The Brain Penetrant CDK4/6 Inhibitor, PRT3645, is Highly Effective in Combination with Targeted Therapies in Preclinical Models of NSCLC, CRC and HER2-positive Breast Cancer

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Background

- Cell cycle deregulation is a hallmark of cancer and the hyperactivation and overexpression of CDKs are often drivers of cancer pathogenesis.[1]
- Cyclin-dependent kinase 4 and 6 (CDK4)/(CDK6) are critical mediators of cellular transition into S phase and important for the initiation, growth, and survival of many cancers.[2,3]
- At present three CDK4/CDK6 inhibitors are approved for the treatment of estrogen receptor-positive (ER+), HER2-negative breast cancer, and are being explored in other cancer indications as well.[4]

Objectives

- To explore the activity of PRT3645 in other tumor types as well as in combination with inhibitors targeting RAS/RAF/MEK/ERK pathway, and with a brain penetrant HER2 receptor tyrosine kinase inhibitor in preclinical models.

Key Findings

- Oral PRT3645 induced tumor regression in tumors progressed on Palbociclib treatment.
- PRT3645 demonstrated synergy in vitro and significantly improved anti-tumor efficacy in vivo when combined with clinically approved KRAS G12C inhibitors in KRAS mutated NSCLC xenograft models that harbor the CDKN2A deletion.
- Combination of PRT3645 and RAS/RAF/MEK/ERK pathway inhibition achieved significant durable tumor growth inhibition or tumor regressions in KRAS or BRAF mutated colorectal xenograft models.
- PRT3645 was highly efficacious in combination with the brain penetrant HER2 inhibitor tucatinib and enhanced median survival significantly in a HER2-positive orthotopic human breast cancer brain metastasis (BCBM) model.

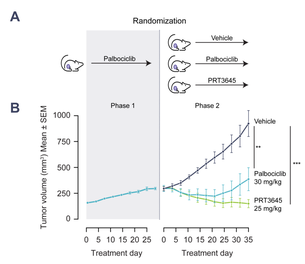
Results

Table 1. PRT3645 is a potent CDK4/6 inhibitor with biased selectivity for CDK4

	PRT3645
Biochemical IC_{50} (CDK4, nM)[1]	3
Biochemical IC_{50} (CDK6, nM)[2]	14
Proliferation IC_{50} (MCF7, nM)	47
Proliferation IC_{50} (U87 MG, nM)	21
Fold selectivity CDK4 vs other CDKs	
CDK1	>1000
CDK2	>1000
CDK3	>500
CDK5	>1000
CDK7	>1000
CDK9	>1000

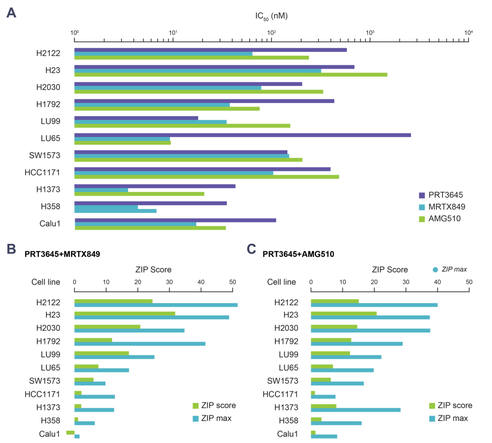
Biochemical TR-FRET assay carried out at 1 mM ATP[2] or 2 mM ATP[1] and 10-day cell proliferation IC_{50} determined by CellTiter-Glo® assay.

