with ICG; tumor specific hyperthermia was then generated with a near infrared laser. The target thermal dose was 42-45°C for 5 minutes to stimulate tumor immunity rather than lethal hyperthermia for ablation. Harvested tissue was then analyzed with immunohistochemistry, flow cytometry, bulk RNA sequencing, and single cell RNA sequencing (scRNAseq).

Results: We found that the anatomic, cellular, and molecular features of this model recapitulate the characteristics of advanced human HCC; transcriptomic evaluation of the cirrhotic tissue using bulk RNAseq revealed upregulation of numerous hallmark gene pathways that are commonly associated with human cirrhosis, and transcriptome analysis of orthotopic HCC tumors clustered closely with advanced human HCC. MTPA as a monotherapy and in combination with immune checkpoint therapy significantly increased intratumoral CD3+ and activated CD8+ T cells while decreasing regulatory T cells relative to control or immune checkpoint therapy alone based on immunohistochemistry, flow cytometry, and single cell RNA sequencing data. Furthermore, we identified evidence of MTPA's influence on systemic tumor immunity, with suppression of remote tumor growth following treatment of orthotopic tumors.

Conclusion: Tumor-specific hyperthermia may help overcome resistance mechanisms to immunotherapy in advanced HCC.

Abstract No. 332

Transcatheter intra-arterial local immunotherapy of hepatocellular carcinoma using high affinity anti-programmed cell death ligand-1 antibody-nanoconjugates

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Purpose: Local immune checkpoint inhibitor (ICI) immunotherapy might be essential to overcome ICI immunotherapy resistance, immune tolerance, and irAEs in immune-suppressive hepatocellular carcinoma (HCC). Herein we evaluate the therapeutic efficacy of intra-arterial (IA) local immunotherapy of HCC using anti-PD-L1 mAb (aPD-L1)-nanoconjugates.

Materials and Methods: An engineered Z-domain (Z), an immunoglobulin G (IgG) Fc-specific binding protein, is introduced to Ferumoxytol (Fer) for Z-Fer and aPD-L1 was conjugated with Z-Fer. Affinity and avidity of engineered Z mediated aPD-L1 nano-conjugates (aPD-L1-Z-Fer) to block the PD-1/PD-L1 axis were evaluated *in vitro* and *in vivo*. For evaluating *in vivo* therapeutic potential of local delivered aPD-L1-Z-Fer, N1S1 rats (n=30) were divided into 5 groups (control, IV injected aPD-L1, IA infused aPD-L1, IA infusion of covalently binding aPD-L1-Fer nano-conjugates, and IA infusion of Z-domain mediated aPD-L1-Fer nano-conjugates (10 mg/kg of aPD-L1). Tumor response was monitored with MRI for 14 days. Immunohistology of tumor tissues was performed with H&E, TUNEL, and CD3 staining. Immune responses including CD3, CD4, CD8, Treg, and inflammatory cytokines were investigated with flowcytometry and cytometric beads array kits.

Results: Engineered aPD-L1-Z-Fer ICI formulation demonstrates enhanced affinity and avidity for effective IC blockade of PD-L1 expressed HCC cells *in vitro*. Transcatheter-directed hepatic IA local aPD-L1-Z-Fer ICI immunotherapy showed superior *in vivo* HCC tumor suppression and immune conversion compared with systemic aPD-L1 or chemically conjugated aPD-L1@Fer. Relative tumor growth volume changes of the group treated with IA infusion of aPD-L1-Z-Fer were each 8.1, 5.3, 6.0, and 3.6 times less than aPD-L1 (IV), aPD-L1 (IA), and aPD-L1@Fer (IA) groups. Anti-cancer immune modulation of the IA infused aPD-L1-Z-Fer induced a decrease of MDSC and Treg with an increase of functional cytotoxic T lymphocytes (CTLs) in tumor-infiltrating lymphocytes (TILs) compared with other groups.

Conclusion: Transcatheter-directed local ICI immunotherapy using aPD-L1-Z-Fer will provide a promising potential of local HCC immunotherapy and opportunity for advanced combinational immunotherapies. Future studies that can validate the therapeutic efficacy of the transcatheter local immunotherapy are warranted for the potential clinical translation.

Abstract No. 333

Improved delivery of a TLR-9 agonist to liver tissue by intravascular pressure enabled drug delivery (PEDD) compared with direct needle injection

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Purpose: Direct injection of the TLR-9 agonist SD-101 oligonucleotide has shown promising results for the treatment of superficial cutaneous melanoma metastatic lesions in combination with checkpoint inhibition. However, this mode of delivery is limited to easily discernable tumors located in superficial locations. Here we compare local infusion of labeled SD-101 into hepatic tissue using the Pressure Enabled Drug Delivery (PEDD) method vs. direct needle injection. PEDD has been shown to increase the concentration and penetration of therapeutics into tumors, while limiting exposure to off-target tissue.

