16th Annual Lorne D. Sullivan Lectureship & Research Day

Date: Tuesday, June 21, 2022
Time: 8:00 AM – 3:00 PM (PDT)
Location: Paetzold Health Education Centre
Vancouver General Hospital
Dr. Anthony Atala is the Link Professor and Director of the Wake Forest Institute for Regenerative Medicine, and the Boyce Professor and Chair of the Department of Urology at Wake Forest University. Dr. Atala's work focuses on growing tissues and organs for patients and for physiological modelling. He is Editor-in-Chief of Stem Cells- Translational Medicine, and BioPrinting. Fifteen applications of technologies developed in his laboratory have been used clinically. He is editor of 25 books, has published over 800 journal articles and has applied for or received over 250 national/international patents. Dr. Atala was elected to the National Academy of Medicine and the National Academy of Inventors. He is a recipient of the US Congress funded Columbus Foundation Award, the Edison Science/Medical Award, the R&D Innovator of the Year Award, and the Smithsonian Ingenuity Award. Dr. Atala’s work was listed twice as Time Magazine’s top 10 medical breakthroughs of the year, and he was named by Nature Biotechnology as one of the top 10 translational researchers in the world. Dr. Atala completed his MD degree at the University of Louisville, and his specialty training at Children's Hospital Boston and Harvard Medical School, where he remained as a faculty member before moving to Wake Forest.
Dr. Lorne D. Sullivan

Dr. Sullivan was raised in Moss Bank, Saskatchewan, and graduated from the University of Saskatchewan, Faculty of Medicine in 1962. After an Internship at Vanderbilt University, he completed his urological training at UBC under the mentorship of Dr. John Balfour. He pursued postgraduate training in Urological Oncology as a Royal College Traveling Fellow at several US Cancer Centres before establishing his practice in British Columbia in 1971. During his years of clinical practice, he developed a highly recognized centre for the surgical management of urological cancer at UBC and was a visionary in the establishment of the Prostate Research Centre. He was a respected teacher and clinician responsible for training an entire generation of British Columbia urologists.

At the national and international level, he served as President of the Canadian Urological Association, the Northwest Urological Society, the Western Section of the AUA, and Chaired the Specialty Committee in Urology and was Chief Examiner for the Royal College of Physicians and Surgeons of Canada. He was Head of the Division of Urology at UBC from 1991-1999. He retired in 1999 to spend time with his family and grandchildren, but continues to grace the Department with his presence at academic and social functions.

Previous Sullivan Lectureship & Research Day Lecturers

2021 - Dr. Christopher Barbieri
2019 – Dr. Christopher P. Evans
2018 – Dr. Robert Reiter
2017 – Dr. James E. Lingeman
2016 – Dr. Michael A.S. Jewett
2015 – Dr. John M. Barry
2014 – Dr. James A. Eastham
2013 – Dr. Paul Lange
2012 – Dr. Ralph V. Clayman
2011 – Dr. David A. Bloom
2010 – Dr. Gerald H. Jordan
2009 – Dr. Peter T. Scardino
2008 – Dr. Inderbir Singh Gill
2007 – Dr. Joao Pippi Salle

Previous Division of Urology Graduation Dinner Guest Speakers

2006 – Dr. John Fitzpatrick
2005 – Dr. Laurence H. Klotz
2004 – Dr. Ralph V. Clayman
2003 – Dr. Denis H. Hosking
2002 – Dr. Anthony E. Khoury
2001 – Dr. Ian Thompson
2000 – Dr. Richard J. Finley
Program

Welcome
8:00 AM – 8:15 AM    Dr. Martin Gleave, CM, MD, FRCSC, FACS
Distinguished Professor and Head, Department of Urologic Sciences, UBC
Executive Director, Vancouver Prostate Centre
British Columbia Leadership Chair in Prostate Cancer Research

Dr. Robert McMaster, PhD
Vice Dean, Research, Faculty of Medicine, UBC

Dr. David Granville, PhD, FAHA
Executive Director, Vancouver Coastal Health Research Institute
Professor and Associate Dean, Research, Faculty of Medicine, UBC

Lorne D. Sullivan Lectureship
8:15 AM – 8:55 AM    Regenerative Medicine New Approaches to Healthcare
Dr. Anthony Atala, MD
George Link, Jr. Professor and Director
Wake Forest Institute for Regenerative Medicine
W. H. Boyce Professor and Chair, Department of Urology
Wake Forest School of Medicine

Session I: Clinical Kidney
(7-minute talk and 3-minute Q&A)
8:55 AM – 9:55 AM    Moderator: Dr. Ryan Paterson

8:55 AM – 9:05 AM    URETEROSCOPY WITH THULIUM FIBER LASER LITHOTRIPSY VERSUS PERCUTANEOUS NEPHROLITHOTOMY FOR THE TREATMENT OF RENAL STONES 15-20MM
Abdulghafour A Halawani, Jessica Que, Victor Wong, Ben Chew

9:05 AM – 9:15 AM    THULIUM FIBER LASER VERSUS HOLMIUM: YAG: A CLINICAL COMPARISON OF LASER LITHOTRIPSY EFFICIENCY IN A RETROSPECTIVE OF 73 PATIENTS AT A TERTIARY STONE CENTRE
Alec Mitchell, Victor Wong, Abdulghafour Halawani, Ryan Paterson, Ben Chew

9:15 AM – 9:25 AM    PREDICTING TRANSPLANT KIDNEY FUNCTION DECLINE FROM ULTRASOUND ONLY USING AN INTERPRETABLE ARTIFICIAL INTELLIGENCE MODEL
Ricky Hu, Rohit Singla, Zoe Hu, Cailin Ringstrom, Victoria Lessoway, Janice Reid, Robert N. Rohlin, Timothy Murray, Christopher Nguan

9:25 AM – 9:35 AM    THE KIDNEYS ARE NOT NORMAL: ULTRASOUND SPECKLE DISTRIBUTIONS OF TRANSPLANTED KIDNEYS
Rohit Singla, Cailin Ringstrom, Ricky Hu, Victoria Lessoway, Janice Reid, Robert N. Rohlin, Christopher Nguan
9:35 AM – 9:45 AM  COMPARISON OF SURGICAL OUTCOMES BETWEEN STAGED VERSUS SIMULTANEOUS NATIVE NEPHRECTOMY FOR AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE
Anthony Emmott, Iman Baharmand, Michael Eng, Christopher Nguan, David Harriman

9:45 AM – 9:55 AM  IN VITRO AND IN VIVO ASSESSMENT OF PATHOGENIC PROPERTIES OF PROTEUS MIRABILIS IN URINARY TRACT INFECTION AND STRUVITE STONE FORMATION
Roman Herout, Sara Khodami, Igor Moskalev, Alina Reicherz, Ben Chew, Chelsie Armbuster, Dirk Lange

State of Art Lecture I
9:55 AM – 10:10 AM  PROMs’ PROMises in Pediatric Urology
Dr. Soojin Kim
Investigator and Pediatric Urologist, BC Children’s Hospital
Clinical Assistant Professor, Department of Urologic Sciences, UBC

Break and Posters 10:10 AM – 10:40 AM

Session II: Andrology
(7-minute talk and 3-minute Q&A)
10:40 AM – 11:10 AM  Moderator: Dr. Dirk Lange

10:40 AM – 10:50 AM  INVESTIGATING INTRATESTICULAR HETEROGENEITY IN NON-OBSTRUCTIVE AZOOSPERMIA USING SINGLE CELL RNA SEQUENCING AND IMMUNE CELL PHENOTYPING

10:50 AM – 11:00 AM  DEEP LEARNING-BASED AUTOMATED SPERM IDENTIFICATION FROM TESTIS BIOPSES

11:00 AM – 11:10 AM  UNDERRECOGNIZED FACTORS AFFECTING PENILE IMPLANT SATISFACTION IN PATIENTS
Julie Wong, Luke Witherspoon, Ryan Flannigan

Session III: Bladder Cancer
(7-minute talk and 3-minute Q&A)
11:10 AM – 11:50 AM  Moderator: Dr. Lucia Nappi

11:10 AM – 11:20 AM  NOVEL 3D PERSONALIZED BLADDER CANCER MODEL ON DEMAND: A NEW ERA FOR PERSONALIZED MEDICINE.
Monjaras-Avila CU, Luque-Badillo AC, Chavez-Munoz C; So AI

11:20 AM – 11:30 AM  PROTEOMICS PROFILING OF MUSCLE INVASIVE BLADDER CANCERS TREATED WITH NEOADJUVANT CHEMOTHERAPY

11:30 AM – 11:40 AM  MULTI-OMIC PROFILING OF METASTATIC UROTHELIAL CARCINOMA PATIENTS EXHIBITING EXCEPTIONAL RESPONSE TO SYSTEMIC CHEMOTHERAPY
A NOVEL CHIMERIC ANTIGEN RECEPTOR SYSTEM THAT TARGETS ONCOFETAL CHONDROITIN SULFATE GLYCOSAMINOGLYCANS IN SOLID TUMORS
Nastaran Khazamipour, Nader Al Nakouzi, Htoo Zarni Oo, Morgan Robert, Tobias Gustavsson, Ali Salanti, and Mads Daugaard

MULTICENTER EVALUATION OF NEOADJUVANT AND INDUCTION GEMCITABINE-CISPLATIN VERSUS GEMCITABINE-CISPLATIN FOLLOWED BY RADICAL CYSTECTOMY FOR MUSCLE-INVASIVE BLADDER CANCER

Lunch and Posters

Session IV: Computational Biology
(7-minute talk and 3-minute Q&A)

12:45 PM – 1:45 PM Moderator: Dr. Alexander Wyatt

12:45 PM – 12:55 PM DEVELOPMENT OF A NOVEL SINGLE CELL METHODOLOGY TO ASAY ENHANCER ACTIVITY
Tunc Morova, Dogancan Ozturan, Chia-Chi Flora Huang, Funda Sar, Nathan A. Lack

12:55 PM – 1:05 PM scTAGGER: FAST AND ACCURATE MATCHING OF CELLULAR BARCODES ACROSS SHORT- AND LONG-READS OF SINGLE-CELL RNA-SEQ EXPERIMENTS
Ghazal Ebrahimi, Baraa Orabi, Meghan Robinson, Cedric Chauve, Ryan Flannigan, Faraz Hach

1:05 PM – 1:15 PM GENION, AN ACCURATE TOOL TO DETECT GENE FUSION FROM LONG TRANSCRIPTOMICS READS
Fatih Karaoglanoglu, Cedric Chauve, Faraz Hach

1:15 PM – 1:25 PM CIRCULATING TUMOUR DNA FRACTION AS AN INDEPENDENT PROGNOSTIC TOOL IN METASTATIC CASTRATION-RESISTANT PROSTATE CANCER

1:25 PM – 1:35 PM PROSPECTIVE IDENTIFICATION OF ACTIONABLE CTDNA ALTERATIONS IN A PHASE II PRECISION ONCOLOGY TRIAL FOR ADVANCED PROSTATE CANCER
Wilson Tu, Edmond M Kwan, Andrew Murtha, Cecily Bernales, Matti Annala, Kim N.Chi, Alexander W. Wyatt

1:35 PM – 1:45 PM A GENERALIZABLE MACHINE LEARNING FRAMEWORK FOR CLASSIFYING DNA REPAIR DEFECTS USING CTDNA EXOMES.
Elie J. Ritch, Cameron Herberts, Sarah W. S. Ng, Jack V. W. Bacon, Evan W. Warner, Gillian Vandekerkhove, Corinne Maurice-Dror, Cecily Q. Bernales, Elena Schönlau, Nicolette M. Fonseca, Steven J. M. Jones, Kim N. Chi, Alexander W. Wyatt

State of Art Lecture II

1:45 PM – 2:00 PM A Puzzle of Complexity: Androgen Receptor-Mediated Transcription in Prostate Cancer
Dr. Nathan Lack
Senior Research Scientist, Vancouver Prostate Centre
Assistant Professor, Department of Urologic Sciences, UBC
Associate Professor, School of Medicine, Koç University (Istanbul, Turkey)
Session V: Prostate

(7-minute talk and 3-minute Q&A)

2:00 PM – 2:10 PM  
MODERATOR: Dr. Xuesen Dong

2:00 PM – 2:10 PM  
SUPER-ENHANCER REMODELLING IN ENZ RESISTANT PROSTATE CANCER IS ASSOCIATED WITH STEMNESS AND MYC FAMILY BINDING
Maxim Kobelev, Dwipayun Ganguli, Takeshi Namekawa, Joshua Scurl, Amina Zoubeidi

2:10 PM – 2:20 PM  
CHAPERONE MEDIATED AUTOPHAGY COORDINATES METABOLIC REPROGRAMMING OF PROSTATE CANCER CELLS PROMOTING TREATMENT RESISTANCE
Nicholas Nikesitch, Eliana Beraldi, Fan Zhang, Kotaro Suzuki, Hans Adomat, Robert Bell, Ladan Fazli, Christopher Wells, Nicholas Pinette, Neetu Saxena, Yuzhuo Wang, Colin Collins, Martin Gleave

2:20 PM – 2:30 PM  
REGULATION AND TARGETING OF CHONDROITIN SULFATE GLYCOSAMINOGLYCANS IN PROSTATE CANCER

2:30 PM – 2:40 PM  
LONGITUDINAL SINGLE CELL RNA SEQUENCING OF A NEUROENDOCRINE PROSTATE CANCER MODEL
Funda Sar, Dong Lin, Tunc Morova, Anne Haegert, Robert Bell, Yen-Yi Lin, Cindy Dong, Hui Xue, Stanislav Volik, Nathan Lack, Yuzhuo Wang, Colin Collins

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URETEROSCOPY WITH THULIUM FIBER LASER LITHOTRIPSY VERSUS PERCUTANEOUS NEPHROLITHOTOMY FOR THE TREATMENT OF RENAL STONES 15-20MM

Abdulghafour A Halawani1, Jessica Que1, Victor Wong1, Ben Chew1

1. Urologic Science, The University of British Columbia, Vancouver, BC, Canada

Introduction:
Endourology has experienced revolutionary inventions in the last few decades. Thulium Fiber Laser (TFL) is the latest laser technology that has showed promising advantages in both pre-clinical and clinical settings, which may allow larger stones to be treated ureteroscopically. This study aimed to compare the effectiveness of TFL ureteroscopy vs percutaneous nephrolithotomy (PCNL) in the management of renal stones 15 to 20 mm.

