



# Efficacy of Intralesional Injection with PV-10 in Combination with Co-Inhibitory Blockade in a Murine Model of Melanoma

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## Introduction

Rose Bengal is a water-soluble xanthene dye that has been previously used in liver function studies and is still in use by ophthalmologists. PV-10 is a 10% solution of Rose Bengal formulated for intralesional (IL) injection. In clinical trials, IL PV-10 therapy induced regression of both injected lesions and uninjected bystander lesions in patients with melanoma. We have previously shown that IL injection of PV-10 into a single subcutaneous B16 melanoma tumor led to regression of both the injected tumor and uninjected B16 lung lesions. Tumor regression correlated with the induction of systemic anti-tumor T cell immunity. In this study, we have measured whether IL PV-10 and co-inhibitory blockade improves anti-tumor immunity and regression of melanoma.

## Methods

**Single flank model:** C57BL/6 mice were injected subcutaneously (s.c.) on with B16 or B16-OVA cells. Tumor was injected IL with 50 uL of PV-10 or PBS on day 7-14 after tumor induction. Three days after IL injection, mice received intraperitoneal (i.p.) injections of 20 mg/kg anti-CTLA4, anti-PD1 or anti-PDL1 antibodies. NrlgG antibodies were used as a control. Antibody injections were continued every 3-4 days.

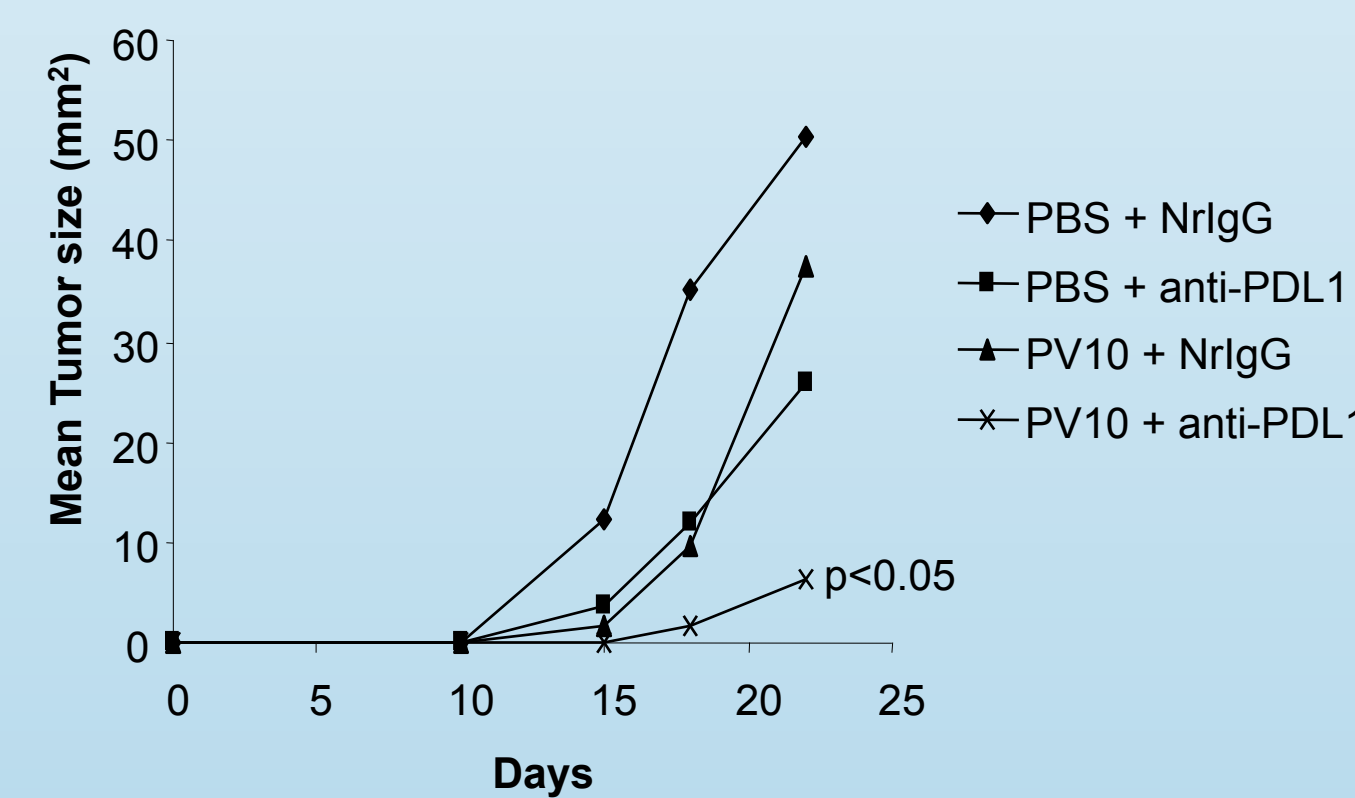
**Bilateral model:** C57BL/6 mice were injected s.c. on both flanks with B16 cells. The tumor on the right flanks was injected IL with 50 uL of PV-10 or PBS on day 7 after tumor induction. Three days after IL injection, mice received i.p. injections of 20 mg/kg NrlgG or anti-PDL1 antibodies. Antibody injections were continued every 3-4 days. Tumor sizes were measured.

