Brain Penetrant CDK4/6 Inhibitor PRT3645 Demonstrates Anti-tumor Activity and Enhances Survival in Glioblastoma and Breast Cancer Brain Metastasis Models

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Background

- Cell cycle deregulation is a hallmark of cancer and cyclin-dependent kinase (CDK) inhibitors specifically inhibit CDK4/6 and block cell transition from the G1 to the S phase of the cell cycle.[1]
- CDK4/6 inhibitors are the first and only class of highly specific CDK inhibitors approved for cancer treatment to date.[2,3]
- CDK4/6 inhibitors have transformed the treatment paradigm of estrogen receptor-positive (ER+), human epidermal growth factor receptor 2 (HER2-) breast cancers, with three CDK4/6 inhibitors currently approved in the US.[2,3]
- Prelude Therapeutics, Inc. has discovered and developed PRT3645, an orally bioavailable brain-penetrant CDK4/6 inhibitor with high potency and selectivity, excellent pharmacokinetic (PK) parameters across species, brain penetrance, favorable tissue distribution relative to brain exposure, and significant anti-tumor efficacy in a subcutaneous model of breast cancer, orthotopic models of glioblastoma (GBM), and breast cancer brain metastasis (BCBM).

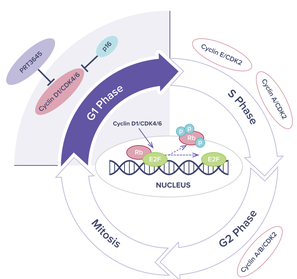
Objective

- To profile the biochemical/pharmacological activity of PRT3645, a brain-penetrant CDK4/6 inhibitor, both *In vitro* and *In vivo* in various cancers, including GBM and BCBM models, as a single agent as well as in combination with standard of care (SoC) agents.

Key Findings

- PRT3645 inhibits cellular phosphorylation of retinoblastoma (Rb) protein with low nanomolar activity.
- PRT3645 treatment resulted in inhibition of cell proliferation in various tumor types with most cell lines having a half-maximal inhibitory concentration (IC_{50}) of <100 nM.
- PRT3645 was well tolerated and highly efficacious in a subcutaneous xenograft model of breast cancer as well as in orthotopic models of human GBM and BCBM.

CyclinD-CDK4/6-Rb-E2F Pathway



Methods

- **Cell cycle analysis:** Cells were harvested, washed with phosphate-buffered saline (PBS), and fixed with ice-cold ethanol (70% v/v). Cells were stained following instructions from a Click-iT™ EdU Pacific Blue™ Flow Cytometry Assay Kit. DNA content was determined by DRAQ5™ DNA dye. Cell cycle distribution was analyzed using flow cytometry and the data were processed using FlowJo software.
- **Cell proliferation assay:** Cells were seeded in a 96- or 384-well plate, incubated overnight at 37 °C to allow adhesion, and then treated with inhibitors, as shown in the figures. Cell proliferation was determined using MTS solution (Promega) or fluorescence staining of nuclei.
- **Western blotting:** Briefly, cells were seeded at 5×10^5 cells/well in 6-well plates, incubated overnight at 37 °C to allow adhesion, and then treated with inhibitors for 24 hours. Cellular proteins were collected and denatured by 1% sodium dodecyl sulfate with β-mercaptoethanol.
- **Xenograft and orthotopic models (*In vivo*):** After tumors were established subcutaneously or orthotopically, treatment was commenced via oral gavage with either vehicle control or drugs, as described in the figures.