Method:
We retrospectively analyzed the medical records of 42 patients who underwent either TFL lithotripsy (n=21) or PCNL (n=21) for 15-20mm renal stones. Stone-free rate (SFR) was assessed with one or combined imaging modalities with either non-contrast CT, KUB-ultrasound, or X-ray. Other variables, including operative time, stone size and density, prior ureteral stenting was recorded. In the case of multiple stones, total stone surface area was measured.

Results:
Characters such as Patients’ age (years), prior ureteral stenting, number of stones, stone density, and total stone surface area (mm2) were similar between the groups (Table 1). The length of surgery was significantly lower in TFL group (p=0.002) (Table 1). Compared to TFL, PCNL group had longer hospital stay (p=0.0001) (Table 1). Our data showed no statistically significant difference in SFR between TFL and PCNL groups.

Conclusion:
This study revealed that TFL lithotripsy produced a similar stone free rate to PCNL with significantly lower operative time and hospital stay. TFL can be an effective alternative to PCNL in the management of 15-20 mm renal stones. However, further randomized trials are warranted.

| Table 1: Stone and Operative Outcome Thulium Fiber Laser Lithotripsy versus PCNL |
|---------------------------------|-------|-------|-------|-------|-------|
|                                | TFL   | PCNL  | p      | p      |
| Number of Subjects             | 21    | 21    | -      |       |
| Age, Years                     | 57.57 | 54    | 0.5019 |       |
| Pre-stented, % Yes             | 33% (7/21) | 19% (4/21) | 0.4876 |       |
| Surgery Length, MM: SS (S.D.)  | 51.48 (23.98) | 93.10 (52.43) | 0.002 |       |
| Number of Stones, Mean (S.D.)  | 2.86 (1.85) | 2.43 (2.20) | 0.4990 |       |
| Total Stone Surface Area (mm2), Mean (S.D.) | 355.63 (341.72) | 569.70 (720.59) | 0.2259 |       |
| Total Stone Density, HU (S.D.) | 1031.24 (443.97) | 1151.71 (299.79) | 0.3089 |       |
| Ureteral/Renal Pelvis Injury, % Yes | 5% (1/21) | 10% (2/21) | 1.000 |       |
| Length of Hospital Stay, Days (S.D.) | 0.52 (1.29) | 2.19 (0.60) | 0.000 |       |
| Stone-Free, % Yes              | 52% (11/21) | 76% (16/21) | 0.1971 |       |
THULIUM FIBER LASER VERSUS HOLMIUM: YAG: A CLINICAL COMPARISON OF LASER LITHOTRIPSY EFFICIENCY IN A RETROSPECTIVE OF 73 PATIENTS AT A TERTIARY STONE CENTRE

Alec Mitchell, Victor Wong, Abdulghafour Halawani, Ryan Paterson, Ben Chew

INTRODUCTION AND OBJECTIVE:
Since its first use by Dr. Denstedt in 1993 the Holmium: YAG laser has been the gold standard laser for lithotripsy. Recently, new emerging laser technology has evolved for clinical use: the Thulium Fiber Laser (TFL), which has shown promising results in several preclinical studies. This new technology may expand the boundaries of laser lithotripsy. This study aimed to compare the efficacy of TFL and Ho: YAG in terms of stone fragmentation rate, operative time, and stone-free rate.

METHODS:
A retrospective analysis was conducted at a tertiary stone center to identify patients treated with Ho: YAG or TFL laser lithotripsy. Seventy-three cases were included, 42 patients in Ho: YAG group and 31 in the TFL group. Operative time was calculated from scope introduction to scope removal. Stone-free rate (SFR) was assessed with one or multiple imaging modalities. These were non-contrast CT, KUB-ultrasound, or X-ray. Other variables, including stone size, density and prior ureteral stenting were recorded. In the case of multiple stones, total stone surface area was measured as well.

RESULTS:
Patients' age (years), prior ureteral stenting, procedure time (min:sec), and total stone surface area were similar between groups. The number of treated stones was higher in the TFL group (p= 0.0015) (Table 1). Compared to Ho: YAG, TFL showed a significantly higher rate of stone fragmentation per mm2 stone surface area (p= 0.02). The data showed a similar SFR between Ho: YAG and TFL groups, as well as the size of residual fragments (classified as < 4mm or > 4mm) (Table 1).

CONCLUSIONS:
This study demonstrates that the TFL has a more efficient lithotripsy effect per mm^2 stone surface area than Ho: YAG. SFR was similar for both Ho: YAG and TFL; however, this is confounded by the fact that a significantly higher number of stones have been treated with TFL. In our study, TFL produced a similar stone free rate to Ho: YAG at a more efficient rate of lithotripsy. Further clinical studies are warranted to tease out the above results and to determine whether Thulium can truly challenge holmium as the default laser in urology.

Source of Funding: No funding

.../continued on page 9
Table 1: Stone and operative outcomes holmium vs thulium.

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<th>Holmium</th>
<th>Thulium</th>
<th>p-value</th>
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<tr>
<td>Number of Subjects</td>
<td>42</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>Age, Years</td>
<td>57.14 (16.00)</td>
<td>58.48 (14.32)</td>
<td>0.72</td>
</tr>
<tr>
<td>BMI (S.D.)</td>
<td>29.49 (5.29)</td>
<td>24.43 (8.36)</td>
<td>0.035*</td>
</tr>
<tr>
<td>Pre-stented, % Yes</td>
<td>14.29% (6/42)</td>
<td>25.81% (8/31)</td>
<td>0.22</td>
</tr>
<tr>
<td>Surgery Length, MM: SS (S.D.)</td>
<td>00:48:32 (18:54)</td>
<td>00:41:12 (25:24)</td>
<td>0.17</td>
</tr>
<tr>
<td>Total Laser Energy, Joules (S.D.)</td>
<td>4100.61 (5616.82)</td>
<td>16850.03 (7523.32)</td>
<td>0.00039*</td>
</tr>
<tr>
<td>Number of Stones, Mean (S.D.)</td>
<td>1.26 (0.69)</td>
<td>2.22 (1.67)</td>
<td>0.0015*</td>
</tr>
<tr>
<td>Total Stone Surface Area (mm²), Mean (S.D.)</td>
<td>96.37 (82.87)</td>
<td>95.13 (67.93)</td>
<td>0.94</td>
</tr>
<tr>
<td>Total Stone Density, HU (S.D.)</td>
<td>860.03 (361.83)</td>
<td>781.95 (368.00)</td>
<td>0.37</td>
</tr>
<tr>
<td>Rate of Stone Fragmentation, mm² /</td>
<td>2.27 (1.67)</td>
<td>3.40 (2.30)</td>
<td>0.02*</td>
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Tests used: Numerical Variables = Student’s T-Test; Categorical Variables = Fisher’s Exact Test
PREDICTING TRANSPLANT KIDNEY FUNCTION DECLINE FROM ULTRASOUND ONLY USING AN INTERPRETABLE ARTIFICIAL INTELLIGENCE MODEL

Ricky Hu¹, Rohit Singla²,³ Zoe Hu¹, Cailin Ringstrom⁴, Victoria Lessoway⁴, Janice Reid⁴, Robert N. Rohling⁴, Timothy Murray⁵, Christopher Nguan⁶

1. School of Medicine, Queen’s University, Kingston, ON, Canada
2. School of Biomedical Engineering, University of British Columbia, Vancouver, BC, Canada
3. Faculty of Medicine, University of British Columbia, Vancouver, BC, Canada
4. Dept. of Electrical & Computer Engineering, University of British Columbia, Vancouver, BC, Canada
5. Dept. of Radiology, University of British Columbia, Vancouver, BC, Canada
6. Dept. of Urologic Sciences, University of British Columbia, Vancouver, BC, Canada

Objective:
Prognostication of kidney function may benefit the optimization of clinical care in post-transplant patients to ensure long-term graft survival. Subtle features in ultrasound imaging may aid in the long-term prognostication. We developed an interpretable artificial intelligence (AI) algorithm to predict a 5-year 30% decline in estimated glomerular filtration rate (eGFR) using only post-transplant ultrasound images.

Materials and Methods:
Post-transplant ultrasound scans and 5-year eGFR values from N=819 renal transplant patients were obtained from Vancouver General Hospital. A multi-stage AI algorithm to segment whole kidney, cortex, and medullary regions. Imaging features related to morphology, intensity, texture, and ultrasound speckle (total 110 features) were computed for each region. A random forest classifier was trained to use the features to predict 5-year eGFR decline. Predictions were compared to actual outcomes with 5-fold cross-validation using a 80/20 train/test split. The Kidney Failure Risk Equation (KFRE) was used as a comparator. Predictive features were identified by computing Gini importance scores. Subgroup analysis by age (>60 and <60) and eGFR (>15 ml/min and >15ml/min) was performed.

Results:
A mean (± standard deviation) prediction accuracy of 0.85 ± 0.05 was achieved using images only. There was no significant difference between the age and chronic kidney disease subgroups (p>0.05). Accuracy was significantly higher than the use of only KFRE (0.62 ± 0.03). In multivariable analysis, cortical elongation (ratio of the cortex thickness to cortex length), cluster shade, and speckle parameters combined were the three most predictive features. Cortical elongation is correlated to thinness and cluster shade represents inhomogeneities in tissue, both of which are known to be associated with pathology.

Conclusions:
A non-invasive eGFR decline prediction tool was created using multi-stage AI. The architecture allows for extraction of predictive features which provide interpretability to the model to serve as a future physician-aid tool for prognostication.
THE KIDNEYS ARE NOT NORMAL: ULTRASOUND SPECKLE DISTRIBUTIONS OF TRANSPLANTED KIDNEYS

Rohit Singla1,2, Cailin Ringstrom3, Ricky Hu4, Victoria Lessoway3, Janice Reid3, Robert N. Rohling3,5, Christopher Nguan6

1. School of Biomedical Engineering, University of British Columbia, Vancouver, BC
2. Faculty of Medicine, University of British Columbia, Vancouver, BC
3. Dept. of Electrical & Computer Engineering, University of British Columbia, Vancouver, BC
4. School of Medicine, Queen's University, Kingston, ON
5. Dept. of Mechanical Engineering, University of British Columbia, Vancouver, BC
6. Dept. of Urologic Sciences, University of British Columbia, Vancouver, BC

Objective:
Ultrasonic speckle is dependent on tissue architecture. In the native kidney, the glomeruli are hypothesized to be the dominant scatter causing speckle. Changes in the speckle may reflect changes in the glomeruli architecture. However, it is unknown which statistical distribution best characterizes the speckle in transplanted kidneys. This includes the compartments of the cortex and medulla. We investigate a series of distributions and assess their ability to discriminate between transplanted kidney compartments.

Materials and Methods:
In a retrospective cohort of transplant ultrasound scans (n=821) from Vancouver General Hospital, a segmentation network was applied to extract the cortex and medullary regions. In each region, a probability distribution is fitted onto the intensity values resulting in distribution-specific parameters. Seven different statistical distributions were used based on whether the vasculature or glomerulus were considered the scatterers. Student’s t-test was used to evaluate differences in distribution parameters between regions in a pair-wise manner. Kullback-Leibler divergence (KL) and goodness of fit were used to identify the distribution with minimal error. Subgroup analysis by age, body mass index (BMI), sex, ethnicity, primary diagnosis, donor age, and type were examined for disparities.

Results:
Only the Rayleigh and Nakagami distributions had parameters that were significantly different in each region (p < 0.05). Both had low fitting errors (<0.05), but the Nakagami had a higher KL value. Recipient age and BMI had significant correlations with Nakagami parameters (age: rho=0.11, p < 0.05, BMI: rho=0.10, p < 0.05). No other demographic had a correlation.

Conclusions:
The Nakagami distribution is able to discriminate between the cortex and medulla of transplanted kidneys regardless of recipient’s sex, ethnicity, primary diagnosis or donor’s age or type. This distribution may be used to track changes in glomerular health, and may aid in segmentation or disease detection.
COMPARISON OF SURGICAL OUTCOMES BETWEEN STAGED VERSUS SIMULTANEOUS NATIVE NEPHRECTOMY FOR AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE

Anthony Emmott¹ MD, Iman Baharmand² BSc, Michael Eng¹ MD, Christopher Nguan¹ MD, David Harriman¹ MD

¹. Department of Urologic Sciences, University of British Columbia
². Faculty of Medicine, University of British Columbia

Introduction and Objectives:
Bilateral native nephrectomy (BNN) for patients with autosomal dominant polycystic kidney disease (ADPKD) is considered in select cases at or around the time of renal transplant (RT). There is no consensus regarding staged versus simultaneous BNN. The purpose of this study is to compare the surgical outcomes between patients with ADPKD undergoing BNN as a staged or simultaneous procedure with RT.

Methods:
Retrospective review of patients with ADPKD who underwent RT between 2009 and 2020 at two related academic institutions was undertaken. Clinical records were reviewed to collect data on intraoperative and post-operative details, including graft survival, blood transfusion rates and post-operative complications. Data were analyzed with one-way analysis of variance (ANOVA) or Pearson’s chi-squared test in SPSS.

Results:
Of the 150 patients identified, 46 underwent BNN (18 simultaneous with RT; 11 prior to RT; 17 post RT). Demographic characteristics between groups were comparable. Patients in the simultaneous group received transplants exclusively from living donors whereas those in the staged groups had a mix of living and deceased donors (p=0.003). There was no difference in graft survival. 4 patients (22%) in the simultaneous group received a blood transfusion, while no patients in the staged groups required transfusion (p=0.069). There were no differences in postoperative incisional hernias, wound dehiscence, ileus, lymphoceles, biopsy confirmed rejection, cardiac event or readmission between groups (p=NS). Post-operative infection was highest in the prior to RT group which coincided with a higher rate of open procedures, while median length of hospital stay was lower in the simultaneous group (all p<0.05).

Conclusions:
Staged or simultaneous BNN with RT are both feasible and safe. Decreased hospital stay and a trend towards increased blood transfusion was seen in the simultaneous group with open surgeries associated with increased infections.
IN VITRO AND IN VIVO ASSESSMENT OF PATHOGENIC PROPERTIES OF PROTEUS MIRABILIS IN URINARY TRACT INFECTION AND STRUVITE STONE FORMATION

Roman Herout¹², Sara Khoddami¹, Igor Moskalev¹, Alina Reicherz¹³, Ben H. Chew¹, Chelsie E. Armbruster⁴, Dirk Lange¹

¹. The Stone Centre at Vancouver General Hospital, Department of Urologic Sciences, University of British Columbia, Vancouver, British Columbia, Canada
². Department of Urology, University Hospital Carl Gustav Carus, TU Dresden, Dresden, Germany
³. Department of Urology, Marien Hospital Herne, Ruhr-University of Bochum, Herne, Germany
⁴. Department of Microbiology and Immunology, University of Buffalo, NY, USA

INTRODUCTION AND OBJECTIVES:

Proteus mirabilis (PM) is a Gram-negative, urease-positive bacterium that is associated with catheter-associated urinary tract infections (CAUTI) and struvite stone formation. The mechanisms of PM invasion are poorly studied, and the role of bacterial surface components (BSCs) remain widely unknown.

