Moderated Poster Session II: Basic Science
Thursday, October 27, 2011, 3:15 pm – 5:00 pm

P21
Effect of Antioxidant Supplements on the Response of Experimental Overactive Bladder to Solifenacin

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Background: One of the most common forms of urological dysfunction is the development of unstable bladder contractions. Solifenacin is a relatively new selective anti-muscarinic agent that is particularly useful in the treatment of overactive bladder (OAB) dysfunctions in men and women. Experimentally, we have demonstrated that OAB is associated with the generation of free radicals and oxidative damage to the bladder. The hypothesis being tested is that Solifenacin + coenzyme Q10 + alpha-lipoic acid (CoQ + LA) will be more effective in the treatment of OAB than either individually.
Methods: 48 male WNZ rabbits were separated into 8 groups of 6 rabbits. The following oral treatments were given: Groups 1 and 5) vehicle (saline); Groups 2 and 6) Solifenacin; Groups 3 and 7) CoQ + LA; Groups 4 and 8) Solifenacin + CoQ + LA. After 3 weeks of treatment, the rabbits in groups 1-4 received partial outlet obstruction. The rabbits continued their treatments for 4 weeks following surgery. The non-surgical rabbits received treatment for 7 weeks. At the end of the experimental time period, each rabbit from all groups received a cystometry and then all rabbits underwent the *in situ* study for inducing OAB.
Results: PBOO caused a significant increase in bladder weight in all groups, however, the bladder weight of the obstructed solifenacin + antioxidant group was significantly lower than all other obstructed groups. Obstruction increased the percentage of rabbits showing OAB in all groups; however, the increase in the antioxidant group and the solefenacin + antioxidants were lower than for the other two groups. Obstruction caused a significant decreases in the response to Ach in all groups; however, the Ach response of the obstructed group treated with the combined solifenacin + antioxidants was significantly greater than the other obstructed groups. The in-vitro contractile responses of the combined solifenacin + antioxidant obstructed group to all forms of stimulation were significantly higher compared to the no-treatment obstructed group. PBOO resulted in a 60% decrease in citrate synthase and ChAT activities. Solifenacin had no effect on the activity of both enzymes, however, pretreatment with both the antioxidants or the combination of solifenacin + antioxidants protected both enzyme activities.
Conclusion: Addition of the antioxidants improves the effectiveness of solifenacin in the treatment of obstructive bladder dysfunction and OAB.

P22
A Pilot Study Evaluating the Efficacy of Traction Therapy for Peyronie's Disease on a Novel Rat Model

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Background: The efficacy of traction therapy for the treatment of Peyronie's disease (PD) is controversial without basic histological support but appears to be gaining in popularity and widespread use. This pilot study evaluates the morphological, histological and functional changes associated with traction therapy on a novel rat model for PD.
Methods: Adult male Sprague-Dawley rats aged 20-24 weeks received intratunical injections of TGF-beta-1 and Tetradecyl Sulphate at Day 0 and Day 7 for induction of a durable penile plaque. At 4 weeks, the rats were divided into a traction group and a control group. The traction group underwent microscopic surgery where 2 plicating horizontal mattress sutures were placed on each side of the stable plaque to exert longitudinal stress. At week 6, an additional plicating suture was placed on each side to

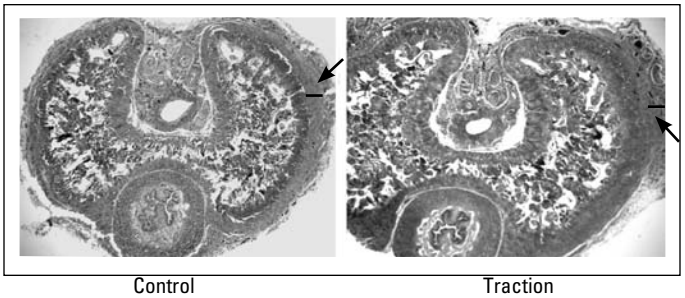

Fig. 1. P22. Histology of Penile Plaque on Control Group versus Traction Group.

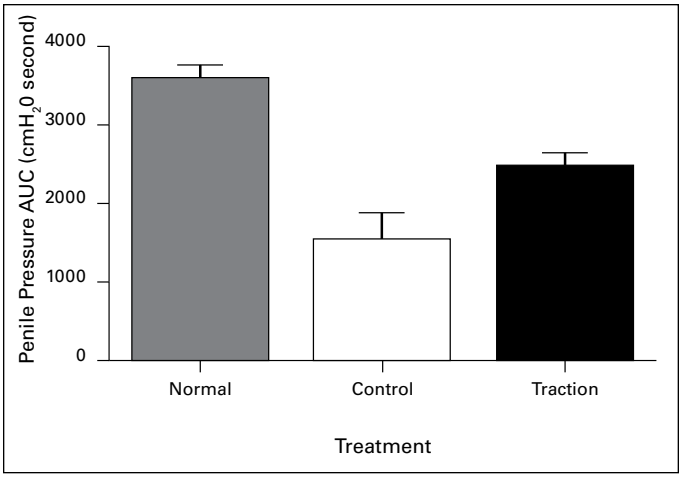

Fig. 2. P22. Penile Pressures of Normal, Control vs. Traction

assure adequate tension. The control group received no interventions. Penile pressures were measured in triplicates on all rats using cavernous nerve electrostimulation. They were sacrificed for gross, histological and immunohistological analysis at week 8.
Results: Gross examination of penile curvature did not show a significant difference between the traction and control groups. On histological examination, the plaque is shown as an area of increased non-polarized collagen deposit within the tunica albuginea on the right (Fig. 1). This area is smaller with less non-polarized collagen in the traction group compared to the control group. Immunohistology showed recovery of smooth muscle alpha actin and decrease of TGF-b1 in the traction group. Lastly, the traction group achieved higher penile pressures than the control group with cavernous nerve electrostimulation (Fig. 2).
Conclusions: Traction therapy is a novel approach for the treatment of Peyronie's disease. The results of this pilot study shows evidence of histological and functional improvement in the traction group. Further study is warranted.

