

**CDK2 INHIBITORS FOR TREATMENT OF CANCER****CROSS-REFERENCE TO RELATED APPLICATIONS**

**[001]** This application claims priority to and the benefit of U.S. Provisional Patent Application No. 63/561,041, filed on March 4, 2024, U.S. Provisional Patent Application No. 63/673,543, filed on July 19, 2024, and U.S. Provisional Patent Application No. 63/765,206, filed on February 28, 2025, the entire contents of each of which are incorporated herein by reference.

**SEQUENCE LISTING**

**[002]** The instant application contains a Sequence Listing which has been submitted electronically in XML format and is hereby incorporated by reference in its entirety. Said XML copy, created on March 1, 2025, is named "BPM-6053WO\_SL.xml" and is 11,924 bytes in size.

**BACKGROUND**

**[003]** Ovarian and endometrial cancers are prevalent gynecologic malignancies and a major cause of both morbidity and mortality (Siegel, R.L. *et al.*, CA Cancer J. Clin. (2023), 73(1): 17-48). Patients with advanced and aggressive high-grade serous ovarian cancer (HGSOC) and endometrial cancer (EMCA) often experience resistance to standard of care systemic therapies, leading to recurrence and poor outcomes (Bejar, F.G. *et al.*, Am. Soc. Clin. Oncol. Educ. Book. (2022), 42: 1-17; Xu, H. *et al.*, Cell. Rep. Med. (2021), 2(9): 100394). Amplification of *CCNE1*, encoding for cyclin E1, is a common oncogenic event present in several aggressive cancers, including ~20% of all ovarian cancers and ~40% of uterine carcinosarcomas (Gorski, J.W. *et al.*, Diagnostics (Basel) (2020), 10(5)). Aberrant, high expression of Cyclin E1 (CCNE1) has been associated with chemoresistance, poor prognosis, and lower disease-free survival (Fagundes, R. *et al.*, Front. Cell. Dev. Biol. (2021), 9: 774845; Gorski, J.W. (2020); Zheng, X. *et al.*, Hereditas. (2023), 160(1): 13). Novel treatments are therefore needed for these cancers to improve treatment response rate and survival.

**[004]** Cyclin E1 is an activating subunit of cyclin-dependent kinase 2 (CDK2), which together regulate the G1 to S-phase transition through phosphorylation and inactivation of retinoblastoma protein 1 (Rb) (Koff, A. *et al.*, Science (1992), 257(5077): 1689-1694). In normal mammalian cells, activation of CDK2 is regulated by intricate modulation of cyclin E levels, first by CDK4/6-cyclin D induced activation of E2F signaling, including synthesis of the cyclin E encoding genes (*CCNE1* and *CCNE2*) in G1, followed by ubiquitin-proteasome pathway-mediated degradation of cyclin E during late S phase (Fagundes, R. (2021)). CDK2 activity is also negatively regulated by the KIP/CIP proteins p21, p27, and p57 (Tadesse, S. *et al.*, Drug Discov. Today (2020) 25(2): 406-413). *CCNE1* amplification and overexpression of cyclin E1 leads to loss of controlled fluctuation of cyclin E1

levels and therefore aberrant CDK2 activity and dysregulated cell cycle progression. High levels of cyclin E1 are associated with increased DNA double-stranded breaks, genomic instability, and dysregulation of genes involved in cellular survival and proliferation (Gorski, J.W. (2020); Brown, V.E. *et al.*, NAR Cancer (2023), 5(3): zcad039). *CCNE1*-amplified cancer cell lines exhibit mutual exclusivity with BRCA mutations and insensitivity to PARP inhibitors (Etemadmoghadam, D. *et al.*, Proc. Natl. Acad. Sci. USA (2013), 10(48): 19489-19494), but are dependent on CDK2 for proliferation (Au-Yeung, G. *et al.*, Clin. Cancer Res. (2017) 23(7): 1862-1874; Etemadmoghadam, D. *et al.*, Clin. Cancer Res. (2013) 19(21): 5960-5971), and sensitive to CDK2 inhibitors (Brown, V.E. *et al.*, Presented at AACR Annual Meeting 2022, April 8–13, 2022, Poster 2306; Dietrich, C. *et al.*, Cancer Discov. (2023), PMID: 38047585). Therefore, CDK2 is an attractive target for a precision oncology approach in CDK2 vulnerable cancers and warrants further clinical development.

**[005]** Since CDKs are key regulators of the cell cycle and their activity is frequently altered in cancer cells, therapeutic strategies using selective CDK inhibitors have long been in development (Asghar, U. *et al.*, Nat. Rev. Drug Discov. (2015), 14(2): 130-146). Early efforts to target CDK family members were with non-specific and pan-CDK inhibitors. The overall lack of selectivity in these early inhibitors was problematic due to off-target activity that led to dose-limiting toxicities. Selective CDK4/6 inhibitors in combination with endocrine therapy have significantly improved metastatic breast cancer patient overall survival (Lin, M. *et al.*, J. Cancer (2020), 11(24): 7127-7136). Beyond breast cancer, CDK4/6 inhibitors have demonstrated pre-clinical and investigational activity in several cancer types, including ovarian cancer (Lim, J.S.J. *et al.*, Cancer Discov. (2016)), 6(7): 697-699; Konecny, G.E. *et al.*, Clin. Cancer Res. (2011), 17(6): 1591-1602; Colon-Otero, G., *et al.*, EMBO Open (2020), 5(5): e000926; Brown, V.E. *et al.*, (2022)). The CDK4-Rb-p16 pathway is known to predict tumor responsiveness to CDK4/6 inhibitors in breast cancer. While *RB* is critical for CDK4/6 inhibitor-mediated arrest, the loss of *RB* and high p16 expression are biomarkers for resistance to CDK4/6 inhibitors (Asghar, U. (2015); Palafox, M. *et al.*, Nat. Commun. (2022), 13: 5258). Aberrant CDK2 activity has been identified as a mechanism of resistance to CDK4/6 inhibitors in some breast cancers; therefore the use of CDK2 inhibitors has been proposed as a strategy to address CDK4/6 inhibitor resistance (Pandy, K. *et al.*, Cancers (Basel) (2020), 12(12): 3566; Al-Qasem, A.J. *et al.*, npj Precision Oncol. (2022), 6: 68; Watt, A.C. and Goel, S., Breast Cancer Res. (2022), 24, 17). Achieving molecular selectivity over CDK1 has historically been particularly challenging in the development of selective CDK2 inhibitors..

**[006]** There is a need to develop improved methods of treating endometrial cancer or ovarian cancer in a subject using amplification and/or levels of *CCNE1*, levels of *Rb*, and levels of p16 as predictive markers for response to CDK2 inhibitors and combinations with CDK2 inhibitors.

## SUMMARY

[007] In one aspect, the present disclosure provides a method of treating endometrial cancer or ovarian cancer in a subject in need thereof, wherein the subject's tumor, plasmas, or other tissue is identified as having each of the following:

- i. an elevated expression level of p16 mRNA or p16 protein as compared to a control sample;
- ii. intact Rb and no loss of function mutations in Rb as compared to a control sample; and
- iii. an increased copy number of CCNE1 gene and/or an elevated expression level of CCNE1 mRNA or CCNE1 protein as compared to a control sample,

comprising administering to the subject a therapeutically effective amount of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, thereby treating the endometrial cancer or ovarian cancer.

[008] In another aspect, the present disclosure provides a method of treating endometrial cancer or ovarian cancer in a subject identified as having all of the following:

- i. an elevated expression level of p16 mRNA or p16 protein;
- ii. intact Rb, which is based on expression level of Rb mRNA or Rb protein, and no loss of function mutations in Rb; and
- iii. an increased copy number of CCNE1 gene and/or an elevated expression level of CCNE1 mRNA or CCNE1 protein

in the subject's cancer, wherein the levels of p16 and Rb are compared to the average mRNA or protein levels of p16 and mRNA or protein levels of Rb in a population of subjects suffering from endometrial cancer or ovarian cancer; and the copy number of CCNE1 is greater than 2 and/or the expression levels of CCNE1 mRNA or protein is elevated as compared to the average mRNA or protein levels of CCNE1 in a population of subjects suffering from endometrial cancer or ovarian cancer, and wherein the subject is in need thereof, the method comprising administering to the subject a therapeutically effective amount of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, thereby treating the endometrial cancer or ovarian cancer.

[009] In another aspect, the present disclosure provides a method of treating endometrial cancer or ovarian cancer in a subject in need thereof comprising:

- i. testing, or having tested, a first biological sample obtained from the subject with endometrial cancer or ovarian cancer, thereby measuring
  - a. levels of p16;
  - b. levels of Rb; and
  - c. amplification and/or levels of CCNE1,

in the subject's cancer;

- ii. comparing the levels of p16 mRNA or protein, the levels of Rb mRNA or protein, and the amplification and/or levels of CCNE1 mRNA or protein, in step i. to the average levels of

p16 mRNA or protein, levels of Rb mRNA or protein, and amplification and/or levels of CCNE1 mRNA or protein in a population of subjects suffering from endometrial cancer or ovarian cancer;

- iii. determining that the subject's cancer is characterized by all of the following:
  - a. an elevated expression level of p16 mRNA or p16 protein as compared to an average level of p16 in a population of subjects suffering from endometrial cancer or ovarian cancer;
  - b. intact Rb, which is based on expression level of Rb mRNA or Rb protein as compared to an average level of Rb in a population of subjects suffering from endometrial cancer or ovarian cancer, and no loss of function mutations in Rb; and
  - c. an increased copy number of CCNE1 gene, which is greater than 2, and/or an elevated expression level of CCNE1 mRNA or CCNE1 protein as compared to an average level of CCNE1 in a population of subjects suffering from endometrial cancer or ovarian cancer; and
- iv. administering to the subject a therapeutically effective amount of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, thereby treating the endometrial cancer or ovarian cancer.

**[0010]** In another aspect, the present disclosure provides a method of treating endometrial cancer or ovarian cancer in a subject in need thereof, wherein the subject's tumor, plasmas, or other tissue is identified as having each of the following:

- i. low or no p16 mRNA or protein expression, dysfunctional p16, or mutated p16 as compared to a control sample;
- ii. intact Rb and no loss of function mutations in Rb as compared to a control sample; and
- iii. an increased copy number of CCNE1 gene and/or an elevated expression level of CCNE1 mRNA or CCNE1 protein as compared to a control sample, and

comprising administering to the subject a therapeutically effective amount of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, and a therapeutically effective amount of a CDK4/6 inhibitor, or a pharmaceutically acceptable salt thereof, thereby treating the endometrial cancer or ovarian cancer.

**[0011]** In another aspect, the present disclosure provides a method of treating endometrial cancer or ovarian cancer in a subject identified as having all of the following:

- i. low or no p16 mRNA or protein expression, dysfunctional p16, or mutated p16;
- ii. intact Rb, which is based on expression level of Rb mRNA or Rb protein expression, and no loss of function mutations in Rb; and
- iii. an increased copy number of CCNE1 gene and/or an elevated expression level of CCNE1 mRNA or CCNE1 protein

in the subject's cancer, wherein the levels of p16 and Rb are compared to the average mRNA or protein levels of p16 and mRNA or protein levels of Rb in a population of subjects suffering from endometrial cancer or ovarian cancer, and the copy number of CCNE1 is greater than 2 and/or the expression levels of CCNE1 mRNA or protein is elevated as compared to the average mRNA or protein levels of CCNE1 in a population of subjects suffering from endometrial cancer or ovarian cancer, and wherein the subject is in need thereof, comprising administering to the subject a therapeutically effective amount of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, and a therapeutically effective amount of a CDK4/6 inhibitor, or a pharmaceutically acceptable salt thereof, thereby treating the endometrial cancer or ovarian cancer.

[0012] In another aspect, the present disclosure provides a method of treating endometrial cancer or ovarian cancer in a subject in need thereof comprising:

- i. testing, or having tested, a first biological sample obtained from the subject with endometrial cancer or ovarian cancer, thereby measuring
  - a. levels of p16;
  - b. levels of Rb; and
  - c. amplification and/or levels of CCNE1,in the subject's cancer;
- ii. comparing the levels of p16 mRNA or protein, the levels of Rb mRNA or protein, and the amplification and/or levels of CCNE1 mRNA or protein, in step i. to the average mRNA or protein levels of p16, mRNA or protein levels of Rb, and amplification and/or mRNA or protein levels of CCNE1 in a population of subjects suffering from endometrial cancer or ovarian cancer;
- iii. determining that the subject's cancer is characterized by all of the following:
  - a. low or no p16 mRNA or protein expression, dysfunctional p16, or mutated p16 as compared to an average level of p16, functional p16, or wild type p16 in a population of subjects suffering from endometrial cancer or ovarian cancer;
  - b. intact Rb, which is based on expression level of Rb mRNA or Rb protein as compared to an average level of Rb in a population of subjects suffering from endometrial cancer or ovarian cancer, and no loss of function mutations in Rb; and
  - c. an increased copy number of CCNE1 gene, which is greater than 2, and/or an elevated expression level of CCNE1 mRNA or CCNE1 protein as compared to an average level of CCNE1 in a population of subjects suffering from endometrial cancer or ovarian cancer; and
- iv. administering to the subject a therapeutically effective amount of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, and a therapeutically effective amount of a

CDK4/6 inhibitor, or a pharmaceutically acceptable salt thereof, thereby treating the endometrial cancer or ovarian cancer.

#### BRIEF DESCRIPTION OF THE FIGURES

**[0013]** **Figure 1** is a graph showing CDK2 cellular potency measured by inhibition of pRbT821/826 by AlphaLISA after 18h dosing in OVCAR-3, a CDK2-sensitive, *CCNE1* amplified ovarian cell line. pLamin S22 inhibition was used as a cellular readout of CDK1 inhibition by AlphaLISA after 3h dosing .

**[0014]** **Figure 2A** shows OVCAR3, FU-OV-1, COV318, and KLE (*CCNE1* amplified (CN  $\geq 6$ , mRNA high)) cell lines treated with BLU-222. Cell confluency was measured by IncuCyte. **Figure 2B** shows ES-2, TOV-21G, MES-SA, and HEC-1-B (*CCNE1* normal (CN  $< 3$ , mRNA normal)) cell lines treated with BLU-222. Cell confluency was measured by IncuCyte. **Figure 2C** shows the antiproliferative effect of BLU-222 GI<sub>50</sub> values measured by CyQuant (5d) across a panel of ovarian and uterine cell lines, grouped by cell CCNE1 copy number. **Figure 2D** shows the antiproliferative effect of BLU-222 GI<sub>50</sub> values measured by CyQuant (5d) across a panel of ovarian and uterine cell lines, grouped by cell CCNE1 mRNA expression level. “Overexpressed” was defined as *CCNE1* mRNA levels in the top quartile across all cell lines with transcriptome data in CCLE. **Figure 2E** shows the mean tumor volume over time in OVCAR-3 T2A model (*CCNE1*-amplified, Rb-intact, p16-high expressor) in NOD-SCID female mice treated with vehicle or varying doses of BLU-222 (as shown). **Figure 2F** shows the mean tumor volume over time in ES-2 model (*CCNE1* normal) xenograft in BALB/c nude female mice treated with vehicle or varying doses of BLU-222 (as shown). **Figure 2G** shows cell cycle profile of OVCAR-3, FU-OV-1, COV318, and KLE cell lines (*CCNE1*-amplified, BLU-222 responder cell lines) treated with BLU-222. Cells were treated with a dose titration of BLU-222 for 24 hours and cell cycle profile determined by Click-iT™ EdU Alexa Fluor™ Flow Cytometry Assay Kit. Error bars represent SEM of at least 2 biological replicates. EdU, 5-ethynyl-2'-deoxyuridine; SEM, standard error of the mean. **Figure 2H** shows cell cycle profile of ES-2, TOV-21G, MES-SA, and HEC-1-B cell lines (*CCNE1*-normal, BLU-222 nonresponder cell lines) treated with BLU-222. Cells were treated with a dose titration of BLU-222 for 24 hours and cell cycle profile determined by Click-iT™ EdU Alexa Fluor™ Flow Cytometry Assay Kit. Error bars represent SEM of at least 2 biological replicates. EdU, 5-ethynyl-2'-deoxyuridine; SEM, standard error of the mean.

**[0015]** **Figure 3A** shows the level of pRbS807/811 over time normalized to  $\beta$ -actin in OVCAR-3 T2A model xenograft bearing NOD-SCID female mice treated with varying doses of BLU-222 (as shown) as percent of vehicle. **Figure 3B** shows the BLU-222 plasma pharmacokinetics over time in the treated groups. **Figure 3C** shows the level of pRbS807/811 over time normalized to  $\beta$ -actin in ES-2 model xenograft bearing Balb/c nude female mice treated with varying doses of BLU-222 (as

shown) as percent of vehicle. **Figure 3D** shows the BLU-222 plasma pharmacokinetics over time in the treated groups.

**[0016]** **Figure 4** shows the effect of BLU-222 at varying concentrations (as shown) on the cell cycle profiling of OVCAR3, FUOV1, COV318, and KLE (*CCNE1*-amplified, CN  $\geq 6$ ) and ES-2, TOV21G, MESSA, and HEC-1B (CN  $< 3$ , RNA not overexpressed) cell lines, as determined by flow cytometry.

**[0017]** **Figure 5** shows cell cycle profiling in *CCNE1* overexpressing but copy number non-amplified ( $< 6$ ) cell lines: of CaOV3, OVMANA, and OAW42 (BLU-222 responder) and A2780, COV434, and SKOV3 (BLU-222 non-responder) cell lines treated with BLU-222 dose titration.

**[0018]** **Figure 6A** is a waterfall plot of gene dependency Z-scores arising from CRISPR whole-genome library deletion screen in BLU-222-treated OVCAR-3. Inactivation of *RBI* and *CDKN2A* were identified as the top markers for loss of BLU-222 sensitivity. CDKN2A, cyclin-dependent kinase inhibitor 2A; CRISPR, clustered regularly interspaced short palindromic repeats. **Figure 6B** shows BLU-222 GI<sub>50</sub> values across a panel of ovarian and uterine cell lines, grouped by the presence of multivariate biomarker signature [Rb intact/not intact; p16 high/low] to demonstrate an enhancement of response within biomarker signature group. CN, copy number; GI<sub>50</sub>, concentration for 50% of maximal inhibition of cell proliferation. **Figure 6C** shows BLU-222 GI<sub>50</sub> values across a panel of high *CCNE1* ovarian and uterine cell lines, grouped by the presence of multivariate biomarker signature [Rb intact/not intact; p16 high/low] to demonstrate an enhancement of response within biomarker signature group. CN, copy number; GI<sub>50</sub>, concentration for 50% of maximal inhibition of cell proliferation. **Figure 6D** shows BLU-222 GI<sub>50</sub> values across a panel of ovarian and uterine cell lines, grouped by the presence of multivariate biomarker signature [CyclinE1-High, Rb-Intact, p16-High] to demonstrate an enhancement of response within biomarker signature group. CN, copy number; OE, overexpression; GI<sub>50</sub>, concentration for 50% of maximal inhibition of cell proliferation. **Figure 6E** is a histogram showing the levels of Cyclin E1, p16, and Rb in ST3052, ST2526, ST1386, ST259, and ST189 PDX models. **Figure 6F** shows the tumor volume over time in ST3052 PDX model treated with vehicle or BLU-222 60 mpk. **Figure 6G** shows the tumor volume over time in ST2526 PDX model treated with vehicle or BLU-222 60 mpk. **Figure 6H** shows the tumor volume over time in ST1386 PDX model treated with vehicle, BLU-222 30 mpk, 60 mpk, and 100 mpk BID. **Figure 6I** shows the tumor volume over time in ST259 PDX model treated with vehicle or BLU-222 60 mpk. **Figure 6J** shows the tumor volume over time in ST189 PDX model treated with vehicle or BLU-222 60 mpk. **Figure 6K** shows the dependency of BLU-222 tumor growth inhibition in endometrial PDX models categorized by *CCNE1*, Rb, and p16 status based on protein expression quantification.

**[0019]** **Figure 7A** is a graph showing the dependency of BLU-222 sensitivity in OVCAR-3 cells on *RBI* expression. **Figure 7B** is a graph showing the dependency of BLU-222 sensitivity in

OVCAR-3 cells on CDKN2A expression. **Figure 7C** shows quantification of proteins normalized to beta-actin signal in OVCAR-3 sgNTC and sgCDKN2A cell lines. **Figure 7D** shows CDK2 dependency in CCLE lines from DepMap RNAi. Cell lines are grouped by *CCNE1*, Rb, and p16 status. **Figure 7E** shows CDK2 dependency in CCLE lines from CRISPR datasets.

[0020] **Figure 8A** shows a weak correlation between CDKN2A mRNA expression and CDKN2A protein abundance signal. **Figure 8B** shows a weak correlation between RB1 mRNA expression and RB1 protein abundance signal.

[0021] **Figure 9A** shows a loss of sensitivity to BLU-222 in OVCAR-3 cells with CDKN2A deletion. **Figure 9B** shows an increase in sensitivity to ribociclib in OVCAR-3 cells with CDKN2A deletion. **Figure 9C** shows that no additional anti-proliferative effect is conferred when ribociclib is added to BLU-222 in OVCAR-3 cell lines without CDKN2A deletion. **Figure 9D** shows restoration of sensitivity to BLU-222 + ribociclib combination in OVCAR-3 cells with CDKN2A-1 deletion. **Figure 9E** shows restoration of sensitivity to BLU-222 + ribociclib combination in OVCAR-3 cells with CDKN2A-2 deletion. **Figure 9F** shows the effect on cell cycle distribution of BLU-222 (250 nM), ribociclib (3  $\mu$ M), or both in CDKN2A1 and CDKN2A2 knockout OVCAR-3 cells compared to OVCAR-3 cells without knockout. **Figure 9G** shows the synergy score of BLU-222+ribociclib in a panel of ovarian and uterine cancer cell lines, grouped by *CCNE1* expression level. **Figure 9H** shows the synergy score of BLU-222+ribociclib in a panel of ovarian and uterine cancer cell lines, grouped by Rb and p16 expression levels. **Figure 9I** shows the synergy score of BLU-222+ribociclib in a panel of high *CCNE1* ovarian and uterine cancer cell lines, grouped by Rb and p16 expression levels. **Figure 9J** shows the antiproliferative effect of BLU-222 and ribociclib, alone and in combination, in OVK18 (cyclin E1 high, Rb intact, p16 low ovarian cells) cells as measured by CyQuant after 5 days of treatment. **Figure 9K** shows the antiproliferative effect of BLU-222 and ribociclib, alone and in combination, in OVCAR-3 (cyclin E1 high (*CCNE1* amplified), Rb intact, p16 high ovarian cells). **Figure 9L** shows the antiproliferative effect of BLU-222 and ribociclib, alone and in combination, in MFE-296. **Figure 9M** shows the tumor volume over time in OVK18 tumor model treated with BLU-222, ribociclib, or BLU-222+ribociclib. **Figure 9N** shows the tumor volume over time in OVCAR-3 T2A tumor model treated with BLU-222, ribociclib, or BLU-222+ribociclib. **Figure 9O** shows the tumor volume over time in MFE-296 tumor model treated with BLU-222, ribociclib, or BLU-222+ribociclib.

[0022] **Figure 10A** shows drug synergy analysis (SynergyFinder) of BLU-222 and ribociclib in uterine cell lines with high and low levels of cyclin E1 and p16. **Figure 10B** shows synergy plots and proliferation curves for BLU-222 and + ribociclib treatment in cyclin E1-high and Rb intact cell lines. Cyclin E1, Rb, and p16 status is noted.

[0023] **Figure 11A** shows the BLU-222 and ribociclib plasma concentrations measured at the indicated times post-final dose in OVK18 tumor bearing CB17 SCID female mice administered BLU-

222, ribociclib, or BLU-222+ribociclib. **Figure 11B** shows the BLU-222 and ribociclib plasma concentrations measured at the indicated times post-final dose in MFE-296 tumor bearing BALB/c nude female mice administered BLU-222, ribociclib, or BLU-222+ribociclib. **Figure 11C** shows the BLU-222 and ribociclib plasma concentrations measured at the indicated times post-final dose in OVCAR-3 T2A tumor bearing BALB/c nude female mice administered BLU-222, ribociclib, or BLU-222+ribociclib.

[0024] **Figure 12A** shows the *in vivo* tumor growth kinetics in *CCNE1*-amplified OVCAR-3 T2A xenograft in NOD-SCID female mice treated with BLU-222, paclitaxel, carboplatin, and combinations thereof (as shown). **Figure 12B** shows the *in vivo* tumor growth kinetics in ES-2 *CCNE1*-normal xenograft in BALB/c nude female mice treated with BLU-222, paclitaxel, carboplatin, and combinations thereof (as shown). **Figure 12C** shows the *in vivo* tumor growth kinetics in ST3052 tumor model treated with BLU-222, paclitaxel, and the combination thereof (as shown). **Figure 12D** shows the *in vivo* tumor growth kinetics in ST2526 tumor model treated with BLU-222, paclitaxel, and the combination thereof (as shown). **Figure 12E** shows *in vivo* tumor growth kinetics in ST1386 tumor model treated with BLU-222, paclitaxel, and the combination thereof (as shown). **Figure 12F** shows the *in vivo* tumor growth kinetics in ST259 tumor model treated with BLU-222, paclitaxel, and the combination thereof (as shown). **Figure 12G** shows the *in vivo* tumor growth kinetics in ST189 tumor model treated with BLU-222, paclitaxel, and the combination thereof (as shown). **Figure 12H** shows strong responders to BLU-222 monotherapy highlighted in blue (proliferative  $GI_{50} \leq 200$  nM) and BLU-222 non-responders (proliferative  $GI_{50} > 200$  nM) with high synergistic potential (ZIP most synergistic area score  $\geq 10$ ) highlighted in red.

[0025] **Figure 13A** is a histogram showing the levels of Cyclin E1, p16, and Rb in ST270 and ST182B PDX models treated with BLU-222 and carboplatin. **Figure 13B** shows the tumor volume over time in ST270 and ST182 PDX models treated with BLU-222, carboplatin, and BLU-222+carboplatin. **Figure 13C** shows the tumor volume over time in OVCAR-3 T2A tumor model treated with BLU-222, gemcitabine, and BLU-222+gemcitabine.

[0026] **Figure 14** shows BLU-222 in combination with chemotherapeutic agents in cell lines. ZIP most synergistic area scores categorized by cell line cyclin E1 and p16 status.

[0027] **Figure 15A** shows the BLU-222 plasma concentration in ST3052 tumor model administered BLU-222 or BLU-222+paclitaxel. **Figure 15B** shows the BLU-222 plasma concentration in ST2526 tumor model administered BLU-222 or BLU-222+paclitaxel. **Figure 15C** shows the BLU-222 plasma concentration in ST1386 tumor model administered BLU-222 or BLU-222+paclitaxel. **Figure 15D** shows the BLU-222 plasma concentration in ST259 tumor model administered BLU-222 or BLU-222+paclitaxel. **Figure 15E** shows the BLU-222 plasma concentration in ST189 tumor model administered BLU-222 or BLU-222+paclitaxel. **Figure 15F** shows phosphorylated Rb (pRb)/total Rb signal normalized to vehicle (AlphaLISA) graphed versus

tumor growth inhibition (TGI) of BLU-222 monotherapy in endometrial PDX models. BLU-222 monotherapy tumor growth inhibition (TGI) correlates with pRbS807/811. **Figure 15G** shows the BLU-222 plasma concentration in ST182B tumor model administered BLU-222 or BLU-222+carboplatin. **Figure 15H** shows the BLU-222 plasma concentration in ST270 tumor model administered BLU-222 or BLU-222+carboplatin. **Figure 15I** shows phosphorylated Rb (pRb)/total Rb signal normalized to vehicle (AlphaLISA) graphed versus tumor growth inhibition (TGI) of BLU-222 monotherapy in ovarian PDX models.

[0028] **Figure 16A** is a diagram showing cell cycle promotion by CDK4/6 and CDK2. **Figure 16B** is a diagram showing cell cycle dependence on CDK2 when CDK4/6 is inactive/inhibited.

[0029] **Figure 17A** shows the antiproliferative effect of Compound 2 GI<sub>50</sub> values measured by CyQuant (5d) across a panel of ovarian and uterine cell lines, grouped by cell CCNE1 copy number. **Figure 17B** shows the antiproliferative effect of Compound 3 GI<sub>50</sub> values measured by CyQuant (5d) across a panel of ovarian and uterine cell lines, grouped by cell CCNE1 copy number. **Figure 17C** shows the antiproliferative effect of Compound 2 GI<sub>50</sub> values measured by CyQuant (5d) across a panel of ovarian and uterine cell lines, grouped by cell CCNE1 mRNA expression level. **Figure 17D** shows the antiproliferative effect of Compound 3 GI<sub>50</sub> values measured by CyQuant (5d) across a panel of ovarian and uterine cell lines, grouped by cell CCNE1 mRNA expression level.

