26th Meeting of the IBCN

September 28th – 30th, 2023

at

Hyatt Centric Montreal

Montreal, Canada

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THURSDAY, SEPTEMBER 28th, 2023

19:00 Welcome Dinner: *Hyatt Centric Montreal, Horizon Bar (Rooftop)*

We will have the traditional "get-together" the evening prior to the meeting. This is an informal gathering with a welcome drink at 7 pm and buffet dinner at 8 pm at the hotel.

FRIDAY, SEPTEMBER 29th, 2023

***Breakfast on your own in the hotel **BEFORE** meeting

	Introduction	
7:30	Registration	
08:00	Welcome to IBCN Meeting	Roman Nawroth
08:05	Welcome to Montreal	Wes Kassouf

Abstract Session I		
	Clinical Trials and Outcomes	**Gautier Marcq & Stephen Williams**
08:15	Long term recurrence risk, metastatic potential, and length of surveillance of low-grade non-muscle invasive bladder cancer	Amy Chan
08:25	Transurethral resections of bladder tumor quality criteria and early recurrence rate: preliminary data from the international RESECT study	Gautier Marcq
08:35	SURE-01: Interim results of a phase 2 study of neoadjuvant sacituzumab govitecan (SG), followed by radical cystectomy (RC), for patients with muscle-invasive bladder cancer (MIBC)	Antonio Cigliola
08:45	Evaluation of molecular residual disease by urinary comprehensive genomic profiling in the SWOG S1605 phase II study of atezolizumab in patients with BCG-unresponsive NMIBC	Marie-Pier St-Laurent

Keynote I		
	Organoid Biology	**Roman Nawroth**
9:15	Keynote: Organoids in translational research and clinical impact	Michael Shen
9:45	Discussion	

10:00	**Introduction to Breakout Groups**	**Peter Black**

10:15 – 10:50 Health Break

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Industry Meets IBCN – Breakout 10:50 – 12:50		
Partner	**Topic**	**Facilitator**
Janssen	The TARIS Drug Delivery System: A novel drug/device treatment approach for localized and biomarker targeted drug delivery in bladder cancer	Stephen Williams & Sam Spigelman
Bristol Myers Squibb	Incorporating Immunotherapy in Bladder Cancer Care: Benefits and challenges in real world clinical settings	Peter Black & Shreya Mitra & Wes Kassouf
Pfizer	On the Horizon: Current and Emerging Therapies to Address BCG-Naïve High-Risk NMIBC	Tilman Todenhöfer & Julia Brinkmann & Bernadett Szabados
CG Oncology	Cretostimogene oncoloytic immunotherapy in Bladder Cancer	Yair Lotan & Vijay Kasturi & Pat Keegan
EMD Serono	Implications for assessment and management of oligometastatic disease in advanced UC patients receiving standard systemic treatment	Peter Chung & Fabio Cury
ImmunityBio	N-803 in NMIBC: Next Steps in the Clinic	Bobby Reddy & John Sfakianos
EnGene	EG-70, an intravesical, non-viral gene therapy designed for localized immunotherapy in NMIBC patients: from preclinical discovery to patients	Rosemary Mazanet & James Sullivan & Anthony Cheung & Ashish Kamat

12:50- 14:00 Lunch Break & Poster View

IBCN Speaker		
14:00	TCGA and Beyond	Seth Lerner
14:30	Discussion	Peter Black

Abstract Session II		
	Tumor Biology and the Microbiome	**David Müller & Sita Vermeulen**
14:50	Field cancerization impacts tumor development, T-cell exhaustion and clinical outcomes in bladder cancer	Trine Strandgaard
15:00	An holistic and integrated approach for investigating the bacterial microbiome, gene expression profile and immune cell profile in the non-muscle invasive bladder cancer tumour microenvironment	Tyler Wooldridge
15:10	Meta-analysis of GWAS data from diverse populations for Bladder Cancer (mapBC) collaboration: initial findings	Jeffrey Damrauer
15:20	Exploring the impact of microbiome in the response of combined radiation with immune checkpoint blockade in muscle invasive bladder cancer (MIBC)	Éva Michaud
15:30	Patient-derived organoids identify tailored therapeutic options and determinants of plasticity in sarcomatoid urothelial bladder cancer	Michele Garioni

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15:40-18:00 Poster Session with Wine & Snacks		
15:40	Short presentations of all posters at podium	Lars Dyrskjøt & Kent Mouw

Instructions:
All poster presenters will have the opportunity to provide a brief **2-minute** "teaser" at the podium. Each poster presenter should prepare 1-3 slides that highlight the poster findings and encourage attendees to visit the poster to learn more.
Important: Please send a PDF file of your slides to Lars Dyrskjøt (lars@clin.au.dk) no later than September 26th. Slides cannot be added during the meeting. Please name your PDF file with your last name and your poster number (which can be found in the program).

Adjourn		
18:00	Closing words	Roman Nawroth

IBCN Dinner: *Vieux-Port Steakhouse, 39 Rue Saint-Paul E, Montréal*	
19:30	Cocktail reception
20:00	Dinner

SATURDAY, Sept 30, 2023

***Breakfast on your own in the hotel **BEFORE** meeting

8:00	IBCN – General Assembly	Members Only

	Aging and Sex: Impact on Immunity and Treatment Response	Roland Seiler & John Sfakianos
9:00	Aging associated immune decline impacts response to BCG immunotherapy in bladder cancer	Madhuri Koti
9:15	Sex-related differences in bladder cancer development	Sean Li
9:30	Transcending Genomics: A Study of Gender-Specific Variations in Oncobiome and Immune Response	Laura Bukavina
9:45	Discussion	

10:00 – 10:15 Health Break

	Keynote II	K. Mouw & L. Prokunina-Olsson
10.15	Keynote: Epigenomics and transcriptional reprogramming	Andrew Hsieh
	Epigenetics	
10:45	Epigenetic programs and subtypes	Isabelle Bernard-Pierrot
11:05	KDM6A alterations in bladder cancer	Byron Lee
11:25	Discussion	

Abstract Session III		
	Molecular subtypes and response evaluation	Trine Strandgaard & Tilman Todenhöfer
11:40	Gene expression-based classification of urothelial carcinoma for research and clinical applications	Elena Aramendía

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11:50	Alignment of molecular subtypes across multiple bladder cancer subtyping classifiers	Ewan A. Gibb
12:00	A composite biomarker approach to spare neoadjuvant chemotherapy in select muscle-invasive bladder cancer patients	Joep J. de Jong
12:10	Molecular signatures predict GEMDOCE responses for BCG-unresponsive non-muscle invasive bladder cancer	Woonyoung Choi
12:20	Proteogenomic Characterization of Chemotherapy Response in Muscle Invasive Bladder Cancer	Mathew V Holt

12:30– 13:30 Lunch & Poster View

	Optimizing bladder preservation in MIBC	**Lars Dyrskjøt & Bernadett Szabados**
13:30	DDR mutations and chemotherapy sensitivity	Kent Mouw
13:50	Liquid biopsies for treatment efficacy monitoring	Phil Abbosh
14:10	Clinical trial status and future plans	Matt Galsky
14:30	Discussion	

