

BLU-222, an investigational, potent, and selective CDK2 inhibitor, demonstrated robust antitumor activity in *CCNE1*-amplified ovarian cancer models

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Background

- A broad range of aggressive cancers harbor cyclin E1 (*CCNE1*) gene amplifications¹ (Figure 1A)
- CCNE1* amplification has been associated with poor survival in ovarian cancer, representing an unmet medical need^{2,3} (Figure 1B)
- Cyclin E1 is the canonical binding partner of cyclin-dependent kinase 2 (CDK2) and the cyclin E1-CDK2 complex drives G1/S progression of the cell cycle⁴ (Figure 2)
- CDKs are a class of enzymes that, along with their regulatory cyclin binding partners, drive cell cycle progression⁴
- Cell lines harboring *CCNE1* amplification are sensitive to CDK2 knockout or catalytic inhibition with ATP-competitive molecules, suggesting CDK2 may be an attractive therapeutic target for *CCNE1*-amplified tumors^{5,6}
- Selectively inhibiting CDK2 for *CCNE1*-amplified tumors may limit off-target CDK-driven toxicities
- BLU-222 is an orally available, selective investigational CDK2 inhibitor⁶
 - The US Food and Drug Administration cleared the investigational new drug application and a phase 1/2 trial (VELA; NCT05252416) of BLU-222 in patients with *CCNE1*-amplified tumors is now enrolling⁷
- We present preclinical validation studies leading to the development of BLU-222 for the treatment of patients with ovarian cancer harboring a *CCNE1* amplification

Figure 1: *CCNE1* amplification is prevalent across various tumor types and correlates with poor overall survival in patients with ovarian cancer¹⁻³

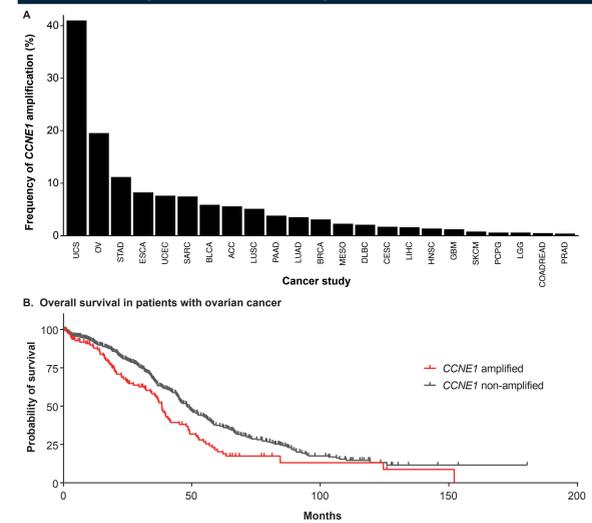
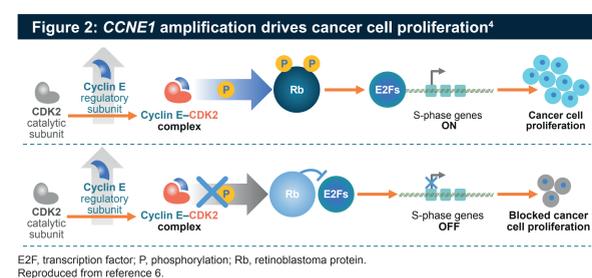


Figure 2: *CCNE1* amplification drives cancer cell proliferation⁴



Methods

- BLU-222 selectivity was measured by enzyme assays and cellular target engagement assays (NanoBRET)
- Data from Project Achilles⁵ and proliferation assays from a panel of cancer cell lines were used to determine CDK2 sensitivity based on *CCNE1* copy number
- In vitro* cellular potency was assessed by phospho-Rb levels
- Mechanism of action was determined using CRISPR-Cas9 generated Rb knockout cells
- In vivo* antitumor activity of BLU-222 as a single agent or in combination with standard of care (SOC) agents was measured in the OVCAR-3 cell line-derived xenograft (CDX) tumor model harboring a *CCNE1* amplification

