# PD-1 blockade potentiates an IGFBP-2-specific anti-tumor immune response in ovarian cancer

## Introduction

- Increased overall survival in ovarian cancer is associated with an increase in tumor infiltrating lymphocytes (TIL), particularly an increase in the CD8+FoxP3+ ratio.
- There can be very few type 1 T-cells detected in ovarian cancers.
- Current immunotherapy strategies, such as anti-PD-1 or -PD-L1 monoclonal antibody therapy, have been used in clinical trials in ovarian cancer to try to modulate the activity of these resident ovarian cancer-specific T-cells.
- Overall response rates with these agents range from 10-17%.
- We hypothesize that ORR with checkpoint blockade could be increased if a greater number of ovarian cancer-specific type I T-cells could be induced as the resident TIL through active immunization.

## Methods

**Vaccination, tumor challenge and anti-PD-1 treatment:** Female Albino C57BL/6 mice (6-8 weeks old) were immunized with IGFBP-2 (1-163) or pUMVC3 vector alone (50 μg plain) as a mixture with mouse IgG3(C3). Three immunizations were given two weeks apart. For tumor challenge, a syngeneic mouse epithelial ovarian cancer cell line stably expressing a cedonitum optimized luciferin gene. CD45-Luc2 was implanted (i.p. 2.5x10^5 in each of the lower right and left quadrants from the supine position). For the preventative vaccine strategy, the tumor was implanted two weeks after the third vaccine. For the therapeutic vaccine strategy, the tumor was implanted three weeks before the start of vaccine. Anti-mouse PD-1 mAb clone 4D5, mouse IgG1 (200μg/mouse) was injected i.p. on the same day as the first vaccine and every other day thereafter for a total of 5 treatments.

**Tumor growth assessment by bioluminescent imaging:** A total of 3mg/ml D-luciferin was injected as described for tumor implant, above. After 20 min, the mice were anesthetized and bioluminescent images were taken from the supine position with a Xenogen IVIS. Images were normalized using Living Image software and is expressed as total flux (photons/sec).

**Flow cytometry:** Receptor expression was determined in the homogenized tumor (14 weeks after implantation) by adding Per-CP-conjugated anti-mouse CD4, PE-conjugated anti-mouse CD3, FITC-conjugated anti-mouse PD-1, APC-conjugated anti-mouse CD4, PE-Cy7-conjugated anti-mouse CD8 and PE-conjugated anti-mouse PDL-1. Flow cytometry was performed on the FACSCanto and data analyzed using FlowJo software. Typically, 10,000 cells were collected per sample.

**Statistical analysis.** The unpaired, two-tailed Student’s t-test, ANOVA or Mantel-Cox tests were used to evaluate differences between groups. p<0.05 was considered significant. All statistical analyses were performed using GraphPad Prism 6.04.

## Results / Conclusions

- **Immunization targeting IGFBP-2** in a prophylactic setting resulted in a 62% decrease in tumor growth compared to controls (p=0.056; Fig.1).
- **Tumors express high levels of PD-L1 and PD-1** in the TIL as compared to the spleen (p=0.011; Fig.2).
- No control of tumor growth was observed if 1. Vaccination was initiated in a therapeutic setting 2. Mouse was treated with anti-PD-1 monotherapy after tumor challenge (Fig.3)
- If vaccination was performed concomitantly with PD-1 blockade, tumor growth was inhibited by 99% in 6/8 of the mice compared to vaccine alone (p=0.048; Fig.3).
- A significant increase in overall survival at 32 weeks was observed in the mice receiving the combination regimen (p=0.028; Fig. 4).
- Efficacy with checkpoint blockade may be improved by combining treatment with active immunization targeting overexpressed ovarian cancer associated antigens.

## Funding

This work was supported by P50 CA083636-15 and the Athena Distinguished Professor of Breast Cancer Research Award (MLD).