Anti-tumor Activity of Palbociclib Is Enhanced by Selinexor in Preclinical Models of HER2+ Breast Cancer

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Abstract

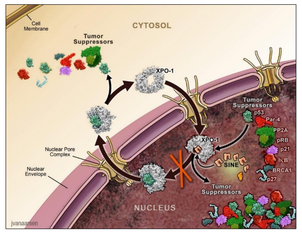
Introduction: XPO1 (exportin-1/ CRM1) inhibitor selinexor (KPT-330) is the first-in-class, orally bioavailable, clinical stage SINE (Selective Inhibitor of Nuclear Export) compound with marked anti-tumor activity towards solid and hematological malignancies. This activity can be further enhanced by other therapeutic agents. We have previously shown strong synergistic preclinical activity of selinexor plus trastuzumab against HER2+ breast cancer. In cancer models of acquired resistance to HER2-targeted therapies, G1/S phase cell cycle regulators Cyclin D1 and CDK4/6 are inappropriately activated. We therefore investigated the combinatorial effect of selinexor plus palbociclib, a CDK4/6 inhibitor, in HER2+ breast cancer models as a treatment option for recurrent and relapsed HER2+ breast cancers.

Methods: The effects of selinexor or palbociclib single agents or in combination were tested *in vitro* with BT474 HER2+ breast cancer cell line. Total RNA and protein was extracted from cell lysates and analyzed by qPCR and immunoblots. *In vivo*, a subcutaneous BT474 xenograft mouse model was treated with selinexor (5 mg/kg or 15 mg/kg; qodx3) or palbociclib (50 mg/kg or 150 mg/kg; qd) single agents or in combination. Tumor growth and body weights were monitored for 60 days. Tumors were harvested and analyzed by immunohistochemistry (IHC).

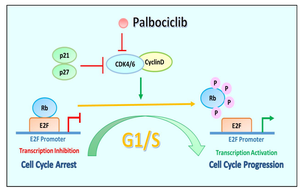
Results: Selinexor plus palbociclib was highly effective *in vitro* and *in vivo* in BT474 breast cancer cells. In *in vitro* assays, selinexor or palbociclib single agents demonstrated inhibitory effects on cell proliferation and showed additive effects when combined. *In vivo*, the combination resulted in significant survival benefit and enhanced tumor growth inhibition compared to vehicle or either single agent. IHC analysis of xenograft tumors showed synergistic inhibition of cell proliferation by selinexor plus palbociclib. The Ki67 proliferation index determined by IHC was 25% for vehicle control, 20% for selinexor, 7% for palbociclib and 2% for the combination. Based on IHC analysis, the synergistic anti-tumor activity of selinexor plus palbociclib was achieved at multiple levels of the CDK4/6 pathway. Selinexor treatment increased p21, p27 and Rb nuclear staining. Both p21 and p27 are inhibitors of CDK4/6 while Rb is a negative regulator of cell cycle progression. CDK4/6 phosphorylates and inactivates Rb, which allows cell cycle progression. In selinexor as well as palbociclib treated samples, phosphorylated Rb in the nucleus decreased, indicating a down-regulation of the CDK4/6 pathway.

Mechanisms of Action of Selinexor and Palbociclib

A. Selinexor is a Selective Inhibitor of Nuclear Export (SINE) compound that blocks XPO1-dependent nuclear export, increasing the nuclear retention of major tumor suppressor proteins. Nuclear retention of XPO1 cargo proteins reactivates their tumor supporessor fuctions, inhibiting proliferation and inducing apoptosis. XPO1 cargo proteins include several important proteins involved in G1/S transition, such as Rb, p21 and p27.



B. Palbociclib selectively inhibits cyclin-dependent kinases CDK4 and CDK6, arresting G1 to S-phase progression. Activated cyclin D-CDK4/6 complexes phosphorylate Rb, releasing it from the Rb-E2F complex and allowing target gene expression that facilitates cell cycle progression. The cyclin dependent kinase inhibitor proteins p21 and p27 are endogenous cellular mechanisms to inhibit CyclinD-CDK4/6 complex and arrest cell cycle progression.



