Moderated Poster Session IV: Basic Science & Education
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The Role of 5-HT2 on Pudendal Inhibition of Micturition Reflex in Cats

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Background: The role of 5-HT2 and opioid receptors in pudendal inhibition of bladder activity induced by intravesical infusion of saline or 0.25% acetic acid (AA) was investigated in anesthetized cats using methysergide (a 5-HT2 receptor antagonist) and naloxone (an opioid receptor antagonist).
Methods: Repeat cystometrograms (CMG's) were performed in 20 alpha-chloralose anesthetized cats by infusing the bladder with saline or 0.25% acetic acid (AA). Pudendal nerve stimulation at multiples of threshold (T) intensity for inducing observable anal twitch was used to suppress AA-induced bladder overactivity. Various doses of methysergide and naloxone were administered prior to CMG's.
Results: AA irritated the bladder and significantly ($p<0.0001$) reduced bladder capacity to 27.0±7.4% of saline control capacity. Pudendal nerve stimulation (PNS) at multiples of threshold (T) intensity for inducing anal sphincter twitching restored bladder capacity to 60.1± 8.0% at 1-2T ($p<0.0001$) and 92.2±14.1% at 3-4T ($p=0.001$) of the saline control capacity. Methysergide (0.03-1 mg/kg, i.v.) suppressed low intensity (1-2T) PNS inhibition but not high intensity (3-4T) inhibition, and also significantly ($p<0.05$) increased control bladder capacity at the dosage of 0.3-1 mg/kg. During saline infusion without AA irritation, PNS significantly increased bladder capacity to 150.8±9.9% at 1-2T ($p<0.01$) and 180.4±16.6% at 3-4T ($p<0.01$) of the saline control capacity. Methysergide (0.1-1 mg/kg) significantly ($p<0.05$) increased saline control bladder capacity and suppressed PNS inhibition at the dosage of 0.03-1 mg/kg. After methysergide treatment (1 mg/kg), naloxone significantly ($p<0.05$) reduced control bladder capacity during AA infusion but had no effect during saline infusion. Naloxone also had no influence on PNS inhibition.
Conclusions: These results suggest that 5-HT2 receptors play a role in PNS inhibition of reflex bladder activity and interact with opioid receptors in micturition reflex pathway. Understanding neurotransmitter mechanisms underlying pudendal neuromodulation is important for the development of new treatments for bladder disorders.

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Urothelial Carcinoma Exosomes Contain Del1/edil-3 And Facilitate Bladder Cancer Progression

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Background Exosomes are 30-200nm membrane-bound vesicles that contain biologically active mRNA, miRNA, and proteins. A growing body of literature has emerged in support of roles for exosomes in normal and pathological processes. Here we show that exosomes isolated from high-grade muscle invasive (HGMI) urothelial cell lines and urine of patients with HGMI disease promotes angiogenesis, invasion and migration.
Methods Exosomes from high-grade urothelial carcinoma (HGUC) cell line TCC-SUP versus control cell lines were subjected to mass spectrometry. The tumour-associated protein EDIL-3/Del-1 was identified and selected for further analysis. Exosomes from TCC-SUP were applied to human umbilical vein endothelial cells (HUVECs) in a tube-forming assay. Migration and invasion was tested by trans-well and scratch migration assays, respectively. Exosomes purified from shEDIL-3 TCC-SUP cells were also tested in angiogenesis, migration and invasion assays. qRT-PCR based pathway focused arrays were used to identify conserved factors that may be affected by EDIL-3 and confirmation of key components were validated. rEDIL-3 was used in a scratch migration assay to test for sufficiency of EDIL-3 activity. Western blot analysis was used to identify the presence of EDIL-3 in exosomes isolated from the urine of patients with HGMI disease versus healthy controls. Urinary exosomes from patients with HGMI were also tested for their ability to promote angiogenesis, migration and invasion.
Results Mass spectrometry of exosomes from TCC-SUP identified EDIL-3/Del-1 in TCC-SUP but not in control SV-HUC exosomes and was confirmed with western blotting. TCC-SUP exosomes promote angiogenesis in a tube-forming assay and facilitate migration of both endothelial and urothelial carcinoma cells. In addition, HGUC exosomes promote invasion in a trans-well assay. Importantly, shEDIL-3 exosomes did not facilitate migration of endothelial or urothelial cells, or angiogenesis. rEDIL-3 applied to cell lines demonstrates that EDIL-3 is sufficient to facilitate migration. Pathway analysis identified several conserved angiogenic and motility factors affected by EDIL-3 via EGFR and ERK1/2 signaling pathways. Western blotting of exosomes isolated from the urine of patients with HGMI disease identified EDIL-3. Moreover, exosomes from the urine of patients with HGMI disease facilitated migration, angiogenesis and invasion.
Conclusions HGUC exosomes contain the tumour-associated protein EDIL-3 and can promote tumour progression. We demonstrate that EDIL-3 is necessary for angiogenesis and sufficient for migration of urothelial cells. Critically, this protein was also identified in urinary exosomes from patients with HGMI disease, suggesting a role for EDIL-3 in bladder cancer progression and may serve as a novel therapeutic target.

