

Optimizing vaccine immunotherapy for prostate cancer

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ABSTRACT

Introduction: Treatment options for metastatic prostate cancer are limited and cures are rare. In other cancers immunotherapy has shown remarkable efficacy in certain patient subsets particularly when combined with other treatment modalities. Our previous work showed that combining radiation and vaccine therapy slows the growth of syngeneic prostate cancer in mice more effectively than single modality treatment arms. Here we tested the hypothesis that addition of immune checkpoint inhibitors targeting the PD1/PD-L1 axis further improves efficacy of the dual radiation/vaccine treatment.

Methods: Mice were injected with tumor cells expressing human prostate specific antigen (TPSA-23). The mice were divided into six groups. One group received no therapy. One group received vaccine and radiation alone. The other four groups received radiation, vaccine, and either a PD-1 or PD-L1 inhibitory antibody or its corresponding isotype control. Tumors were measured twice weekly, growth curves generated, and results were analyzed using a 2-way ANOVA test.

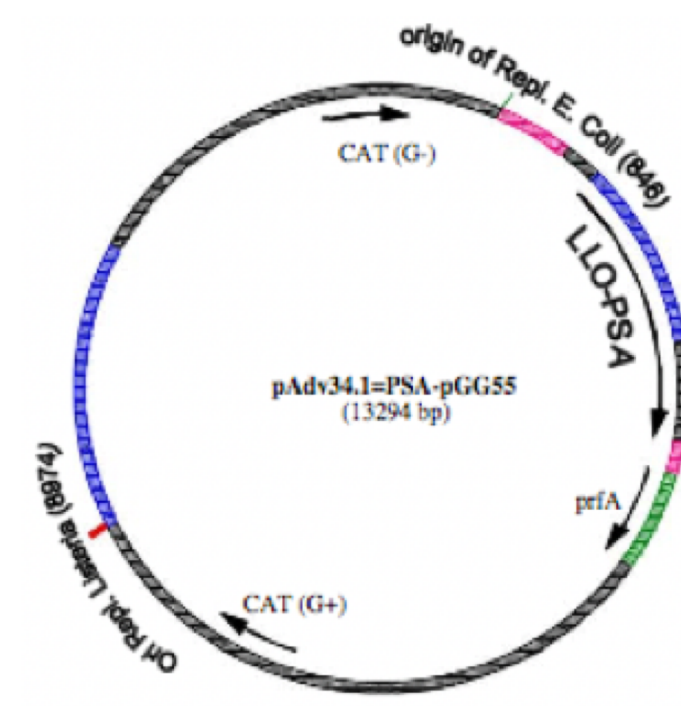
Results: Mice receiving radiation and vaccine therapy demonstrated delayed tumor growth as compared to untreated mice. Addition of the anti-PD-1 antibody was significantly ($p=0.0005$) more effective than dual treatment in delaying tumor growth when compared to corresponding isotype controls at early time points. However, overall survival was not significantly improved by either antibody targeting the PD1/PD-L1 axis beyond the growth inhibition achieved by the radiation/vaccine combination.

Conclusions: Our results suggest that PD1 checkpoint blockade may have utility when added to radiation/vaccine immunotherapy in a murine prostate cancer model. The molecular correlates of these effects are yet to be determined.

BACKGROUND

ADXS-PSA is an attenuated *Listeria monocytogenes* (*Lm*) strain genetically engineered to secrete a fusion protein composed of human PSA and a truncated nonhemolytic form of listeriolysin O LLO (tLLO-PSA).

Lm is taken up by antigen presenting cells (APC) and secretes LLO to escape the phagolysosome and enter the cytosol. The tLLO-PSA fusion protein is processed into peptides, which are subsequently presented to T cells via MHC molecules.



