with this combination immunotherapy strategy in pancreatic cancer.

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454 ONCOLYTIC PARAINFLUENZA VIRUS 5 VECTOR ENHANCES NATURAL KILLER CELL KILLING OF LUNG TUMOR CELLS IN 2D AND 3D SPHEROID CULTURES

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Background Natural killer (NK) cells are innate immune cells with natural cytotoxicity towards both tumor cells and virus infected cells. We have developed a particle-based method for in vitro specific expansion of NK cells that yields highly cytotoxic NK cells (PM21-NK cells). There is intense interest in the use of novel oncolytic viruses with the potential to synergize with immune cells to kill tumor cells. Here we have tested the hypothesis that infection with a tumor-selective cytopathic Parainfluenza virus 5 (PIV5-P/V) vector will enhance PM21-NK cell-mediated killing of lung cancer cells in both 2-dimensional (2D) and 3-dimensional (3D) cultures.
Methods In 2D cultures, live cell time-lapse imaging, flow cytometry and luminescence-based methods were used to assess the killing efficiency of PM21-NK cells against A549 lung tumor cells infected with PIV5-P/V. Blocking antibodies were used to evaluate different NK cell activating receptors involved in recognition of infected tumor cells. IncuCyte live cell imaging system was used to assess real time killing of 3D lung spheroids by a combination of NK cells and PIV5-P/V virus. Z-stack spheroid images were captured using Keyence microscope.
Results In 2D cultures, PM21 NK cells efficiently kill A549 cells that have been infected with P/V CPI- virus and enhance the overall rate of killing compared to uninfected cell targets. Antibody blocking showed that the viral Hemagglutinin-Neuraminidase (HN) glycoprotein and NK cell receptors NKp30, NKp46 and NKG2D were involved in PM21-NK cell recognition of PIV5-P/V infected A549 cells. In 3D cultures of A549 tumor spheroids, PIV5-P/V infection was limited to the outer layer of the spheroid, with restricted spread of the infection to inner compartments. However, addition of PM21-NK cells to PIV5-P/V-infected spheroids resulted in killing of not only the infected surface of the spheroid but continued to the uninfected cells located at the center of the spheroid.
Conclusions Our data support the potential of combining oncolytic virotherapy along with PM21-NK cell adoptive therapy against lung cancer.

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455 IMPACT OF EPHB4 AND PD-1 TREATMENT ON IMMUNE INFILTRATE IN ADVANCED BLADDER CANCER

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Background Bladder cancer is the fourth most common cancer in American men with chances of 1 in 27 developing this form of cancer. Despite the progress in treating these patients with immunomodulatory agents, the vast majority of patients remain refractory to therapeutic intervention. EphB4 and EphrinB2 are induced in the tumor vasculature and modulate immune response within the tumor microenvironment. Intervention blocking Ephrin and PD-1/PD-L1 pathway has shown promising data in preclinical models. These data form the basis of clinical investigation of combined therapy in bladder cancer and other tumor types.
Methods Preclinical mouse models were treated with decoy soluble EphB4 and tumor infiltrating immune cells were profiled by RNA expression analysis post-treatment and compared to control treated mice. Next, patients were treated with soluble Eph4B in combination with anti-PD1 therapy, biopsies were obtained prior to and during the course of treatment. Biopsies were used for analysis of localized protein and RNA expression by GeoMx Digital Spatial Profiling (DSP). DSP analysis focused on tumor rich regions of interest (ROIs), adjacent stromal immune populations and microniches around vascular sites, with emphasis on sites where CD45+ T-cells were observed to be surrounding capillaries within and surrounding the tumor, presumably from extravasation.
Results In preclinical mouse models, EphB4 was found to induce several inflammatory pathways as a monotherapy including key immunomodulatory checkpoints such as PD1, PDL1, PDL2. Similarly, patients enrolled in this study were observed to have elevated T-cell infiltration in primary and secondary tumor sites, resulting in tumor mass reduction in post-treatment observations. DSP between matched samples discovered interesting differences in T-cell populations between both protein and mRNA expression. We observed evidence of tumor-debulking by decreased expression of epithelial markers such as Pan-cytokeratin and S100B within tumor ROIs, and increased infiltration within these ROIs measured by immune cell markers such as CD3 and CD163. Additionally, we observed increased GZMA expression post-treatment in perivascular regions suggestive of higher ongoing response by cells entering the tumor microenvironment. Additional analysis of localized RNA expression provided further support for activation of inflammatory cascades in post-treatment samples.
Conclusions These discoveries provide insights into the mechanism of action of EphB4 combination therapy in bladder cancer, providing support for a role of EphB4 acting as an adjuvant for PD1 therapy. Our results highlight the ability of EphB4 to activate the immune system both in preclinical models and in key structures within the tumor microenvironment during combination therapy.
Trial Registration NA
Ethics Approval The studies were approved by USC IRB Protocol 4B 15-11 and IACUC Protocol 20570.

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456 IMPACT OF ANGIOTENSIN II PATHWAY INHIBITION ON TUMOR RESPONSE TO ANTI PD(L)1 BASED THERAPY

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Background Angiotensin II (Ang II) has been shown preclinically to increase VEGF and TGF-β expression through AT1 receptor signaling but to decrease VEGF and TGF-β through AT2. Thus, we hypothesized that the ang II pathway might have a role in carcinogenesis and immune evasion and selectively inhibiting AT1 via angiotensin receptor blockers (ARBs)

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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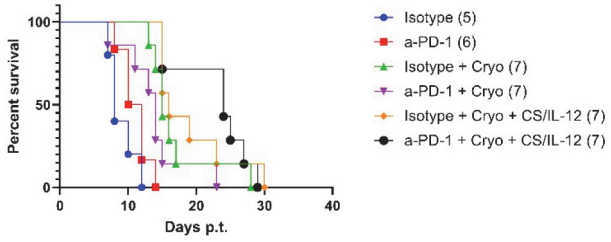
Background Clinically, cryoablation is used to treat certain early stage prostate, liver, and kidney tumors in addition to bone and soft tissue sarcomas. However, for late-stage cancers, ablation is only an auxiliary step before complete resection. This leaves a gap of patients with advanced and inoperable 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