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PS19-10

Novartis Oncology

Preclinical Head-to-Head Comparison of CDK4/6 Inhibitor Activity Toward CDK4 vs CDK6

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Introduction (1 of 2)

- The cyclin-dependent kinase (CDK) 4/6 inhibitors ribociclib, palbociclib, and abemaciclib have been established as effective treatment options for hormone receptor–positive (HR+) human epidermal growth factor receptor 2–negative (HER2–) breast cancer
  - Although these agents have not been evaluated in head-to-head clinical studies, they have all demonstrated significant progression-free survival improvements[1]
  - However, only abemaciclib plus endocrine therapy (ET; MONARCH-2) and ribociclib plus ET (MONALEESA-3 and MONALEESA-7) achieved significant improvements in overall survival to date—prompting closer examination of how these CDK4/6 inhibitors may be distinct[2-4]

CDK4/6, cyclin-dependent kinase 4 or 6; HER2−, human epidermal growth factor receptor-2−negative; HR+, hormone receptor−positive.

Introduction (2 of 2)

- Preclinical studies revealed differences in how these molecules interact with various kinases[5,6]
  - Ribociclib and palbociclib exhibited greater selectivity for CDK4 and CDK6 relative to other human kinases compared with abemaciclib, which may have important implications given that nonspecific binding may lead to off-target dose-limiting effects
  - In addition, these molecules displayed different patterns of activity against CDK4 relative to CDK6 in both biochemical and cellular assays
  - Although intriguing, these preclinical studies either used purified proteins without the accompanying cellular context or used proliferation as a proxy for target engagement

CDK, cyclin-dependent kinase.

Objective

- To extend the body of evidence created by the prior preclinical analyses by constructing cellular model systems with which the effects of ribociclib, palbociclib, and abemaciclib on either CDK4 or CDK6 could be studied in isolation

CDK4/6, cyclin-dependent kinase 4 or 6.

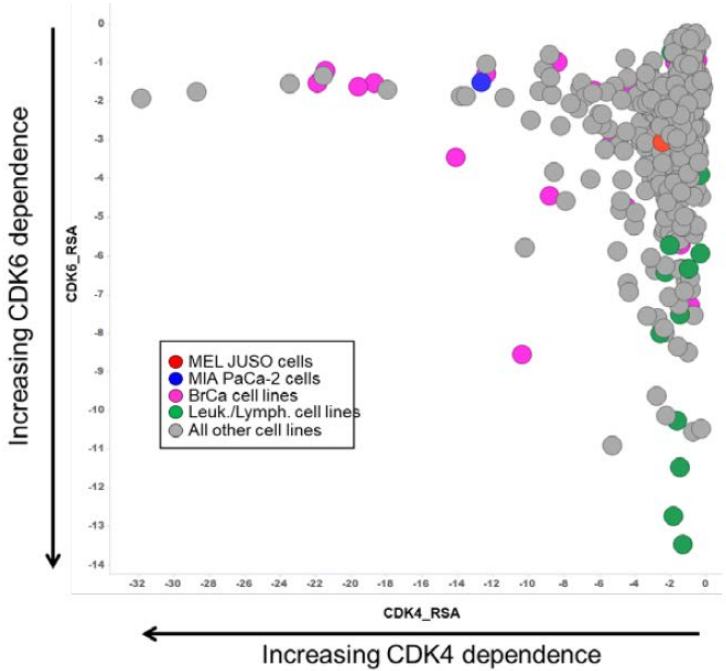
Methods (1 of 4)

- Two cell lines were selected for this analysis after being identified as lacking strong dependence on either CDK4 or CDK6, as indicated by short hairpin RNA knockdown (**Figure 1**)
  - MEL-JUSO, an *NRAS*-mutant melanoma cell line
  - MIA PaCa-2, a *KRAS*-mutant pancreatic ductal adenocarcinoma cell line
- Isogenic variants of each cell line lacking either CDK4 or CDK6 expression were generated by ablating CDK4 or CDK6 expression using CRISPR/CAS9 genome editing techniques and single-cell clonal selection
  - MEL-JUSO CDK4 knockout (KO) clones 1-3 and CDK6 KO clones 2-4 were used for all subsequent experiments (**Figure 2A**)
  - MIA PaCa-2 CDK4 KO clones 1 and 4 and CDK6 KO clones 2 and 3 were used for the experiments reported here (**Figure 2B**)

CDK4/6, cyclin-dependent kinase 4 or 6; CRISPR, clustered regularly interspaced short palindromic repeats; MIA PaCa-2 cells, human pancreatic ductal adenocarcinoma cell line; KRAS, Kirsten rat sarcoma; NRAS; neuroblastoma rat sarcoma; RNA, ribonucleic acid.