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Combination of Foot Stimulation and Tolterodine Treatment Eliminates Bladder Overactivity in Cats

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Background: Recent clinical evidence supports the concept of neuromodulation and antimuscarinic combination therapy as a treatment for overactive bladder (OAB) to increase treatment efficacy and reduce side effects rates due to antimuscarinic treatment. Our previous studies in cats demonstrated transcutaneous foot stimulation is a novel, noninvasive form of neuromodulation to effectively inhibit bladder overactivity. The purpose of our study is to investigate the efficacy of combined low-dose tolterodine, a first-line antimuscarinic, and transcutaneous foot stimulation in cats to noninvasively inhibit bladder overactivity and lower adverse antimuscarinic effects.
Methods: Cystometrograms were performed on alpha-chloralose anesthetized cats (N=6) by infusing 0.25% acetic acid (AA) to induce bladder overactivity. Foot stimulation (5 Hz) was applied at 2 and 4 times the threshold (T) intensity for inducing toe movement to inhibit the bladder overactivity. Cumulative doses of tolterodine (0.003-0.3 mg/kg i.v.) were also administered to determine the effect of combination treatment on bladder overactivity.
Results: AA irritation of the bladder significantly ($p<0.0001$) reduced bladder capacity to 23.6±7.1% of saline control capacity. Foot stimulation alone

at 2T and 4T inhibited bladder overactivity and significantly ($p<0.0001$) increased bladder capacity to 50.7±6.8% and 79.0±11.6% of saline control, respectively. Tolterodine alone at 0.3 mg/kg significantly ($p<0.05$) increased bladder capacity to 65.6±15.5% of saline control. However, when tolterodine at a threshold dose (0.3 mg/kg) was combined with foot stimulation, the bladder capacity was significantly ($p<0.05$) increased to 86.2±6.2% and 107.9±10.6% by 2T and 4T stimulation, respectively. Complete inhibition of bladder overactivity could be achieved at a lower tolterodine dose (0.1 mg/kg) when combined with 4T stimulation (97.0±11.2% of saline control). The amplitude of micturition contraction was not changed by tolterodine treatment.

Conclusions: This study suggests a novel, efficacious, and non-invasive OAB treatment by combining foot stimulation with a lower dose tolterodine to potentially limit the adverse effects due to antimuscarinic therapy and increase patient compliance. It also provides the first objective evidence supporting an additive therapeutic benefit of neuromodulation and antimuscarinic combination treatment. If shown to be clinically efficacious, foot stimulation combined with a low dose tolterodine could significantly improve the treatment for OAB.