“Overexpressed” was defined as *CCNE1* mRNA levels in the top quartile across all cell lines with transcriptome data in CCLE.

[0030] **Figure 18A** shows the mean tumor volume over time in OVCAR-3 T2A model (*CCNE1*-amplified, Rb-intact, p16-high expressor) in NOD-SCID female mice treated with vehicle or varying doses of Compound 3 (as shown). **Figure 18B** shows the mean tumor volume over time in OVCAR-3 T2A model (*CCNE1*-amplified, Rb-intact, p16-high expressor) in NOD-SCID female mice treated with vehicle or varying doses of Compound 4 (as shown).

[0031] **Figure 19A** shows a loss of sensitivity to BLU-222, Compound 2, and Compound 3 in OVCAR-3 cells with RB1 deletion. Proliferation measured by CyQuant (5d). **Figure 19B** shows a loss of sensitivity to BLU-222, Compound 2, and Compound 3 in OVCAR-3 cells with CDKN2A deletion. Proliferation measured by CyQuant (5d treatment).

[0032] **Figure 20** shows a loss of sensitivity to BLU-222 in OVCAR-3 cells with RB1 or CDKN2A deletion KLE, a *CCNE1* amplified uterine cancer cell line. Proliferation measured by CyQuant (5d treatment).

[0033] **Figure 21A** shows Compound 2 GI<sub>50</sub> values measured by CyQuant (5d) across a panel of ovarian and uterine cell lines, grouped by the presence of multivariate biomarker signature [Rb intact/not intact; p16 high/low] to demonstrate an enhancement of response within biomarker signature group. **Figure 21B** shows Compound 3 GI<sub>50</sub> values across a panel of ovarian and uterine

cell lines, grouped by the presence of multivariate biomarker signature [Rb intact/not intact; p16 high/low] to demonstrate an enhancement of response within biomarker signature group.

**[0034]** **Figure 22A** shows Compound 2 GI<sub>50</sub> values measured by CyQuant (5d) across a panel of high CCNE1 ovarian and uterine cell lines, grouped by the presence of multivariate biomarker signature [Rb intact/not intact; p16 high/low] to demonstrate an enhancement of response within biomarker signature group. **Figure 22B** shows Compound 3 GI<sub>50</sub> values measured by CyQuant (5d) across a panel of high CCNE1 ovarian and uterine cell lines, grouped by the presence of multivariate biomarker signature [Rb intact/not intact; p16 high/low] to demonstrate an enhancement of response within biomarker signature group.

**[0035]** **Figure 23A** shows Compound 2 GI<sub>50</sub> values measured by CyQuant (5d) across a panel of ovarian and uterine cell lines, grouped by the presence of multivariate biomarker signature [CyclinE1-High, Rb-Intact, p16-High] to demonstrate an enhancement of antiproliferative response within biomarker signature group. **Figure 23B** shows Compound 3 GI<sub>50</sub> values measured by CyQuant (5d) across a panel of ovarian and uterine cell lines, grouped by the presence of multivariate biomarker signature [CyclinE1-High, Rb-Intact, p16-High] to demonstrate an enhancement of antiproliferative response within biomarker signature group.

**[0036]** **Figure 24A** shows BLU-222 and Compound 3 proliferation IC<sub>50</sub> measured by CyQuant (5d) in OV-90 (ovarian cancer cell line, CCNE1 overexpressing) with or without CDKN2A overexpression to demonstrate that CDKN2A overexpression sensitizes OV-90 to CDK2 inhibitors. **Figure 24B** shows BLU-222 and Compound 3 proliferation IC<sub>50</sub> measured by CyQuant (5d) in AN3CA (uterine cancer cell line, CCNE1 overexpressing) with or without CDKN2A overexpression to demonstrate that CDKN2A overexpression sensitizes AN3CA to CDK2 inhibitors.

#### DETAILED DESCRIPTION

##### *Methods of Treatment*

**[0037]** The disclosure provides predictive markers (e.g., biomarkers and pharmacodynamic markers, e.g., levels, gene copy number, gene sequence, expression levels, phosphorylation levels, or mutations) to identify those human subjects having, suspected of having, or at risk of developing endometrial cancer or ovarian cancer for response to CDK2 inhibitors and combinations with CDK2 inhibitors. The disclosure provides improved methods of treating endometrial cancer or ovarian cancer in a subject using levels of combinations of biomarkers as predictive markers for response to CDK2 inhibitors and combinations with CDK2 inhibitors.

**[0038]** Levels of p16, levels of Rb, and amplification and/or levels of CCNE1 are predictive markers for response to CDK2 inhibitors. In one aspect, expression levels of p16, intact Rb (based on expression levels of Rb), and amplified and/or elevated expression levels of CCNE1, were predictive

markers for response to CDK2 inhibitors. In some embodiments, intact Rb means detectable Rb protein expression and no loss-of-function mutation.

**[0039]** In some embodiments, a comparison of the amplification and/or levels of certain biomarkers is made between a biological sample (*e.g.*, the subject's tumor, plasmas, or other tissue) and a control sample. In some embodiments, the control sample comprises normal endometrial tissue or normal ovarian tissue. In some embodiments, the control sample is from a subject with a normally functioning pathway. In some embodiments, the control sample provides or a comparison is made to an average expression level (*e.g.*, mRNA or protein) of certain biomarkers in a population of subjects suffering from a solid tumor. In some embodiments, the control sample provides or a comparison is made to an average expression level (*e.g.*, mRNA or protein) of certain biomarkers in a population of subjects suffering from endometrial cancer or ovarian cancer.

**[0040]** In one aspect, the present disclosure provides a method of treating endometrial cancer or ovarian cancer in a subject in need thereof, wherein the subject's tumor, plasmas, or other tissue is identified as having each of the following:

- i. an elevated expression level of p16 mRNA or p16 protein as compared to a control sample;
- ii. intact Rb and no loss of function mutations in Rb as compared to a control sample; and
- iii. an increased copy number of CCNE1 gene and/or an elevated expression level of CCNE1 mRNA or CCNE1 protein in the cancer as compared to a control sample,

comprising administering to the subject a therapeutically effective amount of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, thereby treating the endometrial cancer or ovarian cancer.

**[0041]** In some embodiments, the subject's tumor, plasmas, or other tissue is identified as having an increased copy number of CCNE1 gene in the cancer as compared to a control sample. In some embodiments, the subject's tumor, plasmas, or other tissue is identified as having an elevated expression level of CCNE1 mRNA or CCNE1 protein in the cancer as compared to a control sample.

**[0042]** In some embodiments, the present disclosure provides a method of treating endometrial cancer or ovarian cancer in a subject in need thereof, wherein the subject's tumor, plasmas, or other tissue is identified as having each of the following:

- i. an elevated expression level of p16 mRNA or p16 protein as compared to a control sample;
- ii. intact Rb and no loss of function mutations in Rb as compared to a control sample; and
- iii. an elevated expression level of CCNE1 mRNA or CCNE1 protein in the cancer as compared to a control sample,

comprising administering to the subject a therapeutically effective amount of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, thereby treating the endometrial cancer or ovarian cancer.

[0043] In some embodiments, *CCNE1* is normal as compared to a control sample. In some embodiments, *CCNE1* is normal and has a diploid *CCNE1* copy number. In some embodiments, the copy number of *CCNE1* is diploid. In some embodiments, the copy number of *CCNE1* is less than 3. In some embodiments, the copy number of *CCNE1* is less than 3 and/or there is a normal expression level of *CCNE1* mRNA or *CCNE1* protein in the cancer as compared to a control sample.

[0044] In some embodiments, *CCNE1* is amplified as compared to a control sample. In some embodiments, there is an elevated expression level of *CCNE1* mRNA or *CCNE1* protein in the cancer as compared to a control sample. In some embodiments, the copy number of *CCNE1* is greater than 2 and/or there is an elevated expression level of *CCNE1* mRNA or *CCNE1* protein in the cancer as compared to a control sample. In some embodiments, the copy number of *CCNE1* is 3 to 5 and/or there is an elevated expression level of *CCNE1* mRNA or *CCNE1* protein in the cancer as compared to a control sample. In some embodiments, the copy number of *CCNE1* is greater than or equal to 6 and/or there is an elevated expression level of *CCNE1* mRNA or *CCNE1* protein in the cancer as compared to a control sample.

[0045] In another aspect, the present disclosure provides a method of treating endometrial cancer or ovarian cancer in a subject identified as having all of the following:

- i. an elevated expression level of p16 mRNA or p16 protein;
- ii. intact Rb, which is based on expression level of Rb mRNA or Rb protein, and no loss of function mutations in Rb; and
- iii. an increased copy number of *CCNE1* gene and/or an elevated expression level of *CCNE1* mRNA or *CCNE1* protein

in the subject's cancer, wherein the levels of p16 and Rb are compared to the average mRNA or protein levels of p16 and mRNA or protein levels of Rb in a population of subjects suffering from endometrial cancer or ovarian cancer; and the copy number of *CCNE1* is greater than 2 and/or the expression levels of *CCNE1* mRNA or protein is elevated as compared to the average mRNA or protein levels of *CCNE1* in a population of subjects suffering from endometrial cancer or ovarian cancer, and wherein the subject is in need thereof, the method comprising administering to the subject a therapeutically effective amount of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, thereby treating the endometrial cancer or ovarian cancer.

[0046] In another aspect, the present disclosure provides a method of treating endometrial cancer or ovarian cancer in a subject in need thereof comprising:

- i. testing, or having tested, a first biological sample obtained from the subject with endometrial cancer or ovarian cancer, thereby measuring
  - a. levels of p16;
  - b. levels of Rb; and
  - c. amplification and/or levels of *CCNE1*,

in the subject's cancer;

- ii. comparing the levels of p16 mRNA or protein, the levels of Rb mRNA or protein, and the amplification and/or levels of CCNE1 mRNA or protein, in step i. to the average mRNA or protein levels of p16, mRNA or protein levels of Rb, and amplification and/or mRNA or protein levels of CCNE1 in a population of subjects suffering from endometrial cancer or ovarian cancer;
- iii. determining that the subject's cancer is characterized by all of the following:
  - a. an elevated expression level of p16 mRNA or p16 protein as compared to an average level of p16 in a population of subjects suffering from endometrial cancer or ovarian cancer;
  - b. intact Rb, which is based on expression level of Rb mRNA or Rb protein as compared to an average level of Rb in a population of subjects suffering from endometrial cancer or ovarian cancer, and no loss of function mutations in Rb; and
  - c. an increased copy number of CCNE1 gene, which is greater than 2, and/or an elevated expression level of CCNE1 mRNA or CCNE1 protein as compared to an average level of CCNE1 in a population of subjects suffering from endometrial cancer or ovarian cancer; and
- iv. administering to the subject a therapeutically effective amount of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, thereby treating the endometrial cancer or ovarian cancer.

**[0047]** In some embodiments, the methods disclosed herein further comprise testing, or having tested, a first biological sample obtained from the subject with endometrial cancer or ovarian cancer, thereby measuring the levels of phosphorylated Rb protein, the levels of nucleophosmin 1 (NPM1) protein, the levels of phosphorylated NPM1 protein, and the levels of thymidine kinase (TK1) in addition to the levels of p16 protein, the levels of Rb protein, the levels of CCNE1, and the amplification of CCNE1.

**[0048]** In some embodiments, the methods disclosed herein further comprise testing, or having tested, a second biological sample obtained from the subject with endometrial cancer or ovarian cancer, thereby determining whether the levels of p16 protein, the levels of Rb protein, the levels of phosphorylated Rb protein, the levels of CCNE1, the amplification of CCNE1, the levels of NPM1 protein, the levels of phosphorylated NPM1 protein, and the levels of TK1 change after administering a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, thereby monitoring a response in the subject.

**[0049]** In some embodiments, provided herein is a method of monitoring a response in a subject having or at risk of developing endometrial cancer or ovarian cancer comprising:

- i. testing, or having tested, a first biological sample obtained from the subject having or at risk of developing endometrial cancer or ovarian cancer, thereby measuring
  - a. levels of p16;
  - b. levels of Rb and/or levels of phosphorylated Rb protein;
  - c. amplification and/or levels of CCNE1;
  - d. levels of NPM1 and/or the levels of phosphorylated NPM1 protein;
  - e. levels of TK1,in the subject's biological sample;
- ii. comparing the levels of p16 mRNA or protein, levels of Rb mRNA or protein, levels of phosphorylated Rb protein, amplification and/or levels of CCNE1 mRNA or protein, levels of NPM1 mRNA or protein, levels of phosphorylated NPM1 protein, levels of TK1 mRNA or protein, and levels of TK1 protein activity in step i. to the average levels of p16 mRNA or protein, levels of Rb mRNA or protein, levels of phosphorylated Rb protein, amplification and/or levels of CCNE1 mRNA or protein, levels of NPM1 mRNA or protein, levels of phosphorylated NPM1 protein, levels of TK1 mRNA or protein, and levels of TK1 protein activity in a population of subjects suffering from endometrial cancer or ovarian cancer;
- iii. determining that the subject's biological sample is characterized by one or more of the following:
  - a. a changed expression level of p16 mRNA or protein;
  - b. a changed expression level of Rb mRNA or protein or levels of phosphorylated Rb protein;
  - c. a changed expression level of CCNE1 mRNA or protein or a change in the copy number of CCNE1;
  - d. a changed expression level of NPM1 mRNA or protein or levels of phosphorylated NPM1 protein; and
  - e. a changed expression level of TK1 mRNA or protein or a changed level of TK1 protein activity,
- iv. administering to the subject in need thereof a therapeutically effective amount of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof;
- v. testing, or having tested, a second biological sample obtained from the subject, thereby measuring
  - a. levels of p16;
  - b. levels of Rb and/or levels of phosphorylated Rb protein;
  - c. amplification and/or levels of CCNE1;
  - d. levels of NPM1 and/or levels of phosphorylated NPM1 protein;

- e. levels of TK1,  
in the subject's biological sample;
- vi. determining whether the subject having or at risk of developing endometrial cancer or ovarian cancer has responded to treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, if the comparison of the second biological sample analysis in step v. to the first biological sample analysis in step i. shows one or more of the following:
  - a. a changed expression level of p16 mRNA or protein;
  - b. a changed expression level of Rb mRNA or protein or levels of phosphorylated Rb protein;
  - c. a changed expression level of CCNE1 mRNA or protein or a change in copy number of CCNE1;
  - d. a changed expression level of NPM1 mRNA or protein or levels of phosphorylated NPM1 protein; and
  - e. a changed expression level of TK1 mRNA or protein or a changed level of TK1 protein activity.

**[0050]** In some embodiments, the method of monitoring a response in a subject having or at risk of developing endometrial cancer or ovarian cancer comprising:

- i. testing, or having tested, a first biological sample obtained from the subject having or at risk of developing endometrial cancer or ovarian cancer, thereby measuring
  - a. levels of phosphorylated Rb protein;
  - b. levels of phosphorylated NPM1 protein; and
  - c. levels of TK1,  
in the subject's biological sample;
- ii. comparing the levels of phosphorylated Rb protein, levels of phosphorylated NPM1 protein, levels of TK1 protein, and levels of TK1 protein activity in step i. to the average levels of phosphorylated Rb protein, levels of phosphorylated NPM1 protein, levels of TK1 protein, and levels of TK1 protein activity in a population of subjects suffering from endometrial cancer or ovarian cancer;
- iii. determining that the subject's biological sample is characterized by one or more of the following:
  - a. a changed level of phosphorylated Rb protein;
  - b. a changed level of phosphorylated NPM1 protein;
  - c. a changed expression level of TK1 protein; and
  - d. a changed level of TK1 protein activity;
- iv. administering to the subject in need thereof a therapeutically effective amount of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof;

- v. testing, or having tested, a second biological sample obtained from the subject, thereby measuring
  - a. levels of phosphorylated Rb protein;
  - b. levels of phosphorylated NPM1 protein; and
  - c. levels of TK1,in the subject's biological sample;
- vi. determining whether the subject having or at risk of developing endometrial cancer or ovarian cancer has responded to treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, if the comparison of the second biological sample analysis in step v. to the first biological sample analysis in step i. shows one or more of the following:
  - a. a changed level of phosphorylated Rb protein;
  - b. a changed level of phosphorylated NPM1 protein;
  - c. a changed expression level of TK1 protein; and
  - d. a changed level of TK1 protein activity.

**[0051]** In some embodiments, the method of treating endometrial cancer or ovarian cancer in a subject in need thereof (e.g., of paragraph [0046]) further comprises:

- v. further testing, or having tested, a first biological sample obtained from the subject with endometrial cancer or ovarian cancer, thereby measuring
  - a. levels of phosphorylated Rb protein;
  - b. levels of phosphorylated NPM1 protein; and
  - c. levels of TK1,in the subject's cancer;
- vi. testing, or having tested, a second biological sample obtained from the subject, thereby measuring
  - a. levels of phosphorylated Rb protein;
  - b. levels of phosphorylated NPM1 protein; and
  - c. levels of TK1,in the subject's cancer;
- vii. determining whether the subject having or at risk of developing endometrial cancer or ovarian cancer has responded to treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, if the comparison of the second biological sample analysis in step vi. to the first biological sample analysis in step v. shows one or more of the following:
  - a. a changed level of phosphorylated Rb protein;
  - b. a changed level of phosphorylated NPM1 protein;
  - c. a changed expression level of TK1 protein; and
  - d. a changed level of TK1 protein activity,

thereby monitoring a response in a subject having or at risk of developing endometrial cancer or ovarian cancer.

**[0052]** In some embodiments, the levels of p16 mRNA or protein are elevated compared to a control sample. In some embodiments, the levels of p16 mRNA or protein are elevated compared to the average levels of p16 protein measured in normal tissue. In some embodiments, the levels of p16 mRNA or protein are elevated compared to the average levels of p16 protein measured in a population of subjects suffering from solid tumors. In some embodiments, the levels of p16 mRNA or protein are elevated compared to the average levels of p16 protein measured in a population of subjects suffering from endometrial cancer or ovarian cancer.

**[0053]** In some embodiments, the levels of p16 mRNA or protein are low or absent compared to a control sample. In some embodiments, the biological sample has low or no p16 mRNA or protein, dysfunctional p16, or mutated p16 compared to a control sample. In some embodiments, the levels of p16 mRNA or protein are low or absent as compared to the average levels of p16 protein measured in normal tissue. In some embodiments, the levels of p16 mRNA or protein are low or absent as compared to the average levels of p16 protein measured in a population of subjects suffering from solid tumors. In some embodiments, the levels of p16 protein are low or absent as compared to the average levels of p16 protein measured in a population of subjects suffering from endometrial cancer or ovarian cancer. In some embodiments, the subject's endometrial cancer or ovarian cancer is characterized by low or no p16 protein expression, dysfunctional p16, or mutated p16 as compared to the average levels of p16 protein, functional p16, or wild type p16 as measured in a population of subjects suffering from endometrial cancer or ovarian cancer.

**[0054]** In another aspect, the present disclosure provides a method of treating endometrial cancer or ovarian cancer in a subject in need thereof, wherein the subject's tumor, plasmas, or other tissue is identified as having each of the following:

- i. low or no p16 mRNA or protein expression, dysfunctional p16, or mutated p16 as compared to a control sample;
- ii. intact Rb and no loss of function mutations in Rb as compared to a control sample; and
- iii. an increased copy number of CCNE1 gene and/or an elevated expression level of CCNE1 mRNA or CCNE1 protein in the cancer as compared to a control sample,

comprising administering to the subject a therapeutically effective amount of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, and a therapeutically effective amount of a CDK4/6 inhibitor, or a pharmaceutically acceptable salt thereof, thereby treating the endometrial cancer or ovarian cancer.

**[0055]** In another aspect, the present disclosure provides a method of treating endometrial cancer or ovarian cancer in a subject identified as having all of the following:

- i. low or no p16 mRNA or protein expression, dysfunctional p16, or mutated p16;

- ii. intact Rb, which is based on expression level of Rb mRNA or Rb protein expression, and no loss of function mutations in Rb; and
- iii. an increased copy number of CCNE1 gene and/or an elevated expression level of CCNE1 mRNA or CCNE1 protein

in the subject's cancer, wherein the levels of p16 and Rb are compared to the average mRNA or protein levels of p16 and mRNA or protein levels of Rb in a population of subjects suffering from endometrial cancer or ovarian cancer; and the copy number of CCNE1 is greater than 2 and/or the expression levels of CCNE1 mRNA or protein is elevated as compared to the average mRNA or protein levels of CCNE1 in a population of subjects suffering from endometrial cancer or ovarian cancer, and wherein the subject is in need thereof, comprising administering to the subject a therapeutically effective amount of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, and a therapeutically effective amount of a CDK4/6 inhibitor, or a pharmaceutically acceptable salt thereof, thereby treating the endometrial cancer or ovarian cancer.

[0056] In another aspect, the present disclosure provides a method of treating endometrial cancer or ovarian cancer in a subject in need thereof comprising:

- i. testing, or having tested, a first biological sample obtained from the subject with endometrial cancer or ovarian cancer, thereby measuring
  - a. levels of p16;
  - b. levels of Rb; and
  - c. amplification and/or levels of CCNE1,
 in the subject's cancer;
- ii. comparing the levels of p16 mRNA or protein, the levels of Rb mRNA or protein, and the amplification and/or levels of CCNE1, in step i. to the average mRNA or protein levels of p16, mRNA or protein levels of Rb, and amplification and/or mRNA or protein levels of CCNE1 in a population of subjects suffering from endometrial cancer or ovarian cancer;
- iii. determining that the subject's cancer is characterized by all of the following:
  - a. low or no p16 mRNA or protein expression, dysfunctional p16, or mutated p16 as compared to an average level of p16, functional p16, or wild type p16 in a population of subjects suffering from endometrial cancer or ovarian cancer;
  - b. intact Rb, which is based on expression level of Rb mRNA or Rb protein as compared to an average level of Rb in a population of subjects suffering from endometrial cancer or ovarian cancer, and no loss of function mutations in Rb; and
  - c. an increased copy number of CCNE1 gene, which is greater than 2, and/or an elevated expression level of CCNE1 mRNA or protein as compared to an average level of CCNE1 in a population of subjects suffering from endometrial cancer or ovarian cancer; and

- iv. administering to the subject a therapeutically effective amount of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, and a therapeutically effective amount of a CDK4/6 inhibitor, or a pharmaceutically acceptable salt thereof, thereby treating the endometrial cancer or ovarian cancer.

**[0057]** In some embodiments, provided herein is a method of monitoring a response in a subject having or at risk of developing endometrial cancer or ovarian cancer comprising:

- i. testing, or having tested, a first biological sample obtained from the subject having or at risk of developing endometrial cancer or ovarian cancer, thereby measuring
  - a. levels of p16;
  - b. levels of Rb and/or levels of phosphorylated Rb protein;
  - c. amplification and/or levels of CCNE1;
  - d. levels of NPM1 and/or the levels of phosphorylated NPM1 protein;
  - e. levels of TK1,

in the subject's biological sample;

- ii. comparing the levels of p16 mRNA or protein, levels of Rb mRNA or protein, levels of phosphorylated Rb protein, amplification and/or levels of CCNE1 mRNA or protein, levels of NPM1 mRNA or protein, levels of phosphorylated NPM1 protein, and levels of TK1 mRNA or protein, and levels of TK1 protein activity in step i. to the average levels of p16 mRNA or protein, levels of Rb mRNA or protein, levels of phosphorylated Rb protein, amplification and/or levels of CCNE1 mRNA or protein, levels of NPM1 mRNA or protein, levels of phosphorylated NPM1 protein, and levels of TK1 mRNA or protein, and levels of TK1 protein activity in a population of subjects suffering from endometrial cancer or ovarian cancer;
- iii. determining that the subject's biological sample is characterized by one or more of the following:
  - a. a changed expression level of p16 mRNA or protein;
  - b. a changed expression level of Rb mRNA or protein or levels of phosphorylated Rb protein;
  - c. a changed expression level of CCNE1 mRNA or protein or a change in the copy number of CCNE1;
  - d. a changed expression level of NPM1 mRNA or protein or levels of phosphorylated NPM1 protein; and
  - e. a changed expression level of TK1 mRNA or protein or a changed level of TK1 protein activity,

- iv. administering to the subject in need thereof a therapeutically effective amount of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, and a therapeutically effective amount of a CDK4/6 inhibitor, or a pharmaceutically acceptable salt thereof;
- v. testing, or having tested, a second biological sample obtained from the subject, thereby measuring
  - a. levels of p16;
  - b. levels of Rb and/or levels of phosphorylated Rb protein;
  - c. amplification and/or levels of CCNE1;
  - d. levels of NPM1 and/or levels of phosphorylated NPM1 protein;
  - e. levels of TK1,
 in the subject's biological sample;
- vi. determining whether the subject having or at risk of developing endometrial cancer or ovarian cancer has responded to treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, and a CDK4/6 inhibitor, or a pharmaceutically acceptable salt thereof, if the comparison of the second biological sample analysis in step v. to the first biological sample analysis in step i. shows one or more of the following:
  - a. a changed expression level of p16 mRNA or protein;
  - b. a changed expression level of Rb mRNA or protein or levels of phosphorylated Rb protein;
  - c. a changed expression level of CCNE1 mRNA or protein or a change in the copy number of CCNE1;
  - d. a changed expression level of NPM1 mRNA or protein or levels of phosphorylated NPM1 protein; and
  - e. a changed expression level of TK1 mRNA or protein or a changed level of TK1 protein activity.

**[0058]** In some embodiments, the method of monitoring a response in a subject having or at risk of developing endometrial cancer or ovarian cancer comprising:

- i. testing, or having tested, a first biological sample obtained from the subject having or at risk of developing endometrial cancer or ovarian cancer, thereby measuring
  - a. levels of phosphorylated Rb protein;
  - b. levels of phosphorylated NPM1 protein; and
  - c. levels of TK1,
 in the subject's biological sample;
- ii. comparing the levels of phosphorylated Rb protein, levels of phosphorylated NPM1 protein, levels of TK1 protein, and levels of TK1 protein activity in step i. to the average levels of

- phosphorylated Rb protein, levels of phosphorylated NPM1 protein, and levels of TK1 protein, and levels of TK1 protein activity in endometrial cancer or ovarian cancer;
- iii. in response to determining that the subject's biological sample is characterized by one or more of the following:
    - a. a changed level of phosphorylated Rb protein;
    - b. a changed level of phosphorylated NPM1 protein;
    - c. a changed expression level of TK1 protein; and
    - d. a changed level of TK1 protein activity;
  - iv. administering to the subject in need thereof a therapeutically effective amount of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, and a therapeutically effective amount of a CDK4/6 inhibitor, or a pharmaceutically acceptable salt thereof;
  - v. testing, or having tested, a second biological sample obtained from the subject, thereby measuring
    - a. levels of phosphorylated Rb protein;
    - b. levels of phosphorylated NPM1 protein; and
    - c. levels of TK1,
 in the subject's biological sample;
  - vi. determining whether the subject having or at risk of developing endometrial cancer or ovarian cancer has responded to treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, and a CDK4/6 inhibitor, or a pharmaceutically acceptable salt thereof, if the comparison of the second biological sample analysis in step v. to the first biological sample analysis in step i. shows one or more of the following:
    - a. a changed level of phosphorylated Rb protein;
    - b. a changed level of phosphorylated NPM1 protein;
    - c. a changed expression level of TK1 protein; and
    - d. a changed level of TK1 protein activity.

**[0059]** In some embodiments, the method of treating endometrial cancer or ovarian cancer in a subject in need thereof (e.g., of paragraph [0056]) further comprises:

- v. further testing, or having tested, a first biological sample obtained from the subject with endometrial cancer or ovarian cancer, thereby measuring
  - a. levels of phosphorylated Rb protein;
  - b. levels of phosphorylated NPM1 protein; and
  - c. levels of TK1,
 in the subject's cancer;
- vi. testing, or having tested, a second biological sample obtained from the subject, thereby measuring

- a. levels of phosphorylated Rb protein;
- b. levels of phosphorylated NPM1 protein; and
- c. levels of TK1,

in the subject's cancer;

- vii. determining whether the subject having or at risk of developing endometrial cancer or ovarian cancer has responded to treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, and a CDK4/6 inhibitor, or a pharmaceutically acceptable salt thereof, if the comparison of the second biological sample analysis in step v. To the first biological sample analysis in step v. shows one or more of the following:
  - a. a changed level of phosphorylated Rb protein;
  - b. a changed level of phosphorylated NPM1 protein;
  - c. a changed expression level of TK1 protein; and
  - d. a changed level of TK1 protein activity,

thereby monitoring a response in a subject having or at risk of developing endometrial cancer or ovarian cancer.

