4654. Differential immunomodulation following checkpoint blockade in the orthotopic ID8-luc ovarian model

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Introduction

- The availability of syngeneic models for ovarian cancer is extremely limited.
- The ID8-luc ovarian model is established intraperitoneally with disease progression resulting in the accumulation of ascites fluid. However, solid tumors do not reliably form, making assessment of immunomodulation challenging.
- The ID8-luc model was originally developed to assess efficacy and has now been developed as a tool to investigate immuno-modulatory drug candidates by utilizing tissues in the peritoneal cavity of diseased animals for *ex vivo* analyses.
- Specifically, flow cytometry of diseased ovaries and ascites was employed to examine the effects of checkpoint inhibition on immune cells.

Methods

- ID8-luc-mCh-puro cells were implanted intraperitoneally into female C57BL/6BrdCrHsd-*Tyr*^{c} (albino C57BL/6) mice. Once bioluminescence imaging (BLI) indicated established disease, treatment was initiated. Checkpoint inhibitors (anti-mPD-1 (RMP1-14), anti-PD-L1 (10F.9G2) or anti-mCTLA4 (9D9) (Bio X Cell, West Lebanon, NH) were administered at 10 mg/kg twice per week for 3.5 weeks. Cisplatin was administered at 5 mg/kg once weekly for 3 weeks. Disease progression was monitored by BLI.
- All animal work was performed in an AAALAC-accredited facility, in alignment with applicable animal welfare regulations and with predetermined humane euthanasia criteria on all studies.
- Ovaries and ascites were harvested 24 hours after the sixth (Day 34) or seventh dose (Day 37), dissociated (Miltenyi, Germany) and stained for flow cytometry. Data was analyzed using FlowJo® software (FlowJo, LLC, Ashland, OR).
- Tissues were harvested on Day 28 or 35 post-tumor implant, fixed in formalin and embedded into paraffin blocks. Slides from each tissue were stained with H&E for histopathology assessment.

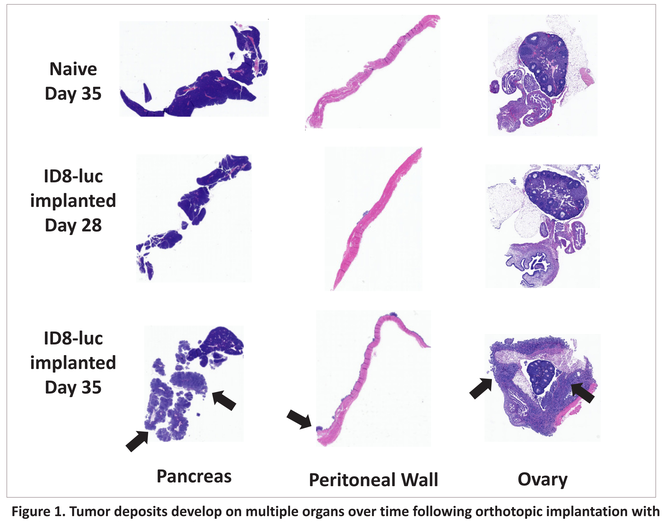

Figure 1. Tumor deposits develop on multiple organs over time following orthotopic implantation with ID8-luc tumor cells. Arrows denote tumor development not seen in naïve organs or those sampled on Day 28.

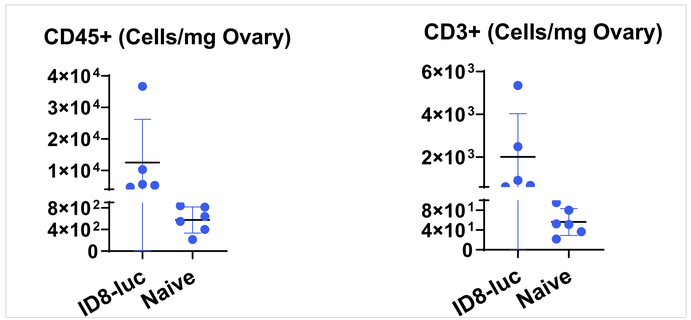

Figure 2. Infiltration of immune cells is dramatically increased in ovaries following orthotopic implant of ID8-luc cells. Data shown was generated from samples collected 33 days post-tumor implant.

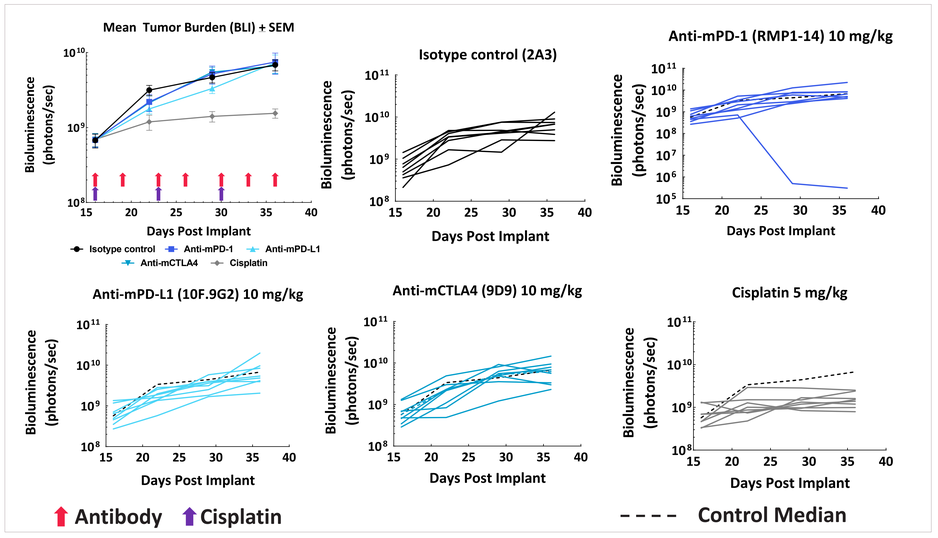

Figure 3. Treatment of checkpoint inhibitors and cisplatin in the ID8-luc murine ovarian tumor model. Checkpoint inhibition does not result in efficacy against ID8-luc. Cisplatin treatment exhibits moderate efficacy against ID8-luc.