METHODS:

In vitro adhesion and invasion assays were performed: kidney (HEK293, A498) and bladder cells (T24) were exposed to wild type (WT) or one of seven different PM mutant strains (MSs) with deficiencies in genes encoding BSCs. Cells were incubated for 2&24h, lysed for CFU counts (adhesion) or treated with antibiotics and then lysed (invasion). Subsequently, we performed these assays in an established CAUTI mouse model: a 4mm catheter piece was percutaneously introduced into the bladder. Proteus WT or MSs were introduced percutaneously (5x10⁵ bacteria) in the bladder the following day. On d3 mice were sacrificed. The number of bacteria in urine, adherent to catheters, and adherent to/invaded into bladder tissue were assessed.

RESULTS:

In vitro, WT showed greater adhesion to all cell types when compared to the MSs. No significant invasion of the MSs was detectable at 24h. In vivo, WT showed greater number of planktonic (urine) bacteria, bacteria adherent to catheters, and bacteria adherent to/invading bladder tissue when compared to the MSs. The number of bacteria in urine for the CEA058 and waaE mutants was lower than that for WT & other MSs. Re-introduction of the knocked out BSC genes resulting in the biggest defects (complemented strains –cCEA and cwaaE) resulted in the return of the invasion phenotype both in vitro and in vivo.

CONCLUSION:

BSCs play an important role in various stages of CAUTI development, including adhesion and biofilm formation on indwelling catheters. Specifically pertaining to the virulence of P. mirabilis and UTI/Struvite pathogenesis, BSCs are essential for the invasion of bladder cells.
INTRODUCING INTRATESTICULAR HETEROGENEITY IN NON-OBSTRUCTIVE AZOOSPERMIA USING SINGLE CELL RNA SEQUENCING AND IMMUNE CELL PHENOTYPING

Arina Piechka1, Ghazal Ebrahimi1, Luke Witherspoon1, Meghan Robinson1, Anton Afanassiev2, Geoffrey Schiebinger2, Eric Belanger3, Katy Milne4, David Bond5, Faraz Hach1, Ryan Flannigan1,5

1. Department of Urologic Sciences, University of British Columbia, Vancouver BC
2. Department of Mathematics, University of British Columbia, Vancouver BC
3. Department of Pathology, University of British Columbia, Vancouver BC
4. Deeley Research Centre, British Columbia Cancer Victoria, Victoria BC
5. Department of Urology, Weill Cornell Medicine, New York NY.

Introduction and Objectives:
The etiology of Non-obstructive azoospermia (NOA) remains largely unknown. Furthermore, significant heterogeneity exists between and within NOA patients' testes. This study aimed to capture heterogeneity within an NOA testis and investigate cell-type-specific abnormalities to normal controls.

Methods:
Two testis biopsies were derived from one patient with NOA with known histologic heterogeneity, and one normal control. ScRNAseq was carried out using 10X Genomics and Illumina sequencing platforms in technical duplicates. Cell clustering, gene expression, Ingenuity Pathway Analysis (IPA), ligand-receptor analyses and TradeSeq pseudotime differential expression analyses were used to evaluate the datasets.

Results:
Cell types detected in our normal control was congruent with the previously published dataset. One NOA sample largely lacked germ cells and was broadly categorized as Sertoli Cell Only (SCO). This sample uniquely demonstrated a large cluster of cells expressing a mixture of Leydig and myoid genes; this same cluster exhibited high expression of NR4A1—known marker of common progenitor of Leydig and myoid cells suggesting incomplete maturation. T cells and granulocytes were also present which were absent among normal controls. In the second NOA sample, we detected all germ cells up to elongated spermatids and labelled this as hypospermatogenesis (HS). In this HS NOA sample, T cells were also present. After aligning the normal germ cells and HS NOA germ cells in pseudo time, differential gene expression and IPA pathway analyses revealed that NOA-derived germ cells had down regulation in RNA and nucleotide base excision repair mechanisms suggesting intrinsic deficiencies. Histology corroborated our scRNAseq results and 6-channel immunofluorescence demonstrated increased HLA-DR+ and CD163+ immune cell density surrounding NOA tubules.

Conclusions:
Differentiating features of the SCO sample were the presence of T cells, granulocytes and immature markers among Myoid and Leydig cell populations. Pathway analyses of NOA germ cells suggest intrinsic abnormalities in RNA processing.
DEEP LEARNING-BASED AUTOMATED SPERM IDENTIFICATION FROM TESTIS BIOPSES

Ryan Lee1,2,3, Luke Witherspoon3,4,5, Meghan Robinson3,4, Jeong Hyun Lee1,2, Simon P. Duffy1,2,6, Erik Lamoureux1,2, Ryan Flannigan3,4,7*, Hongshen Ma1,2,4,8*

1. Department of Mechanical Engineering, University of British Columbia, Vancouver, BC, Canada
2. Centre for Blood Research, University of British Columbia, Vancouver, BC, Canada
3. Department of Urologic Sciences, University of British Columbia, Vancouver, BC, Canada
4. Vancouver Prostate Centre, Vancouver General Hospital, Vancouver, BC, Canada
5. Department of Urology, The Ottawa Hospital, Ottawa, ON, Canada
6. British Columbia Institute of Technology, Burnaby, BC, Canada
7. Department of Urology, Weill Cornell Medicine, New York, NY, USA
8. School of Biomedical Engineering, University of British Columbia, Vancouver, BC, Canada

*Corresponding Authors: hongma@mech.ubc.ca; ryan.flannigan@ubc.ca

Introduction and Objectives:

Non-obstructive azoospermia (NOA) is the most severe form of male infertility. Currently, it is treated using microsurgical sperm extraction (microTESE) to retrieve sperm cells for in vitro fertilization via intracytoplasmic sperm injection (IVF-ICSI). The success rate of this procedure for NOA patients is limited by the ability of andrologists to identify a few rare sperm cells among millions of background testis cells. We sought to improve this procedure’s success rate by applying artificial intelligence and computer vision systems to detect rare sperm from microscopy images.

Methods:

We obtained low-resolution microscopy images of microTESE samples for training and testing of a convolutional neural network (CNN). Our CNN uses the U-Net architecture to perform pixel-based classification on image patches from brightfield microscopy images, followed by a computer vision morphological analysis to detect individual sperm instances. This deep learning model is trained using microscopy images of fluorescently labelled sperm, doped into testis biopsies from NOA patients, and the cells are fixed to eliminate their motility.

Results:

This deep learning algorithm was tested using purified sperm samples at different imaging magnifications to determine the upper bounds of performance. We achieved the best performance at 20X, followed by 10X, and 4X magnifications, as expected. We found a sperm detection F1-score of 85.2% when doping rare sperm cells into testis biopsies from NOA patients.

Conclusions:

These results demonstrate the potential to use automated microscopy to dramatically increase the amount of testis biopsy tissue that can be comprehensively exampled, greatly increasing the chance of finding viable rare sperm, and thereby increasing the success rate of IVF-ICSI for couples with NOA.
Introduction and Objectives: Surgical management via penile prosthesis is an option for patients who have failed medical management. There is a paucity of literature surrounding factors contributing to patient satisfaction after implant surgery. The objective of this study was to characterize patients' and surgeons' attitudes toward factors affecting satisfaction with this procedure.

Methods: Two patient cohorts were identified and contacted via email: a medical management of erectile dysfunction (ED) cohort and a penile implant patient cohort. A third cohort, Canadian urologists who perform penile implant surgeries, were also contacted. The surveys consisted of 5-7 questions, including a rating question regarding the importance of various penile implant factors.

Results: 46 ED patients, 45 post-implant patients, and 12 urologists completed the survey. Mean overall satisfaction on a 10-point scale was 6.49 (SD=2.92). 67% of urologists selected patient satisfaction as one of their least favourite aspects of penile implant surgery. Compared to post-implant patients, ED patients reported greater importance in the areas of appearance (p=0.035), soft glans (p=0.040), and concealment of implant (p=0.007). Urologists ranked natural feel (p=0.019) and generating a discrete erection (p=0.022) as less important than patients.

Conclusions: This is the first study that examines which specific variables of penile implant surgery are associated with satisfaction, while comparing surgeons' understanding of what patients' desire from this surgery. This study identifies several factors deemed important by patients but underrecognized by urologists. This knowledge can aid urologists in optimizing pre-operative counselling and improving patient satisfaction.
NOVEL 3D PERSONALIZED BLADDER CANCER MODEL ON DEMAND: A NEW ERA FOR PERSONALIZED MEDICINE

Monjaras-Avila, CU1,5; Luque-Badillo, AC2,5; Chavez-Munoz, C.4,5*; So, Al3,5*

1. Interdisciplinary Oncology Program, Faculty of Medicine, University of British Columbia, Canada
2. Experimental Medicine Program, Faculty of Medicine, University of British Columbia, Canada
3. Department of Urologic Sciences, Faculty of Medicine, University of British Columbia, Canada
4. Department of Medicine, Faculty of Medicine, University of British Columbia, Canada
5. Vancouver Prostate Centre, Department of Urologic Sciences, University of British Columbia, Canada

Introduction and objectives:
Bladder cancer (BCa) is the most expensive cancer to treat on a per-patient basis because of a 60-70% recurrence rate. Muscle-invasive BCa is currently treated with radical cystectomy and neoadjuvant chemotherapy. One clinical limitation is the inability to predict which patients would benefit from chemotherapy and which tumors are inherently chemoresistant. There are some predictive tumor models to guide individual treatment, unfortunately, they do not fulfill all requirements. The objective of this project is to develop a 3D in vitro patient-derived BCa tumor model as a preclinical platform for drug testing and predicting patients’ outcomes in a personalized manner.

Methods:
To engineer the 3D cancer model, we obtained BCa tumor tissue from VGH. BCa samples were dissociated into single cells and seeded into decellularized pig bladders. For genetic validation, we used targeted sequencing in patients and their corresponding engineered 3D tumors by comparison of 50 driver genes. 3D BCa models from patients were treated with cisplatin and gemcitabine (Gem/Cis). The results were compared to their corresponding BCa patient chemotherapy outcomes.

Results:
We have already established a protocol for decellularizing bladders. Decellularized tissue has been evaluated by histology, SEM and DNA quantification. We have successfully optimized a reseeding protocol using UM-UC3 cells as proof-of-principal and later human BCa patient cells. This model has been tested with Gem/Cis, showing a statistically significant reduction in cell viability as chemotherapy concentrations increase. I am currently working BCa patient samples for genetic validation.

Conclusions:
In conclusion, this 3D patient-derived BCa model will enable us to generate many in vitro avatars to accurately recreate the tumor of a patient, and simultaneously screen for suitable drugs in a personalized manner.
PROTEOMICS PROFILING OF MUSCLE INVASIVE BLADDER CANCERS TREATED WITH NEOADJUVANT CHEMOTHERAPY

Contreras-Sanz A1, Reike MJ1, Negri GL2, Oo HZ1, Spencer Miko SE2, Nielsen K2, Roberts ME1, Scurll J1, Ikeda K1, Wang G1, Seiler R1, Morin GB2, Black PC1

1. Vancouver Prostate Centre
2. BC Cancer Research Institute

Introduction and Objectives:
Neoadjuvant chemotherapy (NAC) followed by radical cystectomy (RC) is recommended for muscle invasive bladder cancer (MIBC). However only ~40% of eligible patients show an objective response. While genomic alterations and transcriptomic classifiers have been shown to predict response to NAC in retrospective studies, proteomic analysis of MIBC in this context is lacking. SP3-Clinical Tissue Proteomics (SP3-CTP) uses formalin-fixed paraffin-embedded tissue (FFPE) to circumvent the need for fresh frozen tissue. Here we profile the proteome of MIBC in the context of NAC to: 1) identify potential novel prognostic biomarkers, and 2) study the biology of NAC-resistant tumors.

Methods:
Pre-treatment tissue was included from 107 MIBC patients who received NAC followed by RC. Residual tumor (≥pT1, pN0-3) in the RC specimen after NAC was available for 55 patients (51%). Benign ureter was used as control. FFPE samples were subjected to multiplex SP3-CTP and bioinformatic analysis.

Results:
We quantified ~10,000 proteins across all samples. Unsupervised clustering of preNAC tissue established four clusters with distinct survival outcomes, but no difference in pathologic stage after NAC: CC1, with high metabolic activity and a luminal profile; CC2, with high nuclear activity; CC3 with high immune infiltration, and basal characteristics; and CC4, with high immune infiltration and increased lipid metabolism. CC3 showed worse overall survival (p<0.01) and aligned with the RNA-based basal subtype. Multivariable analysis adjusting for other prognostic variables identified both favorable (MTIF3, MAPK9) and unfavorable (NES, DVL2) biomarkers. Matched analysis of pre- and postNAC RC tissue identified biomarkers indicative of NAC resistance.

Conclusions:
We identified four proteomic clusters with distinct biology and survival, alongside novel prognostic biomarkers. We are validating these results by immunohistochemistry in a larger NAC cohort. A non-NAC cohort will be used to confirm the prognostic vs. predictive relevance of these findings.
MULTI-OMIC PROFILING OF METASTATIC UROTHELIAL CARCINOMA PATIENTS EXHIBITING EXCEPTIONAL RESPONSE TO SYSTEMIC CHEMOTHERAPY

Scurll J1, Contreras-Sanz A1, Roumiguie M1, Chen E1, Roberts ME1, Oo HZ1, Ikeda K1, Chehroudi C1, Vandekerkhove G1, Bacon JvW1, Wyatt AW1, Black PC1

1. Vancouver Prostate Centre

Introduction and Objectives:
Metastatic urothelial carcinoma (mUC) is an aggressive malignancy with limited therapeutic options and poor prognosis. However, a small subset of mUC patients have durable responses to first or second-line systemic chemotherapy. We molecularly profiled tumors in a cohort of patients with exceptional response (ER) to treatment to understand potential therapeutic vulnerabilities. We aimed to establish the rationale for larger studies focusing on predictive and prognostic profiles of ER.

Methods:
Ten mUC patients with ER (defined as clinical complete or partial response to systemic chemotherapy lasting ≥18 months) were identified. We performed whole-transcriptome and targeted-DNA sequencing, and multi-colour immunohistochemistry (mIHC), on all available specimens (N=19). For all patients we included tumor tissues immediately preceding the metastatic event. For 5/10 patients we included historic (non-metastatic) serial surgical specimens.