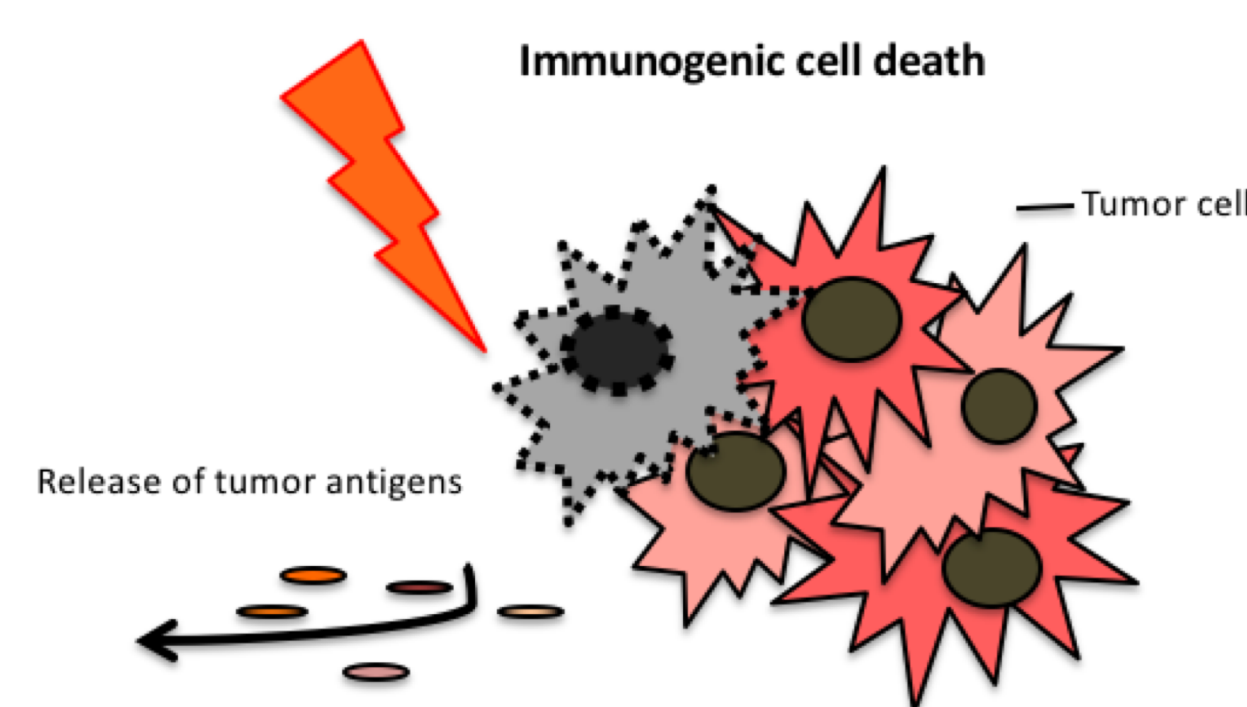
Potential mechanisms of tumor elimination by ADXS-PSA vaccination:

- ↑ Robust innate response to *Lm*
- ↑ Tumor antigen-specific adaptive immunity involving CD8 and CD4 T cells
- ↓ Immune tolerance (Reduced T_{reg} and/or MDSC activity/abundance)

Immuno-radiotherapy utilizes radiation, a common cancer therapeutic modality, to induce immunogenic cell death

The goal of radiation as an immunotherapeutic aid:

- ↑ Tumor antigen release
- ↑ MHC I expression
- ↑ Cell adhesion factors
- ↑ Cytokines and chemokines
- ↑ Recruitment of APCs

