945 *In vivo* anti-tumor activity of onvansertib, a PLK1 inhibitor, combined with gemcitabine or carboplatin in platinum- resistant ovarian carcinoma patient-derived xenograft models

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Background

The standard treatment for high grade ovarian carcinoma (HGOC) is cytoreductive surgery followed by a platinum based therapy. Despite an initial high rate of complete remission, most of the patients will relapse with a much less platinum sensitive disease. Patients with resistant tumors have limited options, including monotherapy with gemcitabine, and new effective therapeutic alternatives are needed. The Polo-like kinase 1 (PLK1) is a master regulator of mitosis and recent evidences suggest its role in interfering with several DNA repair mechanisms. We investigated the effect of onvansertib (ONV), a highly selective ATP-competitor PLK1 inhibitor, in combination with two DNA damaging agents: carboplatin (CARBO) and gemcitabine (GEM) in platinum-resistant HGOC patient-derived xenografts (PDXs).

Aim of the study

The aim of the present work was to test the effect of onvansertib (ONV), a highly selective ATP-competitor PLK1 inhibitor, in combination with carboplatin (CARBO) and gemcitabine (GEM) in platinum-resistant HGOC patient-derived xenografts (PDXs).

Material and Methods

In vitro studies. Ovcar 8 cells were seeded and after 48h were treated with different concentrations of onvansertib and carboplatin/gemcitabine After 5 days cellular viability was evaluated by MTS assay. Data were analyzed using Combenefit software. **OC-PDX models.** The Patient derived xenografst (PDXs) used in this study are part of a human ovarian xenobank, established at the Mario Negri Institute in Milan (IT), and described (Ricci F et al, Cancer Res, 2014). For these studies, 2 high grade serous ovarian carcinomas, *TP53* mutated and cisplatin resistant, were selected (MNHOC266R, #266 and MHNOC315, #315). #266 derived from a cisplatin (DDP)-sensitive PDX made resistant through multiple *in vivo* DDP treatment cycles, while #315 is a model of intrinsic DDP resistance. **Antitumor activity.** The selected PDXs were orthotopically (i.p.-MNHOC266R) or subcutaneously (s.c. -MNHOC315) transplanted in NCr-nu/nu mice and randomized into: 1) Control/ vehicle-treated group; 2) Onvansertib (40mg/kg, per os, p.o., 5 days/week for 4 weeks, *p.o.*); 3) Carboplatin (50mg/kg, *i.v.* q7x4); 4) Gemcitabine (60 mg/kg *i.p.* q7x4) 5) Combination of onvansertib and carboplatin; 6) Combination of onvansertib and gemcitabine. The antitumor activity was evaluated by calculating the survival of mice bearing #266R model and by evaluating the tumor growth inhibition for #315 model. **Pharmacodynamic (PD) studies**. #266R and #C315 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. Tumor samples were both formalin-fixed paraffin-embedded (FFPE) and snap frozen. Tumor protein lysates were obtained and caspase activity measured by the Caspase-Glo®3/7 kit (Promega). **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.

Results

Figure 1. In vitro combination of onvansertib and carboplatin (A) and onvansertib and gemcitabine (B) in OVCAR 8 cells.

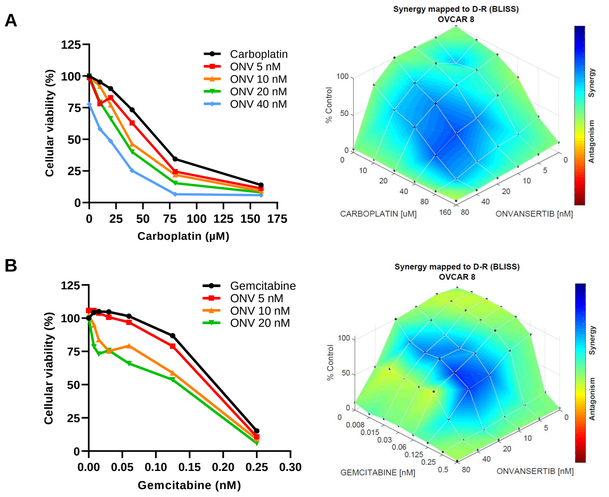


Figure 2. Mice body weight and survival curves of #226R-bearing mice treated with onvansertib, carboplatin and their combination (A) or with onvansertib, gemcitabine and their combination (B). Tumor growth curves and tumor weights at the end of the treatment in #315-bearing mice treated with onvansertib, carboplatin and their combination (C) or with onvansertib, gemcitabine and their combination (D).