P23
Citrate Synthase, Sarcoplasmic Reticular Calcium ATPase, and Choline Acetyltransferase Activities of Specific Pelvic Floor Muscles of the Rabbit

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Introduction and Objectives: While the relationship between pelvic floor muscle dystonia and urinary urgency and frequency, along with pelvic pain, is well described in clinical literature, the exact mechanism and pathways remain elusive. Improved understanding of the relation between the pelvic floor muscles and urinary tissues is clinically relevant in tailoring the treatment of these patients. This study compares three important cellular enzymes in pelvic floor muscles, bladder muscle (body and base) and bladder mucosa. These enzymes were chosen because of their relationship with intracellular calcium uptake and release, mitochondrial energy production, and muscarinic neurotransmission; which have significant implications regarding contractile function.
Methods: Adult female WNZ rabbits were euthanized and the following muscle were excised: pubococcygeus (PC), ischiocavernosus (IC), bladder body muscle and mucosa, and bladder base muscle. The citrate synthase (CS) (mitochondrial biomarker enzyme), sarcoplasmic reticular calcium ATPase (SERCA) (sarcoplasmic reticular biomarker enzyme), and choline acetyltransferase (ChAT) (biomarker for muscarinic neurotransmission) were quantitated and compared (Table 1).
Results: The CS and SERCA activities of the bladder body mucosa were significantly higher than that of the bladder body or base muscle. The CS, SERCA, and ChaT activities of the PC pelvic floor muscle were significantly higher than the IC pelvic floor muscle. Thus, specific pelvic floor muscles do not have the same metabolic properties, the PC pelvic floor muscle should have significantly greater capacity to contract than the IC muscle. Interestingly, the bladder body and base muscles have significantly higher ChaT activities than both pelvic floor muscles.
Conclusions: There are significant differences between specific pelvic floor muscles for the three important intracellular enzymes CS, SERCA, and ChAT.

P24
Acute Androgen Deprivation Activates Notch Signaling in Prostate Cancer Cells: Evidence of a Notch Tumor Suppressor Effect

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Background: Notch signaling plays an important role in tissue development and supports cell fate decisions. Abnormally active Notch signaling is also associated with human malignancy but its role in prostate cancer is controversial. We investigated the effects of acute and chronic androgen deprivation on expression of Notch signaling using the LNCaP cell model of prostate cancer.
Methods: LNCaP cells and an androgen independent (AI) variant were grown in androgen (10 pM R1881) supplemented or androgen-deficient medium. RNAs and proteins were extracted for assessment of androgen's effects on expression of Notch signaling (ligands, receptors and target genes) using human gene Chip-based microarray expression profiling, quantitative real-time RT-PCR (qPCR) assays (for mRNA) and Western blot assays (for protein). Small interfering RNAs targeting Jag1 and Notch3 were used to knockdown expression of these genes during androgen deprivation to test for effects on Notch target gene expression and cell growth.
Results: Comparative gene expression microarray profiling, validated by qPCR and Western blots indicated that the Notch ligand, Jag1, and Notch receptor, Notch3, were significantly and selectively up-regulated by acute or chronic androgen deprivation in LNCaP cells. Similarly, Notch target genes Hes1 and Hey1 were up-regulated. Knockdown of Jag1 and/or Notch3 with siRNA reduced expression of these Notch target genes in androgen deprived cells, and resulted in significantly increased growth of these cells in androgen deficient medium. Expression of Notch3 and Hey1 remained elevated in AI cells and could be reduced by Notch3 siRNA, but these cells were still able to grow in androgen deprived medium.
Conclusions: Androgen deprivation up-regulates Notch signaling mediated by Jag1 and Notch3 in parental LNCaP cells. Knockdown of Jag1 and/or Notch3 expression enabled further growth of these cells in androgen deficient medium suggesting that Notch signaling has a tumor suppressive effect during the initial stages of androgen deprivation therapy.

Table 1. P23.

	Citrate Synthase Nmol CoA / min / mg protein	Total CaATPase µmole Pi/mg protein	SERCA µmole Pi/mg protein	ChAT Pmoles/mg protein
Bladder Body Muscle	29.3 + 7	1.5 + 0.4	0.18 + 0.003	4.9 + 1
Bladder Body Mucosa	56.5 + 8 *	2.0 + 0.4	0.38 + 0.1 *	n/a
Bladder Base Muscle	24.5 + 4	1.9 + 0.3	0.132 + 0.012	3.6 + 0.8
PC Pelvic Floor	54.7 + 5 *x	1.6 + 0.2	0.19 + 0.03 x	1.2 +0.5 *x
IC Pelvic Floor	25.3 + 3.5	1.1 + 0.1	0.06 + 0.004	0.7 + 0.1 *

* = significantly different from bladder body muscle, $p < 0.05$
x = significantly different from IC Pelvic Floor Muscle

P25

Analysis of Tocopherol-associated Protein (TAP) Expression in Prostate Cancer and its Correlation with Clinico-pathologic Features

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Background: Tocopherol-associated protein (TAP) has been reported to act as a tumor suppressor in prostate cancer. We investigated the correlation between TAP immunohistochemical staining of prostate cancer and clinico-pathologic features in patients who had undergone radical retropubic prostatectomy.

Methods: Retrospective review was performed on 114 patients undergoing radical retropubic prostatectomy between 1997 and 2003. Immunohistochemical stains were performed on prostatic adenocarcinoma and benign glands from each specimen with a TAP monoclonal antibody. Seven patients were excluded due to failed staining controls. Analysis of stain intensity was evaluated by a single pathologist. Intensity scores (1+, 2+, 3+) were correlated with clinical (PSA recurrence) and pathologic features (Gleason score, extraprostatic extension, seminal vesicle invasion, lymph node metastasis, surgical margin status).

Results: One hundred seven patients were evaluated with a mean follow-up of 86.5 months (range 4-154). There was a down regulation of TAP staining in prostate cancer compared to benign prostatic glands ($p<0.001$). Kaplan-Meier analysis revealed a relationship between the intensity of TAP staining and recurrence free survival as defined by biochemical recurrence PSA≥0.2 ($P=0.03$) (Fig. 1). Fisher Exact Probability tests showed no statistically significant correlations between the intensity of TAP staining and pathologic features.

Conclusions: TAP immunohistochemical staining revealed a down regulation of intensity in prostate cancer compared to benign glands in radical prostatectomy specimens. This supports the role of TAP as a tumor suppressor in prostate cancer. Loss of TAP expression was associated with a risk of biochemical recurrence. Further investigation of a larger series is required to assess its role as a prognostic marker.

