Combination of the PLK1 Inhibitor Onvansertib and PI3Kα Inhibitor Alpelisib is effective in Palbociclib-Resistant PIK3CA-mutated HR+ Breast Cancer

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Background

Resistance to first-line therapies in HR+/Her2- breast cancer:

- CDK4/6 inhibitors, including palbociclib, in combination with endocrine therapy is the first line standard-of-care for HR+/HER2− breast cancer.
- Though effective, most patients progress due to intrinsic or acquired resistance to CDK4/6 inhibitors or endocrine therapy[1].
- PIK3CA gene mutation is one of the most prevalent (~40%) gene alteration in HR+ breast cancer and is implicated in resistance to therapy[2].
- PI3Kα inhibitor, alpelisib, is approved for PIK3CA-mutated, advanced HR+/HER2− breast cancer. Drug associated toxicities and resistance mechanisms may limit its clinical benefit[3].

Onvansertib - an investigational polo-like kinase 1 (PLK1) inhibitor

- PLK1 is a serine/threonine protein kinase, key regulator of mitosis and cell cycle progression.
- PLK1 is overexpressed in breast cancer and has been shown to mediate resistance to palbociclib in HR+ breast cancer[4].
- Onvansertib is an oral and selective inhibitor of PLK1, currently in clinical development for solid tumors and hematological malignancies.

This study evaluated the potential of onvansertib to increase the efficacy of alpelisib in PIK3CA-mutant HR+ breast cancer preclinical models.

Pre-clinical Models

Mutational status and IC_{50} values of onvansertib and alpelisib in ER+ breast cancer cell lines

Cell line	Mutational Status PIK3CA	Mutational Status PTEN	IC_{50} (nM) Onvansertib	IC_{50} (nM) Alpelisib
MCF7	Mut (E545K)	WT	68 ± 5	273 ± 27
T-47D	Mut (H1047R)	WT	211 ± 4	216 ± 3
EFM-19	Mut (H1047L)	WT	23 ± 2	185 ± 14
CAMA-1	WT	Loss	58 ± 6	Resistant
ZR-75-1	WT	Loss	135 ± 18	Resistant
MCF7/164R-7 (Fulvestrant-resistant)	Mut (E545K)	WT	64 ± 1	288 ± 20

PIK3CA and PTEN mutational status of selected ER+ breast cancer cell lines. The cells were treated with varying doses of onvansertib or alpelisib for 6-7 days and cell viability was assessed using CellTiter-Glow® assay. IC_{50} values are shown.

Overview of the PDX models

PDX Model	Patient's prior treatments	PDX Origin	Mutations	Gene amplification/ gain	Resistance to palbociclib	Response to fulvestrant (ful) ± palbociclib (palbo)
HBCx-134 palboR31	PI3Kα Inhibitor, Letrozole, FEC + Docetaxel	Bone Met	PIK3CA (H1047R)	CCND1, ESR1	Acquired	• Resistant to fulvestrant • Resistant to ful + palbo
HBCx-86	NA	Primary breast tumor	PIK3CA (E545K)	NA	Intrinsic	NA
HBCx-180	FEC, Tamoxifen, Palbociclib + Letrozole	Bone Met	PIK3CA (H1047R)	CCND1	Intrinsic	• Resistant to fulvestrant • Resistant to ful + palbo