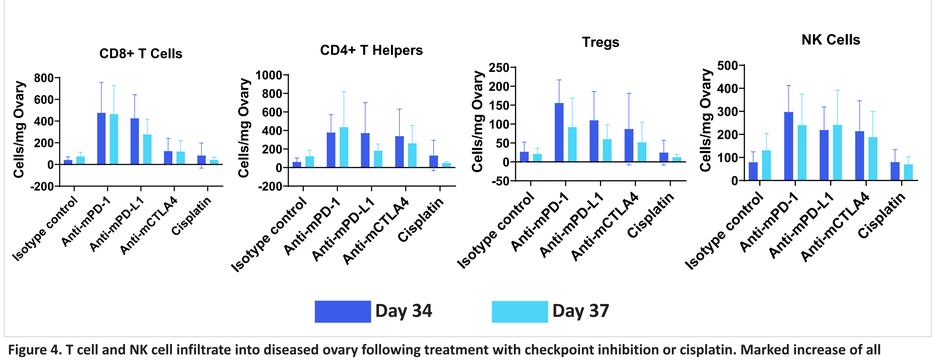

Figure 4. T cell and NK cell infiltrate into diseased ovary following treatment with checkpoint inhibition or cisplatin. Marked increase of all populations compared to isotype control is illustrated. Cisplatin treatment did not result in increased infiltration into the ovaries compared to isotype control. Extent of filtration does not markedly change between Days 34 and 37 post-tumor implant (24h post-antibody dose).

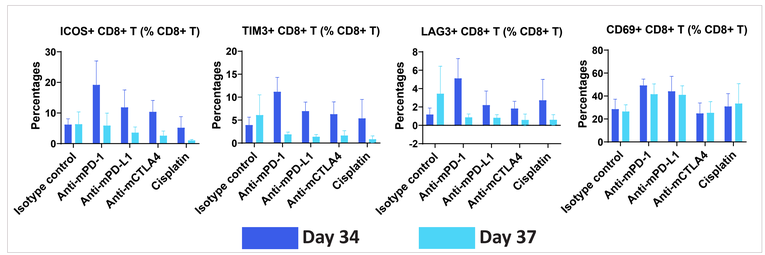

Figure 5. CD8+ cell biomarkers for T cell activation and/or inhibition in ovary following checkpoint inhibitor or cisplatin therapy. Marked increases in ICOS, TIM3 and LAG3 expression are seen with anti-mPD-1 treatment. Marked decreases in checkpoint expression occur between Days 34 and 37 post-implant (24h hours post-antibody dose). Little to no change in expression of CD69 on CD8+ cells in any group.

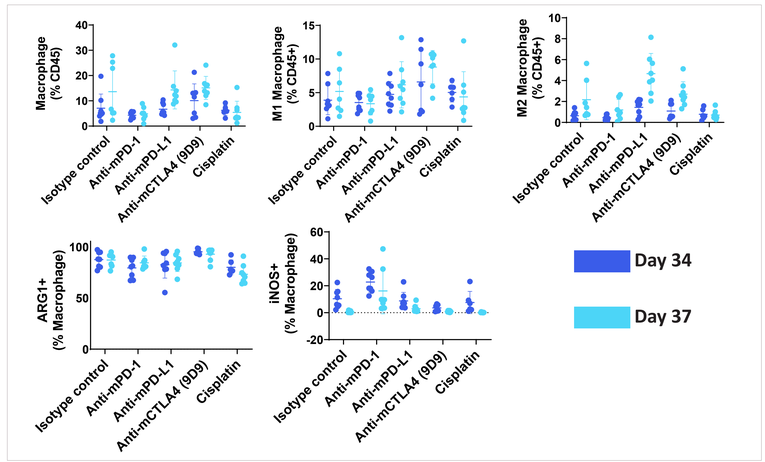

Figure 6. Macrophage content and immunosuppression biomarker expression in ascites fluid. Marked increase in M2 macrophages with anti-mPD-L1 or anti-CTLA4 treatment. Marked decreases in iNOS expression on macrophages between Days 34 and 37 post-implant (24h hours post-antibody dose) that appears unrelated to treatment.

Results and Conclusions

- ID8-luc tumors form on pancreas, peritoneal wall and ovary as disease progresses.
- Marked increases in immune cell infiltrate are demonstrated in ovary from ID8-luc implanted mice compared to naïve mice.
- The ID8-luc model is refractory to checkpoint antibody treatment. However, increases of lymphoid infiltrate into diseased ovary is indicative of a mounting immune response.
- CD8+ T cells in ovary were increased compared to isotype control and CD8+ checkpoint expression decreased across the two timepoints, while CD69 expression remained constant. Significance of this immunomodulation has not been determined.
- Following treatment with anti-mPD-L1, increases in M2 macrophages in ascites along with decreases in iNOS+ macrophages across all groups in late stage disease suggest an immunoresponsive phenotype that may not be related to treatment.
- Checkpoint inhibition in the ID8-luc model may serve to prime the immune system for at least additive efficacy with a combination partner.

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