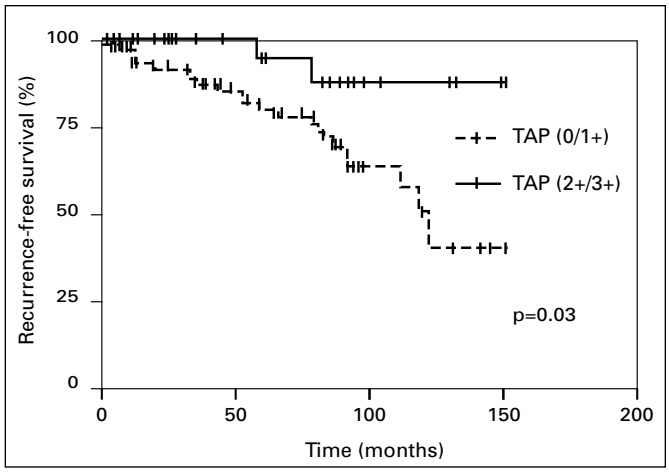

Fig. 1. P25.

P26 – PRIZE WINNING ESSAY

Excess Dietary Folate Intake Promotes Prostate Carcinogenesis in an *in vivo* Model of Tumorigenesis

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Background: Recent studies have demonstrated that folic acid supplementation is associated with an increased incidence of prostate cancer. We have previously reported a positive correlation between serum and prostate tumor folate, and increased cancer cell proliferation in men with higher serum folate concentrations. To determine the effect of dietary folate manipulation on prostate carcinogenesis and progression in an *in vivo* model of tumorigenesis (subrenal prostatic recapitulation).

Methods: Prostate tumor and adjacent normal tissues from patients with prostate cancer were analyzed using the subrenal prostatic recapitulation model to determine the effect of dietary folate on tumor characteristics.

Table 1. P26. Dietary folate supplementation facilitates formation of small atypical glands with features of cancer

	Epithelium (dietary folate intake mg/kg)	Normal	Hyperplasia	PIN	Small atypical foci with features of adenocarcinoma
8 wk	Normal (0)	2/8 (25%)	5/8 (63%)	1/8 (13%)	**0/8 (0%)**
	Tumor (0)	1/6 (17%)	0/6 (0%)	3/6 (50%)	2/6 (33%)
	Normal (2)	2/6 (33%)	3/6 (50%)	1/6 (17%)	0/6 (0%)
	Tumor (2)	0/4 (0%)	0/4 (0%)	2/4 (50%)	2/4 (50%)
	Normal (20)	0/7 (0%)	4/7 (57%)	3/7 (43%)	0/7 (0%)
	Tumor (20)	0/11 (0%)	0/11 (0%)	8/11 (73%)	3/11 (23%)
16 wk	Normal (0)	1/12 (8%)	10/12 (84%)	1/12 (8%)	0/12 (0%)
	Tumor (0)	2/16 (13%)	3/16 (19%)	2/16 (13%)	9/16 (55%)
	Normal (2)	2/12 (17%)	6/12 (50%)	3/12 (25%)	1/12 (8%)
	Tumor (2)	0/8 (0%)	1/8 (12%)	5/8 (63%)	2/8 (25%)
	Normal (20)	2/7 (29%)	4/7 (57%)	0/7 (0%)	1/7 (14%)
	Tumor (20)	0/7 (0%)	0/7 (0%)	6/7 (86%)	1/7 (14%)
24 wk	Normal (0)	0	0	0	0
	Tumor (0)	1/4 (25%)	0/4 (0%)	1/4 (25%)	2/4 (50%)
	Normal (2)	2/4 (50%)	1/4 (25%)	0/4 (0%)	1/4 (25%)
	Tumor (2)	0/6 (0%)	0/6 (0%)	4/6 (67%)	2/6 (33%)
	Normal (20)	0/8 (0%)	5/8 (63%)	3/8 (37%)	0
	Tumor (20)	0	0	0	0

Mice were randomly assigned to receive amino-acid defined diets which contained 0, 2 or 20mg/kg folic acid (folate-deficient, folate-sufficient, or excessively folate-fortified, respectively). Tissue recombinants (n=64) were serially grafted and harvested after 8, 16, and 24 weeks under the renal capsule. Histological and morphological features were assessed based upon 20 fields per recombinant, with the worst histological phenotype assigned to that recombinant.

Results: Mice achieved serum folate levels that were consistent with the levels seen in human patients [i.e. approximately 2x adequate (24nM), 5x adequate (60nM) and 10 times adequate levels (120nM) for the 0, 2 and 20mg/kg folate diets, respectively]. The incidence of normal glands, hyperplasia, PIN, and adenocarcinoma in human prostate cancer tissue recombinants was 6.5%, 6.5%, 50% and 37%, respectively; incidence in normal prostate tissue recombinants was 17%, 62.5%, 18.7%, and 4.7% (Table 1). Among normal prostate tissue recombinants, excess (20 mg/kg) dietary folate intake increased the incidence of PIN and adenocarcinoma when compared to recombinants grown in mice receiving a folate deficient diet (31.3% vs. 7%, respectively; $p<0.05$). An increased incidence of PIN and adenocarcinoma was also observed among prostate cancer recombinants in mice with adequate or excess dietary folate intake when compared to folate deficient mice (97.6% vs. 75.3%, respectively; $p<0.05$).

Conclusions: Modulation of dietary folate in an in vivo human prostate tissue recombinant model of tumorigenesis resulted in exposure to 5-10 fold excess levels of circulating folate, and promoted prostate carcinogenesis.

P27

Arrayed Imaging Reflectometry: Moving Towards the Rapid, Multiplex Analysis of Cancer Biomarkers in Serum

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Background: Currently available techniques for the measurement of serum cancer biomarker expression are relatively slow, expensive, and low throughput. An array analysis that detects and quantifies the expression of putative cancer biomarkers could be used to develop profiles to stratify cancer patients based on risk and likelihood of response to a given treatment. Arrayed Imaging Reflectometry (AIR) is a novel direct protein detection method previously developed in our laboratory. AIR involves immobilization of antibodies to target proteins on a silicon chip carrying an antireflective coating. Binding of the target protein to the antibody probe results in a thickness change on the surface of the chip, destroys the antireflective condition, and allows laser light to be reflected at that specific location in proportion to the amount of target protein in the sample. The goal of the present study was to utilize AIR to simultaneously detect multiple putative cancer biomarker proteins spiked in a solution of dilute serum.