Results

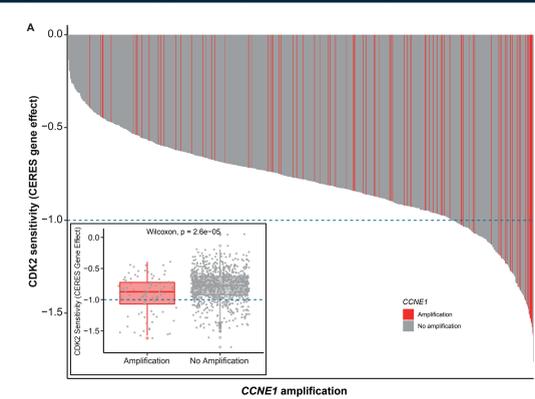
Table 1: BLU-222 is a selective and potent CDK2 inhibitor

Kinome S (10) ^a	Enzyme activity IC ₅₀ (nM) ^b						Cellular activity IC ₅₀ (nM) ^c	
	CDK2	CDK1	CDK4	CDK6	CDK7	CDK9	pRb T821 (CDK2 cell)	pLamin S22 (CDK1 cell)
0.045	2.6	233.6	377.4	275.2	6941.2	6115.1	4.2	380.2
	NanoBRET activity IC ₅₀ (nM) ^d							
	CDK2	CDK1	CDK4	CDK6	CDK7	CDK9		
	17.7	452.3	5104.6	2621.7	6330.4	2697.7		

^aKinome S(10): fraction of kinases with <10 percentage of control at 3 μM among all the kinases tested, measured by KINOME scan platform against 468 kinases. ^bEnzyme activities IC₅₀ were measured at 1 mM ATP using canonical CDK/cyclin pairs: CDK2/Cyclin E1; CDK1/Cyclin B1; CDK4/Cyclin D1; CDK6/Cyclin D3; CDK7/Cyclin H1/MNAT1; CDK9/Cyclin T1. ^cHEK-293T cells were transfected with canonical CDK/cyclin pairs as in the enzyme assay and treated with compound and a tracer for 2 hours before measurements were taken. ^dpRb T821 protein was assessed in synchronized OVCAR-3 cells to reflect CDK2 cellular potency; pLamin S22 was assessed in asynchronous OVCAR-3 cells to reflect CDK1 cellular potency.

- BLU-222 exhibits single-digit nanomolar cellular potency and is selective for CDK2 over other CDK family members

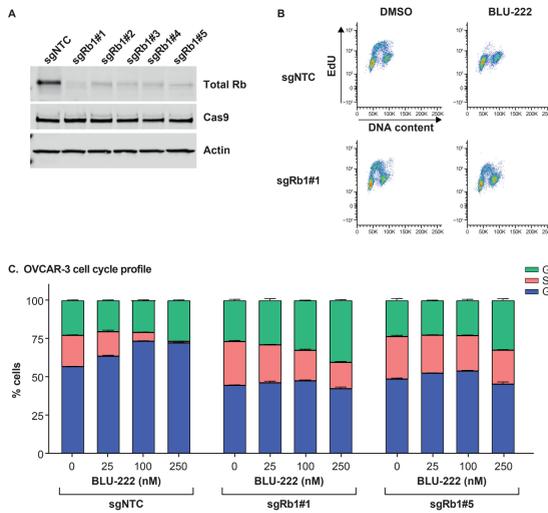
Figure 3: *CCNE1* copy number predicted sensitivity to CDK2 inhibition



(A) CDK2 essentiality scores replotted from Project Achilles. *CCNE1*-amplified cell lines (CN ≥3; red bars) and nonamplified lines (CN <3; gray bars) plotted against CDK2 essentiality score. Blue dotted line represents cut-off for essentiality. Lower insert represents same data as a box plot.

(B) IC₅₀ values in nM across a panel of cell lines. Cells (amplification, CN ≥3; no amplification, CN <3) were treated for 5 days with BLU-222 to determine the dose response. CyQuant was used to measure proliferation relative to DMSO control. Error bars represent SEM.

