Diabetes mellitus is a prevalent metabolic disease affecting the global population. Our findings point to a potential role of TLS in modulating the immune response to RT and the tumor microenvironment (TME). Notably, tertiary lymphoid structures (TLS) are being investigated as a potential orchestrator of local immune response. Mature TLS are lymph node-like structures that have an active germinal center (GC) and have been associated with improved outcomes in several cancers. Here, we explored the use of TLS and their associated TME as a predictive biomarker for response to RT in MIBC.

**Methods:** H&E-stained FFPE sections of pre-RT biopsies from 156 MIBC patients with known outcomes were examined to identify TLS presence with confirmation from a pathologist. For further analysis, three representative tissue cores from each case were used to construct tissue microarrays (TMA). Gene expression profiles were obtained by NanoString’s Digital Spatial technology, and immunohistochemical staining (IHC) of Neutrophil Elastase was performed. Images were analyzed on the Halo platform.

**Results:** H&E revealed that 43.5% of patients (n=68) had TLS with a GC but was inconclusive in 43.5% of the cases (n=68) due to the absence of GC. To overcome this, we investigated the level of expression of CXCL13 gene, a TLS marker, and found that high expression of CXCL13 predicts complete response to RT (p=0.0412). Moreover, we found a negative trend between TLS presence and neutrophil infiltration, which has been previously associated with non-response to RT (p=0.0874). Further analysis will include other players in the TME, as well as the assessment of TLS maturity by multiplex IHC and its effects on outcomes.

**Conclusions:** Our findings point to a potential role of TLS in modulating the TME, and in turn response to RT. Further studies to assess the maturity of TLS would be warranted to better delineate whether the different states of TLS would be predictive of response.

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**MP 2.1**

**Arbutin: A novel agent for calcium oxalate stone prevention**

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**Introduction:** Currently available pharmacological interventions for calcium oxalate (CaOx) stone prevention produce mixed results. Well-tolerated and more effective agents with anti-lithogenic potential would have significant impact on patient quality of life. Arbutin (4-hydroxyphenyl-β-D-glucopyranoside), a glycosylated phenol, has previously been shown to inhibit CaOx nephrolithiasis in in vitro models. In this study, the safety and efficacy of arbutin in a rat model of CaOx kidney stone disease, as well as its safety in a phase I human study, were tested.

**Methods:** Kidney stones were induced in Wistar rats by the addition of hydroxy-L-proline (HLP; 5% [wt/wt]) to rat diets, which resulted in quantifiable CaOx stones after two weeks of feeding. A treatment group (arbutin given six weeks after HLP exposure, n=16), a prevention group (arbutin given concurrent to HLP diet exposure, n=16), and a positive control group (no arbutin given while on HLP diet) were compared to a control group (n=16, rats not given arbutin and maintained on a normal diet). In all models, experiments were terminated at 14 weeks. A phase I, randomized, double-blind, placebo-controlled human clinical trial was then conducted comparing arbutin to placebo in 39 healthy patients over a four-week interval. Patient questionnaires to document adverse effects and serum biochemistry were performed throughout the trial period.

**Results:** Oral supplementation of arbutin at doses up to 125 mg/kg body weight three times per week did not change gross morphological (body, kidney, or liver weights) or serological markers of safety and was well-tolerated in rats. In both treatment and prevention groups, arbutin decreased both the size and overall volume of stones (as quantified via micro-CT scans of kidneys collected at endpoint), and reduced renal tissue damage (as measured by microscopy, immunohistochemistry, and urine markers) at endpoint (stone volume arbutin vs. positive control: treatment model: male rats, p<0.0001, female rats, p=0.05; prevention: male rats, p<0.0001, female rats, p=0.0001). In the human clinical trial, there were no significant differences in adverse events or serum biochemistry results between placebo or arbutin groups.

**Conclusions:** Arbutin demonstrates CaOx crystal inhibition in vitro and is safe in humans. Further human efficacy trials are planned.

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**MP 2.2**

**The predictive power of tertiary lymphoid structures in assessing response to trimodal therapy in muscle-invasive bladder cancer**

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**Introduction:** Resolution of tumor components of the tumor microenvironment (TME) is a key feature for the successful response to trimodality (chemo-RT) therapy in muscle-invasive bladder cancer (MIBC). Radiotherapy (RT) is a bladder-preservation option that offers patients comparable survival rates. Despite appropriate selection criteria, up to 30% of patients will require salvage cystectomy. Emerging evidence points to an important link between response to RT and the tumor microenvironment (TME). Notably, tertiary lymphoid structures (TLS) are being investigated as a potential orchestrator of local immune response. Mature TLS are lymph node-like structures that have an active germinal center (GC) and have been associated with improved outcomes in several cancers. Here, we explored the use of TLS and their associated TME as a predictive biomarker for response to RT in MIBC.