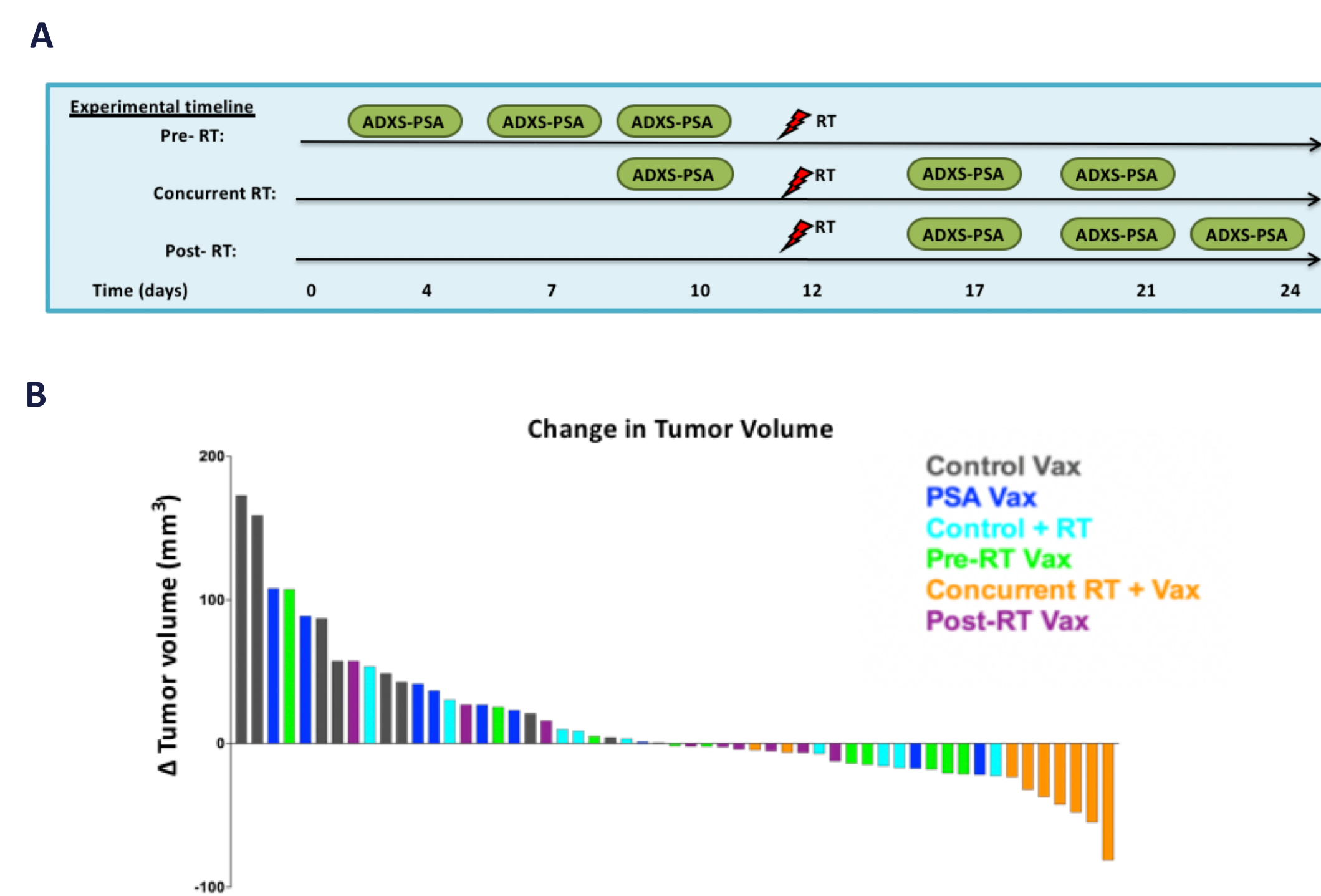


ADXS-PSA and RT combination therapy has shown efficacy in mice bearing TRAMP1 prostate tumor cells engineered to express human PSA (TPSA23).

Hypothesis: Adding checkpoint inhibitors targeting the PD1/PD-L1 axis enhances therapeutic efficacy of dual RT/ADXS-PSA therapy.

