Combination of poly ADP-ribose polymerase (PARP) inhibitor with a T cell receptor β chain-directed antibody fusion molecule in immune-excluded prostate cancer models

Ginette S. Santiago-Sanchez[1], Francesca Rosato[1], Kellsye P. Fabian[1], Michelle R. Padget[1], Jung-Min Lee[3], Fatima Karzai[4], Jeffrey Schlom[1], James L. Gulley[1], Duane H. Hamilton[1], Jonelle K. Lee[1], Andrew Bayliffe[2], Zhen Su[2], Jacques Moisan[2], Madan Katragadda[2], James W. Hodge[1]

[1]Center for Immuno-Oncology, Center for Cancer Research (CCR), National Cancer Institute (NCI), National Institutes of Health (NIH), Bethesda, MD. [2]Marengo Therapeutics, Cambridge, MA [3]Women's Malignancies Branch, CCR, NCI, NIH, Bethesda, MD. [4] Genitourinary Malignancies Branch, CCR, NCI, NIH, Bethesda, MD

Abstract

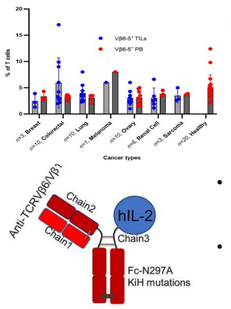
Background. Metastatic castration-resistant prostate cancer (mCRPC) is an aggressive disease with limited response to systemic therapy despite testosterone suppression. STAR0602 is a selective, bifunctional T cell agonist composed of an antibody targeting Vβ6 and Vβ10 T cell receptors (TCRs) fused to human interleukin-2, that selectively expands specific Vβ6$^+$ CD8$^+$ memory T cells and has shown clinical activity as a monotherapy in anti-PDL-1 resistant tumors (NCT05592626). Here, we studied the combination of mSTAR1302, the murine surrogate of STAR0602, with the PARP inhibitor olaparib in immune-excluded prostate cancer models.

Methods. TRAMP-C2 and RM-1, immunologically "cold" murine prostate cancer models, were used to assess antitumor activity and survival benefit after combination therapy treatment. Depletion studies were performed to determine the requirement of a subset of immune cells (natural killer (NK) cells, CD4$^+$, CD8$^+$, and Vβ13$^+$ T cells) or interferon (IFN)-γ for the therapeutic efficacy of combination therapy with olaparib and mSTAR1302. Flow cytometry and RNA expression analyses were performed on tumors and spleens to assess the immune response.

Results. Combination therapy with olaparib and mSTAR1302 elicited significant tumor regression of TRAMP-C2 and RM-1 tumors and improved survival compared to either mSTAR1302 or olaparib alone. Combination therapy with olaparib and mSTAR1302 significantly increased the frequency of tumor-infiltrating lymphocytes (TILs), expanded activated Vβ13$^+$ CD4$^+$ and Vβ13$^+$ CD8$^+$ T cells, decreased immunosuppressive cells, and increased the CD8$^+$ T cell population with stem cell-like properties. Depletion studies demonstrated that Vβ13$^+$ CD4$^+$, Vβ13$^+$CD8$^+$, and NK cells, as well as IFN-γ are required for the antitumor efficacy of combination therapy with olaparib and mSTAR1302. A TRAIL-R2 knockout TRAMP-C2 model demonstrated the critical role of TRAIL-R2 in antitumor efficacy.

Conclusions. In summary, these data support the rationale for a planned clinical trial with olaparib and STAR0602 for mCRPC patients who have progressed on androgen deprivation therapy.

Introduction



Prevalence of Vβ6 TCR T cells in TILs and PBMCs from cancer patients.

STAR0602

- This antibody targets the germline variable Vβ6 and Vβ10 TCRs fused to human interleukin-2 (IL-2).
- Simultaneously engages a nonclonal mode of TCR activation with costimulation to promote activation and expansion of Vβ TCR chains.

Modified from Hsu et al., Sci.Transl.Med. **15**, eadi0258 (2023)

Results

Combination therapy with olaparib and mSTAR1302 induces tumor regression in murine "cold" prostate cancer models