**[0060]** In some embodiments, the CDK4/6 inhibitor is selected from abemaciclib, birociclib (also referred to as XZP- 3287), BGB-43395, BPI-16350, dalpiciclib (also referred to as SHR6390), FLX-925 (also referred to as AMG-925), GLR2007, lerociclib, LY5219, narazaciclib (also referred to as ON-123300), palbociclib, atirmociclib (also referred to as PF-07220060), PF-07224826, RGT-419B, ribociclib, trilaciclib, and UCT-03-008, or a combination thereof. In some embodiments, the CDK4/6 inhibitor is selected from abemaciclib, BPI-16350, dalpiciclib, lerociclib, palbociclib, ribociclib, trilaciclib. In some embodiments, the CDK4/6 inhibitor is selected from abemaciclib, dalpiciclib, palbociclib, and ribociclib. In some embodiments, the CDK4/6 inhibitor is abemaciclib. In some embodiments, the CDK4/6 inhibitor is dalpiciclib. In some embodiments, the CDK4/6 inhibitor is palbociclib. In some embodiments, the CDK4/6 inhibitor is ribociclib.

**[0061]** In another aspect, the present disclosure provides a method of treating endometrial cancer or ovarian cancer in a subject in need thereof, wherein the subject's tumor, plasmas, or other tissue is identified as having each of the following:

- i. intact Rb and no loss of function mutations in Rb as compared to a control sample; and
- ii. an elevated expression level of CCNE1 mRNA or CCNE1 protein, but independent of a copy number of CCNE1 gene as compared to a control sample; and

wherein the subject is in need thereof, comprising administering to the subject a therapeutically effective amount of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, thereby treating the endometrial cancer or ovarian cancer.

[0062] In another aspect, the present disclosure provides a method of treating endometrial cancer or ovarian cancer in a subject in need thereof, wherein the subject's tumor, plasmas, or other tissue is identified as having each of the following:

- i. intact Rb and no loss of function mutations in Rb as compared to a control sample; and
- ii. an increased copy number of CCNE1 gene and/or an elevated expression level of CCNE1 mRNA or CCNE1 protein in the cancer as compared to a control sample; and

wherein the subject is in need thereof, comprising administering to the subject a therapeutically effective amount of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, and a therapeutically effective amount of a chemotherapeutic agent, or a pharmaceutically acceptable salt thereof, thereby treating the endometrial cancer or ovarian cancer.

[0063] In another aspect, the present disclosure provides a method of treating endometrial cancer or ovarian cancer in a subject in need thereof, wherein the subject's tumor, plasmas, or other tissue is identified as having each of the following:

- i. intact Rb and no loss of function mutations in Rb as compared to a control sample; and
- ii. an elevated expression level of CCNE1 mRNA or CCNE1 protein, but independent of a copy number of CCNE1 gene as compared to a control sample; and

wherein the subject is in need thereof, comprising administering to the subject a therapeutically effective amount of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, and a therapeutically effective amount of a chemotherapeutic agent, or a pharmaceutically acceptable salt thereof, thereby treating the endometrial cancer or ovarian cancer.

[0064] In some embodiments, method is a method of treating endometrial cancer or ovarian cancer in a subject identified as having each of the following:

- i. intact Rb, which is based on expression level of Rb mRNA or Rb protein, and no loss of function mutations in Rb; and
- ii. an increased copy number of CCNE1 gene and/or an elevated expression level of CCNE1 mRNA or CCNE1 protein;

in the subject's cancer, wherein the levels of Rb are compared to the average mRNA or protein levels of Rb in a population of subjects suffering from endometrial cancer or ovarian cancer; and the copy number of CCNE1 is greater than 2 and/or the expression levels of CCNE1 mRNA or protein is elevated as compared to the average mRNA or protein levels of CCNE1 in a population of subjects suffering from endometrial cancer or ovarian cancer, and wherein the subject is in need thereof, the method comprising administering to the subject a therapeutically effective amount of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, and a therapeutically effective amount of a chemotherapeutic agent, or a pharmaceutically acceptable salt thereof, thereby treating the endometrial cancer or ovarian cancer.

[0065] In some embodiments, the method further comprises the subject's tumor, plasmas, or other tissue is identified as having certain p16 expression.

[0066] In another aspect, the present disclosure provides a method of treating endometrial cancer or ovarian cancer in a subject in need thereof, wherein the subject's tumor, plasmas, or other tissue is identified as having each of the following:

- i. certain p16 mRNA or protein expression as compared to a control sample;
- ii. intact Rb and no loss of function mutations in Rb as compared to a control sample; and
- iii. an elevated expression level of CCNE1 mRNA or CCNE1 protein, but independent of a copy number of CCNE1 gene as compared to a control sample; and

wherein the subject is in need thereof, comprising administering to the subject a therapeutically effective amount of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, thereby treating the endometrial cancer or ovarian cancer.

[0067] In another aspect, the present disclosure provides a method of treating endometrial cancer or ovarian cancer in a subject in need thereof, wherein the subject's tumor, plasmas, or other tissue is identified as having each of the following:

- i. certain p16 mRNA or protein expression as compared to a control sample;
- ii. intact Rb and no loss of function mutations in Rb as compared to a control sample; and
- iii. an elevated expression level of CCNE1 mRNA or CCNE1 protein, but independent of a copy number of CCNE1 gene as compared to a control sample; and

wherein the subject is in need thereof, comprising administering to the subject a therapeutically effective amount of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, and a therapeutically effective amount of a chemotherapeutic agent, or a pharmaceutically acceptable salt thereof, thereby treating the endometrial cancer or ovarian cancer.

[0068] In some embodiments, the method is a method of treating endometrial cancer or ovarian cancer in a subject in need thereof, wherein the subject's tumor, plasmas, or other tissue is identified as having each of the following:

- i. an elevated expression level of p16 mRNA or p16 protein;
- ii. intact Rb, which is based on expression level of Rb mRNA or Rb protein, and no loss of function mutations in Rb;
- iii. an increased copy number of CCNE1 gene and/or an elevated expression level of CCNE1 mRNA or CCNE1 protein; and

wherein the subject is in need thereof, comprising administering to the subject a therapeutically effective amount of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, and a therapeutically effective amount of a chemotherapeutic agent, or a pharmaceutically acceptable salt thereof, thereby treating the endometrial cancer or ovarian cancer.

[0069] In another aspect, the present disclosure provides a method of treating endometrial cancer or ovarian cancer in a subject identified as having:

- i. certain p16 mRNA or protein expression;
- ii. intact Rb, which is based on expression level of Rb mRNA or Rb protein, and no loss of function mutations in Rb; and
- iii. an elevated expression level of CCNE1 mRNA or CCNE1 protein, but independent of a copy number of CCNE1 gene;

in the subject's cancer, wherein the levels of p16 and Rb are compared to the average mRNA or protein levels of p16 and mRNA or protein levels of Rb in a population of subjects suffering from endometrial cancer or ovarian cancer; and the expression levels of CCNE1 mRNA or protein is elevated as compared to the average mRNA or protein levels of CCNE1 in a population of subjects suffering from endometrial cancer or ovarian cancer, and wherein the subject is in need thereof, comprising administering to the subject a therapeutically effective amount of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, thereby treating the endometrial cancer or ovarian cancer.

[0070] In another aspect, the present disclosure provides a method of treating endometrial cancer or ovarian cancer in a subject identified as having:

- i. certain p16 mRNA or protein expression;
- ii. intact Rb, which is based on expression level of Rb mRNA or Rb protein, and no loss of function mutations in Rb; and
- iii. an elevated expression level of CCNE1 mRNA or CCNE1 protein, but independent of a copy number of CCNE1 gene;

in the subject's cancer, wherein the levels of p16 and Rb are compared to the average mRNA or protein levels of p16 and mRNA or protein levels of Rb in a population of subjects suffering from endometrial cancer or ovarian cancer; and the expression levels of CCNE1 mRNA or protein is elevated as compared to the average mRNA or protein levels of CCNE1 in a population of subjects suffering from endometrial cancer or ovarian cancer, and wherein the subject is in need thereof, comprising administering to the subject a therapeutically effective amount of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, and a therapeutically effective amount of a chemotherapeutic agent, or a pharmaceutically acceptable salt thereof, thereby treating the endometrial cancer or ovarian cancer.

[0071] In some embodiments, method is a method of treating endometrial cancer or ovarian cancer in a subject identified as having all of the following:

- i. an elevated expression level of p16 mRNA or p16 protein;
- ii. intact Rb, which is based on expression level of Rb mRNA or Rb protein, and no loss of function mutations in Rb; and

- iii. an increased copy number of CCNE1 gene and/or an elevated expression level of CCNE1 mRNA or CCNE1 protein;

in the subject's cancer, wherein the levels of p16 and Rb are compared to the average mRNA or protein levels of p16 and mRNA or protein levels of Rb in a population of subjects suffering from endometrial cancer or ovarian cancer; and the copy number of CCNE1 is greater than 2 and/or the expression levels of CCNE1 mRNA or protein is elevated as compared to the average mRNA or protein levels of CCNE1 in a population of subjects suffering from endometrial cancer or ovarian cancer, and wherein the subject is in need thereof, the method comprising administering to the subject a therapeutically effective amount of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, and a therapeutically effective amount of a chemotherapeutic agent, or a pharmaceutically acceptable salt thereof, thereby treating the endometrial cancer or ovarian cancer.

**[0072]** In another aspect, the present disclosure provides a method of treating endometrial cancer or ovarian cancer in a subject in need thereof comprising:

- i. testing, or having tested, a first biological sample obtained from the subject with endometrial cancer or ovarian cancer, thereby measuring
  - a. levels of Rb; and
  - b. amplification and/or levels of CCNE1;in the subject's cancer;
- ii. comparing the levels of Rb mRNA or protein and the amplification and/or levels of CCNE1 mRNA or protein in step i. to the average levels of Rb mRNA or protein and amplification and/or levels of CCNE1 mRNA or protein in a population of subjects suffering from endometrial cancer or ovarian cancer;
- iii. determining that the subject's endometrial cancer or ovarian cancer is characterized by the following:
  - a. intact Rb, which is based on expression level of Rb mRNA or Rb protein as compared to an average level of Rb in a population of subjects suffering from endometrial cancer or ovarian cancer, and no loss of function mutations in Rb; and
  - b. an increased copy number of CCNE1 gene, which is greater than 2, and/or an elevated expression level of CCNE1 mRNA or protein as compared to an average level of CCNE1 in a population of subjects suffering from endometrial cancer or ovarian cancer; and
- iv. administering to the subject a therapeutically effective amount of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, and a therapeutically effective amount of a chemotherapeutic agent, or a pharmaceutically acceptable salt thereof, thereby treating the endometrial cancer or ovarian cancer.

[0073] In another aspect, the present disclosure provides a method of treating endometrial cancer or ovarian cancer in a subject in need thereof comprising:

- i. testing, or having tested, a first biological sample obtained from the subject with endometrial cancer or ovarian cancer, thereby measuring
  - a. levels of p16;
  - b. levels of Rb; and
  - c. levels of CCNE1;in the subject's cancer;
- ii. comparing the levels of p16 mRNA or protein, the levels of Rb mRNA or protein, and the levels of CCNE1 mRNA or protein in step i. to the average levels of p16 mRNA or protein, levels of Rb mRNA or protein, and levels of CCNE1 mRNA or protein in a population of subjects suffering from endometrial cancer or ovarian cancer;
- iii. determining that the subject's endometrial cancer or ovarian cancer is characterized by
  - a. a changed expression level of p16 mRNA or protein as compared to the average level of p16 in a population of subjects suffering from endometrial cancer or ovarian cancer;
  - b. intact Rb, which is based on expression level of Rb mRNA or Rb protein as compared to an average level of Rb in a population of subjects suffering from endometrial cancer or ovarian cancer, and no loss of function mutations in Rb; and
  - c. an elevated expression level of CCNE1 mRNA or protein as compared to an average level of CCNE1 in a population of subjects suffering from endometrial cancer or ovarian cancer; and
- iv. administering to the subject a therapeutically effective amount of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, and a therapeutically effective amount of a chemotherapeutic agent, or a pharmaceutically acceptable salt thereof, thereby treating the endometrial cancer or ovarian cancer.

[0074] In another aspect, the present disclosure provides a method of treating endometrial cancer or ovarian cancer in a subject in need thereof comprising:

- i. testing, or having tested, a first biological sample obtained from the subject with endometrial cancer or ovarian cancer, thereby measuring
  - a. levels of p16;
  - b. levels of Rb; and
  - c. amplification and/or levels of CCNE1;in the subject's cancer;
- ii. comparing the levels of p16 mRNA or protein, the levels of Rb mRNA or protein, and the amplification and/or levels of CCNE1 mRNA or protein in step i. to the average levels of

- p16 mRNA or protein, levels of Rb mRNA or protein, and amplification and/or levels of CCNE1 mRNA or protein in a population of subjects suffering from endometrial cancer or ovarian cancer;
- iii. determining that the subject's endometrial cancer or ovarian cancer is characterized by
- a changed expression level of p16 mRNA or protein as compared to the average level of p16 in a population of subjects suffering from endometrial cancer or ovarian cancer;
  - intact Rb, which is based on expression level of Rb mRNA or Rb protein as compared to an average level of Rb in a population of subjects suffering from endometrial cancer or ovarian cancer, and no loss of function mutations in Rb; and
  - an increased copy number of CCNE1 gene, which is greater than 2, and/or an elevated expression level of CCNE1 mRNA or protein as compared to an average level of CCNE1 in a population of subjects suffering from endometrial cancer or ovarian cancer; and
- iv. administering to the subject a therapeutically effective amount of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, and a therapeutically effective amount of a chemotherapeutic agent, or a pharmaceutically acceptable salt thereof, thereby treating the endometrial cancer or ovarian cancer.

**[0075]** In some embodiments, the method further comprises testing, or having tested, a second biological sample obtained from the subject with endometrial cancer or ovarian cancer, thereby determining whether levels of p16 mRNA or protein, levels of Rb mRNA or protein, levels of CCNE1 mRNA or protein, levels of NPM1 mRNA or protein, levels of phosphorylated NPM1 protein, levels of TK1 mRNA or protein, and levels of TK1 protein activity change after administering a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, and a therapeutically effective amount of a chemotherapeutic agent, or a pharmaceutically acceptable salt thereof, thereby monitoring a response in the subject.

**[0076]** In some embodiments, provided herein is a method of monitoring a response in a subject having or at risk of developing endometrial cancer or ovarian cancer comprising:

- testing, or having tested, a first biological sample obtained from the subject having or at risk of developing endometrial cancer or ovarian cancer, thereby measuring
  - levels of p16;
  - levels of Rb and levels of phosphorylated Rb protein;
  - levels of CCNE1;
  - levels of NPM1 and/or the levels of phosphorylated NPM1 protein; and
  - levels of TK1,in the subject's biological sample;

- ii. comparing the levels of p16 mRNA or protein, levels of Rb mRNA or protein, levels of phosphorylated Rb protein, levels of CCNE1 mRNA or protein, levels of NPM1 mRNA or protein, levels of phosphorylated NPM1 protein, and levels of TK1 mRNA or protein, and levels of TK1 protein activity in step i. to the average levels of p16 mRNA or protein, levels of Rb mRNA or protein, levels of phosphorylated Rb protein, levels of CCNE1 mRNA or protein, levels of NPM1 mRNA or protein, levels of phosphorylated NPM1 protein, levels of TK1 mRNA or protein, and levels of TK1 protein activity in a population of subjects suffering from endometrial cancer or ovarian cancer;
- iii. determining that the subject's biological sample is characterized by one or more of the following:
  - a. a changed expression level of p16 mRNA or protein;
  - b. a changed expression level of Rb mRNA or protein or levels of phosphorylated Rb protein;
  - c. a changed expression level of CCNE1 mRNA or protein;
  - d. a changed expression level of NPM1 mRNA or protein or levels of phosphorylated NPM1 protein; and
  - e. a changed expression level of TK1 mRNA or protein or a changed level of TK1 protein activity,
- iv. administering to the subject in need thereof a therapeutically effective amount of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, and a therapeutically effective amount of a chemotherapeutic agent, or a pharmaceutically acceptable salt thereof;
- v. testing, or having tested, a second biological sample obtained from the subject, thereby measuring
  - a. levels of p16;
  - b. levels of Rb and/or levels of phosphorylated Rb protein;
  - c. levels of CCNE1;
  - d. levels of NPM1 protein and/or levels of phosphorylated NPM1 protein; and
  - e. levels of TK1,
 in the subject's biological sample;
- vi. determining whether the subject having or at risk of developing endometrial cancer or ovarian cancer has responded to treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, and a chemotherapeutic agent, or a pharmaceutically acceptable salt thereof, if the comparison of the second biological sample analysis in step v. to the first biological sample analysis in step i. shows one or more of the following:
  - a. a changed expression level of p16 mRNA or protein;

- b. a changed expression level of Rb mRNA or protein or levels of phosphorylated Rb protein;
- c. a changed expression level of CCNE1 mRNA or protein;
- d. a changed expression level of NPM1 mRNA or protein or levels of phosphorylated NPM1 protein; and/or
- e. a changed expression level of TK1 mRNA or protein or a changed level of TK1 protein activity.

**[0077]** In some embodiments, the method of monitoring a response in a subject having or at risk of developing endometrial cancer or ovarian cancer comprising:

- i. testing, or having tested, a first biological sample obtained from the subject having or at risk of developing endometrial cancer or ovarian cancer, thereby measuring
  - a. levels of phosphorylated Rb protein;
  - b. levels of phosphorylated NPM1 protein; and
  - c. levels of TK1,
 in the subject's biological sample;
- ii. comparing the levels of phosphorylated Rb protein, levels of phosphorylated NPM1 protein, levels of TK1 protein, and levels of TK1 protein activity in step i. to the average levels of phosphorylated Rb protein, levels of phosphorylated NPM1 protein, levels of TK1 protein, and levels of TK1 protein activity in a population of subjects suffering from endometrial cancer or ovarian cancer;
- iii. determining that the subject's biological sample is characterized by one or more of the following:
  - a. a changed level of phosphorylated Rb protein;
  - b. a changed level of phosphorylated NPM1 protein;
  - c. a changed expression level of TK1 protein; and
  - d. a changed level of TK1 protein activity;
- iv. administering to the subject in need thereof a therapeutically effective amount of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, and a therapeutically effective amount of a chemotherapeutic agent, or a pharmaceutically acceptable salt thereof;
- v. testing, or having tested, a second biological sample obtained from the subject, thereby measuring
  - a. levels of phosphorylated Rb protein;
  - b. levels of phosphorylated NPM1 protein; and
  - c. levels of TK1,
 in the subject's biological sample;

- vi. determining whether the subject having or at risk of developing endometrial cancer or ovarian cancer has responded to treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, and a chemotherapeutic agent, or a pharmaceutically acceptable salt thereof, if the comparison of the second biological sample analysis in step v. to the first biological sample analysis in step i. shows one or more of the following:
  - a. a changed level of phosphorylated Rb protein;
  - b. a changed level of phosphorylated NPM1 protein;
  - c. a changed expression level of TK1 protein; and
  - d. a changed level of TK1 protein activity.

**[0078]** In some embodiments, the method of treating endometrial cancer or ovarian cancer in a subject in need thereof (e.g., of paragraph [0072]) further comprises:

- v. further testing, or having tested, a first biological sample obtained from the subject with endometrial cancer or ovarian cancer, thereby measuring
  - a. levels of phosphorylated Rb protein;
  - b. levels of phosphorylated NPM1 protein; and
  - c. levels of TK1,
 in the subject's cancer;
- vi. testing, or having tested, a second biological sample obtained from the subject, thereby measuring
  - a. levels of phosphorylated Rb protein;
  - b. levels of phosphorylated NPM1 protein; and
  - c. levels of TK1,
 in the subject's cancer;
- vii. determining whether the subject having or at risk of developing endometrial cancer or ovarian cancer has responded to treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, and a chemotherapeutic agent, or a pharmaceutically acceptable salt thereof, if the comparison of the second biological sample analysis in step vi. to the first biological sample analysis in step v. shows one or more of the following:
  - a. a changed level of phosphorylated Rb protein;
  - b. a changed level of phosphorylated NPM1 protein;
  - c. a changed expression level of TK1 protein; and
  - d. a changed level of TK1 protein activity,

thereby monitoring a response in a subject having or at risk of developing endometrial cancer or ovarian cancer.

**[0079]** In some embodiments, the method of treating endometrial cancer or ovarian cancer in a subject in need thereof (e.g., of paragraph [0073]) further comprises:

- v. further testing, or having tested, a first biological sample obtained from the subject with endometrial cancer or ovarian cancer, thereby measuring
  - a. levels of p16;
  - a. levels of phosphorylated Rb protein;
  - b. levels of phosphorylated NPM1 protein; and
  - c. levels of TK1,
 in the subject's cancer;
- vi. testing, or having tested, a second biological sample obtained from the subject, thereby measuring
  - a. levels of p16;
  - b. levels of phosphorylated Rb protein;
  - c. levels of phosphorylated NPM1 protein; and
  - d. levels of TK1,
 in the subject's cancer;
- vii. determining whether the subject having or at risk of developing endometrial cancer or ovarian cancer has responded to treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, and a chemotherapeutic agent, or a pharmaceutically acceptable salt thereof, if the comparison of the second biological sample analysis in step vi. to the first biological sample analysis in step v. shows one or more of the following:
  - a. a changed expression level of p16 mRNA or protein;
  - b. a changed level of phosphorylated Rb protein;
  - c. a changed level of phosphorylated NPM1 protein;
  - d. a changed expression level of TK1 protein; and
  - e. a changed level of TK1 protein activity,

thereby monitoring a response in a subject having or at risk of developing endometrial cancer or ovarian cancer.

**[0080]** In some embodiments, the method of treating endometrial cancer or ovarian cancer in a subject in need thereof (e.g., of paragraph [0074]) further comprises:

- v. further testing, or having tested, a first biological sample obtained from the subject with endometrial cancer or ovarian cancer, thereby measuring
  - a. levels of p16;
  - d. levels of phosphorylated Rb protein;
  - e. levels of phosphorylated NPM1 protein; and
  - f. levels of TK1,
 in the subject's cancer;

- vi. testing, or having tested, a second biological sample obtained from the subject, thereby measuring
  - a. levels of p16;
  - b. levels of phosphorylated Rb protein;
  - c. levels of phosphorylated NPM1 protein; and
  - d. levels of TK1,
 in the subject's cancer;
- vii. determining whether the subject having or at risk of developing endometrial cancer or ovarian cancer has responded to treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, and a chemotherapeutic agent, or a pharmaceutically acceptable salt thereof, if the comparison of the second biological sample analysis in step vi. to the first biological sample analysis in step v. shows one or more of the following:
  - a. a changed expression level of p16 mRNA or protein;
  - b. a changed level of phosphorylated Rb protein;
  - c. a changed level of phosphorylated NPM1 protein;
  - d. a changed expression level of TK1 protein; and
  - e. a changed level of TK1 protein activity,

thereby monitoring a response in a subject having or at risk of developing endometrial cancer or ovarian cancer.

**[0081]** In some embodiments, the subject's endometrial cancer or ovarian cancer is characterized by proficient Rb protein expression and no loss of function mutations in Rb. In some embodiments, the subject's endometrial cancer or ovarian cancer is characterized by low or no Rb protein expression or loss of function mutations in Rb.

**[0082]** In some embodiments, the chemotherapeutic agent is selected from 5-fluorouracil, abraxane, camptothecin, capecitabine, carboplatin, cisplatin, cyclophosphamide, dacarbazine, docetaxel, doxorubicin, etoposide, floxuridine, gemcitabine, ifosfamide, irinotecan, methotrexate, mitomycin, oxaliplatin, paclitaxel, temozolomide, topotecan, vinblastine, vinorelbine, or a pharmaceutically acceptable salt thereof, or a combination thereof. In some embodiments, the chemotherapeutic agent is capecitabine, carboplatin, cisplatin, docetaxel, doxorubicin, gemcitabine, paclitaxel, and vinorelbine or a pharmaceutically acceptable salt thereof. In some embodiments, the chemotherapeutic agent is paclitaxel. In some embodiments, the chemotherapeutic agent is cisplatin, carboplatin or oxaliplatin. In some embodiments, the chemotherapeutic agent is carboplatin. In some embodiments, the chemotherapeutic agent is a platinum-based chemotherapy. In some embodiments, the platinum-based chemotherapy is cisplatin, carboplatin or oxaliplatin.

**[0083]** "Treating" or "treatment" refers to obtaining a desired pharmacological and/or physiological effect. The effect can be therapeutic, which includes achieving, partially or

substantially, one or more of the following results: partially or substantially reducing the extent of the cancer; ameliorating or improving a clinical symptom or indicator associated with the endometrial cancer or ovarian cancer; delaying, inhibiting or decreasing the likelihood of the progression of the endometrial cancer or ovarian cancer; or decreasing the likelihood of recurrence of the endometrial cancer or ovarian cancer.

[0084] The terms “administer”, “administering”, “administration”, and the like, as used herein, refer to methods that may be used to enable delivery of compositions to the desired site of biological action. These methods include, but are not limited to, intraarticular (in the joints), intravenous, intramuscular, intratumoral, intradermal, intraperitoneal, subcutaneous, orally, topically, intrathecally, inhalationally, transdermally, rectally, and the like. Administration techniques that can be employed with the agents and methods described herein are found in *e.g.*, Goodman and Gilman, *The Pharmacological Basis of Therapeutics*, current ed.; Pergamon; and Remington’s, *Pharmaceutical Sciences* (current edition), Mack Publishing Co., Easton, Pa.

[0085] As used herein, the term “patient” or “subject” refers to an organism to be treated by the methods of the disclosure. Non-limiting example organisms include mammals, *e.g.*, murines, simians, equines, bovines, porcines, canines, felines, and the like. In some embodiments, the subject is a human. In some embodiments, the subject is an adult human.

[0086] The precise amount of compound administered to provide an “effective amount” to the subject will depend on the mode of administration such as general the route of administration, the time of administration, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular active ingredient employed, the type, and severity of the endometrial cancer or ovarian cancer, the rate of excretion of the particular active ingredient being employed, and on the characteristics of the subject, such as age, sex, body weight, general health and prior medical history of the patient being treated, and tolerance to drugs. The skilled artisan will be able to determine appropriate dosages depending on these and other factors. When administered in combination with other therapeutic agents, *e.g.*, when administered in combination with an anti-cancer agent, an “effective amount” of any additional therapeutic agent(s) will depend on the type of drug used. Suitable dosages are known for approved therapeutic agents and can be adjusted by the skilled artisan according to the condition of the subject, the type of condition(s) being treated and the amount of a CDK2 inhibitor being used by following, for example, dosages reported in the literature and recommended in the *Physician’s Desk Reference* (57th Ed., 2003).

[0087] In general, a suitable daily dose of a compound of the disclosure will be that amount of the compound that is the lowest dose effective to produce a therapeutic effect. Such an effective dose will generally depend upon the factors described above.

[0088] The particular mode of administration and the dosage regimen will be selected by the attending clinician, taking into account the particulars of the case (*e.g.*, the subject, the disease, the

disease state involved, and the particular treatment. Treatment can involve daily or multi-daily or less than daily (such as weekly or monthly etc.) doses over a period of a few days to months, or even years.