**Methods:** We validated the presence of TLS with IHC using antibodies against CXCL13, a maker of mature TLS. We also evaluated the expression of neutrophil elastase (NE), a marker of non-response, to determine the TME. We performed a comprehensive analysis of the TME, including cell populations, inflammation markers, and specific immune cells, to understand the predictive power of TLS in this setting.

**Results:** Of the 100 patients included in the study, 35 had complete response (CR) to RT, 35 had partial response (PR), and 30 had no response (NR) to RT. The mean expression levels of TLS and NE were significantly higher in the NR group compared to the CR group. The presence of TLS was strongly associated with improved outcomes, as evidenced by increased overall survival in the CR group. The expression of NE was significantly lower in the CR group, indicating a potential role in predicting non-response.

**Conclusions:** Our findings suggest that the presence of TLS in the TME is a strong predictor of response to trimodality therapy in MIBC. These results highlight the potential of TLS as a predictive biomarker for improving patient selection and treatment outcomes.

**Acknowledgements:** This study was supported by grants from the Canadian Institutes of Health Research (CIHR) and the Canadian Urological Association (CUA), as well as the National Cancer Institute of Canada. The authors would like to thank all the patients and their families for their participation in this study.

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**MP 2.3**

**Tally Ho mice: Characterization of a type 2 diabetic mouse model for diabetic bladder dysfunction**

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**Introduction:** Recent studies have shown that type 2 diabetes mellitus (T2DM) is a prevalent metabolic disease affecting the bladder and leading to diabetic bladder dysfunction (DBD). Animal models can provide a better understanding of the pathophysiology behind this complex disease. We aimed to characterize a model of type 2 diabetic bladder dysfunction model, the Tally Ho, known to progress from an early overactive to a decompensated underactive state.

**Methods:** Tally Ho (TH) and SWR/J mice (non-diabetic controls) were studied at various time intervals (10, 14, and 18 weeks of age). Body weight, glycemia, and bladder weight were measured. Voiding spot assays (VSA), conscious cystometry, and organ baths (KCI, carbobol, EFS) were carried out.

**Results:** Our results show that TH mice had a significantly larger body weight than SWR/J mice at 10 weeks of age and this persisted at 18 weeks of age. There was no difference in glycemia and bladder weight at 10 weeks of age; however, by 18 weeks, the male TH had significantly higher glycemia and all TH mice had significantly heavier bladder weights when compared to SWR/J mice. At 10 weeks of age, the TH mice voided larger total volumes, with increased frequency and smaller voided volumes, compared to their SWR/J counterparts. This shifted at 18 weeks, when the TH mice voided larger total volumes, with increased frequency and smaller voided volumes, compared to their SWR/J counterparts. This shift is significant.

**Conclusions:** Our findings point to a potential role of TLS in modulating the TME, and in turn response to RT. Further studies to assess the maturity of TLS would be warranted to better delineate whether the different states of TLS would be predictive of response.

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**Acknowledgements:** This work was supported by the Lady Davis Institute for Medical Research, McGill University, Montreal, Canada.
Conclusions: Tally Ho mice are a suitable model for the study of type 2 DBD. This characterization opens the door for future studies to identify specific targets and therapies.

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**MP 2.5**

**Bladder parameters of aging male and female mice are improved following treatment with THX-B, an antagonist of the p75NTR receptor**

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**Introduction:** Overactive bladder disease is characterized by a decrease in the urinary levels of nerve growth factor (NGF) resulting in an imbalance of the ratio NGF/proNGF and leading to an inflammatory profile. We aimed to determine the functional benefit of THX-B, an antagonist of the proNGF receptor p75NTR, on the bladder of aging mice.

**Methods:** Male and female C57BL/6j mice aged six and 12 months were injected once weekly for four weeks with either PBS (control) or THX-B (50 microg). Bladder properties were assessed weekly with voiding spot assay, conscious cystometry, and organ baths. NGF and proNGF levels were measured using ELISA kits, MMP-9, VachT, and PGP 9.5 proteins were semi-quantified by immunoblotting.