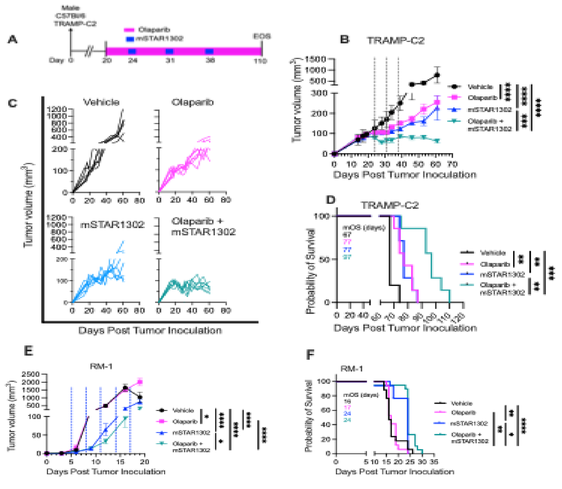


Figure 1. (**A**) Treatment schedule for TRAMP-C2 tumor-bearing male C57BL/6 mice. Starting on day 20, when average tumor volume was between (90-100) mm^3, olaparib treatment (50 mg/Kg once daily) was given intraperitoneally (I.P.). Three doses of mSTAR1302 were given starting on day 24 (1 mg/Kg once weekly) I.P (gray dashed line). (**B**) Tumor growth curve for the TRAMP-C2 tumor model, (**C**) individual tumor growth curves, and (**D**) Survival curve with inset showing median overall survival (mOS) in days. For the RM-1 tumor model, olaparib treatment started at day 3 when the average tumor volume was between 20-50 mm^3 and was given daily until the end of the study (EOS). The first mSTAR1302 dose (blue dashed line; 1 mg/Kg) started at day 5 and given 3 days apart until day 17. Plots showing (**E**) tumor growth curve and (**F**) survival curve for the RM-1 model with inset showing mOS in days. For survival, significance was measured by the Log-Rank Mantel-Cox test. Tumor growth curve data are presented as mean ± SEM and Two-way ANOVA test for group comparison.

Results

Combination therapy with olaparib and mSTAR1302 induces lymphocyte infiltration and a differential gene signaling promoting an activated and cytotoxic T cell phenotype

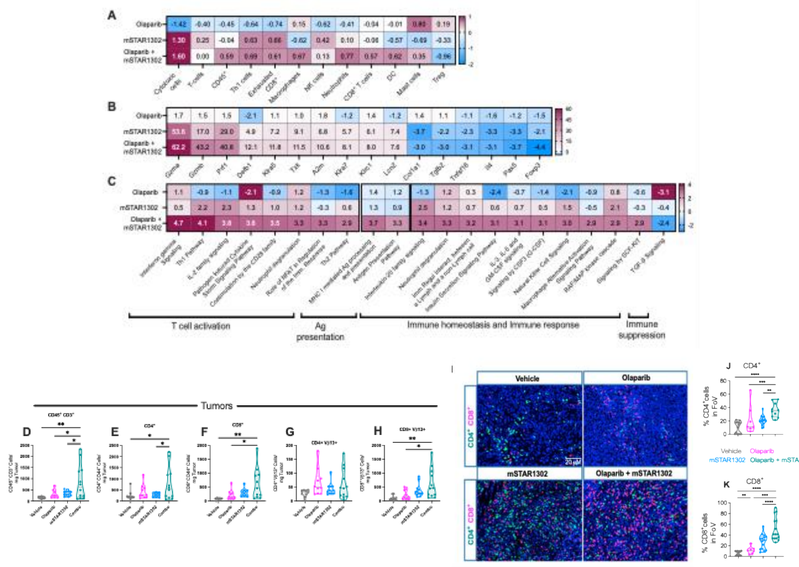


Figure 2. On day 27, as depicted in Fig. 1A, tumors from TRAMP-C2 tumor-bearing male C57Bl/6 mice were harvested and individually processed for gene expression analysis using the NanoString Murine PanCancer Immune Profiling Panel. Heatmaps showing (**A**) cell type profiles based on cell type z-score, (**B**) top 10 upregulated and downregulated transcripts in all treatment groups compared to the vehicle control based on fold-change, and (**C**) pathway enrichment analysis based on z-score. Flow analysis for (**D**) CD45$^+$ CD3$^+$, (**E**) CD4$^+$, (**F**) CD8$^+$, (**G**) CD4$^+$ Vβ13$^+$, and (**H**) CD8$^+$Vβ13+ TILs. At the same time point, tumor tissues were stained for (**I**) CD4$^+$ (teal) and CD8$^+$ T cells (pink) infiltrates and their respective quantifications (**J,K**). FoV: Field of view.

Activated cytotoxic T cells and NK cells are sustained in the periphery by combination therapy with olaparib and mSTAR1302

