Onvansertib Shows Synergistic Efficacy in Combination with Paclitaxel in HR+ Breast Cancer: Mechanistic Insights from Preclinical Models

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Background

Paclitaxel in hormone receptor positive (HR+) metastatic breast cancer:

- Chemotherapy choice for patients who progress on CDK4/6 inhibitors and endocrine therapy (ET).
- Response rates range between 20-40%[1,2].
- Most patients progress due to intrinsic or acquired resistance.

Polo-like kinase 1 (PLK1):

- Serine/threonine protein kinase.
- Key regulator of mitosis and cell cycle progression.
- Overexpressed in breast cancer and associated with poor prognosis [3,4].
- Has been shown to mediate resistance to ET and CDK4/6 inhibitors in HR+ breast cancer[5,6,7].

Onvansertib:

- An orally bioavailable highly selective inhibitor of PLK1, currently in clinical development.
- Showed potent anti-tumor activity in combination with paclitaxel in preclinical models of ovarian cancer and triple-negative breast cancer (TNBC) [8,9].
- A phase 1b/2 clinical trial is ongoing to evaluate the safety and efficacy of onvansertib in combination with paclitaxel in advanced TNBC (NCT05383196).

We investigated the efficacy and mechanisms of action of onvansertib in combination with paclitaxel in HR+ preclinical models resistant to ET and CDK4/6 inhibitors.

Results

Combination of onvansertib and paclitaxel is synergistic in HR+ breast cancer cell lines

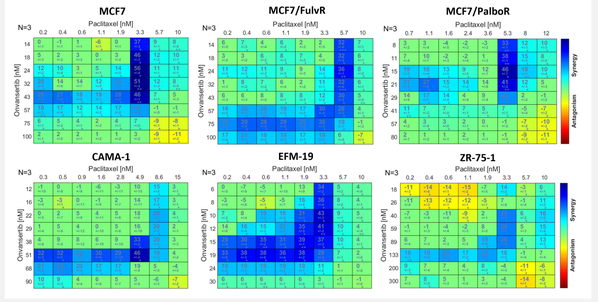


Figure 1. Dose matrix (9x9) evaluation of paclitaxel and onvansertib combination in HR+ breast cancer cell lines. Cell viability was assessed using the CellTiter-Glo® assay after 6 days of treatment at the indicated concentrations. Synergy was determined using the Bliss synergy model and synergistic interactions are indicated in blue on the heatmap (n=3). FulvR: resistant to fulvestrant; PalboR: resistant to palbociclib.

Results

Onvansertib and paclitaxel combination induces mitotic arrest in HR+ breast cancer cell lines

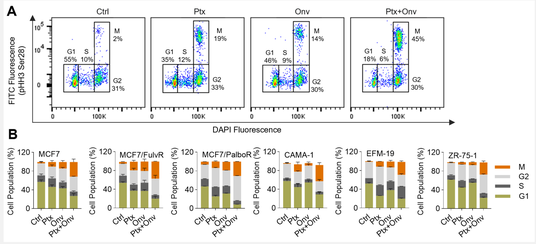


Figure 2. HR+ breast cancer cell lines were treated with DMSO (Ctrl), paclitaxel (Ptx), onvansertib (Onv), or the combination (Ptx+Onv) for 24h and subjected to cell cycle analysis by flow cytometry. Mitotic cells (M) were assessed using anti-phospho-histone H3 (pHH3Ser28) antibody and DNA was stained with DAPI. (**A**) Representative cell cycle distribution analyzed by flow cytometry in MCF7 cells. (**B**) Bar graphs of cell cycle distribution (mean ± SEM, n=3).

