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proliferative response of effector T cells (29, 33). To evaluate whether checkpoint blockade modified the phenotype or functional status of T cells in the BRCA1$^-$ tumor environment *in vivo*, we subjected peritoneal T cells from treated mice to flow-cytometric analysis. In these experiments, BRCA1$^-$ tumor–bearing animals were treated with antibodies to CTLA-4 or PD-1 as monotherapy or combined with PARP inhibition (20 mg/kg/day). CTLA-4 or PD-1 antibody was administered on day 4 after tumor challenge. In addition, a second dose of PD-1 was administered on day 11 because PD-1 signaling is thought to affect peripheral T-cell exhaustion, a late event in the establishment of antitumor immunity. Results from these experiments demonstrated a marked increase in the proportion of CD8$^+$ cells with an effector/memory phenotype in mice receiving CTLA-4 antibody together with the PARP inhibitor (Fig. 2A). This effect was specifically observed among T cells in the peritoneal tumor environment, and not in the spleen (Fig. 2B). In contrast, despite evidence that ovarian tumors express PD-L1 (34, 35), and that tumor-infiltrating lymphocytes upregulate PD-1, blockade of the PD-1–PD-L1 pathway did not significantly increase the proportion of effector/memory cells in this model (Fig. 2A and B).

To determine whether changes in T-cell phenotype following checkpoint blockade in combination with PARP inhibition were associated with increases in local levels of IFNγ or TNFα, we evaluated peritoneal T cells retrieved on days 7, 14, and 21 for intracellular cytokine production by flow cytometry. Cells were restimulated *ex vivo* for 5 hours with PMA and ionomycin in the presence of Golgi transport inhibitors, and then CD4$^+$ and CD8$^+$ T cells were analyzed for IFNγ, and TNFα cytokine production. In these experiments, neither monotherapy with CTLA-4 or PD-1 blockade alone nor PD-1 blockade together with PARP inhibition significantly increased cytokine production by CD4$^+$ or CD8$^+$ T cells in peritoneal samples (Fig. 2C). In contrast, combination therapy with the PARP inhibitor and CTLA-4 antibody did significantly boost both IFNγ and TNFα production in CD4$^+$ and CD8$^+$ T cells in the peritoneal tumor environment on day 21. The percentage of polyfunctional peritoneal CD8$^+$ T cells producing both IFNγ and TNFα was also significantly increased in mice receiving CTLA-4 antibody and PARP inhibitor combined therapy. Like the increase in effector/memory cells, these changes in cytokine production were evident in the peritoneal tumor environment, but similar effects were not seen among splenic T cells (Fig. 2D). From these data, we conclude that CTLA-4 blockade, but not inhibition of PD-1 signaling, induces a Th1 effector phenotype among T cells in the peritoneal tumor environment when combined with PARP inhibition in a BRCA1$^-$ ovarian cancer model.

Increases in IFNγ production in response to combined CTLA-4 blockade and PARP inhibition *in vivo* are sufficient to enhance tumor cell cytotoxicity

Our results demonstrate that combined therapy with the PARP inhibitor and CTLA-4 antibody enhances IFNγ and TNFα production by peritoneal T cells in response to TCR-independent stimulation with PMA and ionomycin. In order to quantify production of these cytokines specifically in response to TCR engagement by peritoneal T cells, we cultured day 21 peritoneal cells with anti-CD3 antibody for 18 hours in culture. In this manner, only T cells were stimulated to express effector cytokines, allowing direct quantification of IFNγ and TNFα (35). The results of this experiment demonstrated that both IFNγ and TNFα levels were highest following anti-CD3 treatment of peritoneal cells from mice receiving combined treatment with the PARP inhibitor and CTLA-4 antibody, with a greater than 20-fold increase in IFNγ and an approximately 5-fold increase in TNFα compared with controls (Fig. 3A and B).

To determine whether high cytokine levels in the peritoneal tumor environment could induce a cytotoxic effect similar to that seen using recombinant cytokines *in vitro* (Fig. 1), tumor cells were cultured with cell-free supernatant retrieved from the T-cell stimulation experiments described above in the presence of increasing concentrations of the PARP inhibitor. As expected, the supernatant from restimulated T cells harvested from BRCA1$^-$ tumor–bearing mice receiving combination therapy had the greatest effect on BRCA1$^-$ tumor cell death when combined with the PARP inhibitor *in vitro* (Fig. 3C and D, black lines). Supporting a role for IFNγ and TNFα in this effect, cell death in response to supernatant from T cells retrieved from mice receiving combination therapy was attenuated when neutralizing antibodies to IFNγ or TNFα were added to cell cultures (Fig. 3C and D, red lines). As seen with recombinant cytokines *in vitro*, this effect was restricted to BRCA1$^-$ tumor cells; BRCA*wt* cells cultured under the same conditions showed no evidence of cytotoxicity (Fig. 3E). Together, these data indicate that combination therapy with the PARP inhibitor and CLTA-4 antibody promotes a strong Th1 T-cell response in the peritoneal tumor environment that results in local increases in effector cytokines that are sufficient to enhance BRCA1$^-$ tumor clearance *in vivo*.

Figure 1.