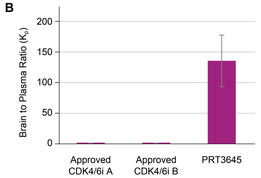
Results

Figure 1. PRT3645 Is a Potent and Selective CDK4/6 Inhibitor With High Brain Penetration Compared With Approved CDK4/6 Inhibitors

A

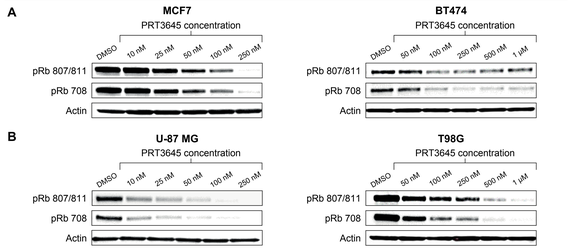
		PRT3645
Biochemical IC_{50} (CDK4, nM)[4]		3
Biochemical IC_{50} (CDK6, nM)[5]		14
Proliferation IC_{50} (MCF7, nM)		47
Proliferation IC_{50} (U-87 MG, nM)		21
Fold selectivity CDK4 vs other isoforms[5]	CDK1	>1000
	CDK2	>1000
	CDK3	>500
	CDK5	>1000
	CDK7	>1000
	CDK9	>1000

B



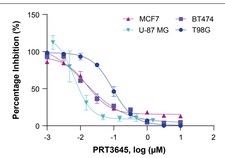
(A) Biochemical TR-FRET assay carried out at 1 mM ATP[5] or 2 mM ATP[4] and 10-day cell proliferation IC_{50} determined by CellTiter-Glo® assay. **(B)** Brain to plasma ratios (K_p) were determined following intravenous (IV) administration (bolus followed by 4-hour IV infusion) in male Sprague-Dawley rats (n=3). Bolus and infusion parameters were based on single bolus dose IV PK data and doses chosen to target a final steady state plasma concentration of 1 µM. ATP, adenosine triphosphate; TR-FRET, time-resolved Förster resonance energy transfer.

Figure 2. PRT3645 Inhibits Cellular Phosphorylation of Rb With Low Nanomolar Activity



Cells were treated in a concentration-dependent manner and western blotting analysis was performed for pRb 807/811 and pRb 708 in cells treated for 24 hours with PRT3645. Actin was used as a loading control. Proteins were visualized on the immunoblot by using LI-COR Odyssey® CLx system. **(A)** Breast cancer cell lines. **(B)** Glioblastoma cell lines. DMSO, dimethyl sulfoxide; pRb, phosphorylated retinoblastoma protein.

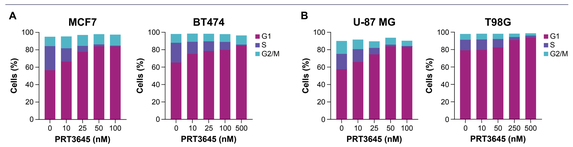
Figure 3. PRT3645 Inhibits Cellular Proliferation of Breast Cancer and Glioblastoma Cell Lines *In Vitro*



Cell Line	IC_{50} (nM)
MCF7	16
BT474	14
U-87 MG	7
T98G	94

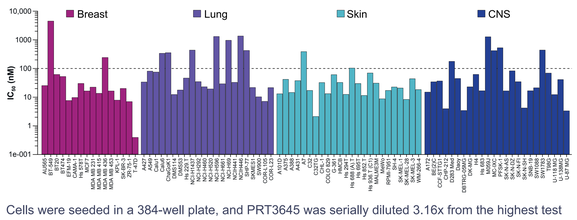
Breast cancer and glioblastoma cells were seeded in a 96-well plate, and PRT3645 was dispensed using a Tecan at ½log serial dilution. After a 10-day incubation, cell viability was measured using a cell counting kit-8 (CCK8) colorimetric assay that measures activity of dehydrogenases in cells which is directly proportional to the number of living cells. IC_{50} values were calculated by using GraphPad Prism 9.3.1 software.

