TTI-621 (SIRPαFc), an Immune Checkpoint Inhibitor Blocking the CD47 "Do Not Eat" Signal, Enhances the Anti-Tumor Effect of Radiation and Targeted Therapy in Ovarian Cancer Models

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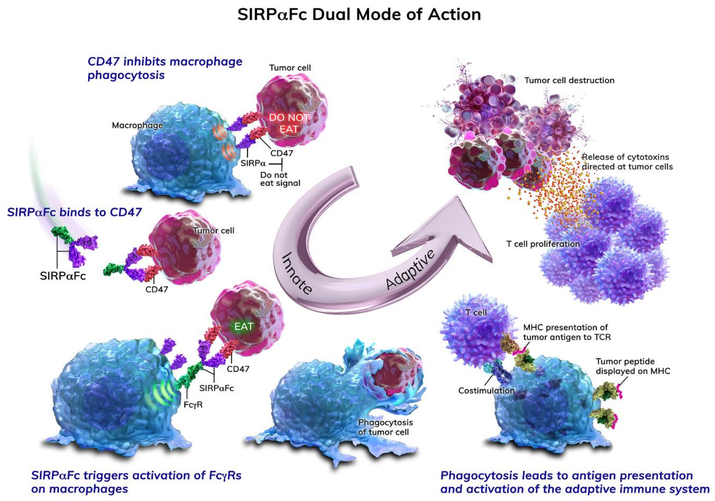
Introduction

- Ovarian cancer (OVCa) is the most lethal gynecologic malignancy. With standard treatment demonstrating a high relapse rate, novel treatment strategies are needed.
- PARP inhibitors are approved as monotherapy agents for BRCA mutated OVCa, and function as radiosensitizers. Both radiotherapy (RT) and PARP inhibitors induce immunogenic cell death, release tumor antigens, and enhance the infiltration of immune cells, including macrophages, to tumor sites. Thus, innate checkpoint inhibition may enhance the anti-tumor effect of DNA damaging agents.
- CD47 is an immune checkpoint that binds signal regulatory protein alpha (SIRPα) and delivers a "do not eat" signal to suppress macrophage phagocytosis. It is frequently overexpressed by tumors to evade macrophage mediated destruction.
- TTI-621 (SIRPαFc), an immune checkpoint inhibitor consisting of the CD47 binding domain of human SIRPα linked to the Fc region of IgG1, blocks the CD47 "do not eat" signal and engages macrophages' Fcγ receptors, thereby enhancing phagocytosis and antitumor activity.
- TTI-621 enables macrophage-mediated killing of tumor cells *in vitro* and *in vivo* and is being evaluated in two clinical studies (NCT02890368 and NCT02663518).
- Here we report the efficacy of the combination of TTI-621 and DNA damaging therapeutics, RT and PARP inhibition, in BRCA1 competent and knock-down OVCa xenografts.

TTI-621 (SIRPaFc): A Novel CD47 Blocking Agent to Promote Innate and Adaptive Anti-Tumor Immunity