Th1-type cytokines enhance the cytotoxic effect of PARP inhibition in BRCA1$^-$ ovarian cancer cells. A, BRCA1$^-$ (BR5-Akt) and BRCA*wt* (T22, ID8) cell lines were cultured in 24-well plates at a starting concentration of 1×10^4 cells per well with or without the PARP inhibitor (PARPi) at the indicated dose, and analyzed at 72 hours for cell viability by flow cytometry. B, peritoneal cells were harvested by PBS wash on day 7 from BRCA1$^-$ tumor–bearing mice treated with the PARPi (40 mg/kg/day; days 3–6) and analyzed by flow cytometry for the percentage of CD45$^-$ tumor cells (left). In addition, total ascites cells were plated at serial dilutions for 72 hours, and the number of tumor colonies among total ascites cells was counted as an estimate of viable tumor burden (right). (Left, $P = 0.0271$; right, $P = 0.0032$, Student *t* test.) C and D, BRCA1$^-$ cells were cultured in 24-well plates for 72 hours in the presence of 0, 0.5, 1, or 2 μg/mL of PARPi and either IFNγ (C, top, PARPi dose effect, $P < 0.0001$; IFNγ dose effect, $P < 0.0001$; interaction, $P < 0.0001$) or TNFα (D, top, PARPi dose effect, $P < 0.0001$; TNFα dose effect, $P < 0.0001$, interaction, $P < 0.0001$) at the concentrations indicated. Cells were then stained with a fixable cell viability dye and analyzed by flow cytometry for the percentage of dead cells. Top dose-response curves show the interaction dose effect between cytokine concentration and PARP inhibitor treatment. Bottom bar graphs illustrate the dose effect independent of interaction. E and F, BRCAwt (T22) cells were assayed for viability as in C and D and show no treatment effect with increasing concentrations of cytokines or the PARP inhibitor. G and H, the fixable viability dye allowed for analysis of intracellular staining for active caspase-3. BRCA$^-$ cells treated with PARPi and IFNγ (G) or TNFα (H) were intracellularly stained and cells positive for active caspase-3 are presented as a percentage of total cells (PARPi dose effect, $P < 0.0001$; IFNγ dose effect, $P < 0.0001$; interaction, $P < 0.0001$). Assays were repeated a minimum of three times. *, $P < 0.05$; **, $P < 0.025$; ***, $P < 0.005$; ****, $P < 0.0001$ by ANOVA and Tukey procedure for multiple comparisons.

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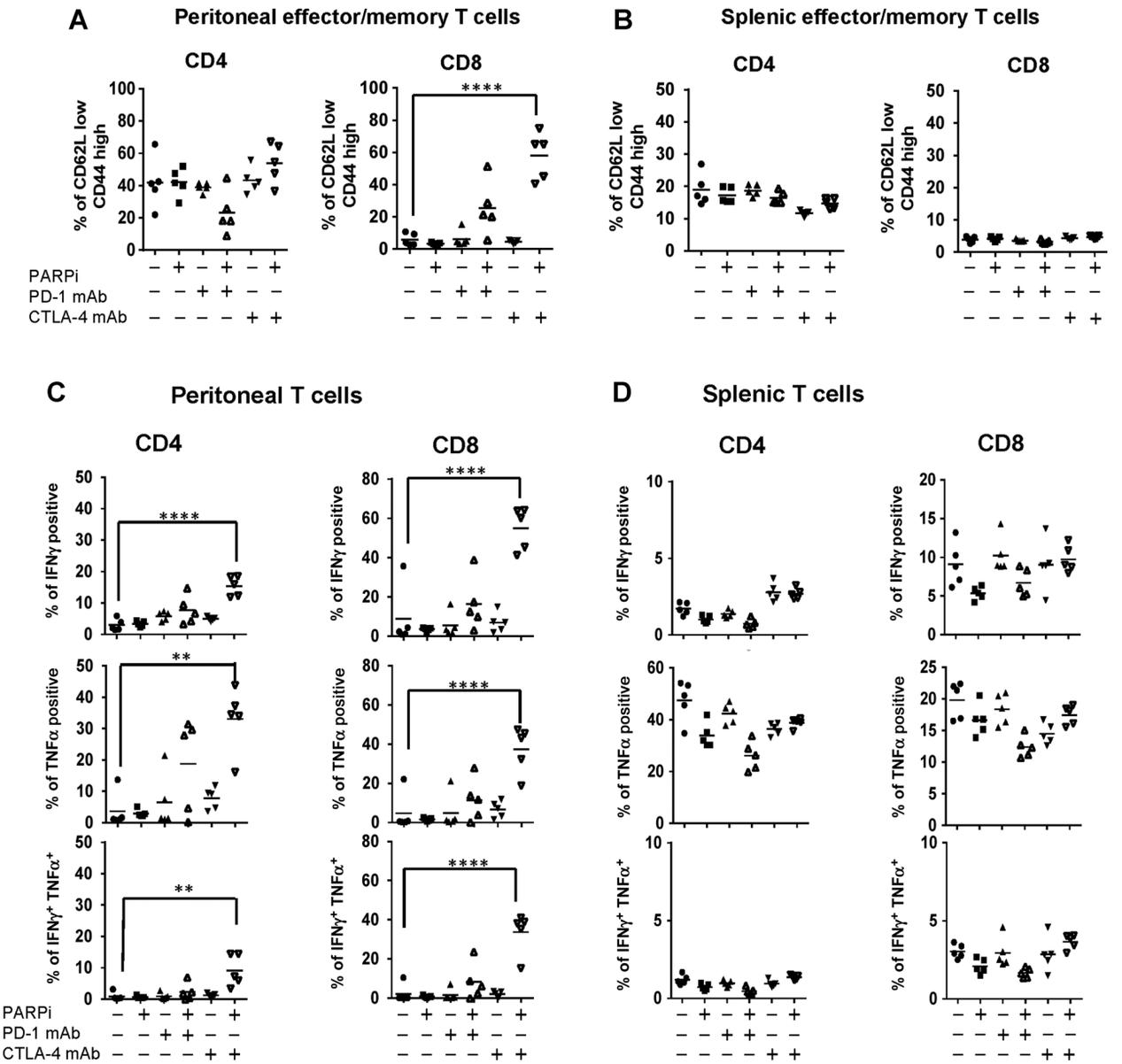