Results:
Targeted sequencing showed mutations or copy number alterations in 27/60 genes across the cohort. Similar to chemo-naive muscle-invasive bladder cancer (MIBC-TCGA2017) and poor-response mUC (prmUC) cohorts, the most commonly mutated genes were TP53, KDM6A, KMT2D and ARID1A. Mutations in DNA damage repair (DDR) genes were more frequent in ER patients compared to MIBC-TCGA2017 and prmUC (50%, 28%, and 26% respectively), with ERCC2 being the most commonly mutated among those. 75% of ER tumors were classed as luminal by RNA analysis, vs. 59% and 44% of the MIBC-TCGA2017 and prmUC cohorts. mIHC showed ER tumors had increased CD8+GzmB+/FoxP3+ cell populations compared to a cohort of muscle-invasive bladder cancer patients treated with neoadjuvant chemotherapy (NAC).

Conclusions:
Our results suggest that ER to systemic chemotherapy may be conferred by multiple molecular mechanisms, including DDR gene alterations, specific transcriptomic subtype and immune microenvironment. Contrary to previous work in the context of NAC, luminal tumors are enriched amongst the mUC patients with ER. Future work will compare this small cohort with a clinically-similar prmUC cohort.
A NOVEL CHIMERIC ANTIGEN RECEPTOR SYSTEM THAT TARGETS ONCOFETAL CHONDROITIN SULFATE GLYCOSAMINOGLYCANS IN SOLID TUMORS

Nastaran Khazamipour1,2, Nader Al Nakouzi2, Htoo Zarni Oo2, Morgan Robert1,2, Tobias Gustavsson3, Ali Salanti3, Mads Daugaard1,2

1. Department of Urologic Sciences, University of British Columbia, Vancouver, British Columbia
2. Vancouver prostate centre
3. University of Copenhagen

The malaria parasite Plasmodium falciparum expresses VAR2CSA proteins on the surface of infected red blood cells that bind a distinct type of chondroitin sulfate (CS) glycosaminoglycan chains exclusively present in the placenta. Placental-type CS becomes re-expressed in human tumors as a secondary oncofetal CS (ofCS) modification to proteoglycans and can therefore be exploited as a tumor target recognized by recombinant VAR2CSA (rVRA2). Here, we report the design and testing of a chimeric antigen receptor (CAR) T cell strategy that utilizes the CS-binding domain of rVAR2.

Incorporation of the rVAR2 sequence into a CAR backbone, produced a universal anti-ofCS CAR (rVAR2-CAR) with affinity for ofCS-positive tumor cells. To improve safety and control the activity of the rVAR2-CAR T cells, a switch-CAR system was designed in which a CAR could be secondarily ‘armed’ with a rVAR2-switch molecule. The population of armed CAR T cells was controlled by the availability of the switch-molecule and halting the addition of the switch molecule during the clonal T cell expansion phase, cleared the armed population of sVAR2-CAR T cells. Co-culturing sVAR2-CAR T cells with human tumor cells led to sVAR2-CAR T cell activation as assessed by activation markers and cytokine production. Notably, sVAR2-CAR T cells effectively eliminated diverse ofCS-positive tumor cells and two treatments of sVAR2-CAR T cells markedly decrease tumor burden in a murine xenograft model of bladder cancer.

In summary, we find that the ofCS-VAR2 binding module can be utilized to develop functional ofCS-reactive CAR T cells for potential treatment of solid tumors.
Background:

Cisplatin-based induction and neoadjuvant chemotherapy is the standard of care for muscle invasive bladder cancer with and without lymph node metastasis, respectively. However, up to 50% of patients are cisplatin-ineligible. Gemcitabine-carboplatin (GCa) represents an alternative chemotherapy regimen for these patients, although it is thought to be less effective than gemcitabine-cisplatin (GC). This study aims to compare pathological response and survival between neoadjuvant/induction GC and GCa followed by RC.
Methods:

A retrospective review was performed on patients who received at least three cycles of neoadjuvant (cT2-4aN0M0) or induction (cTanyN1-3M0) GC or GCa followed by RC at one of 19 centers between 2000 and 2013. Demographic and clinical parameters were compared using Student's t test, chi-squared, or Fisher's exact test. Putative risk factors for cancer-specific and overall survival were analyzed using Cox regression, while predictors of pathological response were investigated using logistic regression.

Results:

Data were available for 747 (147 GCa and 600 GC) patients. Patients treated with GCa were significantly older (67 vs 65 years; p<0.001), had a higher Charlson Comorbidity Index (p=0.016) and had a higher rate of clinical node-positive disease (32% vs 20%; p=0.013) than patients treated with GC. The rate of complete pathological response (pCR; ypT0N0) did not significantly differ between GCa and GC groups (20.7% vs 22.1% respectively; p=0.73). Chemotherapy regimen was not a predictor of overall or cancer-specific survival and was not associated with pCR in the multivariable analysis. Subanalyses comparing the neoadjuvant and induction setting also failed to show significant survival differences between the groups.

Conclusion:

There was no significant difference in pathological response, cancer-specific and overall survival between patients who received induction or neoadjuvant GC versus GCa followed by RC. This suggests that cisplatin-ineligible patients may benefit from GCa chemotherapy prior to RC.
DEVELOPMENT OF A NOVEL SINGLE CELL METHODOLOGY TO ASSAY ENHANCER ACTIVITY

Tunc Morova1, Dogancan Ozturan1, Chia-Chi Flora Huang1, Funda Sar1, Nathan A. Lack1,2

1. Vancouver Prostate Center, Department of Urologic Science, University of British Columbia, Vancouver, Canada
2. Koç University Research Center for Translational Medicine (KUTTAM), Koç University, Istanbul, Turkey

Background:
Enhancers are non-coding DNA elements that act as critical regulators of spatiotemporal gene expression. Yet enhancer usage is markedly heterogeneous and dramatically varies by cell type. Dysregulation of enhancers have been previously associated with various diseases such as multiple cancers, Alzheimer’s disease, and diabetes. Tissue specific activity of enhancers are affected by genomic features such as TF binding, chromatin accessibility and histone marks. Current enhancer quantification assays (STARRseq) can accurately determine active enhancers. However, the gene-enhancer linkage information is lost during the aggregation of the bulk signal. To explore this problem, we are developing the first single-cell enhancer assay, scSTARRseq, that can delineate cellular enhancer activity. Based on 10x Chromium chemistry, this method can be combined with single cell transcriptomics to successfully differentiate the enhancer-gene activation patterns of individual cells. This novel method further will be used for genetic perturbations for critical Androgen Receptor (AR) coregulators and novel drug testing in prostate cancer (PCa).

Methods:
Building on 10x V3 chemistry, we developed a methodology that can selectively amplify mRNA from the STARR transcript. We picked 286 ARBS enhancers as well as 30 positive and 22 negative control sequences. Barcoded AR enhancers oligos are synthesized, cloned into novel scSTARRseq plasmid and validated by next-generation sequencing. LNCaP cells were electroporated with scSTARRseq library and hormone-deprived for 72 hours and treated with androgen for 6 hours. Upon collection of the cells, they are introduced to 10x Genomics microfluidics platform and RNA species are cell barcoded and both enhancer STARR RNA and cellular mRNA information is gained from the same cell.

Results & Conclusions:
Our quality control results showed that scSTARRseq successfully demonstrated the activation of positive control and ARBS enhancer sequences. Preliminary results with respect to bulk STARRseq had high correlation in positive control regions 77% and moderate correlation in ARBS enhancers 55%. During our optimisation we found that scSTARRseq accuracy is affected by transfection amount, PCR cycle and PCR DNA amount which will be in our future development. Along with experimental optimisations, we also developed a streamlined computational pipeline for quality control, enhancer activity measurement.
scTagger: FAST AND ACCURATE MATCHING OF CELLULAR BARCODES ACROSS SHORT- AND LONG-READS OF SINGLE-CELL RNA-SEQ EXPERIMENTS
Ghazal Ebrahimi1, †, Baraa Orabi2, †, Meghan Robinson3, Cedric Chauve5, Ryan Flannigan3,4, Faraz Hach2,3,4

1. Bioinformatics Program, the University of British Columbia, Vancouver, BC, Canada
2. Computer Science Department, the University of British Columbia, Vancouver, BC, Canada
3. Vancouver Prostate Centre, Vancouver, BC, Canada
4. Department of Urologic Sciences, the University of British Columbia, Vancouver, BC, Canada
5. Department of Mathematics, Simon Fraser University, Burnaby, BC, Canada
†: These authors contributed equally to this work

Background:
Single-cell RNA sequencing allows for characterizing the gene expression landscape at the cell type level. However, because of its use of short-reads, it is severely limited at detecting full-length features of transcripts such as alternative splicing. New library preparation techniques attempt to extend single-cell sequencing by utilizing both long- and short-reads. These techniques split the library material, after it is tagged with cellular barcodes, into two pools: one for short-read sequencing and one for long-read sequencing. However, the challenge of utilizing these techniques is that they require matching the cellular barcodes sequenced by the erroneous long-reads to the cellular barcodes detected by the short-reads.

Methods:
We introduce scTagger, a novel computational method that matches cellular barcodes data from long- and short-reads. scTagger performs this matching in three main stages. In the first stage scTagger reduces the size of the cellular barcode whitelist from millions of barcodes to a barcode whitelist of thousands enabling drastically faster computation for the subsequent matching. In the second stage, scTagger exploits the known long-read sequence template that results from the library preparation to extract for each long-read a short segment containing the read’s barcode. Finally, in the third stage, scTagger matches the small list of whitelisted barcodes to the long-read segments using a novel method based on the classic trie data structure.

Results:
We tested scTagger against another state-of-the-art tool on both real and simulated datasets and we demonstrate that scTagger has both significantly better accuracy and time efficiency. We also compare scTagger against a brute-force method that can be considered a baseline for matching barcodes. We show that scTagger is able to achieve as well as the baseline brute-force method in terms of matching accuracy in an order of magnitude less time.
GENION, AN ACCURATE TOOL TO DETECT GENE FUSION FROM LONG TRANSCRIPTOMICS READS

Fatih Karaoglanoglu1,3, Cedric Chauve2, Faraz Hach3,4,5

1. School of Computing Science, Simon Fraser University, Burnaby, BC, Canada
2. Department of Mathematics, Simon Fraser University, Burnaby, BC, Canada
3. Vancouver Prostate Centre, Vancouver, BC, Canada 4. Computer Science Department, the University of British Columbia, Vancouver, BC, Canada 5. Department of Urologic Sciences, the University of British Columbia, Vancouver, BC, Canada

Background:
The advent of next-generation sequencing technologies empowered a wide variety of transcriptomics studies. A widely studied topic is gene fusion which is observed in many cancer types and suspected of having oncogenic properties. Gene fusions are the result of structural genomic events that bring two genes closely located and result in a fused transcript. This is different from fusion transcripts created during or after the transcription process. These chimeric transcripts are also known as read-through and trans-splicing transcripts. Gene fusion discovery with short reads is a well-studied problem, and many methods have been developed. But the sensitivity of these methods is limited by the technology, especially the short-read length. Advances in long-read sequencing technologies allow the generation of long transcriptomics reads at a low cost. Transcriptomic long-read sequencing presents unique opportunities to overcome the shortcomings of short-read technologies for gene fusion detection while introducing new challenges.

Method:
We developed Genion, a computational method that discovers gene fusions. Genion identifies candidate fusion isoforms with a dynamic programming algorithm and statistically filters out false chimeras created during library preparation and sequencing.

Results:
We compare Genion against a recently introduced long-read gene fusion discovery method, LongGF, both on simulated and real datasets. On simulated data, Genion accurately identifies the gene fusions and its clustering accuracy for detecting fusion reads is better than LongGF. Furthermore, our results on the breast cancer cell line MCF-7 show that Genion correctly identifies all the experimentally validated gene fusions.
CIRCULATING TUMOUR DNA FRACTION AS AN INDEPENDENT PROGNOSTIC TOOL IN METASTATIC CASTRATION-RESISTANT PROSTATE CANCER
Nicolette M. Fonseca1, Corinne Maurice-Dror2, Cameron Herbetts1, Andrew J. Murtha1, Catarina Kollmansberger2, Edmond M. Kwan1, Daniel J. Khalaf2, Matti Annala3, William Fan2, Joanna Vergidis2, Krista Noonan2, Daygen L. Finch2, Muhammad Zulfiqar2, Stacy Miller2, Kim N. Chi2 and Alexander W. Wyatt1,4
1. Vancouver Prostate Centre, Department of Urologic Sciences, University of British Columbia
2. BC Cancer, Vancouver, British Columbia
3. Prostate Cancer Research Center, Faculty of Medicine and Life Sciences and BioMediTech Institute, University of Tampere, Tampere, Finland
4. Michael Smith Genome Sciences Centre, BC Cancer, Vancouver, British Columbia, Canada

Introduction:
Metastatic castration resistant prostate cancer (mCRPC) is clinically heterogeneous but current models do not accurately predict patient life expectancy. The levels of circulating tumour DNA (ctDNA) in patient blood plasma can be quantified as the ctDNA fraction (ctDNA%), and prior data suggests a link between ctDNA% and patient prognosis. Our objectives were to: 1) determine the relationship between ctDNA% and clinical markers of disease burden; 2) evaluate the independent prognostic value of ctDNA% for predicting overall survival (OS) and PSA progression-free survival (PSA PFS) in patients prior to first-line therapy for mCRPC.

Methods:
462 treatment-naive mCRPC patients had blood samples drawn prior to first-line therapy. Plasma cell-free DNA was subjected to targeted sequencing and ctDNA% was estimated using validated methods. OS, and PSA PFS were stratified by ctDNA% and compared using Kaplan-Meier and Cox proportional hazards analysis.

Results:
Median follow-up was 20.3 mo (range 0.3-81.64) and median ctDNA% was 5% (range: 0-89%). ctDNA% was positively correlated with alkaline phosphatase (p=0.46, p<0.001), lactate dehydrogenase (p=0.41, p<0.001) and PSA (p=0.3, p<0.001). Stratifying patients into high (ctDNA>30%) and low (ctDNA≤2%) groups showed stronger association with OS [HR: 5.77, 95% CI: 4.27-7.79, p<0.001] and PSA PFS [HR: 5.31, 95% CI: 3.87-7.29, p<0.001] than grouping by median ctDNA% [OS HR: 3.31, 95% CI: 2.64-4.17, p<0.001; PSA PFS HR: 2.76, 95% CI: 2.21-3.45, p<0.001]. In a multivariable model incorporating clinical markers, ctDNA>30% remained strongly associated with OS [HR: 4.24, 95% CI: 2.72-6.61, p<0.001] and PSA PFS [HR: 4.3, 95% CI: 2.77-6.68, p<0.001].