Onvansertib and paclitaxel combination induces DNA damage and apoptosis *in vitro*

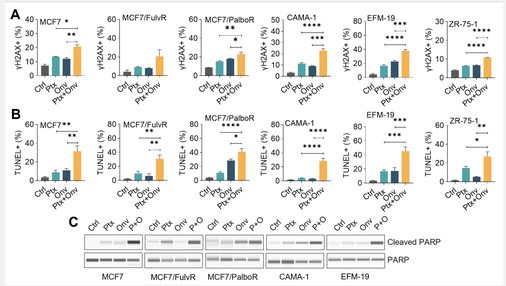
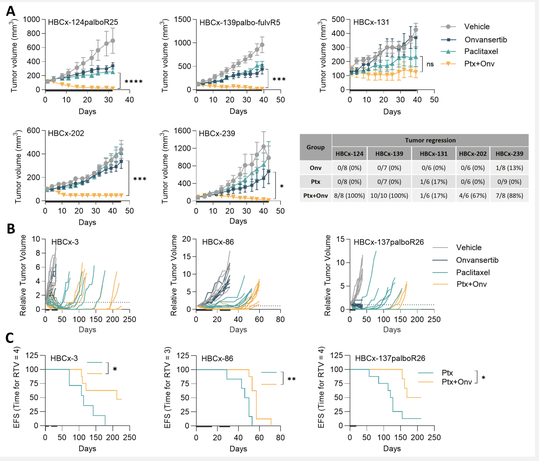


Figure 3. (**A-B**) Cells were treated with DMSO (Ctrl), Ptx, Onv or Ptx+Onv (P+O) for 72-96h and analyzed by flow cytometry for DNA damage using γH2AX marker (**A**) or for apoptosis using the TUNEL assay (**B**). Bar graphs show the percentage of positively stained cells (mean ± SEM, n=3). Statistical significance between combination treatment and single agents was assessed by one-way ANOVA. **p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001*. (**C**) Cells were treated for 24h with the indicated drugs and expression of apoptotic marker cleaved-PARP was analyzed by Simple Western. Representative images of cleaved-PARP and total PARP protein expression are shown.

Overview of the HR+ HER2- breast cancer patient derived xenograft (PDX) models

PDX Model	HBCx-124 palboR25	HBCx-131	HBCx-139 palbo-fulvR5	HBCx-202	HBCx-239	HBCx-3	HBCx-86	HBCx-137 palboR26
PDX Origin	Bone met	Bone met	Bone met	Bone met	Skin met	Primary tumor	Primary tumor	Bone met
Genomic alterations	*CCND1* amp. *FGFR1* amp. *CCNE2* amp.	*CCND1* amp. *FGFR1* amp.	*CCND1* amp. *PIK3CA* mut.	*CCND1* amp.	*ESR1* mut. *PIK3CA* mut. *TP53* mut.	*TP53* mut. *PTEN* del.	*PIK3CA* mut.	*CCND1* amp. *FGFR1* amp. *CCNE2* amp.
Resistant to	Palbociclib + Fulvestrant	*Partial response* • Palbociclib • Fulvestrant • Palbociclib + Fulvestrant	• Palbociclib • Fulvestrant • Palbociclib + Fulvestrant	Palbociclib	• Fulvestrant • Abemaciclib + Fulvestrant	• Palbociclib • Fulvestrant • Palbociclib + Fulvestrant	*Partial response* • Palbociclib • Fulvestrant	• Palbociclib

Onvansertib and paclitaxel combination exhibits robust anti-tumor activity in HR+ breast cancer PDX models resistant to ET and CDK4/6 inhibitors



Tumor regression					
Group	HBCx-124	HBCx-139	HBCx-131	HBCx-202	HBCx-239
Onv	0/8 (0%)	0/7 (0%)	0/6 (0%)	0/6 (0%)	1/8 (13%)
Ptx	0/8 (0%)	0/7 (0%)	1/6 (17%)	0/6 (0%)	0/9 (0%)
Ptx+Onv	8/8 (100%)	10/10 (100%)	1/6 (17%)	4/6 (67%)	7/8 (88%)

Figure 4. PDX models were treated with vehicle (Control), Onv (oral, 45mg/kg, 5times/week), Ptx (IP, 15-25 mg/kg, weekly) or Ptx+Onv for the indicated duration (—) and tumor volumes were measured twice a week. Relative tumor volume (RTV) was calculated as RTV=(tumor volume on measured day)/(tumor volume on day 0). Tumor regression was reported if RTV < 0.5 in at least 1 measurement. (**A**) Mean ± SEM of tumor volumes overtime. Unpaired t-test was used to compare tumor volumes between Ptx+Onv and most effective monotherapy at the last measurement. (**B**) Individual RTV over time. (**C**) Kaplan–Meier of the event-free survival (EFS, time for RTV = 3 or 4). Log-rank Mantel–Cox test was used for statistical analyses. *ns non-significant, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.*

