Ovaries and ascites were harvested 24 hours after the sixth (Day 34) or seventh (Day 35) ID8-luc-mCh-puro cells were implanted intraperitoneally into female C57BL/6BrdCrHsd mice. Once bioluminescence imaging (BLI) indicated established disease, treatment was initiated. Checkpoint inhibitors (anti-mPD-1 (RMPI-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 (Day 35) ID8-luc tumor cells. Arrows denote tumor development not seen in naïve organs or those sampled on Day 28.

### Methods

- **ID8-luc-mCh-puro cells** were implanted intraperitoneally into female C57BL/6BrdCrHsd mice. Once bioluminescence imaging (BLI) indicated established disease, treatment was initiated. Checkpoint inhibitors (anti-mPD-1 (RMPI-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 (Day 35) dose (Day 37), dissociated (Milltenyi, 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.

### 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 with anti-mCTLA4 treatment. Marked increases in iNOS expression on macrophages between Days 34 and 37 post-implant (24 hours post-antibody dose) that appears unrelated to treatment.

**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 mouse 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 Day 34 and 37 post-tumor implant (249 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 decrease in checkpoint expression occur between Days 34 and 37 post-implant (24 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 (24 hours post-antibody dose) that appears unrelated to treatment.