would enhance responses in combination with PD(L)1 blockade.

Methods We pooled data on 597 patients with advanced solid tumors on 20 prospective anti-PD(L)1 based trials. Fisher's exact tests were used to compare objective response rates (ORR) and complete response rates (CRR) in patients receiving ARBs or ACE inhibitors (ACEi) to those in patients not receiving ARBs nor ACEi. Log-rank tests and Kaplan-Meier curves were used to compare overall survival (OS) in these same groups. Data were analyzed in tumor types where at least 5 patients were taking ARBs or ACEi. Multiple logistic regression and Cox regression analyses were performed to assess the effect of ARBs on ORR/CR and OS respectively.

Results In total, 597 patients with dozens of tumor types were pooled. Of these, 71 were taking ARBs and 82 were taking ACEi. Three tumor types had at least 5 patients taking ARBs: bladder, ovarian and prostate. ARB use was associated with improvement in ORR (77.8% vs 30.2% ; p=0.019), CRR (55.6% vs 9.3%; p=0.005) and OS (median: not reached vs 14.2 months (95% CI: 7.1–22.0 months; p=0.005) in patients with bladder cancer (n=52), but not ovarian nor prostate cancer. On multivariable analysis, ARB use remained associated with improved ORR, CRR and OS in patients with bladder cancer. Five tumor types had at least 5 patients taking ACEi: prostate, ovarian, colorectal, cervical and bladder. For all five, no benefit was seen in ORR, CRR nor OS with ACEi use (all p>0.10).

Conclusions ARB use was associated with improvement in ORR, CRR and OS in patients with urothelial or bladder cancer receiving anti PD(L)1 based therapy. No benefit was was seen with ARBs in prostate or ovarian cancer nor with ACEi in any tumor type evaluated. The associated benefit seen in bladder cancer with ARBs but not ACEi may be due to selective AT1 blockade by ARBs versus dual AT1/AT2 blockade by ACEi. This data is hypothesis generating and further study is needed to determine if selective AT1 inhibition can improve outcomes when combined with anti PD(L)1 based therapy in bladder cancer and other tumor types.

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457 INTRATUMORAL INTERLEUKIN-12 ADMINISTERED AFTER CRYOABLATION DOES NOT IMPROVE SURVIVAL IN MULTIPLE BILATERAL MURINE MODELS

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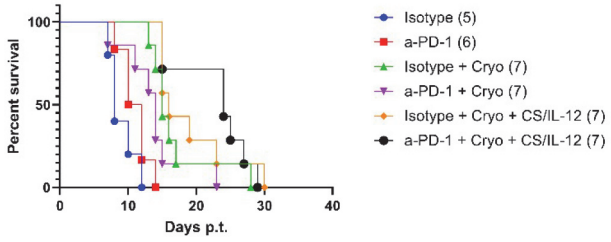
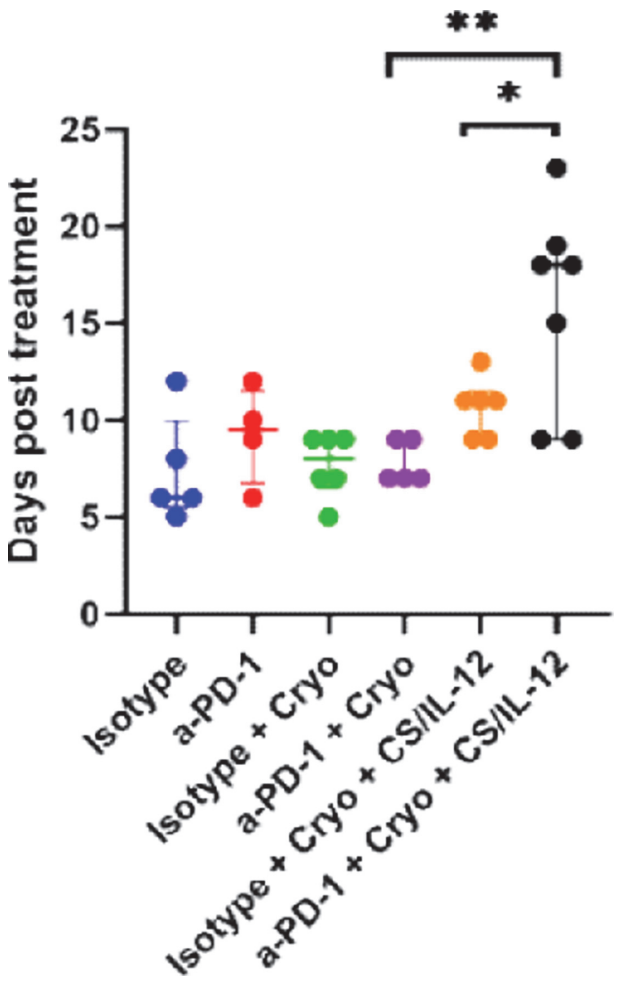
Background Clinically, cryoablation is used to treat certain early stage prostate, liver, and kidney tumors in addition to bone and soft tissue sarcomas. However, for late-stage cancers, ablation is only an auxiliary step before complete resection. This leaves a gap of patients with advanced and inoperable tumors, where up to 90% of all pancreatic, and 80–85% of all prostate cancers are unresectable at diagnosis.[1 2] Because cryoablation can release large amounts of antigen, it is uniquely capable of not only treating advanced, unresectable tumors, but also may induce an in situ vaccination response when combined with the appropriate immunotherapy. Previously, our results in single primary tumor models indicated that the addition of interleukin-12 (IL-12) to cryoablation (cryo) improved tumor burden and survival We hypothesized that intratumoral injection of IL-12 after cryo would activate a strong T cell response and induce systemic immunity in bilateral tumor models.