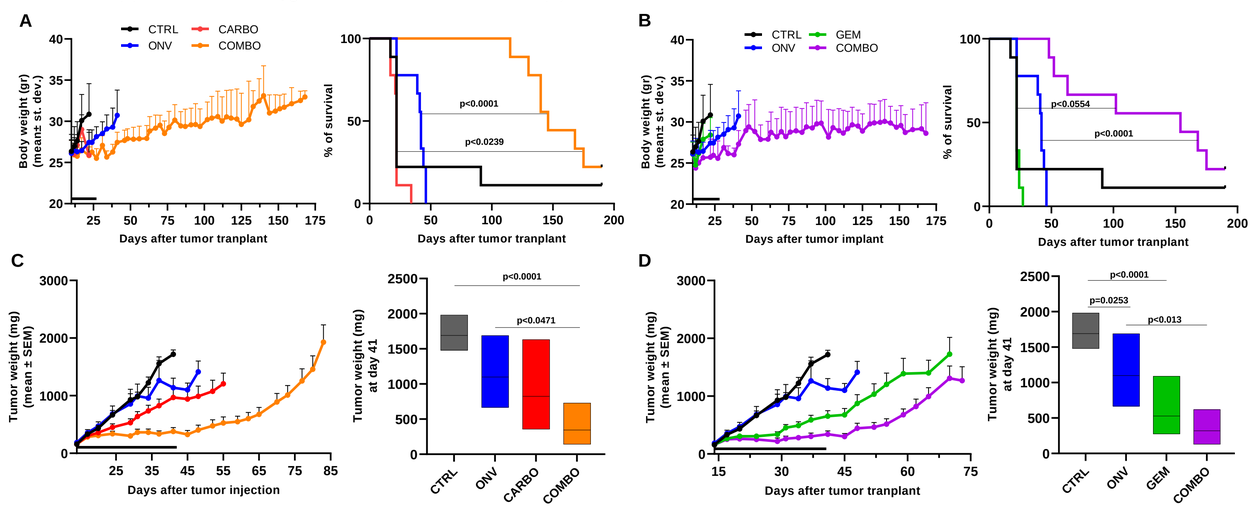
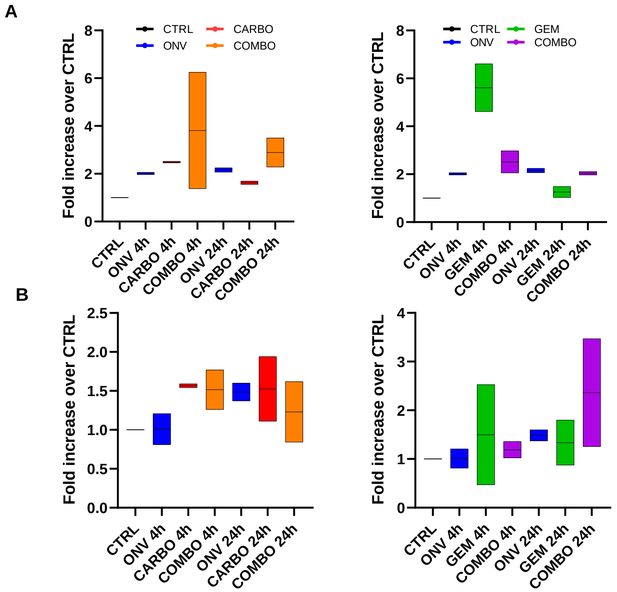


Figure 3. Evaluation of apoptosis in #226R (A) and #315 (B) bearing mice



- Onvansertib was synergistic in combination with carboplatin or gemcitabine in the human ovarian carcinoma cell line OVCAR8 (Figure 1).
- Both combinations were well tolerated *in vivo;* even if a decrease in body weight was observed, it never exceeded 20% and reverted upon drugs withdrawal (Figure 2, panels A and B).
- The selected PDXs were resistant to DDP; onvansertib was slightly active in #266R model, but had no activity in #315. In #266R model both carboplatin and gemcitabine were completely inactive, while a slight activity of both drugs was observed in #315 model. In the two models, both combinations were highly effective as demonstrated by a significant increased survival (#266) and a significant tumor growth inhibition (#315) as compared to controls and single agent treatments (Figure 2).
- Higher caspase induction was observed in #266R tumors treated with ONV and CARBO combinations at 2hrs, and 24hrs (Figure 3A). GEM caused a strong activation of caspase at 4hrs in #266R model that decreased at 24hrs; a slight induction after combination treatment at both time points was observed (Figure 3B). In #315 model all the treatments at both time points, except ONVA 4hr caused a slight increase in caspase activation (Figure 3B).
- Pharmacodynamics assessments of DNA damage pathway are ongoing.

Conclusions

Combinations of the PLK1 inhibitor onvansertib with carboplatin or gemcitabine were very active in both in vitro and in vivo models of platinum-resistant ovarian carcinoma.

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