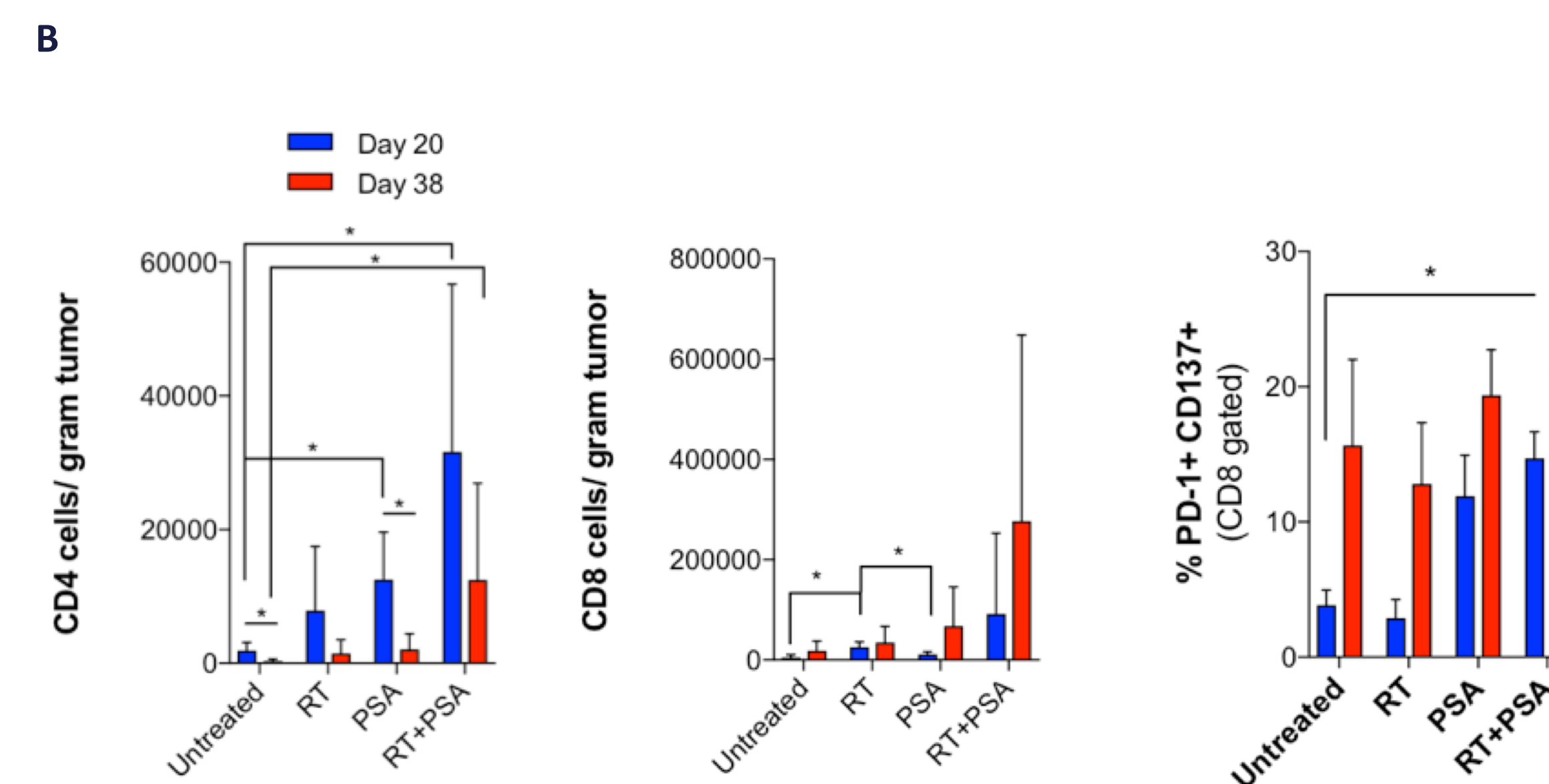