**[0089]** In addition, a CDK2 inhibitor, a pharmaceutically acceptable salt thereof, or a pharmaceutical composition of the disclosure can be co-administered with other therapeutic agents. As used herein, the terms “co-administration”, “administered in combination with”, and their grammatical equivalents, are meant to encompass administration of two or more therapeutic agents to a single subject, and are intended to include treatment regimens in which the agents are administered by the same or different route of administration or at the same or different times. In some embodiments the one or more compounds of the disclosure, a pharmaceutically acceptable salt thereof, or a pharmaceutical composition of the disclosure will be co-administered with other agents. These terms encompass administration of two or more agents to the subject so that both agents and/or their metabolites are present in the subject at the same time. They include simultaneous administration in separate compositions, administration at different times in separate compositions, and/or administration in a composition in which both agents are present. Thus, in some embodiments, a CDK2 inhibitor and the other agent(s) are administered in a single composition. In some embodiments, a CDK2 inhibitor and the other agent(s) are admixed in the composition

*Endometrial Cancer or Ovarian Cancer*

**[0090]** In some embodiments, the endometrial cancer or ovarian cancer is endometrial cancer (including serous endometrial cancer (SEC)). In some embodiments, the endometrial cancer is selected from carcinosarcoma endometrial cancer, clear cell endometrial carcinoma, grade 3 endometrioid endometrial cancer, and SEC. In some embodiments, the endometrial cancer is SEC. In some embodiments, the endometrial cancer or ovarian cancer is ovarian cancer (e.g. ovarian serous cystadenocarcinoma (OV) and high grade serous ovarian cancer (HGSOC)). In some embodiments, the ovarian cancer is OV. In some embodiments, the ovarian cancer is HGSOC.

**[0091]** In some embodiments, the endometrial cancer or ovarian cancer is CCNE1-amplified endometrial cancer or CCNE1-amplified ovarian cancer. In some embodiments, the endometrial cancer or ovarian cancer is CCNE1-amplified endometrial cancer. In some embodiments, the endometrial cancer or ovarian cancer is CCNE1-amplified ovarian cancer.

**[0092]** In some embodiments, the endometrial cancer or ovarian cancer is platinum-resistant and/or platinum-refractory. In some embodiments, the endometrial cancer or ovarian cancer has progressed despite platinum treatment. In some embodiments, the endometrial cancer or ovarian cancer is endometrial cancer (with prior platinum therapy) that has progressed following 2 or more lines of therapies or platinum-resistant or platinum-refractory ovarian cancer. In some embodiments, the endometrial cancer or ovarian cancer is endometrial cancer (with prior platinum-based

chemotherapy) that has progressed following 2 or more lines of therapies. In some embodiments, the endometrial cancer or ovarian cancer is CCNE1 amplified endometrial cancer that has progressed after at least 2 prior lines of therapy, including platinum-based chemotherapy. In some embodiments, the endometrial cancer or ovarian cancer is platinum-resistant or platinum-refractory ovarian cancer. In some embodiments, the endometrial cancer or ovarian cancer is platinum-resistant ovarian cancer, wherein platinum-resistant is a relapse < 6 months after first line platinum-based chemotherapy. In some embodiments, the endometrial cancer or ovarian cancer is platinum-refractory ovarian cancer, wherein platinum-refractory is progression during or within 4 weeks of first line platinum-based chemotherapy. In some embodiments, the endometrial cancer or ovarian cancer is platinum-resistant or platinum-refractory CCNE1 amplified ovarian cancer.

*Biomarkers and Pharmacodynamics Markers*

**[0093]** In some embodiments, the one or more biomarkers are measured based on levels selected from DNA (including cDNA), RNA (including messenger ribonucleic acid (mRNA) and micro ribonucleic acid (miRNA)), protein expression (including protein overexpression), enzyme activity (e.g., for TK1), gene copy number, gene expression, gene sequence, mutations, and phosphorylation.

**[0094]** In some embodiments, additional biological samples are obtained from the subject and compared to the levels of the biomarkers measured in a subject with a normally functioning pathway to continue treating or monitoring. In some embodiments, additional biological samples are obtained from the subject and compared to the levels of the biomarkers measured in the first biological sample to continue treating or monitoring.

**[0095]** In some embodiments, the levels of one or more biomarkers in a biological sample obtained from a subject having or at risk of developing endometrial cancer or ovarian cancer compared to the levels of one or more biomarkers measured in a subject with a normally functioning pathway differ by at least 10%. In some embodiments, the comparison shows a difference of at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, the comparison shows a difference of about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, the difference is an increase. In some embodiments, the difference is a reduction.

*p16*

**[0096]** In some embodiments, the contemplated biomarker is p16 (also known as cyclin-dependent kinase inhibitor 2A, cyclin-dependent kinase 4 inhibitor A, multiple tumor suppressor 1, p16-INK4a, and p16(INK4)), which is encoded by the gene CDKN2A and acts as a negative regulator of the proliferation of normal cells by interacting with CDK4 and CDK6. In some embodiments, the levels of p16 are modulated in response to administration of an effective dose of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, to a subject. In some embodiments, p16 is a biomarker for

CDK2 inhibitor sensitivity. In some embodiments, levels of p16 protein are high, and this is a biomarker. In some embodiments, p16 is absent, and this is a biomarker. In some embodiments, low or no p16 protein expression, dysfunctional p16, or mutated p16 are biomarkers. In some embodiments, the low or no p16 is a biomarker. In some embodiments, dysfunctional p16 is a biomarker. In some embodiments, mutated p16 (e.g., a loss of function mutation) is a biomarker. In some embodiments, p16 or a p16 mutant is functional. In some embodiments, a change in the levels of p16 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, or at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, is indicative/predictive that a subject having or at risk of developing a cancer has responded to treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof.

**[0097]** In some embodiments, the levels of p16 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by at least 10%. In some embodiments, the levels of p16 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, the levels of p16 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, the levels of p16 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are increased. In some embodiments, the levels of p16 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced.

**[0098]** In some embodiments, the levels of p16 at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by at least 10%. In some embodiments, the levels of p16 at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, the levels of p16 at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, the levels of p16 at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are increased. In some embodiments, the levels of p16 at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced.

#### *Retinoblastoma 1 Protein (Rb)*

**[0099]** In some embodiments, the biomarker is retinoblastoma 1 protein (pRb Rb, RB, or RB1), which is encoded by the gene RB transcriptional corepressor 1 (RB1). Rb is a regulator of the cell

cycle and acts as a tumor suppressor. In some embodiments, the levels of Rb are modulated in response to administration of an effective dose of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, to a subject. In some embodiments, the biomarker is proficient Rb protein expression and no loss of function mutations in Rb.

**[00100]** In some embodiments, the biomarker is phosphorylation of Rb at any phosphorylation site. In some embodiments, the biomarker is phosphorylation at the serine corresponding to amino acid position 780 (Ser780 or S780) and/or the serine corresponding to amino acid position 795 (Ser795 or S795). In some embodiments, the contemplated biomarker is phosphorylation of Rb at the serine corresponding to amino acid position 807 (Ser807 or S807) and/or the serine corresponding to amino acid position 811 (Ser811 or S811). In some embodiments, the contemplated biomarker is phosphorylation of Rb at the threonine corresponding to amino acid position 821 (Thr821 or T821). In some embodiments, the contemplated biomarker is phosphorylation of Rb at the threonine corresponding to amino acid position 826 (Thr826 or T826). Rb is activated upon phosphorylation by cyclin D-CDK4/6 at Ser780 and Ser795 and/or at Ser807 and/or Ser811 and by cyclin E/CDK2 at Ser807 and Ser811 and Thr821. In some embodiments, the levels of phosphorylated Rb are modulated in response to administration of an effective dose of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, to a subject. In some embodiments, phosphorylated Rb, Rb, or RB is absent. In some embodiments, the loss of phosphorylated Rb, Rb or RB is a biomarker. In some embodiments, Rb or RB has a mutation (e.g., a loss of function mutation). In some embodiments, Rb, RB, or an RB mutant is functional. In some embodiments, phosphorylated Rb, Rb, or RB is a biomarker for CDK2 inhibitor sensitivity.

**[00101]** In some embodiments, a change in the levels of phosphorylated Rb, Rb, or RB before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, is indicative/predictive that a subject having or at risk of developing a cancer has responded to treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof. In some embodiments, the levels of phosphorylated Rb at any phosphorylation site before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by at least 10%. In some embodiments, the levels of phosphorylated Rb at any phosphorylation site before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, the levels of phosphorylated Rb at any phosphorylation site before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, the levels of phosphorylated Rb at any phosphorylation site or Rb or RB before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are increased. In some embodiments, the levels of phosphorylated Rb at any

phosphorylation site or Rb or RB before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced.

**[00102]** In some embodiments, a change in the levels of phosphorylated Rb or Rb or RB at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, is indicative/predictive that a subject having or at risk of developing cancer has responded to treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof. In some embodiments, the levels of phosphorylated Rb at any phosphorylation site at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by at least 10%. In some embodiments, the levels of phosphorylated Rb at any phosphorylation site at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, the levels of phosphorylated Rb at any phosphorylation site at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, the levels of phosphorylated Rb at any phosphorylation site or Rb or RB at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are increased. In some embodiments, the levels of phosphorylated Rb at any phosphorylation site or Rb or RB at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced.

**[00103]** In some embodiments, the levels of phosphorylated Rb at Ser780 and/or Ser795 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by at least 10%. In some embodiments, the levels of phosphorylated Rb at Ser780 and/or Ser795 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, the levels of phosphorylated Rb at Ser780 and/or Ser795 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, the levels of phosphorylated Rb at Ser780 and/or Ser795 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are increased.

**[00104]** In some embodiments, the levels of phosphorylated Rb at Ser780 and/or Ser795 at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by at least 10%. In some embodiments, the levels of phosphorylated Rb at Ser780 and/or Ser795 at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by at least 10%, 15%, 20%, 25%, 30%, 35%,

40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, the levels of phosphorylated Rb at Ser780 and/or Ser795 at two different timepoints during treatment are changed by about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, the levels of phosphorylated Rb at Ser780 and/or Ser795 at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are increased. In some embodiments, the levels of phosphorylated Rb at Ser780 and/or Ser795 at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced.

**[00105]** In some embodiments, the levels of phosphorylated Rb at Ser780 and/or Ser795 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced. In some embodiments, the levels of phosphorylated Rb at Ser780 and/or Ser795 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, the levels of phosphorylated Rb at Ser780 and/or Ser795 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 10%. In some embodiments, the levels of phosphorylated Rb at Ser780 and/or Ser795 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 20%. In some embodiments, the levels of phosphorylated Rb at Ser780 and/or Ser795 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 30%. In some embodiments, the levels of phosphorylated Rb at Ser780 and/or Ser795 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 40%. In some embodiments, the levels of phosphorylated Rb at Ser780 and/or Ser795 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 50%. In some embodiments, the levels of phosphorylated Rb at Ser780 and/or Ser795 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 60%. In some embodiments, the levels of phosphorylated Rb at Ser780 and/or Ser795 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 70%. In some embodiments, the levels of phosphorylated Rb at Ser780 and/or Ser795 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 80%. In some embodiments, the levels of phosphorylated Rb at Ser780 and/or Ser795 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 90%. In some embodiments, the levels of phosphorylated Rb at Ser780 and/or Ser795 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 100%.

**[00106]** In some embodiments, the levels of phosphorylated Rb at Ser807 and/or Ser811 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by at least 10%. In some embodiments, the levels of phosphorylated Rb at Ser807 and/or Ser811 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, the levels of phosphorylated Rb at Ser807 and/or Ser811 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, the levels of phosphorylated Rb at Ser807 and/or Ser811 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are increased.

**[00107]** In some embodiments, the levels of phosphorylated Rb at Ser807 and/or Ser811 at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by at least 10%. In some embodiments, the levels of phosphorylated Rb at Ser807 and/or Ser811 at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, the levels of phosphorylated Rb at Ser807 and/or Ser811 at two different timepoints during treatment are changed by about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, the levels of phosphorylated Rb at Ser807 and/or Ser811 at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are increased. In some embodiments, the levels of phosphorylated Rb at Ser807 and/or Ser811 at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced.

**[00108]** In some embodiments, the levels of phosphorylated Rb at Ser807 and/or Ser811 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced. In some embodiments, the levels of phosphorylated Rb at Ser807 and/or Ser811 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, the levels of phosphorylated Rb at Ser807 and/or Ser811 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 10%. In some embodiments, the levels of phosphorylated Rb at Ser807 and/or Ser811 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 20%. In some embodiments, the levels of phosphorylated Rb at Ser807 and/or Ser811 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 30%. In some embodiments, the levels of phosphorylated Rb at

Ser807 and/or Ser811 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 40%. In some embodiments, the levels of phosphorylated Rb at Ser807 and/or Ser811 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 50%. In some embodiments, the levels of phosphorylated Rb at Ser807 and/or Ser811 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 60%. In some embodiments, the levels of phosphorylated Rb at Ser807 and/or Ser811 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 70%. In some embodiments, the levels of phosphorylated Rb at Ser807 and/or Ser811 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 80%. In some embodiments, the levels of phosphorylated Rb at Ser807 and/or Ser811 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 90%. In some embodiments, the levels of phosphorylated Rb at Ser807 and/or Ser811 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 100%.

**[00109]** In some embodiments, the levels of phosphorylated Rb at Thr821 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by at least 10%. In some embodiments, the levels of phosphorylated Rb at Thr821 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, the levels of phosphorylated Rb at Thr821 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, the levels of phosphorylated Rb at Thr821 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are increased.

**[00110]** In some embodiments, the levels of phosphorylated Rb at Thr821 at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by at least 10%. In some embodiments, the levels of phosphorylated Rb at Thr821 at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, the levels of phosphorylated Rb at Thr821 at two different timepoints during treatment are changed by about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, the levels of phosphorylated Rb at Thr821 at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are increased. In some

embodiments, the levels of phosphorylated Rb at Thr821 at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced.

**[00111]** In some embodiments, the levels of phosphorylated Rb at Thr821 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced. In some embodiments, the levels of phosphorylated Rb at Thr821 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, the levels of phosphorylated Rb at Thr821 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 10%. In some embodiments, the levels of phosphorylated Rb at Thr821 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 20%. In some embodiments, the levels of phosphorylated Rb at Thr821 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 30%. In some embodiments, the levels of phosphorylated Rb at Thr821 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 40%. In some embodiments, the levels of phosphorylated Rb at Thr821 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 50%. In some embodiments, the levels of phosphorylated Rb at Thr821 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 60%. In some embodiments, the levels of phosphorylated Rb at Thr821 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 70%. In some embodiments, the levels of phosphorylated Rb at Thr821 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 80%. In some embodiments, the levels of phosphorylated Rb at Thr821 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 90%. In some embodiments, the levels of phosphorylated Rb at Thr821 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 100%.

**[00112]** In some embodiments, the levels of phosphorylated Rb at Thr826 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by at least 10%. In some embodiments, the levels of phosphorylated Rb at Thr826 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, the levels of phosphorylated Rb at Thr826 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%,

90%, 95%, or 100%. In some embodiments, the levels of phosphorylated Rb at Thr826 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are increased.

**[00113]** In some embodiments, the levels of phosphorylated Rb at Thr826 at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by at least 10%. In some embodiments, the levels of phosphorylated Rb at Thr826 at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, the levels of phosphorylated Rb at Thr826 at two different timepoints during treatment are changed by about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, the levels of phosphorylated Rb at Thr826 at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are increased. In some embodiments, the levels of phosphorylated Rb at Thr826 at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced.

**[00114]** In some embodiments, the levels of phosphorylated Rb at Thr826 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced. In some embodiments, the levels of phosphorylated Rb at Thr826 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, the levels of phosphorylated Rb at Thr826 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 10%. In some embodiments, the levels of phosphorylated Rb at Thr826 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 20%. In some embodiments, the levels of phosphorylated Rb at Thr826 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 30%. In some embodiments, the levels of phosphorylated Rb at Thr826 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 40%. In some embodiments, the levels of phosphorylated Rb at Thr826 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 50%. In some embodiments, the levels of phosphorylated Rb at Thr826 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 60%. In some embodiments, the levels of phosphorylated Rb at Thr826 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 70%. In some embodiments, the levels of phosphorylated Rb at Thr826 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 80%. In some embodiments, the levels of phosphorylated Rb at Thr826 before and after administration of a CDK2

inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 90%. In some embodiments, the levels of phosphorylated Rb at Thr826 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 100%.

#### *CCNE1*

**[00115]** CCNE1 is a cell cycle factor essential for the control of the cell cycle at the G1/S transition (Ohtsubo et al., 1995, Mol. Cell. Biol. 15:2612-2624). CCNE1 acts as a regulatory subunit of CDK2, interacting with CDK2 to form a serine/threonine kinase holoenzyme complex. The CCNE1 subunit of this holoenzyme complex provides the substrate specificity of the complex (Honda et al., 2005, EMBO 24:452- 463). CCNE1 is encoded by the cyclin E1 ("*CCNE1*") gene (GenBank Accession No. NM\_001238). The amino acid sequence of human CCNE1 is found at GenBank Accession No. NP\_001229 / UniProtKB Accession No. P24864).

**[00116]** In some embodiments, a subject has aberrant expression of the CCNE1 gene. In some embodiments, an aberrant level of CCNE1 that is different than a control level of CCNE1 is indicative/predictive that a human subject having or at risk of developing endometrial cancer or ovarian cancer will respond to a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, administered at an effective dose. In some embodiments, the amplification and/or levels of CCNE1 is characterized by an increased copy number of CCNE1 gene and/or elevated expression level of CCNE1 RNA or CCNE1 protein. In some embodiments, the amplification and/or levels of CCNE1 may be an amplification of CCNE1. In some embodiments, the amplification of CCNE1 is characterized by an increased copy number of CCNE1 gene. In some embodiments, the amplification and/or levels of CCNE1 may be levels of CCNE1. In some embodiments, the levels of CCNE1 are the expression level of CCNE1 RNA. In some embodiments, the levels of CCNE1 are the expression level of CCNE1 mRNA. In other embodiments, the levels of CCNE1 are the expression level of CCNE1 protein. In other embodiments, the levels of CCNE1 are an indirect measure of the level of CCNE1 RNA or CCNE1 protein. In some embodiments, the levels of CCNE1 are modulated in response to administration of an effective dose of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, to a subject.

**[00117]** In some embodiments, CCNE1 or a mutated CCNE1 is functional. In some embodiments, treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, modulates the levels of CCNE1. In some embodiments, a change in the levels of CCNE1 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, or at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, is indicative/predictive that a subject having or at risk of developing endometrial cancer or ovarian cancer has responded to treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof.

**[00118]** In some embodiments, the levels of CCNE1 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by at least 10%. In some embodiments, the levels of CCNE1 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, the levels of CCNE1 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, the levels of CCNE1 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are increased. In some embodiments, the levels of CCNE1 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced.

**[00119]** In some embodiments, the levels of CCNE1 at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by at least 10%. In some embodiments, the levels of CCNE1 at two different timepoints during treatment are changed by at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, the levels of CCNE1 at two different timepoints during treatment are changed by about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, the levels of CCNE1 at two different timepoints during administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are increased. In some embodiments, the levels of CCNE1 at two different timepoints during administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced.

**[00120]** In some embodiments, the levels of CCNE1 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are increased by at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, the levels of CCNE1 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are increased by at least 10%. In some embodiments, the levels of CCNE1 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are increased by at least 20%. In some embodiments, the levels of CCNE1 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are increased by at least 30%. In some embodiments, the levels of CCNE1 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are increased by at least 40%. In some embodiments, the levels of CCNE1 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are increased by at least 50%. In some embodiments, the levels of CCNE1 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are increased by at least 60%. In some embodiments, the levels of CCNE1 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are

increased by at least 70%. In some embodiments, the levels of CCNE1 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are increased by at least 80%. In some embodiments, the levels of CCNE1 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are increased by at least 90%. In some embodiments, the levels of CCNE1 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are increased by at least 100%.

**[00121]** In some embodiments, the levels of CCNE1 at two different timepoints during administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are increased by at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, the levels of CCNE1 at two different timepoints during administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are increased by at least 10%. In some embodiments, the levels of CCNE1 at two different timepoints during administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are increased by at least 20%. In some embodiments, the levels of CCNE1 at two different timepoints during administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are increased by at least 30%. In some embodiments, the levels of CCNE1 at two different timepoints during administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are increased by at least 40%. In some embodiments, the levels of CCNE1 at two different timepoints during administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are increased by at least 50%. In some embodiments, the levels of CCNE1 at two different timepoints during administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are increased by at least 60%. In some embodiments, the levels of CCNE1 at two different timepoints during administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are increased by at least 70%. In some embodiments, the levels of CCNE1 at two different timepoints during administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are increased by at least 80%. In some embodiments, the levels of CCNE1 at two different timepoints during administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are increased by at least 90%. In some embodiments, the levels of CCNE1 at two different timepoints during administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are increased by at least 100%.

*Nucleophosmin 1 (NPM1)*

**[00122]** In some embodiments, the biomarker is nucleophosmin 1 protein (NPM1), which is also known as nucleolar phosphoprotein B23 or numatrin and is encoded by the NPM1 gene. NPM1 is a protein involved in multiple cellular functions, including ribosome assembly and transport, DNA polymerase  $\alpha$  regulation, centrosome duplication, and molecular chaperoning activities. In some embodiments, the levels of NPM1 are modulated in response to administration of an effective dose of

a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, to a subject. In some embodiments, the biomarker is proficient NPM1 protein expression and no loss of function mutations in NPM1.

**[00123]** In some embodiments, the biomarker is phosphorylation of NPM1 at any phosphorylation site. In some embodiments, the biomarker is phosphorylation at the serine corresponding to amino acid position 125 (Ser125 or S125). In some embodiments, the biomarker is phosphorylation at the threonine corresponding to amino acid position 199 (Thr199 or T199). In some embodiments, the contemplated biomarker is phosphorylation of NPM1 at the threonine corresponding to amino acid position 234 (Thr234 or T234) and/or the threonine corresponding to amino acid position 237 (Thr237 or T237). In some embodiments, the levels of phosphorylated NPM1 are modulated in response to administration of an effective dose of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, to a subject. In some embodiments, phosphorylated NPM1 is absent. In some embodiments, the loss of phosphorylated NPM1 is a biomarker. In some embodiments, NPM1 has a mutation (e.g., a loss of function mutation). In some embodiments, NPM1 mutant is functional. In some embodiments, phosphorylated NPM1 is a biomarker for CDK2 inhibitor sensitivity.

**[00124]** In some embodiments, a change in the levels of NPM1 or phosphorylated NPM1 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, is indicative/predictive that a subject having or at risk of developing a cancer has responded to treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof. In some embodiments, the levels of NPM1 or phosphorylated NPM1 at any phosphorylation site before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by at least 10%. In some embodiments, the levels of NPM1 or phosphorylated NPM1 at any phosphorylation site before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, the levels of NPM1 or phosphorylated NPM1 at any phosphorylation site before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, the levels of NPM1 or phosphorylated NPM1 at any phosphorylation site before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are increased. In some embodiments, the levels of NPM1 or phosphorylated NPM1 at any phosphorylation site before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced.

**[00125]** In some embodiments, a change in the levels of NPM1 or phosphorylated NPM1 at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, is indicative/predictive that a subject having or at risk of developing cancer has responded to treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof. In some embodiments, the levels of NPM1 or phosphorylated NPM1 at any phosphorylation site at two different timepoints

during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by at least 10%. In some embodiments, the levels of NPM1 or phosphorylated NPM1 at any phosphorylation site at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, the levels of NPM1 or phosphorylated NPM1 at any phosphorylation site at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, the levels of NPM1 or phosphorylated NPM1 at any phosphorylation site at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are increased. In some embodiments, the levels of NPM1 or phosphorylated NPM1 at any phosphorylation site at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced.

**[00126]** In some embodiments, the levels of phosphorylated NPM1 at Thr199 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by at least 10%. In some embodiments, the levels of phosphorylated NPM1 at Thr199 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, the levels of phosphorylated NPM1 at Thr199 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, the levels of phosphorylated NPM1 at Thr199 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are increased.

**[00127]** In some embodiments, the levels of phosphorylated NPM1 at Thr199 at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by at least 10%. In some embodiments, the levels of phosphorylated NPM1 at Thr199 at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, the levels of phosphorylated NPM1 at Thr199 at two different timepoints during treatment are changed by about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, the levels of phosphorylated NPM1 at Thr199 at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are increased. In some embodiments, the levels of phosphorylated NPM1 at Thr199 at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced.

[00128] In some embodiments, the levels of phosphorylated NPM1 at Thr199 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced. In some embodiments, the levels of phosphorylated NPM1 at Thr199 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, the levels of phosphorylated NPM1 at Thr199 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 10%. In some embodiments, the levels of phosphorylated NPM1 at Thr199 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 20%. In some embodiments, the levels of phosphorylated NPM1 at Thr199 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 30%. In some embodiments, the levels of phosphorylated NPM1 at Thr199 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 40%. In some embodiments, the levels of phosphorylated NPM1 at Thr199 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 50%. In some embodiments, the levels of phosphorylated NPM1 at Thr199 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 60%. In some embodiments, the levels of phosphorylated NPM1 at Thr199 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 70%. In some embodiments, the levels of phosphorylated NPM1 at Thr199 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 80%. In some embodiments, the levels of phosphorylated NPM1 at Thr199 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 90%. In some embodiments, the levels of phosphorylated NPM1 at Thr199 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 100%.

*Thymidine Kinase 1 (TK1)*

[00129] In some embodiments, the contemplated biomarker is TK1 (thymidine kinase 1). TK1 is a direct downstream target of Rb-E2F pathway. It is involved in cellular proliferation through the recovery of the nucleotide thymidine in the DNA salvage pathway. TK1 is important for DNA repair following DNA damage because TK1 is necessary for the formation of nucleotides outside of the S phase. In some embodiments, the levels of TK1 are modulated in response to administration of an effective dose of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, to a subject. In some embodiments, TK1 is absent. In some embodiments, the loss of TK1 is a biomarker. In some embodiments, TK1 has a mutation (e.g., a loss of function mutation or a resistance mutation). In some embodiments, TK1 or a TK1 mutant is functional. In some embodiments, a TK1 mutant has a resistance mutation. In some embodiments, TK1 is differentially methylated. In some embodiments,

TK1 is serum TK1. In some embodiments, TK1 is a biomarker for CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, sensitivity.

**[00130]** In some embodiments, a change in the levels of TK1 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, is indicative/predictive that a subject having or at risk of developing a cancer has responded to treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof. In some embodiments, the levels of TK1 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by at least 10%. In some embodiments, the levels of TK1 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, the levels of TK1 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, the levels of TK1 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are increased.

**[00131]** In some embodiments, a change in the levels of TK1 at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, is indicative/predictive that a subject having or at risk of developing cancer has responded to treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof. In some embodiments, the levels of TK1 at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by at least 10%. In some embodiments, the levels of TK1 at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, the levels of TK1 at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are changed by about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, the levels of TK1 at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are increased.

**[00132]** In some embodiments, the levels of TK1 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced. In some embodiments, the levels of TK1 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, the levels of TK1 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 10%. In some embodiments, the levels of TK1 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 20%. In some

embodiments, the levels of TK1 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 30%. In some embodiments, the levels of TK1 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 40%. In some embodiments, the levels of TK1 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 50%. In some embodiments, the levels of TK1 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 60%. In some embodiments, the levels of TK1 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 70%. In some embodiments, the levels of TK1 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 80%. In some embodiments, the levels of TK1 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 90%. In some embodiments, the levels of TK1 before and after administration of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 100%.

**[00133]** In some embodiments, the levels of TK1 at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced. In some embodiments, the levels of TK1 at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In some embodiments, the levels of TK1 at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 10%. In some embodiments, the levels of TK1 at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 20%. In some embodiments, the levels of TK1 at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 30%. In some embodiments, the levels of TK1 at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 40%. In some embodiments, the levels of TK1 at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 50%. In some embodiments, the levels of TK1 at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 60%. In some embodiments, the levels of TK1 at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 70%. In some embodiments, the levels of TK1 at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 80%. In some embodiments, the levels of TK1 at two different timepoints during treatment with a CDK2 inhibitor, or a

pharmaceutically acceptable salt thereof, are reduced by at least 90%. In some embodiments, the levels of TK1 at two different timepoints during treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, are reduced by at least 100%.

#### *Biological Samples*

**[00134]** Suitable biological samples for the methods described herein include any sample that contains blood or tumor cells obtained or derived from the human subject in need of treatment. For example, a biological sample can contain tumor cells from biopsy from a patient suffering from a solid tumor. A tumor biopsy can be obtained by a variety of means known in the art. Alternatively, a blood sample can be obtained from a patient suffering from a hematological cancer.

**[00135]** A biological sample can be obtained from a human subject having, suspected of having, or at risk of developing, endometrial cancer or ovarian cancer.

**[00136]** Methods for obtaining and/or storing samples that preserve the activity or integrity of molecules (e.g., nucleic acids or proteins) in the sample are well known to those skilled in the art. For example, a biological sample can be further contacted with one or more additional agents such as buffers and/or inhibitors, including one or more of nuclease, protease, and phosphatase inhibitors, which preserve or minimize changes in the molecules in the sample.

