# Selinexor, a Selective Inhibitor of Nuclear Export (SINE) Compound, Shows Enhanced Anti-Tumor Activity in Combination with the PARP Inhibitor, Olaparib, in Models of Triple Negative Breast Cancer (TNBC)

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## ABSTRACT

**Background**: Selinexor is a SINE (Selective Inhibitor of Nuclear Export) compound currently in Phase I and II clinical trails for the treatment of hematological and solid malignancies. Selinexor induces cell death by blocking the key nuclear export protein XPO1 and forcing nuclear retention of tumor suppressor proteins (TSPs), including p53, BRCA1/2, pRB and FOXO3A. Recent work from our lab suggests that selinexor inhibits DNA damage repair by inhibiting Chk1 and Rad51 expression. Olaparib is an FDA approved therapy for BRCA1/2 mutated ovarian cancer, which inhibits Poly-ADP-Ribose Polymerase (PARP) and prevents DNA damage repair. Furthermore, Olaparib is being evaluated for the treatment of Triple Negative Breast Cancer (TNBC). We hypothesized that combination of selinexor and olaparib would enhance cancer cell death by accumulation of DNA damage that cannot be resolved in TNBC. We aimed to test the combination of selinexor and olaparib in TNBC harboring mutated or WT BRCA1 genes.

**Methods:** The effects of selinexor alone or in combination with olaparib were tested on a panel of 7 TNBC cell lines using MTT and soft-agar colony formation assays in parallel with FACS analysis. Combination index (CI) values were determined using the CompuSyn software and treatment was considered synergistic when CI<1. Comparative *in-vivo* efficacy of single-agent vs. combination therapy was evaluated in MDA-MB-468 (BRCA1 wild type, TNBC) and MDA-MB-436 (BRCA1 mut TNBC) xenograft models.

**Results:** The median $IC_{50}$ values for selinexor and olaparib were 1.88 μM (range: 0.27 μM to >10 μM) and 92.6 μM (range: 17.5 μM to >300 μM), respectively. Combination treatment led to synergistic inhibition of proliferation in the 7 TNBC cell lines evaluated. The median CI tested on the panel of cell lines was 0.68 (ranging from 0.4 to 0.96). FACS analysis revealed an additive effect of selinexor and olaparib combination on S-phase inhibition and G2 arrest in both BRCA1 mutated and BRCA1 wild type cells. Furthermore, Annexin V/PI staining showed an additive effect on TNBC cell apoptosis in all cell lines tested. In the MDA-MB-468 xenograft model, 98% tumor growth inhibition (TGI) was observed in the combination group compared to 77% and 81% TGI in single-agent selinexor and olaparib, respectively. This effect is additive by E-Bliss modeling. In contrast, in the MDA-MB-436 model of TNBC (BRCA1 mutant) the effect of the combination was synergistic, with 84% TGI in combination vs. 40% in selinexor and 16% in olaparib.

**Conclusion:** Selinexor and olaparib act additively or synergistically, to induce apoptosis in TNBC cells and robustly enhance anti-tumor effects in TNBC-derived xenograft models. These data provide a rational basis to support the study of selinexor-olaparib combination in clinical trials.

## INTRODUCTION

Olaparib is a PARP inhibitor approved for the treatment of BRCA mutant ovarian cancer. The BRCA1/2 mutant tumors are more sensitive to PARP inhibition due to synthetic lethality. Loss of BRCA1/2 in combination with inhibition of PARP results in accumulation of DNA damage that cannot be repaired leading to cell death.

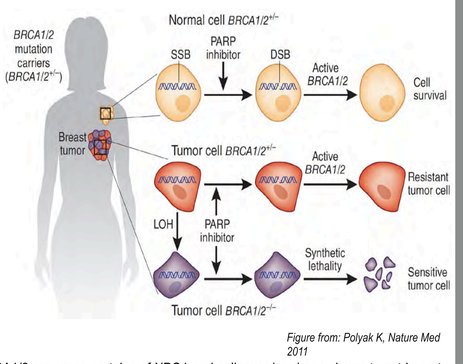
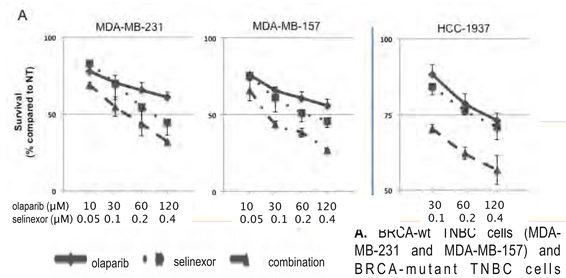



*Figure from: Polyak K, Nature Med 2011*

Considering that BRCA1/2 are cargo proteins of XPO1 and selinexor has been shown to act in part through inhibition of DNA damage repair, we hypothesized that combination of selinexor and olaparib would be synergistic in models of TNBC. The objectives of the current study were to determine whether selinexor would increase sensitivity of TNBC cells (BRCA1/2 WT/mutant) to olaparib and to compare the effect of combining olaparib and selinexor *in-vivo* in models of TNBC BRCA1/2 mutant and BRCA1/2 WT.