Methods: Silicon chips were etched to an oxide layer of 1378 Å using dilute hydrofluoric acid. Antibodies to negative control proteins (green fluorescent protein, fluorescein) and candidate prostate cancer biomarkers (interleukin-6, transforming growth factor-beta, tumor necrosis factor-alpha, vascular endothelial growth factor, and interferon-gamma) were immobilized to the chips using a microarrayer. The protein targets were spiked together at decreasing concentrations (100 ng/mL, 1 ng/mL, 100 pg/mL) in 20% bovine serum in buffer. Control chips were also exposed to a solution containing only 20% bovine serum in buffer. Following a 2 hour incubation, all chips were imaged with a camera capable of detecting reflected laser light from the chip surfaces. The intensities of each probe-target spot were compared to both intra- and inter-chip control spots to determine binding.

Results: Each of the target proteins were detected from the solutions of spiked bovine serum. Detection was achieved at each concentration of target protein, with decreasing reflectance seen with decreasing concentration of the target cocktail.

Conclusions: Arrayed Imaging Reflectometry can detect and quantify multiple potential cancer biomarkers simultaneously, from within a background of serum containing many other proteins. This technology could potentially be used to profile patients with various malignancies, with powerful implications for diagnosis, risk stratification, and tailored therapies. Ongoing investigations include determining the lower limits of target concentrations that can be detected with AIR, as well as the initial profiling of serum samples from prostate cancer patients and men with an elevated PSA.

P28

Investigating Bladder Cancer Risk in Hereditary Non-Polyposis Colorectal Cancer Patients with Mismatch Repair Gene Mutations

Sean C. Skeldon[1], ***Cynthia Kuk***[1], *Melyssa Aronson*[2], *Kara Semotiuk*[2], *Aaron Pollett*[3], *Bas W. G. van Rhijn*[1], *Peter J. Bostrom*[1], *Steven Gallinger*[2], *Zane Cohen*[2], *Neil E. Fleshner*[1], *Michael A. Jewett*[1], *Sally Hanna*[1], *Theodorus H. van der Kwast*[4], *Andrew Evans*[4], *Bharati Bapat*[5], *Alexandre R. Zlotta*[6].

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Background: Hereditary non-polyposis colorectal cancer (HNPCC) is caused by mutations in mismatch repair (MMR) genes. Increased risk for urothelial cell carcinoma (UCC) of the ureter has been described in those with MSH2 gene mutation. We have shown a link between MSH2 mutation and an increased risk of bladder cancer (BC). We aim to confirm previous reports and identify a link between MSH2, upper urinary tract (UUT)-UCC and BC at the tissue level.

Methods: BC and UUT-UCC risk was analyzed in MMR gene mutation carriers within the Familial Gastrointestinal Cancer Registry in Toronto, Canada. Data was obtained of 321 persons with known mutations (MLH1, MSH2, MSH6, PMS2). 177 and 129 patients had germline MSH2 and MLH1 mutations, respectively. Canadian standardized incidence ratios were used to compare cancer risk in patients with confirmed germline MMR mutations to the general population. Microsatellite instability (MSI) analysis and immunohistochemistry (IHC) of the MMR proteins were performed and compared to gender, stage and grade matched sporadic bladder tumours to provide a histological correlation.

Results: Among 177 MSH2 mutation patients, BC was found in 11 (6.21%) patients but only in 3 of 129 patients (2.32%) with MLH1 mutations. No patients with germline MSH6 or PMS2 mutations had a diagnosis of BC. Of the 11 patients with MSH2 mutations, there were 5 men and 6 women, which is in contrast to the expected male to female ratio for BC of 3:1 in Canada. This 6.21% incidence of BC among MSH2 carriers is significantly increased compared to the lifetime risk seen in the Canadian general population. 9 of 11 tumours (81.8%) were MSH2 deficient on IHC and 6 of these were MSI-H, 0% lacked expression of MLH1 while all matched sporadic cases displayed normal expression of MSH2 and MLH1. Among MSH2 carriers, UUT-UCC was found in 7 (3.95%) patients. All 7 tumours were found to be deficient in MSH2 expression and 5 of the 7 (71.4%) tumors were MSI-H.

Conclusions: HNPCC patients with germline MSH2 mutations are at an increased risk for both UUT-UCC and BC. Family members of germline MSH2 mutation carriers should be screened for urinary UCC. Also, sporadic UUT-UCC diagnosed in patients under 60 years old or with a family history of HNPCC-related cancers should be screened for HNPCC by IHC analysis of MMR proteins. Our study suggests that mutations of MMR genes may have an important contribution in the development of a subset of UCC.

P29

The *FGFR3* Mutation Identifies Patients with Favorable Disease at Radical Cystectomy for Bladder Cancer

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Background: Radical cystectomy (RC) is the standard treatment for patients with treatment-refractory non-muscle invasive (NMI) and for muscle invasive (MI) bladder cancer (BC). The *FGFR3* mutation has gained attention as a marker for favorable NMI-BC and it was found to be associated with favorable prognosis. We determined the *FGFR3* mutation status in a cohort of patients who underwent RC and evaluated its potential as a marker for favorable disease.

Methods: We included 290 patients from three university hospitals (Dallas, N=132; Toronto, N=104; Turku, N=54) who underwent radical cystectomy with at least a bilateral pelvic lymph-adenectomy. Patients who received neo-adjuvant treatment were excluded. All cases were reviewed by one uro-pathologist. *FGFR3* mutation status was examined by multiplex PCR-SNaPshot analysis in the 290 cystectomy specimens and in 68 of 92 cancer-positive nodes. *FGFR3* mutation status was correlated to various clinical and pathological parameters using chi-square statistics.