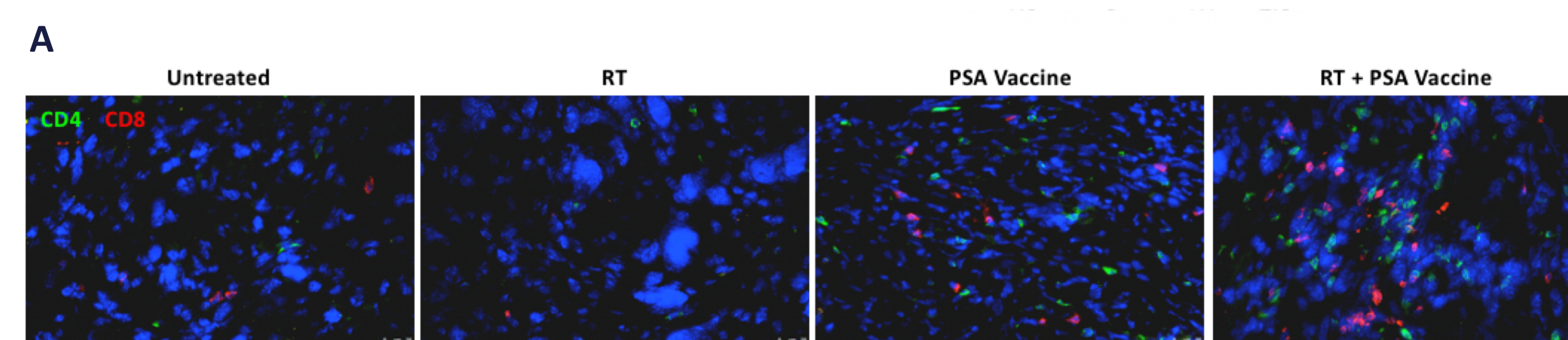
RESULTS

