PARP INHIBITION INCREASES IN THE SUPPRESSIVE CAPACITY OF TUMOR-ASSOCIATED TREGS IN A BRCA1-DEFICIENT MODEL OF OVARIAN CANCER

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Background
Emerging evidence indicates that tumor-directed anticaner agents have immunomodulatory effects that contribute to therapeutic outcomes. Targeted therapy by inhibition of poly(ADP-ribose) polymerase (PARPi) has had a profound effect on disease outcomes in ovarian cancer. The cytotoxic effects of PARPi are attributed to inhibition of single-stranded DNA repair pathways resulting in accumulation of DNA-damage in BRCA-deficient cells. While PARP is commonly associated with DNA repair, it has also been linked to a variety of other processes including cell division and inflammation. Published data using PARP1-KO mice suggests that PARP impairs circulating regulatory T cell (Treg) function through modulation of FoxP3, however, the impact on tumor-associated Tregs is unclear. As in many cancers, Tregs accumulate in the ovarian cancer tumor microenvironment (TME) and represent a key mechanism of immune escape. With growing interest in testing combinations of PARPi and immunotherapy, we sought to examine the impact of PARPi on peripheral and tumor-associated Tregs.

Methods
To isolate viable Tregs we used FoxP3-eGFP co-expressing transgenic mice. Female mice (8-10 wk) were challenged with BRCA1-deficient ID8 cells (i.p.) and treated daily with ABT-888 (40 mg/kg) for six weeks. Tumor-associated Tregs were sorted from total peritoneal cells pre-enriched for CD4+ T cells. In separate experiments, peripheral Tregs were sorted from CD4+ spleen and lymph node cells and pre-treated with ABT-888 for 24 hours prior to use. Standard proliferation assays using naïve T cells were used to assess Treg suppressive function. Flow cytometry was used to measure cell divisions and expression of FoxP3 and CTLA-4.

Results
Ex-vivo treatment of Treg from non-treated, tumor-naïve mice showed that ABT-888 pre-treatment significantly reduced the suppressive capacity of Tregs in a dose-dependent manner (P < 0.05). Similarly, ABT-888 treatment resulted in decreased expression of FoxP3 and CTLA-4 in a dose-dependent manner (P < 0.05). Conversely, tumor-associated Tregs from PARPi-treated mice had superior suppressive capacity compared to those from non-treated mice (P < 0.05). No differences between treated and non-treated groups were observed in Tregs isolated from the spleen.

Conclusions
Taken together these data highlight the immunomodulatory effects of PARPi on tumor-associated Tregs. Here we present evidence that PARPi treatment promotes the suppressive capacity of Tregs in the TME and also identify a potential interaction between PARPi and response to immunotherapy in BRCA-deficient ovarian cancer. Future studies will include PARP inhibitors with varying degrees of DNA trapping ability as well as non-BRCA mutated tumors.