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CDK2 inhibition with BLU-222 in combination with ribociclib demonstrates robust antitumor activity in pre-clinical models of CDK4/6 inhibitor-naïve and -resistant HR+/HER2– breast cancer

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Background

- Cyclins are regulatory proteins that, when partnered with cyclin-dependent kinases (CDKs), are essential for the regulation of cell growth and proliferation[1]
- CDK4/6 inhibitors are standard of care in combination with endocrine therapy in advanced hormone receptor-positive (HR+) and human epidermal growth factor receptor 2 negative (HER2–) breast cancer[2]
- Despite improved progression-free and overall survival, almost all patients with HR+/HER– breast cancer develop CDK4/6 inhibitor resistance and experience disease progression on treatment[3]
- Aberrant activation of CDK2/cyclin E is a key resistance mechanism by which tumors can evade CDK4/6 blockade[4]
  - Patients with HR+/HER2– breast cancer could benefit from treatment with a selective CDK2 inhibitor in combination with CDK4/6 inhibitors, both in the resistant and first-line (1L) settings[4]
- BLU-222 is an investigational novel, potent, and selective small-molecule inhibitor of CDK2 (**Table 1**) with favorable oral pharmacokinetic properties, currently in early-stage clinical development (NCT05252416)[5]
- Here, we investigate the antitumor activity of BLU-222 in pre-clinical models of CDK4/6 inhibitor-naïve and -resistant HR+/HER2– breast cancer

Table 1: BLU-222 is a selective and potent CDK2 inhibitor[6]

Kinome S (10)[a]	Enzyme activity IC_{50} (nM)[b]					
	CDK2	CDK1	CDK4	CDK6	CDK7	CDK9
0.045	2.6	233.6	377.4	275.2	6941.2	6115.1
	NanoBRET activity IC_{50} (nM)[c]					
	CDK2	CDK1	CDK4	CDK6	CDK7	CDK9
	17.7	452.3	5104.6	2621.7	6330.4	2697.7
	Cellular activity IC_{50} (nM)[d]					
	pRb T821 (CDK2 cell)		pRb S807/811 (CDK2 cell)		pLamin S22 (CDK1 cell)	
	4.2		24.0		380.2	

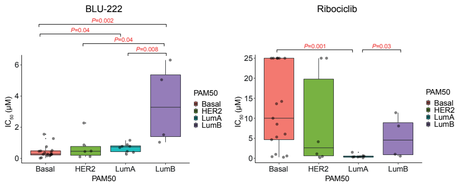
[a]Kinome S(10): fraction of kinases with <10 percentage of control at 3 µM among all the kinases tested, measured by KINOMEscan® platform against 468 kinases. [b]Enzyme activities IC_{50} were measured at 1 mM ATP using canonical CDK/Cyclin pairs: CDK2/Cyclin E1; CDK1/Cyclin B1; CDK4/Cyclin D1; CDK6/Cyclin D3; CDK7/Cyclin H1/MNAT1; CDK9/Cyclin T1. [c]HEK-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. [d]pRb T821 or pRb S807/811 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.

ATP, adenosine triphosphate; CDK, cyclin-dependent kinases; IC_{50}, half-maximal inhibitory concentration; pRB, phosphorylated retinoblastoma protein.

Results

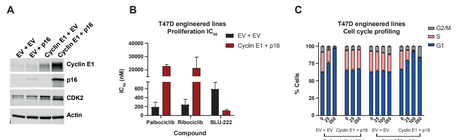
- BLU-222 and ribociclib demonstrated activity in distinct sub-types of breast cancer (**Figure 1**)

Figure 1: IC_{50} of breast cancer cell lines treated with BLU-222 or ribociclib



Cells were treated for 5 days with compound and assayed with CyQUANT™. *P*-values were determined using using Wilcoxon signed-rank test. HER2, human epidermal growth factor receptor 2; IC_{50}, half-maximal inhibitory concentration; Lum, luminal; PAM50, Prediction Analysis of Microarray 50.

Figure 2: Protein expression (A), compound IC_{50} (B) and cell cycle profile (C) in engineered T47D cells

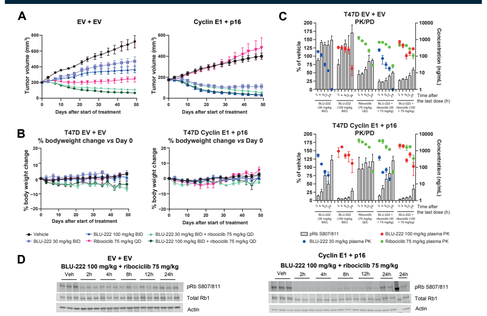


(A) Cells overexpressing cyclin E1 and/or p16 were probed for protein expression of indicated markers. (B) Cells were treated with indicated compounds for 5 days and assayed by CyQUANT™. IC_{50} are averages of at least four independent experiments. Error bars represent SD. (C) Cells were treated for 24 hours with indicated compounds. Cell cycle profile assessed by EdU incorporation (2 h) combined with DNA content (FxCycle™). Error bars represent SD (n=3).