Results: A *FGFR3* mutation was detected in 37 (13%) of RCs. Sixty-two patients were female. The mean age at RC was 65.5 years (range: 39-88 yrs). Pathological stage was <pT2, pT2, pT3 and pT4 in 48, 84, 120 and 38 RCs, respectively. Grade 2 (WHO1973) was found in 64 cases and Low-grade (WHO2004) in 24 cases. The remainder of the RCs were G3 and/or High-grade. Carcinoma in situ (CIS) and lympho-vascular invasion (LVI) were found 133 (46%) and 138 (48%) times, respectively. In 92 (32%) RC, positive lymph-nodes (N1=30, N2=62) were found. The median number of removed nodes was 13 (range: 1-53). Positive surgical margins were found at the bladder in 10, at the ureter in 7 and at the urethra in 6 cases. The presence of a *FGFR3* mutation was associated with lower stage (P<0.001), lower grade (P<0.001), absence of CIS (P=0.005), absence of LVI (P=0.001) and pN0 (P=0.003). We found no correlation for the *FGFR3* mutation to gender or margin status. We found a *FGFR3* mutation in 2 out of 68 analyzed positive nodes. The same mutation was detected in the RC specimen.

Conclusions: The *FGFR3* mutation selectively identifies patients with favorable BC at RC. The mutation was extremely rare in patients with cancer-positive nodes. *FGFR3* mutation status is a promising marker to guide decision making on adjuvant therapy after RC.

P30

FGFR3 Mutation, FGFR3 Expression and *FGFR3* Copy-Number Variation: Only the Mutation is Associated with Favorable Bladder Cancer

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Background: The fibroblast growth factor receptor 3 (FGFR3) is a tyrosine kinase receptor frequently activated by point mutations in bladder cancer (BC). These mutations are associated with genetically stable, pTa and low-grade BC representing the favourable BC pathway. Conversely, FGFR3 over-expression was recently found in 40% of muscle invasive BC. We examined *FGFR3* mutation status, protein expression in patients originally diagnosed as pT1. We also investigated copy-number variations in *FGFR3* as an alternative mechanism to activate FGFR3.

Methods: We included 84 patients with first-diagnosis pT1-BC. An uro-pathologist reviewed the slides for grade and (sub)stage. The *FGFR3* mutation status was examined by PCR-SNaPshot and FGFR3 protein expression by standard immuno-histochemistry (FGFR3 B9). Copy number status was determined in 69/84 cases with nine probes covering 9 exons of the *FGFR3* gene (MLPA).

Results: We found 27 *FGFR3* mutations. FGFR3 over-expression was found in 26 (96%) of these. Of the 57 wild type BC, 27 (47%) BC showed over-expression. Pathological parameters significantly differed (P<.01) between mutant and wild type tumors with the *FGFR3* mutation pointing to more favorable BC. However, if the BC was wild type, FGFR3 protein status had no influence on grade and (sub)stage. We found 6 tumors with >3 copies of *FGFR3*. Only one of 22 wild type tumors with over-expression of FGFR3 had >3 gene copies.

Conclusions: Only *FGFR3* mutant BC was associated with favourable disease characteristics. Almost all *FGFR3* mutant BC show over-expression. In addition, 47% of wild type BC also had over-expression suggesting an alternative mechanism for FGFR3 activation. Increased *FGFR3* copy number is not this alternative mechanism. Nevertheless, the wild type tumors with over-expression may represent a subset that may benefit from FGFR3-targeted therapy.

P31

Involvement of Metabotropic Glutamate Receptor 5 in Pudendal Inhibition of Micturition Reflex in Cats

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Background: Neuromodulation is a useful treatment modality in patients with refractory lower urinary tract symptoms, but the mechanisms of action are poorly understood. Metabotropic glutamate receptors are a promising target for modulating nociception and inflamatory pain and may be involved in the micturition pathway. The goals of this study were, therefore, to determine the involvement of metabotropic glutamate receptor mGlu5 in reflex-induced micturition and inhibitory pudendal neuromodulation using MTEP, a selective mGlu5 allosteric antagonist to block the receptor during saline and acetic acid cystometrograms.

Methods: Effects of MTEP (0.1-50 mg/kg, i.v.) administered in increasing cumulative doses was evaluated in eleven anesthetized cats. Effects of each dose on capacity and inhibition of bladder activity induced by pudendal nerve stimulation were evaluated by cystometrograms (CMG) using saline (n=5) or 0.25% acetic acid (n=6) to irritate the bladder and induce bladder overactivity. The pudendal nerve was stimulated via a nerve cuff electrode at 5 Hz and various voltages to inhibit overactivity.

Results: In the absence of pudendal nerve stimulation, MTEP (1-50 mg/kg) significantly increased saline bladder capacity but not AA capacity. Infusion of AA significantly reduced bladder capacity compared to saline. During saline or AA CMGs, pudendal nerve stimulation significantly inhibited bladder contraction and increased saline capacity to 168.4 +/- 26.9% and 196.0 +/- 37.4% of the control at stimulation intensities of 1T and 2-4T, respectively, and 67.9 +/- 31.3% and 98.4 +/- 55.2% of the control for AA infusion. After administration of MTEP (0.1-50 mg/kg i.v.), the saline bladder capacity was not significantly reduced by pudendal nerve stimulation at low (1T) and higher intensity (3-4T). MTEP doses of 1 mg/kg and greater, however, significantly reduced the AA bladder capacity and eliminated the inhibition of bladder overactivity induced by low intensity (1T) pudendal nerve stimulation but did not significantly antagonize the inhibitory effect of higher intensity (3-4T) stimulation. MTEP did transiently inhibit isovolumetric rhythmic bladder contractions induced by saline and AA infusion.

Conclusions: This study demonstrated that the mGlu5 receptor is involved in bladder antinociception induced by pudendal nerve stimulation during both acetic acid and saline conditions. These results obtained in cats elucidate a possible neurotransmitter mechanism underlying pudendal

neuromodulation and provide a potential new target (mGluR5) for pharmacological treatment of lower urinary tract disorders.

P32

Comparison of Molecular Markers, Sub-Stage and the EORTC Risk-Score to Predict Clinical Outcome of pT1 Bladder Cancer

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Background: We evaluated the impact of sub-stage, clinico-pathological parameters and 4 molecular markers on the clinical outcome of primary pT1 bladder cancer (BC) treated with BCG.