Abstract Session IV		
	Liquid biopsies: techniques and applications	**J Damrauer & G Vandekerkhove**
14:50	Initial results from the prospective randomized UroFollow trial comparing marker-guided vs. cystoscopy-based standard follow-up in patients with low/intermediate risk bladder cancer	Bernd Schmitz-Dräger
15:00	Urinary comprehensive genomic profiling predicts urothelial cancer up to 12 years ahead of clinical diagnosis: an expanded analysis of the Golestan Cohort Study	Florence Le Calvez-Kelm
15:10	Added value of ctDNA testing for identifying FGFR3 alterations in metastatic urothelial cancer eligible for erdafitinib treatment	David Müller
15:20	Simulating the effects of molecular urine markers in follow-up of patients with high risk non-muscle invasive bladder cancer	Bernd Schmitz-Dräger

Awards, Wrap-Up & Closing Remarks	
15:30	Awards Presentation – Peter Black
15:40 -16:00	Closing Remarks – Roman Nawroth

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Poster overview		
#	**Abstract Title**	**Presenter**
1	Investigating the role of Tumor Associated B cells in Bladder Cancer Progression	Sadaf Rahimi
2	The stroma-rich consensus bladder cancer subtype correlates with improved prognosis after neoadjuvant immunotherapy and radical cystectomy	Joep de Jong
3	Impact of Mental Health Illness on Adherence to Surveillance and Treatment Guidelines in Non-Muscle Invasive Bladder Cancer Patients	Valentina Grajales
6	Impact of urinary and gut microbiota on Bacillus Calmette-Guérin-induced response in non-muscle invasive bladder cancer	Dalia Othman
7	Exploring the Landscape of Targetable Mutations in FGFR3 Wild-type Non-Muscle Invasive Bladder Cancer	Muneeb Alam
8	Tumor-immune interactions and cisplatin resistance in localized muscle-invasive bladder cancer	Filipe Carvalho
11	Comparative Analysis of very Reduced vs Full Dose BCG Treatment for Non-Muscle Invasive Bladder Cancer: A Contemporary Experience from Chile	Valentina Grajales
12	A Basality Score to differentiate urothelial bladder cancer genetic susceptibility patterns	Raquel Benítez
15	Bladder cancer tumor evolution and metastasis modeled in PDXs	Carina Bernardo
16	Assessment of circulating tumor DNA status as a prognostic marker for adverse pathologic features and recurrence detection in patients with muscle invasive bladder cancer.	Shivaram Cumarasamy
17	Prognostic value of inflammatory parameters in systemic therapy of urothelial carcinoma: Are early CRP dynamics exclusively immunotherapy-associated?	Philippe Becker
18	NRF2 activation promotes fitness disadvantage in normal urothelium and drives basal-like phenotype	Akihiro Hamada
19	IHC-based molecular subtyping and phenotypic heterogeneity in bulk versus invasive front of pT1 bladder cancer	Gottfrid Sjödahl
20	CDK4/6 inhibitors induce a multistep molecular response in bladder cancer cells	Roman Nawroth
21	Proteogenomic characterization of bladder cancer reveals sensitivity to TRAIL-induced apoptosis in FGFR3-mutated tumors	Isabelle Bernard-Pierrot
22	FBXW7 Loss-of-Function is associated with worse outcomes and leads to the accumulation of MYC in muscle-invasive bladder cancer	Ruiliang Wang
24	Predicting response to intravesical BCG in high risk non-muscle invasive bladder cancer using an artificial intelligence-powered pathology assay: outcomes from a multicenter cohort	Yair Lotan
25	Multi-omic profiling of non-muscle invasive low-grade metastatic Ta bladder tumours	Mathieu Roumiguie
26	Smoking Status is Underreported and Undermeasured in Bladder Cancer Drug Trials; Opportunities for Improvement in Clinical Trial Design	Hannah Kay
27	Development and external validation of an artificial intelligence-based prediction tool for tumour progression in non-muscle invasive bladder cancer: A retrospective multi-institutional cohort study	Jethro Kwong
28	Circulating tumor DNA for characterization of ERBB2 as a predictive biomarker in metastatic urothelial carcinoma	Gillian Vandekerkhove

29	Investigating sex chromosomal influence on tumor progression and response to bacillus Calmette Guérin immunotherapy in the four-core genotype murine model of non-muscle invasive bladder cancer	Gwenaelle Conseil
30	Predicting treatment outcome in muscle invasive bladder cancer using multimodal deep learning models	Simon Grouard
31	Patterns of smoking cessation strategies and perceptions of e-cigarette harm among bladder cancer survivors: findings from a cross sectional convenience sample	Jobin Chandi
32	Longitudinal personalized urinary tumor DNA analysis in muscle invasive bladder cancer from the neoadjuvant immunotherapy trial RJBLC-I2N003	Ruiyun Zhang
34	Novel and non-invasive tool for immunotherapy-response prediction in non-muscle invasive bladder cancer patients based on miRNAs and cytokine detection in urine	Cristian Suárez Cabrera
35	Establishment of the Multiplex IHC panel combined with FGFR3 RNAScope for exploration of tumor immune microenvironment in upper tract urothelial carcinoma.	Tomoko Iwata
36	Unraveling circulating T follicular helper cells profiles in non-muscle invasive bladder cancer	Kartik Sachdeva
37	The 'normal' bladder: A comprehensive investigation into the immune landscape and microbiome in normal bladder.	Madhavi Natarajan
38	Tumor-spatial and immune features are associated with response to neoadjuvant chemo-immunotherapy for muscle-invasive bladder cancer	Wolfgang Beckabir
39	Investigating BCG response associated intra-tumoral profiles of CD79a+ B cells, CD103+ tissue resident memory T cells and tertiary lymphoid structures in non-muscle invasive bladder cancer	Madhuri Koti
40	Analysis of the Tumor Microenvironment and PD-L1 Expression Reveals Myofibroblasts as a Prognostic Biomarker in Non-Invasive Bladder Cancer	Jesus Maria Paramio
41	Investigating the role of atypical B cells in response to BCG immunotherapy in a murine model of non-muscle invasive bladder cancer	Priyanka Yolmo
42	The second-generation histone deacetylase inhibitor quisinostat strongly synergises with cisplatin and the PARP inhibitor talazoparib in cisplatin resistant and naïve bladder cancer cells	Michèle Hoffmann
43	Development and initial evaluation of infigratinib-eluting seeds for localized treatment of non-muscle invasive bladder cancer (NMIBC)	Moritz Maas
44	Combined positive score (CPS) and PD-L1 status in patients with high-risk non-muscle invasive bladder cancer are influenced by an intravesical therapy with Bacillus Calmette-Guérin (BCG)	Moritz Maas
45	DOT1L regulates the expression of key driver genes in luminal muscle invasive bladder cancer	Sudha Kotapalli
47	State tobacco control policies: Opportunities for smoking cessation in urologic health	Hannah Kay
49	Combining Antibody-Drug Conjugates with Radiation in Preclinical Bladder Cancer Models	Yuzhen Zhou

50	The prognostic value of HER expression in the era of HER targeted therapies in metastatic urothelial carcinoma	Bernadett Szabados
51	Missense tumor mutations in the MTAP gene are only present in bladder cancer patients who are active smokers	Piyush Agarwal
52	Round Ligament Vaginal Colpopexy for Prevention of Post Anterior Exenteration Pelvic Organ Prolapse	Piyush Agarwal
53	Gemcitabine and cisplatin with or pembrolizumab in advanced urothelial cancer: Exploratory analysis from the phase 3 KEYNOTE-361 study	Thomas Powles

ORAL ABSTRACTS

FRIDAY, SEPTEMBER 29, 2023

Long term recurrence risk, metastatic potential, and length of surveillance of low-grade non-muscle invasive bladder cancer

Amy Chan, Eunice Villegas[1], Katherine Lajkosz[2], Christian Vitug[1], Shayan Din[1], Peter Black[3], Theodorus H. van der Kwast[4], Michael Jewett[5], Morgan Roupret[6], Eva Comperat[7], Jeffrey L. Wrana[8], Joan Sweet[4], Thomas Seisen[6], Neil E Fleshner[5], Girish S Kulkarni[5], Alexandre R Zlotta[1]

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Introduction: Very few patients with Ta low grade (LG) bladder cancer (BC) are at risk of developing metastases or dying of BC. However, long term follow-up data are scant and length of follow-up is poorly defined, especially when free of recurrence for 5 years. We investigated the incidence of late "recurrence", risk of metastases and death due to BC in patients with TaLG BC.

