Synergistic Anticancer Activity of Eribulin Plus Palbociclib in Patient-Derived Xenograft (PDX) Models of ER+/Her2- Human Breast Cancer

Abstract #191

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Abstract

Eribulin is a synthetic analog of the marine sponge natural product halichondrin B. Its clinical formulation is currently approved in numerous countries for treating certain patients with advanced breast cancer or advanced liposarcoma. Eribulin's anticancer mechanisms include relatively fast antimitotic effects that lead to cell death by apoptosis, and slower complex effects on the tumor microenvironment that include increased vascular perfusion, reduced hypoxia and changes in phenotype associated with decreased migration and invasiveness in vitro. Such changes in tumor phenotype led us to ask whether eribulin could be successfully combined with inhibitors of cyclin dependent kinases 4 and 6 (cdk 4/6), which work at the G1/S cell cycle checkpoint where phenotypic changes are typically regulated. It is now recognized that patient-derived xenograft (PDX) tumor models more faithfully recapitulate human tumor biology and drug responsiveness than standard xenograft models derived from established human cancer cell lines. Accordingly, in the current study, we used two ER+/PR+/Her2– PDX models developed from patients with luminal B breast cancers, OD-BRE-0192 and OD-BRE-0745, to test in vivo combinations of eribulin and the cdk 4/6 inhibitor palbociclib. Eribulin and palbociclib dose levels were chosen that led to only minimal effects when given alone, and were administered on Q7Dx3 and Q1Dx5[x3] schedules, respectively, with palbociclib withheld the day before and the day of weekly eribulin dosing to avoid cell cycle-based antagonism that could occur between mitotic and G1/S blockers. In the OD-BRE-0745 model only, a fourth cycle of eribulin followed by 5 days of palbociclib was administered starting 13 days after the third eribulin dose. Under these conditions, combining eribulin and palbociclib led to markedly superior anticancer activity in both models (minimum T/C values of 29% and 41%) compared to either agent alone (T/C: 55-67% and 88-98%, respectively). These preclinical PDX results support clinical exploration of eribulin and palbociclib combinations for appropriate patients with ER+/Her2- breast cancers.

Introduction

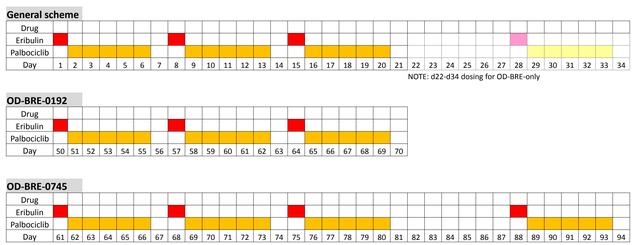
Eribulin mesilate (Halaven®) is currently approved in >60 countries worldwide for treatment of certain patients with advanced breast cancer and/or advanced liposarcoma (or soft tissue sarcoma, in Japan). As a mechanistically novel microtubule dynamics inhibitor, eribulin exerts cytotoxic effects *via* antimitotic mechanisms that trigger cancer cell apoptosis following prolonged and irreversible mitotic blockade (Towle et al., 2001; Kuznetsov et al., 2004; Jordan et al., 2005; Smith et al., 2010; Towle et al., 2011). More recently, preclinical studies have also revealed unexpected effects of eribulin on residual tumors and tumor cells that are not killed by eribulin's cytotoxic antimitotic effects. In preclinical breast cancer models, such effects include tumor vascular remodeling resulting in increased perfusion and mitigation of hypoxia, phenotypic reversal of epithelial-mesenchymal transition (EMT), decreased capacities for migration and invasion, and decreased ability to seed metastases in experimental metastasis models (Funahashi et al., 2014; Yoshida et al., 2014). Similarly, in preclinical models of liposarcoma and leiomyosarcoma, eribulin increases tumor perfusion as well as expression of adipocyte and smooth muscle differentiation markers, respectively (Kawano et al., 2016). Growing clinical evidence suggests that these preclinically-defined non-antimitotic effects of eribulin also occur in patients (Ueda et al., 2016; Goto et al., 2016; Prat et al., 2015, Kobayashi et al., 2016).

