

preceded the development of erythema in our cases, representing a harbinger for the more severe grade of rejection that eventually developed. Our experience was consistent with other VCAs in that donor specific antibodies did not develop, despite a severe Banff Grade. Consistent use of topical calcineurin inhibitor based immunosuppression on the allograft skin may be helpful in warding off future episodes, as our patient has been rejection free now for 18 months. To date, no histologic signs of chronic rejection were present on 2-year protocol surveillance biopsy. We have added rapamycin to the current drug regimen, with concurrent reduction of tacrolimus dosing for renal protection, which has been demonstrated in cardiac transplantation to deter the intimal hyperplasia/vasculopathy associated with chronic rejection.

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Metagenomic characterization of influenza virus: bacteria super-infections associated with death and survival

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OBJECTIVES/SPECIFIC AIMS: To better understand host, viral and bacterial responses underlying disparate outcome. **METHODS/STUDY POPULATION:** We utilized metagenomic analysis of 559 genes, the NanoString nCounter Immunology Panel-Plus kit and FFPE mouse lungs from a published BSI time course. **RESULTS/ANTICIPATED RESULTS:** Results show an overall increased level of gene expression during BSIs associated with survival when compared to gene expression during peak viral titers. Early viral clearance and the presence of *S. pyogenes* in the lungs of TX98 infected lungs 24 hours after BSI was confirmed. Host responses tied to differences in early viral detection and clearance consisted of RIG-I, OAS, TLR3, TLR8 and TLR9. Key changes were noted in the expression of type I and II interferons, complement proteins, cytokines, chemokines, Fc receptors, scavenger receptors, the immunoproteasome and genes associated with T, B, Treg and NK cell activation. Interestingly, there was no significant increase in host antimicrobial peptides during BSIs associated with survival while CAMP and *Nos2* were significantly increased 24hrs prior to death in lethal BSIs. **DISCUSSION/SIGNIFICANCE OF IMPACT:** To our knowledge this is the first side by side metagenomics study of influenza BSIs associated with death and survival. Results offer mechanistic insight into clinical outcomes.

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Modulation of Hedgehog Signaling Alters Immune Infiltration in Pancreatic Cancer

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OBJECTIVES/SPECIFIC AIMS: Pancreatic ductal adenocarcinoma (PDA) has a dismal 5-year survival rate of 9%, making this disease one of the deadliest human malignancies (<https://seer.cancer.gov/>). Primary barriers to the treatment of pancreatic cancer include extensive stromal interactions and sustained immune suppression. Aberrant Hedgehog (HH) pathway activity is a hallmark of pancreatic tumorigenesis. Tumor-derived HH ligands signal in a paracrine fashion to the surrounding stroma to influence tumor growth. Expression of HH ligands increases during PDA progression, and previous work has shown that genetic deletion of Sonic HH (*Shh*)

from the epithelium of mice with pancreatic tumors results in increased Indian HH (*Ihh*) expression. This research aims to investigate the translational impact of changes in immune infiltration following deletion of *IHH* in a preclinical mouse model of pancreatic cancer. **METHODS/STUDY POPULATION:** *Ihh* was deleted in tumor cell lines (*IhhKO*) derived from a genetically engineered mouse model of pancreatic cancer (*LSL-KrasG12D/+;LSL-TrpR270H;P48-Cre*), using CRISPR/Cas-9 gene editing to assess the role of *Ihh* in the tumor microenvironment. The level of HH signaling was determined using tumor cell co-cultures with *Gli1lacZ* fibroblasts (derived from mice with a *lacZ* reporter allele knocked into the *Gli1* locus), in which Beta Galactosidase activity serves as a readout for HH signaling. WT and *IhhKO* tumor cells were orthotopically transplanted into the pancreas of syngeneic C57BL/6 mice. Human pancreas samples were obtained from surgical resection of pancreatic adenocarcinoma, or fine needle biopsy procedure (FNB). Immune profiling of mouse and human pancreatic tumors was performed using Cytometry Time-of-Flight analysis (CyTOF), and tumor composition was analyzed by single-cell RNA sequencing (scRNA seq). In vitro cultures with pancreatic fibroblasts treated with either WT or *IhhKO* tumor cell conditioned media (CM) were cultured with bone-marrow derived macrophages to assess tumor crosstalk. **RESULTS/ANTICIPATED RESULTS:** Tumor cells lacking *Ihh* were generated through CRISPR/Cas-9 deletion, and this was confirmed by qRT-PCR. Co-culture of *IhhKO* tumor cells with *Gli1lacZ* fibroblasts results in decreased *Gli1* expression both in vitro and in vivo. Immune profiling revealed that tumors lacking *Ihh* have significantly fewer tumor associated macrophages (*CD11b+/F4/80+/CD206+*), resulting in decreased presence of immunosuppressive factors such as arginase 1 and PDL1. Immune phenotyping of human pancreatic tissues revealed similar populations of immunosuppressive myeloid cells present in tumors. In vitro co-cultures demonstrated that, in the presence of bone-marrow derived macrophages, immunosuppressive IL-6 production was reduced in pancreatic fibroblasts cultured with *IhhKO*-CM, as compared to fibroblasts cultured with WT-CM, providing mechanistic insight into the in vivo phenotype observed. Further, scRNA seq analysis suggests that modulation of HH signaling in the tumor microenvironment alters chemokine and immunomodulatory signaling pathways driven by fibroblasts in the pancreatic tumor microenvironment. **DISCUSSION/SIGNIFICANCE OF IMPACT:** HH signaling in pancreatic fibroblasts contributes to the establishment of an immune suppressive environment in pancreatic cancer. Combining methods to target HH signaling and immune checkpoint therapy has translational potential in treating pancreatic cancer patients.