TTI-621 Inhibits Tumor Growth in Ovarian Cancer Xenografts

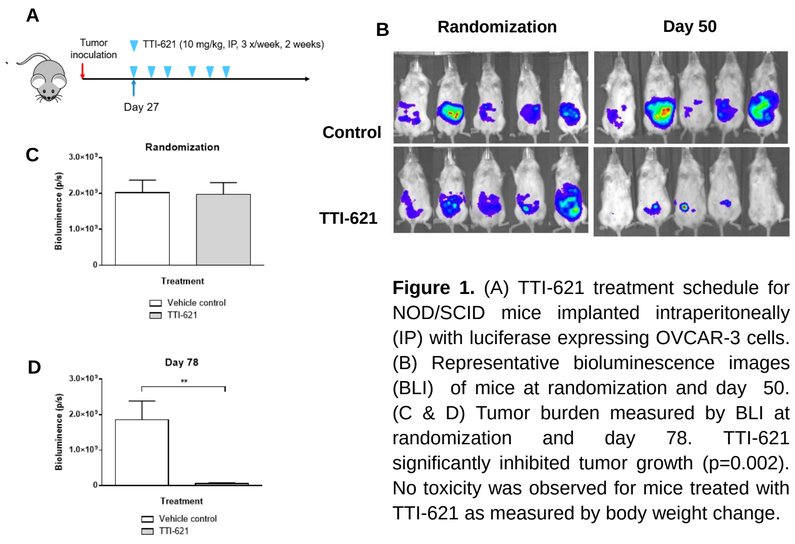


Figure 1. (A) TTI-621 treatment schedule for NOD/SCID mice implanted intraperitoneally (IP) with luciferase expressing OVCAR-3 cells. (B) Representative bioluminescence images (BLI) of mice at randomization and day 50. (C & D) Tumor burden measured by BLI at randomization and day 78. TTI-621 significantly inhibited tumor growth (p=0.002). No toxicity was observed for mice treated with TTI-621 as measured by body weight change.

TTI-621 Improves Tumor Control and Survival in SKOV-3 Xenografts

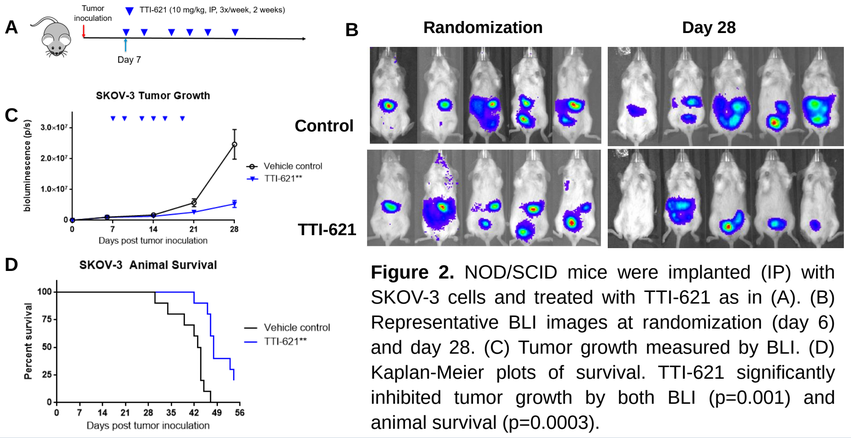


Figure 2. NOD/SCID mice were implanted (IP) with SKOV-3 cells and treated with TTI-621 as in (A). (B) Representative BLI images at randomization (day 6) and day 28. (C) Tumor growth measured by BLI. (D) Kaplan-Meier plots of survival. TTI-621 significantly inhibited tumor growth by both BLI (p=0.001) and animal survival (p=0.0003).

BRCA1 Knock Down in SKOV-3 Cells

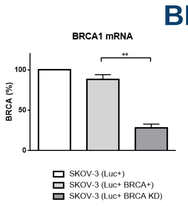


Figure 3. mRNA levels of BRCA1 in SKOV-3 (Luc+) cells. BRCA1 was knocked down using lentiviral shRNA constructs (gene target sequence GAGAGTGCTTGGGA-TCGAT). Control shRNA was used to generate a BRCA1 competent cell line (Luc+ BRCA+). BRCA1 knock-down cells (Luc+ BRCA KD) have significantly lower BRCA1 expression compared to SKOV-3 (Luc+ BRCA1+) (p=0.0016).

TTI-621 Enhances the Anti-Tumor Effects of PARP Inhibition in a SKOV-3 BRCA1 Knock Down Tumor Model

