Abstract No. 244

Characterization of Intratumor Heterogeneity in a Porcine Hepatocellular Carcinoma Model

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Purpose: In 2022, there are expected to be ~41,000 new liver cancer cases and ~30,500 deaths, of which 75% will be due to hepatocellular carcinoma (HCC). The Oncopig is a promising translational large animal cancer model that can recapitulate human HCC phenotypes due to its similarities with humans in terms of size, anatomy, liver metabolism, and genetics. Previously, with whole genome sequencing, we identified Oncopig HCC intratumor heterogeneity that resembled signatures observed in human HCC tumors. In this study, we designed a whole exome sequencing kit to decrease sequencing costs and aimed to further investigate genes and relevant pathways implicated in HCC carcinogenesis in our Oncopig HCC model.

Materials and Methods: An Oncopig HCC tumor was induced following intrahepatic autologous injection of Oncopig HCC cells into a cirrhotic liver microenvironment. The Oncopig HCC tumor was biopsied at 5 different locations within the tumor to characterize intratumor heterogeneity. Tumor fragments, the injected HCC cell line, and a control kidney sample were sequenced and aligned to the pig reference genome (Sscrofa11.1) using BWA MEM. Duplicate reads were removed using the GATK MarkDuplicates function. Strelka 2.9 was used to call somatic single nucleotide variants (SNVs). Variants with a sufficient empirical variant score in at least one sample passed the filter. The functional impact of identified SNVs was predicted using SnpEff. Ingenuity Pathway Analysis was used to identify pathways enriched for genes impacted by SNVs.

Results: 10,735 total SNVs were identified within different regions of the Oncopig HCC tumor associated with 39,634 effects with approximately 671 (1.7%) high impact effect. 8,456 (66.2%) effects were missense and 650 (5.1%) were nonsense. 13,042 (32.9%) effects were identified in exon regions. We identified variants in the *TP53BP1*, *CDKN2AIP*, *TERT*, and *RPS6KA3* genes all found to be previously related to HCC driver genes. Over 1000 genes with moderate to high impact variants were identified to be involved with hepatic fibrosis, liver hyperplasia, hepatitis, hepatic steatosis, type II diabetes mellitus signaling, and cell death and survival. TP53 and KRAS were identified as potential upstream regulators of 271 and 113 genes with moderate to high variant effects respectively.

Conclusion: In this study, we showed intratumor heterogeneity in the Oncopig recapitulates common phenotypes expressed in human HCC proving further validation of our model with the goal of studying HCC tumor biology and testing therapeutic options.

Abstract No. 245

Pancreatic Retrograde Venous Infusion (PRVI) Significantly Enhances Delivery of NearIR Labeled SD-101 TLR9 Agonist to Targeted Regions of the Porcine Pancreas

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Purpose: Advanced pancreatic ductal adenocarcinoma (PDAC) has an overall 5-year survival rate of only 10%. Systemically delivered conventional chemotherapy and immunotherapy have had limited success in locally advanced PDAC patients, in part, due to high intratumoral pressure which restricts blood flow and therapeutic absorption. Here we describe a novel trans-venous approach for delivering labeled SD-101 (SD-101^{L}), a novel immune modulating TLR9 Type C agonist, into pancreatic tissues under elevated pressure.

Materials and Methods: The study was conducted on normal 40-60kg female swine. A fixed dosage of nearIR labeled SD-101^{L} was administered to each animal. The therapeutic was dispersed in either 10 mL ($n = 5$) or 20 mL ($n = 5$) of solution to evaluate how infusion volume impacts delivery efficiency. A third cohort was evaluated to determine if allowing the therapeutic to "dwell" within the vessel at stasis for 20 minutes impacted uptake (10 mL infusion, 20-min dwell, $n = 5$). Infusion was conducted through a TriSalus Infusion System (TIS-21120-60) at a rate of 2 mL/min into a volume drained by a single vein (target tissue) for all cohorts. A 35mm Hg maximum vascular pressure threshold was established after device deployment prior to infusion. The remainder of the pancreas remained untreated (nontarget tissue). Quantification of tissue uptake within the pancreases was performed by nearIR fluorescence imaging on sequential 1-cm-thick sections of tissue.

Results: Target tissues displayed 11.5-fold (10 mL, 0-min dwell, $P = 0.003$), 10.8-fold (10 mL, 20-min dwell, $P = 0.001$) and 11.2-fold (20 mL, 0 min, $P = 0.018$) enrichment in fluorescent signal relative to nontarget tissues. Single vessel infusions resulted in a mean treated volume of 4.5 ± 0.6 cm^3. Infusion volume and dwell time did not significantly impact target tissue fluorescence intensity or treated tissue volume. Vascular pressure was significantly increased relative to normal venous pressure after device deployment (21.6 ± 1.5 mm Hg vs 30.2 ± 1.0 mm Hg, $P = 0.000$) and during infusion (21.6 ± 1.5 mm Hg vs 33.7 ± 1.5 mm Hg, $P = 0.000$).

Conclusion: PRVI methodology significantly increased the concentration of SD-101^{L} within a targeted infusion region of the pancreas. The observed 10.8 to 11.5-fold enrichment may be attributed to exposing tissues to concentrated therapeutic solution under elevated pressure, promoting convective transport. PRVI methodology may allow physicians to improve therapeutic delivery to locally advanced PDAC tumors. These data formed the preclinical foundation for the PERIO-03 clinical trial at MD Anderson Cancer Center.

Abstract No. 246

High-Throughput Drug Screening of Patient-Derived Cell Lines Identifies Novel Potential Treatment Options for Hepatocellular Carcinoma

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Purpose: The purpose of this study was to assess the ability of high throughput drug screening (HTDS) performed on patient-