Figure 2.

Checkpoint blockade with CTLA-4 antibody combined with PARP inhibition enhances T-cell effector function in the peritoneal tumor environment. Using the protocol depicted in Supplementary Fig. S1, BRCA1^{-} tumor–bearing mice were sacrificed on day 21 for analysis ($n = 5$/group). Peritoneal (A) and splenic (B) CD4^{+} and CD8^{+} T cells were analyzed for CD44 and CD62L expression, and percentage of CD62L^{low}/CD44^{hi} cells was gated to determine the percentage of effector/memory CD4^{+} and CD8^{+} T cells of total CD4^{+} or CD8^{+} T cells, respectively. Peritoneal (C) cells and splenocytes (D) were restimulated in 24-well plates with PMA and ionomycin for 5 hours in the presence of Golgi transport inhibitors. CD4^{+} and CD8^{+} T cells were then analyzed by flow cytometry for intracellular cytokine expression. The percentages of T cells from treated animals producing IFNγ (top), TNFα (middle), or both (bottom) were compared with untreated controls. Data are representative of two experiments: *, $P < 0.05$; **, $P < 0.025$; ****, $P < 0.0001$ by ANOVA and Tukey procedure for multiple comparisons.

Combined treatment with a PARP inhibitor and CTLA-4 antibody promotes IFNγ-mediated tumor rejection in a BRCA1^{-} tumor model

With evidence that IFNγ enhances the cytotoxic effect of PARP inhibition *in vitro*, and that PARP inhibition combined with CTLA-4 blockade increased IFNγ production and an effector phenotype among peritoneal T cells *in vivo*, we evaluated the impact of this regimen on survival in the BRCA1^{-} tumor model. To do this, animals inoculated with BRCA1^{-} cancer cells were treated with checkpoint blockade monotherapy or combined treatment with the PARP inhibitor (40 mg/kg/day), and survival was measured from the day of tumor challenge until animals reached a weight of 30 g due to ascites accumulation (Supplementary Fig. S1). Results from these experiments demonstrated limited benefit from CTLA-4 monotherapy, in keeping with prior reports (4, 5); however, treatment with the CTLA-4 antibody

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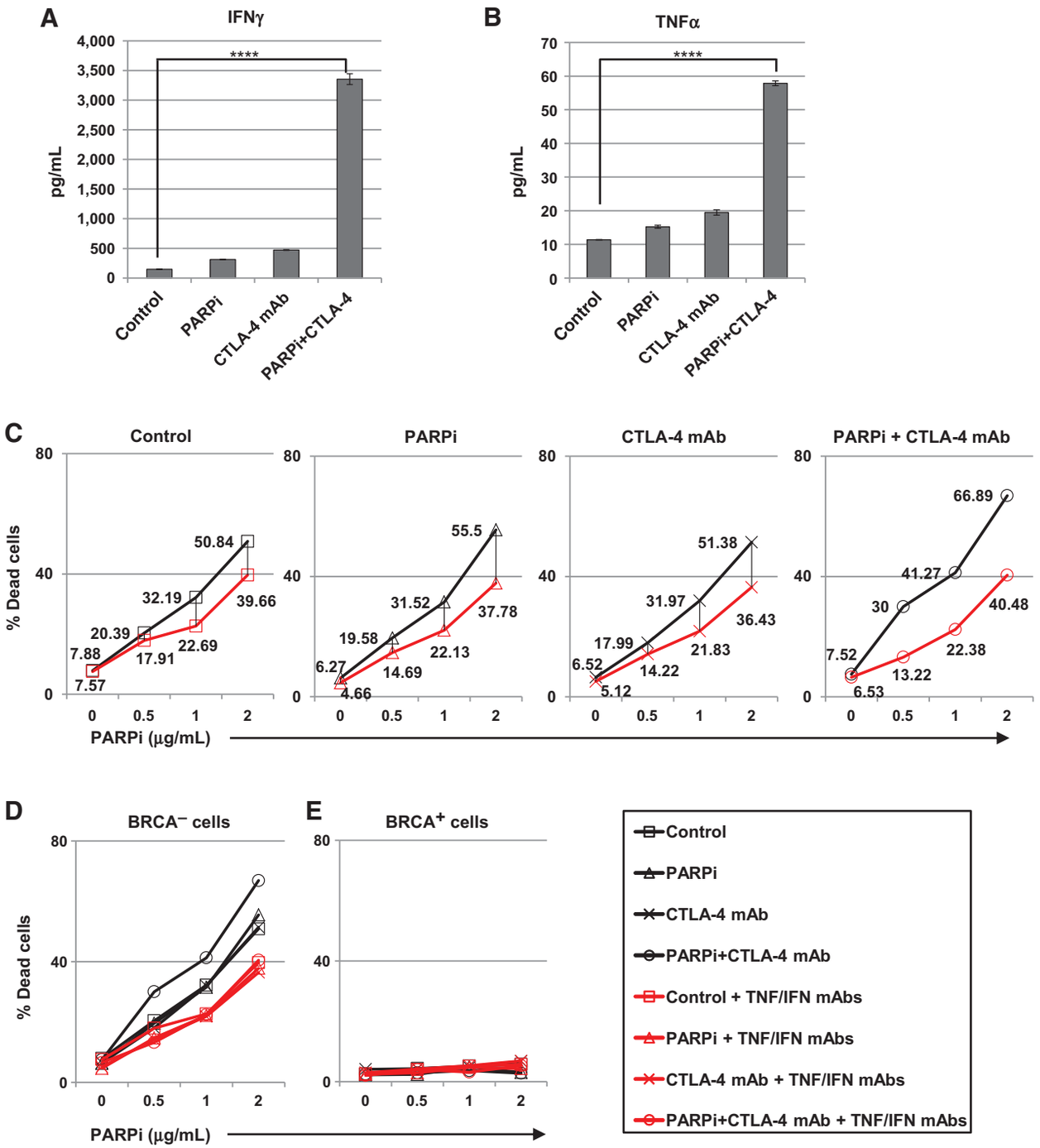