For analysis of T cell activation, splenocytes were collected on day 7-14 after PV-10 injection after at least 2 i.p. antibody treatments. Splenocytes were co-cultured with irradiated B16 or B16-OVA cells and MC-38 colorectal cells. Supernatants were collected after 48 hours IFN-gamma was measured by ELISA.

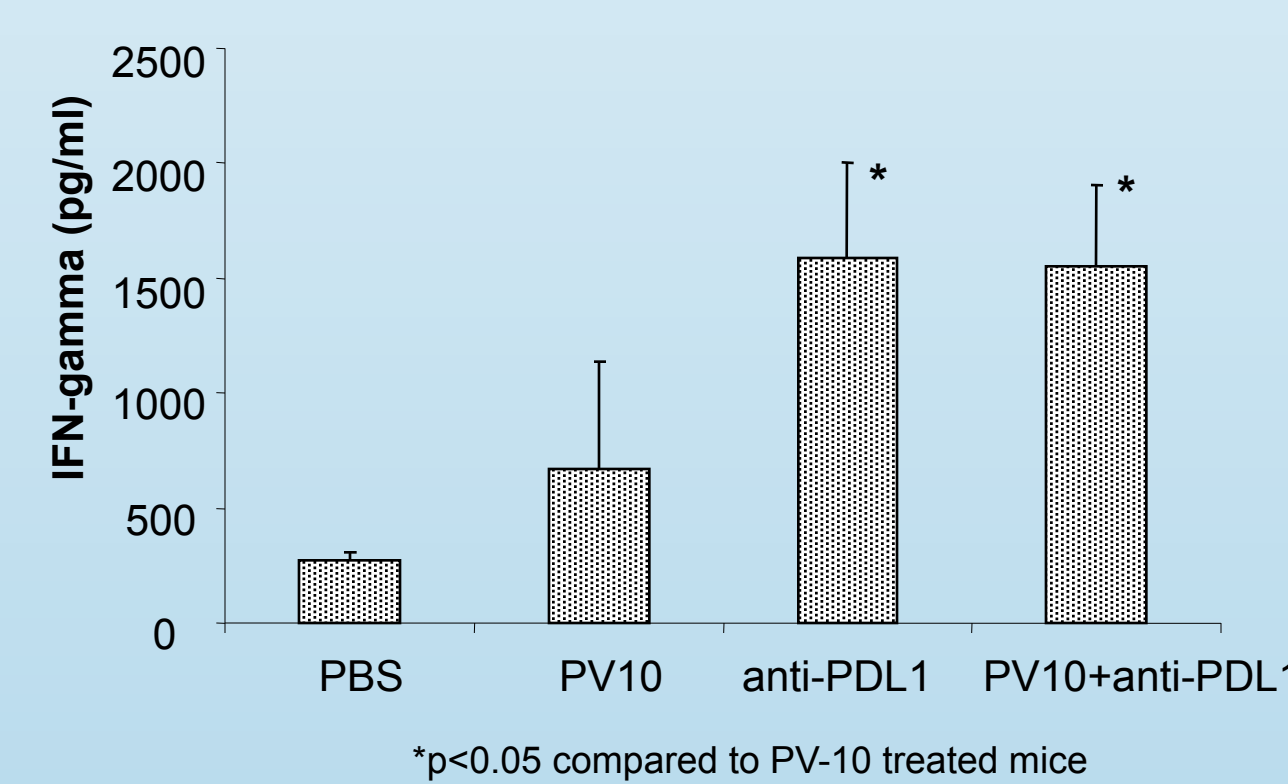
## Results

### Combination Therapy with IL PV-10 and anti-PDL1 antibodies in B16 bearing mice

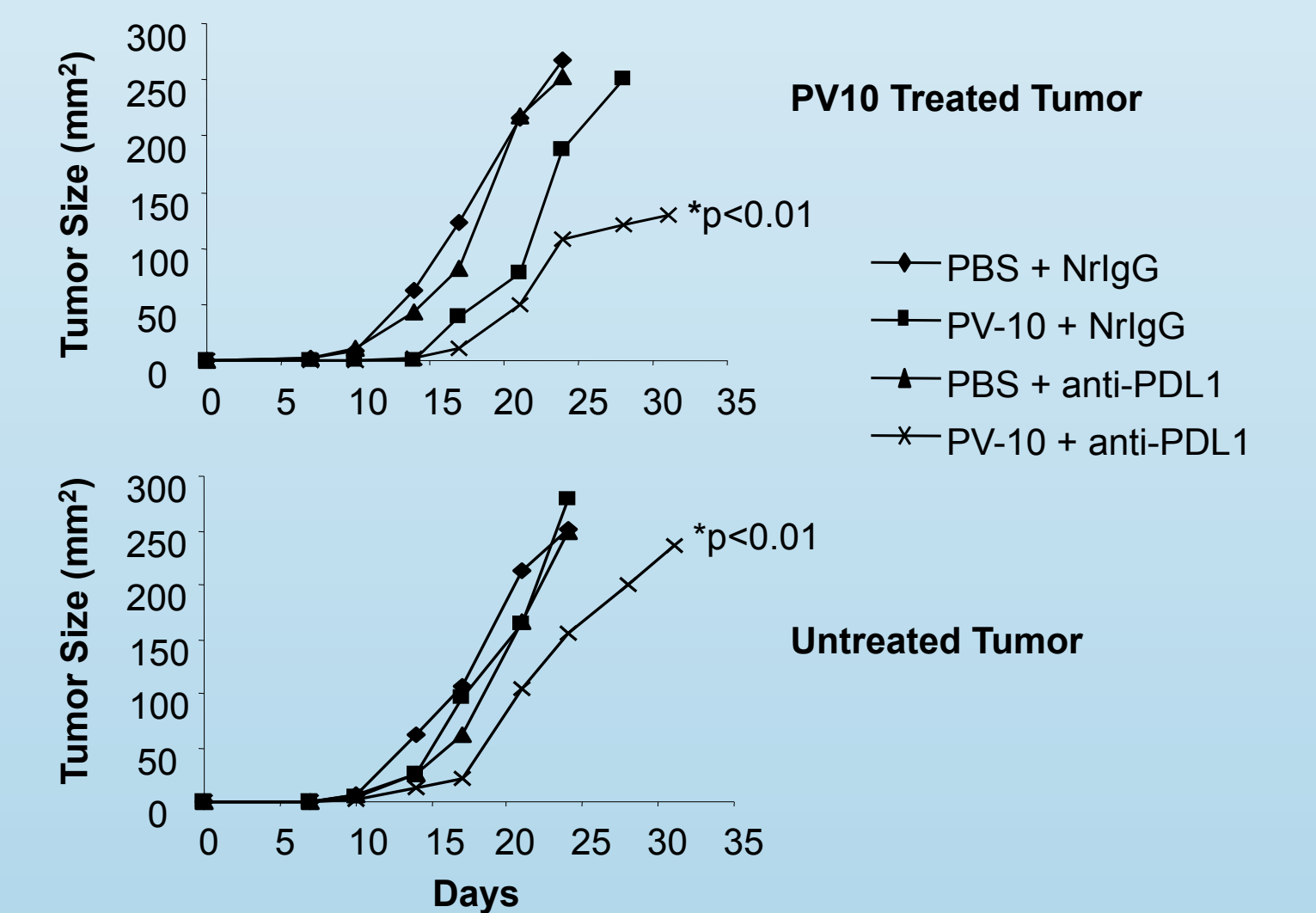
Treatment with anti-PDL1 antibodies in combination with IL PV-10 results in reduced growth of B16 tumor