P50

Involvement of 5-HT_3 Receptor In Pudendal Inhibition of Bladder Overactivity in Cats

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Background: Pudendal neuromodulation is an effective treatment for refractory overactive bladder (OAB) that initial studies have shown to be superior to sacral neuromodulation, however the neurotransmitter mechanism behind pudendal neuromodulation is currently unknown. The purpose of this study was to examine the involvement of the 5-HT_3 receptor, which has been shown to mediate neuromodulation therapy for somatic pain, in both pudendal neuromodulation and the micturition reflex in anesthetized cats using the 5-HT_3 antagonist, ondansetron. Understanding the neurotransmitter mechanism behind pudendal neuromodulation and the micturition reflex is important for the development of new drugs for the treatment of OAB and to enhance current neuromodulation therapies.

Methods: Cystometrograms (CMGs) were performed in a total of 18 adult under alpha-chloralose anesthesia under both normal and overactive bladder conditions induced by intravesical infusion of saline and 0.25% acetic acid (AA), respectively. Pudendal nerve stimulation (PNS) was applied (5 Hz) at multiples of the threshold intensity (T) for inducing anal twitching via a tripolar cuff electrode at low intensity (1.5-2T) and high intensity (3-4T) stimulation. In both the saline (N=6 cats) and acetic acid (N=12 cats) groups, CMGs were performed with and without PNS under increasing cumulative doses (0.003 to 3mg/kg, i.v.) of intravenous ondansetron, a 5-HT_3 antagonist.

Results: AA irritation significantly reduced bladder capacity to 16.5±3.3% of saline control capacity, while PNS restored the capacity to 82.0±12% ($p=0.0001$) and 98.6±15% ($p<0.0001$) at 1.5-2T and 3-4T, respectively. Ondansetron, a 5-HT_3 receptor antagonist, (1-3mg/kg, i.v.) eliminated low intensity (1.5-2T) PNS inhibition and reduced high intensity (3-4T) PNS inhibition of bladder overactivity. During saline distention, PNS significantly increased bladder capacity to 173.2±26.4% ($P=0.036$) and 193.2±22.5% ($p=0.008$) of saline control capacity at 1.5-2T and 3-4T, respectively, but ondansetron (0.003-3 mg/kg, i.v.) had no effect on PNS inhibition. Ondansetron also significantly ($p<0.05$) and dose-dependently increased control bladder capacity during both AA irritation (0.3-3 mg/kg) and saline distention (0.1-3 mg/kg) without stimulation to a maximum capacity of 70.3±15.5% and 230.8±46.6% of saline control, respectively.

Conclusions: This study reveals that 5-HT_3 receptors partially mediate PNS inhibition of bladder overactivity. In addition ondansetron alone significantly inhibited bladder overactivity indicating the 5-HT_3 receptor is excitatory in the micturition reflex pathway and may be used as a potential treatment for OAB. Understanding neurotransmitter mechanisms underlying pudendal neuromodulation will help to find novel targets for drug development and improve current neuromodulation therapies for bladder disorders.

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Reduction in Hospital Admissions with the Addition of Prophylactic IM Ceftriaxone Prior to Transrectal Ultrasound-Guided Prostate Biopsies

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Background: This IRB-approved retrospective study evaluated the sepsis rates in the two pre-prostate biopsy antibiotic protocols.

Methods: Prior to October 2011, the prophylactic protocol required ciprofloxacin 500mg bid starting one day pre-biopsy and continuing for 3 days post-biopsy (4 days total) (CiproAlone). Diabetic patients were prescribed ciprofloxacin for 4 days post-biopsy. Regional infection rates and bacterial sensitivities were studied and the antibiotic protocol was changed. To reduce infection rates, patients were prescribed one dose of ciprofloxacin 500 mg PO one hour prior to the biopsy and Ceftriaxone 1 g IM at the time of the biopsy (CiproCeft). No additional doses of antibiotics were given. Both protocols required a Fleet enema the night before or morning of the biopsy. Data were collected from biopsies performed from October 2010 through September 2012. Hospitalization rates between the CiproAlone versus CiproCeft protocols were examined. Post-biopsy hospitalizations were reviewed for relevant clinical history, laboratory results, antibiotic resistance testing, and other details of hospitalization. Sepsis was identified based on Standard Systemic Inflammatory Response Syndrome (SIRS) criteria.

