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

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Abstract

Introduction: XP01 (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 hematologic malignancies. This activity can be further enhanced by other therapeutic agents. We have previously shown strong synergistic preclinical activity of selinexor plus trastuzumab toward 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 immunoblot. In vivo, a subcutaneous BT474 xenograft mouse model was treated with selinexor (5 mg/kg or 15 mg/kg, qdivic) or palbociclib (50 mg or 150 mg/kg) 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 BT474 breast cancer cells. 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 inhibitory effects of selinexor by selinexor plus palbociclib. The K67 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 the nuclear export of transcription factors, reducing tumor growth and proliferation. Nuclear retention of XP01 cargos proteins inactivates their tumor suppressor functions, inhibiting proliferation and inducing apoptosis. XP01 cargos 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 CDK4/6 complexes phosphorylates Rb, releasing it from the Rb-E2F complex and allowing target gene expression that facilitates cell cycle progression. The cyclin-dependent kinase inhibitors proteins p21 and p27 are endogenous cellular mechanisms to inhibit Cyclin/CDK complexes and arrest cell cycle progression.

Figure 1. 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 inhibitors proteins p21 and p27. Selinexor also induced cleavage of Caspase 3, a marker for apoptosis.

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

Figure 3. K67 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 K67 proliferation index (% positive cells).

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 inhibitors proteins p21 and p27. Selinexor also induced cleavage of Caspase 3, a marker for apoptosis.

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

Figure 5. Selinexor enhances the activity of palbociclib on the BT474 xenograft. All drugs administered PO on study days indicated.

Table 1. Palbociclib Combination Treatment Prolongs Survival of Mice Carrying BT474 Xenografts

Table 2. Effects of Selinexor and Palbociclib on BT474 Xenografts in vivo

Table 3. Synergistic Anti-proliferation Effects of Selinexor and Palbociclib on BT474 Xenografts in vitro

Table 4. Selinexor-Palbociclib Combination Treatment Shows Additive Effects on BT474 Cell Cycle Arrest in vitro

Figure 6. 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 phospho-Rb 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 XP01-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 with xenograft in multiple combinations and preclinical models.
- This combination warrants further investigation as an effective treatment option for recurrent and relapsed HER2+ breast cancer.

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