Materials and Methods: The study was conducted on female swine (n=8, 45-65kg). The first cohort (n=4) received a hepatic arterial (HA) PEDD infusion of the TLR-9 agonist oligonucleotide ODN 2395 conjugated to an IRD800 nearIR fluorophore (ODN2395F), using a TriNav device (TNV-21120-35, TriSalus Life Sciences). This was followed by needle injection of the TLR-9 agonist oligonucleotide SD-101 conjugated to a Cy5.5 fluorophore (SD-101F). The second cohort (n=4) received HA PEDD infusion of SD-101F followed by injection of ODN2395F. Needle injections and PEDD infusions were delivered to contralateral hepatic lobes. NearIR imaging was performed on 1 cm thick sections of the organ using the Pearl Trilogy Imaging System to quantify the volume and the relative concentration of oligo present in the tissue as determined by magnitude of fluorescence.

Results: The volume of liver tissue containing the oligo was significantly greater using PEDD ($99.0\pm28.8 cm^3$ SE, n=8) than that by injection ($13.5\pm2.9 cm^3$ SE, n=8) ($P=0.011$). PEDD distributed the therapeutic within the targeted vascular network, producing a measurable uptake throughout the infusion zone. Direct injection of the oligos often resulted in a confined 1-2 cm diameter regions of treated tissue. PEDD also resulted in a

significantly higher concentration of oligo retained in the tissue relative to direct injection. Quantification of tissue luminous intensity (kiloluminous units, klu) revealed 49.0±10.1 klu SE in PEDD treated tissues vs 19.3±6.35 klu SE for direct injection (P=0.015).

Conclusion: The results of this study illustrate the advantage of the PEDD method for HA infusion of therapeutics such as SD-101 directly into hepatic tissue. A 7-fold increase in treated tissue volume and a 2.5-fold increase in therapeutic delivery using PEDD in the normal porcine liver relative to direct needle injection suggest that PEDD offers a potentially effective means of treating diffuse intrahepatic disease through targeted delivery.

Abstract No. 334

Drug distribution maps with CT after direct co-injection with contrast agent in ex vivo tissue

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Purpose: Intratumoral injections pressurize fluid that can distribute a drug over a large volume, but the distribution is highly variable because fluid may rupture tissue and take a path of least resistance. This study was designed to inform technique in a clinical trial injecting a recombinant immunotoxin with a prescribed 4mL injection volume. A secondary goal was to determine if drug distribution may be approximated from contrast distribution on CT to facilitate future intra-procedural evaluation.

Materials and Methods: E*x vivo* bovine liver was injected with 1, 2, or 4 mL (n=3, rate = 1 mL/min) solutions of 32 mg/mL iodixanol mixed with 2.5 mg/mL fluorescently labeled (FITC) albumin, a surrogate of similar size to the drug of interest. After an Injection the liver was frozen, sectioned through widest cross-section in triplicate, and imaged with fluorescent microscopy. The FITC-albumin distribution volume was defined by outlining the fluorescent region and approximated as a sphere. The iodixanol distribution volume was determined using 3DSlicer by thresholding above baseline liver radiodensity. Concentration was quantified with calibration standards. Relative concentration was defined as the concentration within tissue divided by the pre-injection concentration.

Results: CT and fluorescence imaging of injected tissue showed iodixinal distribution over a larger volume than FITC-albumin. However, there is a strong correlation (r^2 = 0.92) between the distribution volumes of both molecules. Unique transport behaviors of each particle were shown by analysis of the distribution volume and concentration profiles as a function of distance from the centroid. Iodixanol dilutes within tissue (relative conc. < 1) and its concentration exhibits a strong radial dependence with a maximum at the centroid. In contrast, FITC-albumin is more concentrated than its pre-injection concentration (relative conc. > 1) with a weak radial dependence.

Conclusion: CT imaging of a contrast agent may roughly predict distribution of a co-injected drug in tissue despite different physiochemical properties. FITC-albumin and iodixanol have a strong correlation between distribution volumes, but differences in concentration and spatial distribution may inform clinical injection techniques. Drug surrogate became concentrated centrally at injection sites. These differences may depend on the injection parameters (e.g., volume, rate), drug properties (e.g., size, charge, binding affinity) and tissue properties (e.g., permeability, interstitial pressures) and will be the subject of future investigation.

Abstract No. 335

Use of metformin and survival in patients with hepatocellular carcinoma (HCC) undergoing liver directed therapy: a SEER-Medicare analysis

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Purpose: Metformin is increasingly recognized for its anti-tumor properties in hepatocellular carcinoma (HCC). This study examines the association of metformin use with overall survival (OS) among patients with HCC undergoing image-guided liver-directed therapy (LDT): thermal ablation, transarterial chemoembolization (TACE), or Y-90 radioembolization (Y-90 RE).

Materials and Methods: Using linked National Cancer Institute Surveillance, Epidemiology, and End Results (SEER) registry and Medicare claims databases between 2007-2015, we identified patients with a diagnosis of HCC at age ≥ 66 who underwent LDT within 30 days of diagnosis. Patients who had a liver transplant, surgical resection, or other malignancy were excluded. Metformin use was identified by at least two Medicare Part D prescription claims within 6 months prior to LDT. Overall survival (OS) was measured by the time between the first LDT and date of death or last documented Medicare observation. Comparisons of baseline demographics, clinical characteristics, and OS after LDT were performed between all patients on and not on metformin, as well as diabetics on and not on metformin. Demographics and clinical characteristics were compared using chi-square testing. OS was compared using Kaplan-Meier estimation with a log-rank test. Hazard ratio (HR) of death using multivariate analysis with Cox-proportional modeling was used to identify predictors of prolonged OS (P=0.05).