Methods Panc02 cells were purchased from ATCC; MC38 and MB49 cells were acquired from the NIH. Female C57BL/6 mice were purchased from Jackson Laboratory. For primary tumor implantation, 1×10^6 Panc02 cells and 3×10^5 MC38 cells were injected subcutaneously (s.c.) in the right flank. For rechallenge, the same dose of cells was implanted on the left flank of cured mice. For bilateral models, in both the MB49 and MC38 models, 3×10^5 and 1.5×10^5 cells were injected s.c. in the right and left flanks respectively on the same day. For the Panc02 model, 1×10^6 cells were implanted s.c. on both the right and left flanks on the same day. Tumor volume was calculated as $0.5*a*b^2$ given the perpendicular long (a) and short (b) dimensions. Tumors measuring between 150–300 mm^3 were cryoablated with three cycles of freeze/thaw using the Visual-ICETM Cryoablation System (Boston Scientific). The dose of IL-12 was 1 µg/mouse in 1.5% (w/v) chitosan acetate (CS) dissolved in DPBS, and then injected intratumorally within an hour after cryoablation. For the anti-PD-1 and isotype antibodies (BioXCell, clone: RMP1.14). 300µg was injected intraperitoneally every 3 days starting on the day of cryoablation for a total of 4 doses.

Results In the bilateral MB49 mouse bladder cancer model, the median survival for the cryo alone group was 20 days post treatment (p.t.) compared to 23 days p.t. for cryo + CS/IL-12, which was not significant, and 12 days for the untreated control group. In the bilateral Panc02 model, the median survival for both the cryo alone and cryo + CS/IL-12 groups was the same at 20.5 days p.t., compared to 10 days p.t. for the untreated control. In the bilateral MC38 model, the addition of anti-PD-1 to cryo + CS/IL-12 did not significantly improve survival compared to isotype + cryo + CS/IL-12, with a median survival of 24 days p.t. and 16 days p.t. respectively (p=0.53, Log-rank test) (figure 1). However, addition of anti-PD-1 did significantly delay abscopal tumor growth up to 500 mm^3 when compared to the isotype + cryo + CS/IL-12 (p=0.0398, Unpaired t test) (figure 2). Finally, the addition of IL-12 worsens memory in the MC38 model, where 100% of rechallenged cryo alone mice



Abstract 457 Figure 1 Survival of bilateral MC38
MC38 cells were implanted s.c. in the right and left flanks of C57/BL6 mice at 3×10^5 and 1.5×10^5 cells per 100 uL respectively. The tumor was allowed to grow to between 150 to 300 mm^3 prior to cryoablation. CS/IL-12 was injected intratumorally within an hour after cryoablation. Criteria for euthanasia was a tumor burden greater than or equal to 2000 mm^3 or if mouse became moribund. The median survival for each group was 8 days p.t. (Isotype), 11 days p.t. (a-PD-1), 15 days (Iso + Cryo), 14 days p.t. (a-PD-1 + Cryo), 16 days p.t. (Iso + Cryo + CS/IL-12), 24 days p.t. (a-PD-1 + Cryo + CS/Il-12), p < 0.0001 (Log-rank test). The number of subjects for each group is given in parenthesis in the legend. Abbreviations: CS, chitosan acetate; p.t., post treatment