Results

Onvansertib and alpelisib are synergistic in ER+ breast cancer cell lines

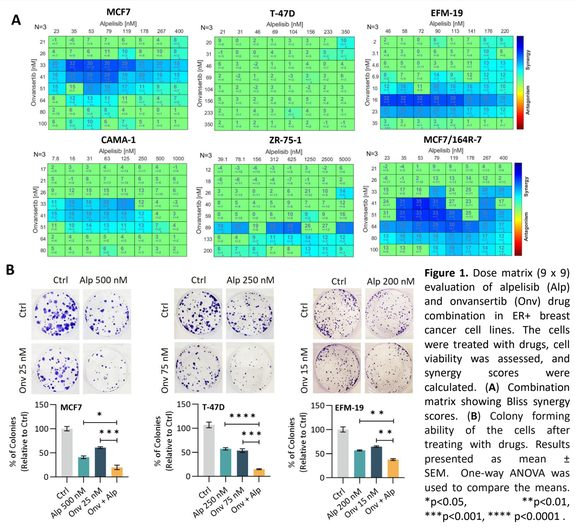


Figure 1. Dose matrix (9 x 9) evaluation of alpelisib (Alp) and onvansertib (Onv) drug combination in ER+ breast cancer cell lines. The cells were treated with drugs, cell viability was assessed, and synergy scores were calculated. (**A**) Combination matrix showing Bliss synergy scores. (**B**) Colony forming ability of the cells after treating with drugs. Results presented 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 and alpelisib combination induces G2/M arrest *in vitro*

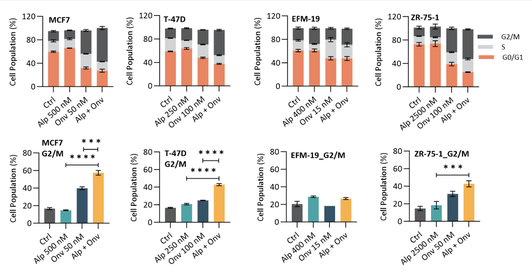


Figure 2. Effect of alpelisib (Alp) and onvansertib (Onv) on cell cycle distribution of ER+ breast cancer cell lines. The cells were treated with the drugs for 72-96h, stained with DAPI and cell cycle distribution was assessed by flow cytometry. The percentage of cells in G1, S and G2/M phases are plotted. Results are the mean of three experiments and are presented as mean ± SEM. One-way ANOVA was used to compare the means. ***p<0.001, **** p<0.0001.

Onvansertib and alpelisib induces apoptosis in ER+ breast cancer cell lines

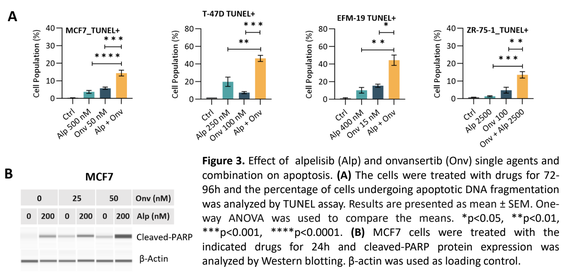
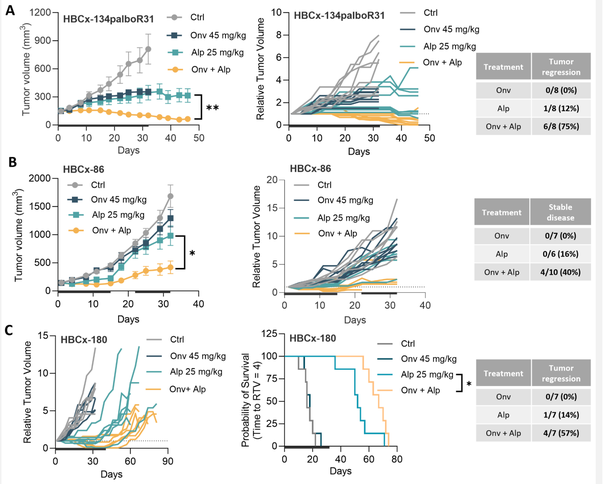


Figure 3. Effect of alpelisib (Alp) and onvansertib (Onv) single agents and combination on apoptosis. **(A)** The cells were treated with drugs for 72-96h and the percentage of cells undergoing apoptotic DNA fragmentation was analyzed by TUNEL assay. Results are presented as mean ± SEM. One-way ANOVA was used to compare the means. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. **(B)** MCF7 cells were treated with the indicated drugs for 24h and cleaved-PARP protein expression was analyzed by Western blotting. β-actin was used as loading control.