Methods: This retrospective study analyzed 521 patients with primary TaLG diagnosed between 1989-2019 at a university center (Toronto, Canada). Risk of recurrence, progression to high grade Ta/T1, muscle invasion, metastases and death due to BC at 5, 10 and 15 years were assessed. RNAseq analysis compared the transcriptomic profiles of 4 LG tumors that metastasized to non-progressing tumors. Inter-observer variability in pathological grade by expert pathologists was assessed in 80 cases.

Results: Mean and median follow-up was 9.9 and 8.5 years, respectively. Among 521 patients (72.9% men, median age 67.0 years), 350(67.2%) recurred, 57(10.9%) progressed in stage, 20(3.8%) developed metastases and 15(2.9%) died from BC. Of the patients recurrence-free for the first 5 years, 50 (50/251, 9.6%) developed BC within 20 years and 2 died of BC (2/521, 0.4%). Cancer-specific survival was 99.1%, 97.8% and 96.3% at 5, 10 and 15 years, respectively. Transcriptomic profiles between metastasized Ta LG and non-recurring tumors were different, despite appearing phenotypically similar. Moderate concordance was observed using the 1973 WHO grading system (Kappa=0.41;95%CI=0.32-0.50), improved with the 2004 system (0.78; 95%CI=0.65-0.90).

Conclusions: Our results challenge the assumption that LG Ta BC nearly never progress to potentially lethal disease, with 2.9% dying from BC. However, the risk of BC-related mortality is extremely low in patients recurrence-free for the first 5 years. Efforts are needed to minimize inter-observer variability in pathological grading as surprisingly even experts sometimes disagree between low and high grade.

Transurethral resections of bladder tumor quality criteria and early recurrence rate: preliminary data from the international RESECT study

Marcq G.[1], Gallagher K.M.[2], Szostek A.[1], Piazza P.[3], Del Guidice F.[4], Bhatt N.R.[5], Clement K.D.[6], Zimmermann E.[7], Khadhouri S.[8], Kulkarni M[9], Gaba F.G.[10], Anbarasan T.[11], Ng A.[12], Light A.[13], Asif A.[12], Chan V.[9], Nathan A.N.[12], Rossie S.H.R.[14], Cooper D.[15], Aucott L.[15], O'Brien T.[16], Maclennan S.[17], Nielsen M.[18], Mariappan P.[19], Kasivisvanathan V.[12], RESECT study global collaborators (BURST)[20]

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Introduction: Transurethral resection of the bladder tumors (TURBT) is essential in the management of bladder cancer (BC). The objective of our study was to compare TURBT quality criteria and to analyze the rate of early recurrence between sites taking part in the RESECT study.

Methods: RESECT is an ongoing international, multicentre, observational study including all consecutive new cases of BC scheduled for TURBT (> 17,000 patients included as of June 1st 2023). Patients were excluded if they presented a preoperative imaging of a muscle-invasive bladder cancer (MIBC), missing data or if they had not yet had their first cystoscopic check-up after TURBT. The quality criteria used were: presence of detrusor muscle (DM+), use of single instillation intra-vesical chemotherapy given within 24 hours (SI-IVC-24), completeness of resection documented, and if all of tumour number, size and location were documented in the operation note. A mixed-effects multivariate logistic

regression was used for the analysis of the early recurrence rate (defined by the first cystoscopy post TURBT). NCT05154084

Results: Data from 4597 patients having TURBT for first tumour were extracted from 186 sites. Regarding the TURBT quality criteria the median achievement rate per site was 71.4% for DM+, 43.8% for SI-IVC-24, 72.4% for documenting resection completeness and 79.6% for documenting all tumour features. with a significant difference in achievement between the sites (< 0.05). The median recurrence rate by site was 12% and 27% for low-grade and high-grade tumors, respectively. After adjusting for tumor size, number, stage and grade (significantly and independently associated with early recurrences), a significant difference in the early recurrence rate between the centers (< 0.0001) was shown.

Conclusions: There is significant variation in TURBT quality criteria and in the early recurrence rate of NMIBC after TURBT surgery between sites that could not be explained by currently understood tumor features.

SURE-01: Interim results of a phase 2 study of neoadjuvant sacituzumab govitecan (SG), followed by radical cystectomy (RC), for patients with muscle-invasive bladder cancer (MIBC)

Antonio Cigliola[1], Chiara Mercinelli[1], Daniele Raggi[1], Damiano Patanè[1], Emanuele Crupi[1], Andrea Necchi[2]

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Introduction: For patients (pts) with MIBC who cannot receive or refuse cisplatin-based chemotherapy there is no standard-of-care neoadjuvant therapy. SG is an antibody-drug conjugate (ADC) targeting Trop-2, with SN-38 as a payload. In SURE-01 (NCT05226117) we aim to evaluate the efficacy of neoadjuvant SG before RC.Patients and

Methods: In SURE-01, pts have a histopathologically-confirmed predominant urothelial carcinoma (UC), a clinical stage T2-T4N0M0 MIBC, and should be ineligible or refuse to receive cisplatin-based chemotherapy. Pts receive 4 cycles of 10 mg/Kg SG IV, on days 1, 8, of each 21-day cycle, before RC. After surgery patients are managed according to standard practice. The primary endpoint is the proportion of ypT0N0. The assumptions include a ypT0N0 ≤20% as H0 and ≥45% as H1 in a single-stage design, accounting for a total of 56 pts.

Results: From 03-06/2022, 8 pts were enrolled. Median age was 77y, 4 pts had a cT3-4N0, 4 a cT2N0. Four pts (50%) had a mixed variant histology. All pts received at least 1 cycle of SG: Grade 3-4 neutropenia was seen in 6/8 pts (75%), Grade 3-4 diarrhea in 4/8 (50%). Two pts (25%) had treatment discontinuation after C1D8 and died: one due to treatment-related sepsis and diarrhea, one due to complications following a treatment-related sepsis. The remaining pts had dose reduction to 75% since C2D1. Toxicity was unrelated to UGT1A1 polymorphism. 3/6 pts (50%) receiving RC achieved a ypT0N0 response, 2 pts ypT3N0, one an ypT3N1. All pts with a residual disease revealed a ctDNA-negative Signatera test post-RC. All 6 patients are currently alive without relapse.

Conclusions: Based on interim safety results, SURE-01 was amended and a dose-reduction to 7.5 mg/Kg SG is now administered since C1D1, added to primary prophylaxis with G-CSF.Encouraging preliminary efficacy data with SG monotherapy were seen.