Conclusions:
The levels of ctDNA in blood plasma measured prior to first-line systemic therapy for mCRPC outperforms established clinical factors for estimating patient prognosis. Since ctDNA% is increasingly reported by commercial test providers, we posit that this variable could be easily leveraged to improve models that predict patient life expectancy.
PROSPECTIVE IDENTIFICATION OF ACTIONABLE CTDNA ALTERATIONS IN A PHASE II PRECISION ONCOLOGY TRIAL FOR ADVANCED PROSTATE CANCER

Wilson Tu1, Edmond M Kwan1, Andrew Murtha1, Cecily Bernales1, Matti Annala1,2, Kim N. Chi1,3, Alexander W. Wyatt1

1. Vancouver Prostate Centre, Department of Urologic Sciences, University of British Columbia, British Columbia, Canada
2. Faculty of Medicine and Health Technology, Tampere University and Tays Cancer Centre, Tampere, Finland
3. BC Cancer, Vancouver Centre, Vancouver, British Columbia, Canada

Introduction:

Precision oncology trials in metastatic castration-resistant prostate cancer (mCRPC) rely on genomic profiling of archival prostate tissue biopsies despite failure rates of 30-40%. We report findings from the first 500 plasma samples screened on a phase II, multicentre, eight-arm umbrella trial (NCT03385655) using circulating tumour DNA (ctDNA) to match mCRPC patients to biomarker (BM)-informed targeted therapies.

Methods:

mCRPC patients progressing after androgen receptor inhibitor therapy were eligible. Plasma cell-free DNA underwent targeted sequencing with an exon-limited panel (n=315) or expanded panel integrating select introns and a genome-wide copy number grid (n=183). A molecular tumour board (MTB) assigned patients to treatment arms based on prespecified BM criteria (BM+), or by randomization if BM negative (BM-). We report tumour content (ctDNA%), alterations, BM status, and serial sample analysis for screened samples.

Results:

503 samples from 469 patients were screened from 11/2017-11/2021. Median time from blood draw to MTB decision was 20 days and decreased over time (27 days in year 1-2 vs 17 days in year 3-4). 339 samples (68%) had ctDNA detected (ctDNA+), of which the median ctDNA was 18.7% (IQR 6-35%). 51% of ctDNA+ samples were BM+ (37% of all screened samples). Driver alterations influencing BM status included AR (74%), PI3K pathway (36%; PTEN 29%, PIK3CA 6%, AKT 3%), and DNA repair defects (26%; mismatch repair 6%, BRCA2 5%, ATM 9%, CDK12 9%, other 10%). The expanded panel detected additional intronic structural variants, notably in PTEN (2% vs 17%) and BRCA2 (0.6% vs 5%). In 47 patients with >1 sample, 32 (68%) experienced an increase in ctDNA% over time (median +8%), and 13 of 46 (28%) changed from BM- to BM+.

Conclusions:

Prospective centralized screening of ctDNA in mCRPC is highly feasible for guiding precision oncology initiatives. Our framework can be used in new biomarker-driven trials to stratify patients according to genomic alteration status.
A GENERALIZABLE MACHINE LEARNING FRAMEWORK FOR CLASSIFYING DNA REPAIR DEFECTS USING CTDNA EXOMES

Elie J. Ritch1, Cameron Herberts1, Sarah W. S. Ng1, Jack V. W. Bacon1, Evan W. Warner1, Gillian Vandekerkhove1, Corinne Maurice-Dror2, Cecily Q. Bernales1, Elena Schönlau1, Nicolette M. Fonseca1, Steven J. M. Jones3, Kim N. Chi2, Alexander W. Wyatt1,2,3

1. Vancouver Prostate Centre, Department of Urologic Sciences, University of British Columbia
2. Department of Medical Oncology, BC Cancer
3. Michael Smith Genome Sciences Centre, BC Cancer

Introduction:
DNA damage repair (DDR) deficiency causes sensitivities to certain targeted therapies for prostate cancer. However, current approaches based on sequencing coding regions of candidate DDR genes do not accurately predict treatment benefit. We aimed to develop a machine-learned framework that uses minimally-invasive blood collections to identify prostate cancers with different classes of DDR deficiency, without requiring specific gene-specific mutation information or whole-genome sequencing.

Methods:
We used deep whole-exome DNA sequencing (WES) of liquid-biopsies to develop a novel computer software: DARC Sign (DnA Repair Classification SIGNatures). DARC Sign combines mutations and copy number signature spectra to create XGBoost models that can classify prostate cancers with deficiencies in BRCA2, CDK12 or the mismatch repair (MMR) pathway. Models for each category were trained and evaluated in 129 prostate cancer WES liquid-biopsy samples where DDR gene alteration status was known from prior targeted sequencing. Models were also tested in WES data from 26 bladder cancers.

Results:
DARC Sign identified and differentiated deficiencies in BRCA2, CDK12 and MMR from each other as well as from samples with proficient DDR. Unique strengths included learned differentiating features rather than preselected features, and the use of more practical WES versus whole-genome data. The resulting models were interpretable and robust: BRCA2 AUC = 0.99 (accuracy=0.98), CDK12 = 0.99 (accuracy = 0.98) and MMR AUC = 1.00 (accuracy=1.00).

Conclusions
This study demonstrates that mutation and copy number features in practical liquid biopsy data can be used to classify different types of DDR deficiencies in patients with prostate cancer. Our new software is publicly available and may help in future clinical trials that aim to optimize response rates to targeted therapies through improved patient selection.
SUPER-ENHANCER REMODELLING IN ENZ RESISTANT PROSTATE CANCER IS ASSOCIATED WITH STEMNESS AND MYC FAMILY BINDING

Maxim Kobelev1, Dwaipayan Ganguli1, Takeshi Namekawa1, Joshua Scurll1, Amina Zoubeidi1

1. Vancouver Prostate Centre, Department of Urologic Sciences, University of British Columbia, Vancouver, BC, CA

Introduction:
The implementation of highly potent next-generation AR pathway inhibitors has prolonged survival of men with advanced prostate cancer. However, prolonged AR pathway inhibition (ARPI) can alter the archetypal course of the disease, leading to emergence of particularly aggressive variants that have lost their luminal identity and have gained stem-like and neuroendocrine characteristics. With the exception of RB1 and TP53 loss, genomic analyses have not defined genomic features that reliably distinguish plasticity phenotypes, suggesting that epigenetic events are key contributing factors for cell plasticity and treatment resistance. The objective of this study is to explore changes in active epigenetic marks with a particular focus on super-enhancers and to identify factors that regulate these active regions in castrate resistant prostate cancer (CRPC) versus neuroendocrine prostate cancer (NEPC).

Methods:
ChIPseq for active histone marks (H3K27ac) and various transcription factors was performed in CRPC and ENZ resistant (ENZR) NEPC-like cells. RNA-seq was integrated with ChIP-seq to identify potential drivers and to create a signature which were applied to patient data sets. Publicly available H3K27ac data in cell lines and PDX models were leveraged to validate our findings.

Results:
We observed extensive remodelling of super-enhancers in our NEPC-like model compared to CRPC. Genes annotated to these regions are associated with stem cell pathways and are strongly upregulated as a result of ARPI in vitro, in vivo, and in patients. Factor enrichment analysis at NEPC-like super-enhancers showed enrichment for MAX and MYC family binding that was also found in small cell lung cancer (SCLC) cells, suggesting that these super-enhancers are common between NEPC and SCLC. ChIP-seq for MYC and MAX revealed an expansion of MAX cistrome and contraction of MYC cistrome in NEPC-like cells compared to CRPC. In particular, we saw increased binding of MAX at ENZR super-enhancers and neuronal associated genes.

Conclusions:
Our analysis of super-enhancers revealed significant remodelling in NEPC-like ENZR cell lines. These regions show particularly strong association with stem signatures and ARPI. MAX binding was expanded in the ENZR model and showed increased binding at super-enhancers. MYC was mostly absent from unique ENZR MAX sites suggesting the involvement of MYCN/MYCL. However, our ENZR model does not express MYCN, suggesting a potential role for MYCL in treatment resistant prostate cancer.
Androgen deprivation therapy (ADT) and androgen receptor pathway inhibition (ARPI) remains the standard of care for advanced prostate cancer (PCa). The androgen receptor (AR) plays an important role in PCa metabolism, with ARPI subjecting PCa cells to acute metabolic stress caused by reduced biosynthesis and energy production. The upregulation of ARPI stress response mechanisms is essential in protecting PCa cells from acute ARPI induced stress which compromises their survival and proliferation. This requires PCa cells undergoing rapid phenotypic changes to adapt to their environment and circumvent lethal outcomes. By profiling proteomic pathway alterations associated with the ARPI stress response in LNCaP cells, we identified the upregulation of chaperone mediated autophagy (CMA), a stress response mechanism yet to be defined in PCa. CMA, a selective protein degradation pathway, specifically targets protein substrates via a CMA recognition motif and is an essential survival mechanism in cancer cells during energy depleted metabolic stress. During acute ARPI induced stress, CMA is upregulated and coordinates proteome remodeling of PCa cells to circumvent abrupt environmental changes caused by treatment. The upregulation of CMA leads to an enrichment of gene sets associated with AR-independent PCa growth, which coincides with in-vitro and in-vivo modeling, where the overexpression of L2A (mediator of CMA activity) promotes PCa growth during treatment using Enzalutamide. This appears to be mediated through metabolic reprogramming, reflected through changes in metabolic and biosynthetic pathways identified through CMA gain- and loss-of-function experiments. Interestingly, the upregulation of CMA coincides with the concomitant upregulation of metabolic enzymes involved in replenishing the TCA cycle. Inhibition of these enzymes, in combination with Enzalutamide, re-sensitizes L2A overexpressing cells to Enzalutamide treatment. In summary, our data illustrates the importance of CMA in mediating the ARPI stress response in PCa, providing novel insights into the mechanisms of ARPI treatment resistance.
REGULATION AND TARGETING OF CHONDROITIN SULFATE GLYCOSAMINOGLYCANS IN PROSTATE CANCER

Zakir Tahiry1,2, Irina Nelepcu1,2, Chris Kedong Wang1,2, Htoo Zarni Oo1,2, Nada LaLlous1,2, Charlotte B. Spliid3,7, Nastaran Khazamipour1,2, Joey Lo1,2, Sarah Truong1,2, Colin Collins1,2, Desmond Hui2, Shaghayegh Esfandnia2, Hans Adomat2, Thomas Mandel Clausen3,7, Tobias Gustavsson3,8, Swati Choudhary3,8, Robert Dagil1,8, Eva Corey9, Yuzhuo Wang2, Anne Chauchereau6, Ladan Fazli1,2 & Mads Daugaard1,2

1. Department of Urologic Sciences, University of British Columbia, Vancouver, BC, Canada
2. Vancouver Prostate Centre, Vancouver, BC, V6H 3Z6, Canada
3. Centre for Medical Parasitology at Department for Immunology and Microbiology, Faculty of Health and Medical Sciences, University of Copenhagen and Department of Infectious Disease, Copenhagen University Hospital, Copenhagen, Denmark
4. Department of Urology, University of Washington, Seattle, WA 98195
5. Fred Hutchinson Cancer Centre, Seattle, WA 98109-1024, United States
6. Prostate Cancer Group, INSERM UMR981, Gustave Roussy, University of Paris-Saclay, F-94805, Villejuif, France.
7. Department of Cellular and Molecular Medicine, University of California San Diego, La Jolla, CA, USA.
8. VAR2pharmaceuticals Ole Maaløes Vej 3, 2200 København, Denmark

Introduction and background:
Chondroitin sulfate (CS) chains are secondary glycosaminoglycan modifications to CS proteoglycans (CSPGs) that are expressed on the exterior of plasma membranes as part of the cellular glycocalyx. CS chains are comprised of alternating GalNAc and GlcAc sugar rings that can be sulfated on different atomic positions. The sulfation patterns of CS chains dictate how CSPGs interact with signalling molecules in the microenvironment and how they engage intracellular pathways to control cell behaviour. Elevated levels of CS have been observed in prostate cancer for more than three decades. CS in PC display high levels of GalNAc carbon-4 (C4S) sulfation across the CS chains. While cancer-associated CS alterations are currently being pursued as therapeutic targets in PC, the mechanisms governing CS expression and roles in PC progression remain elusive.

Results:
PC progression is associated with resistance to androgen receptor (AR) pathway inhibition (ARPI) and supported by a reactive tumor microenvironment. Here we show that changes in CS chains are AR-regulated and promote the adaptive progression of castration-resistant prostate cancer (CRPC) after ARPI. AR directly represses transcription of the 4-O-sulfotransferase gene CHST11 under basal androgen conditions, maintaining steady-state CS in early-stage prostate adenocarcinomas. When AR signaling is inhibited by ARPI or lost during progression to non-AR-driven CRPC as a consequence of lineage plasticity, CHST11 expression is unleashed, leading to elevated C4S levels. Moreover, we show that CHST11 expression correlates with metastases in PC patients and inhibition of the tumor cell CS glycocalyx delays CRPC progression and impairs growth and motility of PC.

Conclusion:
The PC cell glycocalyx is AR-regulated and promotes disease progression and metastases development. The glycocalyx CS-composition shift towards a 4-O-dominated CS signature can be targeted by recombinant lectins with high specificity for drug-delivery. Thus, CS glycocalyx represents a therapeutic opportunity in tumors with elevated presentation of C4S CS-glycosaminoglycans.
LONGITUDINAL SINGLE CELL RNA SEQUENCING OF A NEUROENDOCRINE PROSTATE CANCER MODEL

Funda Sar1*, Dong Lin1*, Tunc Morova1, Anne Haegert1, Robert Bell1, Yen-Yi Lin1, Cindy Dong1, Hui Xue1, Stanislav Volik1, Nathan Lack1, Yuzhuo Wang2-3**, Colin Collins1**

*equally contributed
**co-corresponding

1. Vancouver Prostate Centre, Vancouver, BC, Canada.
2. Department of Urologic Sciences, Faculty of Medicine, University of British Columbia, Vancouver, BC, Canada
3. Department of Experimental Therapeutics, BC Cancer Agency, Vancouver, BC, Canada

Adenocarcinoma of the prostate (PCa) is the third leading cause of death from cancer in Canada. It is well established that advanced PCa growth depends on reactivation androgen receptor signaling. Potent treatments such as enzalutamide, are used to treat men with advanced PCa. While these potent drugs are effective at prolonging life, ultimately progression to castrate-resistant prostate cancer is inevitable. It has been observed that these drugs drive 10-17% of adenocarcinomas to transdifferentiate into a highly aggressive androgen-independent variant, neuroendocrine PCa (NEPC). Patients with NEPC generally live less than a year because there are no effective therapies for this aggressive disease. To study the emergence of NEPC, we have developed a clinically relevant patient-derived xenograft, LTL331. LTL331 is derived from a prostatectomy specimen of therapy-naive PCa. While the tumor initially responds to host castration, shrinking in size with a decrease in AR and PSA expression, later it transdifferentiates to NEPC just as it did in the donor. The ability of LTL331 to recapitulate the clinical course of the patient makes it an ideal system to study transdifferentiation of PCa into NEPC.