CDK, cyclin-dependent kinases; EV, empty vector; IC_{50}, half-maximal inhibitory concentration; SD, standard deviation.

- In *vitro* proliferation assays, co-expression of cyclin E1 and p16 sensitized T47D cells to BLU-222 by approximately 10-fold compared with the parental control (110 nM *vs* 1078 nM, respectively) (**Figure 2**)
- CDK4/6 inhibition with palbociclib or ribociclib had no antiproliferative effect in the cyclin E1-overexpressing cells (**Figure 2**)

Figure 3: Antitumor activity (A), body weight (B), and PK/PD (C, D) in T47D engineered xenograft models

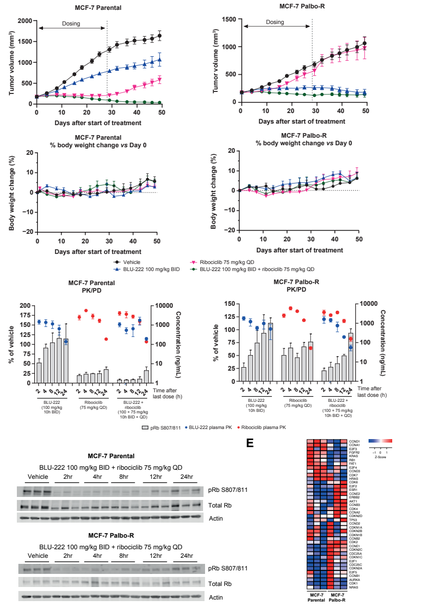


(A) Mice inoculated subcutaneously with engineered T47D cells (10×10^6 cells). Drug treatment was initiated when tumors reached ~150–250 mm³. (B) Body weight of mice treated in panel A. Bodyweight was measured twice a week over the course of 49 days. Five mouse deaths were observed across ribociclib (n=3), BLU-222 + ribociclib (n=2) treatment groups over both models combined. (C) Following three days of treatment, tumors were lysed and probed for pRb S807/811. Data is plotted as percent of vehicle-treated animals (gray bars). Plasma PK for indicated agents was measured at indicated time points (n=3). (D) Representative Western blots are shown for combination treatment groups (BLU-222 100 mg/kg BID + ribociclib 75 mg/kg QD) for indicated markers.

BID, two times a day; CDK, cyclin-dependent kinases; EV, empty vector; IC_{50}, half-maximal inhibitory concentration; PD , pharmacodynamics; PK, pharmacokinetics; pRB, phosphorylated retinoblastoma protein; QD, once daily; Rb1, retinoblastoma protein 1; Veh, vehicle.

- *In vivo* treatment of the empty vector control T47D xenografts with ribociclib led to tumor stasis, while ribociclib in combination with BLU-222 led to tumor regression (**Figure 3A**)
- T47D xenografts overexpressing both cyclin E1 and p16 were resistant to ribociclib. However, treatment with single-agent BLU-222 or its combination with ribociclib led to tumor regression (**Figure 3A**)
  - No significant change in body weight was observed in any treatment group (**Figure 3B**)
- Treatment with BLU-222 and ribociclib reduced retinoblastoma protein phosphorylation (pRb) S807/811 in T47D xenografts overexpressing both cyclin E1 and p16 compared with empty vector control (**Figure 3C, 3D**)

Figure 4: Antitumor activity (A), body weight (B), PK/PD (C, D), and gene expression (E) in MCF-7 parental and palbociclib-resistant xenograft models

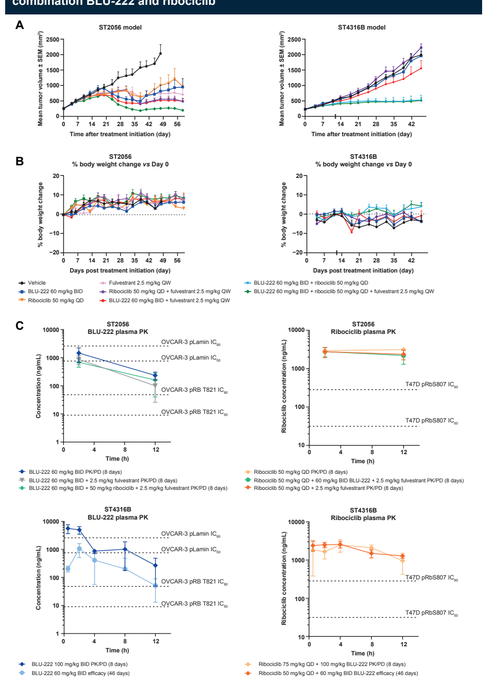


(A) Mice inoculated subcutaneously with MCF-7 parental or palbociclib-resistant cells (10×10^6 cells). Drug treatment (indicated by double-ended arrows) was initiated when tumors reached ~150–250 mm³ and was stopped after 28 days. The regrowth of the remaining tumors was monitored in the absence of drug treatment for three additional weeks. (B) Body weight was measured twice a week over the course of 49 days. No significant body weight change observed in any treatment group. Five mouse deaths were observed across ribociclib (n=1), BLU-222 (n=2), BLU-222 + ribociclib (n=2) treatment groups over both models combined. (C) Following three days of treatment, tumors were lysed and probed for pRb S807/811. Data is plotted as % of vehicle-treated animals (gray bars). Plasma PK for indicated agents was measured at indicated time points (n=3). (D) Representative Western blots are shown for combination treatment groups (BLU-222 100 mg/kg BID + ribociclib 75 mg/kg QD) for indicated markers. (E) Heatmap showing the expression of genes involved in cell cycle regulation in MCF-7 parental and MCF-7 palbociclib resistant models (n=3 tumors/model).

