurement (n=1) or as an average for multiple measurements (n>1).

* * *

[00237] Although specific embodiments and examples have been described above, these embodiments and examples are only illustrative and do not limit the scope of the disclosure. Changes and modifications can be made in accordance with ordinary skill in the art without departing from the disclosure in its broader aspects as defined in the following claims. For example, any embodiment described herein can be combined with any other suitable embodiment described herein to provide additional embodiments.

What is claimed is:

1. A crystalline form of *N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide.

2. A crystalline form of (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)pyrrolidine-1-sulfonamide.

3. The crystalline form of claim 2 characterized by a powder X-ray diffraction pattern comprising one, two, three, or four peaks selected from the group consisting of 8.1 ± 0.2° 2θ, 13.9 ± 0.2° 2θ, 16.1 ± 0.2° 2θ, 16.7 ± 0.2° 2θ, and 22.6 ± 0.2 °2θ.

4. The crystalline form of claim 3, wherein the powder X-ray diffraction is carried out using Cu radiation.

5. The crystalline form of claim 3 or claim 4, wherein the powder X-ray diffraction is carried out using a Bruker D8 Advantage diffractometer operating in reflection geometry, a tube voltage of 45 kV, and filament emission of 40 mA.

6. The crystalline form of any of claims 3 to 5 further characterized by a differential scanning calorimetry curve comprising a melting endotherm having an onset temperature of about 122 °C ± 5 °C.

7. The crystalline form of any of claims 3 to 5 further characterized by a differential scanning calorimetry curve comprising a melting endotherm having a peak at about 128 °C ± 5 °C.

8. The crystalline form of any of claims 3 to 5 further characterized by a differential scanning calorimetry curve comprising a melting endotherm having an onset temperature of about 122 °C ± 5 °C and a peak at about 128 °C ± 5 °C.

9. The crystalline form of any of claims 6 to 8, wherein the differential scanning calorimetry is conducted on a TA Instruments Differential Scanning Calorimeter, model Q2000, with a sample placed in an aluminum pan and heated under nitrogen at a rate of 10 °C/minute to a temperature of 300 °C.

10. The crystalline form of any of claims 3 to 9 further characterized by a thermogravimetric analysis thermogram wherein the crystalline form exhibits a weight loss of less than about 1.0 weight % from about 25 ºC to about 100 ºC.

11. The crystalline form of any of claims 3 to 10 further characterized by a gravimetric vapor sorption plot wherein the crystalline form exhibits a reversible moisture uptake of less than about 1.0 weight % from about 0% relative humidity to about 80% relative humidity at 25 ºC ± 0.1 ºC.

12. The crystalline form of any of claims 3 to 5, wherein the crystalline form is further characterized by the following:

a differential scanning calorimetry curve comprising a melting endotherm having an onset temperature of about 122 ºC ± 5 ºC; and

a gravimetric vapor sorption plot wherein the crystalline form exhibits a reversible moisture uptake of less than about 1.0 weight % from about 0% relative humidity to about 80% relative humidity at 25 ºC ± 0.1 ºC.

13. The crystalline form of any of claims 3 to 5, wherein the crystalline form is further characterized by the following:

a thermogravimetric analysis thermogram wherein the crystalline form exhibits a weight loss of less than about 1.0 weight % from about 25 ºC to about 100 ºC; and

a gravimetric vapor sorption plot wherein the crystalline form exhibits a reversible moisture uptake of less than about 1.0 weight % from about 0% relative humidity to about 80% relative humidity at 25 ºC ± 0.1 ºC.

14. The crystalline form of any of claims 3 to 5, wherein the crystalline form is further characterized by the following:

a differential scanning calorimetry curve comprising a melting endotherm having an onset temperature of about 122 °C ± 5 °C;

a thermogravimetric analysis thermogram wherein the crystalline form exhibits a weight loss of less than about 1.0 weight % from about 25 °C to about 100 °C; and

a gravimetric vapor sorption plot wherein the crystalline form exhibits a reversible moisture uptake of less than about 1.0 weight % from about 0% relative humidity to about 80% relative humidity at 25 °C ± 0.1 °C.

15. The crystalline form of any of claims 3 to 14 further characterized by a solid-state ^{13}C NMR spectrum comprising one, two, three, or four peaks selected from the group consisting of 161.2 ±0.2 ppm, 137.6 ±0.2 ppm, 113.1 ±0.2 ppm, 72.3 ±0.2 ppm, and 60.0 ±0.2 ppm.

16. The crystalline form of any of claims 3 to 15, wherein the crystalline form is a crystalline anhydrate.

17. The crystalline form of any of claims 3 to 16, wherein the crystalline form comprises less than 5 weight % of any other crystalline form of (*S*)-*N*-ethyl-3-((9-ethyl-2-(((2*R*,3*S*)-2-hydroxypentan-3-yl)amino)-9*H*-purin-6-yl)amino)-pyrrolidine-1-sulfonamide.

18. A pharmaceutical composition comprising the crystalline form of any of claims 1 to 17, and one or more pharmaceutically acceptable excipients.

19. A method of treating or preventing a CDK2-mediated condition in a subject suffering from or susceptible to the CDK2-mediated condition, the method comprising administering to the subject a therapeutically effective amount of the crystalline form of any of claims 1 to 17.

20. The use of the crystalline form of any of claims 1 to 17 for the manufacture of a medicament for treating or preventing a CDK2-mediated condition.

21. A kit comprising a unit dosage form comprising the crystalline form of any of claims 1 to 17 contained within a packaging material, and a label or package insert which indicates that the unit dosage form can be used for treating a CDK2-mediated condition.