Figure 4: Treatment with BLU-222 arrested cells at G1/S in an Rb-dependent manner

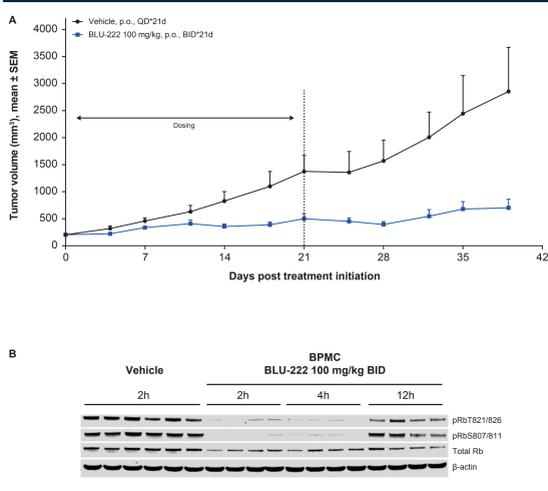


(A) Rb1 knockout confirmation in OVCAR-3 cells. Five sgRNAs targeting Rb1 were tested for knockout efficiency in OVCAR-3 cells. Cells lysed after 2 weeks of selection and evaluated by Western blot.

(B) Representative flow cytometry plots of OVCAR-3 cells after treatment. OVCAR-3 sgNTC and sgRb1#1 cells were treated with DMSO or BLU-222 (250 nM) for 24 hours. DNA content was measured by FxCycle (X-axis) and S-phase cells were measured by EdU incorporation (Y-axis).

(C) Cell cycle profile of OVCAR-3 cells. OVCAR-3 sgNTC, sgRb1#1, and sgRb1#5 were treated with a dose titration of BLU-222 for 24 hours and the cell cycle profile was determined by FxCycle and EdU incorporation. Error bars represent SEM in two independent experiments.

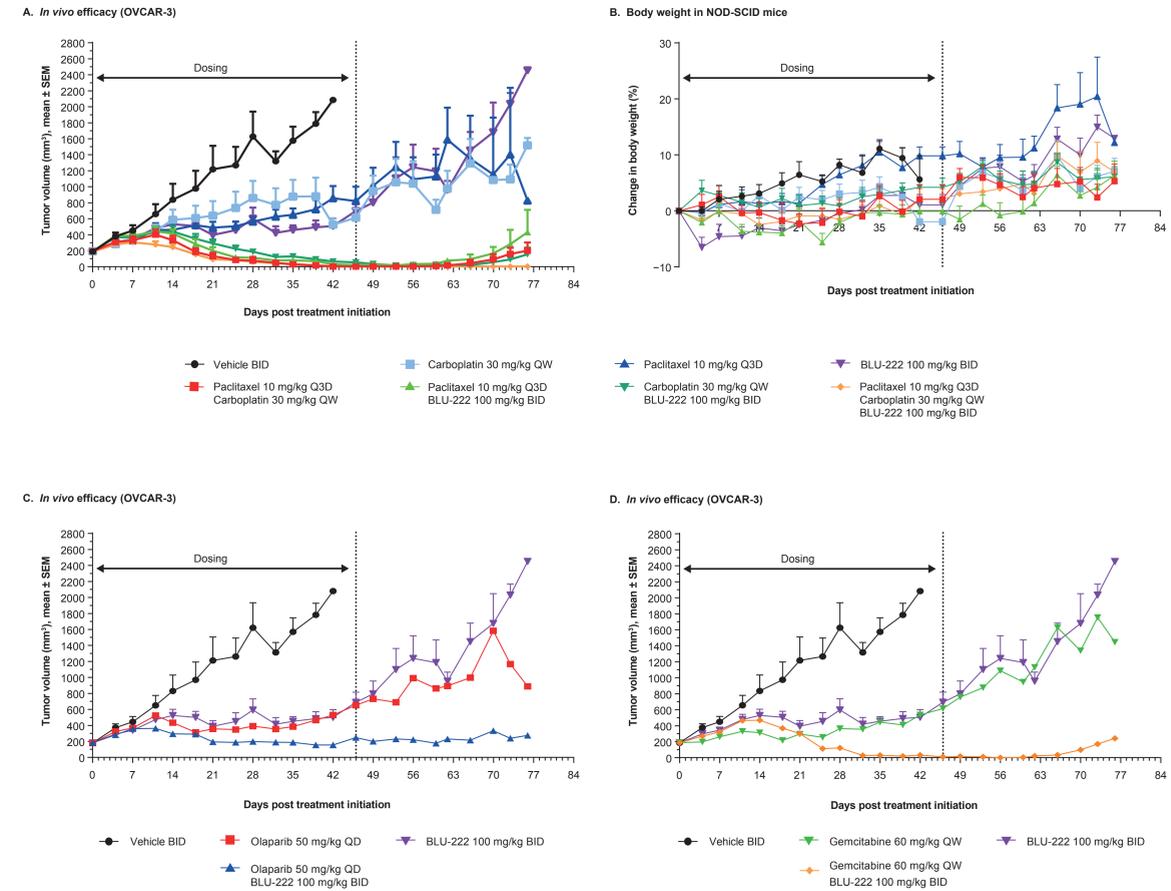
Figure 5: BLU-222 showed single-agent antitumor activity *in vivo* in a *CCNE1*-amplified tumor model