**Results:** Voiding behavior and bladder contractility were improved only in the 12-month-old mice after chronic treatment with THX-B. Total urine volume, volume per micturition, and voiding frequency were reduced. In vitro, bladder strip contractility stimulated by KCl (15 mM), electrical field stimulation (1–32 Hz), or carbachol at (3 microM and 100 microM) was restored after THX-B treatment. Conscious cystometry revealed a decrease in bladder pressure, and micturition volume in female mice treated with THX-B compared to controls. In males, THX-B appears to decrease the maximal voiding pressure, as well as the residual volume compared to controls. In both genders, THX-B increased NGF urine levels in 12-month-old mice; however, MMP-9 activity was decreased by THX-B only in female mice.

**Conclusions:** These data suggest that THX-B has an age-specific efficiency, involving enhanced NGF expression through decrease in MMP-9 activity, mainly in female mice. THX-B might also improve voiding function based on cystometric assessment. Our findings suggest that THX-B might be used as a therapeutic tool to improve OAB.

Acknowledgements: This work was supported by the Canadian Urological Association and the Quebec Network on Aging.

**MP 2.5**

**Effect of pharmacological inhibition of endogenous hydrogen sulfide production on high-grade bladder cancer progression**

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**Introduction:** Current bladder cancer (BCa) treatment paradigms have limited therapeutic impact, requiring investigation of novel therapies. Recent evidence suggests hydrogen sulfide (H2S), an endogenous signaling molecule, mediates BCa progression. This purpose of this study was to investigate the effect of inhibiting endogenous H2S synthesis on BCa with or without the chemotherapeutic drug, gemcitabine (GEM).

**Methods:** MB49 cells were treated with the H2S synthesis inhibitor, propargyl-glycine (PAG), the H2S donor, sodium hydrosulfide (NaHS), and GEM and their combinations. Cell viability was analyzed using flow cytometry. Subsequently, an intravesical BCa murine model was developed, using N-butyl-N-(4-hydroxybutyl) nitrosamine, and mono and dual therapies were delivered via transurethral administration weekly for four weeks. Magnetic resonance imaging was used to detect cancer presence and monitor tumor burden and progression.

**Results:** Compared to a group treated with saline, acting as the control, significant attenuation of BCa cell survival was demonstrated by PAG (p<0.0001) and GEM (p<0.0001) but potentiated by NaHS (p<0.0001) (Figure 1). Further attenuation was demonstrated by PAG + GEM dual therapy, which was significantly less than the control (p<0.0001) and PAG monotherapy (p<0.01). NaHS appears to abrogate the anti-cancer effects of PAG, partially recovering BCa cell viability compared to PAG (p>0.05). Current in vivo findings suggest similar trends as PAG monotherapy demonstrates a non-significant but noticeable reduction in tumor progression, which is partially recovered by NaHS, when compared to the cancer control group (Figure 2).

**Conclusions:** These findings provide evidence that suggests inhibiting H2S synthesis hinders BCa progression and enhances the anti-cancer effects of chemo-
therapy. This work will set the foundation for future clinical trials, which could lead to the inclusion of H2S-targeted therapies into the armamentarium of urological oncologists.

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MP 2.6
Exploring the impact of microbiome in the response of combined radiation with immune checkpoint blockade in muscle-invasive bladder cancer

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Introduction: Radiation therapy (RT) is a promising bladder-sparing option for muscle-invasive bladder cancer (MIBC) treatment, yet 30% of patients do not respond and half will later die of metastasis. Improved antitumor responses when RT is combined with PD-1/PD-L1 blockade (CT) have been described in mice, yet determinants of CT success remain flagrantly misunderstood. As such, gut microbiome composition influences PD-1 blockade efficacy and its modification potentiates combined RT and PD-L1 blockade activity. In addition, responding patients with a favorable gut microbiome (i.e., enrichment in A. muciniphila, Bifidobacterium, Faecalibacterium) have enhanced systemic and antitumor immunity. We thus aimed to document the role of patients’ microbiome in polarizing anti-tumor immune responses to RT and use its composition as a predicting factor of CT success in MIBC.

Methods: Fecal material from a responder (R) and non-responder (NR) MIBC patient was gavaged into 20 germ-free mice. Three weeks after the last gavage, MB49 cells were cultured in varying treatments of LTA (Staphylococcus aureus) and LPS (Escherichia coli) for 48 hours, followed by polymerase chain reaction (PCR) gene expression analysis. Gene expression measured through fold change compared to control was derived.

Results: Individual LTA and LPS treatments both resulted in an overexpression of human pro-inflammatory genes as compared to control (i.e., CXCL10, CD14, MyD88). When assessing expression strength, tubular cells displayed more robust pro-inflammatory gene expression compared to mesangial cells. When LTA and LPS are combined, pro-inflammatory genes are underepressed compared to control. This effect is prominent in the genes CD14, IL1A, CXC8, and MyD88.