Figure 4. PRT3645-Treated Cancer Cells Show Cell Cycle Inhibition With a Strong Reduction in the S-Phase



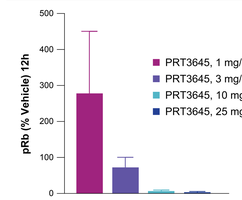
Cells were treated with PRT3645 in a concentration-dependent manner for 24 hours. Cell cycle phases (G1, S, and G2/M) were measured by EdU (5-ethynyl-2'-deoxyuridine) incorporation combined with DNA dye (DRAQ5™) for 2 hours. Samples were analyzed by flow cytometry, and the data were processed using FlowJo software. **(A)** Breast cancer cell lines. **(B)** Glioblastoma cell lines.

Figure 5. PRT3645 Is Highly Effective in Reducing Cell Viability of Various Tumor Types in an OncoPanel™ Cell Proliferation Assay



Cells were seeded in a 384-well plate, and PRT3645 was serially diluted 3.16x from the highest test concentration of 30 µM and assayed (OncoPanel™ cell proliferation assay) over 10 concentrations with a maximum assay concentration of 0.1% DMSO. Automated fluorescence microscopy was carried out using a Molecular Devices ImageXpress® Micro XL high-content imager, and images were analyzed with MetaXpress® 5.1.0.41 software.

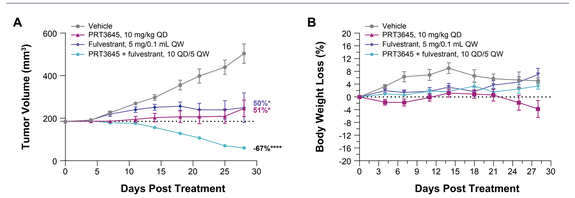
Figure 6. PRT3645 Is Highly Effective in Reducing Tumor pRb in a Single-Dose PK/PD Study in a U-87 MG Subcutaneous GBM Model



A U-87 MG subcutaneous GBM xenograft model was established by injecting tumor cells (3.5×10^5 cells/mouse, with 50% Matrigel™) in 0.1 mL media at the right flank by subcutaneous administration. PRT3645 was administered orally at 1, 3, 10, and 25 mg/kg as a single dose and tumor pRb levels were detected at the 12-hour timepoint.

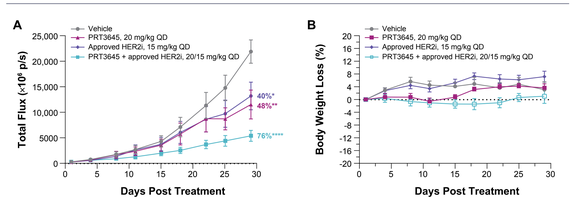
SD, single dose; PD, pharmacodynamics.

Figure 7. PRT3645 Is Highly Effective in Combination With Fulvestrant in a Subcutaneous ER+/HER2- MCF7 Breast Cancer Model and Shows Tumor Regressions



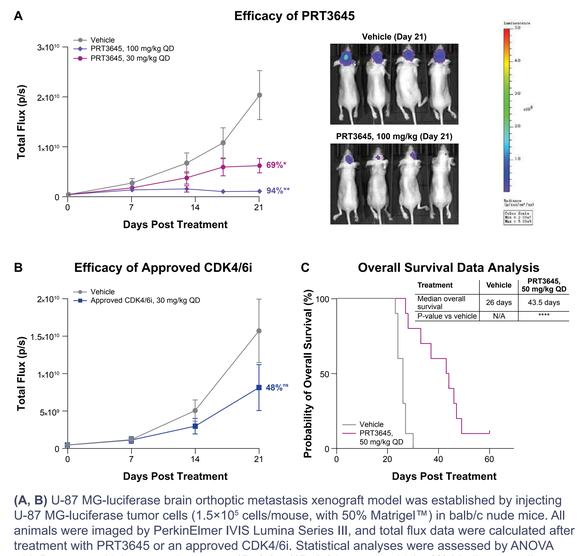
(A) Mice were inoculated subcutaneously at the right flank with MCF7 human breast tumor cells (10×10^6 cells) in 0.2 mL of PBS mixed with matrigel™ (1:1 v/w) for tumor development and 3 days before cell inoculation; β-estradiol sustained release tablets (0.36 mg) were implanted on the left back of each mouse. **(B)** Body weight changes post treatment. *P<0.05, ****P<0.0001 versus vehicle at day 28 post treatment. Statistical analyses were assessed by one-way analysis of variance (ANOVA) with Dunnett multiple comparisons test. QD, once daily; QW, once weekly.

