Antitumor activity of potent and selective CDK2 inhibitors as monotherapy and in combination with chemotherapy in models of small cell lung cancer

Nealia House,[1] Liang Yuan[1], Christophe Lemetre[1], Maxine Chen[1], Philip Ramsden[1], Scott Ribich[1], Lakshmi Muthuswamy[1], Kerrie Faia[1]

[1]Blueprint Medicines Corporation, Cambridge, MA, USA

Abstract 5330

Poster and supplementary methods available for download at

Background

- Cyclin-dependent kinase 2 (CDK2) is a key mediator of cell cycle progression. In cancer, dysregulation of the cell cycle can render cells dependent on CDK2 activity, making CDK2 an attractive target for selective inhibition in certain cancers[1–4]
- BLU-222 is a potent and highly selective CDK2 inhibitor (**Figure 1**) that has been evaluated in a phase I/II clinical trial (NCT05252416)[5–7]
- BLU-956 is a potent, selective, and orally bioavailable CDK2 inhibitor, with an improved preclinical pharmacokinetic (PK) and selectivity profile compared with BLU-222
- Here, we explore BLU-222 and BLU-956 activity in CDK2-dependent preclinical models of ovarian, breast, and small cell lung cancer (SCLC). Further, we demonstrate in SCLC that antitumor activity is enhanced when combining CDK2 inhibition with cisplatin

Drug potency profile — **Figure 1: BLU-222 and BLU-956 are potent and selective CDK2 inhibitors**

BLU-222[8]

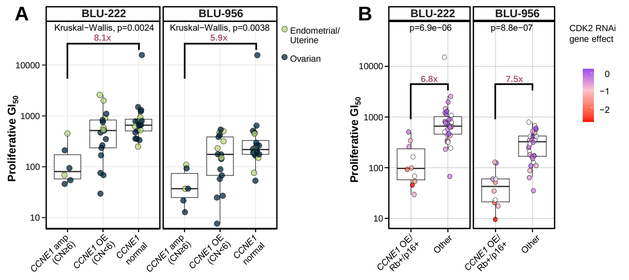
Kinome selectivity	Enzymatic assay IC_{50} (nM) [fold selectivity]							Cellular assay IC_{50} (nM)
S(10) @ 3μM	**CDK2/E1**	**CDK2/A2**	**CDK1/B**	**CDK4/D1**	**CDK6/D3**	**CDK7/H**	**CDK9/T1**	**pRb T821 (CDK2)**
0.045	2.6	15.5 [6x]	234 [90x]	377 [145x]	275 [106x]	6941 [2670x]	6115 [2352x]	4.2

BLU-956

Kinome selectivity	Enzymatic assay IC_{50} (nM) [fold selectivity]							Cellular assay IC_{50} (nM)
S(10) @ 3μM	**CDK2/E1**	**CDK2/A2**	**CDK1/B**	**CDK4/D1**	**CDK6/D3**	**CDK7/H**	**CDK9/T1**	**pRb T821 (CDK2)**
0.040	0.8	16.1 [20x]	163 [203x]	116 [146x]	393 [491x]	4826 [6032x]	9063 [11328x]	3.7

CDK, cyclin-dependent kinase; IC_{50}, 50% inhibitory concentration; pRb, phosphorylated retinoblastoma.

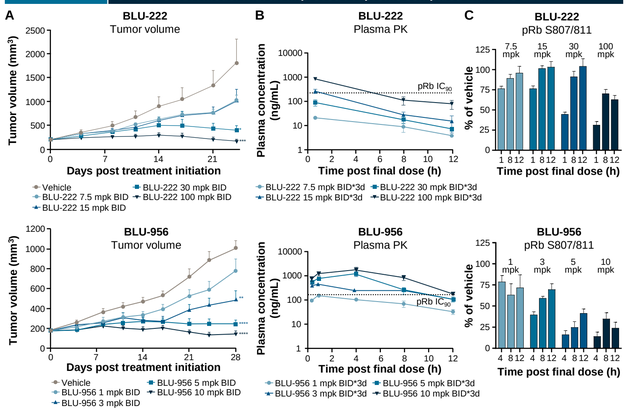
Ovarian/uterine cancer — **Figure 2: BLU-222 and BLU-956 induce strong antiproliferative effect in *CCNE1* OE/Rb+/p16+ cell lines**



BLU-222[8] and BLU-956 GI_{50} values measured by CyQUANT (5d) in a panel of ovarian and uterine cell lines. Fold differences between median GI_{50} are noted. **(A)** Cell lines categorized by *CCNE1* status. **(B)** Cell lines categorized by *CCNE1* OE/Rb+/p16+ versus "other" (cell lines that do not meet triple biomarker criteria).