We applied single-cell RNA sequencing across a time series to determine the changes in transcriptional programs underlying the transdifferentiation. Here, we will present the preliminary results, where we first have employed a trajectory inference method to combine physical time-points with pseudotime to order and anchor the cells on the temporal axis. This allowed us to identify early transdifferentiating (eTD) cells. We then were able to compare changes in gene expression from PCa, eTD to fully differentiated NE cells. To complement, we determined the changes in pathways and analyzed transcription factor activation. For genome analysis copy number was inferred from expression data. This revealed unexpected copy number dynamics during transdifferentiation. Finally, we inferred interactions of tumor cells to their microenvironment.
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EXPRESSION AND CHARACTERIZATION OF HOXB13 A CRITICAL PROSTATE-SPECIFIC TRANSCRIPTION FACTOR

Ugur Meric Dikbas1,2, Brittany Rufenach-Barber3, Joseph Lee2, Nada Lallous1,2, Filip Van Petegem3, Nathan A. Lack1,2,4.

1. Department of Urologic Sciences, University of British Columbia, Vancouver, British Columbia
2. Vancouver Prostate Centre, University of British Columbia, 2660 Oak Street, Vancouver, British Columbia V6H 3Z6, Canada
3. Department of Biochemistry and Molecular Biology, Life Sciences Institute, University of British Columbia, Vancouver, Canada
4. School of Medicine, Koç University, Rumelifeneri Yolu, Istanbul 34450, Turkey

Introduction and Objectives:
HOX family of proteins are master regulators of embryonic development that share a highly conserved DNA binding domain. One of the members of this family, HOXB13 is a tissue-specific transcription factor that is essential for prostate organogenesis. Mutations or aberrant expression of HOXB13 is linked to prostate cancer (PCa). Even though HOXB13 is essential for the growth and proliferation of almost all prostate cancer models, there is little known about its unique co-regulator binding domain. While DNA-binding domain is highly conserved, the co-regulator domain of HOXB13 offers a promising pharmacological target potentially treat late stage PCa. In this study, we aim to exogenously express and characterize HOXB13 to support the design of small-molecule compounds for the treatment of CRPC.

Method and Results:
Utilizing a bacterial expression system, we optimized the exogenous expression of HOXB13 with different purification tags and truncations. From this, we found that both the presence of a maltose binding protein (MBP) tag and DNA greatly improved the solubility and stability of exogenously expressed truncated co-activator domains. Using these findings, I was able to successfully purify both the HOXB13 DNA binding domain (HBX) and full length (FL) protein with good yields and purity. To our knowledge, this is the first time that FL HOXB13 has been purified.

Conclusions:
In this study, we, for the first time, were able to purify FL HOXB13 with high yield and purity. This will help guide development of small molecule inhibitors that target HOXB13 and potentially treat late stage PCa. Moreover, this study will help us understand how homeobox proteins function within the cell.
UP-REGULATION OF THIOREDOXIN REDUCTASE 1 EXPRESSION MEDIATES CYTOPROTECTION OF FLAVAN-3-OLS AGAINST HYPOXIA-INDUCED CELL DEATH OF HUMAN KIDNEY PROXIMAL TUBULAR CELLS

Jixiao Zhu, Manqin Fu, Guoyu Dai, Qiunong Guan, Caigan Du
Department of Urologic Sciences, University of British Columbia, Vancouver, BC, Canada

Background and Objective:
Renal hypoxia and it-aggravated oxidative stress are a common final pathway to the development of a variety of kidney dysfunction including kidney transplant failure, and using of dietary antioxidants such as flavan-3-ols to prevent kidney failure has received much attention. This study was designed to investigate the mechanisms by which flavan-3-ols prevent hypoxia-mediated renal cell death.

Methods:
Human kidney proximal tubular cell (HKC-8) cultures were exposed to hypoxia (1% O2) for a period of 72 h in the presence of flavan-3-ols (i.g. catechin, epicatechin, and procyanidin B1-B2). Cell death was examined using flow cytometric analysis. Gene expression was determined using PCR array and Western blotting, and their networks were analyzed using STRING databases.

Results:
Catechin had the highest protection among these flavan-3-ols against hypoxia-induced cell death in cultured HKC-8 cells at the same dose. Exposure of HKC-8 cells to hypoxia stimulated oxidative stress mainly by up-regulation of DUOX2, NOX4, CYBB and PTGS2 and downregulation of antioxidant TXNRD1 and HSP90AA1, and treatment with catechin or other flavan-3-ols prevented the down-regulation of TXNRD1 expression in hypoxic HKC-8 cells. Overexpression of TXNRD1 prevented hypoxia-induced cell death, and inactivation of TXNRD1 with TRI-1, a specific TXNRD1 inhibitor, resulted in the loss of catechin cytoprotection against hypoxia-induced HKC-8 cell death.

Conclusion:
Our data demonstrated that the cytoprotection of antioxidant flavanols (catechin, epicatechin, and procyanidin B1-B2) against hypoxia was mediated by the stimulation of TXNRD1 expression in HKC-8 cells, suggesting that enhancing TXNRD1 expression or activity may become a novel therapeutic strategy to prevent hypoxia-induced kidney damage - a common pathway for the progression of kidney disease to the end-stage.
LONG-PULSE PHOTOTHERMAL THERAPY OF SOLID TUMORS AS PRECONDITIONING FOR IMMUNOTHERAPY

Negin Farivar1,2, Nader Al Nakouzi1,2, Morgan Roberts2, Irina Nelepcu2, Joey Lo2, and Mads Daugaard1,2,3

1. Department of Urologic Sciences, Faculty of Medicine, Gordon & Leslie Diamond Health Care Centre, 2775 Laurel Street, Vancouver, BC Canada V5Z 1M9
2. Vancouver Prostate Centre, Jack Bell Research Centre, 2660 Oak Street, Vancouver, BC V6H 3Z6
3. Experimental Medicine | Department of Medicine, Faculty of Medicine, 2775 Laurel Street

Photothermal therapy (PTT) refers to the use of photothermal agents (plasmonic nanoparticles) to convert electromagnetic radiation to heat and kill the target (tumor) cells. Due to the high efficacy and minimal side effects, this therapy has gained clinical traction and has been studied extensively in recent years. In addition to direct killing of the tumor cells, PTT can also induce an immunostimulatory response including, secretion of cytokines, infiltration and activation of immune effector cells. This immune response can be reinforced in combination with cancer immunotherapy. Here, we report the engineering and validation of a novel high-power pulsed laser device able to induce selective and localized heating of tumors while reducing the heat affected zone (HAZ) and unwanted damage to surrounding tissue. Long-pulsed PTT induces acute necrotic cell death in heat affected areas and the release of tumor associated antigens. This antigen release is immunostimulatory and triggers maturation of dendritic cells. Therefore, PTT can potentially offer enhanced therapeutic responses when combined with immune checkpoint inhibitors.
A 360 DEGREE VIEW OF SEXUAL HEALTH SERVICES AT SELECTED INSTITUTIONS ACROSS CANADA: THE NEED FOR THE CANADIAN ONCOLOGY SEXUAL HEALTH INITIATIVE (COSHI)

Ryan Flannigan, MD1,2, Andrew Matthew, PhD3, Eugenia Wu, BSc2, Steven Guirguis, MA3, and Monita Sundar, MA2, Celestia Higano, MD1,2

1. Department of Urologic Sciences, UBC
2. Prostate Cancer Supportive Care Program, VPC
3. Department of Surgical Oncology, Princess Margaret Cancer Centre, UHN

Introduction:
Sexual health (SH) is compromised by cancer diagnosis and treatment. Prevalence rates of sexual dysfunction are 90% in prostate/gynecological, 73% in breast, 30% in colorectal, and 20% in non-breast/non-pelvic cancers. SH clinics in oncology settings are the exception in Canada. As such, we formed COSHI which is comprised of a multi-disciplinary group of SH experts with the following goals:
• Develop a SH virtual resource repository for participating Cancer Centres
• Develop standardized treatment protocols and access for SH across Canada
• Develop a series of cancer-type-specific guidelines for SH treatment
• Establish a national SH database, inclusive of clinical and patient reported outcomes (PROs)

Methods:
The authors contacted selected Canadian clinicians to participate in the development of COSHI. Purposeful effort was made to ensure regional and multi-disciplinary representation. All clinicians contacted agreed to be “site-champions” for COSHI. In 6/2021 a meeting was held to define the scope, mission, and governance of COSHI. A 360° survey was distributed to 13 participating Cancer Centres to characterize SH care in oncology in Canada. Responses were collated and descriptive results reported.

Results:
12/13 institutions responded to the survey. All sites reported some form of SH care: 7 have cancer-specific clinics (gyne, prostate, colorectal); 3 offer SH care for all cancers; and 5 offer SH education classes. Nine sites have in-person clinics and 3 offer virtual services. MDs (urologists) deliver SH care at 9 sites, RNs at 6, and psychologists at 4. At least some SH-related PROs are collected at 8 sites. 8 sites reported that SH was a "gap in care" and all reported limited to no community-based SH resources.

Conclusions:
The 360° survey confirms gaps, discrepancies, or absence of SH care across selected Canadian Cancer Centres. Results underscore the need for an organization such as COSHI with its goals to improve SH care across Canada.
POTENTIAL OF R-SULFURPHANE FOR TREATMENT OF DIABETIC NEPHROPATHY

Jian Gao1,2, Qiunong Guan1, Hongwei Cheng2, Caigan Du3
1. Department of Urologic Sciences, University of British Columbia, Vancouver, British Columbia
2. Pharmaplanter Technology, Burnaby, British Columbia

Introduction and Objectives:
Sulforaphane (SFN) is a natural compound found in crucifer vegetables, and it has potential for treating chronic diseases. SFN suppresses inflammation as well as lowers the level of oxidative stress. Our aims are to evaluate the therapeutic potential of SFN for the treatment of chronic kidney disease, particularly diabetic nephropathy, and also to determine the mechanisms behind SFN actions. Our hypothesis is that SFN could protect the kidney from damages via both direct or indirect mechanisms, and one of indirect pathways is to protect the intestine, so that gut bacteria-producing uremic toxins such as indoxyl sulphate, p-cresyl sulphate and trimethylamine N-oxide are not absorbed by the body and cause damages in the kidney.

Methods:
Human intestinal epithelial Caco2 cell line was used as an in vitro model. Cell death was determined using MTT and flow cytometric analysis with Annexin-V and 7-AAD staining. Protein expression was examined using Western blot.

Results:
Here, we showed that cell death in cultured Caco 2 cells was induced by high levels of glucose in a dose-dependent manner. MTT assay showed a decrease in cell viability from 100% with 25 mM glucose (medium control) to 75±5% in cultures with 100 mM glucose and 50±6% with 200 mM glucose (P < 0.0001). Similar results were seen in these cultures by the flow cytometric analysis. Western blot confirmed that the cell death was mediated by apoptosis (caspase 3 cleavage), but not by autophagy (LC3 activation). Incubation with SFN protected intestinal epithelial cells from glucose-induced cell death at the high levels, which was showed more significant at concentration of 10 μM.

Conclusions:
Our data suggest that a high glucose level may induce intestinal injury via apoptosis, and SFN prevents intestinal barrier dysfunction in hyperglycemia condition. The effects of SFN on diabetic nephropathy will be investigated in an in vivo model.
THE ROLE OF THE GASTROINTESTINAL MICROBIOME IN OXALATE TRANSPORT AND PROCESSING IN KIDNEY STONE FORMATION

Sarah Hanstock1, Demian Ferreira1, Hans Adomat1, Genelle Healey2, Alina Reicherz1, Aaron Miller3, Ben Chew1, Dirk Lange1

1. Department of Urologic Sciences, University of British Columbia, Vancouver, British Columbia
2. Department of Pediatrics, University of British Columbia, Vancouver, British Columbia
3. Lerner Research Institute, Cleveland Clinic, Cleveland, Ohio

Introduction:
Kidney stones remain a global problem with limited effective treatments and high rates of recurrence. A primary cause of kidney stones is hyperoxaluria. Dietary oxalate can be absorbed through the gastrointestinal tract. One factor that may modulate this intestinal oxalate absorption pathway is the gastrointestinal microbiome. A few studies, including one in our lab, have reported that in a clinical population, intestinal butyrate-producing bacteria are of lower abundance in those with urinary stones.

Aims and hypothesis:
Our study aims to examine the role of microbe-derived short chain fatty acids (SCFA), such as butyrate, in oxalate processing, manifesting as both local changes at the intestine, and extraintestinal changes using both in vitro and in vivo models. We hypothesize that butyrate modulates oxalate transport and processing at both intestinal and renal sites and may reduce hyperoxaluria.

Methods:
Preliminary in vitro experiments will determine the effect of SCFA on oxalate transport and transporters using a Caco-2 transport assay. To further test our hypothesis, an in vivo murine model will be fed combinations diets supplemented with inulin (prebiotic), tributyrin (a precursor for butyrate production), and oxalate. During experiments, urine, blood, and stool samples will be collected for oxalate measurements, the latter also being used for microbiome analyses. Renal and intestinal tissue will be collected to measure expression of oxalate transporters, expression of inflammatory markers, and histology.

Results:
Preliminary results from a pilot study indicate efficacy of our in vivo model for future use in experiments.