(A) BLU-222 antitumor activity in the OVCAR-3 CDX model. Mice inoculated SC with OVCAR-3 (6 × 10⁶) cells. Drug treatment (indicated by double-headed arrows) was initiated when tumors reached ~150–250 mm³ and continued through Day 21. The regrowth of the remaining tumors was monitored in the absence of drug treatment.

(B) Pharmacodynamic inhibition. Tumor lysates were assessed by Western blots at the indicated time points 3 days post treatment.

Figure 6: Combination treatments with BLU-222 and standard of care therapies induced tumor regression



(A) BLU-222 + chemotherapy combination in the OVCAR-3 CDX model. Mice inoculated SC with OVCAR-3 (6 × 10⁶) cells. Drug treatment (indicated by double-headed arrows) was initiated when tumors reached ~150–250 mm³ and continued through Day 46. The regrowth of the remaining tumors was monitored in the absence of drug treatment.

(B) Body weight measurement in mice treated with BLU-222 + chemotherapy. Mice were monitored over the course of the study and body weight measurements were taken twice weekly.

(C) BLU-222 + olaparib and (D) BLU-222 + gemcitabine combinations in the OVCAR-3 CDX model. Mice inoculated SC with OVCAR-3 (6 × 10⁶) cells. Drug treatment (indicated by double-headed arrows) was initiated when tumors reached ~150–250 mm³ and continued through Day 46. The regrowth of the remaining tumors was monitored in the absence of drug treatment.

Conclusions and future directions

- CCNE1* copy number increase was a strong predictor of response to CDK2 inhibition across tumor types in cellular systems
- BLU-222 is a selective and potent CDK2 inhibitor that arrested cells at the G1/S boundary in an Rb-dependent manner
- BLU-222 as monotherapy showed antitumor activity in a *CCNE1*-amplified CDX tumor model
- The combinations of BLU-222 with carboplatin (SOC first-line treatment), BLU-222 with olaparib, and BLU-222 with gemcitabine all induced tumor regression that was sustained even after treatment cessation
- Taken together, this evidence provides scientific rationale for the clinical development of BLU-222 as a monotherapy and in combination with SOC agents in *CCNE1*-amplified cancers

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Disclosures

All authors, except R Lobbari, N Bifulco, M Maynard, S Wenglowky, and YJ Choi, are current employees and shareholders of Blueprint Medicines Corporation. R Lobbari, N Bifulco, M Maynard, and S Wenglowky were former employees of Blueprint Medicines Corporation at the time of the study and do not still receive stock or options.