Treatment with anti-PDL1 antibodies alone or in combination with PV-10 leads to the induction of B16-specific T cells

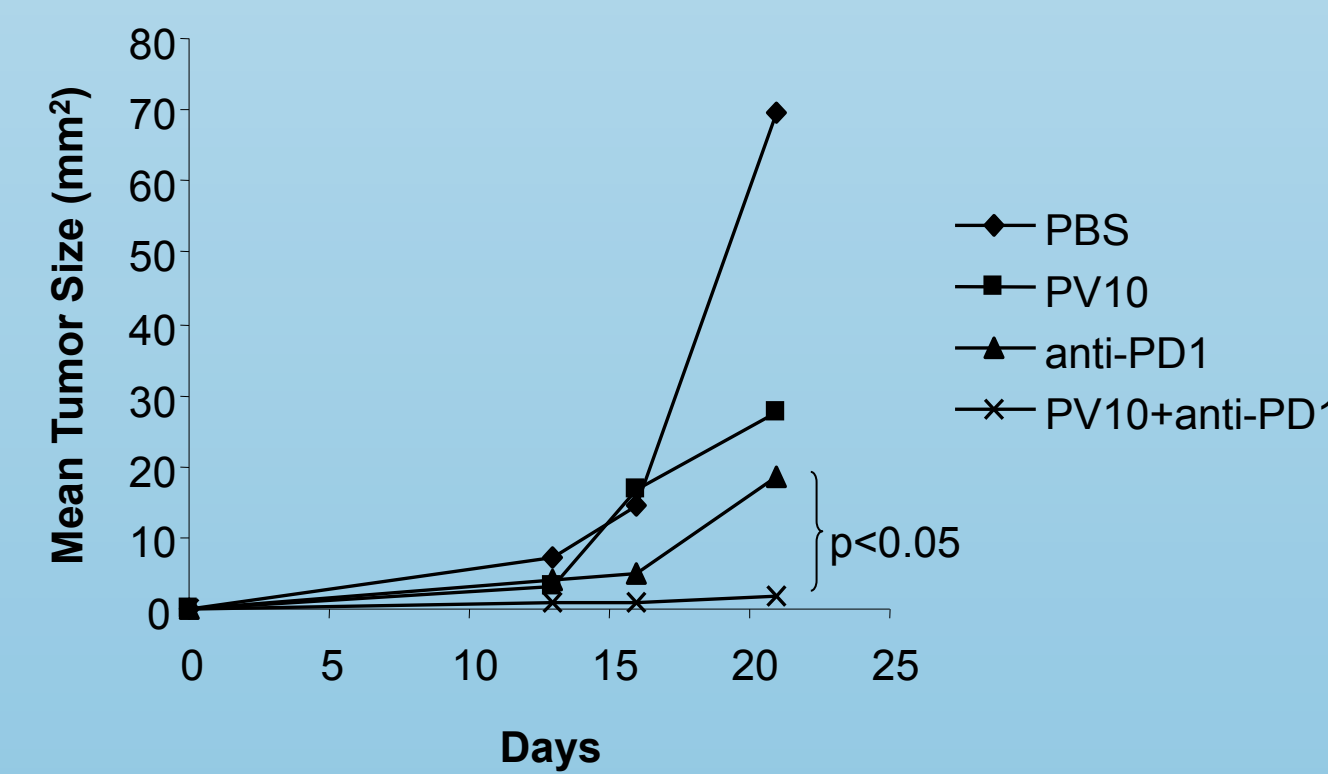


Treatment with IL PV-10 and anti-PDL1 antibodies slows growth of injected and uninjected tumors in a bilateral B16 tumor model