Concurrent administration schedule of ADXS-PSA vaccine and RT effectively inhibits growth of TPSA23 cells transplanted to syngeneic C57BL/6 mice



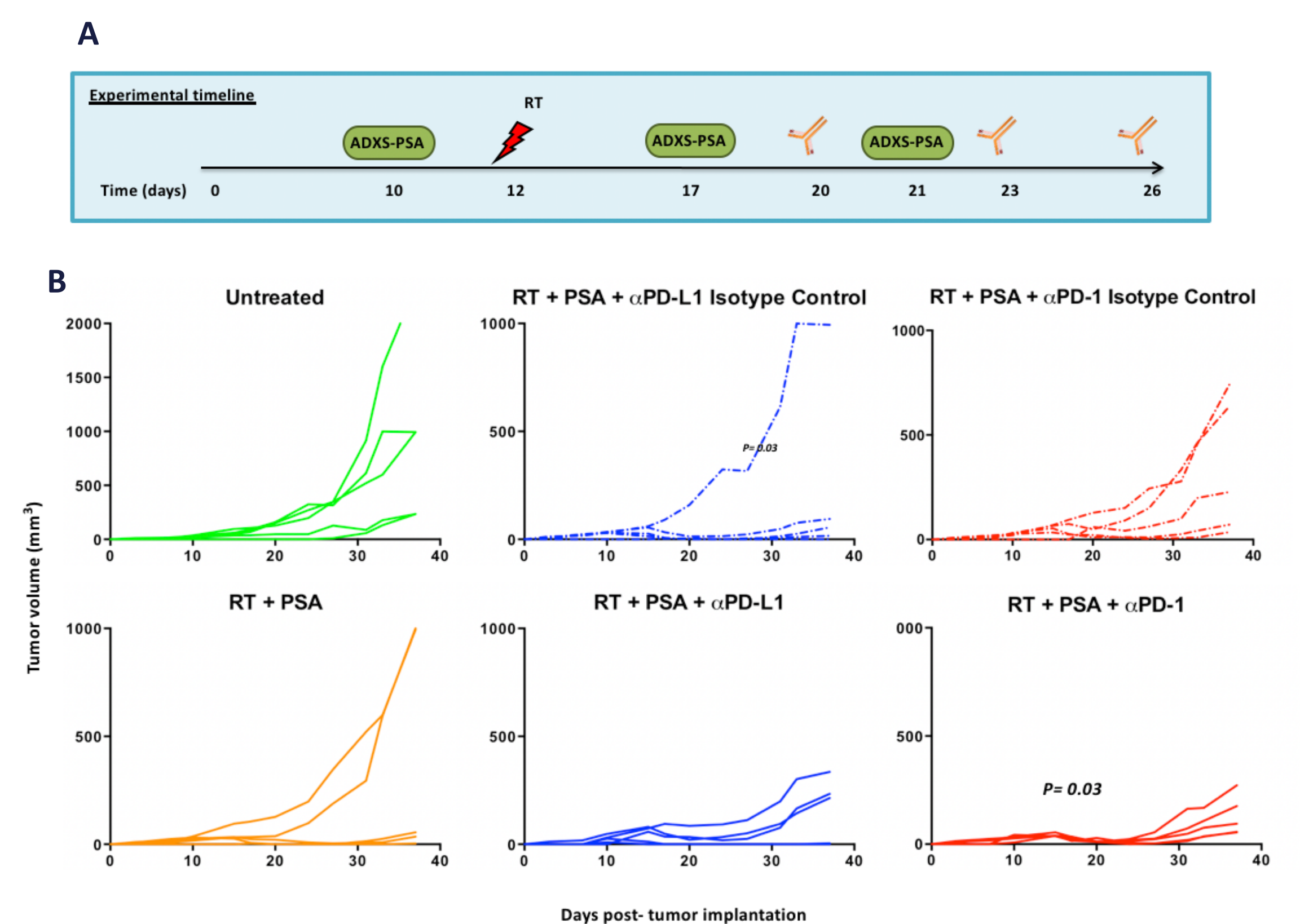
A. Schedules for dual treatment (radiation and PSA vaccine) sequenced differently. B. Concurrent administration of radiation and vaccination most effectively reduces tumor volume measured at day 25 after tumor cell inoculation. Concurrent dual treatment served as a baseline for the treatment schedule used in triple combination therapy experiments (RT/PSA vaccine/PD-1/PD-L1 inhibitors). These results also establish the superiority of dual therapy over single treatment modalities.

Concurrent administration of ADXS-PSA vaccine and RT enhances infiltration of T lymphocytes in tumors and induces PD1 expression in a subset of those T cells