## RESULTS

### Selinexor Sensitizes *BRCA*-WT and *BRCA*-Mutant TNBC Cells to Olaparib

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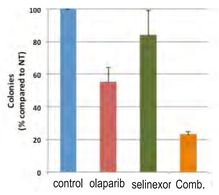
| | IC50 olaparib (μM) | IC50 selinexor (μM) | CI |
|---|---|---|---|
| HCC-1937 | >300 | >10 | 0.45 |
| MDA-MB-436 | 17.5 | 0.3 | 0.58 |
| MDA-MB-157 | 135 | 0.28 | 0.68 |
| MDA-MB-231 | 225 | 0.27 | 0.74 |
| MDA-MB-468 | 38.7 | 1.88 | 0.4 |
| BT-549 | 92.6 | 2.26 | 0.78 |
| MDA-MB-453 | 67 | 2.3 | 0.96 |
| **Median** | **92.6** | **1.88** | **0.68** |

**A.** BRCA-wt TNBC cells (MDA-MB-231 and MDA-MB-157) and BRCA-mutant TNBC cells (HCC-1937) were treated with olaparib and/or selinexor for 72 h. Survival was determined using the MTT assay. Data represent mean ± standard deviation (SD) of 3 experiments.

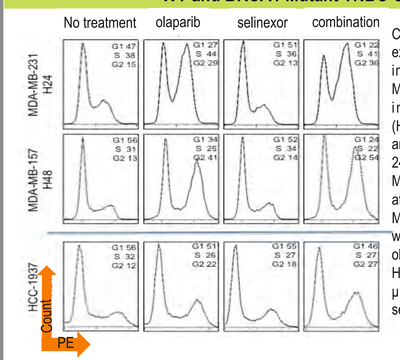
**B.** A panel of TNBC cells were treated with olaparib and/or selinexor. $IC_{50}$ for each drug was determined using the MTT assay after 72 h and synergism was determined by calculating the combination index (CI) using the Chou-Talalay method. CI <1 is considered synergistic.

### Selinexor-Olaparib Combination Inhibits Colony Formation of TNBC Cells Compared to Single Agents



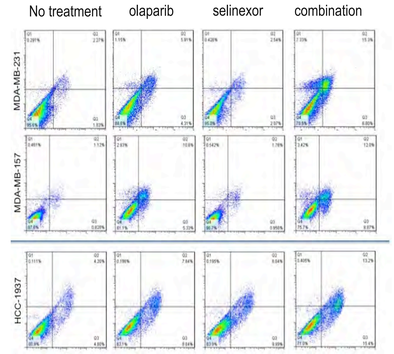

MDA-MB-157 cells were treated with Olaparib (4 μM) and/or Selinexor (0.1 μM) for 15 days in a soft agar colony formation assay. Results demonstrate enhanced inhibition of colony formation in the combination group. Data represent mean % of colonies relative to control ± SD. Two separate experiments were performed, each in triplicate.

### Selinexor-olaparib Combination Induces G2 Cell Cycle Arrest in BRCA1 WT and BRCA1 Mutant TNBC Cells



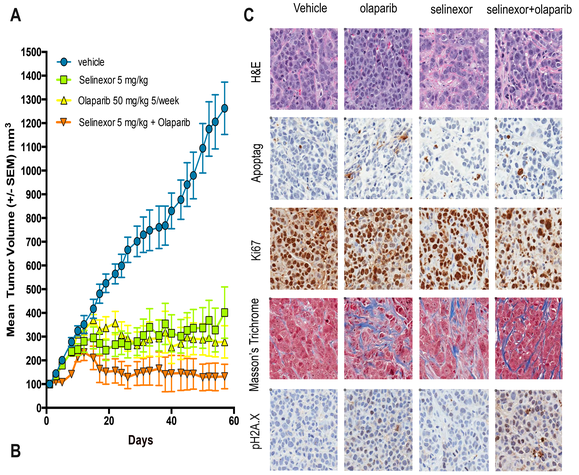

Cell cycle analysis following exposure to olaparib ± selinexor in BRCA1-wt cells (MDA-MB-231 and MDA-MB-157) and in BRCA1-mutated cells (HCC-1937). Induction of G2-arrest was observed as early as 24 h after treatment in MDA-MB-231 and HCC-1937, and after 48 h in MDA-MB-157.
MDA-MB-231 and MDA-MB-157 were treated with 10 μM olaparib and 0.05 μM selinexor; HCC-1937 were treated with 30 μM olaparib and 0.8 μM selinexor.