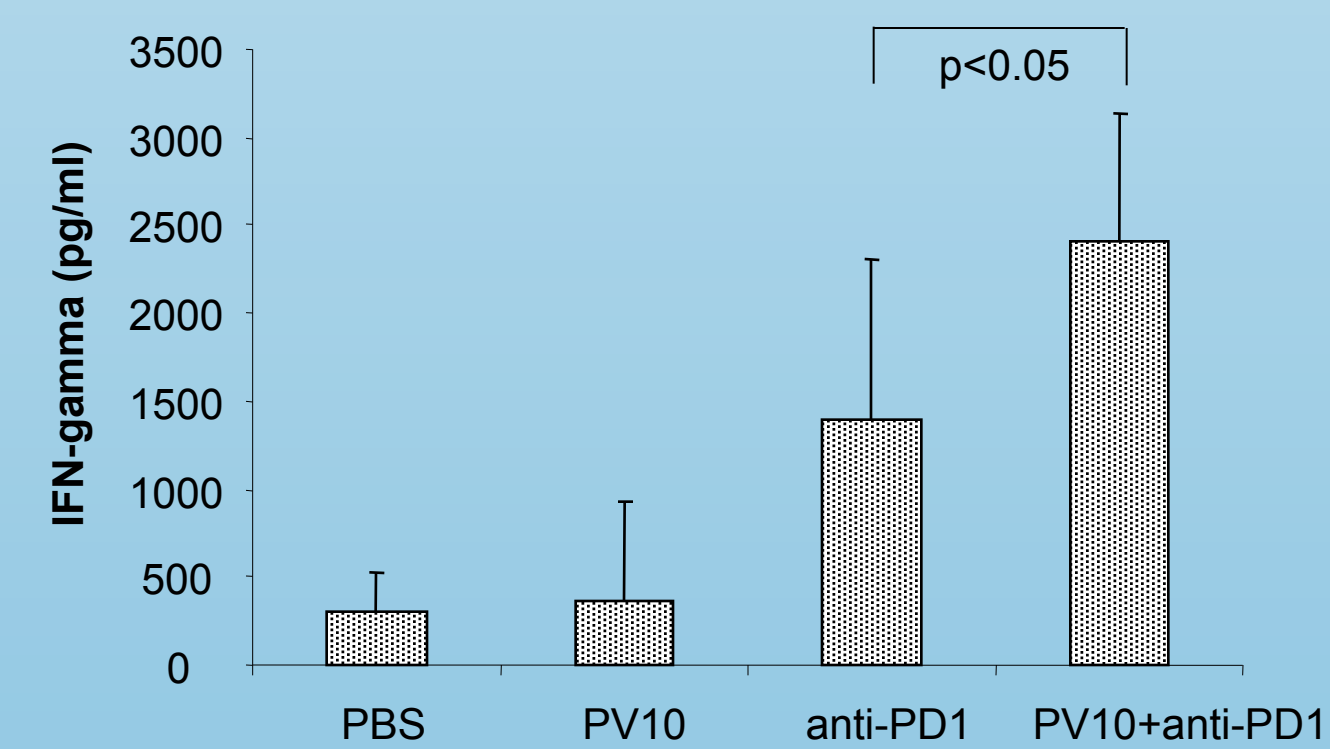


### Combination Therapy with IL PV-10 and anti-PD1 antibodies in B16-OVA bearing mice

Treatment with anti-PD1 antibodies in combination with IL PV-10 results in reduced growth of B16-OVA tumor