Results: 4128 biopsies included- 2093 in the CiproAlone cohort and 2035 in the CiproCeft cohort. The post-prostate biopsy infection hospitalization rate was 0.6% (14 patients) in the CiproAlone group versus 0.0% (0 patients) in the CiproCeft group ($p<0.0001$ using Fisher's exact test). Of hospitalized patients, 64% fit SIRS criteria. Five hospitalized patients fit the Sepsis (SIRS and source of infection) criteria. Positive cultures (urine and/or blood) were obtained from 64% (n=8) of hospitalized patients. Of patients with positive cultures hospitalized on CiproAlone, 75% (6 of 8) had Fluoroquinolone resistant Escherichia coli (E. coli), and one had a strain resistant to Cephalosporins. Other antibiotic resistances included one gentamicin resistant E. coli strain, and 3 of 8 strains with TMP-SMX resistant E. coli. Diabetes mellitus was also associated with an increased risk of infectious complications after prostate biopsy ($p=0.041$) in our study population, but there was no difference between the two groups in the rates of diabetes mellitus ($p=0.43$, Fisher Exact Test). Patient age, PSA, number of biopsy cores obtained, and race were not found to be independent predictors of post-TRUS biopsy hospitalization for infection using a multivariate regression analysis.

Conclusions: A prophylactic pre-biopsy protocol including two classes of antibiotics reduced post-biopsy sepsis and hospitalization rates. Additional analysis of additional factors involved in the success of the new protocol (patient compliance, resistance rates for specific antibiotics) is warranted.

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Valproic Acid Decreases Thrombospondin-1 Expression In A Mouse Model Of Superficial Bladder Cancer

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Background: The primary challenge in treating superficial bladder cancer is the risk of recurrence which requires intense surveillance. Valproic acid (VPA) is a seizure medication and histone deacetylase inhibiter that has been potential as an anti-neoplastic agent. We have previously demonstrated reduction of bladder tumour volume in mice treated with VPA. Bladder cancer cell lines treated with VPA have reduced proliferation and increased expression of RNA encoding the anti-angiogenic protein thrombospondin-1 (TSP-1). We examined TSP-1 expression in UPII-SV40T mice in response to VPA administration.

Methods: UPII-SV40T transgenic mice express the oncogenic simian virus 40 T protein in the urothelium and develop exophytic bladder tumours.