Impact:
Findings from this study may provide insight into the etiology of kidney stone disease, and inform better prevention of kidney stone recurrence.
IDENTIFICATION AND VALIDATION OF RARE GERMLINE VARIANTS MODULATING PROSTATE CANCER METASTASIS

Yen-Yi Lin\textsuperscript{1,2}, Stanislav Volik\textsuperscript{1}, Anne Haegert\textsuperscript{1}, Robert Bell\textsuperscript{1}, Funda Sar\textsuperscript{1}, Stéphane LeBihan\textsuperscript{1}, Colin Collins\textsuperscript{1,2}

\textsuperscript{1} Vancouver Prostate Centre, Vancouver, Canada, V6H 3Z6
\textsuperscript{2} Department of Urologic Sciences, University of British Columbia, Vancouver, Canada, V5Z 1M9

Metastatic PCa is invariably fatal, killing \textasciitilde 40,000 men in Canada and the US in 2020. The underlying mechanisms of progression of localized prostate cancer to metastatic stage remain poorly understood. Paradoxically, some high-risk localized PCa can be cured by surgery and/or radiation while low-risk tumors may give rise to metastasis. It has been proposed that germline variants may play a larger role in tumor progression to the metastatic stage than appreciated, and that germline modifiers of cancer progression can be identified in extreme phenotype cohorts that are smaller than a typical cancer risk study. We hypothesized that \textit{nonsynonymous rare germline variants (nsRVs, < 1.5\% population frequency)} that potentiate metastasis can be detected using this approach. To this end, we assembled a cohort of 52 localized high-grade (Gleason Score (GS) > 4+3) clinically annotated FFPE prostate cancer (PCa) tumor specimens, 26 of which had metastasized to bone and the rest did not. All have a minimum of 10 years follow-up and are treatment naïve. After pathology review and selection, the tumor and the corresponding pathologically benign tissue, obtained from a distant prostate quadrant, were whole exome sequenced. Through improved machine learning algorithms for large datasets, I will focus on accurately detecting germline signatures for metastasis in primary tumors. Preliminary, germline nsRVs specific for metastatic samples were identified in more than 900 genes, including some known for involvement in cancer progression and metastasis (e.g. BRCA1/2, ATM, FOXM1, POLB, MYC, TP53, and WRN). That gave us confidence in the validity of this approach. Once validated, these germline modulators will set the stage for future large-scale studies aimed at functionalizing the genes and rare variants as biomarkers and drug targets. This would have enormous implications for the clinical management of PCa patients and could extend to other cancers.
MODULAR BIOREACTOR FOR ENGINEERING A KIDNEY

Luque-Badillo, AC1,5; Monjaras-Avila, CU2,5; Chavez-Munoz, C.4,5*; So, AI3,5*

1. Experimental Medicine Program, Faculty of Medicine, University of British Columbia, Canada
2. Interdisciplinary Oncology Program, Faculty of Medicine, University of British Columbia, Canada
3. Department of Urologic Sciences, Faculty of Medicine, University of British Columbia, Canada
4. Department of Medicine, Faculty of Medicine, University of British Columbia, Canada
5. Vancouver Prostate Centre, Department of Urologic Sciences, University of British Columbia, Canada

Introduction and objectives:
In Canada, end-stage renal disease (ESRD) has been rising, becoming the 10th leading cause of death and representing a healthcare burden to our system. Kidney transplant is the gold standard treatment for ESRD. Unfortunately, the number of available organs for transplantation is not enough. Tissue engineering has emerged as an alternative solution for organ shortage; the extracellular matrix (ECM) obtained from decellularized organs maintain the organ's microarchitecture and bioactive molecules that aid in the recellularization process. However, complete organ repopulation has not been accomplished. Therefore, I propose to develop a modular bioreactor capable of repopulating decellularized kidneys, that can potentially be functional for transplantation.

Methods:
Pig kidneys will be decellularized using our established protocol. Tangential flow filters and acoustic resonance will be used to develop a filtration system that will allow us to separate cells with the objective of recirculating only viable cells into the organ. Moreover, a sterilizable chamber will be developed to create negative pressure in order to increase the recellularization percentage.

Results:
I have successfully decellularized a whole pig kidney in 46 hours and have preliminary data on reseeding this whole organ by perfusion using an in vitro bioreactor applying negative pressure and using ethylene oxide sterilization (ETO). A complete cell removal was obtained preserving the organ's ECM and <50ng dsDNA/mg of dry tissue. In the histological evaluation of the recellularized kidney, it can be observed a higher density of cells in less culture time when compared to previous publications. Furthermore, we were able to show cell colonies in almost all areas of the kidney including the cortex and medulla.

Conclusions:
The development of this modular bioreactor could be translated into reseeding human kidneys, significantly impacting the regenerative medicine perspective for the treatment of patients with ESRD.
MULTI-FOCAL GENOMIC DISSECTION OF SYNCHRONOUS PRIMARY AND METASTATIC TISSUE FROM DE NOVO METASTATIC PROSTATE CANCER

Andrew J. Murtha†a, Evan Warner†a, Kim Van der Eecken†b, Edmond M. Kwana, Sarah W.S. Ng, Emilia Chena, Nicolette Fonsecaa, Cameron Herbersta, Cecily Q. Bernalesc, Gráinne Donnellana, Elena Schönlausb, Sofie Verbekeb, Nicolaas Lumenb, Jo Van Dorpeb, Joonatan Spolad, Ellie Ritcha, Bram De Laerea, Gillian Vandekerkhovea, Matti Annalad, Piet Ostc, Alexander W Wyatt*b

†co-first authors; *co-corresponding authors

Vancouver Prostate Centre, Department of Urologic Sciences, University of British Columbia, British Columbia, Canada; bDepartment of Pathology, Ghent University Hospital, Ghent, Belgium; cDepartment of Human Structure and Repair, Ghent University, Belgium; dProstate Cancer Research Center, Faculty of Medicine and Life Sciences and BioMediTech Institute, University of Tampere, Tampere, Finland.

Background:
10% of newly diagnosed prostate cancer presents with metastases. Known as de novo metastatic castrate-sensitive prostate cancer (mCSPC), it is disproportionately responsible for >50% of prostate cancer deaths. Cancer genotyping can identify vulnerabilities exploitable by targeted therapies. However, tissue from de novo mCSPC is scarce; neither prostatectomy nor metastatic biopsy is standard, and it is unknown if diagnostic biopsies or plasma circulating tumour DNA (ctDNA) are representative of dominant metastatic disease.

Methods:
We performed pathological and genomic assessment of all spatially or phenotypically-distinct tumour foci and liquid biopsies (n=560) in 43 patients with de novo mCSPC who underwent prostatectomy, pelvic lymph node dissection, and plasma collection.

Results:
91% (478/523) of tissue foci had evidence of prostate cancer by targeted DNA sequencing, with a median tumour fraction of 48%. When modelling selection of a single index focus, low tumour fraction precludes complete genomic profiling in 19% of patients. Only 48% of plasma cell-free DNA samples prior to systemic therapy had a ctDNA fraction above 0.3% (median fraction 5%). Primary genomic heterogeneity was pervasive, including clonally distinct prostate cancer populations in 21% of patients. Primary site heterogeneity was observed in at least one of TP53, RB1, or PTEN in 51% of the cohort. The two patients with compound disruption of TP53, PTEN, and RB1 experienced rapid progression to castration resistance and death within two years of diagnosis, despite initial low-risk clinical features.

Conclusions:
Many tumours exhibit spatial heterogeneity within the prostate. This data raises concerns about accurate tumour genotyping in routine clinical practice where needle biopsy specimens are the only available tissue for profiling. Nevertheless, some de novo mCSPC are marked by aggressive genomics and experience rapid progression to lethal disease, suggesting that tailored multi-focal genomic profiling can further segment the disease.
THE ROLE OF THE URINARY MICROBIOME IN DETERMINING RESPONSE TO INTRAVESICAL BCG IN PATIENTS WITH HIGH-RISK NON-MUSCLE INVASIVE BLADDER CANCER

Dalia Othman1,2, Igor Moskalev2, Ali Hussein2, Demian Ferreira1,2, Alberto Contreras-Sanz2, Aaron Miller3, Peter Black2 and Dirk Lange1,2

1. The Stone Centre at Vancouver General Hospital, Department of Urologic Sciences, University of British Columbia
2. Vancouver Prostate Centre, Department of Urologic Sciences, University of British Columbia
3. Lerner Research Institute, Department of Cardiovascular and Metabolic Sciences, Cleveland Clinic

Introduction:
Bladder cancer (BCa) is the fifth most common cancer in Canada and with a recurrence rate of 80%, BCa is the most expensive cancer to treat on a per patient basis. 70% of BCa patients are diagnosed with non-muscle invasive bladder cancer (NMIBC). Following tumor resection, gold-standard treatment of Bacillus Calmette-Guérin (BCG) acts as an immunostimulatory therapeutic. While effective, it fails in 40% of patients. Recent work illustrated the colonization of the bladder with the urinary microbiome. Given pre-existing evidence that commensal microbiomes can affect inflammatory and immune responses, we hypothesize that the urinary microbiome may have a similar function in the bladder, affecting the degree of BCG-induced anti-tumor immune response.

Objectives:
To determine whether the urinary microbiome composition impacts BCG responsiveness in patients and understand how its composition affects the immune response to BCG in the bladder using an in vivo mouse model.

Methodology:
Collection of patient specimens, before and after treatment, to identify the urinary microbiome composition through metagenomic sequencing as a biomarker of BCG responsiveness. Mice underwent ultrasound-guided introduction of BCG into the bladder lumen, in presence and absence of a urinary microbiome, to assess the inflammatory response and bladder lumen microenvironment. Tissue and urine were collected to examine change in inflammatory response and urinary microbiome composition.

Current Progress:
Specimens have been collected from a total of 35 patients, reaching >33% of the recruitment goal. Current samples collected are to be sent for preliminary sequencing and analysis. The pilot in vivo studies have been successful, and all mice survived through the proposed methodology.

Impact:
Improve on the current knowledge regarding the role of the urinary microbiome in immune regulation. Identify clinically relevant bacterial targets to help advance current therapeutic strategies targeting bladder cancer.
FUNCTIONAL CHARACTERIZATION OF ANDROGEN RECEPTOR-MEDIATED TRANSCRIPTION

Doğancan Özturan¹, Bengul Gökbayrak¹, Nathan A. Lack¹,²

¹. Vancouver Prostate Centre, Department of Urologic Sciences, University of British Columbia, Vancouver, Canada
². Koç University Research Center for Translational Medicine (KUTTAM), Koç University, Istanbul, Turkey

Introduction:

The androgen receptor (AR) is considered a master transcription factor of prostate cancer (PCa) growth and differentiation, and exerts its effects by influencing the transcriptional regulation of its target genes. AR-mediated transcription is driven by distal enhancer elements that are required for gene expression, as they "enhance" the transcription of target genes (Zhang et al., 2012). Recently, we showed that these AR binding sites (ARBS) work in concert to drive target gene activation. However, the exact enhancer-promoter pairs and how many "true" enhancers elements are involved in the activation of a given AR-targeted gene is poorly understood. Using a gene locus which contains 16 ARBS and the canonical AR target gene PSA/KLK3 as a model, we interrogated gene expressions of all 15 KLK genes upon the genomic perturbation of all ARBS in the KLK locus by CRISPR-mediated inhibition system.

Methods:

Hormone-sensitive PCa cells (LNCaP) transduced with dCas9-KRAB plasmid were transfected with guide RNAs (n=3) targeting each ARBS individually. After transfection, the cells are androgen-deprived for 24 hours and 1nM dihydrotestosterone is introduced for 24 hours. Upon collection of the cells, quantitative PCR has been conducted to assess the expression of all KLK genes. Stable knockout cell lines for selected ARBS were created for downstream AR ChIP and chromosome conformation capture assays.

Results & Discussion:

From this systematic screen, we showed that only a subset of ARBS contributes to AR-mediated gene transcription. Specifically, PSA/KLK3 and KLK2 share the same ARBS as enhancers via chromatin looping. Upon inactivation of PSA/KLK3 promoter, we observed enhancer rewiring that directed an AR-regulated enhancer to KLK2, increasing the KLK2 transcriptional output. Interestingly, using ARBS-null stable cell lines, we measured the AR occupancy in other KLK ARBS and found that a single ARBS perturbation causes a significant decrease in nearby occupancy which suggests that AR binding could be dependent on nearby binding sites that creates a specific 3D conformation which leads to stable gene activation. Overall our work suggests a co-operative action of AR-mediated gene transcription.
IN VITRO EFFICACY OF A NOVEL DUAL PARP-HDAC INHIBITOR

Louise Ramos1,2, Sarah Truong1,2, Beibei Zhai1,2, Fariba Ghaidi1,2, Jay Joshi1,2, Dennis Brown1, Neil Sankar1, John Langlands1, Jeffrey Bacha1, Wang Shen1, Poul H Sorensen3,4, Nada Lallous1* & Mads Daugaard12*

1. Rakovina Therapeutics, Vancouver, BC, Canada
2. Vancouver Prostate Centre, Vancouver, BC, Canada
3. Department of Molecular Oncology, British Columbia Cancer Research Centre, Vancouver, BC, Canada
4. Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, Canada
5. Department of Urologic Sciences, University of British Columbia, Vancouver, BC, Canada

*Co-senior authors

Introduction:
In preclinical studies, poly-adenosine diphosphate-ribose polymerase (PARP) inhibitors demonstrated therapeutic potential in multiple cancer types. Though effective treatments, PARP inhibitors can develop resistance over time. Combination therapies such as, inhibition with both histone deacetylases (HDACs) inhibition and PARP inhibition has been shown to sensitize cells to treatment in vitro. Clinically, there are challenges due to different pharmacokinetic profiles and overlapping toxicities. Here, we evaluate the activity and efficacy of a novel bifunctional small-molecule compound designed to have both PARP and HDAC inhibiting activity.

Methods:
PARP1 and PARP2 activity were measured using colorimetric assay kits. HDAC activity was measured using HeLa nuclear extracts and a fluorogenic peptide-based biochemical assay. Cell survival EC50s were determined using live cell imaging with an Incucyte S3 system and the CellTiter Glo viability assay. Accumulation of DNA damage was detected by western blot using anti-phospho histone H2AX antibody.

Results:
A representative compound from the kt-3000 series showed potent inhibition of PARP1 and PARP2 with IC50 values in the low nM range, comparable to FDA-approved PARP inhibitors. The compound also showed inhibition of HDAC enzymes with IC50 values in the low μM range. Cell survival EC50 values were superior to Olaparib in various cell lines in vitro. Treatment also increased accumulation of pH2AX by western blot and increased S and G2/M cell cycle arrest compared to Olaparib. A functional recombinant PARP1 has also been purified for future binding analysis.