BID, two times a day; Palbo-R, palbociclib-resistant; PD, pharmacodynamics; PK, pharmacokinetics; pRB, phosphorylated retinoblastoma protein; QD, once daily; Rb1, retinoblastoma protein 1.

- Combination treatment with BLU-222 and ribociclib led to a strong and sustained antitumor response in both MCF-7 parental and palbociclib-resistant xenografts compared with the single agents (**Figure 4A**)
  - No significant change in body weight was observed in any treatment group (**Figure 4B**)
- Combination treatment with BLU-222 and ribociclib substantially reduced pRb S807/811 phosphorylation in MCF-7 parental xenografts, while both single-agent treatment of BLU-222 and the combination with ribociclib reduced this marker in the palbociclib-resistant xenografts (**Figure 4C, 4D**)
- Differential gene expression in MCF-7 parental *vs* palbociclib-resistant tumors suggests changes in core cell cycle machinery may be contributing to CDK4/6 inhibitor resistance (**Figure 4E**)

Figure 5: Antitumor activity (A), body weight (B), and plasma exposure (C) in PDX models of CDK4/6 inhibitor-resistant HR+/HER2– breast cancer, treated with combination BLU-222 and ribociclib



(A) Antitumor activity in CDK4/6 inhibitor PDX model ST2056, derived from a patient that progressed on 1L abemaciclib + fulvestrant, 2L fulvestrant, and 3L investigational therapy, and in model ST4316B, derived from a patient that progressed on 1L palbociclib + fulvestrant and 2L abemaciclib + fulvestrant. Drug treatment was initiated when tumors reached ~150–250 mm³. For the ST4316B model, BLU-222 was reduced from 100 mg/kg BID to 60 mg/kg BID on day 12 as indicated by tick mark, and ribociclib was reduced from 75 mg/kg to 50 mg/kg. (B) Body weight of mice treated in (A). Bodyweight was measured twice a week over the course of the study. (C) Plasma exposure over time for BLU-222 and ribociclib was measured in the OVCAR-3 and T47D CDX tumor models in mice bearing ST2056 xenografts treated for 8 days and ST4316B xenografts treated for 8 and 46 days, respectively. Cellular IC_{50} or IC_{90} for indicated phosphorylation marks corrected for plasma protein and FBS binding are represented by dotted lines.

1L, first-line; 2L, second-line; 3L, third-line; BID, two times a day; CDX, cell-derived xenograft; FBS, fetal bovine serum; IC_{50}, half-maximal inhibitory concentration; HR+/HER2–, hormone receptor-positive and human epidermal growth factor receptor 2 negative; IC_{90}, 90% inhibitory concentration; PD, pharmacodynamics; PDX, patient-derived xenograft; PK, pharmacokinetics; QD, once daily; QW, once weekly.

- In two patient-derived xenograft models of CDK4/6 inhibitor-resistant HR+/HER2– breast cancer, BLU-222 in combination with ribociclib led to tumor stasis (**Figure 5A**)
  - No significant change in body weight was observed in any treatment group (**Figure 5B**)
  - Plasma exposure for both agents suggests target coverage over the treatment period (**Figure 5C**)

Conclusions

- This study supports that CDK2/cyclin E activation is a key vulnerability in CDK4/6 inhibitor-resistant, HR+/HER2– breast cancer
- The improved durability of response when BLU-222 is combined with CDK4/6 inhibitors in the CDK4/6-naïve setting supports the study of the combination of these agents in 1L treatment
- BLU-222 is currently under investigation as a single agent and in combination therapy in VELA (NCT05252416), a phase 1/2, first-in-human trial in patients with cyclin E-amplified ovarian, endometrial, and other cancers as well as HR+/HER2– breast cancer with disease progression on a CDK4/6 inhibitor.[5] Phase 1 and 2 cohorts include patients treated with:
  - BLU-222 monotherapy in *cyclin E1 (CCNE1)*-amplified ovarian cancer and endometrial cancer
  - BLU-222 in combination with carboplatin in *CCNE1*-amplified ovarian cancer
  - BLU-222 with fulvestrant in HR+/HER2– breast cancer
  - BLU-222 with ribociclib and fulvestrant in HR+/HER2– breast cancer with disease progression on a CDK4/6 inhibitor

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Disclosures

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