Figure 1. PRT3645 induces tumor regression in MCF7 breast tumor xenografts progressing on Palbociclib treatment



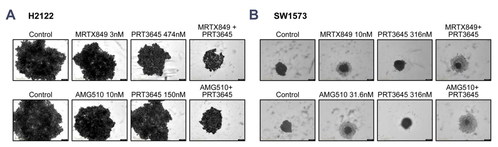
A) Schematic of the study design with two phases. **B)** Animals bearing MCF7 xenografts were treated with Palbociclib 30mg/kg daily for 28 days (phase 1). When tumors progressed, animals were randomized and treated daily with vehicle, Palbociclib, and PRT3645 (phase 2). **P<0.01, ***P<0.001

Figure 2. PRT3645 and KRAS G12C inhibitors show strong synergy potential in KRAS G12C NSCLC Cell Lines *in Vitro*



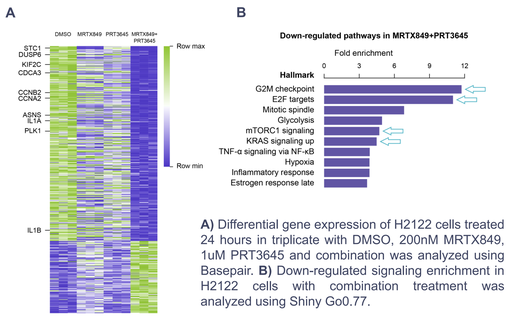
A) Single agent activity. KRAS G12 NSCLC cells were treated with PRT3645, MRTX849, AMG510 for 10 days. **B,C) Synergistic effects of drug combination of PRT3645 and KRAS G12C specific inhibitor, MRTX849 or AMG510.** Cells were treated with a combination of PRT3645 with either MRTX849, or AMG510 for 10 days. Synergy zip scores were calculated via Synergy Finder.

Figure 3. PRT3645 + MRTX849 combination induces spheroid growth inhibition in KRAS G12C NSCLC



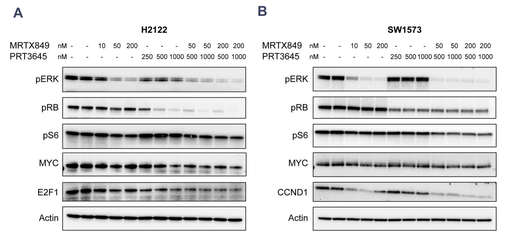
The 3D spheroid assay was measured using the IncuCyte® System in **A)** H2122 and **B)** SW1573 NSCLC cell lines with MRTX849, PRT3645 and the combination treatments for 10 days.

Figure 4. PRT3645 + MRTX849 combination enhances single agent activity by the down-regulating cell cycle, KRAS, mTOR and other signature pathways



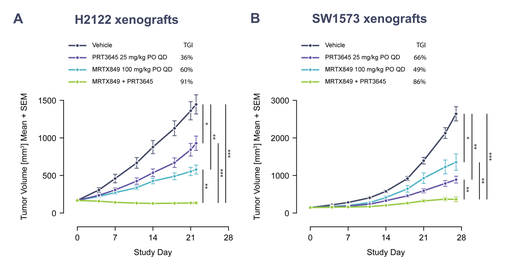
A) Differential gene expression of H2122 cells treated 24 hours in triplicate with DMSO, 200nM MRTX849, 1uM PRT3645 and combination was analyzed using Basepair. **B)** Down-regulated signaling enrichment in H2122 cells with combination treatment was analyzed using Shiny Go0.77.

Figure 5. PRT3645 + MRTX849 combination down regulates cell cycle, KRAS and mTOR signaling



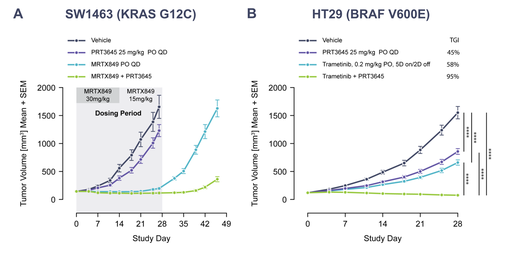
Western blot analysis of cell cycle, KRAS and mTOR downstream pathway proteins in **A)** H2122 and **B)** SW1573 cells with MRTX849, PRT3645 and the combination treatments for 24 hours. pERK Thr^{202}/Tyr^{204}, pRB Ser^{807} /^{811}, pS6 $Ser^{235/236}$, MYC, E2F1, CCND1, and Actin were measured.