Methods (2 of 4)

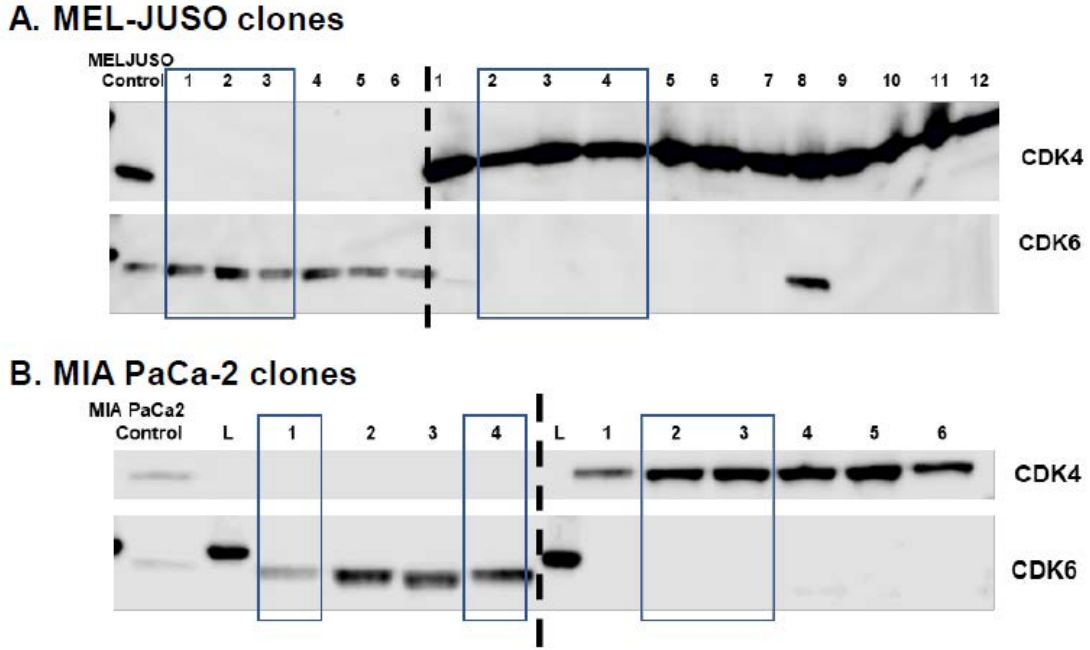
Figure 1. Cell Line Selection by Sensitivity to shRNA Knockdown[7]



BrCa, Breast Cancer; CDK4/6, cyclin-dependent kinase 4 or 6; Leuk, leukocytes; Lymph, lymphocytes; MIA PaCa-2 cells, Human Pancreatic Ductal Adenocarcinoma cell line; shRNA, short hairpin RNA; RNA, ribonucleic acid.

Methods (3 of 4)

Figure 2. Isolation of CDK4 or CDK6 Knockout Clones



CDK4/6, cyclin-dependent kinase 4 or 6; MIA PaCa-2, human pancreatic ductal adenocarcinoma cell line.

Methods (4 of 4)

- Levels of phosphorylated RB (phospho-RB807/811) protein were used as a readout for target inhibition and were measured by FastScan phospho-RB807/811 enzyme-linked immunosorbent assay (ELISA)
- Half-maximal inhibitory concentrations (IC_{50}) for each CDK4/6 inhibitor were calculated in parental cells as well as variant cell lines expressing only CDK4 or CDK6
- Measurement of phospho-RB807/811 was performed in T47-D cells (an estrogen receptor–positive breast cancer cell line) to confirm results of prior analyses

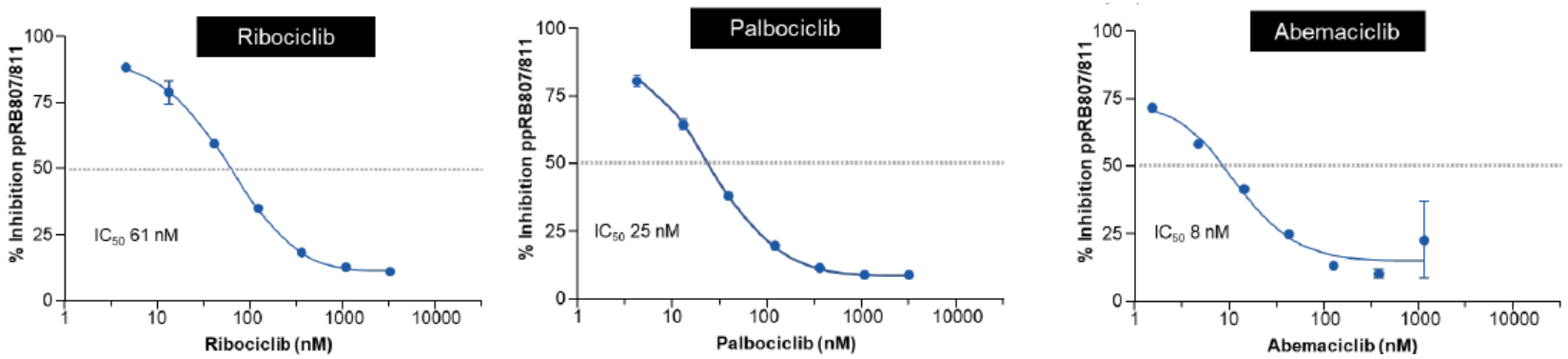
CDK4/6, cyclin-dependent kinase 4 or 6.