Evaluation of molecular residual disease by urinary comprehensive genomic profiling in the SWOG S1605 phase II study of atezolizumab in patients with BCG-unresponsive NMIBC

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7 Convergent Genomics, Research and Development

Introduction: BCG-unresponsive (BU) non-muscle invasive bladder cancer (NMIBC) is associated with high rates of recurrence and progression. Methods to assess recurrence risk and monitor therapeutic response are needed. Here, we conducted urinary comprehensive genomic profiling (uCGP) in patients treated with atezolizumab.

Methods: Urine samples from 134 patients with BU NMIBC were collected at baseline and after four cycles of treatment. uCGP was completed using UroAmp (Convergent Genomics). Recurrence risk was calculated at baseline, and molecular response was classified based on change in molecular residual disease (MRD) between the two time points. Comparison of mutations was made to a non-study cohort of high-grade BCG-naïve (BN) NMIBC (n=101). Clinical variables were not available for analysis.

Results: Baseline samples demonstrated high genomic diversity. The number of mutations ranged from 0–20, with a median of 4. Among samples with mutations, allele frequencies ranged from < 1% up to 80%. Copy number gains up to 22x were observed. The most frequently mutated genes in baseline samples included TERT promoter (43%), TP53 (32%), ARID1A (21%), KMT2D (21%), and ERBB2 (15%). NIT1 and SOX4 were frequently amplified (12% and 10%). Compared to BN, BU patients were enriched in: SNVs in ERBB3 (OR=4.6, p=0.01), ERBB2 (OR=2.3, p=0.09), ARID1A (OR=2.2, p=0.05), TP53 (OR=1.9, p=0.05), and TERT promoter (OR=1.8, p=0.05); gain in NIT1 (OR=3.2, p=0.05); and high aneuploidy (OR=3.2, p=0.01). At baseline, 68% of patients were predicted to be at high risk of clinical recurrence. After treatment, 8% showed complete MRD response, 14% partial response, 25% stable MRD, and 46% exhibited MRD expansion.

Conclusions: This study suggests uCGP can identify genomic patterns associated with BU NMIBC and assess response to treatment via MRD measurements. Ongoing correlation with clinical characteristics and outcomes will test the utility of uCGP to monitor therapeutic response

Field cancerization impacts tumor development, T-cell exhaustion and clinical outcomes in bladder cancer

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Introduction: Field cancerization, describing areas of normal tissue affected by mutated clones, may affect treatment response and outcome in bladder cancer. High urinary tumor DNA (utDNA) levels in patients with non-muscle-invasive bladder cancer (NMIBC) have been associated with worse clinical outcomes, and utDNA may be used for real-time assessment of residual disease.

Methods: Using comprehensive genomic- and proteomic analyses, we analyzed 751 urothelial bladder biopsies, including 662 normal-appearing biopsies, and 234 urine samples from 136 NMIBC patients. The median follow-up time was 7.7 years. Samples were procured at multiple time points during the disease courses. Field cancerization was assessed in normal-appearing biopsies using deep-targeted sequencing (mean coverage: 1,359X) with application of unique molecular identifiers and error correction models. DNA from urine was sequenced (mean coverage: 2,153X) and urine proteomics were analyzed using Olink.

Results: We detected mutations in 458 out of 751 analyzed bladder biopsies. High levels of field cancerization were associated with high tumor mutational burden (p=0.007), high tumor neoantigen load (p=0.029), and high tumor-associated CD8 T-cell exhaustion (p=0.017). Additionally, high field cancerization was associated with worse short term outcome (p=0.029) and increased with age (p=0.0027). Non-synonymous mutations in bladder cancer driver genes such as KDM6A, ARID1A and TP53 were identified as early disease drivers found already in the normal-appearing bladder biopsies.utDNA levels reflected the bladder disease status and originated from field cancerization and tumors. High levels of utDNA after BCG were associated with worse clinical outcomes for the patients (p=0.027) and with disease progression (p=0.003). High field cancerization resulted in high urinary levels of proteins associated with angiogenesis, apoptosis and tumor immunity.

Conclusions: Field cancerization of the bladder may affect tumor development, immune responses, and clinical outcomes. utDNA measurements may have the potential to be used to monitor disease status and treatment response in patients with NMIBC.

An holistic and intergrated approach for investigating the bacterial microbiome, gene expression profile and immune cell profile in the non-muscle invasive bladder cancer tumour microenvironment

Tyler Wooldridge[1], Nicola Annels[2], Hardev Pandha[2]

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Introduction: Bladder cancer (BC) is the 10th most common cancer world-wide with an estimated 570,000+ people being diagnosed in 2020. Communities of bacteria exist in both normal and cancerous tissues and can impact treatment efficacy (e.g. metabolising chemotherapeutics). We investigated the bacterial microbiome in formalin-fixed paraffin-embedded (FFPE) tumour tissue across different stages of BC compared to adjacent normal tissue to investigate differentially expressed (DE) bacteria between Bacillus-Calmette-Guerin (BCG) treatment responders vs non-responders. These findings were correlated to host gene expression, infiltrating immune cell profiles and urinary cytokine within the tumour microenvironment (TME).

Methods: Bacterial signatures within urine (n=56) and FFPE tissues (n=66), (matching patients n=44), derived from the Royal Surrey County Hospital, Guildford, UK, were determined using 16s rRNA sequencing (V3-V4). Sequencing data were processed through QIIME2 and clustered into amplicon sequence variants (ASVs). Alpha (Shannon and observed) and Beta (Bray-Curtis, weighted/unweighted uniFrac) diversity analysis was performed. RNA extracted from FFPE BC tissues generated gene expression data using the Nanostring IO360 panel (770 gene CodeSet) and was correlated to tumour microbiome. 9-colour multiplex immunohistochemistry (mIHC) using Phenoimager HT (Akoya Biosciences) was performed to investigate and spatially define immune cell types (CD4, CD8, CD68, CD57, FOXP3, GRZB, PD-L1, PANCK, DAPI), within the TME (n=71), which were correlated to gene expression, bacterial signatures, and clinical patient information.

Results: BC bacterial microbiome showed decreased diversity with disease progression ($P<0.01$). The bacterial profiles between urine and FFPE cancer tissues revealed independent groups ($P<0.01$), showing urine is not an accurate proxy. DE bacteria were found between BCG responders vs non responders. Immune cell counts and spatial relationships determined via mIHC, showed differences between disease staging. Analysis has also shown links between bacteria, immune gene expression and immune cells populations.

Conclusions: Bacteria may influence patient BCG treatment responses and pose as an interesting target for modulation to improve therapeutic outcomes.

Meta-analysis of GWAS data from diverse populations for Bladder Cancer (mapBC) collaboration: initial findings

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Introduction: To date, the largest genome-wide association study (GWAS) of bladder cancer susceptibility (Koutros, Eur Urol, 2023, NCI-meta) involved a meta-analysis of 13,790 individuals with bladder cancer and reported 24 genome-wide significant ($p<5x10^{-8}$) loci. While this was a sizable increase over the prior studies, estimates of heritability suggest more loci are yet to be identified.

Methods: Using a fixed effects inverse variance weight meta-analysis to combine summary statistics from participating cohorts, case-control studies, and case-series (NCI-meta) and large population biobanks (Million Veterans Program [MVP], UKB, FinnGen), we performed a GWAS comprised of 30,136 individuals with and 1,576,084 individuals without bladder cancer.

Results: By doubling the number of individuals with bladder cancer from the previous GWAS, we identified a total of 50 genome-wide significant loci. We replicated all previous risk loci and identified 26 novel loci. Of note, a variant tagging the pathogenic deletion in CHEK2 1100delC (T367Mfs*15) achieved genome-wide significance. Smaller studies had previously implicated CHEK2 in familial bladder cancer, however this is the first large-scale population-based study to validate this finding in GWAS.

