



Intralesional Injection of Melanoma with Rose Bengal Induces Regression of Untreated Synchronous Melanoma In a Murine Model

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Introduction

Rose Bengal is a xanthene dye initially used to induce direct anti-tumor responses by producing reactive oxygen species in the presence of light. Serendipitously, injectable Rose Bengal (PV-10) was found to ablate tumors without light and regress uninjected bystander lesions. A Phase I trial of 20 patients with Stage III-IV melanoma who received intralesional injection of PV-10 led to locoregional control (CR+PR+SD) of injected lesions for 75% of patients and locoregional control of uninjected, bystander lesions for 55% of patients. In a Phase II trial, 80 patients were treated with intralesional PV-10 and locoregional control of the injected lesion was achieved in 71% of patients. Of the 38 patients with bystander lesions, 55% had locoregional control. This study was undertaken to confirm the apparent systemic bystander effect of PV-10 and to determine if this systemic effect is mediated by an immunologic anti-tumor response.

Methods

Two models were used to investigate the systemic effects of PV-10. In the initial model, mice received subcutaneous (SQ) B16-F10 melanoma cells to establish a solitary flank tumor and intravenous B16-F10 to establish multiple lung metastases. On day 7, the SQ tumor was treated with intralesional PV-10; intralesional phosphate buffered saline (PBS) was used in control mice. The mice were sacrificed on day 21 to enumerate the lung metastases. In a second model, mice were injected in their bilateral flanks with B16-F10 cells, and the right tumor injected with PV-10 or PBS on day 7. Tumor sizes were measured for both the right (treated) and left (untreated/ bystander) SQ tumors. Splenocytes collected on day 14 were processed for FACS and co-cultured prior to an interferon- γ ELISA.

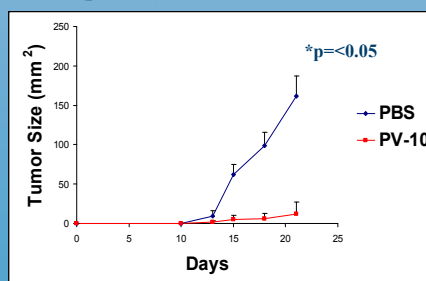
Results

In the first model, C57BL/6 mice treated with PV-10 had significantly fewer lung metastases than mice treated with PBS ($p < 0.05$). Three out of five mice treated with PV-10 had three or less lung metastases; all control mice treated with PBS had over 250 lung metastases.

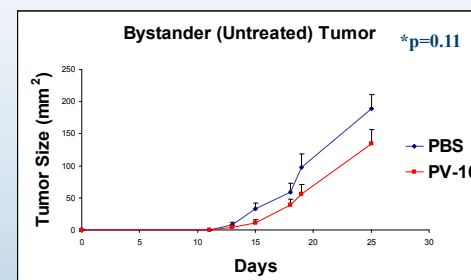
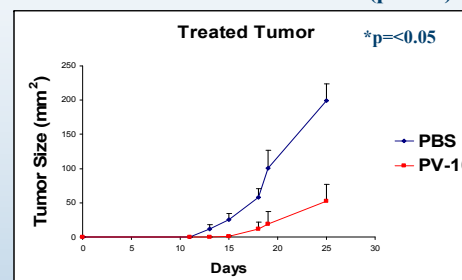
	Number of Lung Metastases
Controls	>250
	>250
	>250
	>250
	>250
PV-10	3
	0
	1
	>250
	>250

$*p < 0.05$

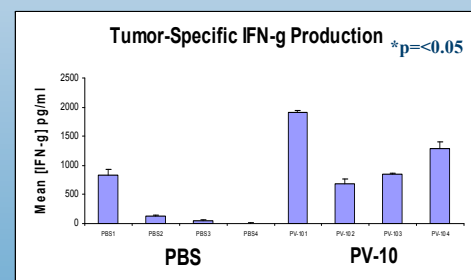
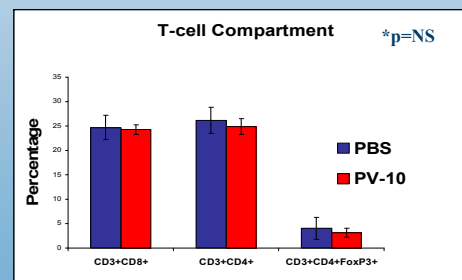
Mice bearing a solitary flank tumor with lung metastases had a decrease in SQ tumor size when treated with intralesional PV-10 ($p < 0.05$).



C57BL/6 mice with bilateral flank tumors had a significant regression of tumors injected with PV-10 ($p < 0.05$), and there was a trend in size reduction in the untreated bystander flank tumor for mice treated with PV-10 ($p = 0.11$).



Splenocytes were analyzed by flow cytometry. There was no difference in T cell subsets between control and treated mice. Splenocytes from PV-10 treated mice produced interferon- γ in response to B16-F10 ($p < 0.05$).



Conclusions

PV-10 has shown promise in treating metastatic melanoma in previous clinical trials. PV-10 has led to objective responses of both injected and uninjected tumors, suggesting a systemic effect. These murine studies confirm that PV-10 chemoablation results in both a direct effect on injected melanoma lesions as well as a systemic response that leads to regression of synchronous lung metastases. Intralesional PV-10 treatment leads to the induction of tumor-specific immunity.

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