Figure 3.
Increases in IFNγ production in response to combined CTLA-4 blockade and PARP inhibition *in vivo* are sufficient to enhance tumor cell cytotoxicity. Mice were treated as in Supplementary Fig. S1 and euthanized on day 21 followed by retrieval of peritoneal cells ($n = 5$/group). A total of 5×10^6 peritoneal cells were restimulated *ex vivo* with 10 μg/mL of anti-CD3 for 18 hours. Cell-free supernatants were harvested and pooled by treatment group, and levels of IFNγ (A) and TNFα (B) were determined by ELISA. C–E, supernatants were added to BRCA^{-} cells at a ×4 dilution with DMEM-C in the presence of PARPi at indicated concentrations and cultured 72 hours in the absence (black lines) or presence (red lines) of IFNγ and TNFα neutralizing mAbs (10 μg/mL). C, cells were analyzed for viability by flow cytometry, and values shown are the percentages of dead cells with or without neutralizing antibody treatment. D, overlay of data in C. E, the same as D using BRCA*wt* cells (T22). *, $P < 0.05$; **, $P < 0.025$; ***, $P < 0.005$; ****, $P < 0.0001$ by ANOVA and Tukey procedure for multiple comparisons.

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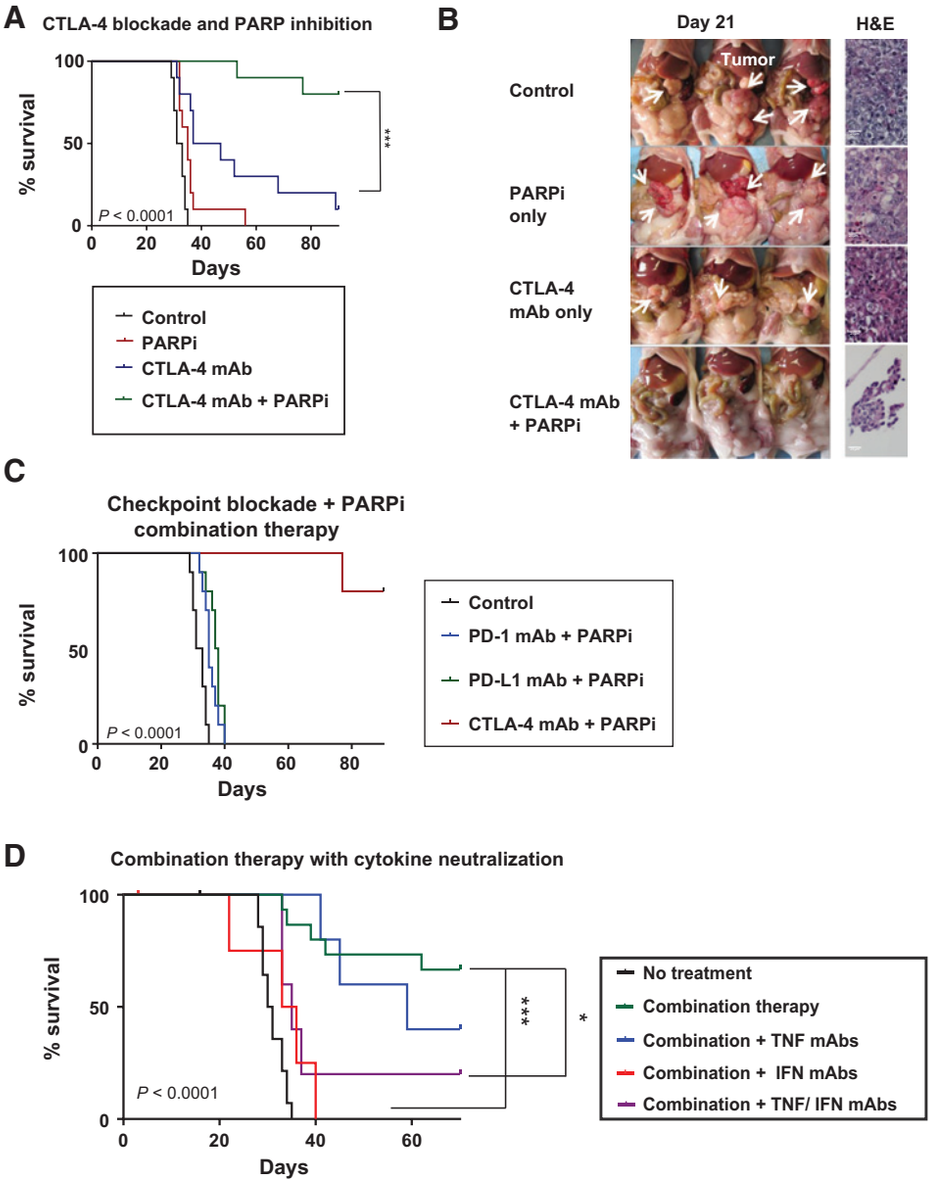