Methods: The slides of 129 primary BC from Rotterdam, NL (n=60) and Toronto, Canada (n=69) were reviewed and the pT1 diagnosis was confirmed. Sub-staging was done in two separate rounds, using a new system, i.e. pT1micro-invasive (pT1m) and pT1extensive-invasive (pT1e), and according to invasion of the muscularis mucosae (pT1a/pT1b/pT1c). Uni- and multivariate analyses for recurrence and progression were performed with clinical- (size, multiplicity, hospital, gender, age), pathological- (sub-stage, CIS, grade1973 & 2004) and molecular markers (*FGFR3* mutation and MIB-1, P53, P27 expression). The EORTC risk-scores for recurrence and progression were calculated.

Results: Median follow-up was 6.5 years (range 0.3-21.6), 24/129 patients were female. CIS was found in 45 (35%) cases. Seventy-seven (60%) patients had a solitary pT1 lesion. Sixty-two (48%) lesions were bigger than 3cm. The EORTC score for recurrence was intermediate in 122/129 (95%), 7 cases were high risk. The EORTC score for progression was intermediate in 16 cases, 113/129 (88%) were high risk. Forty-two patients remained recurrence-free (33%). Progression to pT2 or metastasis was observed in 38 (30%) patients. Sub-stage was as follows: 40 pT1m and 89 pT1e; 79 pT1a, 17 pT1b and 33 pT1c. Grade review resulted in 55 G2 and 74 G3 (WHO1973) and 26 low-grade and 103 high-grade (WHO2004). We found 37 *FGFR3* mutations and aberrant expression of MIB-1, P53 and P27 was found in 85, 69 and 48 BCs, respectively. Significant in univariate analysis for recurrence were multiplicity (P<.001) and CIS (P=.026). In multivariate analysis for recurrence, multiplicity (P<.001, RR 2.0, 95%CI: 1.4-3.0) was the only significant variable. Significant in univariate analysis for progression were gender (P=.036), substage (m/e) (P=.004), substage (a/b/c) (P=.009), CIS (P=.029), *FGFR3* (P=.031), MIB-1 (P=.034), P27 (P=.048), MIB-1/P27 (P=.012), *FGFR3*/MIB-1 (P=.033) and *FGFR3*/P27 (P=.018). In multivariate analysis for progression, female gender (P=.014, RR 2.7, 95%CI: 1.3-5.8), sub-stage (m/e) (P=.003, RR 2.8, 95%CI: 1.4-5.7) and CIS (P=.015, RR 2.1, 95%CI: 1.2-4.0) were the significant variables. Grade and the EORTC risk-scores were never significant.

Conclusions: Multiplicity was the strongest predictor of recurrence while CIS, female gender and sub-stage (pT1m / pT1e) were the most important variables for progression in pT1 bladder cancer. The additional value of molecular markers was modest. The value of the EORTC risk-score was limited in this primary pT1-BC group.

P33

Heat Shock Protein 70 (HSP70): A Marker for Recurrence in Patients with pT1 Bladder Cancer

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Background: HSPs is a class of functionally related proteins whose expression is increased when cells are exposed to elevated temperatures or other stress. In neoplasms, HSPs function in the regulation of apoptosis, as a modulator of p53, and in the immune response against tumors. Recently, they have been reported to be aberrantly expressed in a number of cancers, including bladder cancer (BC) where HSP60 and HSP90 have been proposed as prognostic factors. Previously, we found an association between FGFR3 mutation and expression with BC prognosis in a series of patients with primary T1 BC. Here, we examined HSP60 and HSP70 expression levels and their relationship to pathological and clinical parameters in the same group of primary T1 BC patients treated conservatively with BCG.

Methods: We included 69 patients from the University Health Network, Toronto, Canada with primary (first diagnosis) pT1-BC. Mean age was 71.1 years (SD:8.5). Microarrays were built and HSP60 and HSP70 protein expression was determined by standard immunohistochemistry (HSP70 Antibody, StressMarq Biosciences Inc, Victoria, BC, Canada; HSP90 Antibody, Santa Cruz, CA, USA). Slides were co-reviewed with an experienced uro-pathologist with staining scores dependent on the expression and intensity of the marker. HSPs expression was correlated with pathological, clinical outcomes and with the expression of FGFR3, Ki67, P27, and P53 markers. FGFR3 mutation was analyzed by SNaPshot analysis and expression by standard immunohistochemistry. Statistical analyses included Kaplan-Meier method and multivariate Cox-regression analysis.

Results: HSP70 was found to be expressed in 29/54 (54%) high-grade tumors and in 9/14 (64%) low-grade tumors. Kaplan-Meier survival analysis demonstrated that the lack of HSP70 expression was a significant predictor for disease recurrence ($p<0.05$). In the multivariate model adjusting for grade, size and concomitant CIS, lack of HSP70 expression remained a significant predictor for recurrence (HR of 1.952, 95% CI 1.02-3.75; $p = 0.045$). HSP70 was shown to correlate with FGFR3 expression and mutation ($p<0.05$). HSP60 was not associated with any pathological or clinical outcomes and did not correlate with the expression of any marker.

Conclusions: Both HSP70 and FGFR3 may play an important role in T1 BC. Further investigations will analyze the exact place of HSP70 in the dual pathway of BC carcinogenesis; one of them involving the FGFR3 pathway.

P34

Effects of Water Avoidance Stress and Isolation on Mice

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Background: Water avoidance stress (WAS) in rats may induce voiding and pathology similar to human interstitial cystitis (IC). However, findings with WAS are not well described in mice. This study is intended to determine if WAS can be linked to bladder specific changes in mice, which make ideal study animals due to ease of genetic manipulation.

