and colon cancer lines, CRISPR/Cas9-mediated gene editing was used to differentiate the transcriptomic profile driven by HIF-2α from that of HIF-1α or HIF-3α, allowing for the derivation of a HIF-2α-specific gene signature. Cancer cell and macrophage-derived signatures were applied to publicly available datasets to investigate cancer types, other than ccRCC, in which HIF-2α may play an important pathological role. **Conclusions** Collectively, these data support the development of our novel and selective HIF-2α inhibitors for the treatment of cancer and expand the indications that may benefit beyond ccRCC.

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**584 THERAPEUTIC VASCULAR NORMALIZATION TO PROMOTE TUMOR-ASSOCIATED TERTIARY LYMPHOID STRUCTURES**

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**Background** Tertiary lymphoid structures (TLS) are non-encapsulated immune cell aggregates that form at sites of chronic inflammation, including in and around tumors. Recent studies have shown that the presence of TLS in human tumors is an indicator of positive clinical outcome. However, due to dysregulated angiogenesis, many tumors have a poorly-organized and leaky vasculature that impedes entry of immune effector cells into tumors and consequently, TLS formation. It has been shown in pre-clinical studies that low doses of antiangiogenic agents normalize the tumor vasculature, leading us to hypothesize that treating tumors with low-doses (well below drug MTD) of vascular normalizing (VN) therapies will improve immune cell infiltration and TLS formation within the tumor microenvironment (TME).

**Methods** To test this hypothesis, melanoma-bearing mice were treated intratumorally with VN agents. Five days post-treatment, tumors were digested into single cell suspensions and RNA was isolated and used for RT-PCR. Transcript levels of TLS-promoting factors (CCL19, CCL21, CXCL13) and markers of vascular normalization (HIF1α, GLUT1) and inflammation/immune cell infiltration (CXCL10, VCAM1, CD8A) were measured and compared to PBS treated controls. Changes in tumor vasculature were evaluated using immunofluorescence microscopy (IFM) of tumor sections stained with CD31, PNAd, and PDGFRβ. Fluorescently-labeled lectin was injected into the mice to observe tumor perfusion. TLS formation was evaluated in tumor sections using IFM, with TLS being defined as PNAd+ vessels surrounded by clusters of CD45+ cells.

**Results** We observed that the VN agents dasatinib, STING agonist, bevacizumab, and agonist anti-TNFR1 antibody each induced global changes in the TME that are consistent with enhanced immune cell infiltration and TLS formation. These changes include increases in expression of CCL19, CCL21, and VCAM1. Dasatinib and STING agonists were shown to promote VN, as demonstrated by improved lectin perfusion into the tumor and increased pericyte coverage of blood vessels on-treatment.

**Conclusions** VN agents induce global changes in immune cell infiltration and TLS-promoting factors in the TME. In vivo, these agents induce VN in the TME and promote TLS formation. This knowledge can be used to develop optimal combination immunotherapy designs in the cancer setting.

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**585 INTRALESIONAL INJECTION OF ROSE BENGAL IMPROVES THE EFFICACY OF GEMCITABINE CHEMOTHERAPY AGAINST PANCREATIC CANCER**

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**Background** Chemotherapy regimens that include gemcitabine are considered standard of care in patients with advanced pancreatic ductal adenocarcinoma (PDAC). However, most patients with PDAC die within 2 years of diagnosis, even with these standard of care regimens. In this study, we explored the ability of intratumoral injections of PV-10, a 10% solution of rose bengal, to induce lesion-specific ablation and control of metastatic pancreatic tumors in a murine model.

**Methods** PV-10 was cultured with human pancreatic cancer cell lines overnight and cell death was measured via Annexin-V and DAPI staining. Murine pancreatic tumor cells (Panc02) were injected subcutaneously in one flank to establish a single tumor model; to establish a bilateral tumor model, Panc02 tumor cells were implanted in the opposite flanks. On day 7, a single tumor was treated with intralesional PV-10. Gemcitabine (60 mg/kg) was injected intraperitoneally twice per week for 2 weeks. These experiments were repeated using Panc02 cells modified to overexpress the neoantigen ovalbumin (OVA). Control mouse tumor were directly injected with PBS. Tumor growth of PV-10 injected tumors and non-injected bystander tumors on the opposite flank were measured. Damage associated molecular patterns (DAMPs) in serum and immune cell frequencies within the spleens of tumor-bearing mice were measured to identify an associated systemic response with tumor lytic treatment regimen.

**Results** We established that less than 50% of human and murine pancreatic cells were alive after a 24 hour incubation with 200μM PV-10 in vitro. The combination of intralesional PV-10 with the systemic administration of gemcitabine delayed the growth of treated tumors and non-injected distal tumors. In contrast, gemcitabine monotherapy failed to delay tumor growth in bilateral Panc02 tumor models. We observed that this treatment strategy was markedly more successful in immunogenic Panc02OVA tumors resulting in lesion-specific ablation in 5/8 mice compared to 0/8 mice that were treated with gemcitabine monotherapy. This suggests that the combination therapy enhanced the immune-mediated clearance of tumors. Moreover, regression of tumors in mice that received PV-10 in combination with gemcitabine was associated with the depletion of splenic CD11b+Gr-1+ cells and increases in damage associated molecular patterns HMGB1, S100A8, and IL-1α.

**Conclusions** Together, these results demonstrate that intraleisional therapy with PV-10 can enhance the efficacy gemcitabine against pancreatic tumors.

**Ethics Approval** Studies were performed under approved Institutional Review Board (IRB) laboratory protocols at the H. Lee Moffitt Cancer Center (Tampa, FL).

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