Selinexor-Palbociclib Combination Treatment Shows Additive Effects on BT474 Cell Cycle Arrest *in vitro*

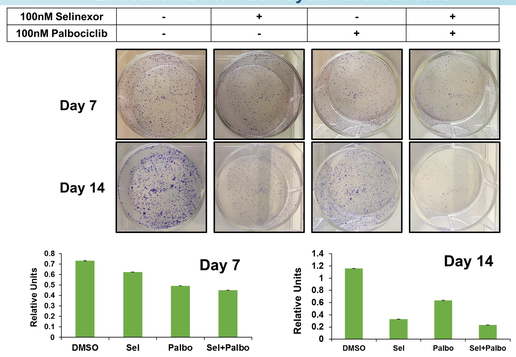


Figure 2. The effects of selinexor or palbociclib alone or in combination on cell cycle arrest were tested on BT474 (breast cancer) cells using a colony formation assay. After 14 days of continuous treatment, selinexor + palbociclib combination treated cells show less colony formation than either single agent alone.

Synergistic Anti-proliferation Effects of Selinexor and Palbociclib on BT474 Xenografts *in vivo*

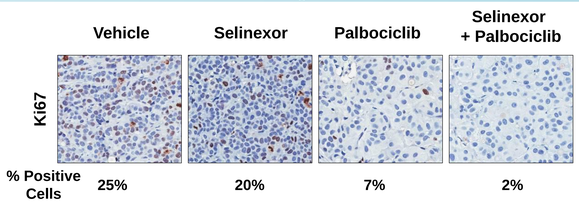


Figure 3. Ki67 staining of tumor tissue from BT474 xenografts treated with selinexor or palbociclib alone or in combination. The combination of selinexor and palbociclib shows a synergistic effect of Ki67 proliferation index (% positive cells).

Selinexor and Palbociclib Induce G1/S Arrest through Up-regulation of p21 and p27 and Down-regulation of Phospho-Rb *in vitro*

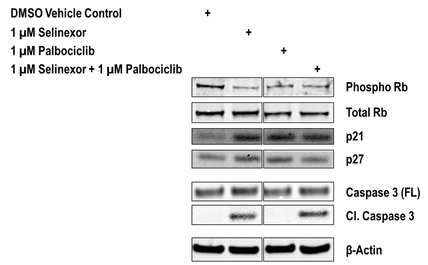
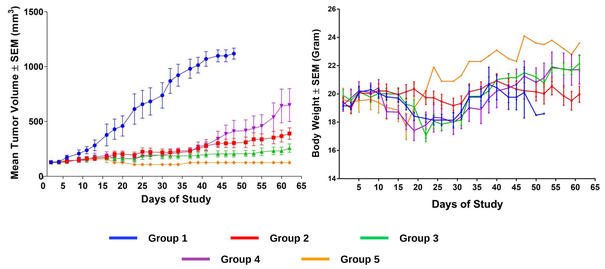


Figure 4. Western blot analysis of BT474 cells treated with selinexor or palbociclib single agents or in combination. Both agents decreased phospho-Rb levels, and increased cyclin-dependent kinase inhibitor proteins p21 and p27. Selinexor also induced cleavage of Caspase 3, a marker for apoptosis.

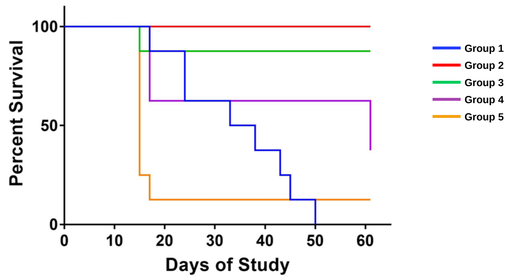
Selinexor Enhanced the Anti-Tumor Activity of Palbociclib in BT474 Xenograft Models