Animals were treated between 4 and 6 months of age, when median time to detectable tumour is reached. Wild-type and UPII-SV40T mice were implanted with osmotic pumps delivering VPA for two weeks at a daily dose of approximately 50 mg/kg. The whole bladders were harvested to liquid nitrogen. RNA was isolated using Qiagen RNeasy kit and subjected to quantitative real-time PCR. TSP-1 expression was analyzed relative to sex, UPII-SV40T genotype, age, and VPA.
Results: There was no treatment related toxicity. Levels of TSP-1 mRNA did not differ between wild-type, UPII-SV40T untreated and wild-type treated mice. UPII-SV40T mice treated with VPA showed TSP-1 RNA levels decreased significantly. Relative expression of TSP-1 for the treated mice was 2 fold lower than other animals. On both univariate and multivariate analysis, VPA administration was the only factor associated with TSP-1 expression.
Conclusions: In contrast to our in vitro studies with bladder cancer cell lines VPA administration lowered TSP-1 RNA levels in the bladders of UPII-SV40T mice. Lower TSP-1 gene expression in mice could be due to feedback regulation in long duration treatment (compared to short-term tissue culture) or muscle and stromal versus tumour response. Further work will be needed to dissect the anti-tumour activity of VPA in bladder cancer.
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Distinguishing Insignificant versus Aggressive Prostate Cancer Based on sentinel ncRNAs
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Background: Active surveillance (AS) for prostate cancer is becoming an increasingly popular treatment option. Eligibility for AS protocols is often based on prostate biopsies, PSA, and PSA kinetics, and each has its own shortcomings. The emergence of non-coding RNAs (ncRNA) as novel biomarkers in prostate cancer may provide powerful diagnostic and prognostic tools. Two important classes of ncRNAs are microRNAs (miRNA) which negatively regulate gene expression and small nucleolar RNAs (snoRNA) which target the modification of ribosomal RNA (rRNA) and small nuclear RNA (snRNA), affecting translational efficiency and mRNA splicing. We hypothesize that changes in ncRNAs precede the morphological changes in tumour pathology and that combining ncRNA profiling with pathological staging of core biopsies will refine AS selection criteria.
Methods: Formalin fixed paraffin embedded (FFPE) prostate tissue was obtained from patients undergoing diagnostic core biopsies. The biopsy material included cores with pathological evidence of prostate cancer; cores with no evidence of prostate cancer from the same patients; and cores from patients with no histological evidence of prostate cancer which were used as benign controls. FFPE sections were de-paraffinized and RNA extracted using the miRNeasy FFPE Kit (Qiagen). The presence of small RNA (<200 nucleotides) was quantitated on the Bioanalyzer 2100. All samples were interrogated on Affymetrix GeneChip miRNA 3.0 Arrays. Data were analyzed using Partek Genomics Suite software. Entities with fold changes greater than 1.5 were considered different and selected for further analysis
Results: A training set of 40 biopsy samples have been interrogated on the Affymetrix 3.0 arrays. Out of a total of 5639 ncRNA probes we found 202 ncRNA that are significantly different between benign and tumour core biopsies, including 129 miRNAs, 24 H/ACA box snoRNAs, and 49 C/D box snoRNAs. These ncRNAs can be binned into two categories:
1. ncRNAs that correlate tightly with Gleason grade, and provide molecular correlates of the Gleason score, but cannot be used to distinguish between insignificant and aggressive prostate cancer.
2. ncRNAs that are poorly correlated with Gleason Score, and therefore may be indicative of molecular progression in prostate cancer prior to histological changes.
Conclusions: We have identified a cohort of sentinel ncRNAs that may be associated with aggressive prostate cancer. The data generated in this training set can now be used as a testing set to establish the ability of ncRNA profiles to distinguish between insignificant and aggressive prostate cancer, improving patient selection for active surveillance.

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The Emerging Role of NOX and ATM Proteins in Renal Fibrosis
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Background: Regardless of etiology, renal fibrosis is a common pathological process in the progression of chronic kidney disease. The role of TGF-β1 mediated SMAD dependent pathways has been studied in fibrosis; however, the role of non-SMAD dependent pathways in TGF-β1 signaling in fibrosis is yet to be elucidated. We investigated the relationship between free radical generation (ROS) and TGF-β1 as well as the role of NADPH oxidases (NOX), sources of ROS generation, and ataxia-telangiectasia mutated kinase (ATM), a downstream target of ROS in renal injury, in TGF-beta induced gene changes.
Methods: Using the normal rat kidney fibroblast cell line (NRK 49f), ROS generation in response to TGF-β1 was tested using carboxy 2-7,dichlorofluorescein (DCFDA) assay. In separate follow-up studies diphenyleneiodonium chloride (DPI), a NOX inhibitor, and N-acetylcysteine, an inhibitor of ROS generation, were used to pretreat the cells. In a further study the cells were treated with an ATM inhibitor (KU55933) before stimulation with TGF-β1. Western immunoblotting was performed to evaluate the effect of these conditions on markers of fibrosis. Immunohistochemical staining of wild type mouse kidneys (WT) with surgically induced unilateral ureteral obstruction (UUO), sham operated kidneys (SHAM), and the contra lateral kidney (CON) was performed for $pATM^{Ser\ 1981}$ staining.
Results: We determined that TGF-β1 induces ROS generation rapidly in a sustainable fashion in NRK49f cells. Pretreatment with DPI demonstrated dose dependent inhibition of TGF-β1 mediated PAI-1 expression suggestive of NOX involvement. Pretreatment with N-acetylcysteine dose dependently suppressed PAI-1 expression, supporting the role of ROS in TGF-β1 induced fibrosis. ATM inhibition with KU55933 also dose dependently suppressed PAI-1 expression in renal fibroblasts suggesting involvement of ATM proteins. Immunohistochemical staining of UUO kidneys showed an increased staining of $pATM^{Ser\ 1981}$ in the fibrotic kidney compared to contralateral controls suggestive of activated ATM pathway in TGF-β1 driven fibrosis.
Conclusions: These findings support further investigation of non-SMAD dependent pathways for TGF-β1 signaling in the progression of fibrosis, specifically that of ROS and ATM downstream of TGF-β1. These alternative pathways may prove useful as future therapeutic targets for amelioration of renal fibrosis.

