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Combining PARP inhibition with the Polo-like kinase (PLK1) inhibitor onvansertib overcomes PARP inhibitor resistance

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Background

- Half of ovarian carcinomas (OC) harbors defects in homologous recombination (HR)-mediated DNA repair and this is likely to be responsible for the high sensitivity to poly(ADP-ribose) polymerase inhibitors (PARPi). PARPi have been approved as maintenance in recurrent and in first-line settings in high grade OC with very exciting results. However, also for PARPi resistance is inevitable. Identifying combination treatments to sensitize tumors cells to PARPi and/or overcome PARPi resistance is critical to expand the benefit of these therapies.
- The Polo-like kinase 1 (PLK1) is a Ser/Thr kinase and a master regulator of mitosis. It has been recently involved in the HR-mediated DNA repair and in the recovery from the G2/M checkpoint. Based on PLK1 role in HR, we hypothesized that PLK1 inhibition may be combined with olaparib, a PARPi, to reverse PARPi resistance.
- Onvansertib is a highly specific PLK1 inhibitor under clinical investigation.

Aims of the study

The aim of the present work was to test *in vivo* the combination of the PLK1 inhibitor onvansertib and olaparib (a PARPi) in different OC-patient-derived xenograft (PDX) models resistant to olaparib.

Material and Methods

OC-PDX models. The patient-derived xenografts (PDXs) used in this study are part of a human ovarian xenobank, recently established at the Mario Negri Institute in Milan (IT), and described in [1]. For these studies, 3 models whose molecular and pharmacological characteristics are reported in Table 1, were selected. MNHOC316DDP derived from a cisplatin (DDP)-sensitive PDX made resistant through multiple *in vivo* DDP treatment cycles.

Antitumor activity. The selected PDXs were orthotopically transplanted in NCr-nu/nu mice and randomized into: 1) Control/ vehicle-treated group; 2) Olaparib (100mg/kg- MNHOC22 and MNHOC316DDP- or 80mg/kg- MNHOC266- *per os*); 3) Onvansertib (50mg/kg, *per os*); 4) Combination (Combo), 5 days/week for 4 weeks. For MNHOC316DDP, DDP treated mice (5mg/kg q7x3) were considered as control. The antitumor activity was evaluated by calculating the increase in life span (ILS%)= [(median survival control group-median survival treated group)- median survival treated group]x100.

Pharmacodynamic (PD) studies. MNHOC22 and MNHOC266 bearing mice were treated with the doses previously reported for four consecutive days, and then euthanized at 2 hrs and 24 hrs after the last treatment. Ascitic cells were both formalin-fixed paraffin-embedded (FFPE) and snap frozen for PD studies. PD studies included: proliferation measured by Ki67 IHC stain, apoptosis measured by the Caspase-Glo®3/7 kit (Promega), mitosis quantified by mitotic events count on FFPE and anti-pH3-Ser10 expression by WB, DNA damage/apoptosis quantified by WB using an anti-γH2AX antibody. RAD51- foci were quantified by using an IF-based method as recently described [2] and by scoring in blind the % of RAD51/geminin (GMN)-positive tumor cells.

Statistical analyses: For survival analyses, Kaplan-Mayer curves are reported and Mantel-Cox test was used; unpaired-t test was performed for all the other comparisons. p-value<0.05 was considered significant.

Table 1. Molecular and pharmacological characteristics of the PDXs under study.

#ID PDX	Platinum sensitivity	Olaparib sensitivity	*TP53* status	*BRCA1* status
MNHOC22	+++	-	mut	mut
MNHOC266	+++	-	mut	mut
MNHOC316DDP	-	-	mut	wt

+++: very sensitive; -: resistant

Results

Figure 1. Mice body weight of MNHOC22 model (A), and MNHOC22 (B), MNHOC266 (C) and MNHOC316DDP (D) bearing mice survival curves.