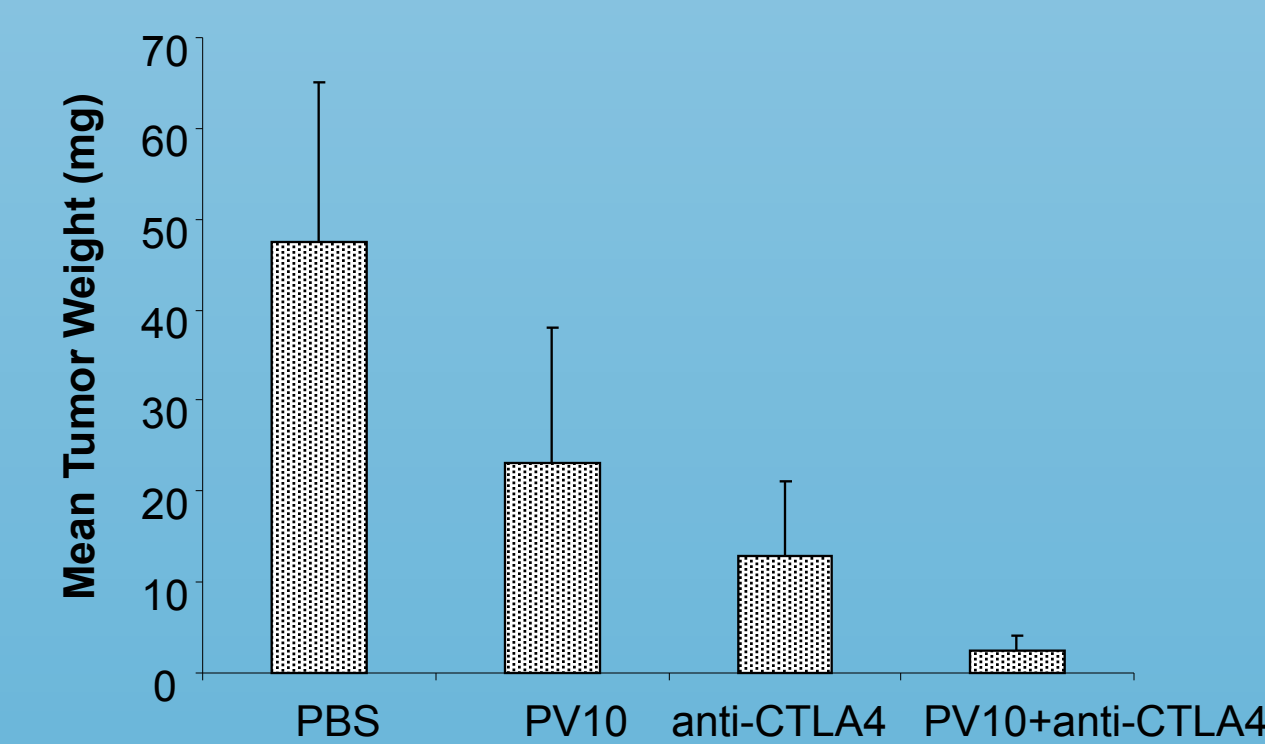


Treatment with anti-PD1 antibodies in combination with IL PV-10 leads to the induction of B16-OVA-specific T cells