P55
A Novel Survival Model of Pelvic Floor Dysfunction after Rabbit Pelvic Floor and Transvaginal Electrical Stimulation
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Background: Existing data supports a relationship between the levators and pelvic organ function, however there is a paucity of animal models for chronic pelvic floor dysfunction. We previously developed an acute model of pelvic floor dysfunction in the rabbit and demonstrated that direct electrical needle stimulation of the pubococcygeous muscle resulted in cystometry (CMG) and electromyography (EMG) changes consistent with dysfunctional voiding; larger bladder capacity, longer interval between contractions and prolonged contraction duration. The current experiment seeks to explore the in vivo effects of needle and transvaginal electrical stimulation using a survival model.
Methods: Twelve female adult virgin white New Zealand rabbits were housed in metabolic cages to record baseline voiding and defecation for 3-days. Anesthetized CMG/EMG was performed before and after treat-

ment animals (n=9) received bilateral tetanizing needle stimulation (4 trains 10s apart, 15mA, 25Hz, 0.2ms, 10 pulses/train) to the pubococcygeous muscle and controls (n=3) sham needle placement. After 7-days of metabolic recording, all animals were subjected to tetanizing transvaginal stimulation (5 minutes, 6.5mA, 10Hz, 0.1ms repetitive) and CMG/EMG. After 5-days, a final CMG was performed and bladder with pelvic floor muscles collected.
Results: Throughout the experiment mean fecal weight and urine production were similar between groups. At baseline, animals demonstrated heterogeneous voided volume and frequency behavior. Needle tetanizing stimulation of the pubococcygeous muscle significantly prolonged interval between CMG contractions with mean time to third contraction rising from 38 to 53 minutes (p=0.008 vs. pre-stimulation), representing a significant mean increase of 15 minutes (p=0.022 vs. 1 minute for control). Vaginal stimulation also significantly increased time to third contraction from 37 to 47 minutes (p=0.015 vs. pre-stimulation). On linear regression analysis of cage parameters, needle stimulation resulted in larger voided volumes and less frequent voids. Of the rabbits that underwent needle stimulation 7/9 (78%) demonstrated voiding dysfunction versus 6/12 (50%) after transvaginal stimulation, with little change in cage parameters seen one day after vaginal stimulation.
Conclusions: Both direct pubococcygeous and transvaginal electrical stimulation resulted in prolonged intervals between CMG contractions. Changes in cage parameters were primarily seen after direct stimulation of the pelvic floor, with larger volume and less frequent voids noted. This model supports the findings of our prior experiment, with changes after stimulation consistent with dysfunctional voiding behavior, thus reiterating the central role of the pelvic floor in coordinated voiding function.