CDK2, cyclin-dependent kinase 2; CN, copy number; GI_{50}, concentration for 50% of maximal inhibition of cell proliferation; OE, overexpression; Rb, retinoblastoma.

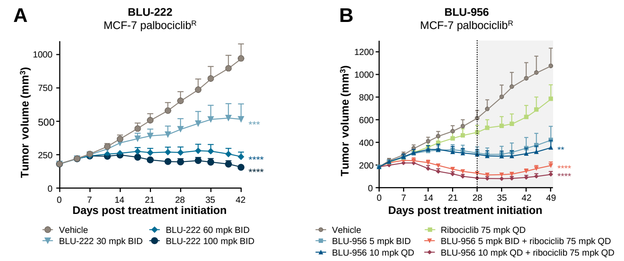
Ovarian cancer — **Figure 3: BLU-956 induces strong antiproliferative effect in *CCNE1*-amplified OVCAR-3 CDX model with an improved PK profile compared with BLU-222**



(A) *In vivo* tumor growth kinetics in the *CCNE1*-amplified OVCAR-3 T2A CDX model. Mean tumor volume ± SEM is plotted. 2-way ANOVA to vehicle: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. **(B)** Plasma PK from tumor-bearing mice dosed for 3 days and plasma drawn at indicated timepoints. Mean plasma concentration ± SEM is plotted. **(C)** pRb S807/811 inhibition in treated (3 days) OVCAR-3 T2A tumors. Mean ± SEM pRb signal normalized to β-actin is plotted relative to percent of vehicle.

BID, twice daily; CDX, cell-derived xenograft; IC_{90}, 90% inhibitory concentration; mpk, mg/kg; PK, pharmacokinetics; pRb, phosphorylated retinoblastoma; SEM, standard error of the mean.

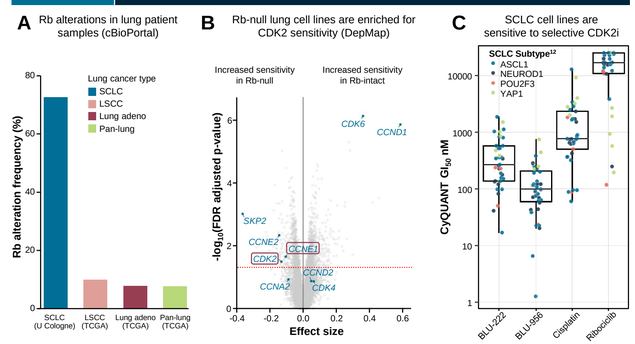
Breast cancer — **Figure 4: CDK2i monotherapy induces a strong antitumor effect in a CDK4/6i-resistant MCF-7 breast model**



(A-B) *In vivo* tumor growth kinetics in a palbociclib-resistant breast model treated with (A) BLU-222 and (B) BLU-956 and/or ribociclib. Mean tumor volume ± SEM is plotted. Statistical deviation from vehicle is noted: 2-way ANOVA, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

BID, twice daily; CDK2i, cyclin-dependent kinase 2 inhibitor; CDK4/6i, cyclin-dependent kinase 4/6 inhibitor; mpk, mg/kg; palbociclib^R, palbociclib-resistant; QD, once daily; SEM, standard error of the mean.

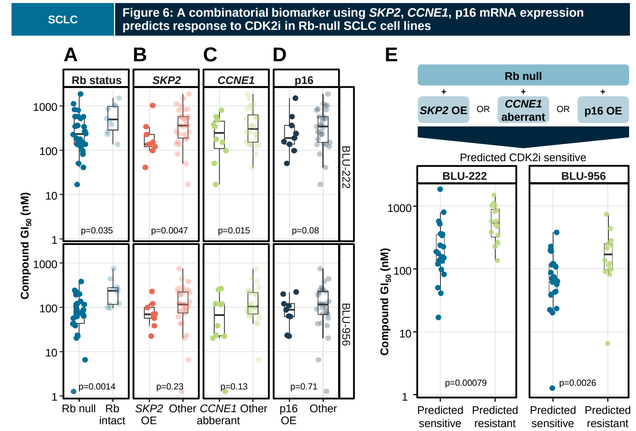
Lung cancer — **Figure 5: Rb-null lung cell lines are enriched for CDK2 sensitivity**