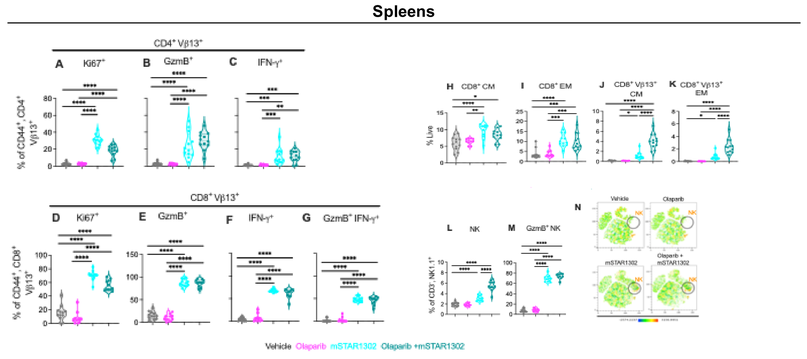


Figure 3. On day 27, as depicted in Fig. 1A, spleens from TRAMP-C2 tumor-bearing male C57BL/6 mice were isolated and stained for flow analysis for the expression of (**A**) Ki67$^+$, (**B**) GzmB$^+$,(**C**) IFN-γ$^+$ on CD4$^+$ Vβ13$^+$ T cells and (**D**) Ki67$^+$, (**E**) Granzyme B (GzmB)$^+$, (**F**) IFN-γ$^+$, (**G**) GzmB$^+$ IFN-γ$^+$ on CD8$^+$ Vβ13$^+$ T cells. Flow analysis was also performed to determine (**H**) central memory (CM), (**I**) effector memory (EM) CD8$^+$ T cells, (**J**) CM, (**K**) EM CD8$^+$ Vβ13$^+$ T cells, (**L**) NK cells, and (**M**) NK GzmB$^+$ cells. (**N**) t-SNE projections of CD45$^+$ cells (138,000 cells/treatment group) depicting the NK population (orange). One-way ANOVA was used to assess significance.

Combination therapy with olaparib and mSTAR1302 elicits a reduction of immune-suppressive cells and the expansion of a cytotoxic T cell pool

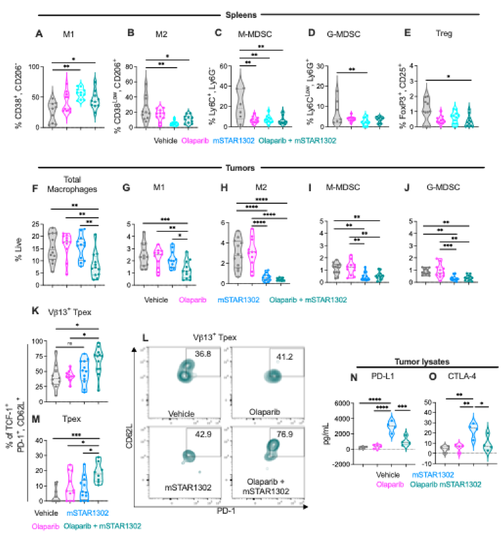


Figure 4. On day 27, as depicted in Fig. 1A, spleens from TRAMP-C2 tumor-bearing male C57BL/6 mice were isolated and stained for flow analysis for (**A**) M1 and (**B**) M2 macrophages, (**C**) monocyte-myeloid-derived suppressor cells (M-MDSC), (**D**) granulocytic myeloid-derived suppressor cells (G-MDSC), and (**E**) regulatory T cells (Tregs). Tumors digested were stained for (**F**) total macrophages, (**G**) M1, (**H**) M2 (**I**) M-MDSC, (**J**) G-MDSC, (**K-L**) CD8$^+$ Vβ13$^+$ progenitor exhausted T cells (Tpex) (programmed cell death protein 1 (PD-1)$^+$, TCF-1$^+$, CD62L$^+$),) and (**M**) CD8$^+$ Tpex. O-link proteomic analysis was performed on tumor lysates to quantify soluble (**N**) programmed death ligand 1 (PD-L1; CD274) and (**O**) cytotoxic T-lymphocyte-associated protein 4 (CTLA-4). One-way ANOVA assessed significance.

CD4$^+$ Vβ13$^+$, CD8$^+$ Vβ13$^+$, and NK cells are required to drive antitumor and antigen cascade response elicited by combination therapy