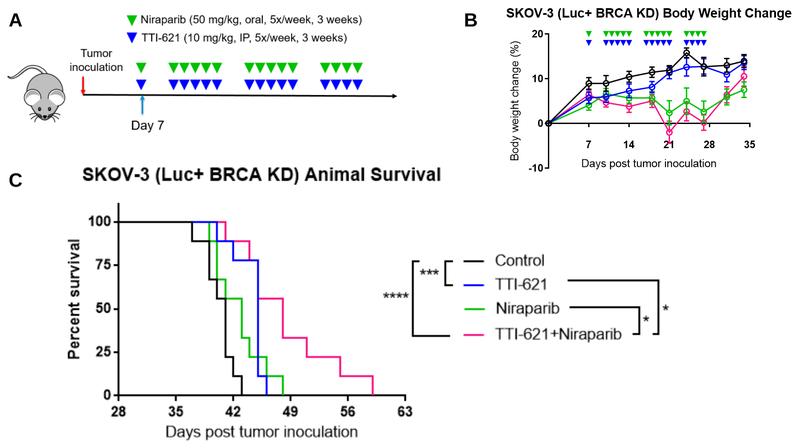
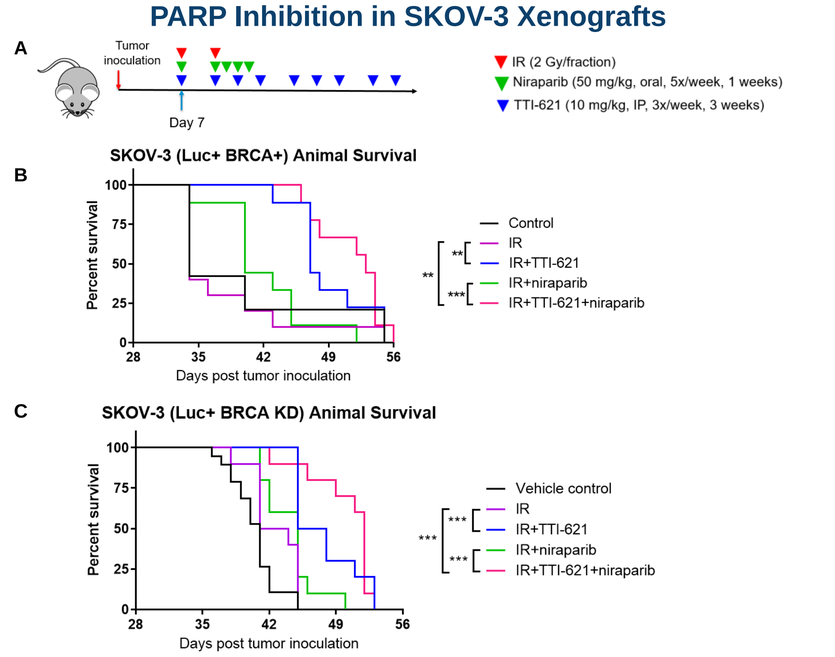


Figure 4. (A) NOD/SCID mice implanted (IP) with SKOV-3 (Luc+BRCA1 KD) and treated with TTI-621, niraparib, or TTI-621+niraparib. (B) Body weight change of animals. (C) TTI-621 significantly inhibited tumor growth (***, p≤0.001) compared to vehicle control. TTI-621 + niraparib is superior to either monotherapy (p = 0.0436 compared to TTI-621 alone, and p = 0.0105 compared to niraparib alone).

TTI-621 Enhances the Anti-Tumor Effects of Radiation and PARP Inhibition in SKOV-3 Xenografts



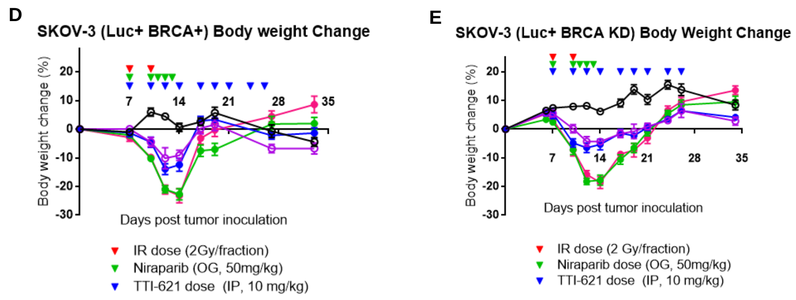


Figure 5. (A) Mice bearing SKOV-3 (Luc+ BRCA+) or SKOV-3 (Luc+ BRCA KD) tumors were treated with whole abdomen ionizing radiation (IR), IR+TTI-621, IR+niraparib, or IR+TTI-621+niraparib starting on day 7. (B & C) Survival of animals. (D & E) Body weight change of animals following treatment. In both tumor models, TTI-621 significantly enhanced the efficacy of IR (p = 0.0085 in SKOV-3 (Luc+ BRCA+) and p = 0.0009 in SKOV-3 (Luc+ BRCA KD). The triple combination of IR+TTI+niraparib significantly prolonged animal survival compared to IR+niraparib (p = 0.0004). No chronic toxicity was observed for all treatment as assessed by body weight.

