mimic physiological TME to dissect the combinational role of Nivolumab and Blinatumomab (figure 2).

**Results** The result suggest that combinatory therapy is highly depend on the dosage of Blinatumomab and also T cell number in the TME, which might give an instruction for ongoing clinical trial design. Finally, we have employed humanized mouse models bearing Raji or Daudi tumor cells to further validate this combination treatment in vivo. Both In-vivo and In-vitro data support that Blinatumomab is dominant in activating T cell and Nivolumab can only exhibit synergistic effect under suboptimal dosage of Blinatumomab.

**Abstract 781 Figure 1** Establishment of In vitro co-culture system for CD3 BiTE establish in vitro human PBMC based system to validate CD3 BiTE function

**Abstract 781 Figure 2** Opdivo and CD3 BiTE Combination Opdivo could further promote T cell activation under the treatment of CD3 BiTE

**Conclusions** Successfully establish in vitro system to evaluate the function of CD3 BiTE and also take advantage of MLR/tumor co-culture system to demonstrate PD1 antibody could further promote T cell activation under appropriate dosage of CD3 BiTE.

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**782** ALDH1A INHIBITION AS ADJUVANT TO OVARIAN CANCER IMMUNOTHERAPY

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**Background** Despite some recent advances, new therapeutic approaches for ovarian cancer (OvCa), the 5th leading cause of cancer deaths in women, are clearly needed. Aldehyde dehydrogenase-1A (ALDH1A) enzymes represent a novel therapeutic target for OvCa. ALDH1A is upregulated in OvCa initiating cells and mediate the biosynthesis of retinoic acid (RA) to regulate numerous cellular processes, including proliferation, metastasis, and chemotheraphy resistance. We recently identified novel pan-ALDH1A family inhibitors (ALDHi) that induce necroptosis in OvCa stem-like cells and synergize with chemotherapy, leading to tumor eradication in vivo. Here, we hypothesize that, in addition to controlling tumor progression, ALDHi trigger immunogenic cell death (ICD) via necroptosis and can potentiate anti-tumor immunity.

**Methods** We performed RNA-Seq on four human OvCa cell lines (A2780, CAOV-3, OVCAR-5, OVSAHO) treated for 8 hours with two different ALDHi. To measure the impact on T cell immunity we performed flow cytometry to measure cell proliferation assays and CD4 naïve differentiation into Th1/Th17/Treg subsets. Molecular targets in the RA pathway were confirmed by western blot.

**Results** ALDHi triggered significant changes in (i) ER stress unfolded protein response and regulators of the ER stress response, such as ATF4 and EIF2aK3 (PERK), (ii) inflammatory pathways, (iii) cell death, survival, and (iv) gene transcription-RAR signaling. Treatment of cancer cell lines with ALDHi induced expression of Phospho-eIF2a, a marker for the ICD, along with increased expression of ATF3 and ATF4, and calreticulin, suggesting cancer cells undergoing ICD.

Using polyclonal stimulation of murine splenocytes and human PBMC, we observed that ALDHi promote T cell proliferation, especially of CD8 T cells. Furthermore, exposure of naïve CD4 cells to Th1 and Treg differentiation conditions leads to increased production of INFγ and reduced number of Foxp3+ iTregs, respectively. Further, in a co-culture of iTreg and stimulated splenocyte, ALDHi treatment diminishes the iTreg’s capacity to induce immune suppression. Ex vivo treatment of ovarian cancer ascites cells with various ALDHi leads to significant decrease of CD14+ cells, an effect associated with downregulation of NR4A1 (NUR77), a nuclear receptor that interacts RAR/RXR, downstream of RA signaling.

**Conclusions** ALDHi induce immunogenic cell death in cancer cells. Immune cells respond to ALDHi in a cell specific manner. ALDHi support CD8 T cell proliferation and CD4 Th1 induction, while inhibiting iTregs. Exposure to ALDHi leads to downregulation of NR4A1 and reduction in suppressive macrophage numbers. Our results support the use of ALDHi as immune modulators in ovarian cancer and adjuvants to immunotherapy.

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**785** ESTROGEN-DEPRIVATION PROMOTES TH1 POLARIZATION OF TUMOR-ASSOCIATED T CELLS IN A MOUSE MODEL OF HIGH GRADE SEROUS OVARIAN CANCER

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**Background** Immunotherapy has achieved long-term survival in patients with melanoma and other tumors, introducing a new paradigm in cancer treatment. Differential outcomes among men and women receiving immune checkpoint inhibitors implicate sex steroids as modulators of treatment response. Estrogen signaling has a profound impact on T cell function...
and has been shown to upregulate FoxP3 expression, promoting a suppressive regulatory phenotype. Conversely, estrogen deprivation promotes Th1 skewing, including increased IFN-γ production in response to antigen-specific stimulation. We hypothesize that immunomodulatory effects of estrogen deprivation will enhance immunotherapy outcomes.