Methods: Female CRL:CD1 mice were used in this study. Nine mice were maintained in their cages (housed 3 to a cage), nine were placed in dry chambers in isolation and six were placed in WAS chambers in isolation for 2 hours every morning for 20 days. The mice were monitored with video cameras and were weighed daily. Fecal pellet counts were obtained from the WAS and Dry Chamber groups. On day 11, voiding patterns of 3 mice from the home and 3 dry chambers were evaluated by placing them individually on filter paper in microisolator cages. Afterwards, bladders

were harvested for pathology and blood was collected for serum cortisol testing. The rest of the mice continued until day 21, when voiding patterns of the mice were evaluated and bladders were harvested for pathology.
Results: The dry chamber mice had statistically significant lower weight compared to the other groups by Day 10 of the study ($p<0.05$). The wet chamber mice had statistically significant higher fecal pellet production compared to the dry chamber by Day 10 of the study ($p<0.05$). Serum cortisol was not statistically different between the dry and home mice. Video analysis shows behavioral variability among the mice. Void counts ranged from 0 to 41 and were highly skewed precluding comparison using parametric tests, but an association of increased spotting and behavior was noted. No bladder specific or treatment specific pathology was noted in the bladder specimens.
Conclusions: Despite similar cortisol profiles as home cage controls, dry chamber mice lost weight. The water avoidance stress mice had increased fecal production. Mice displaying higher activity levels also exhibited high void counts. The mice displayed variable behavior and higher activity levels may be associated with developing a cystitis like voiding pattern although no bladder pathology was observed.

P35
Epigenetic Analysis of the Kallikrein Gene Family in Search for Novel Diagnostic and Prognostic Biomarkers for Prostate Cancer
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Background: Prostate cancer (PCa) is the most common malignancy affecting men in North America. Currently, a blood test for serum Prostate Specific Antigen (PSA), encoded by the kallikrein 3 (*KLK3*) gene is used for PCa diagnosis. However, it has a poor sensitivity and specificity. Therefore, there is a need to identify more effective PCa biomarkers. Evidence suggests that members of the 15-member *KLK* gene family are likely candidates since many *KLK*s are aberrantly expressed in primary PCa and PCa derived cell lines. Epigenetic mechanisms such as DNA methylation may regulate this aberrant *KLK* expression. Nonetheless, few studies have examined the epigenetic regulation of *KLK*s and its implications to prostate carcinogenesis. Aberrant DNA methylation is a well recognized hallmark of carcinogenesis and can serve as a diagnostic and prognostic biomarker for many cancers, including PCa. In this study, *KLK*10 was selected for analysis of DNA methylation based on its significant signal on Agilent Human CpG Island microarrays and EpiTYPER.
Methods: To investigate whether methylation mediated gene silencing and regulates *KLK*10 expression, we treated the PCa cell lines PC3 and 22RV1 with the demethylating drug 5-aza-2′deoxycytidine. Subsequently, using quantitative, high-throughput methylation-specific real-time PCR (MethyLight) technology, we evaluated the relationship between *KLK10* promoter methylation and clinicopathological parameters such as Gleason Score (GS) and pathological stage in a series of 150 radical-prostatectomies and adjacent normal prostate tissue. This series of 150 primary PCas spans the entire spectrum of tumor characteristics from 36 GS5+6 tumors, 75 GS7 tumors and 38 GS8+9 tumors. Also, the sample series contains 87 low stage, 42 locally advanced and 20 high stage tumors to allow for a robust analysis of the significance of *KLK10* methylation as a prognostic biomarker.
Results: Following treatment with 5-aza-2′deoxycytidine, 9.5-fold and 6-fold increase in *KLK*10 transcript expression were observed in PC3 and 22RV1 cells, respectively, establishing that methylation plays a role in regulating gene *expression*. Further, *KLK10* methylation levels were significantly higher in cancerous tissue vs. normal (P-value<0.001). Additionally, *KLK10* methylation levels were significantly increased in pT3b+pT4 vs. pT3a vs. pT2 tumors (P-value=0.005). The prevalence of high *KLK10* methylation was also significantly greater in cancerous tissue (70%) vs. normal (18%), (P-value <0.001) and in pT3b +pT4 (17/20-85%) vs. pT3a (34/42-81%) vs. pT2 tumors (53/87-61%), (P-value =0.019).
Conclusions: Our results suggest that tumor-specific increase in *KLK*10 methylation may be associated with PCa progression and its potential as a prognostic biomarker can be explored in future studies.

P36
Sub-Inhibitory Antibiotic Concentrations Enhance Surface Attachment, Survival and Host Immune Evasion in the Uropathogen Staphlococcus saprophyticus
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Background: Staphylococcus saprophyticus represents the most prominent Gram-positive uropathogen and frequently causes both uncomplicated and recurrent urinary tract infections (UTI) in young females. Although the effects of sub-inhibitory concentrations of antimicrobials on other organisms have been studied, little is known regarding S. saprophyticus' response under such conditions. This may represent an important oversight as transiently low antimicrobial concentrations are present in patients undergoing prophylactic therapy for recurrent UTI management.
Materials and Methods: Initially, we investigated the effects of sub-Minimum Inhibitory Concentrations (MIC) of ciprofloxacin, ampicillin and gentamicin on S. saprophyticus attachment to glass microscope slides. Following this, adherence to ureteral stent material and T24 bladder cells, as well as the effects on S. saprophyticus-induced pro-inflammatory cytokine expression in bladder cells was assessed using sub-MIC ciprofloxacin. Lastly, the ability of sub-MIC antibiotics to enhance survival against subsequent bactericidal challenge was considered.
Results: Adherence to microscope slides, ureteral stent material and T24 bladder cell monolayers were all significantly increased in the presence of sub-MIC levels of antibiotics applied. While S. saprophyticus challenge of T24 bladder cell monolayers alone significantly upregulated both IL-6 and IL-8 expression, the addition of sub-MIC ciprofloxacin abrogated these effects, returning their secretion to control levels. Finally, pre-treatment of S. saprophyticus with sub-MIC antibiotics improved its ability to survive subsequent treatment with typically lethal concentrations of the same agent.
Conclusions: Our results demonstrate that exposure to sub-MIC antibiotics increases S. saprophyticus adherence to both abiotic and biotic surfaces including urinary device material and cultured bladder cells, while inducing survival tolerance to those agents. In addition, low levels of ciprofloxacin downregulate S. saprophyticus-stimulated pro-inflammatory cytokine secretion in bladder cells. These changes may improve its ability to colonize the urinary tract, highlighting the need for clinicians to consider the impact of sub-inhibitory concentrations of antimicrobials when treating recurrent UTI.