Conclusions: This study, which is the largest bladder cancer GWAS to date, represents an advancement in the understanding of germline genetic susceptibility for bladder cancer. Analyses are underway to explore possible biological effects of novel GWAS loci. Future efforts will also aim to increase the proportion of individuals of non-European ancestries, that are underrepresented in current efforts.

Exploring the impact of microbiome in the response of combined radiation with immune checkpoint blockade in muscle invasive bladder cancer (MIBC)

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Introduction: Radiation therapy (RT) is a promising bladder-sparing option for MIBC treatment, yet 30% of patients do not respond and half later die of metastasis. Improved antitumor responses when RT is combined with PD-1/ PD-L1 blockade (CT) have been described in mice, yet determinants of CT success remain flagrantly misunderstood. As such, gut microbiome composition influences PD-1 blockade efficacy and its modification potentiates combined RT and PD-L1 blockade activity. To add, responding patients with favorable gut microbiomes have enhanced antitumor immunity. We thus aim to document the role of patients' microbiome in polarizing anti-tumor immune responses to CT and predicting CT success in MIBC.

Methods: Fecal material from a responder (R) and non-responder (NR) MIBC patient was gavaged into 20 germ-free mice. 3 weeks after the last gavage, MB49 cells were delivered subcutaneously. Once tumors reached 0.1-0.15cm3, mice were randomized into 4 groups : control; anti-PD-L1; RT; RT+anti-PD-L1. 7 days later, tumors were dissociated and stools collected for for single-cell immun and 16S sequencing. Correlation networks were built (TransNet, Microbiome R packages) and visualized in Cytoscape.

Results: We show feasibility and robust engraftment of human FMT to germ-free mice in a MIBC tumor model. FMT from NR lessened the known beneficial effects of RT in the MB49 model compared to FMT from R. Transkingdom analysis of immune and microbe data shows robust statistical interactions between immunosuppression and enrichment in microbes associated to poor outcome humans.

Conclusions: To our knowledge, this is the first study to use FMT as a modulator of response in the context of radiation therapy combinations in MIBC. These findings could be used to select patients who will benefit most from a personalized therapeutic approach.

Patient-derived organoids identify tailored therapeutic options and determinants of plasticity in sarcomatoid urothelial bladder cancer

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Introduction: Sarcomatoid Urothelial Bladder Cancer (SARC) is a rare and aggressive histological subtype of bladder cancer. Here, we established the first long-term 3D organoid-like model derived from a SARC patient (SarBC-01) and used it to identify potential therapeutic strategies and factors driving bladder cancer progression.

Methods: Samples were collected from one SARC patient (SarBC-01) undergoing transurethral resection of the bladder (TURB) and three conventional urothelial carcinoma (UroCa) patients undergoing cystectomy or TURB, and were subsequently processed to generate organoids. Organoids were characterized using H&E staining, immunohistochemistry (IHC), immunofluorescence, and whole-exome sequencing and subjected to in vitro invasion and in vivo tumorigenicity assays. A library of 1567 drugs was tested at single concentration and 26 drugs were validated in dose response analysis. Glucocorticoid receptor expression was assessed using a bulk RNA sequencing public dataset and IHC in an in-house cohort. Single-cell RNA sequencing was performed following the protocol of Chromium GEM v3.1 (10 xGenomics).

Results: SarBC-01 emulated morphological and phenotypical features of SARC and harbored somatic mutations in genes frequently altered in sarcomatoid tumors such as TP53 and RB1. SarBC-01 exhibited significant higher invasive capacity in vitro and faster tumorigenicity in vivo than UroCa-derived organoids, consistent with a more aggressive phenotype. High-throughput drug screening identified drug candidates active against SARC cells exclusively, UroCa cells exclusively, or both. Agents targeting the Glucocorticoid Receptor (GR) pathway were specifically effective in SARC cells. In two independent cohorts, GR expression was significantly higher and more frequent in SARC versus UroCa samples, suggesting that high GR expression represents a hallmark of SARC tumors. Further, glucocorticoid treatment abrogated the invasive ability of SARC cells and led to transcriptomic changes associated with reversion of epithelial-to-mesenchymal transition.

Conclusions: Our study highlights the power of organoids for precision oncology and for providing key insights into factors driving rare tumor entities

ORAL ABSTRACTS

SATURDAY, SEPTEMBER 30, 2023

Gene expression-based classification of urothelial carcinoma for research and clinical applications

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Introduction: Transcriptomic subtype prediction of urothelial carcinomas can be affected by the choice of expression platform, cohort composition, data preprocessing, and tumor purity. To address this, a single-sample-predictor (SSP) based on binary gene-pair rules was developed. The rule-based RandomForest (RF) SSP was trained on minimally preprocessed microarray and RNA-sequencing data and validated in four external bladder cancer datasets. The predicted subtype assignments captured cohesive gene expression signature patterns and significant associations with mutations concordant with the Lund Taxonomy.

Methods: An initial 5-class (Uro, GU, Basal, Mes-like, and ScNE) Lund Taxonomy RF classifier was trained on 307 samples with microarray gene-expression data (preprocessed with both RMA and SCAN using fixed BrainArray version 25 annotations). A new model was trained after identification and removal of outlier samples, which was then applied to two additional uniformly preprocessed microarray datasets and 265 RNA-sequenced samples (summarized to TPM using both Kallisto and Salmon). Prediction results were in good agreement with both previous subtype classifications of these cohorts and with immunohistochemistry-based subtype assignments. A final predictor was built using all four datasets (each with two preprocessing versions) as training data. A separate classifier for substratification of Uro into UroA, UroB, and UroC was designed in a similar manner.

Results: Application of the classifier to the TCGA, IMvigor210, UC-Genome, and Robertson et al. T1 datasets gave confident subtype predictions which recapitulated the expression patterns of the training cohort. A significant enrichment of FGFR3 and RB1 mutations were seen in UroA+UroB and GU+ScNE respectively.

Conclusions: The rule-based single sample classification approach performed well across multiple external datasets with minimal preprocessing requirements. Evaluation across additional datasets (RNA-seq and microarrays) is ongoing, with final validation planned in a cohort of prospectively RNA-sequenced tumors with paired IHC.

Alignment of molecular subtypes across multiple bladder cancer subtyping classifiers

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Introduction: Guideline-recommended treatment for muscle-invasive bladder cancer (MIBC) patients includes cisplatin-based neoadjuvant chemotherapy (NAC) followed by radical cystectomy (RC). There is a need for markers to predict response to NAC and previously published reports demonstrated a high degree of inconsistency among reported subtype-specific outcomes.

Methods: We analyzed gene expression data generated from transurethral resection of MIBC from a previously assembled and published meta-cohort, NACmeta (N=601, 247 treated with NAC+RC and 354 RC without NAC), where extended follow-up was available. Molecular subtypes were assessed using Genomic Subtyping Classifier (GSC), the Consensus Classifier, The Cancer Genome Atlas (TCGA) and the Lund Classifier. For survival analysis, inverse probability weighting was used to balance the clinical characteristics of the NAC and non-NAC patient groups.