### Combination of Selinexor and Olaparib Has Additive Effects on TNBC Cell Apoptosis in Both Wt and Mutant BRCA1




BRCA1-wt cells (MDA-MB-231 and MDA-MB-157) were treated with 30 μM olaparib and/or 0.05 μM selinexor for 72 h. Apoptotic cells were quantified after staining with PI and Annexin V. BRCA1-mutated cells (HCC-1937) were treated with 30 μM olaparib +/- 0.4 μM selinexor.

### Additive Effect of Selinexor-Olaparib Combination on Tumor Growth of MDA-MB-468 Xenografts

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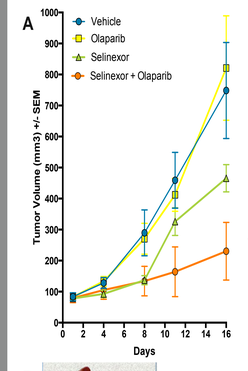
(A) Tumor growth of engrafted MDA-MB-468 cells in immunodeficient mice. Tumor growth inhibition (TGI) of selinexor alone at suboptimal dose of 5 mg/kg, 3 times a week was 77%. Olaparib at 50 mg/kg 5X/wkly showed 81% TGI. Combination of selinexor and olaparib showed 98% TGI and the combination was additive (E-bliss).
(B) Resected Tumors (day 59)
(C) Immunohistochemistry and H&E: Pleomorphic nuclei and cytoplasm observed to similar extent in all sections. Scattered necrotic individual cells and mitotic figures throughout the sections. Apoptag: Scattered apoptotic cells in vehicle and all treatment groups. Ki67: Reduced number of positive cells in selinexor-olaparib treatment group compared to vehicle and single agent-treated groups. Mason's Trichrome: Increase in mature collagen (dark blue) in all treatment groups compared to vehicle. pH2A.X: Increased number of positive cells in the selinexor-olaparib treatment group compared to single agent-treated groups and no staining with vehicle.

### Synergistic Effect of Selinexor–Olaparib Combination on Tumor Growth of MDA-MB-436-derived Xenografts (BRCA1 Mutated)

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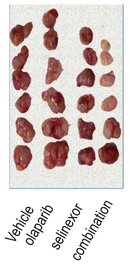
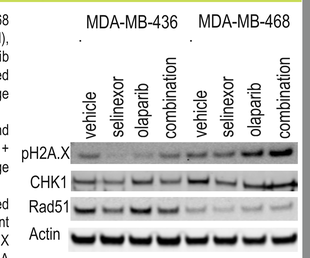



(A) Tumor growth of MDA-MB-436 xenografts in immunodeficient mice. Tumor growth inhibition (TGI) of selinexor alone at suboptimal dose of 7.5 mg/kg, 3 times a week was 40%. Olaparib at 50 mg/kg 5 times a week showed TGI of 16%. Combination of selinexor and olaparib showed 84% TGI and the combination is synergistic (E-bliss).
(B) Resected tumors (day 16)
(C) Immunohistochemistry, H&E: large, pale cells in all treated groups vs. small, dark cells in vehicle. Apoptag: increased positive staining in selinexor-olaparib treatment group compared to single agent-treated groups. Ki67 reduced number of positive cells in selinexor-olaparib treatment group compared to vehicle and single agent-treated groups.

### Selinexor Inhibits DNA Damage Response in MDA-MB-436 Cells

TNBC cell lines MDA-MB-436 and MDA-MB-468 were treated with either vehicle, selinexor (1 μM), olaparib (30 μM), or selinexor + olaparib combination for 24 h. Cell lysates were blotted with antibodies to detect changes in DNA damage response.
MDA-MB-436 showed inhibition of Chk1 and Rad51 expression in cells treated with selinexor + olaparib, suggesting inhibition of the DNA damage response.
DNA damage response proteins were not altered post- selinexor + olaparib combination treatment in MDA-MB-468. However, an increase in pH2A.X expression, suggests an overall increase in DNA damage.




## CONCLUSIONS

- Combination of selinexor and olaparib induces robust anti-tumor activity *in-vitro* and *in-vivo*.
- Selinexor–olaparib combination has led to an additive effect on tumor growth inhibition in MDA-MB-468 xenografts (BRCA1 wt TNBC). The combination showed robust synergistic effect in xenograft model of MDA-MB-436 (BRCA1 mutant cells). Mechanisms leading to the different effects of the combination in the two models are yet to be explored.
- Our pre-clinical data support further investigation of selinexor and olaparib therapy for BRCA1/2 mutant and BRCA1/2 wt TNBC.

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