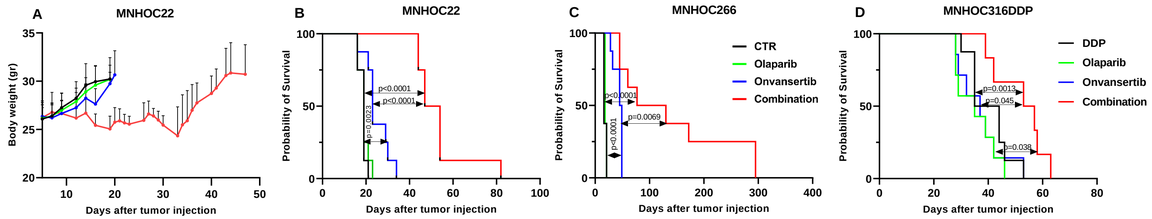
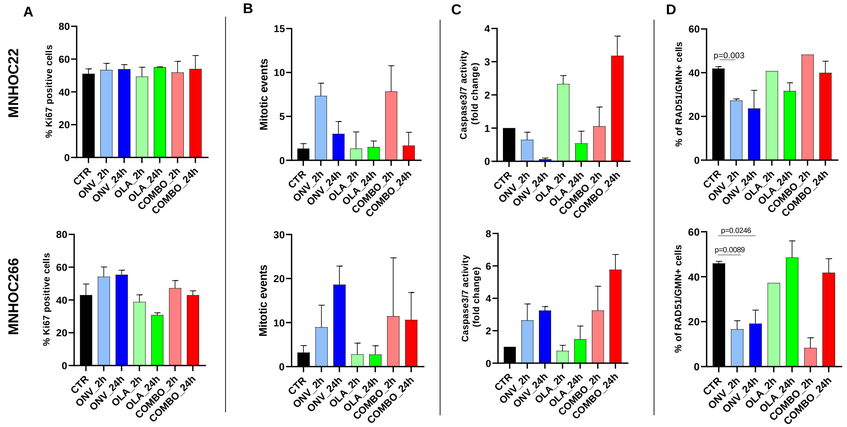


Table 2. Median survival times and ILS in the different PDXs used.

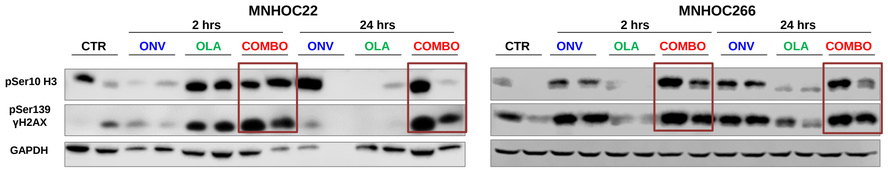
	MNHOC22		MNHOC266			MNHOC316DDP	
	Median survival (days)	%ILS	Median survival (days)	%ILS		Median survival (days)	%ILS
Control	19		16		DDP	39,5	
Olaparib	19	0	18	12,5	Olaparib	37	0
Onvansertib	23	21	47	194	Onvansertib	38	0
Combination	52	173	103	544	Combination	57	44

Figure 2. Evaluation of Ki67 positivity (A), mitosis (B), apoptosis (C) and RAD51-foci formation (D) in PDXs MNHOC22 and MNHOC266.



- **Olaparib/onvansertib combination was well tolerated *in vivo;* even if a decrease in body weight was observed, it never exceeded 20% and reverted upon drugs withdrawal (Figure 1, panel A).**
- **All the selected PDXs were resistant to olaparib; onvansertib was slightly active in MNHOC266 model, but had no activity in both MNHOC22 and MNHOC316DDP. Conversely, the combination was highly effective as demonstrated by a significant increased survival as compared to controls and single agent treatments (Figure 1 panels B, C, D, and Table 2).**
- **No differences in the % of Ki67 positive cells among the different groups were observed (Figure 2, panel A); onvansertib treatment induced an increase in mitotic events at 2 hrs both as single agent and in combination with olaparib (Figure 2, panel B); a higher apoptosis could be seen in both models at 24 hrs (Figure 2, panel C).**
- **As already reported [2], a high basal level of RAD51-foci positive cells was observed in both models. A trend toward a decrease in RAD51-foci positive cells after onvansertib treatment could be observed (Figure 2, panel D).**
- **Higher levels of pSer10-H3 and pSer139-γH2AX were observed in the combination group at both 2 and 24 hrs in both models (Figure 3), suggesting an increased G2/M block and apoptotic death/DNA damage, corroborating the data on mitotic counts and caspase3/7 activities (Figure 2, panels B and C).**

Figure 3. Levels of pSer10-H3 and pSer139-γH2AX in tumors treated or not with onvansertib, olaparib and their combination.



Conclusions

Our data clearly show a strong therapeutic efficacy of the olaparib/ onvansertib combination in olaparib resistant ovarian carcinoma PDXs. The combination seems to induce higher G2/M block and apoptosis/DNA damage. However, further studies are required to clarify the molecular mechanisms underlying the antitumor activity observed *in vivo*, including interference with tumor DNA damage response.

References: [1] Ricci F. *et al.*, Cancer Res. 2014; [2] Guffanti F. *et al.*, BJC 2022.

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