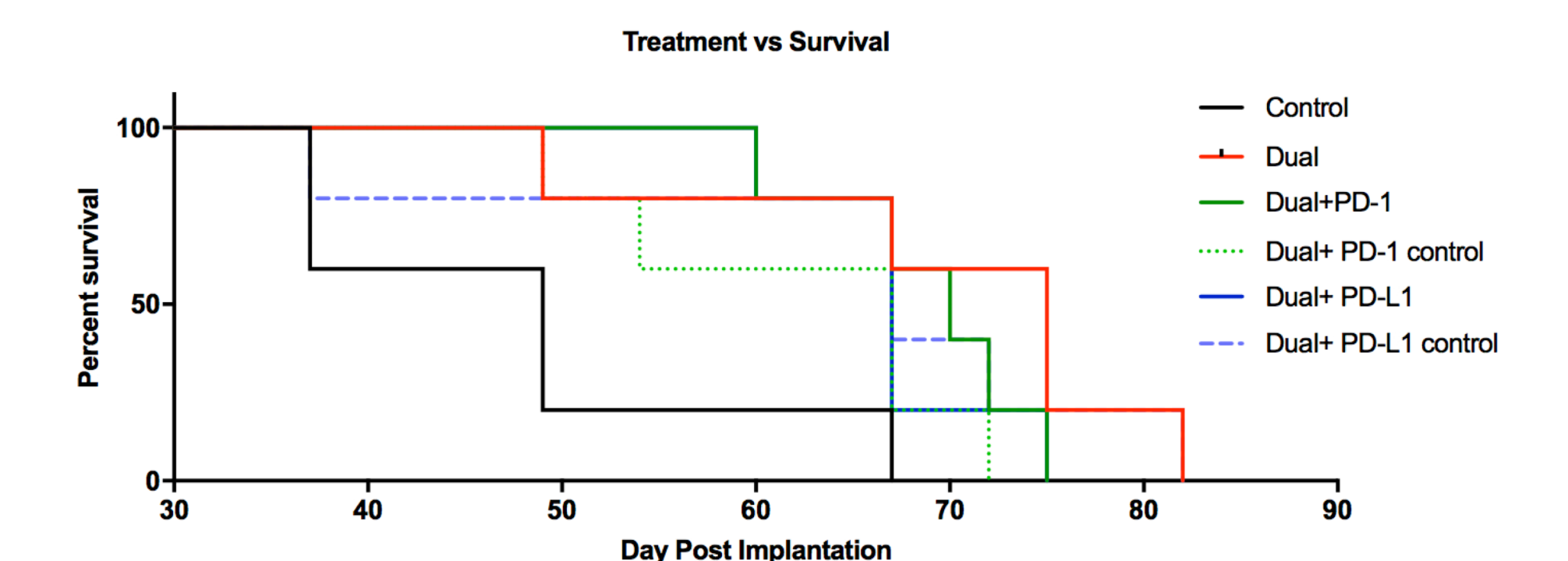
A. Detection of intratumoral CD4+ and CD8+ T cells in tumors by immunofluorescence microscopy. Representative images are shown from tumors in mice receiving no treatment, radiation alone, vaccine alone, or dual radiation and vaccine therapy. Radiation therapy combined with vaccine cooperatively increased intratumoral infiltration with CD4+ and CD8+ T cells. B. Quantitative analysis of intratumoral T cell infiltrates as determined by FACS analysis. Vaccine and dual RT plus vaccine treatment increased abundance of T cells coexpressing PD1 and CD137 on day 20. These cells were also found at high frequency in tumors recurring at day 38 regardless of treatment. These results supported the rationale to target the PD-1 axis following dual treatment (starting on day 20).

Tumor growth inhibition by trimodal radio-immunotherapy targeting the PD1 immune checkpoints upregulated by dual therapy



A. Schedule of treatment for triple combination therapy experiment. TPSA23 tumors were treated with PSA vaccine on days 10, 17, and 21 and irradiated (10 Gy) on day 12. Additionally, an α PD-1 (RMP1-14) or an α PD-L1 antibody (10F.9G2), or an appropriate isotype control, were administered ip on day 20, 23, and 26. B. Spaghetti plots show tumor volume over time. Each line represents tumor volume progression in one mouse. Trimodal radio-immunotherapy including α PD-1 was more effective than dual combination therapy in inhibiting tumor growth at early time points, although all tumors eventually developed resistance and recurred.

Survival of mice treated with dual or triple immunotherapy



Kaplan-Meier representation of survival of mice in different treatment groups. Mice were sacrificed when surrogate endpoints were reached. Despite early tumor growth inhibition, triple treatment did not extend survival when compared to dual therapy.

CONCLUSIONS

- Combination therapy with sub-therapeutic doses of RT and PSA vaccine cooperatively reduces tumor burden compared to single modality treatments, confirming prior observations.
- Survival improved for all groups receiving dual therapy when compared to controls.
- Concurrent administration of vaccine and radiation provides the greatest reduction in tumor growth.
- Therapeutic effects are associated with increased T cell content of tumors.
- Expression of the immune checkpoint receptor PD-1 is increased in recurring tumors.
- Targeting either PD1 or its ligand PD-L1 transiently enhances efficacy of the dual vaccine/RT combination. This effect is statistically significant for anti-PD1.
- However, targeting PD1 or its ligand PD-L1 does not significantly increase overall survival.

ACKNOWLEDGMENTS

This work was supported by Advaxis Immunotherapies and by the Prostate Cancer Foundation (PCF).