Figure 4.
Combined treatment with a PARP inhibitor and CTLA-4 antibody promotes IFNγ-mediated tumor rejection in a BRCA1^{-} tumor model. A, mice were treated as depicted in Supplementary Fig. S1 (40 mg/kg/day PARPi), and survival was compared with that of untreated controls (n = 10/group). B, on day 21, the peritoneal cavity was exposed for evaluation of macroscopic solid tumor burden, and histologic sections of omentum were examined for microscopic tumor implants. Representative samples are shown. C, combination therapy with PARP inhibition (40 mg/kg/day) and antibody blockade of CTLA-4, PD-1, or PD-L1 as in Supplementary Fig. S1 (n = 5–10 per group). D, survival of mice treated with PARPi (20 mg/kg/day) and CTLA-4 mAb combination therapy and neutralizing antibodies to TNFα, IFNγ, or both every 4 days beginning on day 7 (n = 5–15/group); survival comparisons are the Kaplan–Meier curves. Differences among groups were determined with the log-rank (Mantel–Cox) test: *, $P < 0.05$; ***, $P < 0.005$. Each experiment was repeated at least twice, with representative data shown.

together with PARP inhibition resulted in a synergistic therapeutic effect with long-term tumor-free survival in a majority of animals (Fig. 4A). As early as day 21, all control mice and animals receiving monotherapy had bulky solid tumors in the abdominal cavity; however, among mice receiving combination therapy, only 1 mouse had visible tumor and 2 additional mice had evidence of microscopic omental implants (Fig. 4B). When experimental animals receiving combined CTLA-4 blockade and the PARP inhibitor were examined by necropsy on day 90, no gross tumor was evident in surviving mice. A similar effect was observed when tumors were inoculated orthotopically under the ovarian bursa and solid tumor growth was examined on day 21 (Supplementary Fig. S2). In contrast with CTLA-4, no survival benefit was observed when PARP^{-} inhibition was combined with PD-1 or PD-L1 blockade (Fig. 4C). This is in agreement with prior data (36) and with our T-cell functional analyses, indicating PD-1/PD-L1 blockade in combination with PARP inhibition had limited effects on T-cell activation and cytokine induction (Fig. 2). Animals inoculated with BRCA*wt* tumors showed that no significant survival benefit was observed following treatment with the PARP inhibitor alone or in combination with CTLA-4 antibody, which was associated with a lack of any effect on peritoneal T-cell populations in these mice (Supplementary Fig. S3).

With evidence that combined therapy with the PARP inhibitor and CTLA-4 mAb enhances IFNγ and TNFα production by local T cells in BRCA1^{-} tumor–bearing mice, we next tested whether the therapeutic efficacy of PARP inhibition together with CTLA-4 blockade was dependent on elevations in effector cytokines *in vivo*. To do this, mice were treated with combination therapy using the PARP inhibitor (20 mg/kg/day) and CTLA-4 antibody together with neutralizing antibodies to IFNγ, TNFα, or both, and monitored for survival. This experiment demonstrated that

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neutralization of TNFα had a minimal effect on outcomes; however, IFNγ was required for the survival effect of combined treatment *in vivo* (Fig. 4D). Thus, CTLA-4 checkpoint blockade and targeted cytotoxicity with PARP inhibition promoted immune-mediated rejection of high-grade BRCA1$^-$ tumors, resulting in long-term tumor-free survival.

The survival benefit of combined treatment with the PARP-inhibitor and CTLA-4 antibody is due to lasting effects on peritoneal T cells

One of the most remarkable effects of checkpoint blockade has been the lasting therapeutic effects exhibited among patients who respond to treatment (4). Using sarcoma tumor models, Gubin and colleagues (37) have demonstrated that CTLA-4 blockade can induce protective immunologic memory. With evidence that tumor clearance following combined CTLA-4 blockade and PARP inhibition is dependent on local changes in T-cell phenotype and cytokine production, and that it can produce a lasting treatment benefit, we evaluated peritoneal T cells from long-term survivors for evidence of a memory response. To do this, peritoneal and splenic T cells were harvested from mice surviving past day 90 after treatment with CTLA-4 antibody and the PAPR inhibitor and analyzed for cytokine production. Following *ex vivo* stimulation with PMA and ionomycin, very high levels of IFNγ production were observed in both peritoneal and splenic CD4$^+$ and CD8$^+$ T cells from combination treated mice (Fig. 5A and B). Thus, although initial changes in T-cell phenotype and functional status were specifically seen in the peritoneal tumor environment, treated animals did develop evidence of a systemic memory response, which was associated with long-term survival following combined exposure to the CTLA-4 antibody and the PARP inhibitor.