Results (1 of 5)

Phospho-RB807/811 Inhibition in T47-D Cells

- The level of phospho-RB807/811inhibition in T47-D cells was similar to that described previously[6] (in which IC_{50}values for ribociclib, palbociclib, and abemaciclib in T47-D cells were 73, 21, and 10 nM, respectively; **Figure 3**)

Figure 3. FastScan Phospho-RB807/811 ELISA Analysis in T47-D Cells



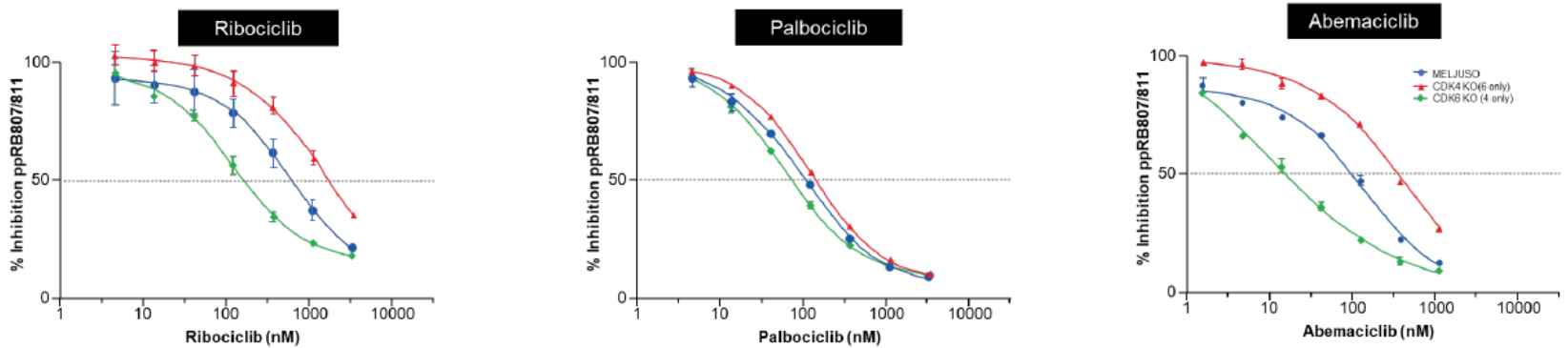
ELISA, enzyme-linked immunosorbent assay; IC_{50} , Half-maximal inhibitory concentrations; T47-D cells, an estrogen receptor–positive breast cancer cell line

Results (2 of 5)

CDK4/6 Inhibition in MEL-JUSO and MIA PaCa-2 Cells

- In MEL-JUSO cells, ribociclib, palbociclib, and abemaciclib inhibited CDK4 at 11-, 2-, and 22-fold lower drug concentrations than CDK6, respectively **(Figure 4, Table 1)**

Figure 4. Fast Scan Phospho-RB807/811 ELISA Analysis in MEL-JUSOCDK4 and CDK6 Knockout Clones



CDK4/6, cyclin-dependent kinase 4 or 6; ELISA, enzyme-linked immunosorbent assay; KO, knockout; MIA PaCa-2, Human Pancreatic Ductal Adenocarcinoma cell line.

Results (3 of 5)

Table 1. IC_{50} Values for Ribociclib, Palbociclib, and Abemaciclib in MEL-JUSO CDK4 and CDK6 Knockout Clones