Onvansertib and alpelisib combination exhibits robust anti-tumor activity and prolongs the benefit of alpelisib in palbociclib-resistant PIK3CA-mutant ER+ breast cancer PDX models



Treatment	Tumor regression
Onv	0/8 (0%)
Alp	1/8 (12%)
Onv + Alp	6/8 (75%)

Treatment	Stable disease
Onv	0/7 (0%)
Alp	0/6 (16%)
Onv + Alp	4/10 (40%)

Treatment	Tumor regression
Onv	0/7 (0%)
Alp	1/7 (14%)
Onv + Alp	4/7 (57%)

Figure 4. (**A**) HBCx-134palboR31 (**B**) HBCx-86 and (**C**) HBCx-180 PDX models were treated with vehicle (Ctrl), onvansertib (Onv), alpelisib (Alp) or combination of Alp and Onv for the indicated duration (▬). Tumors were measured, and relative tumor volume (RTV) was calculated as RTV = (tumor volume on measured day)/(tumor volume on day 0). Individual RTV over time is shown. Tumor regression is reported if RTV < 0.5 in at least 1 tumor measurement. Unpaired t-test was used to compare relative tumor volume at last measurement. (**C**) Kaplan-Meier survival curve for event-free survival (time for RTV = 4) was calculated. Log-rank Mantel-Cox test was used for survival analyses *p<0.05, **p<0.01.

Onvansertib and alpelisib combination induces apoptosis *in vivo*

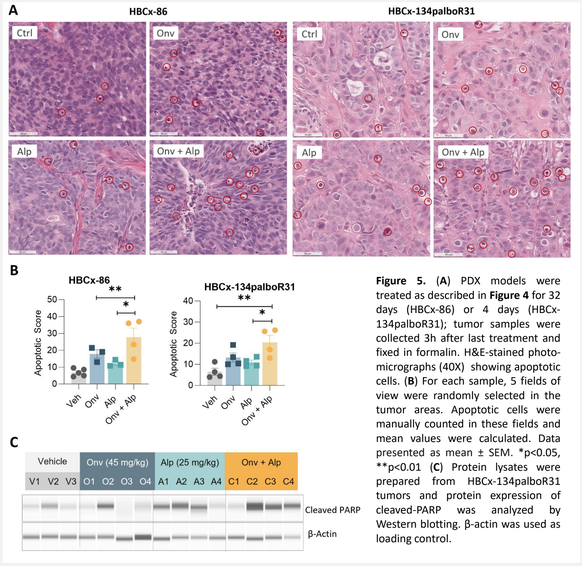


Figure 5. (**A**) PDX models were treated as described in **Figure 4** for 32 days (HBCx-86) or 4 days (HBCx-134palboR31); tumor samples were collected 3h after last treatment and fixed in formalin. H&E-stained photo-micrographs (40X) showing apoptotic cells. (**B**) For each sample, 5 fields of view were randomly selected in the tumor areas. Apoptotic cells were manually counted in these fields and mean values were calculated. Data presented as mean ± SEM. *p<0.05, **p<0.01 (**C**) Protein lysates were prepared from HBCx-134palboR31 tumors and protein expression of cleaved-PARP was analyzed by Western blotting. β-actin was used as loading control.

Conclusions

- Onvansertib and alpelisib synergistically inhibited the cell viability and/or colony forming ability of PIK3CA-mutant and PTEN-loss ER+ breast cancer cell lines.
- Compared to monotherapies, the combination induced more pronounced G2/M arrest and apoptosis in ER+ breast cancer cell lines.
- The combination of onvansertib and alpelisib exhibited robust anti-tumor activity in PIK3CA-mutant palbociclib-resistant ER+ breast cancer PDX models.
- An increase in apoptosis was observed in tumors from mice treated with the combination compared to the monotherapies.

Co-targeting PLK1 and PI3Kα with onvansertib and alpelisib respectively, may constitute a promising therapeutic combination strategy for patients with PIK3CA-mutant HR+ breast cancer failing to respond to first-line standard of care therapies.

References

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3. Mishra et al., Int J Mol Sci. 2021 Apr; 22(7): 3464.
4. Guerrero-Zotano et al., Clin Cancer Res.2023. 14;29(8)1557-68.