To confirm that the extended therapeutic benefit of combined treatment with the PARP inhibitor and CTLA-4 antibody was T-cell mediated, adoptive transfer experiments were performed. To do this, 2×10^5 CD8$^+$ splenocytes retrieved from mice surviving greater than 90 days following combined treatment were transferred to recipient animals, followed by intraperitoneal tumor challenge after 12 hours. In this experiment, a majority of mice receiving CD8$^+$ cells pooled from three long-term survivors were themselves protected from intraperitoneal tumor challenge: All experimental animals survived longer than the control group, and 3 of 5 had no evidence of tumor at day 90 (Fig. 5C). We interpret these results as confirmation that combined therapy using CTLA-4 blockade and PARP inhibition induced a population of effector T cells that increased local levels of IFNγ and promoted the establishment of protective immunologic memory.

IFNγ enhances cytotoxicity in human BRCA1$^-$ tumor cells exposed to a PARP-inhibitor *in vitro*

To test the translational potential of this treatment protocol, we examined the effect of PARP inhibition with increasing doses of recombinant IFNγ or TNFα on human ovarian cancer cells *in vitro*. For these studies, we used the BRCA1$^-$ UWB1.289 cell line and UWB1.289 transfected to express wild-type BRCA1 (BRCA1*wt*), as previously described (21). The results demonstrated that IFNγ, but not TNFα, increased cytotoxicity in BRCA1$^-$ cells treated with the PARP inhibitor *in vitro* (Fig. 6A and B). As in the murine experiments, this effect was not observed in BRCA1*wt* cells (Fig. 6C and D). These data suggest that PARP inhibition in combination with CTLA-4 blockade may have similar therapeutic benefit in patients with BRCA1$^-$ ovarian cancers and support further study of this combination.

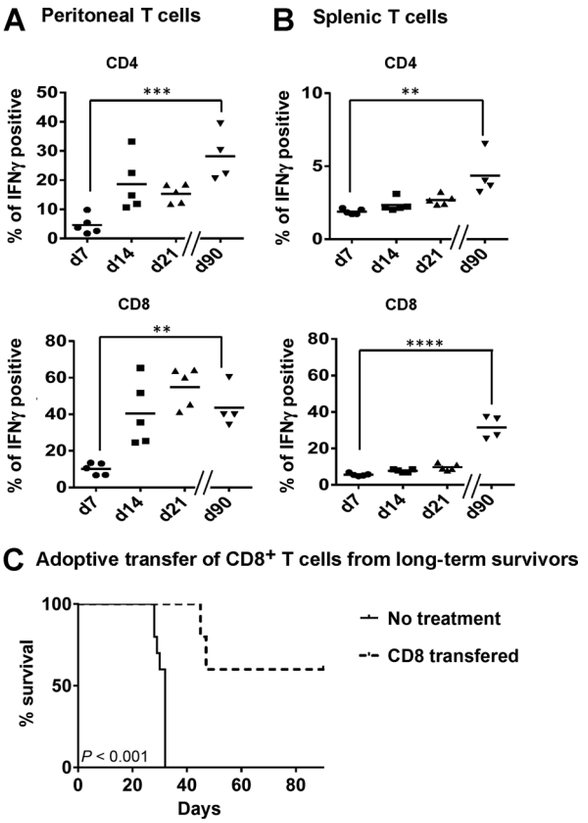

Figure 5.
Combined treatment with the PARP inhibitor and CTLA-4 antibody induces protective immunity. Peritoneal cells (A) and splenocytes (B) were retrieved on days 7, 14, and 21 from combination therapy—treated mice, and from long-term survivors on day 90, and restimulated *ex vivo* with PMA and ionomycin for analysis by flow cytometry for intracellular IFNγ production by CD4$^+$ and CD8$^+$ T cells. *, $P < 0.05$; **, $P < 0.025$, Tukey multiple comparisons test. C, adoptive transfer of CD8$^+$ T cells from long-term survivors protects recipients from tumor development. CD8$^+$ T cells were isolated by MACS-negative selection from long-term combination therapy survivors, pooled, and 2×10^5 cells were adoptively transferred to recipient mice 12 hours prior to challenge with 2×10^5 BRCA$^-$ tumor cells. Mice were monitored for survival ($n = 5$/group) log-rank (Mantel–Cox) test.

Discussion

Here, we demonstrate that CTLA-4 antibody combined with targeted therapy using a PARP inhibitor promotes long-term survival in a BRCA1$^-$ ovarian cancer model, and that this effect is mediated by local increases in IFNγ production by T cells in the tumor environment. Combination therapy rapidly increases T-cell recruitment, activation, and cytokine production in the peritoneal cavity, and this is followed by the induction of lasting systemic effector/memory T-cell immunity. Our finding of a similar trend in human BRCA1$^-$ cells treated with the PARP inhibitor in the presence of elevated levels of IFNγ, and evidence that ascites T cells from women with ovarian cancer express CTLA-4, support the translational potential of this treatment strategy.