The observations of phenotypic effects of eribulin in both breast cancer and soft tissue sarcoma models led us to speculate that eribulin might be affecting cellular differentiation pathways that typically operate at the G1/S cell cycle checkpoint. This led us to further ask whether eribulin could be successfully combined with anticancer agents that specifically exert their effects at the G1/S checkpoint. One such drug is palbociclib (Ibrance®), a cyclin dependent kinase (cdk) 4/6 inhibitor currently approved in the US for certain patients with hormone receptor (HR) positive, human epidermal growth factor receptor 2 (HER2) negative breast cancers in combination with aromatase inhibitors or fulvestrant. In the current study, we examined combinations of eribulin and palbociclib in two patient derived xenograft (PDX) models of HR+/HER2- breast cancers grown in immunosuppressed mice. Using dose levels that elicited only minimal antitumor effects when each agent was given as monotherapy, results from both PDX models showed robust synergistic activity when eribulin and palbociclib were combined. These results suggest a scenario in which both drugs exert effects at the G1/S cell cycle checkpoint in ways that are mechanistically synergistic and that lead to therapeutically favorable outcomes. These preclinical results in PDX models thus support clinical exploration of the combination of eribulin plus palbociclib in appropriate breast cancer patients.

Table 1: Breast Cancer PDX Models

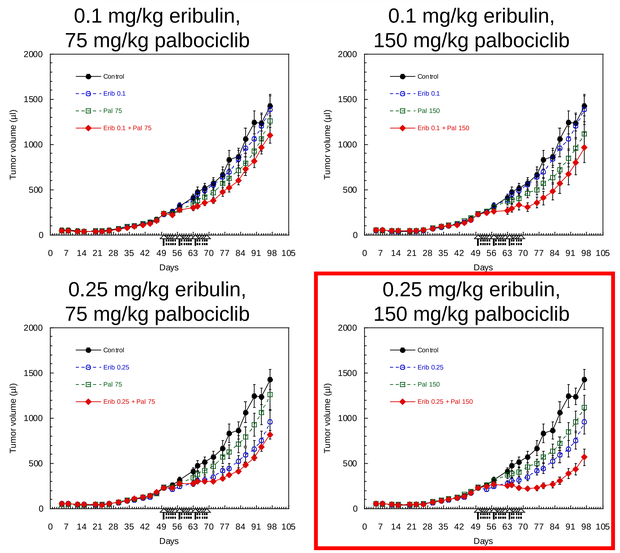
Designation	ER / PR / HER2 Status	Patient and Cancer Details	Patient Prior Therapies
OD-BRE-0192	ER+/PR+/Her2-	45 year old female patient with luminal B invasive lobular breast carcinoma, with lymph node metastases	Patient responded poorly to prior therapies including epirubicin, 5FU, cyclophosphamide, taxotere, paclitaxel, bevacizumab and gemcitabine
OD-BRE-0745	ER+/PR+/Her2-	78 year old female patient with luminal B poorly differentiated infiltrating ductal adenocarcinoma, with lymph node metastases	Patient had no prior chemotherapies or radiotherapies prior to surgery
All clinical samples used for PDX generation were collected under written informed consent (according to the Declaration of Helsinki) and a declaration for commercial use of the samples from the Consultative Committee for Patient Protection in Biomedical Research of Dijon University Hospital (CCPPRB) under authorization by the French Research Ministry for human tissue collection, storage and re-distribution according to L 1243-3, L 1243-4, and L 1245-5 CSP articles.			