Results: A high consistency in transcriptomic expression patterns and nomenclature was observed between luminal-like subtypes, defined as GSC-Luminal, Consensus-Luminal Papillary (LumP), TCGA Luminal-papillary (LumP) and Lund-UroA, but not for basal-like subtypes such GSC-Basal, Consensus Basal/Squamous, TCGA-Basal/Squamous and Lund-Basal/Squamous. Patients with luminal-like subtypes demonstrated no differences in 3-year survival whether they had received NAC (p=0.7 for GSC, p=0.94 for Consensus, p=0.87 for TCGA and p=0.66 for Lund-UroA, respectively). Patients with ≥pT3 disease at RC who were classified as GSC-Luminal did not show a significant survival improvement with NAC (p=0.21).

Conclusions: Luminal-like molecular subtypes, including the GSC-Luminal, Consensus-LumP, TCGA-LumP and the Lund-UroA, identify a subgroup of MIBC patients who do not appear to benefit from current NAC regimens even for locally advanced disease. In addition, we were able to illustrate differences in subtyping nomenclature that are not reflected in the underlying biological definition of the subtypes

A composite biomarker approach to spare neoadjuvant chemotherapy in select muscle-invasive bladder cancer patients

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Introduction: Clinical guidelines recommend neoadjuvant chemotherapy (NAC) prior to radical cystectomy (RC) for the management of muscle-invasive bladder cancer (MIBC), despite treatment-related toxicity and a modest survival benefit. Here, we identified MIBC patients who may avoid NAC using a composite biomarker approach of circulating tumor cell status and molecular subtypes.

Methods: TURBT samples were collected from clinical stage T2-T4aN0-N1M0 cases included in the prospective CirGuidance study (NL3954) that analyzed circulating tumor cell (CTC) status in patient blood using the CELLSEARCH system. For the present study, transcriptome-wide expression profiling was performed on 234 TURBT samples using an array-based approach. Molecular subtypes, long non-coding RNA (lncRNA) based FGFR3+ status and gene signatures were determined as described previously (PMID 31619281). The primary endpoint of this study was cancer-specific mortality (CSM), calculated as the date of study inclusion till date of bladder cancer related death. Median follow-up was 28.8 (IQR: 16.6-40.2) months.

Results: Of 234 patients, 21 (9%) were treated with NAC and RC, while 213 (91%) received RC alone. A CTC-negative status was observed in 172 (81%) of RC-only cases. Molecular subtyping identified 28 luminal FGFR3+ cases with high FGFR3, SHH and p53 pathway activity, and lower EMT hallmark scores. Adjusting for clinical risk factors, both CTC and FGFR3+ status were significant predictors for cancer specific mortality on MVA ($P<0.05$). Of interest, the subgroup of FGFR3+ cases that were CTC-negative (N=26) showed most favorable outcomes with only one CSM event after a median of 33.4 (IQR: 24.8-44.5) months of follow-up.

Conclusions: Using a composite biomarker approach of blood-based CTC status and molecular subtyping, we identified a biologically distinct subgroup of MIBC with favorable prognosis after RC-only, validating the performance of a previously developed lncRNA based FGFR3+ classifier. Clinical trials which withhold NAC from CTC-negative, FGFR3+ MIBC patients are warranted.

Molecular signatures predict GEMDOCE responses for BCG-unresponsive non-muscle invasive bladder cancer

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Introduction: Intravesical combination chemotherapy (gemcitabine and docetaxel [GEMDOCE]) is commonly used to treat patients with BCG-unresponsive non-muscle invasive bladder cancer (NMIBC). However, potential biomarkers of GEMDOCE response in BCG-unresponsive NMIBC has not been fully evaluated. Microtubule targeting agent, Docetaxel and DNA damaging agent, Gemcitabine have shown therapeutic effects by inducing chromosomal instability, cell cycle arrest, and apoptosis. Therefore, we explored whether these gene signatures were associated with GEMDOCE response in patients with BCG-unresponsive NMIBC tumors.

Methods: Whole transcriptome RNAseq and DNA panel sequencing were performed on a cohort of patients with BCG-unresponsive NMIBC who underwent intravesical GEMDOCE. Cluster analysis and gene set variant analysis (GSVA) were used to assess whether gene set signatures were associated with therapeutic response and recurrence-free survival.

Results: Cluster analysis with early and late cell cycle signatures (CCND1, WEE1, CCNA2, CCNB1, CCNE1, etc) generated two main clusters that expressed high levels of late (cluster 1) or early (cluster 2) cell cycle signatures. Cluster 1, which was associated with enrichment of late cell cycle gene signatures showed significant recurrence-free survival benefit compared to cluster 2 (enriched with high level of early cell cycle gene signatures). Additionally, cluster 1 was enriched with the expression of chromosome instability gene signatures such as chromosome segregation (AURKA, CENPA, PLK1, etc) and DNA damage response (p53, ATR, BRCA1/2, etc). TP53 alterations tended to be enriched in cluster 1.

Conclusions: Our data suggests that cell cycle and chromosome instability gene signatures may stratify response to GEMDOCE in patients with BCG-unresponsive tumors. Future studies with larger cohorts will be required to validate the clincial relavance of these observasions.

Proteogenomic Characterization of Chemotherapy Response in Muscle Invasive Bladder Cancer

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Introduction: To further understand the underlying mechanisms of NAC resistance and identify alternative treatments for NAC-resistant MIBCs, we performed comprehensive proteogenomic profiling of pre- and post-NAC tumors with carefully curated response information.

Methods: We processed OCT-embedded and flash frozen tissue samples from 143 patients and samples containing >45% tumor, <10% muscle, and >2,000 protein identifications in a single-shot QC assay were selected for deep-scale proteomic and phosphoproteomic profiling. A final cohort of 58 tumors (44 pre-treatment and 14 post-treatment) were multiplexed using tandem mass tags (TMT-11), fractionated by basic reverse phase chromatography, and analyzed by liquid chromatography and tandem mass spectrometry (LC-MS/MS). Complimentary WES, RNASeq, and SureQuant targeted proteomics were performed with from the same samples. Fragpipe, Skyline and SEPepQuant were used to identify and quantify peptides, protein isoforms and gene abundance.

Results: Over 12,000 proteins and 28,729 phospho-sites were identified, with 8,353 proteins in all samples, including 425 kinases and 77 targets of FDA approved therapies. Despite a median correlation of 0.48 between RNA and Protein abundances, subtyping assignments were mostly in agreement (81%) with an over-representation of neuronal subtypes. Multi-omics subtyping with NMF resulted in 4 clusters which are largely representative of previous subtypes. Resistant tumors had upregulated KRT20 protein while immune related proteins were elevated in sensitive tumors. GSK3B-S9 phosphorylation, which inhibits GSK3B activity, was significantly elevated in the sensitive group while the GSK3B protein abundance showed no difference. SEPepQuant identified specific isoforms which were differential between sensitive and resistant tumors while the gene level quantification was not associated with resistance, suggesting alternative splicing as a potential resistance mechanism. Integrating human tumor data with genetic screen data in bladder cancer cell lines identified druggable protein targets significantly associated with subtypes and resistance.

Conclusions: Our analysis identified multiple avenues for therapeutic engagement for which additional studies are require

Initial results from the prospective randomized UroFollow trial comparing marker-guided vs. cystoscopy-based standard follow-up in patients with low/intermediate risk bladder cancer

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Introduction: A growing body of evidence suggests that current follow-up strategies for patients with non-muscle invasive bladder cancer (NMIBC) result in overdiagnosis and may trigger overtreatment. The goal of the UroFollow trial was to explore the efficacy and safety of a marker-guided follow-up in patients with low/intermediate risk NMIBC.