Our lab has previously demonstrated that IFN-γ levels in the TME predict response to immune checkpoint blockade (ICB) regimens in ovarian cancer models. CTLA4 but not PD1/PDL1 ICB combined with PARP inhibition (PARPi), an oral chemotherapeutic, significantly increased IFN-γ in the TME. Furthermore, IFN-γ was required for the durable survival benefit achieved with PARPi/anti-CTLA4. Here we test whether estrogen deprivation enhances IFN-γ production in the TME and response to PARPi/anti-PD1.

Methods Five-week-old female FVB mice underwent oophorectomy, laparotomy without oophorectomy (sham), or no surgery (n = 5 per group). On day 10, mice were intraperitoneally challenged with 200,000 BR5-Akt syngeneic OC cells and randomly assigned to receive either PARPi/anti-CTLA4, PARPi/anti-PD1 or vehicle control treatment. PARPi (40mg/kg/day) was administered days 13-30 and 100 μg of anti-CTLA4 or 300 μg anti-PD1 was administered on D14. A second dose of anti-PD1 was given on D24. On day 30, peritoneal cells were analyzed by flow cytometry. Tumor burden was measured by IVIS.

Results Oophorectomy was associated with a significant increase in IFN-γ production by tumor-associated CD4+ T cells [30.4% vs 8.2%, p = 0.016] and an increase in the proportion of central memory CD8+ T cells [39.3% vs 34.9%, p = 0.007] in response to PARPi/anti-PD1 compared with sham and no-surgery controls. In contrast, no differences in T cell phenotype or function was noted among groups receiving PARPi/anti-CTLA4. These changes were associated with a decrease in tumor burden in response to PARPi/anti-PD1 on D30.

Conclusions Estrogen deprivation promotes Th1 polarization among tumor-associated T cells in response to PARPi/anti-PD1 treatment. With evidence that high levels of IFN-γ in the TME strongly correlate with survival, we predict that these effects will enhance treatment outcomes in response to PARPi/anti-PD1. This work presents a rationale for testing estrogen receptor modulators in combination with immune therapy agents and provides a potential mechanism to account for observed differences in patient outcomes.

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Abstract 786 Figure 1 aPD-L2 treats B16 in aged but not young mice

Abstract 786 Figure 2 aPD-1, aPD-L1 and aPD-L2 elicit distinct TCSC

Abstract 786 Figure 3 Treatment efficacy correlate with increased TCSC

786 DISTINCT EFFICACY AND IMMUNOLOGICAL RESPONSES TO APD-1, APD-L1 AND APD-L2 IMMUNOTHERAPY IN B16 MELANOMA IN AGED VERSUS YOUNG HOSTS INCLUDES T-CELL STEM CELL EFFECTS AND PD-L2 EXPRESSION DIFFERENCES

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Background Aging is the biggest risk factor for cancer, yet little is known about cancer immunotherapy effects. ?PD-1 can block PD-L1 and PD-L2 while ?PD-L1 blocks PD-1 and CD80. A recent key finding in young hosts including humans is that melanoma response to aPD-1/aPD-L1 correlates with CD8+TCF-1+ T cell stem cell (TCSC) generation.

Methods We tested aPD-1 (100 or 200 μg/mouse), aPD-L1 (100 μg/mouse) or aPD-L2 (200 μg/mouse) in aged (18-33 months) and young (3-8 months) mice challenged orthotopically with B16 (WT or PD-L1ko) melanoma (SQ) or ID8agg ovarian cancer (IP). Tumors were analyzed by flow. Bone marrow-derived DC were generated with GM-CSF.

Results We reported that aPD-1 treats young and aged with B16 and aPD-L1 treats young not aged. aPD-L2 treated B16 in aged but, remarkably, not young, the first anti-cancer single agent immunotherapy exhibiting this property (figure 1). Efficacy in young (aPD-1, aPD-L1) and aged (aPD-L2) correlated with increased tumor TCSC (figure 3), but TCSC differed by age and treatment (e.g., distinct CCR2, CXCR5, CXCR3) (figure 2). aPD-L2 efficacy against B16 in aged mice required host IFN-g and IL-17 (figure 4). IP ID8agg ovarian cancer did not respond to aPD-L2 in aged or young mice. Aged expressed up to 40-fold more PD-L2 versus young on various immune cells suggesting high PD-L2 helps aPD-L2 response (figure 6). Host IFN-g contributed to aged PD-L2 expression, which did not appear cell-autonomous (figure 6). PD-L1KO aged but not young mice challenged with PD-L1KO B16 responded to aPD-1 (figure 5), consistent with PD-L2 block as a mechanism.