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Ketamine Induced Cystitis: Man To Mouse?
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Background: Interstitial cystitis or bladder pain syndrome is a chronic pelvic/perineal pain syndrome with unknown cause. Clinically these patients experience urinary urgency, frequency, and pelvic pain. These symptoms are usually waxing and waning, but are debilitating in nature. Symptoms and physical findings vary between patients, making diagnosis and treatment difficult. Interstitial cystitis is seen sporadically in cats, but no reliable inducible animal model exists. Ketamine is a widely used anesthetic that has become a popular drug of abuse. Chronic users develop a small capacity bladder with symptoms of bladder pain, frequency and urgency, similar to what is seen in patients with interstitial cystitis. Hypothesis: Chronic ketamine will induce a small volume, high frequency voiding pattern in mice similar to that seen in human interstitial cystitis.
Methods: Mice were acclimated to metabolism cages and baseline voiding function assessed for four weeks in three hour sessions two to three times per week for four weeks. Urine production was continuously recorded allowing calculation of void volume and frequency. Group one mice were initially started on daily injections of ketamine at a dose of 40 mg/kg then escalated to 80 mg/kg over 13 weeks. They were then transitioned back to saline injections for two weeks. Group 2 mice were injected with saline for seven weeks and then switched to 80 mg/kg of ketamine for six weeks. Group 3 mice were injected with saline only for the entire study to serve as a control. At least twice weekly, the mice were placed in metabolism cages and offered 6 mL of sweetened water to trigger urine production. Bladders were harvested at necropsy for histological evaluation.
Results: Individual mice displayed characteristic voiding patterns throughout the study that were not significantly influenced by ketamine. However, a trend toward an increase in voided volume with associated decrease in frequency in mice on ketamine was noted. Group 1 mice had evidence of fibrosis throughout most specimens. Subepithelial lymphocytic infiltrates organized in follicles as well as smooth muscle hypertrophy was noted in Group 2, while Group 3 mice showed no evidence of disease.
Conclusions: Chronic ketamine administration did not produce signs and symptoms characteristic of human IC. Results do suggest ketamine may decrease sensation of bladder fullness in mice. Ketamine triggered bladder inflammation and fibrosis.

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Nuclear Localization of Fatty Acid Synthase Correlates with Gleason Grade in Prostate Cancer
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Background: Fatty acid synthase (FASN) acts as an oncogene in prostate carcinogenesis. FASN is thought to be a primarily cytoplasmic protein; however we have recently observed expression of FASN in the nucleus of prostate cancer patient tissue. We investigated whether nuclear localization of FASN correlates with Gleason scores of prostate cancer.
Methods: Prostate specimens were obtained from 30 patients who had undergone radical retropubic prostatectomy for prostate cancer and from two organ donors. Immunohistochemical staining for FASN was performed on 28 specimens while four were exposed to the secondary antibody only, as negative controls. Within the most representative regions of cancer, the percentage of cells with strong nuclear staining was quantified using automated image analysis software. Benign glands were similarly analyzed in the donor prostate tissue. Pathology of all analyzed areas was confirmed through review with our institution's urologic pathologist. The scoring output by the image analysis software was converted into a composite nuclear (c-nuclear) staining score in order to allow appropriate comparison between specimens. A two-sample Wilcoxon rank-sum test was performed to detect a difference in the median percentages of strongly staining nuclei between different cancer grades.
Results: Out of the 30 cancer specimens, 27 had cancer regions of acceptable quality to include in the analysis. Of these 27, four were used as negative controls with secondary antibody only staining. There were two Gleason score (GS) 3+3= 6 negative controls and two GS 3+4=7 negative controls. The remaining 23 specimens composed six GS=6, ten GS=7, and seven GS >= 8, with two additional specimens from benign tissue donors. The median c-nuclear score for the benign glands, GS = 6, GS = 7, and GS>=8 were 0.43, 3.39, 19.87, and 43.59, respectively. The median c-nuclear scores for GS=6 and GS=7 negative controls were 0.25 and 1.6, respectively. The Wilcoxon rank-sum test demonstrated a significant increase in nuclear FASN staining between GS=6 and cancers that were GS=7 and above (p=0.0078). Additionally, a significant increase in nuclear FASN staining existed between only GS=6 compared to GS=7 cancers (p=0.0067).
Conclusions: To our knowledge, this is the first report demonstrating a correlation between nuclear FASN staining and Gleason grade. Nuclear-specific FASN, rather than previously reported cytoplasmic staining, suggests a potential novel role for FASN as a marker of clinical progression and warrants further investigation.