Methods: 213 Patients with pTa/low and high grade (G1-2) NMIBC were included in the prospective randomized multicenter UroFollow trial comparing biannual marker-based follow-up (algorithm comprising UroVysion and NMP22 assays, urine cytology and ultrasound) against the current standard of care (SOC). After a 3-months cystoscopy, in the marker arm (MA) only patients with a positive algorithm result underwent cystoscopy. An end-of-study cystoscopy was recommended to all patients after 3 years.

Results: The study was conducted between 4/2016 and 4/2022. 102 and 111 patients were randomized to the marker arm and SOC, respectively. There were no significant differences between the two arms regarding tumor stage, grade, size, focality, and recurrent lesions of the base line tumors. Median follow-up interval was 2.5 years. Tumor recurrence rates were 30.4% vs. 29.7% in the SOC and MA, respectively. Sensitivity of tumor detection in both arms based upon ITT were 96.8% vs. 78.8% with 1 vs. 5 pTa LG tumors being overlooked in the SOC and MA, respectively. App. 20% of all recurrences were observed at the 3-months UC. One patient (SOC arm) was diagnosed with progression to MIBC at the 3 months UC.

Conclusions: UroFollow is the first randomized trial using the results of molecular urine markers for clinical decision making in patients with low/intermediate risk NMIBC. With all restrictions applying to a small-sized trial we conclude that marker-guided follow-up in this cohort is a safe strategy if including the 3 months UC as an integral part of surveillance.

Urinary comprehensive genomic profiling predicts urothelial cancer up to 12 years ahead of clinical diagnosis: an expanded analysis of the Golestan Cohort Study

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Introduction: Detecting pre-clinical urothelial carcinoma (UC) using urinary comprehensive genomic profiling (uCGP) may providea valuable opportunity for early detection and screening of high-risk populations. The UroAmp (Convergent Genomics) uCGP test uses next-generation sequencing to identify mutations across 60 genes. We informatically narrowing the data to consider 10 genes with the highest performance and test the potential of modified uCGP to detect preclinical UC.

Methods: A UC screening model was developed using uCGP data from a training cohort consisting of 140 urology controls and96 tumors (56 de novo, 40 recurrent). Model validation was performed in two studies: first a multi-institutionalcase-control design with 96 controls and 70 UC cases (22 de novo, 48 surveillance); a second using a nested case-controldesign within the population-based prospective Golestan Cohort Study (50,045 participants), which consisted of 29 asymptomatic individuals who subsequently developed primary UC (median time to UC 7.3 years) and 98 matched controls (median f/u 6.1 years).

Results: The UC screening model was trained to a sensitivity of 88% (97% sensitivity for High-Grade (HG)) and specificity of 94%. In the first validation, a sensitivity of 86% in de novo (87% for HG), 71% overall, and specificity 94% was observed. In the Golestan cohort, baseline uCGP had a prediction sensitivity of 66% (71% for HG) and a specificity of 94%. In contrast baseline TERT predicted 48% of cancers with a specificity of 100%. Cancer-free survival was significantly worse in uGCP-predicted positives vs. negatives (HR 8.5, 95% CI 3.8 – 18.4, $p<0.0001$). When limited to UC diagnosis within seven years, UroAmp detected pre-clinical UC in 86% of future cancers, compared to a sensitivity of 57% using TERT mutations alone.

Conclusions: Our results provide the first evidence from a population-based prospective cohort study of potential pre-clinical urothelial carcinoma detection with urinary comprehensive genomic profiling.

Added value of ctDNA testing for identifying FGFR3 alterations in metastatic urothelial cancer eligible for erdafitinib treatment

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Introduction: Fibroblast growth factor receptor (FGFR) inhibitors (e.g., erdafitinib) are increasingly important in the management of FGFR-altered metastatic urothelial carcinoma (mUC). Archival tissue testing for specific FGFR alterations is now implemented as a companion diagnostic but somatic FGFR status may change during disease progression and also after erdafitinib exposure. Our ongoing study aims to evaluate the role of cell-free DNA (cfDNA) compared to tissue testing for FGFR alteration detection and to evaluate genomic mechanisms of erdafitinib resistance.

Methods: Patients with progressing mUC undergoing archival tissue testing for FGFR alterations were eligible. Blood plasma cfDNA and matched leukocyte DNA were subjected to deep-targeted sequencing with a custom panel covering the most relevant urothelial cancer (UC)-specific gene loci. To evaluate the feasibility of a rapid and low-cost alternative to our deep-targeted sequencing approach, we designed a quantitative (q)PCR assay covering the most frequent FGFR3 mutations and applied it to cfDNA.

Results: To date, 163 patients have enrolled, with matched tissue and cfDNA results available for 77 patients. 58 of these 77 cfDNA screening samples had detected circulating tumor DNA (ctDNA) and could be evaluated for FGFR alterations. Concordance for FGFR status between evaluable ctDNA and tissue samples was 89%. Overall, 9% of tissue-negative patients were cfDNA-positive for FGFR alterations linked to the erdafitinib label, indicating that additional qualifying patients can be identified using cfDNA testing. Our rapid cfDNA qPCR assay demonstrated high concordance (92%) with targeted sequencing, correctly assigning FGFR3 status for all evaluable hotspot mutations. Among the patients providing cfDNA samples at progression on erdafitinib, we identified two patients with multiple new FGFR3 variants supporting polyclonal resistance.

Conclusions: Our study suggests that cfDNA is a valuable adjunct to tissue-based assays for the detection of FGFR alterations to identify patients eligible for FGFR inhibitor therapy and to monitor for mechanisms of resistance.

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Simulating the effects of molecular urine markers in follow-up of patients with high-risk non-muscle invasive bladder cancer

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Introduction: A plethora of urine markers for diagnosis and follow-up of patients with bladder cancer (BC) has been developed and studied in the past. However, the clinical impact of urine testing on patient management remains obscure until today. The goal of this manuscript is to identify scenarios for a potential use of molecular urine markers in the follow-up of patients with high risk non muscle-invasive BC (NMIBC) and estimate potential risks and benefits.

Methods: Information on the course of disease of patients with high risk NMIBC and performance data of a point-of-care test (UBC rapid™), an MCM-5 directed ELISA (ADXBLADDER™) and 2 additional novel assays targeting alterations of mRNA expression and DNA methylation (Xpert bladder cancer monitor™, Epicheck™) were retrieved from high quality trials and/or meta-analyses. In addition, sensitivity of white light cystoscopy (WLC) and the impact of a urine marker result on performance of WLC were estimated based on fluorescence cystoscopy data and information from the CeFub trial. These informations were applied to different scenarios in patient follow-up and sensitivity, estimated number of cystoscopies and the numbers needed to diagnose were calculated.

Results: The sensitivity of guideline-based regular follow-up (SOC) at 1 year was calculated with 91.7%. For different marker supported strategies, sensitivities were estimated ranging from 77 to 97.9%. Calculations suggests that most strategies are equieffective or even

superior to SOC. While for the SOC a number needed to diagnose (NND) of 25.9 per tumor detected was observed, the NND dropped to 4.2 in some marker-supported strategies.

Conclusions: Based on the results of this simulation, a marker-supported follow-up of patients with HR NMIBC is safe and offers the option to significantly reduce the number of WLC. Further research focusing on prospective randomized trials is needed to pave the way towards inclusion of urine markers into clinical decision making.