Abstract 457 Figure 2 Days p.t. for untreated MC38 to reach 500 mm^3

Days post treatment to reach 500 mm^3 was determined as the day on which the untreated tumor volume measurement exceeded 500 mm^3 for the first time and growth continued to increase afterward for at least two days. Statistics: One-way ANOVA * p<0.05, ** p<0.01

were protected (5/5) compared to only 43% protected of the cryo + CS/IL-12 group (3/7).

Conclusions Conclusions: While cryoablation in combination with immunotherapy has the potential to treat advanced, unresectable primary tumors and distant untreated tumors, the addition of a single injection of IL-12 is not enough to induce a strong abscopal effect. Furthermore, it may actually worsen the establishment of effector memory cells. The addition of anti-PD-1 only slows abscopal tumor growth. Future work is needed to understand the mechanism of T cell priming in the context of the post-ablative tumor.

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458 **ANTITUMOR MECHANISMS OF LOCAL RADIATION AND COMBINATION IMMUNOTHERAPY IN AN IMMUNOLOGICALLY COLD MODEL OF NEUROBLASTOMA**

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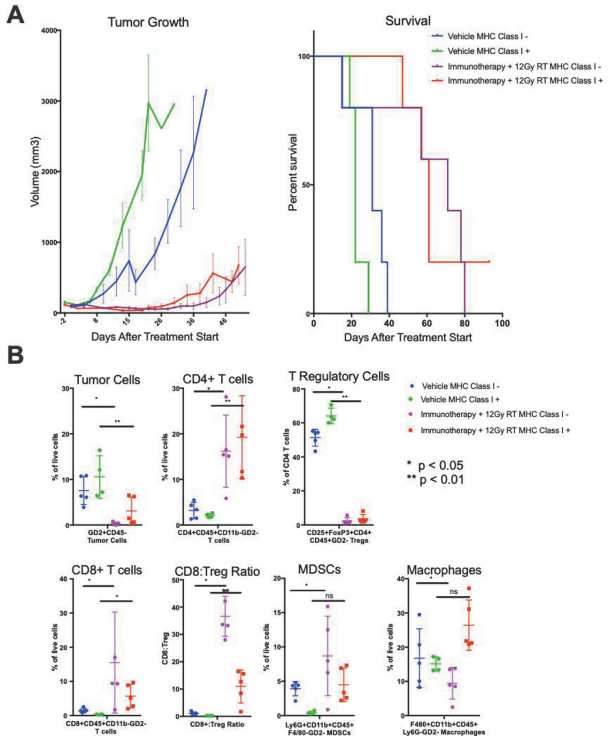
Background The standard treatment for high-risk neuroblastoma includes a combination immunotherapeutic approach consisting of IL-2, GM-CSF, and monoclonal antibodies directed against GD2, a disialoganglioside preferentially expressed in neuroblastoma and melanoma.[1] We recently described an effective a preclinical in-situ vaccination strategy combining local radiation therapy (RT), IL-2-linked to anti-GD2 monoclonal antibody (intratumoral immunocytokine, IT-IC), checkpoint inhibition (anti-CTLA4), and drivers of innate immunity (anti-CD40 and CpG).[2] This strategy is effective in curing mice with immunologically-cold neuroblastoma. We sought to better characterize the anti-tumor mechanisms that mediate this effect.

Methods Mice bearing GD2-expressing, immunologically-cold neuroblastoma tumors (9464D-GD2) were treated with 12Gy RT and combination immunotherapy (IT-IC, anti-CTLA-4, CpG, anti-CD40) over 12 days as previously described.[2] Depletion of individual immune cell sets during treatment was



Abstract 458 Figure 1 Effect of MHC class I expression on response to RT and combination immunotherapy (IT-IC, anti-CTLA4, anti-CD40, CpG). A) Increased MHC class I expression in 9464D-GD2 derived tumors did not alter tumor growth or survival following treatment. B) Increased MHC class I expression did not alter immune subsets following treatment of 9464D-GD tumors with radiation and combination immunotherapy. Increased numbers of CD8+ and CD4+ T-cells was observed with both moderate and absent MHC class I expression. T regulatory cells were also effectively depleted in both treated groups