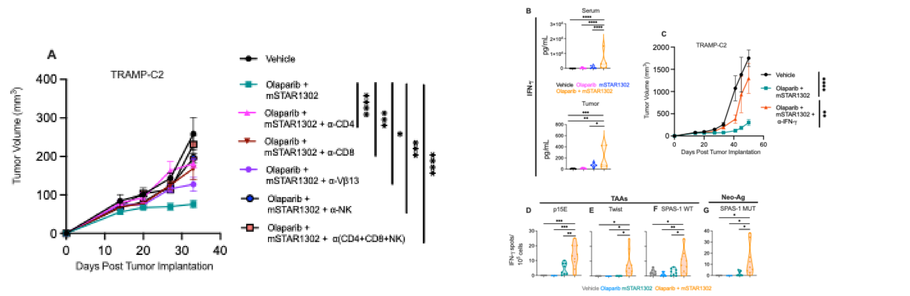


Figure 5. Depletion studies: TRAMP-C2 tumor-bearing mice were treated with α-CD4, α-CD8, and α-NK depleting antibodies (100 µg/mouse) before giving any treatments. (**A**) Tumor growth curve showing mice treated with vehicle, combination therapy with olaparib and mSTAR1302, and combination therapy plus CD4$^+$, CD8$^+$, Vβ13$^+$, and NK depleting antibodies. (**B**) Quantification of IFN-γ levels in serum and tumor lysates of treated mice, 3 days after the first mSTAR1302 dose and after 5 consecutive days of olaparib. (**C**) Tumor growth curve showing mice treated with vehicle, combination therapy, and combination therapy plus an α-IFN-γ depleting antibody. IFN-© ELISpot showing mouse splenocytes stimulated with one of the following H-2D^b or H-2K^b restricted peptides: (**D**) P15e, (**E**) Twist, (**F**) stimulator of prostatic adenocarcinoma-specific T cells-1 wild-type (SPAS-1 WT), and (**G**) SPAS-1 mutant (MUT).

Upregulation of TRAIL-R2 on TRAMP-C2 cells by olaparib is critical for antitumor efficacy

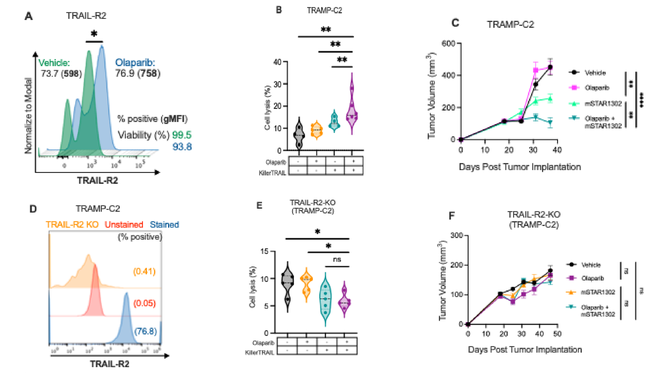


Figure 6. (**A**) Flow cytometry plot showing phenotypic changes on TRAIL-R2 expression after *in vitro* incubation of TRAMP-C2 cells with olaparib (20 µM) (light blue) and untreated cells (green) for 72 hours. (**B**) Violin plots showing % lysis of olaparib-pretreated TRAMP-C2 cells upon incubation with soluble murine, recombinant SuperKillerTRAIL. (**C**) Tumor growth curves of TRAMP-C2 tumor-bearing mice treated with vehicle, mSTAR1302, and combination therapy with olaparib and mSTAR1302. (**D**) Flow cytometry plot showing TRAIL-R2 knockout (KO) TRAMP-C2 cells. (**E**) Violin plots showing % lysis of olaparib-pretreated TRAIL-R2-KO TRAMP-C2 cells upon incubation with SuperKillerTRAIL. (**F**) Tumor growth curve for TRAIL-R2 KO TRAMP-C2 cells *in vivo*.

TRAIL-R2 has low expression in ovarian and prostate cancers compared to healthy tissue, but is induced by olaparib in ovarian cancer patients

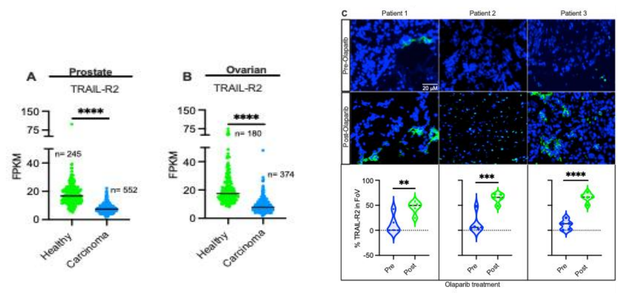


Figure 7. Plots showing TRAIL-R2 expression in **(A)** healthy prostate tissue (Genotype-Tissue Expression (GTEx) dataset) versus prostate carcinomas (The Cancer Genome Atlas Program (TCGA) dataset) and **(B)** healthy ovarian tissue (GTEx dataset) versus ovarian carcinoma (TCGA dataset). Percutaneous biopsies of ovarian tumor tissues from three patients enrolled in the phase 2 clinical trial NCT02484404 were obtained by an interventional radiologist before treatment and on day 15 of cycle one of olaparib treatment. **(C)** TRAIL-R2 expression was determined by immunofluorescence and quantified in paired fresh biopsies from the three patients enrolled in this trial.

Conclusion / Future directions

- Vβ13$^+$ CD4$^+$ and Vβ13$^+$ CD8$^+$ T cells, and NK cells are required for tumor regression in the TRAMP-C2 mouse model.
- Olaparib upregulates TRAIL-R2 on TRAMP-C2 cells, sensitizing them to killing.
- Data support the rationale to develop a clinical trial with olaparib and STAR0602 for mCRPC patients who have progressed on androgen deprivation therapy.