Dosing Scheme Using 48 h Palbociclib Holiday to Avoid Cell Cycle-based Antagonism

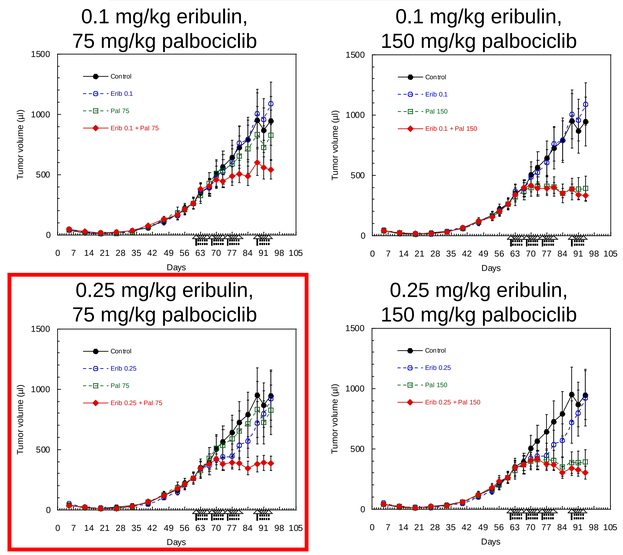


Dosing scheme to avoid possible cell cycle-based antagonism. Previous cell-based *in vitro* studies examining combinations of eribulin and palbociclib (not shown) suggested the possibility that simultaneous exposure to the two drugs could result in cell cycle based antagonism, in which eribulin's antimitotic activity prevented cells from reaching the G1/S cell cycle checkpoint where palbociclib exerts its cdk 4/6 inhibitory activity, and palbociclib's cdk 4/6 inhibitory activity at the G1/S checkpoint prevented cells from reaching mitosis where eribulin exerts its antimitotic activity. To prevent such antagonism in the *in vivo* PDX studies, a 'palbociclib holiday' scheme was used wherein palbociclib was never given the day before or the day of eribulin administration. In this way, eribulin was never administered less than 48 hours after the last palbociclib dosing, allowing sufficient time for G1/S cell cycle blockage by palbociclib to recover. The asymmetric nature of the holiday (48 h recovery from palbociclib's G1/S cell cycle block before eribulin versus 24 h recovery from eribulin's antimitotic effects before palbociclib) was based on the presumption that re-entering the cell cycle from a G1/S block (essentially G0) would inherently be a slower process than resumption and completion of mitosis after levels of eribulin had dropped below threshold levels required to induce mitotic blocks.

Eribulin + Palbociclib, OD-BRE-0192



Eribulin + Palbociclib, OD-BRE-0745



***In vivo* combinations of eribulin and palbociclib in OD-BRE-0192 and OD-BRE-0745 PDX models.** Tumor volumes as shown. All doses are below MTD based on <20% reversible body weight loss and/or <10% lethality. Dosing days indicated by arrows below x-axes: eribulin, arrows with solid lines; palbociclib, arrows with dashed lines. Dose levels resulting in optimal synergy for each model are indicated by red boxes around graphs.

Comments and Conclusions

- Eribulin and palbociclib show synergistic anticancer activity in two patient-derived xenograft (PDX) models of ER+/PR+/HER2- luminal B human breast cancer.
- All doses and combinations were at or below empirically determined MTD dose levels (based on standard criteria of <20% reversible body weight loss and <10% lethality).
- In both models, synergy was seen with doses intentionally selected to show only minimal anticancer activity when administered as single agents.

  A 48 h 'palbociclib holiday' dosing strategy was employed to avoid potential cell cycle-based antagonism. Further studies are currently underway to ascertain whether the palbociclib holiday is required to see synergy.
- In the OD-BRE-0192 PDX model, synergy was optimally seen with 0.25 mg/kg eribulin plus 150 mg/kg palbociclib.
- In the OD-BRE-0745 PDX model, synergy was optimally seen with 0.25 mg/kg eribulin plus 75 mg/kg palbociclib.

The current results provide a preclinical foundation for exploring an eribulin plus palbociclib combination strategy in appropriate breast cancer patients.

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