#### *CDK2 Inhibitors*

#### **Definitions**

**[00137]** The term “alkyl” used alone or as part of a larger moiety, such as “alkoxy” and the like, refers to a saturated aliphatic straight-chain or branched monovalent hydrocarbon radical. Unless otherwise specified, an alkyl group typically has 1, 2, 3, 4, 5, or 6 carbon atoms, *i.e.* C<sub>1</sub>-C<sub>6</sub>alkyl. As used herein, a “C<sub>1</sub>-C<sub>6</sub>alkyl” group means a radical having from 1 to 6 carbon atoms in a linear or branched arrangement. Examples include methyl, ethyl, *n*-propyl, *iso*-propyl, and the like.

**[00138]** The term “alkoxy” refers to an alkyl radical attached through an oxygen linking atom, represented by –O–alkyl. For example, “C<sub>1</sub>-C<sub>4</sub>alkoxy” includes methoxy, ethoxy, propoxy, and butoxy.

**[00139]** The term “aromatic ring system” is art-recognized and refers to a monocyclic, bicyclic or polycyclic hydrocarbon ring system, wherein at least one ring is aromatic.

**[00140]** The term “aryl” refers to a radical of a 6- to 12-membered aromatic ring system. Representative aryl groups include fully aromatic ring systems, such as phenyl, cyclooctatetraene, indene, and naphthyl, and ring systems where an aromatic carbon ring is fused to one or more non-aromatic carbon rings, such as indanyl, phthalimidyl, naphthimidyl, or tetrahydronaphthyl, and the like. The number of ring members designates the number of ring members in the fused ring system. An aryl group may be described as, e.g., a 6-10-membered aryl,

wherein the term “membered” refers to the non-hydrogen ring atoms within the moiety.

**[00141]** The term “cycloalkyl” refers to a saturated hydrocarbon ring system. Unless otherwise specified, a cycloalkyl group typically has 3, 4, 5, 6, 7, 8, 9, or 10 carbon atoms, *i.e.* C<sub>3</sub>-C<sub>10</sub>cycloalkyl. In some embodiments, cycloalkyl has from 3-6 carbon atoms. For example, a C<sub>3</sub>-C<sub>10</sub>cycloalkyl includes cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, cyclononyl, cyclodecyl, bicyclo[1.1.1]pentane, bicyclo[2.1.1]hexane, bicyclo[2.2.1]heptane, bicyclo[3.1.1]heptane, bicyclo[3.2.1]octane, bicyclo[4.1.1]octane, spiro[2.2]pentane, spiro[2.3]hexane, spiro[2.4]heptane, spiro[2.5]octane, spiro[2.6]nonane, spiro[2.7]decane, spiro[3.3]heptane, spiro[3.4]octane, spiro[3.5]nonane, spiro[3.6]decane, spiro[4.4]nonane, spiro[4.5]decane, and the like.

**[00142]** The term “cycloalkoxy” refers to a -O-cycloalkyl group.

**[00143]** The term “halo” refers to a halogen and includes chloro, fluoro, bromo and iodo.

**[00144]** The term “heteroaryl” refers to a radical of a 4- to 12-membered monocyclic or bicyclic aromatic ring system having ring carbon atoms and 1 to 4 ring heteroatoms, wherein each heteroatom is independently selected from oxygen, sulfur, nitrogen, and quaternary nitrogen. In some embodiments, a heteroaryl group is a 5- to 6-membered heteroaryl having ring carbon atoms and 1 to 4 ring heteroatoms (typically 1 to 2). In heteroaryl groups that contain one or more nitrogen atoms, the point of attachment can be a carbon or nitrogen atom, as valency permits. Heteroaryl bicyclic ring systems can include one or more heteroatoms in one or both rings. “Heteroaryl” also includes ring systems wherein the heteroaryl ring is fused with one or more aryl groups, wherein the point of attachment is either on the aryl or heteroaryl ring, and in such instances, the number of ring members designated the number of ring members in the fused (aryl/heteroaryl) ring system. Representative heteroaryl groups include ring systems where each ring comprises a heteroatom and is aromatic, *e.g.*, imidazolyl, oxazolyl, thiazolyl, triazolyl, pyrrolyl, furanyl, thiophenyl pyrazolyl, tetrazolyl, pyridinyl, pyrazinyl, pyridazinyl, pyrimidinyl, triazinyl, indoyl, indolizinyl, benzothiophenyl, purinyl, pyrido[4,3-d]pyrimidine, naphthyl, naphthyridinyl, quinazolinyl, oxadiazolyl, thiadiazolyl, cinnoliny, indazyl, and pteridinyl.

**[00145]** The term “heterocyclyl” or “heterocyclic” refers to a radical of a 3- to 12-membered non-aromatic ring system having ring carbon atoms and 1 to 4 ring heteroatoms, wherein each heteroatom is independently selected from oxygen, sulfur, nitrogen, and quaternary nitrogen (“3- to 12-membered heterocyclyl”). In some embodiments, a heterocyclyl group is a 4- to 12-membered non-aromatic ring system having ring carbon atoms and 1 to 4 (typically 1 to 2) ring heteroatoms, wherein each heteroatom is independently selected from oxygen, sulfur, nitrogen, and quaternary nitrogen (“4- to 12-membered heterocyclyl”). In some embodiments, a heterocyclyl group is a 3- to 6-membered non-aromatic ring system having ring carbon atoms and 1 to 4 (typically 1 to 2) ring heteroatoms, wherein each heteroatom is independently selected from oxygen, sulfur, nitrogen, and quaternary nitrogen (“3-

to 6-membered heterocyclyl”). In heterocyclyl groups that contain one or more nitrogen atoms, the point of attachment can be a carbon or nitrogen atom, as valency permits. Exemplary heterocyclyl groups include azetidiny, oxetanyl, thietanyl, tetrahydrofuranyl, pyrrolidinyl, pyrrolidin-2-onyl, piperidinyl, tetrahydropyranyl, piperazinyl, morpholinyl, azepanyl, oxepanyl, thiepanyl, tetrahydropyridinyl, 2-oxabicyclo[2.1.1]hexane, 5-oxabicyclo[2.1.1]hexane, 3-oxabicyclo[3.1.0]hexane, 2-oxabicyclo[2.1.1]heptane, 7-oxabicyclo[2.2.1]heptane, 3-oxabicyclo[3.1.1]heptane, 6-oxabicyclo[3.1.1]heptane, 2-oxabicyclo[2.2.2]octane, 7-oxabicyclo[4.1.1]octane, 8-oxabicyclo[3.2.1]octane, and the like.

**[00146]** The term “pharmaceutically acceptable salt” refers to a pharmaceutical salt that is, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, and allergic response, and is commensurate with a reasonable benefit/risk ratio. For example, S. M. Berge *et al.* describes pharmacologically acceptable salts in *J. Pharm. Sci.*, 1977, 66, 1–19.

**[00147]** In some embodiments, in the methods of treatment disclosed herein, a CDK2 inhibitor is administered as the free base. In some embodiments, in the methods of treatment disclosed herein, a pharmaceutically acceptable salt of a CDK2 inhibitor is administered.

**[00148]** A CDK2 inhibitor has a biological effect to inhibit or significantly reduce or down-regulate the biological activity of the CDK2 gene and/or CDK2 protein. A CDK2 inhibitor can be used to treat endometrial cancer or ovarian cancer. As used herein, the term “selective CDK2 inhibitor” means a compound which selectively inhibits CDK2 over other CDKs and the kinome. Said another way, a selective CDK2 inhibitor has no or low activity against other CDKs, most notably CDK1, and the kinome. A selective CDK2 inhibitor’s inhibitory activity against CDK2 is more potent in terms of IC<sub>50</sub> value (*i.e.*, the IC<sub>50</sub> value is subnanomolar) when compared with its inhibitory activity against other CDKs and many other kinases. Potency can be measured using known biochemical assays. Advantages associated with such selectivity may include facilitating efficacious dosing and reducing CDK1-mediated on-target toxicities. A CDK2 inhibitor has a favorable toxicity profile related to other non-kinase targets.

**[00149]** Comparison of the CDK2 and CDK1 active-sites showed L32, H84, and Q85 are CDK2-specific, while these residues are M32, S84, and M85 in CDK1. However, these residues are located outside of the catalytic pocket and are inaccessible for binding within the ATP-binding pocket, resulting in very high homology in the active site between these two enzymes. This high homology created a significant challenge for finding a CDK2 inhibitor with high levels of selectivity over CDK1.

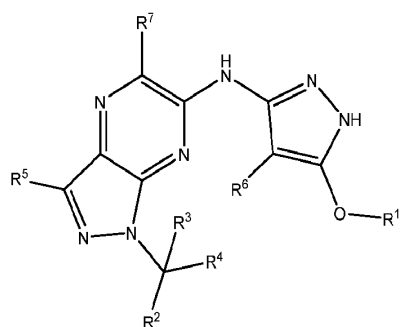
**[00150]** A CDK2 inhibitor is selective against CDK2 versus CDK1. In some such embodiments, a CDK2 inhibitor shows at least 10-fold selectivity for CDK2 versus CDK1. In other embodiments, a CDK2 inhibitor shows at least 20-fold selectivity for CDK2 versus CDK1. In specific embodiments, a

CDK2 inhibitor shows at least 30-fold selectivity for CDK2 versus CDK1. In certain embodiments, a CDK2 inhibitor shows at least 40-fold selectivity for CDK2 versus CDK1. In other embodiments, a CDK2 inhibitor shows at least 50-fold selectivity for CDK2 versus CDK1. A CDK2 inhibitor is selective against CDK2 versus CDK4 and/or CDK6. In some such embodiments, a CDK2 inhibitor shows at least 10-fold selectivity for CDK2 versus CDK4 and/or CDK6. In other embodiments, a CDK2 inhibitor shows at least 20-fold selectivity for CDK2 versus CDK4 and/or CDK6. In specific embodiments, a CDK2 inhibitor shows at least 30-fold selectivity for CDK2 versus CDK4 and/or CDK6.

**[00151]** A CDK2 inhibitor has the advantage of good metabolic stability. One indicator of good metabolic stability is high microsomal stability. Hepatic metabolism is a predominant route of elimination for small molecule drugs. The clearance of compounds by hepatic metabolism can be assessed in vitro using human liver microsomes (HLMs) or human hepatocytes. Compounds are incubated with HLMs plus appropriate co-factors or human hepatocytes and compound depletion is measured to determine an in vitro intrinsic clearance (Cl<sub>int</sub>). The Cl<sub>int</sub> is scaled to total body clearance (CL), and a hepatic extraction ratio (ER) is determined by dividing CL to standard human hepatic blood flow. Compounds that have a low hepatic extraction ratio are considered to have good metabolic stability. In some embodiments, a CDK2 inhibitor has a calculated ER of <0.3, <0.4, <0.5, <0.6.

**[00152]** Several selective CDK2 inhibitors have shown promise either in pre-clinical studies or in early clinical development, including ARTS-021, INCB123667, INX-315, PF-7104091, and BLU-222 among others (Liang, J. *et al.*, Cancer Res. (2022), 82(12 Supplement): 2568; Chand, S. *et al.*, Cancer Res. (2022), 83(7 Supplement): 1143; Dietrich, C. (2023); Hoffman, R.L. *et al.*, “New Drugs on the Horizon sessions offer first disclosures of novel agents, Part 3: PF-07104091: A CDK2 selective inhibitor for the treatment of cyclinE amplified cancers”, AACR Annual Meeting News (2021), online). Cancers with evidence of CDK2 dependencies are a focus for monotherapy strategies, including *CCNE1* amplified cancers, *C-MYC* overexpressing colon cancers, and *KRAS* mutant lung cancers (Tadesse, S. (2020)). In addition to the potential for CDK2 inhibitors as monotherapy, combination with other anti-cancer agents has been explored in pre-clinical models, with synergistic effects being observed in combinations with radiation, taxanes, and PI3K inhibitors. However, the results are confounded by the use of non-selective CDK2 inhibitors (Tadesse, S. (2020)). A promising strategy with extensive pre-clinical evidence is combining CDK2 inhibition with CDK4/6 inhibition in hormone-dependent breast cancer as a means to overcome CDK4/6 inhibitor resistance (Pandy, K. (2020)). Biomarkers of response to combination therapy remain to be identified, but is critical for a precision oncology approach that will capture sensitive populations.

**[00153]** In some embodiments, the CDK2 inhibitor is represented by a compound of Formula (I):



(I), or a pharmaceutically acceptable salt thereof, wherein

R<sup>1</sup> is C<sub>1</sub>-C<sub>4</sub>alkyl optionally substituted with 1 to 4 groups each independently selected from halo and D;

R<sup>2</sup> is C<sub>1</sub>-C<sub>4</sub>alkyl or Ring A, wherein the C<sub>1</sub>-C<sub>4</sub>alkyl is optionally substituted with 1 to 4 groups each independently selected from halo, D, CN, and OH and/or 1 group of 5- to 6-membered heteroaryl having 1 to 3 ring heteroatoms each independently selected from O, S, N, and NR<sup>d</sup>;

R<sup>3</sup> is selected from H, D, C<sub>1</sub>-C<sub>4</sub>alkyl, C<sub>3</sub>-C<sub>10</sub>cycloalkyl, and 4- to 12-membered heterocyclyl, wherein the C<sub>1</sub>-C<sub>4</sub>alkyl and C<sub>3</sub>-C<sub>10</sub>cycloalkyl are each optionally substituted with 1 to 4 R<sup>c</sup>, wherein the 4- to 12-membered heterocyclyl has 1 to 4 ring heteroatoms each independently selected from O, S, N, and NR<sup>d</sup> and then is optionally substituted on a ring carbon with 1 to 4 R<sup>c</sup>; or

R<sup>2</sup> and R<sup>3</sup> are taken together with the carbon atom to which they are attached to form Ring B, wherein Ring B is C<sub>3</sub>-C<sub>10</sub>cycloalkyl or 4- to 12- membered heterocyclyl, wherein the C<sub>3</sub>-C<sub>10</sub>cycloalkyl is optionally substituted with 1 to 4 R<sup>b</sup>, wherein the 4- to 12- membered heterocyclyl has 1 to 4 ring heteroatoms each independently selected from O, S, N, and NR<sup>d</sup> and then is optionally substituted on a ring carbon by 1 to 4 R<sup>b</sup>;

Ring A is selected from C<sub>3</sub>-C<sub>10</sub>cycloalkyl, phenyl, naphthyl, 4- to 12-membered heterocyclyl, and 4- to 12-membered heteroaryl, wherein the C<sub>3</sub>-C<sub>10</sub>cycloalkyl, phenyl, and naphthyl are each optionally substituted with 1 to 4 R<sup>a</sup>, wherein the 4- to 12-membered heterocyclyl and 4- to 12-membered heteroaryl have 1 to 4 ring heteroatoms each independently selected from O, S, N, and NR<sup>d</sup> and then are optionally substituted on a ring carbon with 1 to 4 R<sup>a</sup>;

Each R<sup>a</sup> is independently selected from D, halo, OH, CN, C<sub>1</sub>-C<sub>4</sub>alkyl, and C<sub>1</sub>-C<sub>4</sub>alkoxy, or two R<sup>a</sup>, attached to the same atom, form a =O, wherein the C<sub>1</sub>-C<sub>4</sub>alkyl and C<sub>1</sub>-C<sub>4</sub>alkoxy are each optionally substituted with 1 to 4 groups each independently selected from halo, OH and CN;

Each R<sup>b</sup> is independently selected from D, halo, OH, CN, C<sub>1</sub>-C<sub>4</sub>alkyl, and C<sub>1</sub>-C<sub>4</sub>alkoxy, or two R<sup>b</sup>, attached to the same atom, form a =O, wherein the C<sub>1</sub>-C<sub>4</sub>alkyl and C<sub>1</sub>-C<sub>4</sub>alkoxy are each optionally substituted with 1 to 4 groups each independently selected from halo, OH and CN;

Each R<sup>c</sup> is independently selected from D, halo, OH, CN, C<sub>1</sub>-C<sub>4</sub>alkyl, and C<sub>1</sub>-C<sub>4</sub>alkoxy, or two R<sup>c</sup>, attached to the same atom, form a =O, wherein the C<sub>1</sub>-C<sub>4</sub>alkyl and C<sub>1</sub>-C<sub>4</sub>alkoxy are each optionally substituted with 1 to 4 groups each independently selected from halo, OH, and CN;

Each R<sup>d</sup> is independently selected from H, D, and C<sub>1</sub>-C<sub>6</sub>alkyl;

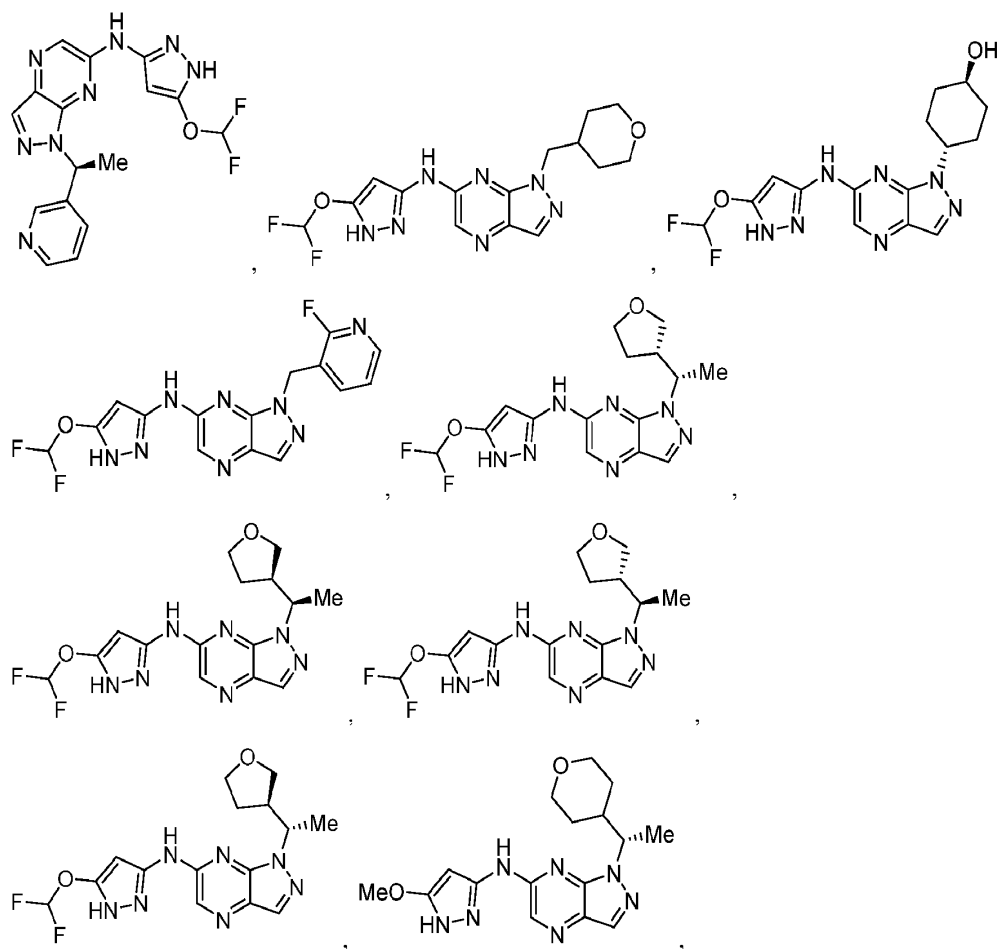
$R^4$  is selected from H, D, and  $C_1$ - $C_4$ alkyl optionally substituted with 1 to 4 groups each independently selected from halo, D and OH;

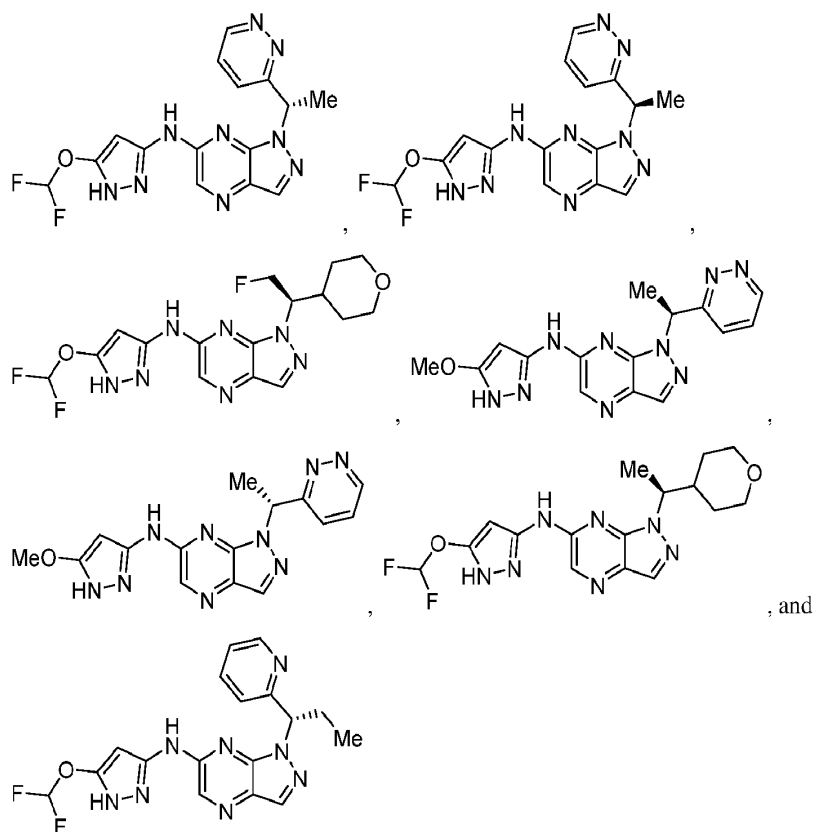
$R^5$  is selected from H, D, halo, CN, and  $C_1$ - $C_4$ alkyl, wherein the  $C_1$ - $C_4$ alkyl is optionally substituted with 1 to 4 groups each independently selected from halo and OH;

$R^6$  is H or D; and

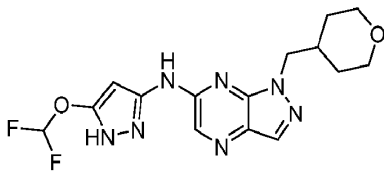
$R^7$  is H or D.

**[00154]** In some embodiments, the CDK2 inhibitors are compounds of Formula (I) and are described in International Application Publication No. WO 2023/278326, the entire teachings of which are incorporated herein by reference. In some embodiments, the CDK2 inhibitor is a compound, or a pharmaceutically acceptable salt thereof, selected from:



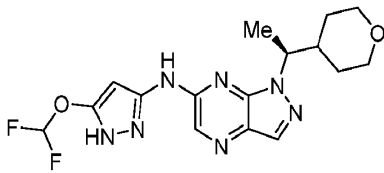


**[00155]** In some embodiments, the CDK2 inhibitor is a compound, which is N-(5-(difluoromethoxy)-1H-pyrazol-3-yl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-pyrazolo[3,4-b]pyrazin-6-amine (also referred to herein as “Compound 1” or “BLU-222”):



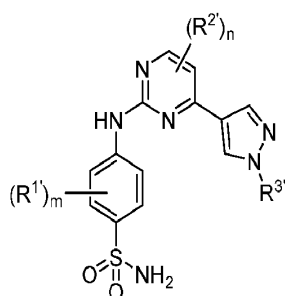
or a pharmaceutically acceptable salt thereof.

**[00156]** In some embodiments, the CDK2 inhibitor is a compound, which is (S)-N-(5-(difluoromethoxy)-1H-pyrazol-3-yl)-1-(1-(tetrahydro-2H-pyran-4-yl)ethyl)-1H-pyrazolo[3,4-b]pyrazin-6-amine (also referred to herein as “Compound 2”):



or a pharmaceutically acceptable salt thereof.

[00157] In some embodiments, the CDK2 inhibitor is represented by a compound of Formula (II):



(II), or a pharmaceutically acceptable salt thereof, wherein

each R<sup>1'</sup> is independently selected from halo, OH, CN, C<sub>1</sub>-C<sub>4</sub>alkyl, and C<sub>1</sub>-C<sub>4</sub>alkoxy, wherein the C<sub>1</sub>-C<sub>4</sub>alkyl and C<sub>1</sub>-C<sub>4</sub>alkoxy are each optionally substituted with 1 to 3 halo;

each R<sup>2'</sup> is independently selected from halo, OH, CN, C<sub>1</sub>-C<sub>4</sub>alkyl, and C<sub>1</sub>-C<sub>4</sub>alkoxy, wherein the C<sub>1</sub>-C<sub>4</sub>alkyl and C<sub>1</sub>-C<sub>4</sub>alkoxy are each optionally substituted with 1 to 3 halo;

R<sup>3'</sup> is C<sub>1</sub>-C<sub>6</sub>alkyl optionally substituted with 1 or 2 groups each independently selected from halo, OH, C<sub>3</sub>-C<sub>6</sub>cycloalkyl, and 3- to 6-membered heterocyclyl, wherein the C<sub>3</sub>-C<sub>6</sub>cycloalkyl is optionally substituted with OH, wherein the 3- to 6-membered heterocyclyl has 1 to 4 ring heteroatoms each independently selected from O, S, N, and NR<sup>a</sup> and then is optionally substituted on a ring carbon with OH; or

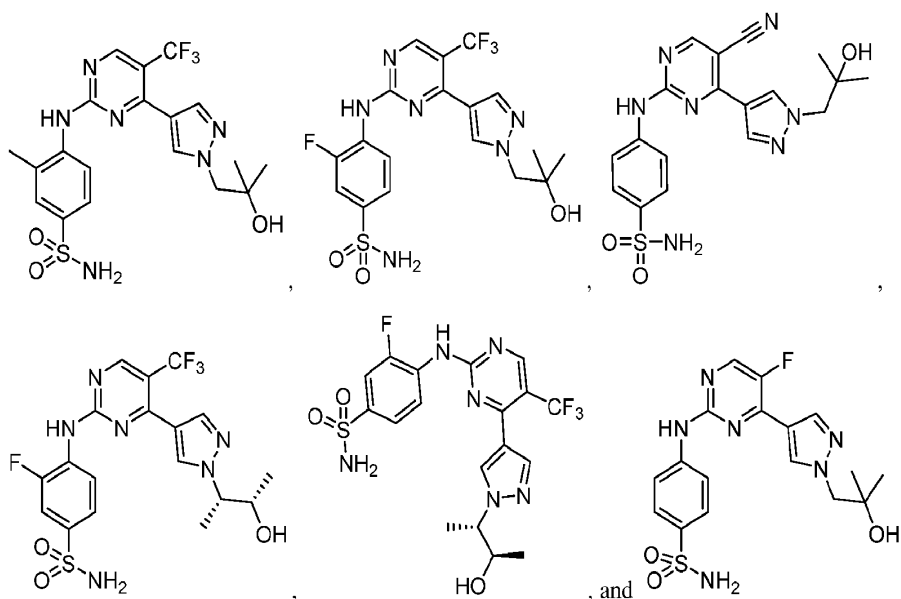
R<sup>3'</sup> is C<sub>3</sub>-C<sub>6</sub>cycloalkyl or 3- to 6-membered heterocyclyl, wherein the C<sub>3</sub>-C<sub>6</sub>cycloalkyl is optionally substituted with OH or -CH<sub>2</sub>OH, wherein the 3- to 6-membered heterocyclyl has 1 to 4 ring heteroatoms each independently selected from O, S, N, and NR<sup>a</sup> and then is optionally substituted on a ring carbon with OH or -CH<sub>2</sub>OH;

each R<sup>a</sup> is independently H or C<sub>1</sub>-C<sub>6</sub>alkyl;

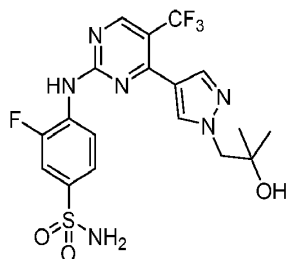
m is selected from 0, 1, 2, 3, and 4; and

n is selected from 0, 1, and 2.