Figure 6. PRT3645 + MRTX849 combination enhances tumor growth inhibition in KRAS G12C NSCLC xenograft models that harbor p16 loss



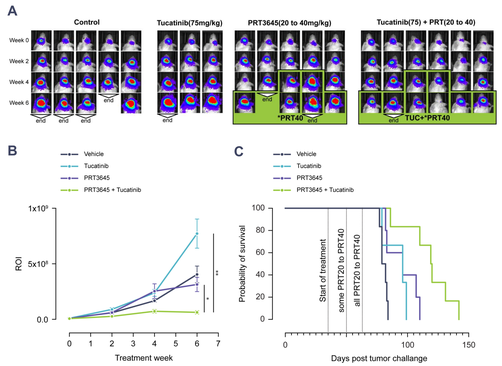
PRT3645 + MRTX849 combination shows anti-tumor efficacy in the H2122 **(A)** and SW1573 **(B)** xenograft models. Drug treatment was initiated when tumors reached ~150–250 mm^3. *P<0.05, **P<0.01, ***P<0.001

Figure 7. Combination of PRT3645 and RAS/RAF/MEK/ERK pathway inhibition achieves significant durable tumor growth inhibition or tumor regressions in colorectal xenograft models



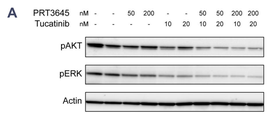
A) PRT3645 + MRTX849 combination in SW1463 xenograft model. Drug treatment was initiated when tumors reached ~150–250 mm3 and continued through Day 28. The tumor growth delay was evaluated in the absence of drug treatment. **B)** PRT3645 + Trametinib (MEK inhibitor) combination in the HT29 xenograft model. Drug treatment was initiated when tumors reached ~150–250 mm3. ****P<0.0001

Figure 8. PRT3645 + HER2 inhibitor, Tucatinib combination attenuates tumor growth in the HER2+ orthotopic human breast cancer brain metastasis model



PRT3645 + Tucatinib combination shows anti-tumor efficacy in the DFBM-355 PDX model. All animals were imaged **A)** and total flux data were calculated **B). C)** The Kaplan-Meier plot showed that combination therapy significantly prolonged median overall survival. *P<0.05, **P<0.01

Figure 9. PRT3645 + Tucatinib combination down regulates PI3K and MAPK signaling in HER2+ breast cancer cell line BT474 *in vitro*



A) Western blot analysis of pAKT Ser^{473} and pERK Thr^{202}/Tyr^{204} in BT474 cells with PRT3645, Tucatinib and the combination treatments for 24 hours.

Conclusions

- PRT3645 induced tumor regression in tumors progressing on Palbociclib treatment in the MCF7 breast cancer xenograft model.
- In KRAS G12C NSCLC cell lines, PRT3645 achieved high synergy scores with two KRAS G12C inhibitors *in vitro.* Combination of PRT3645 and KRAS G12C inhibitors enhanced effects of either single agent as manifested by down-regulating genes involved in cell cycle, KRAS and mTOR signaling pathway.
- In KRAS G12C NSCLC xenograft models, the combination of PRT3645 + approved KRAS G12C inhibitors MRTX849 and AMG510 enhanced anti-tumor efficacy in H2122 and SW1573 NSCLC models that harbor p16 deletion.
- In colorectal xenograft models, PRT3645 + MRTX849 combinations achieved significant durable tumor growth inhibition in SW1463 tumor xenografts with KRAS G12C mutation. PRT3645 + Trametinib combinations induced tumor regression in HT29 tumor xenografts with BRAF V600E mutation.
- In a HER2-positive orthotopic human BCBM model, PRT3645 was highly efficacious in combination with the HER2 inhibitor, tucatinib and increased median survival significantly, potentially due to enhanced co-inhibition on PI3K/AKT and MAPK signaling.

References

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Disclosures

All authors (except Jing Ni and Jean Zhao) are or were employees of Prelude Therapeutics, Inc at the time of research, and may own equity in the Company.