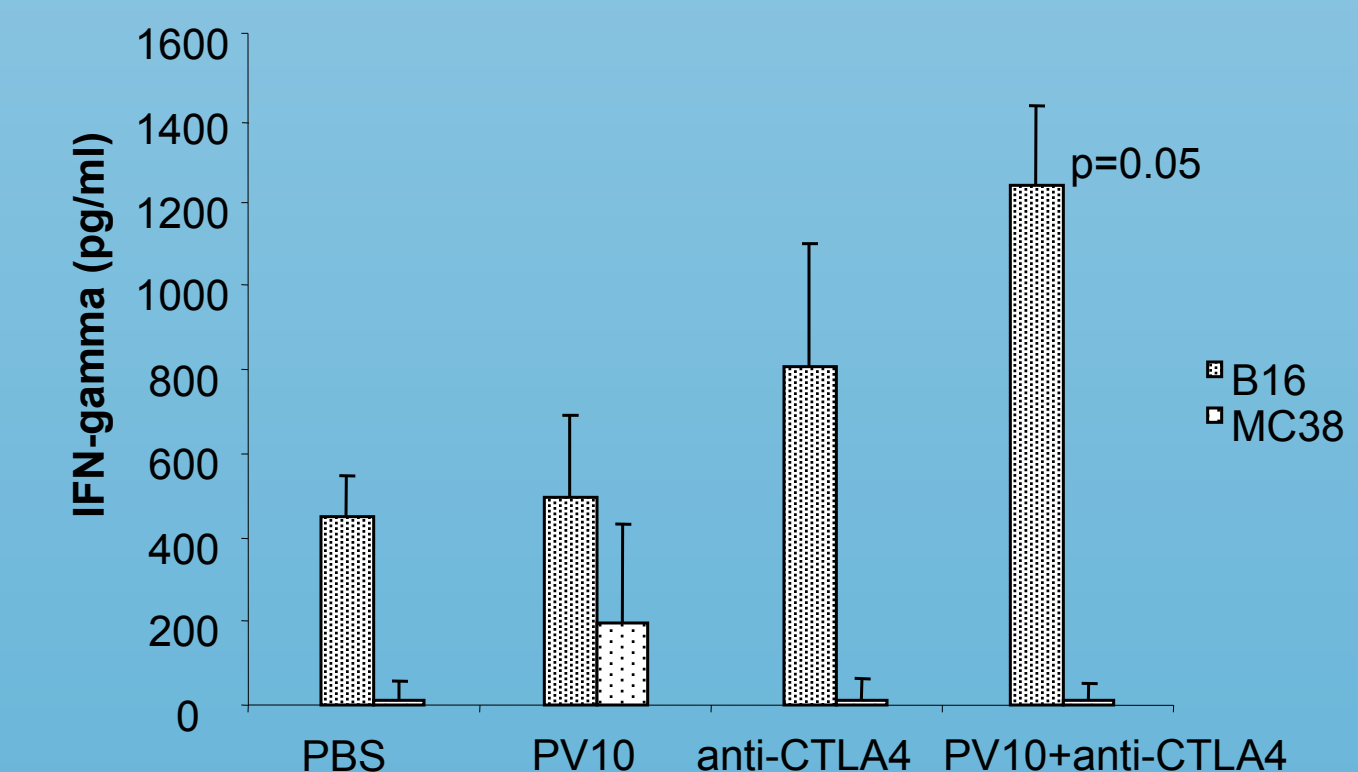


### Combination Therapy with IL PV-10 and anti-CTLA4 antibodies in B16-OVA bearing mice

Treatment with anti-CTLA4 antibodies in combination with IL PV-10 results in smaller B16-OVA tumors



Treatment with anti-CTLA4 antibodies alone in combination with IL PV-10 leads to the induction of B16-specific T cells



## Conclusions

- These murine studies support combination therapy with IL PV-10 and co-inhibitory blockade.
- Combination therapy with IL PV-10 and anti-PDL1 antibodies led to reduced tumor growth in both injected and uninjected bystander lesions. Increased anti-tumor immunity was measured in mice treated with anti-PDL1 antibodies.
- Combination therapy with IL PV-10 and anti-PD1 antibodies led to a reduction in tumor growth and increased tumor-specific T cell activity.
- Combination therapy with IL PV-10 and anti-CTLA4 antibodies led to a trend in tumor reduction and increased tumor-specific T cell activity.

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