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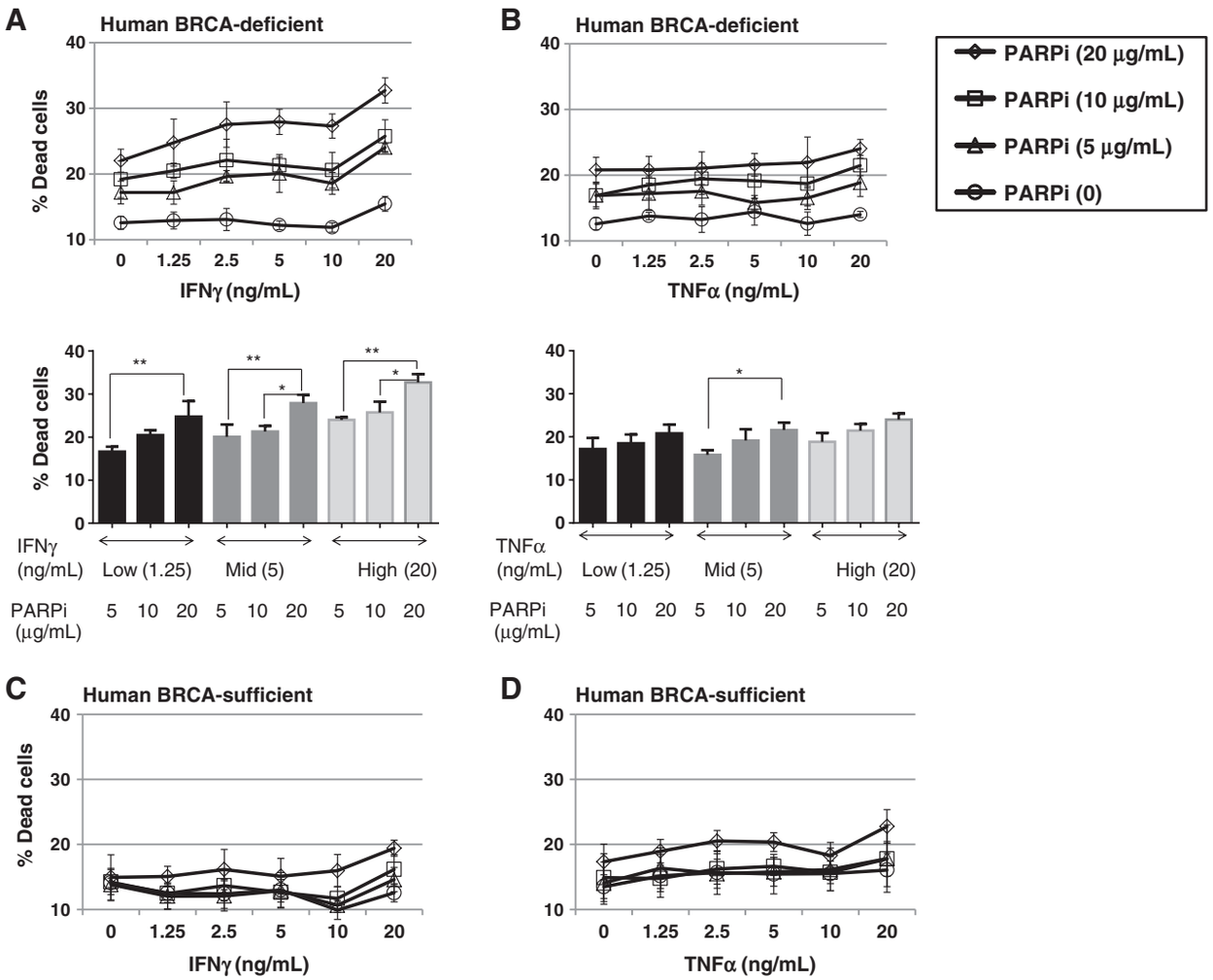

Figure 6.

IFNγ enhances cytotoxicity in human BRCA1^{-} tumor cells exposed to a PARP inhibitor *in vitro*. BRCA1^{-} UWB1.289 cells or UWB1.289 cells transfected with competent BRCA1 were cultured with titrated doses of the PARP inhibitor in the presence of recombinant human IFNγ or TNFα as indicated. After 72 hours, cells were analyzed for viability by flow cytometry. A, BRCA1^{-} UWB1.289 cells exposed to IFNγ and PARP inhibition (top, PARPi dose effect, $P < 0.0001$; IFNγ dose effect, $P < 0.0001$; interaction, $P = 0.3872$ based on two-way ANOVA; bottom, *, $P < 0.05$; **, $P < 0.025$ by Tukey multiple comparisons test). B, BRCA1^{-} UWB1.289 cells exposed to TNFα and PARP inhibition (top, TNFα dose effect, $P = 0.0499$; interaction, $P = 0.9655$ by two-way ANOVA; bottom, *, $P < 0.05$, Tukey multiple comparisons test). C and D, BRCA1-transfected UWB1.289 cells exposed to IFNγ or TNFα and PARP inhibition (no statistically significant dose effect for IFNγ or TNFα).