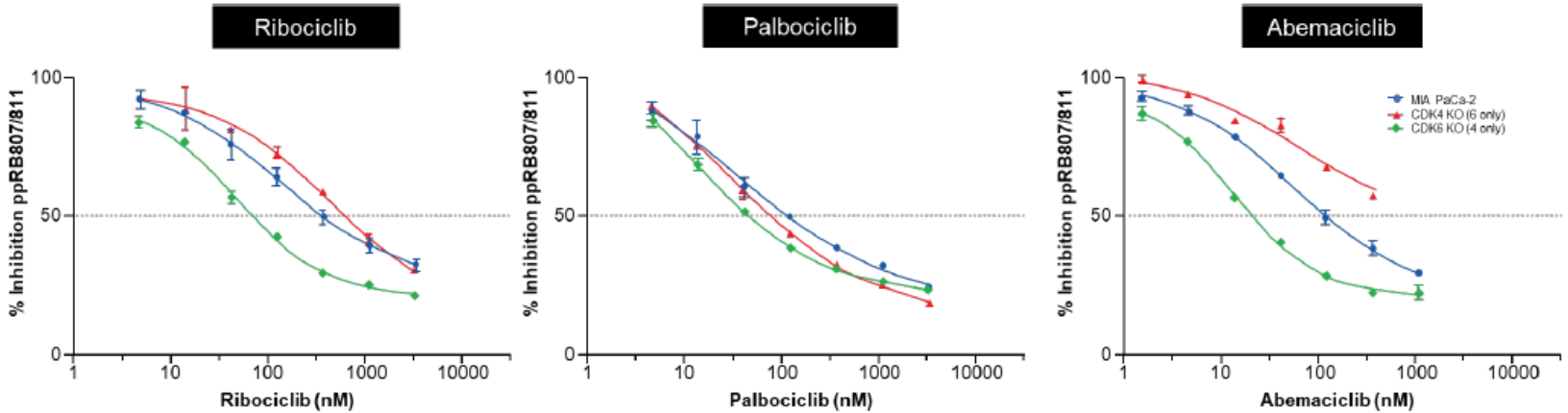
	Ribociclib IC_{50}, nM	Palbociclib IC_{50}, nM	Abemaciclib IC_{50}, nM
MEL-JUSO parent line	602	109	101
MEL-JUSO CDK4 KO (CDK6 only)	1704	146	359
MEL-JUSO CDK6 KO (CDK4 only)	159	75	16

CDK4/6, cyclin-dependent kinase 4 or 6; IC_{50}, Half-maximal inhibitory concentrations; MIA PaCa-2, human pancreatic ductal denocarcinoma cell line.

Results (4 of 5)

- In MIA PaCa-2 cells, ribociclib, palbociclib, and abemaciclib inhibited CDK4 at 9-, 2-, and > 47-fold lower drug concentrations than CDK6, respectively **(Figure 5, Table 2)**

Figure 5. FastScanPhospho-RB807/811 ELISA Analysis in MIA PaCa-2 CDK4 and CDK6 Knockout Clones



CDK4/6, cyclin-dependent kinase 4 or 6; ELISA, enzyme-linked immunosorbent assay; IC_{50}, Half-maximal inhibitory concentrations; KO, knockout; MIA PaCa-2, Human Pancreatic Ductal Adenocarcinoma cell line.

Results (5 of 5)

Table 2. IC_{50} Values for Ribociclib, Palbociclib, and Abemaciclib in MIA PaCa-2 CDK4 and CDK6 Knockout Clones

	Ribociclib IC_{50}, nM	Palbociclib IC_{50}, nM	Abemaciclib IC_{50}, nM
MIA PaCa-2 parent line	375	121	126
MIA PaCa-2-CDK4 KO (CDK6 only)	633	82	NA
MIA paCa-2 CDK6 KO (CDK4 only)	71	48	21

CDK4/6, cyclin-dependent kinase 4 or 6; IC_{50} , Half-maximal inhibitory concentrations; MIA PaCa-2, Human Pancreatic Ductal Adenocarcinoma cell line, NA, not applicable.

Conclusions (1 of 2)

- Consistent with prior biochemical studies and cell-proliferation assays, our findings indicate that both ribociclib and abemaciclib more potently inhibit CDK4 than CDK6, whereas palbociclib has similar activity against both targets in cells[5,6]
- Understanding the importance of CDK4 levels relative to CDK6 in the etiology of breast cancer and possible implications for increased CDK4 target engagement is an emerging topic of discussion in the field

CDK4/6, cyclin-dependent kinase 4 or 6.

Conclusions (2 of 2)

- The clinical exposures achieved by ribociclib at its approved dosage and the differential activity toward CDK4 vs CDK6 described here suggest that ribociclib may drive robust CDK4 inhibition in patients
- Given that CDK4 has been shown to be expressed at higher levels in breast tumor samples, and many breast cancer cell lines have demonstrated greater dependence on CDK4 vs CDK6, the differential inhibition of the CDK4/6 inhibitors demonstrates a potentially important consideration in the treatment of patients with these compounds

CDK4/6, cyclin-dependent kinase 4 or 6.

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Disclosures

SD: employment: Novartis; **GC:** employment, stock ownership: Novartis

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