TTI-621 and Niraparib Enhance the Efficacy of Radiotherapy via Distinct Mechanisms

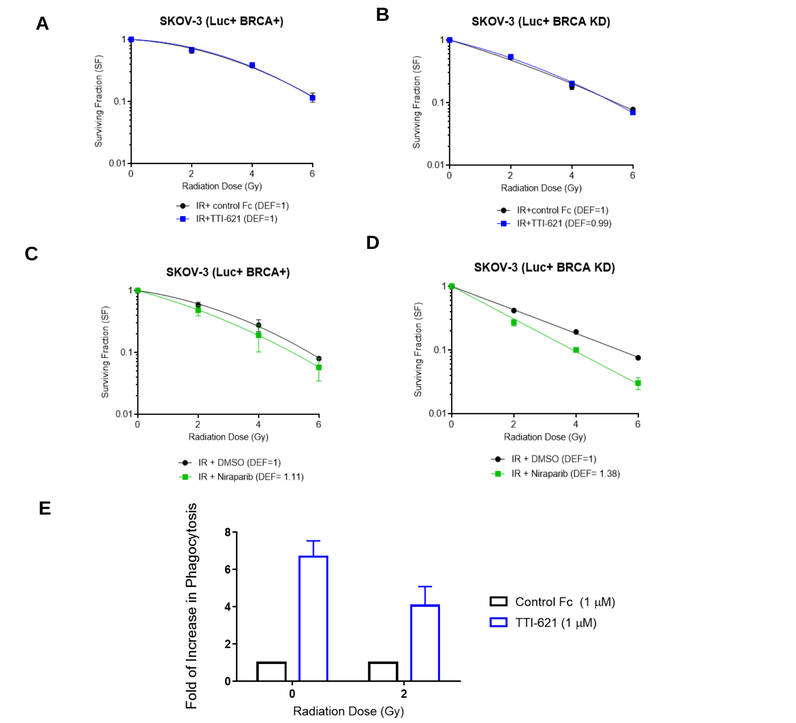


Figure 6. Radiation dose response curve of SKOV (Luc+ BRCA+) and SKOV (Luc+ BRCA KD) cells following treatment with TTI-621 or control Fc (A,B) or niraparib (C,D). A greater radiosensitizing effect of niraparib was observed in SKOV-3 (Luc+ BRCA KD) cells, with a larger dose enhancement factors (DEF) value of 1.38 compared to that of SKOV-3 (Luc+ BRCA+) cells. TTI-621 did not directly increase the cytotoxicity of IR in both cells. (E) Following IR, PKH67 labelled SKOV-3 cells were co-cultured with IFN-γ primed macrophages in the presence of control Fc or TTI-621. TTI-621 enhanced macrophage mediated phagocytosis of both irradiated and non-irradiated SKOV-3 cells.

Conclusions

- TTI-621 monotherapy significantly inhibits the growth of ovarian cancer in xenograft models.
- TTI-621 enhances the anti-tumor effect of PARP inhibition in a BRCA1 knockdown SKOV-3 model.
- The combination of TTI-621 and radiation leads to prolonged survival of animals bearing BRCA1 competent or knockdown tumors. The triple combination of TTI-621+IR+PARP inhibition further enhances treatment efficacy.
- TTI-621 and niraparib enhance the efficacy of radiotherapy via distinct mechanisms.
- These *in vivo* studies suggest that the combination of radiation and CD47 blockade with TTI-621 may enhance tumor control. Patients with BRCA1 mutated tumors may benefit from the combination of TTI-621 with niraparib.
- In addition, triple modality therapy with TTI-621, niraparib and RT may be employed to optimize treatment outcomes for both BRCA-competent and mutated patients.