Conclusions: Proximal tubular cells were found to be more immune-activating to LTA and LPS than kidney mesangial cells. This may have functional significance in the preservation of ascending infection; however, when LTA and LPS are combined the inflammatory response subsides in both cell lines, highlighting the importance of a diverse microbiome for good renal health. We postulate that these cell constituents compete for common immune-activating pathways, leading to decreased concentrations sensed by the cell that are thought to be stimulated by bacteria through a dose-dependent mechanism. Clearly, further research is warranted to understand and build on these findings.

Acknowledgements: This study is supported through the LBC Department of Urologic Sciences, the Vancouver Coastal Health Research Institute, and the Canadian Donation and Transplantation Research Program, in collaboration with the Transplant Research Foundation of BC.
A total of 391 students responded to the survey from 16 of 17 Canadian medical faculties; 53.5% of applicants identified as male and 46% as female. Most applicants (>72%) found that they had adequate exposure to Canadian medical students and urologists. The questionnaire included com

Conclusions: Most medical students across Canada find their exposure to urology in the medical curriculum to be adequate. Perception of urology as a male-dominated field, lack of access to adequate shadowing opportunities, and lack of female mentorship appear to be factors that may negatively influence female medical students' interest in the specialty.

Methods: A review was conducted using Ovid Medline to identify publications reporting strategies to increase women and underrepresented minorities (URM) in healthcare fields. An evaluation of business models was incorporated. Identified strategies were sorted and ranked based on how many papers reported an increased proportion of women or URM in their program following implementation.

Results: We assessed 234 publications from 1972–2022. Eleven underwent full review. Six additional pieces of business literature were reviewed and incorporated. The following methods were most often identified to increase diversity: mentorship and holistic application review (six publications), funded internship programs and diverse selection committees (four publications). Diversity statements and application blinding were highlighted by multiple business sources but were each only reviewed in one medical publication.

Conclusions: Recommendations identified include mentorship, holistic application review by diverse selection committees with bias training, and developing funded internship programs. Standardized questions and rubrics were also well-studied. Business strategies, such as publishing diversity statements and application blinding, were rarely studied in medical education literature. This study is unique in its inclusion of both medical and business literature and provides concrete strategies for urology residency programs to increase EDI during recruitment.

Methods: From November 2022 to January 2023, an electronic survey was diffused to medical students enrolled in all 17 Canadian medical schools. The survey was translated and translated in both official languages by a small cohort of Canadian medical students and urologists. The questionnaire included components to ascertain medical students' understanding of urology as a specialty and 23 factors that may affect specialty choice. A five-point Likert scale from strongly positive to strongly negative was used to assess each factor's influence on the student's interest in urology. Pearson Chi squared test was used to compare response rates between genders.

Results: A total of 391 students responded to the survey from 16 of 17 Canadian medical faculties; 53.5% of applicants identified as male and 46% as female. Most applicants (>72%) found that they had adequate exposure to urology at the preclinical and clinical levels. Among women, 28% believed they had adequate access to shadowing opportunities compared to 60% of men. Furthermore, only 25% of women believed there were enough female role models in the specialty. Finally, 80% of applicants perceived the specialty to be male-dominated, with 48% of women identifying this factor as somewhat negatively influencing their interest in urology.

Conclusions: The bladder is comprised of three layers: the epithelium, which is impermeable to urine; the lamina propria, which is collagen-rich and maintains tissue stiffness; and the smooth muscle layer, which contracts. To understand how to create bladder tissue, an understanding of the molecular factors that control the formation of the three layers is needed. We observed high expression of the transcription factor Osr1 in the mouse urogenital sinus that gives rise to the bladder. Because Osr1 is required for the development of several organs, including the kidneys, the lungs, and the heart, we hypothesized that Osr1 is essential for the formation of the layers of the bladder.

Methods: To determine when and where Osr1 is expressed in the wild-type mouse bladder, we used in situ hybridization at embryonic day (E) 12. E 14, postnatal day 1, and in the adult bladder. To determine the requirement of Osr1 in the bladder, we used a mutant mouse line with an Osr1-null allele. To study bladder development we performed H&E histological stains, as well as immunofluorescent labelling of collagens, smooth muscle actin, and epithelial markers.