(A) Frequency of Rb alterations in lung cancer samples using patient data in cBioPortal.[9–11] Rb alteration is defined as mutation, deep deletion, structural variants, or multiple aberrations. **(B) Gene dependency enrichment comparing Rb-null with Rb-intact lung cell lines in CCLE/DepMap. (C) Compound antiproliferative effects in SCLC cell lines.**

Adeno, adenocarcinoma; CCLE, Cancer Cell Line Encyclopedia; CDK, cyclin-dependent kinase; CDK2i, cyclin-dependent kinase 2 inhibitor; FDR, false discovery rate; GI_{50}, concentration for 50% of maximal inhibition of cell proliferation; LSCC, lung squamous cell carcinoma; Rb, retinoblastoma; SCLC, small cell lung cancer; *SKP2*, S-phase kinase-associated protein 2; TCGA, The Cancer Genome Atlas; U Cologne, University of Cologne.

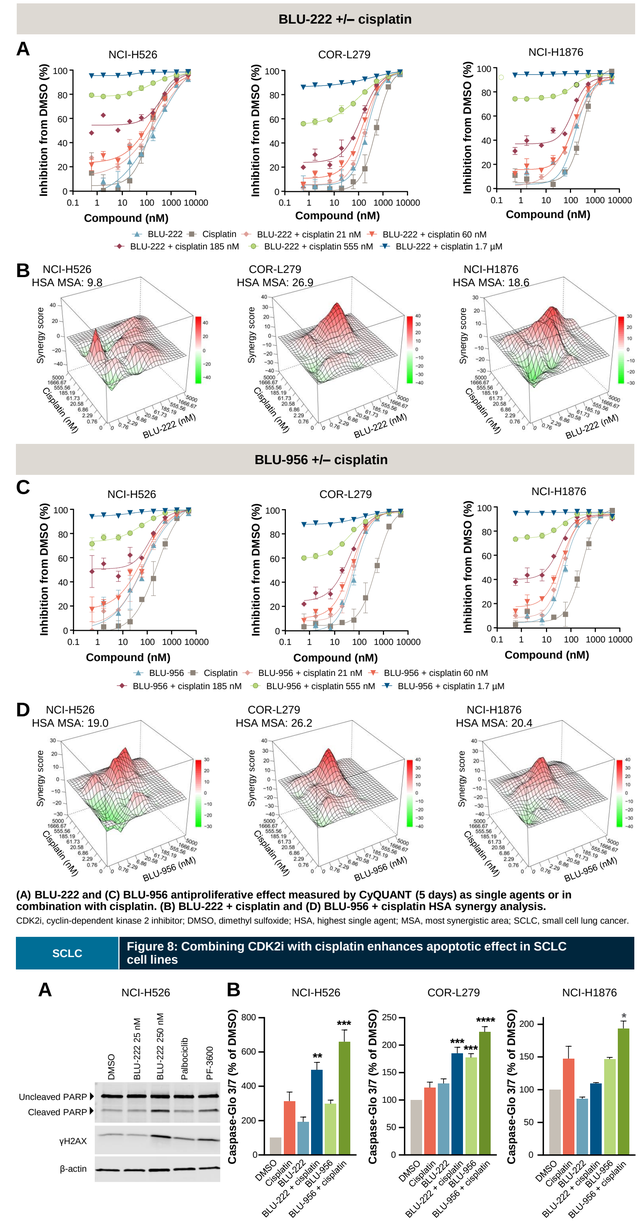
SCLC — **Figure 6: A combinatorial biomarker using *SKP2*, *CCNE1*, p16 mRNA expression predicts response to CDK2i in Rb-null SCLC cell lines**



(A-D) Evaluating CDK2i sensitivity by single biomarkers. (E) Predicting CDK2i sensitivity in SCLC using a composite biomarker signature. Rb-null + high *SKP2*, or aberrant *CCNE1* (CN increase and/or overexpressed), or high *CDKN2A* (p16) expression enriches for sensitivity to CDK2i.

CDK2i, cyclin-dependent kinase 2 inhibitor; CN, copy number; GI_{50}, concentration for 50% of maximal inhibition of cell proliferation; OE, overexpression; Rb, retinoblastoma; SCLC, small cell lung cancer; *SKP2*, S-phase kinase-associated protein 2.