Conclusion:
Our kt-3000 compound shows potent inhibition of PARP1, PARP2, and HDAC, as well as induction of DNA damage and cell cycle arrest. Further development of these bifunctional single molecule inhibitors is underway including establishing direct binding between the compounds and PARP1, PARP2 and HDAC via traditional and novel methods. The lead molecule will be next evaluated in various cancer models including prostate, bladder and kidney cancer.
RinsLR: DISCOVERY OF MID RANGE NOVEL SEQUENCE INSERTIONS USING LONG-READ SEQUENCING

Armita Safa1,2, Can Alkan3, and Faraz Hach1,4

1. Vancouver Prostate Centre, Vancouver, Canada
2. Bioinformatics Program, the University of British Columbia, Vancouver, Canada
3. Department of Computer Engineering, Bilkent University, Ankara, Turkey
4. Department of Urologic Sciences, the University of British Columbia, Vancouver, Canada

Introduction:
Two decades after the assembly of the human genome, the current reference is still not sufficiently representative. Recent studies led by Human Genome Structural Variation Consortium (HGSVC) and Genome in a Bottle Project (GiaB) aim to characterize structural variations (SV). Still, there are genomic sequences that are missing from assemblies. Termed non-repetitive, non-repeat (NRNR) variants, or novel sequence insertions, these sequences need to be discovered to characterize human genome diversity. Furthermore, NRNR variants discovered to date have shown to harbor genes and other functional elements, therefore they may be relevant to diseases.

Methods:
Unlike short-read sequencing (SRS), long-read sequencing (LRS) has a higher basepair error rate, but it is less prone to mapping ambiguity. On the other hand, SRS reads are almost error-free, therefore could provide better breakpoint prediction. Here we introduce RinsLR (Rapid identification of novel sequences through Long Reads), an algorithm to characterize NRNR variants using both long- and short-reads. RinsLR is a hybrid approach as it utilizes LRS to generate more contiguous assemblies with better repeat resolution and SRS for insertion breakpoint resolution.

Results:
Using simulation, we demonstrated that RinsLR achieves high precision (100%) and recall (95%). In comparison, an SRS-based tool, Pamir, and an LRS-based tool, Sniffles, showed relatively lower precision (98% and 75%) and recall (93% and 88%), respectively. Furthermore, when we compared Sniffles and RinsLR on a biological dataset from Telomere-to-Telomere (T2T) Consortium, we observed that RinsLR provided a better precision. Through RinsLR, we discovered 277 novel sequences.
ULTRASOUND-SPECIFIC PHYSICS-BASED DATA AUGMENTATION FOR KIDNEY SEGMENTATION

Rohit Singla1,2, Cailin Ringstrom3, Ricky Hu4, Victoria Lessoway3, Janice Reid3, Robert N. Rohling3,5, Christopher Nguan6

1. School of Biomedical Engineering, University of British Columbia, Vancouver, BC
2. Faculty of Medicine, University of British Columbia, Vancouver, BC
3. Dept. of Electrical & Computer Engineering, University of British Columbia, Vancouver, BC
4. School of Medicine, Queen's University, Kingston, ON
5. Dept. of Mechanical Engineering, University of British Columbia, Vancouver, BC
6. Dept. of Urologic Sciences, University of British Columbia, Vancouver, BC

Objective: Machine learning techniques utilize data augmentation to avoid overfitting, improve generalizability, and overcome data scarcity. In ultrasound kidney segmentation, where there is limited data and a lack of high-quality fine-grained annotations, there may be a high algorithmic uncertainty. The use of ultrasound-specific characteristics in data augmentation may reduce this issue. By improving segmentation, downstream tasks of kidney disease detection and/or prognostication may improve through enhanced morphological measurements or regional analysis. We present time-gain compensation (TGC) and artificial shadowing (AS) as augmentations to the multi-class kidney segmentation problem.

Materials and Methods: Both native and transplant ultrasound scans (n=514) were obtained retrospectively from Vancouver General Hospital over a five-year period. Two expert sonographers manually created fine-grained annotations for four classes (kidney capsule, cortex, medulla, and sinus). These images and labels were used to train a fully supervised segmentation network (nnU-net). Each of the augmentations were applied separately to the network, and compared to the use of only basic augmentations. A 80/20 train/test split was used. Uncertainty was reported as averaged variance (AV) after Monte Carlo dropout for 500 iterations.

Results: With no new augmentations, the AV across all four classes was 1.8e-6. Using TGC significantly reduced AV in three of four classes (cortex: 1.3e-6 to 6.0e-7, medulla: 1.5e-6 to 4.8e-7, and sinus: 9.7e-7 to 5.3e-7) but the overall AV was not statistically significantly different from baseline at 1.6e-6. Using AS yielded no significant benefit.

Conclusions: The use of time-gained compensation as a data augmentation technique for kidney ultrasound segmentation demonstrates a reduction in uncertainty. Using these techniques for enhanced segmentation may yield improvements in regional analysis (cortex vs. medulla) for kidney disease detection and prognostication. Further exploration of the benefits and cons of this technique are warranted.
DEVELOPING MACHINE LEARNING ALGORITHMS TO ADVANCE PEYRONIE’S DISEASE MANAGEMENT AROUND THE BEND

Reza Soltani\textsuperscript{1,3}, Luke Witherspoon\textsuperscript{2}, Ryan Flannigan\textsuperscript{2,3}, Faraz Hach\textsuperscript{2,3}

\textsuperscript{1} Bioinformatics Program, the University of British Columbia, Vancouver, BC, Canada
\textsuperscript{2} Department of Urologic Sciences, the University of British Columbia, Vancouver, BC, Canada
\textsuperscript{3} Vancouver Prostate Centre, Vancouver, BC, Canada

Background:  
Approximately 15% of adult males suffer from penile deformities which often impair their ability to be sexually active, yet only 0.5% present to a healthcare provider. Standard of care evaluation for those who seek medical attention includes performing an office-based penile curvature assessment that utilizes a goniometer and ruler to evaluate the curve, length, and other deformities after a stimulated erection by injecting a medical agent. In this project, we developed a set of guided diagnostic tools that can be utilized by healthcare providers as well as the general public. This artificial intelligence-powered toolkit provides information regarding the deformities and measurements of patients. It improved the accuracy of physician evaluation globally, and the public web/mobile application eliminated barriers to objective assessments and accessing care.

Methods:  
Our automatic curvature assessment pipeline has two major stages. The first stage is the identification of the penis shaft, the head of the penis, and the sizing sticker. We fine-tuned a Mask R-CNN deep neural network over a dataset of penis images. In the second stage, we perform a series of geometric algorithms on the detected mask to find the point of maximal curvature (PMC), its distance from the head of the penis, and the degree of curvature. We perform the following steps in order: 1. Boundary Detection, 2. Corner detection, 3. Detection of PMC, and 4. Measurement of the angle and location of PMC.

Results:  
During the collection of the images in the clinic, our clinicians also record the degree of curvature and the location of maximal curvature using a goniometer and ruler. In addition, we provided the de-identified images to another clinician to do the same assessment by annotating the images. Our model’s results lie between these two sources of assessments suggesting that our model has accurate measurements.
GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIAL SURFACE CONSTITUENTS HINDER KIDNEY CELL VIABILITY IN VITRO

Ruixuan Yang, Dirk Lange, Caigan Du, David Harriman
Department of Urologic Sciences, University of British Columbia

Introduction and Objectives:
Human urine is now known to harbour diverse microbial species. The effect of these microbes on kidney tissue is poorly understood. Lipopolysaccharide (LPS) are gram-negative bacterial surface membrane components that trigger inflammation, while lipoteichoic acid (LTA) are thought to be the functional equivalent in gram-positive bacteria. The purpose of this project is to assess the effects of LPS and LTA on kidney cell viability as an initial study of the urinary microbiome and renal allograft health.

Methods:
In vitro models using two kidney cell lines, Human Mesangial Cells (T-HMC) and Human Kidney Proximal Tubular Cells (HKC-8), were designed to assess the cellular impact of LPS and LTA. Cells were cultured in vitro with LPS from Escherichia coli and LTA from Staphylococcus aureus in varying concentrations for 48 hours. FACS and MTT tests were performed to assess cell apoptosis and metabolic activity, respectively.

Results:
LPS and LTA resulted in comparable degrees of kidney cell apoptosis, but this effect was independent of increasing concentration. In contrast, kidney cell metabolic rates steadily declined as treatment concentration increased. HKC-8 MTT readings fluctuate more with changes in treatment concentration, while also showing overall greater decline in metabolic rate compared to T-HMC cells. Combining LPS and LTA into one treatment led to higher metabolic rates, close to control.

Conclusions:
LPS and LTA both act as stressors to kidney cells. Increasing concentration decreased kidney cell metabolism while apoptosis rates remained unchanged. Combining LPS and LTA seems to have a modulating effect which decreases their joint toxicity, lending credence to the potential benefits of urinary microbes. Our next steps will be to analyze the inflammatory response of these cells to LPS and LTA and also incorporate other known helpful bacterial species into the study.
HARNESSING PROXIMITY LIGATION TO PROBE THE ANDROGEN RECEPTOR VARIANT 7 INTERACTOME

Ivan Yu\(^1\,2\), Shreyas Lingadahalli\(^1\), Tunc Morova\(^1\), Bengul Gokbayrak\(^1,3\), Dogancan Ozturan\(^1\), Nathan Lack\(^1,2,3\)

\(^1\). Vancouver Prostate Centre, University of British Columbia, 2660 Oak Street, Vancouver, British Columbia V6H 3Z6, Canada
\(^2\). Department of Urologic Sciences, University of British Columbia, Vancouver, British Columbia V6T 1Z4 Canada
\(^3\). School of Medicine, Koç University, Rumelifeneri Yolu, Istanbul 34450, Turkey

Introduction

Inhibiting androgen receptor (AR) signalling is a common treatment for recurrent or metastatic prostate cancer (PCa). Yet while initially effective, response is almost always temporary and the cancer inevitably develops resistance, resulting in the more aggressive castration resistant prostate cancer (CRPC). Interestingly, AR signalling is still critical in the majority of CRPC. Several resistance mechanisms have been shown to maintain AR signalling including the expression of constitutively active AR splice variants. Yet how these variants drives resistance remains poorly understood. Recent work has demonstrated that the AR variants (AR\(^{-V7}\)) and full length protein (flAR) largely share similar binding sites but have very different transcriptional activity. Taken together, this suggests that there may be differential co-regulatory proteins recruited by the two isoforms that potentiate their divergent transcriptional activity.

Methods

To characterize the global interactomes of flAR and AR\(^{-V7}\), we utilized TurboID, a promiscuous biotin ligase that biotinylates all nearby proteins. After expressing these fusion proteins, we isolated the putative flAR/AR\(^{-V7}\) interactors by streptavidin pulldown and identified them via mass spectrometry (MS). Next, to elucidate the proteins uniquely associated with AR\(^{-V7}\) homodimers and flAR/AR\(^{-V7}\) heterodimers, we developed and optimized a split version of TurboID that only biotinylates proteins following dimerization.

Results and conclusions

Characterization of the global interactomes for flAR and AR\(^{-V7}\) yielded roughly 100 high confidence flAR or AR\(^{-V7}\) interactors, which included well-characterized AR-interacting proteins, such as HOXB13 and FOXA1. We are currently in the process of validating the common and unique proteins identified as bona fide interactors, as well as generating stable cell lines to characterize the complex-specific interacting partners. Overall, this work will further our understanding of AR\(^{-V7}\) mediated therapeutic resistance.
DYNAMIC PHASE SEPARATION OF THE ANDROGEN RECEPTOR AND ITS COACTIVATORS TO REGULATE GENE EXPRESSION

Fan Zhang1, Maitree Biswas1, Shabnam Massah1, Joseph Lee1, Shreyas Lingadahalli1, Samantha Wong1, Christopher Wells1, Nabeel Khan1, Jane Foo1, Neetu Saxena1, Bei Sun1, Ana Karla Parra-Nuñez1, Christophe Sanchez1, Novia Chan1, Lauren Ung1, Jennifer M. Bui1, Umut Berkay Altintaş3,4, Yuzhuo Wang1, Ladan Fazli1, Paul S. Rennie1, Nathan Lack1,3,4, Artem Cherkasov1, Martin Gleave1, Jörg Gsponer2 and Nada Lallous1

1. Vancouver Prostate Centre, Department of Urologic Sciences, University of British Columbia, 2660 Oak St., Vancouver, BC, V6H 3Z6, Canada; 2. Michael Smith Laboratories, Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, BC, Canada, V6T 1Z4 3. School of Medicine, Koç University, Rumelifeneri Yolu, Istanbul, 34450, Turkey 4. Koç University Research Centre for Translational Medicine (KUTTAM), Koç University, Rumelifeneri Yolu, Istanbul, 34450 Turkey

Background:
Cancers, including prostate cancer (PCa), are addicted to transcription programs driven by specific genomic sites known as superenhancers (SEs). The robust transcription of genes at such SEs is enabled by the formation of phase-separated condensates by transcription factors and coactivators with intrinsically disordered regions. The androgen receptor (AR), the main oncogenic driver in PCa, contains large disordered regions and is co-recruited with the transcriptional coactivator MED1 to SEs in androgen-dependent prostate cancer cells, thus promoting oncogenic transcriptional programs.

Methods:
The ability of AR to form condensates was investigated in PCa cells using fluorescent confocal microscopy, immunofluorescence, and fluorescence recovery after photo bleaching (FRAP). The correlation between foci formation and AR transcriptional activity was measured using qPCR of AR targeted genes. The ability of recombinant full-length AR protein to form condensates was also validated in vitro by using confocal microscopy and turbidity assay.

Results and discussion:
In this work, we show that AR undergoes liquid-liquid phase separation both in prostate cancer models upon androgen stimulation and in vitro. We demonstrate that MED1 plays an essential role in the formation of AR foci and reveal that foci formation correlates with AR transcriptional activity. We also show that AR antagonists that block cofactor recruitment or DNA binding hinder foci formation and thus AR transcriptional activity. These results suggest that enhanced compartmentalization of AR and coactivators at SEs may play an important role in the activation of oncogenic transcription programs.
Organizing Committee

- Dr. Amina Zoubeidi
- Dr. Ben H. Chew
- Dr. Kourosh Afshar
- Dr. Peter C. Black
- Dr. Xuesen Dong

Learning Objectives

- To inform members of the types of clinical and basic science research being conducted in the Department of Urologic Sciences.
- To familiarize members with new innovative research techniques.
- To foster an atmosphere of collaborative research within the Department of Urologic Sciences.

Contact Us

Department of Urologic Sciences
2775 Laurel Street, 6th Floor
Gordon & Leslie Diamond Health Care Centre
Vancouver, BC V5Z 1M9
Phone: 604-875-4301
Fax: 604-875-4637
Email: urology.adminasst@ubc.ca

Vancouver Prostate Centre
2660 Oak Street
Jack Bell Research Centre
Vancouver, BC V6H 3Z6
Phone: 604-875-4818
Fax: 604-875-5654
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