[00158] In some embodiments, the CDK2 inhibitors are compounds of Formula (II) and are described in International Application Publication No. WO 2022/266190, the entire teachings of which are incorporated herein by reference. In some embodiments, the CDK2 inhibitor is selected from:

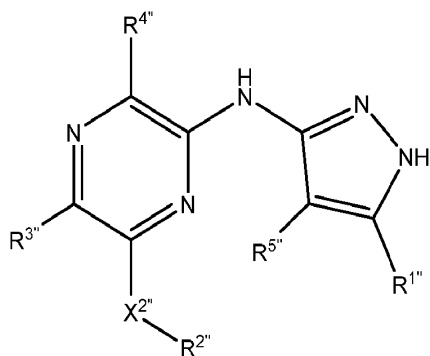


**[00159]** In some embodiments, the CDK2 inhibitor is a compound, which is 3-fluoro-4-((4-(1-(2-hydroxy-2-methylpropyl)-1H-pyrazol-4-yl)-5-(trifluoromethyl)pyrimidin-2-yl)amino)benzenesulfonamide (also referred to herein as “Compound 3”):



or a pharmaceutically acceptable salt thereof.

**[00160]** In some embodiments, the CDK2 inhibitor is represented by a compound of Formula (III):



(III), or a pharmaceutically acceptable salt thereof,

wherein

- $R^{1''}$  is selected from the group consisting of CN,  $C_1$ - $C_4$ alkyl,  $C_1$ - $C_4$ alkoxy,  $-(C(R^{d1})_2)_{p''}$ - $C_3$ - $C_{10}$ cycloalkyl,  $-O(C(R^{d1})_2)_{p''}$ - $C_3$ - $C_{10}$ cycloalkyl,  $-(C(R^{d1})_2)_{p''}$ -4 to 12-membered heterocyclyl,  $-O(C(R^{d1})_2)_{p''}$ -4 to 12-membered heterocyclyl,  $-(C(R^{d1})_2)_{p''}$ -6 to 12-membered aryl, and  $-(C(R^{d1})_2)_{p''}$ -4 to 12-membered heteroaryl, wherein the  $C_1$ - $C_4$ alkyl,  $C_1$ - $C_4$ alkoxy,  $C_3$ - $C_{10}$ cycloalkyl,  $C_3$ - $C_{10}$ cycloalkoxy, 6 to 12-membered aryl are each optionally substituted with 1 to 4  $R^{a1}$ , wherein the 4 to 12-membered heterocyclyl and 4 to 12-membered heteroaryl have 1 to 4 ring heteroatoms each independently selected from the group consisting of O, S, N, and  $NR^{b1}$  and then are optionally substituted on a ring carbon with 1 to 4  $R^{a1}$ ;
- $X^{2''}$  is selected from the group consisting of  $-O-$ ,  $-(C(R^{d2})_2)_{n''}$ -,  $-NR^{b2}$ -,  $-NR^{b2}-(C(R^{c1})_2)_{m''}$ -,  $-O(C(R^{c1})_2)_{m''}$ -, and  $-(C(R^{d2})_2)_{n''}$ - $O-$ ;
- $R^{2''}$  is selected from  $C_1$ - $C_4$ alkyl and Ring A, wherein the  $C_1$ - $C_4$ alkyl is optionally substituted with 1 to 4 groups each independently selected from the group consisting of D, halo, CN, and OH and/or 1 group of 5 to 6 membered heteroaryl having 1 to 3 ring heteroatoms each independently selected from the group consisting of O, S, N, and  $NR^{b3}$ ; or
- $X^{2''}$  and  $R^{2''}$  together form Ring B bonded to the pyrazine, wherein Ring B is  $C_3$ - $C_{10}$ cycloalkyl or 4 to 12-membered heterocyclyl, wherein the  $C_3$ - $C_{10}$ cycloalkyl is optionally substituted with 1 to 4  $R^{a2}$ , wherein the 4 to 12-membered heterocyclyl has 1 to 4 ring heteroatoms each independently selected from the group consisting of O, S, N, and  $NR^{b4}$ , and then is optionally substituted on a ring carbon with 1 to 4  $R^{a2}$ ;
- Ring A'' is selected from the group consisting of  $C_3$ - $C_{10}$ cycloalkyl, 4 to 12-membered heterocyclyl, 6 to 12-membered aryl, and 4 to 12-membered heteroaryl, wherein the  $C_3$ - $C_{10}$ cycloalkyl and 6 to 12-membered aryl are each optionally substituted with 1 to 4  $R^{a3}$ , wherein the 4 to 12-membered heterocyclyl and 4 to 12-membered heteroaryl have 1 to 4 ring heteroatoms each independently selected from the group consisting of O, S, N, and  $NR^{b5}$  and then is optionally substituted on a ring carbon with 1 to 4  $R^{a3}$ ;
- $R^{3''}$  is selected from the group consisting of H, D, halo, OH, CN,  $C_1$ - $C_4$ alkyl,  $-SO_2C_1$ - $C_4$ alkyl, and  $-S(O)NR^{b6}$ - $C_1$ - $C_4$ alkyl;
- $R^{4''}$  is selected from the group consisting of H, D,  $C_1$ - $C_4$ alkyl, and  $C_3$ - $C_{10}$ cycloalkyl;
- $R^{5''}$  is selected from the group consisting of H, D, and  $C_1$ - $C_4$ alkyl;
- Each  $R^{a1}$ ,  $R^{a2}$ , and  $R^{a3}$  is independently selected from the group consisting of D, halo, OH, CN,  $N(R^{b7})_2$ ,  $C_1$ - $C_4$ alkyl, and  $C_1$ - $C_4$ alkoxy, or two  $R^a$  attached to the same atom, form a  $=O$ , wherein the  $C_1$ - $C_4$ alkyl and  $C_1$ - $C_4$ alkoxy are each optionally substituted with 1 to 4 groups each independently selected from the group consisting of D, halo, OH, and CN;
- Each  $R^{b1}$ ,  $R^{b2}$ ,  $R^{b3}$ ,  $R^{b4}$ ,  $R^{b5}$ ,  $R^{b6}$ ,  $R^{b7}$ , and  $R^{b8}$  is independently selected from the group consisting of H, D, and  $C_1$ - $C_4$ alkyl, wherein the  $C_1$ - $C_4$ alkyl is optionally substituted with 1 to 4 D;

Each  $R^{c1}$  is independently selected from the group consisting of H, D,  $C_1$ - $C_4$ alkyl,  $C_1$ - $C_4$ alkoxy,  $C_3$ - $C_{10}$ cycloalkyl, and 4 to 12-membered heterocyclyl, wherein the  $C_1$ - $C_4$ alkyl,  $C_1$ - $C_4$ alkoxy, and  $C_3$ - $C_{10}$ cycloalkyl are each optionally substituted with 1 to 4 groups each independently selected from the group consisting of halo, OH, and CN, wherein the 4 to 12-membered heterocyclyl has 1 to 4 ring heteroatoms each independently selected from the group consisting of O, S, N, and  $NR^{b8}$  and then is optionally substituted on a ring carbon with 1 to 4 groups each independently selected from the group consisting of halo, OH, and CN;

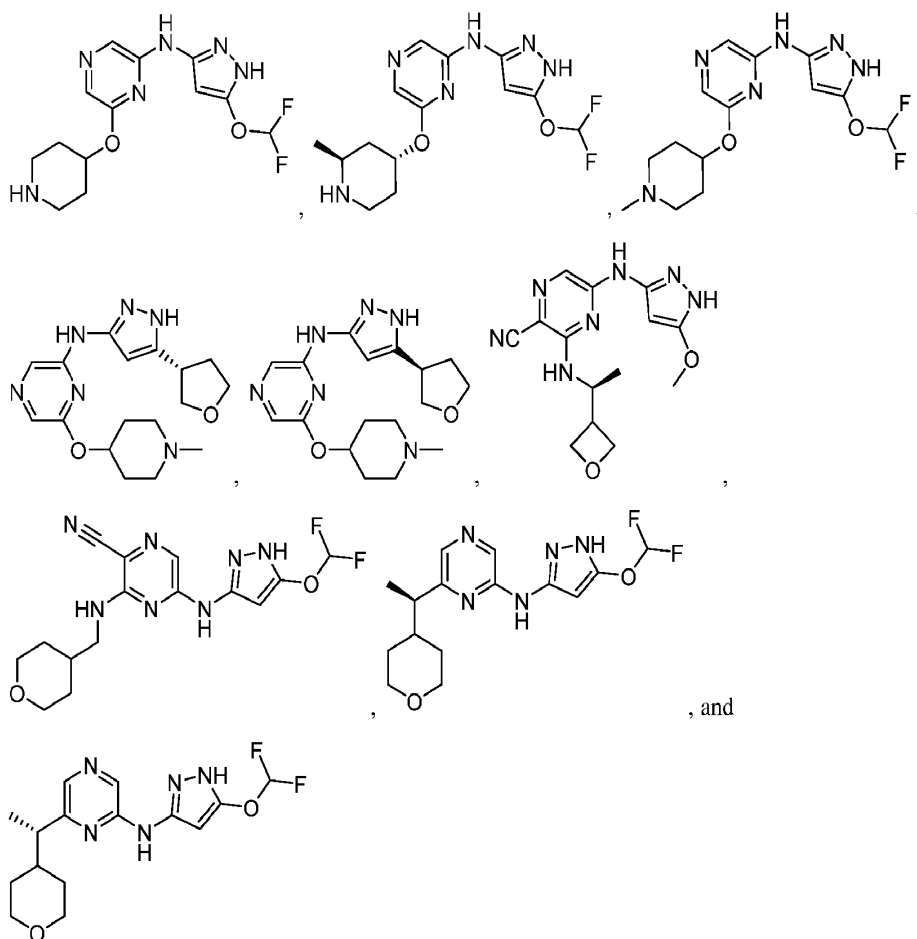
Each  $R^{d1}$  and  $R^{d2}$  is independently selected from the group consisting of H, D, and  $C_1$ - $C_4$ alkyl;

$m''$  is 1 to 4;

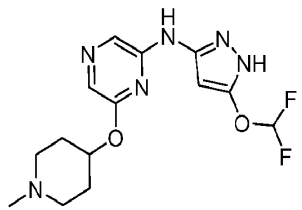
$n''$  is 1 to 4; and

$p''$  is 0 to 3.

**[00161]** In some embodiments, the CDK2 inhibitors are compounds of Formula (III) and are described in International Application Publication No. WO 2024/216154, the entire teachings of which are incorporated herein by reference. In some embodiments, the CDK2 inhibitor is selected from:



[00162] In some embodiments, the CDK2 inhibitor is a compound, which is N-(5-(difluoromethoxy)-1H-pyrazol-3-yl)-6-((1-methylpiperidin-4-yl)oxy)pyrazin-2-amine (also referred to herein as “Compound 4”):



or a pharmaceutically acceptable salt thereof.

[00163] In some embodiments, the CDK2 inhibitor is selected from 7-hydroxy-staurosporine, abemaciclib, AG-24322, alvocidib (also referred to as flavopiridol), AMG 925, AT7519, AVZO-021 (also referred to as ARTS-021), AZD5438, AZD8421, BG-68501 (also referred to as ENS-791), BLU-222 (also referred to as Compound 1), Compound 2, Compound 3, Compound 4, couropitine B, CVT-313, dinaciclib (also referred to as SCH727965), fadraciclib (also referred to as CYC-065), FN-1501, GW8510, INCB123667, INX-315, milciclib (also referred to as PHA-848125AC), NKT3447, NU6140, NUV-422, PHA-690509, PHA-793887, PF-06873600, PF-07224826, R547, RGB-2886638, RGT-419B, RLY-2139, roniciclib (also referred to as BAY1000394), seliciclib (also referred to as CYC202 or (*R*)-roscovitine), SNS-032 (also referred to BMS-387032), SU9516, tagtociclib (also referred to as PF-07104091 or PF-4091), TG02, TP-1287, trilaciclib (also referred to as G1T28), voruciclib (also referred to as P1446A), and ZK-304709, or a pharmaceutically acceptable salt thereof. In some embodiments, the CDK2 inhibitor is selected from AVZO-021, AZD8421, BG-68501, BLU-222, Compound 2, Compound 3, Compound 4, fadraciclib, INCB123667, INX-315, NKT3447, PF-07224826, R547, RLY-2139, SNS-032, and tagtociclib. In some embodiments, the CDK2 inhibitor is selected from AVZO-021, AZD8421, BG-68501, BLU-222 (see NCT05252416), INCB123667, INX-315, NKT3447, PF-07224826, and tagtociclib, or a pharmaceutically acceptable salt thereof. In some embodiments, the CDK2 inhibitor is selected from BLU-222, Compound 2, Compound 3, and Compound 4, or a pharmaceutically acceptable salt thereof.

#### *Pharmaceutical Compositions*

[00164] Pharmaceutical compositions of the disclosure (also referred to herein as the “disclosed pharmaceutical compositions”) comprise one or more pharmaceutically acceptable carrier(s) or diluent(s) and a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof.

[00165] “Pharmaceutically acceptable carrier” and “pharmaceutically acceptable diluent” refer to a substance that aids the formulation and/or administration of an active agent to and/or absorption by a subject and can be included in the pharmaceutical compositions of the disclosure without causing a significant adverse toxicological effect on the subject. Non-limiting examples of pharmaceutically acceptable carriers and/or diluents include water, NaCl, normal saline solutions, lactated Ringer’s,

normal sucrose, normal glucose, binders, fillers, disintegrants, lubricants, coatings, sweeteners, flavors, salt solutions (such as Ringer's solution), alcohols, oils, gelatins, carbohydrates such as lactose, amylose or starch, hydroxymethylcellulose, fatty acid esters, polyvinyl pyrrolidone, and colors, and the like. Such preparations can be sterilized and, if desired, mixed with auxiliary agents such as lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, coloring, and/or aromatic substances and the like that do not deleteriously react with or interfere with the activity of a CDK2 inhibitor. One of ordinary skill in the art will recognize that other pharmaceutical excipients are suitable for use with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof.

**[00166]** The pharmaceutical compositions of the disclosure optionally include one or more pharmaceutically acceptable carriers and/or diluents therefor, such as lactose, starch, cellulose and dextrose. Other excipients, such as flavoring agents, sweeteners, and preservatives, such as methyl, ethyl, propyl and butyl parabens, can also be included. More complete listings of suitable excipients can be found in the Handbook of Pharmaceutical Excipients (5<sup>th</sup> Ed., Pharmaceutical Press (2005)). A person skilled in the art would know how to prepare formulations suitable for various types of administration routes. Conventional procedures and ingredients for the selection and preparation of suitable formulations are described, for example, in Remington's Pharmaceutical Sciences (2003 - 20th edition) and in The United States Pharmacopeia: The National Formulary (USP 24 NF19) published in 1999. The carriers, diluents and/or excipients are "acceptable" in the sense of being compatible with the other ingredients of the pharmaceutical composition and not deleterious to the recipient thereof.

**[00167]** The following examples are intended to be illustrative and are not intended to be limiting in any way to the scope of the disclosure.

## EXEMPLIFICATION

### General Methods

**[00168]** Cell lines and culture

**[00169]** Cell line and culturing information can be found in Table 1.

**[00170]** Table 1. Human cell lines and culture conditions

| Cell Line   | Lineage | Catalog No. | Complete Medium                                 | CN*  | CCNE1/<br>Rb/p16 |
|-------------|---------|-------------|-------------------------------------------------|------|------------------|
| A2780       | Ovary   | 93112519    | RPMI-1640 + 10% FBS                             | 2.0  | OE/+/-           |
| SK-OV-3     | Ovary   | HTB-77      | McCoy's 5A + 10%FBS                             | 2.1  | OE/+/-           |
| UWB1.289    | Ovary   | CRL-2945    | MGEM:1640+3%FBS                                 | 2.6  | OE/+/+           |
| NIH:OVCAR-3 | Ovary   | HTB-161     | RPMI-1640 + 0.01 mg/mL bovine insulin + 20% FBS | 12.3 | OE/+/+           |
| OVMANA      | Ovary   | JCRB1045    | RPMI-1640 + 10% FBS                             | 2.7  | OE/+/+           |

| Cell Line | Lineage     | Catalog No.  | Complete Medium                                                                               | CN*  | CCNEI/<br>Rb/p16 |
|-----------|-------------|--------------|-----------------------------------------------------------------------------------------------|------|------------------|
| OAW42     | Ovary       | 85073102     | DMEM + 2mM Glutamine + 1mM Sodium Pyruvate + 20 IU/l Bovine Insulin + 10% FBS                 | 2.0  | OE/+/-           |
| RMG-I     | Ovary       | IFO50315     | Ham's F12 + 10% FBS                                                                           | 3.8  | Normal/+/-       |
| OVK18     | Ovary       | RBRC-RCB1903 | MEM + 10% FBS                                                                                 | 1.9  | OE/+/-           |
| RMUG-S    | Ovary       | IFO50320     | Ham's F12 + 10% FBS                                                                           | 3.3  | OE/+/-           |
| COV434    | Ovary       | 7071909      | DMEM + 2mM Glutamine + 10% FBS                                                                | 2.1  | OE/+/+           |
| Caov-3    | Ovary       | HTB-75       | DMEM + 10%FBS                                                                                 | 1.9  | OE/-/+           |
| OV-90     | Ovary       | CRL-11732    | 1:1 MCDB 105 + 1.5 g/L sodium bicarbonate: Medium 199 + 2.2 g/L sodium bicarbonate, + 15% FBS | 3.2  | OE/+/-           |
| COV504    | Ovary       | 7071902      | DMEM + 2mM Glutamine + 10% FBS                                                                | 1.4  | Normal/+/-       |
| TOV-112D  | Ovary       | CRL-11731    | 1:1 MCDB 105 + 1.5 g/L sodium bicarbonate: Medium 199 + 2.2 g/L sodium bicarbonate, + 15% FBS | 1.7  | Normal/+/-       |
| OV56      | Ovary       | 96020759     | DMEM:HAMS F12 (1:1) + 2mM Glutamine + 5% FBS + 0.5 µg/mL hydrocortisone + 10µg/mL insulin     | 2.0  | Normal/+/-       |
| SNU-840   | Ovary       | 840          | RPMI 1640 + 10% FBS                                                                           | 2.4  | Normal/+/-       |
| OV7       | Ovary       | 96020764     | DMEM:HAMS F12 (1:1) + 2mM Glutamine + 5% FBS + 0.5 µg/mL hydrocortisone + 10µg/mL insulin     | 2.1  | Normal/-/+       |
| COV644    | Ovary       | 7071908      | DMEM + 2mM Glutamine + 10% FBS                                                                | 2.4  | Normal/+/-       |
| Kuramochi | Ovary       | JCRB0098     | RPMI-1640 + 10% FBS                                                                           | 3.8  | OE/+/+           |
| TOV-21G   | Ovary       | CRL-11730    | 1:1 MCDB 105 + 1.5 g/L sodium bicarbonate: Medium 199 + 2.2 g/L sodium bicarbonate, + 15% FBS | 2.0  | Normal/+/+       |
| COV362    | Ovary       | 7071910      | DMEM + 2mM Glutamine + 10% FBS                                                                | 2.1  | Normal/-/+       |
| OVISe     | Ovary       | JCRB1043     | RPMI-1640 + 10% FBS                                                                           | 3.4  | OE/+/+           |
| ES-2      | Ovary       | CRL-1978     | McCoy's 5A + 10%FBS                                                                           | 1.5  | Normal/+/+       |
| PA-1      | Ovary       | CRL-1572     | EMEM + 10% FBS                                                                                | 2    | Normal/+/+       |
| TYK-nu    | Ovary       | JCRB0234.0   | EMEM + 10% FBS                                                                                | 1.7  | Normal/+/-       |
| KGN       | Ovary       | RCB1154      | 1:1 DMEM:Ham F12 + 10% FBS                                                                    | 2.1  | Normal/+/-       |
| MCAS      | Ovary       | JCRB0240     | EMEM + 20% FBS                                                                                | 1.8  | Normal/+/-       |
| OVSAHO    | Ovary       | JCRB1046     | RPMI-1640 + 10% FBS                                                                           | 3.9  | OE/+/+           |
| OVTOKO    | Ovary       | JCRB1048     | RPMI-1640 + 10% FBS                                                                           | 1.8  | Normal/+/-       |
| FU-OV-1   | Ovary       | ACC444       | 1:1 DMEM:Ham F12 + 10% FBS                                                                    | 19.4 | OE/+/+           |
| COV318    | Ovary       | 7071903      | DMEM+ 10% FBS                                                                                 | 6.8  | OE/+/+           |
| 59M       | Ovary       | 89081802     | DMEM + 20 IU/l Bovine Insulin + 10% FBS                                                       | 2.4  | Normal/+/+       |
| OVCAR-4   | Ovary       | SCC258       | RPMI-1640 + 10% FBS                                                                           | 4.3  | OE/+/+           |
| JHOS-4    | Ovary       | RCB1678      | DMEM:F12 + 10% FBS                                                                            | 3.1  | OE/+/-           |
| RKN       | Soft Tissue | IFO50317     | Ham's F12 + 10% FBS                                                                           | 1.3  | Normal/+/-       |
| MFE-296   | Uterus      | 98031101     | MEM + 2mM Glutamine + 10% FBS                                                                 | 2.0  | Normal/+/-       |
| RL95-2    | Uterus      | CRL-1671     | DMEM:F12 + 0.005 mg/ml human insulin + 10%FBS                                                 | 2.0  | Normal/+/-       |
| KLE       | Uterus      | CRL-1622     | DMEM:F12 + 10% FBS                                                                            | 8.4  | OE/+/+           |
| COLO 684  | Uterus      | 87061203     | RPMI-1640 + 2mM Glutamine + 10% FBS                                                           | 2.7  | OE/-/+           |
| MFE-280   | Uterus      | 98050131     | MEM + 2mM Glutamine + 10% FBS                                                                 | 5.1  | OE/-/+           |
| HEC-1-B   | Uterus      | TCHu 115     | EMEM + 10% FBS                                                                                | 2.5  | Normal/+/-       |
| HEC-1-A   | Uterus      | TCHu 149     | McCoy's 5A + 10% FBS                                                                          | 2.7  | Normal/+/-       |
| HEC-151   | Uterus      | JCRB1122     | EMEM + 15% FBS                                                                                | 2.0  | Normal/+/-       |
| SNG-M     | Uterus      | IFO50313     | Ham's F12 + 10% FBS                                                                           | 2.0  | Normal/+/-       |

| Cell Line              | Lineage | Catalog No.             | Complete Medium                                                                                                                           | CN* | CCNE1/<br>Rb/p16 |
|------------------------|---------|-------------------------|-------------------------------------------------------------------------------------------------------------------------------------------|-----|------------------|
| SK-UT-1                | Uterus  | HTB-114                 | MEM + 2 mM L-glutamine + Earle's BSS + 1.5 g/L sodium bicarbonate, 0.1 mM non-essential amino acids, and 1.0 mM sodium pyruvate + 10% FBS | 2   | OE/-/+           |
| AN3 CA                 | Uterus  | HTB-111                 | EMEM + 10% FBS                                                                                                                            | 1.9 | OE/+/-           |
| MES-SA                 | Uterus  | CRL-1976                | McCoy's 5A + 10% FBS                                                                                                                      |     | Normal/+/-       |
| SK-UT-1B               | Uterus  | HTB-115                 | EMEM + 10% FBS                                                                                                                            |     | OE/+/+           |
| sgNTC<br>OVCAR-3       |         | Engineered<br>cell line |                                                                                                                                           |     |                  |
| sgCDKN2A-<br>1 OVCAR-3 |         | Engineered<br>cell line |                                                                                                                                           |     |                  |
| sgCDKN2A-<br>2 OVCAR-3 |         | Engineered<br>cell line |                                                                                                                                           |     |                  |
| sgRb1-1<br>OVCAR3      |         | Engineered<br>cell line |                                                                                                                                           |     |                  |
| sgRb1-5                |         | Engineered<br>cell line |                                                                                                                                           |     |                  |
| AN3 CA<br>+NTC         |         | Engineered<br>cell line |                                                                                                                                           |     |                  |
| AN 3CA +<br>CDKN2A     |         | Engineered<br>cell line |                                                                                                                                           |     |                  |
| OV-90 +NTC             |         | Engineered<br>cell line |                                                                                                                                           |     |                  |
| OV-90 +<br>CDKN2A      |         | Engineered<br>cell line |                                                                                                                                           |     |                  |
| KLE +NTC               |         | Engineered<br>cell line |                                                                                                                                           |     |                  |
| KLE<br>+CDKN2A         |         | Engineered<br>cell line |                                                                                                                                           |     |                  |

\*CN refers to *CCNE1* copy number; OE refers to CCNE1 overexpression

**[00171] Stable cell line generation**

**[00172] Cas9 stable cell line generation**

**[00173]** OVCAR-3 or COV318 were transduced with Cas9 pR-EF1-Cas9-2A-Blast (Cellecta, Lot# SVC9EB-VS) at a 1:1 multipliity of infection (MOI) with 5 µg/mL polybrene and media refreshed after 18 h. Blasticidin (10 µg/mL) was added 48 hour post-transduction to select for construct-containing cells for 14 days. Cas9 expression was measured by Western blot after 14 days selection.

**[00174] sgRNA stable cell line generation**

**[00175]**  $5.0 \times 10^6$  HEK-293T ( $5.0 \times 10^6$ ) cells were transfected with 1 µg sgRNA and 5 µg viral packaging mix (Cellecta CPCP-K2A) in Lipofectamine 2000 (ThermoFisher Scientific 11668-019). Cells were incubated for 24 h and virus was harvested by media filtration using 0.45 µm vacuum filters (Corning 430514). OVCAR-3 Cas9-containing cells ( $2 \times 10^5$  cells) were transduced with sgRNA viral particles with 5 µg/mL polybrene. Media was refreshed after 18 h. Polybrene was

removed at 24h post-transduction, and at 48h post-transduction, 10 µg/mL blasticidin + puromycin (2 µg/mL) was added to select for transductants.

**[00176]** Table 2. sgRNA (small guide RNA) sequences used to target Cas9 cutting to the CDKN2A, p16(INK4) locus.

| Target      | Source   | Sequence                                   |
|-------------|----------|--------------------------------------------|
| sgNTC       | Cellecta | 5'-GGCAGTCGTTTCGGTTGATAT-3' (SEQ ID NO: 1) |
| sgCDKN2A #1 | Cellecta | 5'-CGGGTCGGGTGAGAGTGGCG-3' (SEQ ID NO: 2)  |
| sgCDKN2A #2 | Cellecta | 5'-CACCGAATAGTTACGGTCGG-3' (SEQ ID NO: 3)  |
| sgCDKN2A #3 | Cellecta | 5'-GATGATGGGCAGCGCCCGAG-3' (SEQ ID NO: 4)  |
| sgCDKN2A #4 | Cellecta | 5'-GTGGCCAGCCAGTCAGCCGA-3' (SEQ ID NO: 5)  |
| sgCDKN2A #5 | Cellecta | 5'-TGTCGCACGGTACCTGCGCG-3' (SEQ ID NO: 6)  |
| sgRB1 #1    | Cellecta | 5'-GGTTCTTTGAGCAACATGGG-3' (SEQ ID NO: 7)  |
| sgRB1 #2    | Cellecta | 5'-TGAATACTTACGAAGTGGT-3' (SEQ ID NO: 8)   |
| sgRB1 #3    | Cellecta | 5'-AAACAATCAAAGGACCGAGA-3' (SEQ ID NO: 9)  |
| sgRB1 #4    | Cellecta | 5'-AACATCTAATGGACTTCCAG-3' (SEQ ID NO: 10) |
| sgRB1 #5    | Cellecta | 5'-TCGCTTGTATTACCGAGTAA-3' (SEQ ID NO: 11) |

**[00177]** Overexpression construct stable cell line generation

**[00178]** Cells (2x10<sup>5</sup>) were transduced with OE construct viral particles (Genscript) with 5 µg/mL polybrene. Media were refreshed after 18 hours, and at 48 hours post-transduction, G418 (800 µg/mL) was added to select for transductants over a period of 10-14 days.