SCLC — **Figure 7: Combining CDK2i with cisplatin enhances antiproliferative effect in SCLC cell lines**



(A) BLU-222 and (C) BLU-956 antiproliferative effect measured by CyQUANT (5 days) as single agents or in combination with cisplatin. (B) BLU-222 + cisplatin and (D) BLU-956 + cisplatin HSA synergy analysis.

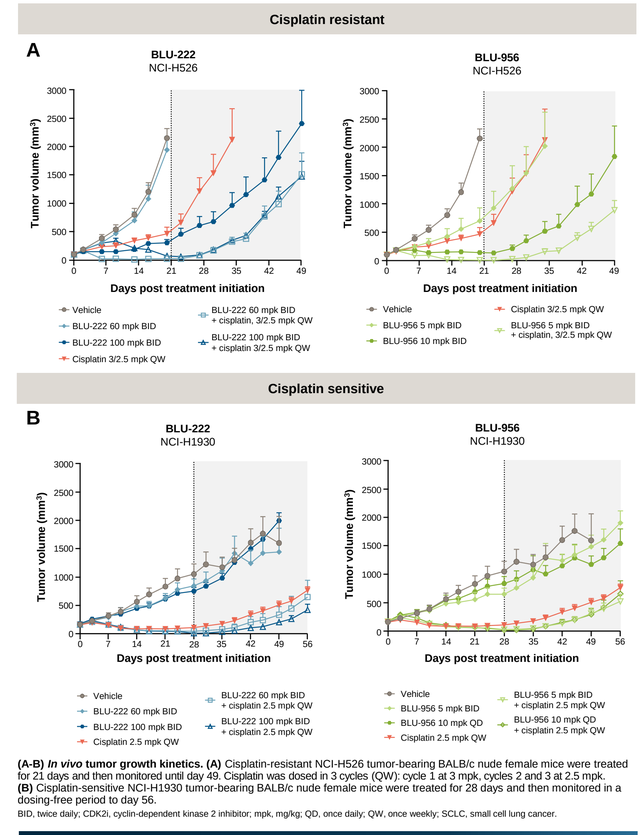
CDK2i, cyclin-dependent kinase 2 inhibitor; DMSO, dimethyl sulfoxide; HSA, highest single agent; MSA, most synergistic area; SCLC, small cell lung cancer.

SCLC — **Figure 8: Combining CDK2i with cisplatin enhances apoptotic effect in SCLC cell lines**

(A) Detection of PARP cleavage and DNA damage marker γH2AX. (B) Caspase cleavage measured by Caspase-Glo 3/7. Assessment conducted in NCI-H256, COR-L279, and NCI-H1876 after a 48h treatment with cisplatin (600 nM), BLU-222 (185 nM), BLU-956 (185 nM), or the indicated combination. Statistical deviation from DMSO assessed by one-way ANOVA with Tukey's multiple comparisons test: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

CDK2i, cyclin-dependent kinase 2 inhibitor; DMSO, dimethyl sulfoxide; PARP, poly (ADP-ribose) polymerase; SCLC, small cell lung cancer.

SCLC — **Figure 9: CDK2i + cisplatin induced combination benefit in a chemotherapy-resistant SCLC model**



(A-B) *In vivo* tumor growth kinetics. (A) Cisplatin-resistant NCI-H526 tumor-bearing BALB/c nude female mice were treated for 21 days and then monitored until day 49. Cisplatin was dosed in 3 cycles (QW): cycle 1 at 3 mpk, cycles 2 and 3 at 2.5 mpk. **(B)** Cisplatin-sensitive NCI-H1930 tumor-bearing BALB/c nude female mice were treated for 28 days and then monitored in a dosing-free period to day 56.

BID, twice daily; CDK2i, cyclin-dependent kinase 2 inhibitor; mpk, mg/kg; QD, once daily; QW, once weekly; SCLC, small cell lung cancer.

Conclusions

- CDK2 inhibition represents a novel therapeutic approach in Rb-null SCLC, an aggressive cancer with high unmet medical need
- Aberrant *CCNE1* or overexpression of *SKP2* or *CDKN2A* were predictive biomarkers for CDK2i single-agent response in Rb-null SCLC lines
- BLU-956 achieved equal or superior antitumor activity compared to BLU-222 in preclinical models of SCLC as well as in preclinical models of ovarian and breast cancer
- BLU-222 and BLU-956 effects were deepened in combination with cisplatin in models of SCLC, potentially via enhanced induction of apoptosis
- These findings provide rationale for targeting CDK2 in combination with cisplatin treatment in SCLC and suggest that specific biomarkers could be useful to predict response

References

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