Onvansertib and paclitaxel combination induces apoptosis *in vivo*

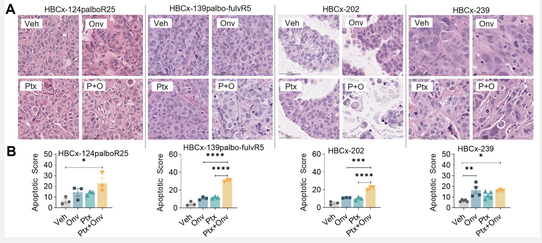


Figure 5. Representative H&E-stained photomicrographs of tumors treated as described in Figure 4. Scale bar = 50 µm. Apoptotic cells were scored by a board-certified pathologist. Graphs represent the scores as mean ± SEM. One-way ANOVA was used to compare the means. **p < 0.05, **p < 0.01, ***p < 0.001 ****p<0.0001.*

Onvansertib + paclitaxel decreases c-MYC protein levels in cell lines and xenograft tumors

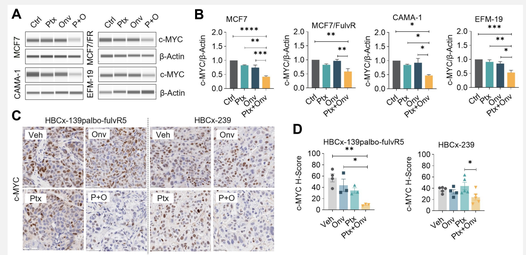


Figure 6. (**A**) Cells were treated with DMSO (Ctrl), Onv, Ptx or P+O for 24h and c-MYC expression analyzed by Simple Western. (**B**) Graphs represent c-MYC/β-Actin densitometric ratios normalized to Ctrl (n=3). (**C**) Representative anti-c-MYC immunohistochemical images of tumors from Fig. 4 treatments; scale bar = 50 µm. (**D**) Graphs show c-MYC H-Score (mean ± SEM). One-way ANOVA compared the means.**p< 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.*

c-MYC protein stability is decreased by onvansertib and paclitaxel combination

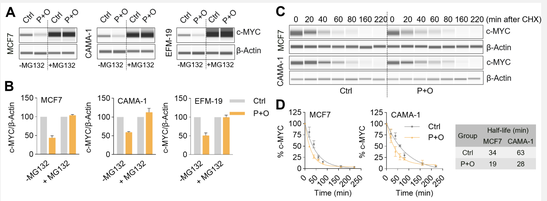


Figure 7. (**A**) Cells were treated with proteasomal inhibitor (MG132, 10 µM), and then with DMSO (Ctrl), or P+O for 24h. c-MYC expression was analyzed by Simple Western. (**B**) Graphs represent densitometric ratios of c-MYC to β-Actin normalized to Ctrl. (**C**) Cells were treated with DMSO or P+O for 6-8h followed by cycloheximide (CHX, 50 µg/ml), and c-MYC protein degradation was assessed by chase assay. (**D**) Half-life of c-MYC was calculated based on 1-phase decay.

Conclusions

Onvansertib and paclitaxel combination:

- Synergistically inhibited cell viability and induced mitotic arrest, DNA damage and apoptosis in HR+ breast cancer cell lines sensitive and resistant to first-line therapies.
- Resulted in enhanced anti-tumor activity compared to monotherapies in 8 HR+ breast cancer PDX models resistant to ET and/or CDK4/6 inhibitors.
- Overcame paclitaxel resistance and delayed tumor relapse in paclitaxel-sensitive PDX models.
- Reduced c-MYC expression in both cell lines and xenograft tumors by decreasing its protein stability, likely contributing to the enhanced apoptosis observed *in vitro* and *in vivo*.

Collectively, these data support that onvansertib in combination with paclitaxel represents a promising therapeutic strategy for HR+ breast cancer patients after progression on endocrine therapy and CDK4/6 inhibitors.

References

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