**[00179]** p16<sub>pLVX-EF1α-IRES-Neo</sub> insert sequence:

ATGGATTACAAGGATGACGACGATAAGGAACCTGCCGCCGAAGCAGCATGGAACCCA  
GCGCCGATTGGCTGGCTACAGCCGCTGCTAGGGGCAGAGTGGAAGAGGTGCGGGCCCTG  
CTGGAAGCCGGAGCTCTGCCTAACGCCCTAACAGCTACGGCCGCAGACCTATCCAGGT  
GATGATGATGGGCTCCGCCAGAGTGGCCGAGCTGCTGCTGCCACGGCGCCGAGCCCA  
ACTGCGCCGACCCCGCCACACTGACCCGGCCAGTTCATGATGCCGCCAGAGAGGGCTTC  
CTGGACACCCTGGTGGTGTGCTGCACCGGGCCGGCGCCCGCTGGATGTGCGGGACGCCTG  
GGGCAGACTGCCAGTGGACCTGGCCGAGGAGCTGGGCCACAGAGATGTCGCCAGATACC  
TGAGAGCCGCTGCAGGCGGCACCAGAGGCTCTAATCACGCCAGAATCGACGCCGCTGAG  
GGACCTAGCGACATCCCTGACTGA (SEQ ID NO: 12)

**[00180]** CyQuant proliferation assay

**[00181]** Cells were seeded in black, clear bottom, 384-well (Corning CLS3571) or 96-well (Corning 3904) plates for a starting confluency of 5-10% using the conditions in Table 1 and allowed to adhere overnight at 37°C/5% CO<sub>2</sub>. For single agent treatment, cells were treated with compound in a 10-point dose titration, top dose 25 µM, 1:4 dilutions. For combination treatment, cells were dosed with compound in a 10-point dose titration matrix, top dose 5 µM, 1:4 dilutions. Cells were treated for 120 hours (5d) and the CyQuant Direct Proliferation Assay was performed according to the

manufacturer's instructions (Invitrogen, C35012). To calculate IC<sub>50</sub> values, percent inhibition was determined relative to DMSO and staurosporine controls at 120 h. To calculate GI<sub>50</sub> values, percent inhibition relative to DMSO and Day 0 was calculated. Synergy scores were determined using SynergyFinder 3.0 (Ivanovski, A. *et al.*, Nucleic Acids Res. (2022) 50(W1): W739-W743).

**[00182] IncuCyte proliferation**

**[00183]** Cells were seeded in 96-well or 6-well plates for a starting confluency of 5-10% using the conditions in Table 1 and allowed to adhere overnight at 37°C/5% CO<sub>2</sub>. Cells were then either dosed with BLU-222 and/or ribociclib at the indicated doses or transfected with 40 nM ON-TARGETplus Non-targeting Control pool siRNA (Horizon Discovery D-001810-10) or ON-TARGETplus Human CDK2 siRNA SMARTPool (Horizon Discovery L-003236-00) with Lipofectamine 2000 Transfection Reagent (Invitrogen 11668027). Percent confluency was measured at least until controls reached 100% confluency, as calculated using IncuCyte 2021A or 2022B software (Sartorius).

**[00184] Clonogenic Assays**

**[00185]** OVCAR3 Cas9 sgNTC, sgCDKN2A-1, and sgCDKN2A-2 cells were in seeded at a density of 1500 cells/well in six-well plates, in duplicate, and allowed to adhere overnight. Cells were treated with 50, 100, 250, 500, and 1000 nM BLU-222, 3000 nM ribociclib, and BLU-222 and ribociclib combinations. Culture medium and compounds were refreshed every four days. On day 15, the cells were gently washed with PBS and stained with 0.125% crystal violet staining solution (Sigma-Aldrich, V5265) in 10% ethanol. For quantitative analysis, the colonies were captured by a scanner and the surviving fraction of individual well which takes both colony number and size into account was measured and analyzed by ImageJ (64-bit, Java 8).

**[00186] AlphaLISA assays**

**[00187]** RB phosphorylation was determined using the AlphaLISA SureFire Ultra Phospho-Rb (Thr821/826) Detection Kit (Revvity ALSU-PRB-B50K) and/or the AlphaLISA SureFire Ultra Human and Mouse Phospho-Rb (Ser807/811) Detection Kit (ALSU-PRB-A10K). OVCAR-3 cells were maintained in RPMI with 20% FBS and plated in serum-free DMEM at 25,000 cells per well in a white bottom 96-well plate (Corning 353296). After 30h starvation, serum-free media was replaced with DMEM with 10% FBS and cells were treated with BLU-222, ribociclib, palbociclib, or abemaciclib in a 9-point dose titration, top dose 10 µM, 1:4 dilution, for 18 hours. AlphaLISA was performed according to the manufacturer's instructions and plates were read on an EnVision plate reader (Revvity). IC<sub>50</sub> was determined in GraphPad Prism using nonlinear regression, variable slope, four-parameter fit. For tumor analysis, tumors were lysed in PhosphoSafe (EMD Millipore, 71296) and 5 µg lysate was added to 384-well OptiPlates (Revvity 6007290).

**[00188] Differential gene expression analysis of BLU-222 treated cell lines**

**[00189]** Ovarian cells at 60-70% confluency were treated at the BLU-222 IC<sub>50</sub> or with 0.1% DMSO for 24h. Cells were collected in triplicate, 2-3 x 10<sup>6</sup> cells per sample. Cell pellets were washed twice with PBS and snap frozen. Library preparation and mRNA sequencing was performed at Azenta following the recommended protocol, with a yield of ~10 Gb per sample. Data was processed through the nf-core/rnaseq pipeline, with read counts generated through Salmon (Ewels, P.A. *et al.*, Nat. Biotech. (2020) 38: 275-278). All sample data passed internal quality control and quantity metrics. DESeq2 was used to perform differential gene expression analysis between the treatment group and DMSO for each cell line. The Wilcoxon Rank Sum test was used to compare changes in gene expression (log<sub>2</sub>(fold change) from DESeq2 analysis) between treatment groups across cell lines. The Benjamini-Hochberg method was used to adjust p-values for multiple testing. As an orthogonal approach, hierarchical clustering was also used to assess gene signatures that could differentiate responders from non-responders.

**[00190] CRISPR/Cas9 resistance screen**

**[00191]** ~100M OVCAR3-Cas9 cells were transduced at MOI=0.5 with 80K shRNA CRISPR-KO lentiviral library (Cellecta KOHW-80K-P). Three days post lentiviral infections, 2 µg/mL puromycin was added to cell media to select and enrich for transduced cells for 14 days. Cells were then treated for 7 days with DMSO or 200 nM BLU-222, ~50 million cells per sample in duplicate. sgRNA enrichment was detected by next-generation sequencing. Raw sgRNA counts were scaled to total sgRNA for each gene per sample, fold change against plasmid was calculated and Z-transform was applied. The RSA method was also used to estimate RSA scores for each gene per sample (König, R. *et al.*, Nat. Methods (2007), 4: 847-849). The minimum value of either the 5<sup>th</sup> or 95<sup>th</sup> percentile of the normalized RSA score distribution was used as the significance threshold and the difference in Z-score between the BLU-222 treated gene and the DMSO treated gene quantified the direction and magnitude of effect.

**[00192] Immunoprecipitation**

**[00193]** Cells were washed with PBS after trypsinization (1 million cells per IP) and lysed on ice in Pierce IP lysis buffer (ThermoFisher 87787) with HALT protease and phosphatase inhibitor mix (ThermoFisher 78430). Lysates were centrifuged at 14,000 rpm for 10 min and supernatant was incubated with 10 µg anti-CDK4 antibody (Bethyl A304-225A), anti-CDK2 antibody (SC-6248), isotype control (Cell Signaling 3900), or 20 µL twice washed anti-FLAG M2 magnetic beads (Sigma M8823) overnight at 4°C with gentle rotation. Lysates were incubated with 50 µL of twice washed Protein A/G magnetic beads (Thermo Fisher Cat# 88802) for 2 hours at room temperature with gentle rotation. Beads were washed three times with lysis buffer and samples were eluted with 1X Laemmli buffer with 2-ME (BioRad). Protein signals were detected by Western blot.

**[00194] Western blot**

**[00195]** Cells were lysed in PhosphoSafe Lysis Buffer (EDM millipore, 71296). Lysates were run in 4–20% Tris-Glycine, SDS-PAGE gels (Bio-Rad 671094) and transferred using the Bio-Rad dry transfer system (Trans-Blot Turbo) (Nitrocellulose membrane, 0.2  $\mu$ M). Membranes were blocked with LI-COR Odyssey Blocking Buffer (927-70001) and incubated with primary antibodies in Odyssey Blocking Buffer with 0.1% Tween-20 at 4°C with agitation: Cyclin E1 1:500 (SC-247), p16 1:1000 (CST 80772), CDK2 1:1000 (SC-6248), CDK4 1:1000 (CST 12790), Cyclin D1 1:1000 (SC-450), TK-1 1:1000 (CST 28755), Rb 1:1000 (CST 9309), pRbS780 1:1000 (CST 9307), pRbS807/811 1:1000 (CST 8516), pRbT821/826 1:500 (SC-271930), Actin (R) 1:2000 (CST 8457), Actin (M) 1:5000 (CST 3700). Secondary antibodies (LI-COR 926-33212, 926-33213, 926-68070, 926-68071, 926-68076) were diluted to 1:10000 in LICOR Blocking Buffer with 0.1% Tween-20. Signals were detected and quantified using LI-COR Odyssey CLx Imaging Studio.

**[00196] Cell cycle profiling**

**[00197]** Cells at 60-70% confluency were treated with BLU-222 for 22h and pulsed with 10  $\mu$ M EdU for 2h. Cell cycle phase was detected by flow cytometry using the Click-it EdU Alexa Fluor 488 Flow Cytometry Kit (Invitrogen C10420) or Alexa Fluor 647 kit (Invitrogen C10634) following the manufacturer's instructions. FACS analysis was performed using LSR Fortessa (BD Biosciences) and analyzed using FlowJo v10.8.1 software (BD Life Sciences).

**[00198] Identifying and evaluating biomarker signatures in publicly available data**

**[00199]** Data was retrieved from the Cancer Cell Line Encyclopedia's DepMap 22Q4 public release (DepMap, Broad, 2021) to interrogate potential biomarkers of BLU-222 response. Specifically, the correlation between CRISPR gene effect (Chronos Score) or RNAi gene effect (DEMETER2 score) with copy number and mutation data from whole exome sequencing or gene expression data from mRNA sequencing was tested. The Kruskal-Wallis test was used for correlations between categorical and continuous variables while the Wilcoxon Rank Sum test was used for correlations between binary and continuous variables. Patient population biomarkers were evaluated using publicly available data from the Cancer Genome Atlas (TCGA).

**[00200] OVCAR-3 T2A CDX model development**

**[00201]** NIH OVCAR-3 cells (ATCC HTB-161) were passaged through mice once and the "T2A" cell line was rederived from the tumor at Shanghai ChemPartner. OVCAR-3 T2A cells were cultured in RPMI 1640 media supplemented with 20% FBS and 0.01 mg/mL insulin. To establish the OVCAR-3 xenograft model,  $6 \times 10^6$  cells were suspended in 50% Matrigel, 50% RPMI1 media (serum-free) and implanted subcutaneously into the flank of NOD-SCID female mice.

**[00202] *In vivo CDX studies***

**[00203]** Cell lines were maintained as a monolayer in appropriate media (Table 1). Cells were implanted 1:1 with Matrigel (Corning 354248) into the flanks of 6-8 week old female mice: OVCAR3-T2A (6 x 10<sup>6</sup> cells; NOD-SCID; Shanghai ChemPartner), OVK18 (5 x 10<sup>6</sup> cells; CB17 SCID; Shanghai ChemPartner) or ES2 (2x10<sup>6</sup> cells; BALB/c nude; Pharmaron). Tumor size was measured twice weekly using a caliper and volume was calculated using the formula  $V = 0.5 \times [\text{shortest diameter}] \times [\text{longest diameter}]^2$ . OVCAR-3 T2A study dosing began when mean tumor volume reached 150-250 mm<sup>3</sup> for efficacy and 400-600 mm<sup>3</sup> for PK/PD. OVK18 study dosing began when mean tumor volume reached 150-250 mm<sup>3</sup> for efficacy and 300-600 mm<sup>3</sup> for PK/PD. ES2 efficacy study dosing began when mean tumor volume reached 150 mm<sup>3</sup>. BLU-222 (10 mg/mL) was sonicated in 0.5% (w/v) CMC-Na, 1% (v/v) Tween-80 to make a homogenous suspension and dosed BID (12h/12h), p.o. Ribociclib (7.5 mg/mL) was sonicated in 0.5% MC to make a homogeneous suspension, dosed QD, p.o. Paclitaxel (1 mg/mL) was sonicated in 5% cermaphor EL + 5% ethanol + 90% saline, dosed Q3D, i.p. Carboplatin (3 mg/mL) was sonicated in saline and dosed QW, i.p. For combination BLU-222 and ribociclib treatment groups, ribociclib was administered 30 minutes prior to BLU-222.

**[00204]** Blood for plasma PK analysis was collected by serial microsampling (~100 or ~200  $\mu$ L blood volume) at the indicated timepoints. Blood was collected into EDTA-2K tubes, placed on ice, and centrifuged at 2000xg, 5 minutes, 4°C, to obtain plasma sample within 15 minutes of collection. Plasma samples were stored at -80°C prior to bioanalytic analysis. Tumors were collected at the indicated timepoints; half was snap-frozen, half was preserved in formalin-fixed, paraffin-embedded (FFPE) blocks. Tumor PD was assessed by Western blot from snap-frozen tumors.

**[00205] *In vivo PDX studies***

**[00206]** Patient derived xenograft (PDX) studies were run at XenoSTART. Models were selected from the XenoSTART database using model baseline genomics and expression of biomarkers of interest was confirmed by Western blotting in study vehicle tumors. Tumor fragments (~70 mg) were subcutaneously implanted into the flanks of 6-12 week old female athymic nude, outbred homozygous (CrI:NU(NCr)-Foxn1<sup>nu</sup>) mice. Tumors and body weights were measured twice weekly over study period. Mice were randomized into treatment groups when mean tumor volume (TV) reached 150-300 mm<sup>3</sup> and mice were treated for 60 days or until tumors reached terminal TV (2000 mm<sup>3</sup>). BLU-222 was prepared as a homogenous suspension in 0.5% (w/v) CMC/1% Tween-80 in water, dosed BID (12h/12h) p.o. Paclitaxel and carboplatin were prepared in 0.9% sterile saline and dosed QW, i.p. Percent TGI was calculated on the latest day at which vehicle and/or treatment groups remained intact.

**[00207]** Whole blood for plasma PK analysis was collected at the indicated timepoints into K<sub>2</sub>EDTA tubes, centrifuged 1700 RCF for 10 minutes at 4°C, then flash frozen and stored at -80°C. Bioanalytic analysis was performed at Charles River Laboratories. Tumors were collected at the indicated timepoints; half was snap-frozen, half was preserved in formalin-fixed, paraffin-embedded (FFPE) blocks. Tumor PD was assessed by Western blot or AlphaLISA.

**[00208] Enzyme activity assays**

**[00209]** Inhibitory effects of the compounds were measured by enzymatic phosphorylation activity of CDK enzyme in complex of cyclin proteins phosphorylates, 7.5 micromolar fluorescently labelled peptide substrate, 5-FAM-QSPKKG-CONH<sub>2</sub> (FL-Peptide 18, Perkin Elmer, 760362), in the presence of adenosine-5'-triphosphate (ATP) and varying concentrations of the test compound in 100 mM 2-[4-(2-hydroxyethyl)piperazin-1-yl] ethanesulfonic acid (HEPES), pH 7.5, 10 mM MgCl<sub>2</sub>, 0.015% Brij-35, 1 mM dithiothreitol (DTT), 1.0% dimethylsulfoxide (DMSO). Assays were performed at 1.0 mM ATP or at ATP K<sub>m</sub> of the CDK enzymes in complex with Cyclin proteins. Reactions proceeded until between 10% to 20% total peptides were phosphorylated at room temperature (25 °C) and were terminated with 35 mM 2,2',2''-(ethane-1,2-diyl)dinitrilo)tetraacetic acid (EDTA). Product was detected using the Caliper mobility shift detection method where the phosphorylated peptide (product) and substrate were electrophoretically separated and measured. Percent activity was plotted against log concentration of compound and points to generate an apparent IC<sub>50</sub>. Kinome selectivity tested at 3 μM; S(10) is the number of kinases inhibited at <10 POC divided by the total number of human wildtype kinases. Biochemical assays were conducted at 1 mM ATP in presence of appropriate cyclin (indicated). Fold selectivity is noted.

**[00210] Table 3. Enzymatic and cellular potency of BLU-222**

| Enzymatic Assay                   |                       |                  |
|-----------------------------------|-----------------------|------------------|
| CDK enzyme/cyclin protein complex | IC <sub>50</sub> (nM) | Fold selectivity |
| CDK2/Cyclin E1                    | 2.6                   |                  |
| CDK2/Cyclin A2                    | 15.5                  | 6x               |
| CDK1/Cyclin B                     | 234                   | 90x              |
| CDK4/Cyclin D1                    | 377                   | 145x             |
| CDK6/Cyclin D3                    | 275                   | 106x             |
| CDK7/Cyclin H                     | 6941                  | 2670x            |
| CDK9/Cyclin T1                    | 6115                  | 2352x            |
| Kinome Selectivity                |                       |                  |
| S(10)                             | 0.045                 |                  |
| Cellular Assay                    |                       |                  |
| Marker                            | IC <sub>50</sub> (nM) | Fold selectivity |
| pRb T821/826 (CDK2)               | 4.2                   |                  |
| pLamin (CDK1)                     | 380                   | 90x              |

**Example 1. *CCNE1* amplification predicted sensitivity to exemplary CDK2 inhibitors in ovarian and endometrial cancer cells**

**[00211]** Ovarian and endometrial are among the cancer types with the highest prevalence of *CCNE1* amplification and have similar clinical, pathologic, and molecular features based on publicly available data from the Cancer Genome Atlas. In ovarian and endometrial cell lines, *CCNE1* copy number (CN) amplification ( $CN \geq 6$ ) strongly correlated with CDK2 dependency in the RNAi DEMETER ( $p = 3.3 \times 10^{-7}$ , Kruskal-Wallis) and CRISPR Chronos datasets from DepMap ( $p = 3.3 \times 10^{-7}$ , Kruskal-Wallis). *CCNE1* copy number amplification also predicted CDK2 dependency broadly across all cell lines, consistent with previously published analyses (Cowley, G.S. *et al.*, Sci. Data. (2014), 1:140035; McDonald, E.R., III, *et al.*, Cell (2017), 170(3): 577-592 e10). Sensitivity of *CCNE1* amplified ovarian and endometrial but not *CCNE1* normal (diploid;  $CN < 3$ ) cell lines was confirmed by siRNA-mediated knockdown of CDK2, consistent with published data (Cowley, G.S. (2015)). CDK2 knockdown was confirmed by Western blot. In ovarian and endometrial cell lines with  $CN 3-5$ , CDK2 dependency in DepMap was highly variable. This suggested that in the *CCNE1*  $CN < 6$  setting, additional biomarkers in conjunction with *CCNE1* may be necessary to robustly predict CDK2-sensitivity.

**[00212]** With selective CDK2 inhibitors entering the clinic, identification of specific biomarkers to predict CDK2 vulnerable cancers is critical to enable targeting responsive tumors. Ovarian, endometrial/uterine, gastric and some breast cancers harbor high frequencies of *CCNE1* amplification and have been the focus of single agent activity in pre-clinical and clinical investigation of CDK2 inhibitors.

**[00213]** BLU-222 is a potent, highly selective, orally bioavailable, investigational CDK2 inhibitor in clinical development. BLU-222 displayed nanomolar cellular potency on pRbT821/826, CDK2-preferential phosphorylation sites, and was selective over CDK family members (1/4/6/7/9) with excellent kinome selectivity (Figure 1 and Table 3). BLU-222 demonstrated robust anti-tumor activity in select *CCNE1* high ovarian and endometrial cancer models. *CCNE1* amplification sensitized endometrial cell lines to BLU-222. BLU-222 showed monotherapy efficacy by potently inhibiting proliferation in *CCNE1*-amplified ovarian and endometrial cancer cell lines in a dose-dependent manner (Figure 2A) while sparing *CCNE1* normal (diploid, mRNA not overexpressed) cell lines (Figure 2B). To evaluate *CCNE1* CN as a predictive biomarker for BLU-222 response, a panel of ovarian and endometrial cancer cell lines ( $n = 42$ ) that harbored *CCNE1* amplification ( $CN \geq 6$ ), *CCNE1* CN gain (3-5), or diploid *CCNE1* CN ( $< 3$ ) was tested (Table 1). The strong responder GI-50 threshold of 200 nM was determined through a Gaussian Mixture Model analysis fitted by the EM algorithm based on the measured GI-50s of all treated cell lines. At this dose in OVCAR-3, pRbT821/826 inhibition (a measure of CDK2 activity) plateaus and pLamin (a measure of CDK1 activity) is not significantly inhibited (Figure 1). In agreement with the genetic knockdown of CDK2

and CDK2-dependency results from DepMap, *CCNE1* amplification predicted a strong CDK2 inhibitor antiproliferative response (Figure 2C, Figure 17A, Figure 17B). *CCNE1* CN gain demonstrated variable CDK2 inhibitor sensitivity, while diploid *CCNE1* CN cell lines were largely BLU-222 insensitive (Figure 2C, Figure 17A, Figure 17B). High *CCNE1* mRNA was tested as a predictor of BLU-222 sensitivity. The threshold for *CCNE1* overexpression was set at the top quartile of mRNA expression from Cancer Cell Line Encyclopedia (CCLE) data. All *CCNE1* amplified cell lines were mRNA overexpressed. *CCNE1* overexpression alone did not enrich for BLU-222 sensitivity and was not a good predictor for BLU-222 response (Figure 2D, Figure 17C, Figure 17D). Therefore, while *CCNE1* CN amplification predicted sensitivity to BLU-222, a high level of *CCNE1* mRNA expression alone was insufficient.

**[00214]** To evaluate BLU-222 in pre-clinical models of ovarian cancer, the activity was compared between *CCNE1* amplified and diploid *CCNE1* CN cell derived xenografts (CDX). OVCAR-3 was a *CCNE1* amplified (CN = 12), high-grade serous ovarian cancer (HGSOC) cell line. To develop the OVCAR-3 T2A model, OVCAR-3 tumors were passaged once through mice to improve *in vivo* growth kinetics. OVCAR-3 T2A was strongly responsive to CDK2 inhibition by BLU-222 *in vivo*, reaching stasis at doses where sustained inhibition of pRbT821/826 and pRbS807/811 was achieved for at least 12 hours (Figure 2E and Figures 3A and 3B). OVCAR-3 was also strongly responsive to Compound 3 (Figure 18A) and Compound 4 (Figure 18B). ES-2, a *CCNE1* normal (CN < 3, mRNA normal), ovarian clear cell carcinoma CDX was insensitive to BLU-222 (Figure 2F) and BLU-222 treatment did not lead to inhibition of pRb (Figures 3C and 3D). This supported that CDK2 inhibition suppressed tumor growth in a *CCNE1* amplified model *in vivo* and that anti-tumor activity was correlated with pRb inhibition. Robust pre-clinical monotherapy activity for BLU-222 in *CCNE1* amplified ovarian and endometrial models was demonstrated (Figures 2A-2H and 6A-6K).

#### **Example 2. An exemplary CDK2 inhibitor arrested cell cycle progression**

**[00215]** To promote the G1/S transition, CDK4/6 and CDK2-cyclin E phosphorylate Rb in late G1, leading to Rb inactivation and release of the E2F transcription factors that promote S-phase related gene expression. The modulation of pRb upon BLU-222 treatment in *CCNE1* amplified or *CCNE1* normal cell lines was measured. Treatment for 24 hours inhibited pRb in *CCNE1* amplified cells, but not *CCNE1* normal cell lines. The expression of TK1, an E2F target gene and measure of downstream activity of the CDK2-Rb signaling axis, was evaluated. TK1 was a proliferation marker and has been used as both a prognostic and pharmacodynamic biomarker for CDK4/6 inhibitors. While TK1 was significantly inhibited by BLU-222 treatment in *CCNE1* amplified cell lines, no change was observed in the insensitive *CCNE1* diploid cell lines.

**[00216]** Consistent with the role of CDK2 promoting the G1/S transition in CDK2-dependent cell lines, BLU-222 treatment led to G1 arrest in *CCNE1*-amplified cells (Figure 2G). In some cell lines, BLU-222 at higher doses induced an accumulation in G2/M. Consistent with other CDK inhibitors

(Dietrich, C. (2023)), and without being bound by any theory, this was interpreted as a result of inhibition of the CDK2-cyclin A complex, or alternatively of CDK1, which occurs at concentrations of BLU-222 well above that needed to selectively inhibit CDK2-cyclin E. BLU-222 treatment had no significant impact on cell cycle distribution in *CCNE1* normal cell lines (Figure 2H and Figure 4). The cell cycle distribution was evaluated in BLU-222 treated *CCNE1* overexpressed, but CN < 6 cell lines. In BLU-222 responder cell lines (GI-50 < 200 nM), BLU-222 treatment resulted in a G1 or G2/M arrest (Figure 5). Cell cycle distribution was not affected by BLU-222 treatment in *CCNE1* overexpressing, BLU-222 non-responder cell lines (Figure 5). Without being bound by any theory, together, pRb inhibition, TK1 downregulation, and accumulation in G1 after BLU-222 treatment supported that the CDK2 inhibition mechanism of action of BLU-222 in *CCNE1* amplified cells was through inhibition of the Rb-E2F axis.

### **Example 3. Biomarker combination for sensitivity to exemplary CDK2 inhibitors in OVCAR-3 and KLE cells**

[00217] To identify genes that modulate the BLU-222 anti-proliferative response in *CCNE1* amplified HGSOC, a genome-wide CRISPR/Cas9 pooled knockout screen was performed in OVCAR-3 cells. OVCAR-3 was sensitive to BLU-222 both *in vitro* (Figure 2A) and *in vivo* (Figure 2E) and therefore gene knockouts that conferred a change in proliferative capacity in the presence of BLU-222 represented markers for response. Five genes were identified that when deleted led to significant loss of BLU-222 sensitivity in OVCAR-3 cells: *RBI*, *CDKN2A*, *SAFB*, *KLH14*, and *HLA-DQAI* (Figure 6A). The loss of BLU-222 sensitivity was validated in the top hits, *RBI*, which encoded the retinoblastoma protein, and *CDKN2A*, specific to the p16 transcript variant, the INK4 family member and endogenous inhibitor of CDK4/6. In OVCAR-3, CRISPR/Cas9 knockouts were generated for *RBI* or *CDKN2A*, each with five sgRNAs; the two sgRNAs that achieved the greatest loss of target gene expression were selected for further evaluation. CDK2 inhibitor induced antiproliferative activity was measured by CyQuant: both sgRBI (Figure 7A, Figure 19A, Figure 20) and sgCDKN2A (Figure 7B, Figure 19B, Figure 20) resulted in loss of BLU-222 and Compound 3 sensitivity in OVCAR-3 and KLE (*CCNE1* amplified ovarian and endometrial cell lines, respectively). Interestingly, nearly all *CCNE1* amplified cell lines in CCLE are positive for Rb and have high p16 expression. These markers converged on modulation of the Rb pathway as being important for BLU-222 response in HGSOC.

### **Example 4. Biomarker combination for *in vitro* and *in vivo* sensitivity to exemplary CDK2 inhibitors**

[00218] Cells with high *CCNE1* mRNA expression in the absence of gene amplification (CN < 6) had variable response to CDK2 inhibition by BLU-222 (Figure 2D). Since Rb or p16 loss were markers of resistance in a *CCNE1* amplified setting, the possibility of using Rb and/or p16 expression

as biomarkers of response in *CCNE1* high ovarian and endometrial cell lines was investigated, regardless of *CCNE1* CN. Cell lines were categorized using Rb and p16 expression from Western blot analysis, since RNA and protein expression by Western blot were not strongly correlated under the experimental conditions used (Figures 8A and 8B). Rb intact was defined as detectable Rb protein expression and no loss-of-function mutations, and p16 high was determined by evaluating the distribution of p16 protein abundance across all ovarian and endometrial cell lines. The CDK2 inhibitor response across all ovarian and endometrial cell lines was evaluated in the context of Rb and p16 expression. Even without selecting based on *CCNE1* status, loss of Rb or p16 trended towards non-response to BLU-222 (Figure 6B), Compound 2 (Figure 21A), and Compound 3 (Figure 21B). Next, narrowing to *CCNE1* high cell lines, CDK2 inhibitor responders were enriched when Rb was intact and p16 was high, and cells with p16 loss or Rb loss were non-responders (Figure 6C, Figure 22A, Figure 22B). Selecting cells using all three biomarkers, the *CCNE1* high/Rb intact/p16 high signature strongly enriched for response, distinguishing responders from non-responders (BLU-222, Wilcoxon rank sum test: BLU-222,  $p = 1.3 \times 10^{-6}$ ; Compound 2,  $p = 5.0 \times 10^{-7}$ ; Compound 3,  $p = 2.8 \times 10^{-6}$ ) (Figure 6D, Figure 23A, Figure 23B). This is in agreement with analysis of cell line CDK2-dependency in DepMap/CCLE (Figures 7C and 7D). Further, RNA-Seq analysis upon BLU-222 treatment in a panel of 18 BLU-222 responder and non-responder ovarian cell lines demonstrated clustering of the *CCNE1* high, Rb intact, p16 high cell lines. BLU-222 induced a reduction in E2F pathway genes in responder cell lines. Combining the CRISPR screen data with the cell line proliferation panel, a combinatorial biomarker signature has been generated to predict CDK2-dependency and CDK2 inhibitor sensitivity in ovarian and endometrial cancer cells with high *CCNE1* expression, even in the absence of *CCNE1* CN amplification. Using a combination of CRISPR whole genome screens coupled with targeted genetic and pharmacological approaches in ovarian and endometrial cell lines, *CCNE1* amplified lines identified as Rb intact and p16 high, and that Rb and p16 expression can be used as biomarkers to enrich for BLU-222 sensitivity in *CCNE1* overexpressed but non-amplified cells.