P58
Biodistribution of Indocyanine Green formulation in Mice after Intravenous and Intraperitoneal injections
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Background: Indocyanine Green (ICG) has been used in medical diagnostics, including angiography. We have developed formulations of ICG that lead to improved fluorescence and demonstrated its utility when enhanced with milk and other embodiments in imaging of the upper urinary tract to identify ureteral injuries. It is known that ICG is excreted via the bile ducts after intravenous (IV) administration. In this study we sought to identify the biodistribution of different ICG-formulation in mice after intravenous injection and intraperitoneal (IP) injection.
Methods: Nine Wild type mice were obtained for this study; the mice were divided into four groups (G1-4). Under anesthesia, a depilatory cream was applied to the ventral side of the mice to remove hair and

facilitate imaging. The first group received IV injections which were done via the mice tail. Near-infrared Fluorescence imaging (NIRF) was then used to observe the initial profile of the ICG dispersion. G1 was injected IV with 0.2 mL of a formulation of ICG and DMSO (2.5 g/mL ICG). G2 received IV ICG in intralipid enhanced fluorescent composition (0.2 mL of 5 µg/mL ICG) G3 got IP injections of ICG+DMSO, while G4 got IP injections of ICG in intralipid. The mice were then allowed to regain consciousness and additional NIRF imaging done at 4, 18, and 24 hours.
Results: G1 (2 mice) showed immediate uptake into the liver after IV injection, at 4 hours the GI tract and the gall bladder were fluorescent confirming that this is the primary means of excretion, At 18 hours only the GI tract was fluorescent, and at 24 hours there continued to be fluorescence of the GI tract in fecal pellets. In G2 (2 mice) the fluorescence was concentrated in the tail at 4 and 18 hours but by 24 hours, fluorescence was noted to be in similar distribution to G1. G 3 (2 mice) and G4 (3 mice) had similar results. In the IP injections, fluorescence was initially concentrated within the peritoneum and injection site at 4 hours. By 18 and 24 hours, the gut fluoresced similar to G1.
Conclusions: We have previously demonstrated that the fluorescence of ICG is enhanced on NIRF by the addition of sterile milk, Intralipid and DMSO. We have now demonstrated that metabolism of our ICG formulations remain the same and it is excreted through the gastrointestinal tract. We hope to demonstrate safety and efficacy of our ICG formulations in humans.

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Urology Residency and Cloud Computing: Enhancing Learning Experience with a Scalable and Modifiable Education Resource Base Specific to Training Program

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Background: Surgical education in residency is a continuously evolving and dynamic process that must be adapted to the specific needs of trainees. The current availability of online resources facilitates the dissemination and accessibility of information. The vast amount of easily accessible information can prove to be overwhelming, particularly for the junior trainee. Cloud computing is hosted outside a defined and controlled home network, and allows access to all resident staff regardless of physical location. Box.com offers content management security, uptime guarantee, and high-grade SSL encryption on transit and 256-bit AES encryption at rest, thereby offering secure, scalable content-sharing. We have set out to develop a program specific online resource database to serve as an adjunct to the current urological training program at our institution.
Methods: Using a commercially available online storage "cloud", a program specific resource database was created.
Results: Full privilege access was given to all residents and staff. Over the course of 5 years, this database was continually grown and updated with open input and contribution from users. This includes surgical videos, staff specific OR outlines, round presentations, and study guides. All of the content is continually updated as new procedures and techniques are introduced so as to cover the entire scope of surgical practice at our institution. Access patterns and utility of content is reviewed.
Conclusions: There has been tremendous use and support of this resource within our program. We have found this to be an excellent adjunctive resource for surgical education not only as an introduction to surgical techniques for junior trainees, but also as a means for dynamic academic collaboration.