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Naltrexone as a Diagnostic Tool in Ocular Neuropathic Pain

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OBJECTIVES/SPECIFIC AIMS: The study aims to track and correlate ocular neuropathic symptoms, corneal sensitivity and dry-eye like pain, after scleral buckle and posterior vitrectomy surgeries. The goal is to identify a population of patients that receive these retinal surgeries that experience ocular neuropathic pain. **METHODS/STUDY POPULATION:** Methods - Prospective and Retrospective cohort studies were designed with the follow cohorts: scleral buckle, posterior vitrectomy, and control. Typical follow up for SB/PV

surgeries are: 1 day, 1 week, 1, 3, 6, 12 months post surgery. CS and DELP metrics are measured at each visit. For study interventions, all subjects (from both arms) will undergo the same series of tests, in the same sequence at each visit. Phase 1 of the visit focuses on CS and phase 2 on DELP. These interventions are as follows: first, subjects will receive Drop A; Drop A will be administered in a randomized, double-blinded manner at each visit to either balanced salt solution (control) or Muro 128 5% hypertonic saline (experimental). Drop A will be administered to both eyes. After receiving the drops, subjects will complete a visual analog scale questionnaire to grade their corneal sensitivity. Next, subjects will undergo a five minute washout. After the washout, subjects will receive Drop B; Drop B will be whichever drop was not administered in the Drop A phase. After Drop B is given, subjects will complete the visual analog scale. To begin phase 2, subjects will be given the Ocular Surface Disease Index to record dry eye signs and symptoms. Finally, tear film parameters will be collected using Schirmer's tear production test and tear film breakup time. Study Population. - Inclusion criteria: For retrospective cohort studies, subjects who have undergone unilateral SB or PV in the past year. For prospective cohort studies, subjects who will undergo unilateral SB or PV in the near future, and age-matched controls. Exclusion criteria: For both retrospective and prospective arms, the same exclusion criteria apply. They include: a previous diagnosis of dry eye; current use of neuropathic pharmacotherapies (including gabapentin, pregabalin, TCAs, SNRIs, carbamazepine, and opioids). RESULTS/ANTICIPATED RESULTS: As of 11/15/18, only the scleral buckle retrospective study arm had enough subjects for any meaningful preliminary report; the arm currently has 8 subjects. Of these 8 subjects, 5/8 subjects report increased surgical-eye corneal sensitivity and 6/8 show discordant dry eye symptoms and tearfilm parameters. Our power analysis showed that N=16 subjects in a group are required to detect a statistical significant difference in corneal sensitivity response. We expect to see a relapsing and remitting pattern of pain (as measured by corneal sensitivity and dry eye questionnaire), as is typical of neuropathic pain. Regarding dry eye symptoms, we anticipate subjects will have prominent dry eye symptoms (as measured by a validated questionnaire), but show no abnormalities in tearfilm parameters. DISCUSSION/SIGNIFICANCE OF IMPACT: To our knowledge, this is the first observational study of neuropathic pain symptoms of corneal sensitivity and dry-eye like pain, in post retinal surgery patients. We recognize the challenge of diagnosing neuropathic pain; currently the gold standard is clinical. However, symptoms of neuropathic pain are non-specific and subtle. Identification of a population suffering from post-retinal surgery ocular neuropathic pain will provide a foundation to test topical naltrexone as a diagnostic tool. If our hypothesis is correct, topical naltrexone could serve as a cheap, easy, and quick diagnostic test for ocular neuropathic pain. We envision this diagnostic test would allow many misdiagnosed and mistreated post-surgical patients to be treated with appropriate therapies aimed at neuropathic etiologies.