An important finding from these experiments is the effect of combined treatment on the peritoneal tumor environment. Prior studies using checkpoint inhibition antibodies have demonstrated a discrepancy between receptor occupancy among peripheral T cells and tumor-infiltrating T cells (36). As checkpoint inhibition is a locally mediated effect, any therapeutic benefit of checkpoint blockade requires engagement of lymphocytes in the tumor microenvironment (4). Our results demonstrate that effector/memory T cells were specifically increased in the peritoneal cavity in response to combination therapy, indicating that this regimen can effectively modulate T cells in the ovarian tumor environment. Because the ovarian tumor environment is considered tolerogenic (3), these changes indicate a significant reversal in the local immune conditions that is associated with long-term survival benefit. Furthermore, we expect that the localized increase in IFNγ following combined treatment may prove clinically advantageous by limiting systemic toxicity (38).

It is notable that despite expression of PD-1 on ascites T cells and PD-L1 on ovarian tumor cells, inhibition of this pathway had no significant impact on survival in the BRCA1^{-} model. This finding was unexpected in light of a recent report describing PD-1 pathway blockade as a strategy to enhance the efficacy of tumor vaccines in an ovarian cancer model (33). Despite differences in the dose and schedule of PD-1 antibody administration in this study, the effect of PD-1 blockade on T-cell function was similar to our results, with approximately 2% of CD8^{+} T cells from treated animals producing IFNγ, which in both cases was not significantly different from controls. We interpret the selective efficacy of CTLA-4 blockade we observed as evidence that activation of new lymphocyte clones, rather than reversal of T-cell

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exhaustion, was responsible for immune-mediated tumor clearance and long-term survival in the BRCA1^{-} model (1, 36, 39).

Strengths of this study include our use of an immunocompetent model that reliably develops high-grade serous tumors that replace the omentum and implant on serosal surfaces of the bowel and peritoneal cavity, mimicking the most common pattern found in patients. The fact that parallel results were demonstrated with BRCA1^{-} human tumor cells supports further testing of this combination for the treatment of clinical disease. In addition, the long-term survival we observed has not been demonstrated previously in other studies using this model (40). Although our study is focused on ovarian cancer, we expect that our results can be extended to other BRCA^{-} cancers, such as breast, prostate, or pancreatic tumors, that are vulnerable to the targeted effect of PARP inhibition. We are also currently investigating strategies to extend similar benefits to patients with sporadic ovarian cancer, using metronomic chemotherapy to sensitize tumors to checkpoint inhibition (41).

Finally, these results document a novel mechanism for BRCA1^{-} tumor cell death driven by an interaction between the PARP inhibitor and IFNγ *in vitro*. As a highly conserved, ubiquitous molecule required for posttranslational protein modification, PARP is involved in many cellular processes in addition to DNA repair, including roles in apoptotic cell death and metabolic pathways (42–45). The observation that only a portion of tumor cell death *in vitro* was due to apoptosis, as shown by caspase-3 cleavage, indicates that additional cytotoxic pathways are engaged in response to IFNγ and the PARP inhibitor. Accumulating reports describe a role for PARP inhibitors in the prevention of catalytic function and PARP trapping, and it is postulated that different forms of cell death may be induced by specific mechanisms of PARP inhibition (46–48). Therefore, it will be important to evaluate specific PARP inhibitors in combination with immune checkpoint blockade to better parse the mechanisms of cell death and to optimize treatment protocols for patients.

On the basis of these data, we propose a two-phase model to describe distinct roles for the PARP inhibitor in promoting tumor regression when combined with CTLA-4 blockade. In phase I, PARP inhibition directly induces tumor cell damage, which primes and diversifies an antitumor T-cell response, a process that is amplified by CTLA-4 blockade (49). In phase II, local T cells activated in the presence of CTLA-4 blockade produce increased levels of IFNγ above a threshold required to enhance the cytotoxic efficacy of PARP inhibition, resulting in additional therapeutic benefit through cell-intrinsic pathways. This model indicates that the therapeutic benefit of PARP inhibition can be significantly amplified by inclusion in immunotherapeutic protocols.

In summary, these data show that long-term survival can be achieved in a BRCA1^{-} ovarian tumor model using a PARP inhibitor combined with CTLA-4 checkpoint blockade. The fact that both PARP inhibitors and CTLA-4 antibodies have been well tolerated as monotherapy in women with ovarian cancer, together with our *in vitro* data using human BRCA1^{-} cancer cells, supports the rapid translation of this treatment protocol for clinical testing. In addition, our results add to prior work suggesting that BRCA mutation status can be used to identify candidates for immunotherapeutic protocols, improving our ability to identify a treatment effect for selected patients (50). Finally, we anticipate that further evaluation of PARP inhibitors in combination with specific immune checkpoint pathways will uncover optimal pairings that will expand the clinical utility of this strategy for the treatment of patients with other BRCA^{-} cancers, or with tumors deficient in alternate DNA-repair pathways.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: T. Higuchi, D.B. Flies, L. Ronner, S.F. Adams
Development of methodology: T. Higuchi, D.B. Flies, G. Mantia-Smaldone, S.F. Adams
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): T. Higuchi, D.B. Flies, N.A. Marjon, G. Mantia-Smaldone, S.F. Adams
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): T. Higuchi, D.B. Flies, N.A. Marjon, P.A. Gimotty, S.F. Adams
Writing, review, and/or revision of the manuscript: T. Higuchi, D.B. Flies, N.A. Marjon, G. Mantia-Smaldone, L. Ronner, P.A. Gimotty, S.F. Adams
Study supervision: S.F. Adams

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