**[00219]** The contribution of these biomarkers in tumors was evaluated by testing BLU-222 monotherapy response in endometrial PDX models. All models had high *CCNE1* mRNA levels, as defined by the top quartile of model baseline expression in the XenoSTART model database; ST2526, ST3052, and ST1386 were *CCNE1* CN aberrant. The models had varying levels of expression of Rb and/or p16, as measured by Western blot (Figure 6E). BLU-222 60 mpk BID dosing resulted in PK exposure that was sufficient to predict single agent activity in responder tumors, exceeding the OVCAR-3 cellular pRb IC<sub>90</sub> for at least 12 h (Figures 15A-15I). ST3052 (*CCNE1* CN = 8, Rb+, p16+) and ST2526 (*CCNE1* CN = 3, Rb+, p16+) were strongly responsive to BLU-222, reaching stasis (Figures 6F and 6G). ST1386 was *CCNE1* amplified (CN = 8) but had lower levels of p16 expression that relative to beta-actin were on the threshold for “p16 high” as determined by the *in*

*vitro* cell line panel. This model was not responsive to BLU-222 at 60 mpk but was responsive to BLU-222 at 100 mpk (Figure 6H). ST259 and ST189 were *CCNE1* CN normal but high *CCNE1* mRNA expressors. ST259 had low levels of p16 expression and did not respond to BLU-222 monotherapy (Figure 6I). ST189 lacked both Rb and p16 expression and did not respond to BLU-222 monotherapy (Figure 6J). Therefore, in these *CCNE1* high endometrial PDX models, *CCNE1* status combined with p16 and Rb status better predicted response to BLU-222 monotherapy than aberrations in *CCNE1* alone (Figure 6K). This was consistent with the cellular *in vitro* data which indicated that Rb and p16 were key predictors for BLU-222 monotherapy activity (Figure 2A-D). Extending beyond the *CCNE1* CN amplification setting, it was demonstrated that using *CCNE1* expression in addition to Rb and p16 expression captures CDK2-vulnerable ovarian and endometrial cancer cells (Figure 6D).

#### **Example 5. Inactivation of CDK4/6 rendered p16-low cells vulnerable to an exemplary CDK2 inhibitor**

[00220] *CDKN2A* (p16) alterations are common oncogenic events and high p16 expression is a biomarker for CDK4/6 inhibitor resistance (Palafox, M. *et al.*, Nat. Commun. (2022), 13(1): 5258). The isogenic *CDKN2A* CRISPR/Cas9 cell lines were used to further investigate the role of p16 in modulating response to BLU-222. *CDKN2A* deletion led to the increased total and phosphorylated Rb, as well as increased TK1, indicating functional upregulation of CDK4 activity compared to the control cell line (Figure 7C). sgCDKN2A knockout also led to increased CDK4-cyclin D1 association (Figure 9A), consistent with restoration of CDK4 to an active state upon p16 loss in OVCAR-3. Reciprocally, induced overexpression of *CDKN2A* in OV-90 and AN3CA, p16 low, BLU-222 non-responder cell lines, sensitized to BLU-222 single agent (Figure 24A, Figure 24B).

[00221] In the *CDKN2A* knockout cells, whether pharmacological inhibition of CDK4/6 could restore sensitivity to BLU-222 was tested. Using a clonogenic assay approach, OVCAR-3 sgNTC and sgCDKN2A cell lines were treated with BLU-222 alone or in combination with ribociclib. While BLU-222 and ribociclib conferred no additional anti-proliferative effect compared to BLU-222 alone in the sgNTC line, BLU-222 sensitivity was restored in the sgCDKN2A cell lines in the presence of 3  $\mu$ M ribociclib (Figures 9D and 9E). In assessing cell cycle response, treatment with BLU-222 led to G1 accumulation in control cells, with no additional effect upon co-treatment with ribociclib (Figure 9F). In the *CDKN2A* knockout cells, BLU-222-induced G1 accumulation was lost (Figure 9F). Co-treatment of BLU-222 and ribociclib in the *CDKN2A* knockout cells restored BLU-222 induced G1 accumulation (Figure 9F). These data are consistent with derepressed CDK4/6 compensating for the loss of CDK2 activity in regulating the G1/S phase transition. This further suggests a plasticity in CDK2 and CDK4/6 driving cell cycle progression. Therefore, in a *CCNE1* high setting where CDK2 and CDK4/6 were active, both CDK2 and CDK4/6 must have been inhibited to elicit an antiproliferative response.

[00222] p16 levels were evaluated as a biomarker for BLU-222 and ribociclib combination treatment broadly across *CCNE1* high ovarian and endometrial cells. Without being bound by any theory, it was proposed that cell lines with high *CCNE1* expression and low p16 expression, and therefore intact CDK4/6 activity, would be sensitive to CDK2 inhibition in combination with CDK4/6 inhibition. Using synergy analysis on ovarian (n=34) and uterine cell lines (n=13), *CCNE1* expression alone was insufficient to predict a synergistic response with a combination of BLU-222 and ribociclib (Figure 9G; Table 1). Categorizing cell lines by Rb and p16 expression, synergistic potential trended towards cell lines with intact Rb and low p16 expression (Figure 9H). Evaluating Rb and p16 within the *CCNE1* high cell setting, cells with high *CCNE1*, intact Rb, and low p16 expression were most predictive of a BLU-222 and ribociclib combination effect (Figure 9I).

[00223] To evaluate the contribution of p16 levels to the BLU-222 and ribociclib combination effect *in vivo*, the OVK18 ovarian CDX model that is *CCNE1* high, Rb intact, and p16 low was used. OVK18 was insensitive to BLU-222 and ribociclib single agents *in vitro* but demonstrated a strong synergistic potential (Figures 9J; Figure 10B). In mice, BLU-222 monotherapy did not induce significant anti-tumor activity while ribociclib monotherapy led to a moderate response (Figures 9M and 10A). The BLU-222 and ribociclib combination had a robust antitumor effect in OVK18 (Figure 9M), as predicted by the *in vitro* data, and led to tumor stasis. The BLU-222 and ribociclib combination effect was also shown in MFE-296, an endometrial cancer model that is *CCNE1* high, Rb intact, and p16 low (Figure 9O). The BLU-222 and ribociclib combination response was evaluated in OVCAR-3, a *CCNE1* high, Rb intact, and p16 high model. In this setting, the BLU-222 and ribociclib combination was not predicted to have additional benefit over BLU-222 alone due to pre-existing CDK4 inhibition via high levels of endogenous p16. *In vitro* proliferation and synergy analysis in OVCAR-3 showed no BLU-222 and ribociclib combination effect (Figure 9K). The OVCAR-3 T2A CDX model was refractory to ribociclib monotherapy and the BLU-222 and ribociclib combination showed no additional anti-tumor activity compared to BLU-222 alone (Figure 9N), as predicted by the *in vitro* data. Further, the BLU-222 and ribociclib combination was well tolerated in mice and the plasma exposure of each agent was unaffected in the combination (Figures 11A-11C). Together, these data demonstrated that the combination of a CDK2 inhibitor (*i.e.*, BLU-222) and a CDK4/6 inhibitor had a strong anti-tumor effect in cyclin E1 high, Rb intact models when p16 levels are low. Without being bound by any theory, with the biomarker analysis for response to BLU-222 single agent in cell lines, this suggested that in the low p16 setting, inactivating CDK4/6 pharmacologically induced a cellular state that was similar to settings in which p16 was expressed at high levels.

[00224] Studies with CDK inhibitors have helped expand the understanding of cell cycle regulation, particularly the role of each CDK and their impact on tumor initiation and progression. Recent studies suggest a compensatory relationship between CDK4/6 and CDK2 under conditions of acute CDK2 inhibition (Figure 16A). The examples above revealed aberrations in cancer cells that led

to CDK2-dependent, dysregulated cell cycle. In addition to high cyclin E1 levels that led to constitutive activation of CDK2, CDK4/6 must also be inactivated or inhibited for CDK2 to be essential for the G1/S phase transition (Figure 16B).

[00225] Other than endogenous or pharmacological inhibition of CDK4/6 activity, Rb loss-of-function mutations abrogated the ability of CDK4/6 to control the cell cycle and these cells were insensitive to CDK4/6 inhibition. In ovarian and endometrial cancers, loss of Rb was relatively rare. However, loss of Rb and its role as a potential resistance biomarker for CDK4/6 inhibitors in HR-positive metastatic breast cancers had been previously reported (Palafox, M. (2022); Ashgar, U.S. *et al.*, JCO Precis. Oncol. (2022), 6: e2100002). Loss of Rb could render ovarian and endometrial cancer cells resistant to BLU-222 (Figure 6A-6K). CDK2-cyclin E mediated cell cycle progression through phosphorylation and inactivation of Rb activity suggested that while Rb was present the Rb circuit could be inhibited to halt cell cycle progression, but that upon loss of Rb aberrant by-pass mechanisms that were CDK2-independent were activated.

[00226] In addition to Rb, the endogenous CDK4/6 inhibitor p16 was identified as a critical player in modulating BLU-222 sensitivity in cyclin E1 high ovarian and endometrial cancer cells (Figure 6A). In a wildtype Rb setting, CDK2 compensated for low CDK4/6 activity when p16 was highly expressed, rendering cells vulnerable to pharmacologic CDK2 inhibition by BLU-222; whereas in cells with low p16 expression, CDK4/6 could drive the cell cycle, even in the presence of high cyclin E1 levels, and was therefore a driver of resistance to CDK2 inhibitors. In ovarian cell lines treated with BLU-222, p16 (*CDKN2A*) transcript levels were not altered after short-term (24h) treatment, suggesting that p16 might not be a dynamic marker of resistance to BLU-222. Instead, basal levels of p16 might dictate response to BLU-222 as a single agent.

[00227] While high p16 levels could predict response to single agent BLU-222 in *CCNE1* high and Rb intact ovarian/endometrial cells, the data presented in the Examples above suggested that low p16 levels could be used to predict response to the BLU-222 with CDK4/6 inhibitor combination.

#### **Example 6. An exemplary CDK2 inhibitor was a chemosensitizing agent in cyclin E1 high ovarian models**

[00228] Given the encouraging combinability of an exemplary CDK2 inhibitor (*i.e.*, BLU-222) and a CDK4/6 inhibitor (*i.e.*, ribociclib) that achieved strong anti-tumor effects in pre-clinical ovarian and endometrial models, combinations of an exemplary CDK2 inhibitor (*i.e.*, BLU-222) in combination with standard of care chemotherapeutic agents were assessed. *CCNE1* amplification was associated with homologous recombination (HR)-proficiency and resistance to standard chemotherapeutic agents, such as platinum and taxane agents. In *CCNE1* amplified cells, CDK2 was critical for aberrant HR to stabilize collapsed replication forks, leading to DNA damaging agent

resistance (Brown, V.E. *et al.*, (2023)). The effect of inhibiting CDK2 in *CCNE1* high tumors on restoring sensitivity to chemotherapeutic agents was tested.

[00229] To evaluate pre-clinical combination activity *in vivo*, BLU-222 was dosed with carboplatin or paclitaxel in the OVCAR-3 T2A CDX model. Both combinations were well tolerated. Mice were dosed for 46 days, and tumors were monitored for regrowth to assess durability of response. As single agents, BLU-222, paclitaxel, or carboplatin demonstrated antitumor activity during the dosing period, but tumors grew after treatment cessation (Figure 12A). BLU-222 in combination with carboplatin or paclitaxel induced robust tumor regression during the dosing period, which was durable upon treatment cessation (Figure 12A). To test BLU-222 combined with carboplatin or paclitaxel in a *CCNE1* normal setting, the ES-2 CDX model was used. This model was refractory to BLU-222, carboplatin, and paclitaxel. Combination of BLU-222 and carboplatin or BLU-222 and paclitaxel had no additional anti-tumor effect (Figure 12B). BLU-222 combined with carboplatin activity was measured in *CCNE1* high ovarian PDX models. A *CCNE1* mRNA high (CN=2) PDX model derived from a treatment naïve patient was carboplatin sensitive (TGI=75.5%) with no additional anti-tumor benefit in the BLU-222 and carboplatin combination (TGI=65.2%, Figure 13A and 13B). In a *CCNE1* amplified (CN=8) PDX model derived from a carboplatin and paclitaxel progressed patient (ST182B), combining BLU-222 with carboplatin significantly improved the anti-tumor effect from the single agents, leading to tumor stasis (TGI=71.4%, Figure 13B). Together, these data supported that BLU-222 in combination with chemotherapy was most robust in chemotherapy resistant, CDK2 dysregulated tumors, such as in the *CCNE1* amplified setting. To evaluate biomarkers of response to the BLU-222 and chemotherapy response, a high-throughput *in vitro* approach in cell lines was taken to measure potential synergy (Figure 12H). Interestingly, *CCNE1*, Rb, and p16 did not significantly predict synergistic potential (Figure 14), which suggested that the chemotherapy combination response may be more broadly acting and independent of *CCNE1*/Rb/p16.

[00230] *CCNE1* amplification and high levels of cyclin E1 were associated with increased levels of replication stress. In the *CCNE1* amplified setting, pre-clinical treatment regimens that exacerbated replication stress and the DNA damage load, such as with ATR, Wee1, or PKMYT1 inhibition, could elicit strong anti-tumor effects. The combination of BLU-222 with gemcitabine was tested in the OVCAR-3 T2A model. Gemcitabine alone did not elicit a strong anti-tumor effect, but the BLU-222 and gemcitabine combination induced a stronger response than either single agent. However, the response was not durable, as tumors regrew post treatment cessation (Figure 13C). While the BLU-222 and gemcitabine combination induced strong anti-tumor activity while on-treatment, this

combination did not induce as strongly durable a response as BLU-222 combined with carboplatin or paclitaxel.

**Example 7. Response to a combination of an exemplary CDK2 inhibitor and paclitaxel in cyclin E1 high, Rb intact endometrial PDX models**

[00231] The CDK2 inhibitor (*i.e.*, BLU-222) and chemotherapy agent combinations were evaluated in *CCNE1* high endometrial PDX models (as tested in Figure 6F-K). ST2526 was derived from a patient who had progressed on fluorouracil and radiation, while all remaining models were derived from treatment naïve patients. All models were resistant to paclitaxel single agent treatment (Figure 12C-G).

[00232] The BLU-222 and paclitaxel combination was well tolerated and the plasma exposure in combination was not impacted from monotherapy treatment (Figures 15A-15E). In the *CCNE1* copy number aberrant, Rb intact, p16 high models (ST3052 and ST2526), BLU-222 induced tumor stasis (TGI = 102% and 107%, respectively, Figures 12C and 12D). While the combination with paclitaxel did not significantly improve the anti-tumor activity from BLU-222 monotherapy in ST3052 (TGI = 107%, Figure 12C), the combination led to tumor regression in ST2526 (TGI = 113%, Figure 12D). A third *CCNE1* copy number aberrant, Rb intact, but p16 low model (ST1386) did not respond to BLU-222 60 mpk (TGI = 17%) or paclitaxel (TGI = 9%) monotherapy, however, stasis was achieved with the BLU-222 and paclitaxel combination (TGI = 78%; Figure 12E). Therefore, in *CCNE1* CN aberrant PDX models, BLU-222 and paclitaxel induced a profound anti-tumor response.

[00233] A combination benefit for BLU-222 with paclitaxel was tested in *CCNE1* mRNA high but CN normal models. ST259 (*CCNE1* CN 2, Rb+, p16-) and ST189 (*CCNE1* CN 2, Rb-, p16-) were refractory to BLU-222 single agent treatment (Figures 12F and 12G). In ST259, a p16 low model, BLU-222 and paclitaxel had a strong anti-tumor effect (TGI = 80%; Figure 12F). In ST189, a model lacking Rb expression, the BLU-222 and paclitaxel combination led to tumor stabilization after two weeks of treatment (TGI = 52%; Figure 12G). Together, these results revealed a combination benefit for BLU-222 and paclitaxel in cyclin E1-high models to overcome chemotherapy resistance, even beyond the *CCNE1* CN amplified setting.

[00234] Beyond combination of CDK inhibitors, a role for BLU-222 was demonstrated as a chemosensitizer in cyclin E1 high tumors using combinations with standard of care chemotherapeutic agents. Analogous to CDK4/6 inhibitor use in metastatic breast cancer, where CDK4/6 inhibitors and endocrine therapy deepen efficacy, CDK2 inhibition combined with chemotherapeutic agents demonstrated a strong combination benefit in the *CCNE1* high setting (Figures 12A, 12C, 12D, 12E, 13B, and 13C-). Even in the *CCNE1* high, Rb intact, p16 high setting where BLU-222 showed strong monotherapy activity, combination with paclitaxel or carboplatin deepened the anti-tumor response, converting a static response to regression. Further, pre-clinical models of chemoresistant cancers that

were non-responders to BLU-222 could be converted to a strongly efficacious response with chemotherapy combination (Figures 12F and 12G). Collectively the data suggest that BLU-222 may work as a chemosensitizer in the *CCNE1* aberrant setting, independent of p16 expression.

**[00235]** BLU-222 is currently being studied in a Phase 1/2 clinical trial as a monotherapy in advanced solid tumors and in combination with ribociclib and fulvestrant in ER+/HER2- breast cancer. Using a combination of *in vitro*, *in vivo*, and bioinformatic approaches, a multivariate *CCNE1*/Rb/p16 biomarker signature to enrich for CDK2 dependency and sensitivity to CDK2 inhibitors (*e.g.*, BLU-222) has been identified in Cyclin E1 high ovarian and endometrial cancers. These biomarkers expanded the mechanistic understanding of cell cycle dysregulation that led to aberrant CDK2 activity and might aid in the interpretation of emerging clinical data in *CCNE1* high tumors. Further, these data provided a scientific rationale for potential future clinical trial patient selection criteria for monotherapy and combination therapy strategies.

**CLAIMS**

1. A method of treating endometrial cancer or ovarian cancer in a subject in need thereof, wherein the subject's tumor, plasmas, or other tissue is identified as having each of the following:
  - i. an elevated expression level of p16 mRNA or p16 protein as compared to a control sample;
  - ii. proficient retinoblastoma 1 protein (Rb) expression and no loss of function mutations in Rb as compared to a control sample; and
  - iii. an increased copy number of cyclin E1 (CCNE1) gene and/or an elevated expression level of mCCNE1 RNA or CCNE1 protein as compared to a control sample, andwherein the subject is in need thereof, comprising administering to the subject a therapeutically effective amount of a cyclin-dependent kinase 2 (CDK2) inhibitor, or a pharmaceutically acceptable salt thereof, thereby treating the endometrial cancer or ovarian cancer.
2. A method of treating endometrial cancer or ovarian cancer in a subject identified as having all of the following:
  - i. an elevated expression level of p16 mRNA or p16 protein;
  - ii. intact Rb, which is based on expression level of Rb mRNA or Rb protein, and no loss of function mutations in Rb; and
  - iii. an increased copy number of CCNE1 gene and/or an elevated expression level of CCNE1 mRNA or CCNE1 proteinin the subject's cancer, wherein the levels of p16 and Rb are compared to the average mRNA or protein levels of p16 and mRNA or protein levels of Rb in a population of subjects suffering from endometrial cancer or ovarian cancer; and the copy number of CCNE1 is greater than 2 and/or the expression levels of CCNE1 mRNA or protein is elevated as compared to the average mRNA or protein levels of CCNE1 in a population of subjects suffering from endometrial cancer or ovarian cancer, and wherein the subject is in need thereof, the method comprising administering to the subject a therapeutically effective amount of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, thereby treating the endometrial cancer or ovarian cancer.
3. A method of treating endometrial cancer or ovarian cancer in a subject in need thereof comprising:
  - i. testing, or having tested, a first biological sample obtained from the subject with endometrial cancer or ovarian cancer, thereby measuring
    - a. levels of p16;
    - b. levels of Rb; and
    - c. amplification and/or levels of CCNE1,

in the subject's cancer;

- ii. comparing the levels of p16 mRNA or protein, the levels of Rb mRNA or protein, and the amplification and/or levels of CCNE1 mRNA or protein, in step i. to the average levels of p16 mRNA or protein, levels of Rb mRNA or protein, and amplification and/or levels of CCNE1 mRNA or protein in a population of subjects suffering from endometrial cancer or ovarian cancer;
- iii. determining that the subject's cancer is characterized by all of the following:
  - a. an elevated expression level of p16 mRNA or p16 protein as compared to an average level of p16 in a population of subjects suffering from endometrial cancer or ovarian cancer;
  - b. intact Rb, which is based on expression level of Rb mRNA or Rb protein as compared to an average level of Rb in a population of subjects suffering from endometrial cancer or ovarian cancer, and no loss of function mutations in Rb; and
  - c. an increased copy number of CCNE1 gene, which is greater than 2, and/or an elevated expression level of CCNE1 mRNA or CCNE1 protein as compared to an average level of CCNE1 in a population of subjects suffering from endometrial cancer or ovarian cancer; and
- iv. administering to the subject a therapeutically effective amount of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, thereby treating the endometrial cancer or ovarian cancer.

4. The method of claim 3 further comprising:

- v. further testing, or having tested, a first biological sample obtained from the subject with endometrial cancer or ovarian cancer, thereby further measuring
  - a. levels of phosphorylated Rb protein;
  - b. levels of phosphorylated nucleophosmin 1 (NPM1) protein; and
  - c. levels of thymidine kinase 1 (TK1),

in the subject's cancer;

- vi. testing, or having tested, a second biological sample obtained from the subject with endometrial cancer or ovarian cancer, thereby measuring
  - a. levels of phosphorylated Rb protein;
  - b. levels of phosphorylated NPM1 protein; and
  - c. levels of TK1,

in the subject's cancer;

- vii. determining whether the subject having or at risk of developing endometrial cancer or ovarian cancer has responded to treatment with a CDK2 inhibitor, or a pharmaceutically

acceptable salt thereof, if the comparison of the second biological sample analysis in step vi. to the first biological sample analysis in step v. shows

- a. a changed level of phosphorylated Rb protein;
- b. a changed level of phosphorylated NPM1 protein;
- c. a changed expression level of TK1 protein; and
- d. a changed level of TK1 protein activity,

thereby monitoring a response in a subject having or at risk of developing endometrial cancer or ovarian cancer.

5. A method of treating endometrial cancer or ovarian cancer in a subject in need thereof, wherein the subject's tumor, plasmas, or other tissue is identified as having each of the following:
- i. low or no p16 mRNA or protein expression, dysfunctional p16, or mutated p16 as compared to a control sample;
  - ii. intact Rb, which is based on expression level of Rb mRNA or Rb protein, and no loss of function mutations in Rb as compared to a control sample; and
  - iii. an increased copy number of CCNE1 gene and/or an elevated expression level of CCNE1 RNA or CCNE1 protein as compared to a control sample

comprising administering to the subject a therapeutically effective amount of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, and a therapeutically effective amount of a CDK4/6 inhibitor, or a pharmaceutically acceptable salt thereof, thereby treating the endometrial cancer or ovarian cancer.

6. A method of treating endometrial cancer or ovarian cancer in a subject identified as having all of the following:
- i. low or no p16 protein expression, dysfunctional p16, or mutated p16;
  - ii. proficient Rb expression and no loss of function mutations in Rb; and
  - iii. an increased copy number of CCNE1 gene and/or an elevated expression level of CCNE1 RNA or CCNE1 protein

in the subject's cancer, wherein the levels of p16 and Rb are compared to the average mRNA or protein levels of p16 and mRNA or protein levels of Rb in a population of subjects suffering from endometrial cancer or ovarian cancer; and the copy number of CCNE1 is greater than 2 and/or the expression levels of CCNE1 mRNA or protein is elevated as compared to the average mRNA or protein levels of CCNE1 in a population of subjects suffering from endometrial cancer or ovarian cancer, and wherein the subject is in need thereof, comprising administering to the subject a therapeutically effective amount of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof,

and a therapeutically effective amount of a CDK4/6 inhibitor, or a pharmaceutically acceptable salt thereof, thereby treating the endometrial cancer or ovarian cancer.

7. A method of treating endometrial cancer or ovarian cancer in a subject in need thereof comprising:
  - i. testing, or having tested, a first biological sample obtained from the subject with endometrial cancer or ovarian cancer, thereby measuring
    - a. levels of p16;
    - b. levels of Rb; and
    - c. amplification and/or levels of CCNE1,in the subject's cancer;
  - ii. comparing the levels of p16 mRNA or protein, the levels of Rb mRNA or protein, and the amplification and/or levels of CCNE1 mRNA or protein, in step i. to the average levels of p16 mRNA or protein, levels of Rb mRNA or protein, and amplification and/or levels of CCNE1 mRNA or protein in a population of subjects suffering from endometrial cancer or ovarian cancer;
  - iii. determining that the subject's cancer is characterized by all of the following:
    - a. low or no p16 mRNA or protein expression, dysfunctional p16, or mutated p16 as compared to an average level of p16, functional p16, or wild type p16 in a population of subjects suffering from endometrial cancer or ovarian cancer;
    - b. intact Rb, which is based on expression level of Rb mRNA or Rb protein as compared to an average level of Rb in a population of subjects suffering from endometrial cancer or ovarian cancer, and no loss of function mutations in Rb; and
    - c. an increased copy number of CCNE1 gene, which is greater than 2, and/or an elevated expression level of CCNE1 mRNA or protein as compared to an average level of CCNE1 in a population of subjects suffering from endometrial cancer or ovarian cancer; and
  - iv. administering to the subject a therapeutically effective amount of a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, and a therapeutically effective amount of a CDK4/6 inhibitor, or a pharmaceutically acceptable salt thereof, thereby treating the endometrial cancer or ovarian cancer.
8. The method of claim 7 further comprising:
  - v. further testing, or having tested, a first biological sample obtained from the subject with endometrial cancer or ovarian cancer, thereby further measuring
    - a. levels of phosphorylated Rb protein;

- b. levels of phosphorylated NPM1 protein; and
    - c. levels of TK1,

in the subject's cancer;
  - vi. testing, or having tested, a second biological sample obtained from the subject, thereby measuring
    - a. levels of phosphorylated Rb protein;
    - b. levels of phosphorylated NPM1 protein; and
    - c. levels of TK1,

in the subject's cancer;
  - vii. determining whether the subject having or at risk of developing endometrial cancer or ovarian cancer has responded to treatment with a CDK2 inhibitor, or a pharmaceutically acceptable salt thereof, and a CDK4/6 inhibitor, or a pharmaceutically acceptable salt thereof, if the comparison of the second biological sample analysis in step vi. to the first biological sample analysis in step v. shows
    - a. a changed level of phosphorylated Rb protein;
    - b. a changed level of phosphorylated NPM1 protein;
    - c. a changed expression level of TK1 protein; and
    - d. a changed level of TK1 protein activity,

thereby monitoring a response in a subject having or at risk of developing endometrial cancer or ovarian cancer.
9. The method of any one of claims 5 to 8, wherein the CDK4/6 inhibitor is selected from abemaciclib, biraciclib (XZP- 3287), BGB-43395, BPI-16350, dalpiciclib (SHR6390), FLX-925 (AMG-925), GLR2007, lerociclib, LY5219, narazaciclib (ON-123300), palbociclib, atirmociclib (PF-07220060), PF-07224826, RGT-419B, ribociclib, trilaciclib, and UCT-03-008, or a combination thereof.
10. The method of any one of the preceding claims, wherein the endometrial cancer or ovarian cancer is CCNE1-amplified ovarian cancer or CCNE1-amplified endometrial cancer.
11. The method of any one of claims 1 to 10, wherein the endometrial cancer or ovarian cancer is endometrial cancer (with prior platinum therapy) that has progressed following 2 or more lines of therapies or platinum-resistant or platinum-refractory ovarian cancer.