Figure 8. PRT3645 Is Highly Effective in Combination With an Approved Small Molecule Brain Penetrant HER2 Inhibitor in ER-/HER2+ BT-474 Luciferase Orthotopic BCBM Model



(A) A BT-474 luciferase brain orthotopic metastasis xenograft model was established by implantation of 2.5 µL of cell suspension (1×10^5 cells/mouse with Matrigel™) into right caudate nucleus of female balb/c nude mice brain; tumors were measured with Xenogen IVIS® imaging and total flux data were calculated. **(B)** Body weight changes post inoculation. *P<0.05, **P<0.01, ****P<0.0001 versus vehicle at day 29 post treatment. Statistical analyses were assessed by ANOVA with Dunnett multiple comparisons test. HER2i, human epidermal growth factor receptor 2 inhibitor; QD, once daily.

Figure 9. PRT3645 Is Highly Efficacious and Enhances Survival in the U-87 MG-Luciferase Orthotopic GBM Model



(A, B) U-87 MG-luciferase brain orthoptic metastasis xenograft model was established by injecting U-87 MG-luciferase tumor cells (1.5×10^5 cells/mouse, with 50% Matrigel™) in balb/c nude mice. All animals were imaged by PerkinElmer IVIS Lumina Series III, and total flux data were calculated after treatment with PRT3645 or an approved CDK4/6i. Statistical analyses were assessed by ANOVA with Dunnett multiple comparisons test; *P<0.05, **P<0.01 versus vehicle. **(C)** Median overall survival analysis and statistical analysis performed by using log-rank (Mantel-Cox test); ****P<0.0001 versus vehicle. ns, nonsignificant; QD, once daily.

Conclusions

- PRT3645 is an orally bioavailable, brain penetrant, and potent CDK4/6 inhibitor with >1000-fold selectivity against other CDK family members (CDK1, CDK2, and CDK9).
- Downregulation of pRb, reduction in S-phase of the cell cycle, and potent inhibition of cell proliferation in glioblastoma and breast cancer cell lines were observed with PRT3645 treatment *In vitro*.
- Across various tumor types, PRT3645 reduced cell viability with the majority of cell lines showing an IC_{50} value of <100 nM.
- PRT3645 single-dose administration in a subcutaneous U-87 MG GBM model showed dose-dependent reduction in tumor pRb at 12 hours.
- PRT3645 demonstrated significant efficacy in an ER+/HER2- MCF7 breast cancer xenograft model and resulted in tumor regression when combined with fulvestrant.
- PRT3645 was highly effective in reducing tumor burden in an ER-/HER2+ BT-474 luciferase orthotopic BC brain metastasis model and demonstrated significant combinatorial benefit with an approved small molecule brain-penetrant HER2 inhibitor in this model.
- PRT3645 was highly effective in reducing tumor burden in a U-87 MG-luciferase GBM orthotopic model and demonstrated enhanced median survival benefit.

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Acknowledgments

We would like to thank Yun Guan (Medicilon), Jin Hongyue (Wuxi AppTec), and Julian Wang (ChemPartner) for conducting the *In vivo* studies presented in this poster.

Disclosures

All authors were employees of Prelude Therapeutics, Inc., Wilmington, DE at the time of research and may own stocks or shares. Yang Zhang and Dave Rominger are now employees of Quanta Therapeutics.

This study was funded by Prelude Therapeutics Inc., Wilmington, DE.

Editorial support was provided by Russell Craddock, PhD, of Parexel International, Uxbridge, UK, and was funded by Prelude Therapeutics Inc., Wilmington, DE.