P37
A Durable Novel Rat Model for Peyronie's Disease and the Evaluation of the Efficacy and Histologic Changes of Repeated Intralesional Verapamil Injections in Peyronie's Disease
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Background: Peyronie's Disease (PD) is a benign disease of localized fibrous plaque formation affecting approximately 5% of the male population with a significant impact on sexual health. Many therapies have been suggested and intralesional verapamil has demonstrated clinical benefits, but the histological effects of treatment have not been investigated. Further, a durable and practical animal model for further study does not exist. This is a multi-phase study to develop a novel model and examine the response to treatment.
Methods: Our model used intratunical Tromboject (Tj), a sclerosing agent, with transforming growth factor beta-1 (TGFb1), compared to the accepted model of TGFb1 intratunical injections. 22 male Sprague-dawley rats were injected with Tj, TGFb1 or both (9, 3 and 10 respectively) and repeated 1 week later. Rats were then sacrificed at 1, 3 and 6 weeks in the Tj group, 6 weeks in the TGFb1, and 9 weeks in the combined group. The

combined group was divided into controls (2), intralesional saline (3) and verapamil (5) therapy performed 3 times per week for 2 weeks. Penile pressure studies and histologic analysis was performed.
Results: Gross curvature was noted at 3 and 6 weeks in the Tj group. The combined controls demonstrated gross curvature and palpable scar at 9 weeks. No difference was seen between controls and saline injection but the verapamil group showed a decrease in plaque size and gross curvature. Trichrome stains demonstrate increased disorganized collagen most pronounced in the combined group followed by the Tj group and TGFb1 group with significantly improved histologically in the verapamil group.
Conclusions: Combination Tj with TGFb1 is a superior model for severe PD in the rat. Plaque formation is more severe, and gross deviations were identified which has not been previously reported. Durability has been demonstrated up to 9 weeks whereas previous models have been shown to resolve spontaneously. Gross and histologic improvements were identified in the verapamil group compared to controls and saline, supporting the pharmacologic role of verapamil and disputing the role of mechanical plaque disruption in plaque remodeling.

P38

Post-stimulation Inhibitory Effect on Reflex Bladder Activity Induced by Activation of Somatic Afferent Nerves in the Foot

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Background: Overactive bladder symptoms are often difficult to manage with medications. It is known that electrical stimulation of somatic afferent pathways in the pudendal and posterior tibial nerves, or sacral spinal roots can inhibit bladder activity in both humans and animals, and is clinically effective in treating overactive bladder symptoms. The purpose of this study is to determine if transcutaneous electrical stimulation of somatic afferent nerves in the foot can induce a post-stimulation increase in bladder capacity in cats.
Methods: In cats (N=12) anesthetized with alpha chloralose, electrical stimulation (5 Hz) was applied to the skin of the hind foot for two periods of 30 minutes via dual pad electrodes attached on the plantar and dorsal surfaces (combination 1-2), or at two sites on the plantar surface (combination 1-3). In the control group (N=12) the isovolumetric contractions were allowed to continue during each 30 minute period without stimulation. The post-stimulation effect was evaluated by performing repeated cystometrograms (CMGs) following 30 minute stimulation. After inducing the post-stimulation effect, foot stimulation was then applied during CMGs via electrode combinations 1-2 or 1-3.
Results: Foot stimulation inhibited isovolumetric rhythmic bladder contractions. The bladder capacity was not increased after the first 30 minute foot stimulation via electrode combination 1-2, but was significantly increased by 47.5 +/- 2.9% after the second 30 minute stimulation via electrode combination 1-3. Bladder capacity was further increased by 23.3 +/- 17.6% and 20.1 +/- 18.6% for electrode combinations 1-2 and 1-3 respectively when the foot stimulation was applied during CMGs.
Conclusions: This study shows that the transcutaneous plantar electrical stimulation of somatic afferent nerves in the foot of anesthetized cats can induce a post-stimulation increase in bladder capacity, suggesting that an intermittent stimulation pattern rather than a continuous stimulation might be effective in clinical applications to treat overactive bladder symptoms.

P39

Immunotherapeutic Applications of Phosphodiesterase Inhibition for Prostate Cancer

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Background: The cyclic nucleotide phosphodiesterases (PDEs) are a family of enzymes that play a central role in controlling cyclic nucleotide action and subsequent regulation of cell function. Specifically in tumor biology, previous studies have ascribed a protective role of cyclic guanosine monophosphate (cGMP) mediated signaling on hypoxia-mediated cancer progression. Herein we describe the expression levels of the known PDE variants in prostate cancer as well as explore the functional role that they play in cancer immune escape.
Methods: cGMP phosphodiesterase assays were used to measure the PDE activity in human prostate cancer cell lines DU145 and PC3. Western Blot analysis was performed to determine the presence of the PDEs in human tissue samples. The effect of PDE inhibitors on cancer cell apoptosis was measured using a flow cytometry-based TUNEL assay. The effect of PDE inhibition on hypoxia-mediated immune escape mechanisms was determined after pre-incubating cells in 0.5% or 20% O2 in the absence or presence of Zaprinast (10-7 - 10-6 M) and subsequent fow cytometric analysis of MICA, an essential tumor-associated antigen required for innate immune responses. A NK-competent murine model was used to measure the in vivo effects of Zaprinast on the growth of human prostate cancer xenografts.
Results: PDE activity assays indicated that the majority of cGMP PDE activity in prostate cancer cell lines is made up from a combination of PDE5 and PDE11 (64-86% of PDE activity). The same two PDE variants also predominated in human malignant prostate tissue as detected using Western blot. TUNEL assays revealed some increased apoptosis when prostate cancer cells were treated with a PDE inhibitor in both hypoxic and standard oxygen conditions. MICA expression was reduced when prostate cancer cells were exposed to hypoxia; however, immunogenicity could be restored by incubating hypoxic samples with a PDE inhibitor (see figure, $p<0.01$). Finally, growth of human prostate tumor xenografts in mice was inhibited by PDE inhibition compared to controls ($p<0.05$).
Conclusion: PDE 5 and 11 are present in human prostate cancer tissue and contribute to the majority of cGMP PDE activity in prostate cancer cell lines. Inhibition of PDE activity, reestablishing cGMP cell signaling in the hypoxic tumor environment, would appear to have beneficial effects including modulating hypoxia-induced cancer immune escape. These results indicate that PDE inhibition may represent a novel therapeutic or adjuvant target for men with prostate cancer.