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Neural connectivity mechanisms linking off-time pubertal development and depression risk in adolescence

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OBJECTIVES/SPECIFIC AIMS: Earlier pubertal timing has been associated with risk for depression, particularly in girls (e.g., Keenan et al., 2014). Evidence suggests pubertal timing in girls also

relates to alterations in the microstructural properties of brain white matter tracts in late adolescence (Chahal et al., 2018), and structural connectivity of cingulate and frontal regions (Chahal et al., in prep), though differences in pubertal development in both boys and girls have not been examined in the context of brain functional connectivity (FC). Individual differences in the course of puberty may have enduring effects on functional coupling among brain regions that may contribute to the risk for psychopathology. To address this question, we explored the relation between pubertal timing and tempo with depression symptoms (age 16). Then, we examined whether brain network FC (age 16) associates with pubertal indices and predicts concurrent and later depressive symptoms (age 18). METHODS/STUDY POPULATION: Sixty-eight adolescents (37 females) completed the Mini-Mood and Anxiety Symptom Questionnaire (MASQ; Clark & Watson, 1995) at ages 14-18. Gompertz growth curve modelling of pubertal development (age 10-15; Waves 1-6) was used to estimate pubertal timing and tempo per individual, separately for males and females (e.g., Chahal et al., 2018). Resting-state MRI data (age 16) were parcellated into 264 cortical and subcortical regions to create region-to-region FC matrices based on correlations of time-series. Individual matrices were fed to the GraphVar program (Kruschwitz et al., 2015) to assess the interaction of pubertal timing and pubertal tempo with functional network connectivity using Network-based statistic (NBS; Zalesky et al., 2010). Subnetworks showing alterations in relation to pubertal timing and tempo were then examined in association with concurrent (age 16) symptoms and used to predict future depressive symptoms (age 18). RESULTS/ANTICIPATED RESULTS: In all youth, earlier pubertal timing was associated with higher depressive symptoms at age 16 ($p < .018$). This association was stronger in girls with slower pubertal tempo ($p < .039$). Interregional connectivity analyses revealed that the interaction of earlier pubertal timing and slower tempo was associated with lower FC between the left cingulate gyrus and right precuneus ($p < .0001$), regions implicated in emotion processing (i.e., Affective Processing Network) and self-referential thinking (i.e., Default Mode Network). FC of the three other emotion- and self-referential processing network regions (i.g., left insula, superior parietal lobule, and precuneus) was lower in youth with greater age 16 depressive symptoms ($p < .0001$). Finally, lower FC of the left and right inferior parietal lobule predicted greater depressive symptoms at age 18 ($p < .0001$). In summary, FC of overlapping affective and default mode network areas was related to earlier pubertal timing and higher concurrent and future depressive symptoms. DISCUSSION/SIGNIFICANCE OF IMPACT: These findings demonstrate individual differences in pubertal maturation are associated with depressive symptoms and differences in brain connectivity in mid-adolescence. Early pubertal development was associated with greater depression symptoms and lower FC of brain regions involved in emotion regulation and self-referential processing. Further, FC between these regions predicted higher depression symptoms two years later. These neurobiological mechanisms may, in part, underlie the link between off-time pubertal development and the risk for depression. These findings also have important implications for precision psychiatry, as we show that a risk-factor of depression (early pubertal timing) may manifest in developing neurobiology in region-specific ways. Previous network models of depression (e.g., Li et al., 2018) implicated affective network connectivity in sustained negative mood and the default mode/ self-referential network in rumination. Other networks implicated in these past models include the reward network, which may be involved in anhedonia and loss of pleasure. Our study only found associations between affective and self-referential regional connectivity, pubertal