Treatment Group	Drug(s)	Dose (mg/kg)	Administration	TGI (%) (Day 59)**
Group 1	Vehicle	-	QoDx3	-
Group 2	Selinexor	5	QoDx3	72.7
Group 3	Selinexor Selinexor + Palbociclib	15 5 50	QoDx3 to Day 15 QoDx3 from Day 19 to end QD from Day 19 to end	88.2
Group 4	Palbociclib	150 50	QD to Day 15 QD from Day 19 to end	40.9
Group 5	Selinexor + Palbociclib	5 150 50	QoDx3 to Day 15 QoDx3 from Day 19 to end QD to Day 15 QD from Day 19 to end	NC*

Figure 5. Selinexor enhances the activity of palbociclib on the BT474 xenograft.
All drugs administered PO on study days indicated
*Not calculated due to small animal number
**Relative to vehicle control tumor volume data from Day 40

Selinexor-Palbociclib Combination Treatment Prolongs Survival of Mice Carrying BT474 Xenografts



Treatment Group	Drug(s)	Median Survival (days)
Group 1	Vehicle	35.5
Group 2	Selinexor	Undefined
Group 3	Selinexor → Selinexor + Palbociclib	Undefined
Group 4	Palbociclib	61
Group 5	Selinexor + Palbociclib	NC*

Figure 6. Selinexor enhances the activity of palbociclib on the BT474 xenograft.
Median survival of the mice presented in Figure 5.
*Not calculated due to small animal number

Cell Cycle Regulators are Reduced by Selinexor and Palbociclib in BT474 Xenografts

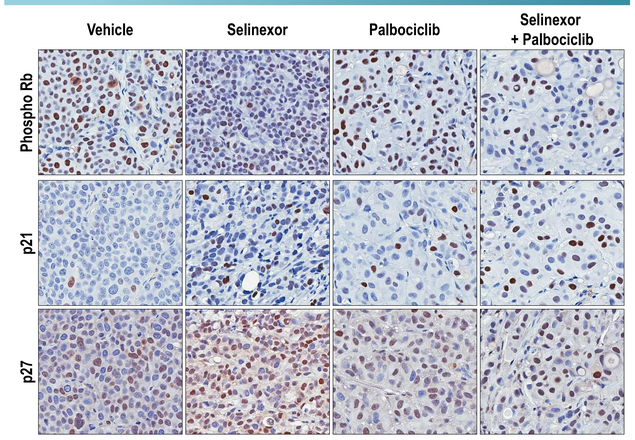


Figure 7. Immunohistochemistry staining of BT474 mouse xenograft tumor samples collected from mice treated with selinexor or palbociclib alone or in combination. Consistent with Western blotting studies (Figure 4), both selinexor and palbociclib treatment reduced phospho-Rb levels in the tumor cells as compared to vehicle control treated samples. Nuclear staining of p21 and p27 levels was also increased by both single agents as compared with vehicle. A similar trend was observed in samples treated with selinexor plus palbociclib combination.

Summary

- Aberrant CDK4/6-CyclinD1 activity often contributes to tumor progression and drug resistance in HER2+ breast cancer by phosphorylating and inactivating the tumor suppressor Rb, allowing G1 to S phase transition.
- Palbociclib is a CDK4/6 inhibitor that blocks CDK4/6-Cyclin D1 activity, reducing phospho-Rb and inducing cell cycle arrest.
- Selinexor inhibits XPO1-dependent nuclear export, increases nuclear tumor suppressor proteins and induces tumor cell apoptosis.
- The combination of selinexor and palbociclib demonstrated additive anti-tumor activity and improved overall survival in a BT474 xenograft model.
- Both selinexor and palbociclib increased phospho-Rb, induced expression of CDK4/6 inhibitors p21 and p27 and suppressed BT474 tumor cell proliferation while only selinexor increased Caspase-3 cleavage.

Conclusions

- **In addition to direct inhibition of CDK4/6, palbociclib might also inhibit CDK4/6 through upregulation of p21 and p27.**
- **Selinexor plus palbociclib shows additive inhibition of cell proliferation *in vivo* and *in vitro* in HER2+ breast cancer cell line BT474 in part by combined down-regulation of CDK4/6 pathway.**
- **This combination warrants further investigation as an effective treatment option for recurrent and relapsed HER2+ breast cancer.**

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