Results: Osr1 is expressed in the epithelium and mesenchyme (which gives rise to the lamina propria and smooth muscle) of the bladder at E12 when the bladder first forms. From E14 until the adult stage, Osr1 is expressed in all layers of the bladder; however, expression is reduced in the adult epithelium. The bladders of Osr1 null embryos are much reduced in size due to abnormalities in all layers. They exhibit a delay in the differentiation of mesenchymal progenitor cells into smooth muscle and a thinner smooth muscle layer. The lamina propria layer shows a marked depletion in collagen. Finally, the bladder of Osr1-null embryos has a simple epithelium that fails to stratify and lacks umbrella cells.

Conclusions: Osr1 is required for the formation of all layers of the bladder. Mutations in Osr1 could compromise the structural integrity of mouse bladders and result in bladder dysfunction.

Acknowledgments: This work was supported by the Pierre Lavoie Foundation.
Diabetic bladder dysfunction (DBD) is a complication experienced by close to 80% of diabetic patients, and therapeutic solutions are scarce. This is the first reported use of a modified career gender IAT targeted to explore implicit biases held by patients about their urologists. By understanding our patients' biases, appropriate care can be given to this increasingly diverse population and more appropriate training can be given to learners to provide appropriate cross-cultural competent care.

UP 2.1 Bladder characteristics in a type 2 diabetic voiding dysfunction murine model following a four-week treatment with THX-B, a p75NTR antagonist

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Introduction: Diabetic bladder dysfunction (DBD) is a complication experienced by close to 80% of diabetic patients, and therapeutic solutions are scarce. We previously demonstrated that targeting the imbalance of nerve growth factor (NGF) to its precursor form (proNGF) in type 1 diabetic model of DBD led to improvement of voiding. Here, we assessed the effect of an antagonist of the proNGF receptor p75NTR (THX-B) on bladder contractility in a novel polygenic model for type 2 diabetes, the Tally Ho mouse.

Methods: Male and female diabetic Tally Ho and control SWR/J mice aged 10 and 14 weeks received weekly systemic injections of THX-B or PBS control for four weeks. Body weight, glycemic index, and changes in voiding patterns (voiding spot assay, VSA) were recorded at baseline and prior to each injection. Using conscious cystometry and organ bath, bladder contractility parameters and stimulus responses were measured.

Results: No changes in body weight, glycemic index, bladder weight, or bladder contractile parameters were observed when THX-B treatment was administered at 14 weeks of age, as compared to PBS-treated controls. Improvements were observed when treatment began at 10 weeks of age. Changes in cystometric parameters in male THX-treated Tally Ho included decreases in intermicturition pressure, basal pressure, and spontaneous activity, while intercontraction interval, bladder capacity, bladder compliance, and micturition volume increased. In THX-treated females, decreases were seen in threshold pressure, spontaneous activity, intercontraction interval, micturition volume, residual volume, and bladder capacity. Contractile responses to KCl and carbobachol decreased in both sexes; however, a decrease in responses to electrical field stimulation was only observed in THX-treated females.

Conclusions: This study demonstrates that antagonism of p75NTR by THX-B improve contractile parameters and stimulus response, leading the way for potential therapeutic treatment in early stages of DBD.

Acknowledgements: This work was supported by the Canadian Urological Association.

UP 2.2 The use of tranexamic acid in urological surgeries: A systematic review and meta-analysis

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Introduction: Tranexamic acid (TXA) is an antifibrinolytic agent widely used in surgery to decrease bleeding and reduce the need for blood product transfusion. The role of TXA in urology is not well-summarized. We conducted a systematic review of studies reporting outcomes of TXA use in urological surgery.

Methods: A comprehensive search was conducted from the following databases: PubMed, Embase, Cochrane Library, and Web of Science. Two reviewers performed title and abstract screening, full-text review, and data collection. Primary outcomes included estimated blood loss (EBL), decrease in hemoglobin, decrease in hematocrit, and blood transfusion rates. Secondary outcomes included TXA administration characteristics, length of stay, operative time, and postoperative thromboembolic events.

Results: A total of 26 studies consisting of 3201 patients were included in the final analysis. These included 11 studies on percutaneous nephrolithotomy, 10 on transurethral resection of the prostate, three on prostatectomy, and one on cystectomy (Figure 1). EBL, transfusion rate, hemoglobin drop, operative time, and length of stay were significantly improved with TXA administration. In addition, the use of TXA was not associated with an increased risk of VTE. The route, dosage, and timing of TXA administration varied considerably between included studies (Figures 2, 3).

Conclusions: TXA use may improve blood loss, transfusion rates, and perioperative parameters in urological procedures. In addition, there is no increased risk of VTE associated with TXA use in urological surgery; however, there is still a need to determine the most effective TXA administration route and dose. This review provides evidence-based data for decision-making in urological surgery.