POSTER ABSTRACTS

Investigating the role of Tumor Associated B cells in Bladder Cancer Progression

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Introduction: Bladder cancer can be broadly categorized into non-muscle invasive bladder cancer (NMIBC) including 75% incident cases and 25% present with de novo muscle invasive bladder cancer (MIBC). High-risk NMIBC patients often progress to secondary MIBC. Our recent study on whole transcriptome analysis of tumors from 460 patients showed increased expression of B cell-associated genes in high-grade tumors. Spatial immune profiling of 332 NMIBC tumors demonstrated increased density of intratumoral B cells in patients with shorter recurrence free survival. We hypothesize that specific anergic B cell subsets expand due to carcinogen-induced chronic inflammation in the bladder mucosa promoting tumor progression.

Methods: Aging mice were exposed to N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN) carcinogen with simultaneous B cell depletion. Systemic immune profiling was conducted at multiple time points using flow cytometry. Whole bladder sections were subjected to H&E and multiplex immunofluorescence staining. Tumor sections from 16 patients who experienced post BCG progression and underwent radical cystectomy were evaluated for immune cell infiltration and presence of tertiary lymphoid structures.

Results: Increased recruitment of atypical B cells (ABCs) was observed following continuous exposure to BBN. B cell depletion during BBN exposure reduced the inflammation and delayed cancer progression with a pronounced effect in female mice. B cell depleted mice exhibited histologically benign or close to normal urothelium whereas untreated and Isotype group showed reactive atypia or dysplasia. Both systemic and local immune profiling depicted sex differences with female mice having a higher number of splenic total and atypical B cells and increased density of both populations in the bladder TIME compared to males.

Conclusions: These results suggest that long term depletion of B cells in aging mice leads to reduced inflammation in the bladder mucosa and delays disease progression. This study is foundational to the development of biomarkers and therapeutics along the B cell exhaustion axis

The stroma-rich consensus bladder cancer subtype correlates with improved prognosis after neoadjuvant immunotherapy and radical cystectomy

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Introduction: In patients with muscle-invasive bladder cancer (MIBC), molecular alterations that predict benefit from neoadjuvant immunotherapy remain largely unstudied. This study aims to evaluate the ability of molecular signatures to predict outcomes after neoadjuvant atezolizumab plus radical cystectomy (RC) and to explore the biology of atezolizumab-resistant tumors.

Methods: RNA-seq data from RC specimens (n=64, post-atezolizumab) from the ABACUS trial were available for analysis (PMID 31686036). Consensus molecular subtypes were assigned using gene expression data from post-treated samples. Unsupervised consensus clustering (CC) was performed to categorize the RC samples and each cluster was characterized using gene signatures. The Kaplan-Meier method estimated differences in patient outcomes. Additional RC cohorts (PURE-01 (post-pembrolizumab; PMID 34301456), post-NAC (PMID 30224344), TCGA (RC only; PMID 30096301) and USTW (RC only; PMID 31619281)) were characterized using the consensus model. Significance testing used a two-sided t-test at a threshold of 0.05.

Results: Unsupervised CC revealed three distinct post-atezolizumab clusters (luminal, scar-like & basal-immune). The scar-like tumors (n=19) expressed genes associated with wound healing/scarring and only 1/19 patients relapsed. Comparing the clustering solution to the consensus subtypes showed the scar-like cluster had considerable overlap with the stroma-rich consensus subtype (n=12) at RC. The stroma-rich subtype showed favorable prognosis with no relapse (median follow-up 13.1 months). Improved outcomes for the stroma-rich consensus subtype at RC were also observed within the PURE-01 and post-NAC cohorts. However, tumors with the stroma-rich subtype in patients who underwent RC without systemic therapy (TCGA and UTSW cohorts) were not associated with improved prognosis.

Conclusions: This study expands our understanding of the biology of atezolizumab resistant MIBC and contributes to the framework for defining molecular subtypes at RC. These results further support the hypothesis that residual bladder cancer with a scar-like / stroma-rich profile may predict improved patient prognosis after neoadjuvant treatment and RC.

Impact of Mental Health Illness on Adherence to Surveillance and Treatment Guidelines in Non-Muscle Invasive Bladder Cancer Patients

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Introduction: The high occurrence of anxiety, depression, and other mental health illness (MHI) in bladder cancer patients has been linked to negative impacts on their well-being and outcomes. We sought to identify the impact of MHI on adherence to surveillance and treatment guidelines on NMIBC patients

Methods: We used the SEER-Medicare linked data set to select patients with NMIBC diagnosed during the years 2005-2019. We divided our patient cohort into two groups: low-risk NMIBC and high-risk NMIBC patients. We defined low-risk (LR) NMIBC as low-grade Ta pathology and high-risk (HR) as high-grade T1. We followed the AUA guidelines for adherence. Univariate and multivariable Cox regression models were used to assess factors associated with adherence.

Results: From a total of 14,201 patients; 9,444 had low risk and 4,757 had high-risk NMIBC. In the LR group 1,625 had MHI. In the HR group 738 had MHI. In the HR group, patients with MHI had lower odds of adhering to cystoscopy (odds ratio [OR] 0.8, 95% confidence interval [CI] 0.65, 0.99; p=0.047), urine biomarker tests (OR 0.56, 95% CI 0.44, 0.72; p<0.0001), and intravesical treatment (OR 0.76, 95% CI 0.64, 0.89; p=0.001. In the LR group, patients MHI had lower odds of adhering to upper tract imaging (OR 0.88, 95% CI 0.79, 1.0; p=0.041) and higher odds of adhering to urine biomarker tests (OR 1.17, 95% CI 1.05, 1.31; p=0.006).

Conclusions: In conclusion, our study revealed that the presence of MHI in high-risk NMIBC patients was associated with reduced adherence to cystoscopy, urine biomarker tests, and intravesical treatment, while in low-risk NMIBC patients, MHI was linked to decreased adherence to upper tract imaging but increased adherence to urine biomarker tests. These findings emphasize the importance of addressing mental health conditions in bladder cancer patients to improve their adherence to surveillance and treatment guidelines.

Impact of urinary and gut microbiota on Bacillus Calmette-Guérin-induced response in non-muscle invasive bladder cancer

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Introduction: Intravesical Bacillus Calmette-Guérin (BCG) remains the first line treatment for high and intermediate risk non-muscle invasive bladder cancer (NMIBC). However, 40% of patients recur despite optimal BCG therapy. Since the commensal microbiota affect immune responses, we hypothesize that they modulate BCG-induced anti-tumor responses. The study's objective is to examine the role of urinary and gut microbiota in BCG-induced immune responses.

Methods: Urine and stool samples were collected from patients with NMIBC before and after BCG treatment to identify microbiota composition through metagenomic sequencing. To assess the role of commensal urinary microbiota on BCG-induced immune responses, mice were treated with BCG through intravesical instillation. Mice had either a healthy or a disrupted urinary microbiota. Disruption was achieved through gentamicin instillation prior to BCG treatment. Fluorescence-activated cell sorting (FACS) and histology was conducted to examine immune cells.

Results: 16s rRNA sequencing from 32 patients demonstrated BCG non-responders were associated with Pseudomonas, which metabolize Polycyclic Aromatic Hydrocarbons (PAH). In responders, Corynebacterium, which colonize healthy urinary microbiota, is more apparent. In vivo FACS analysis of spleen and bladder tissue observed shifts in immune cells. Gentamicin instillation induced a pro-inflammatory response through elevated M1 and reduced M2 macrophages, compared to control. Subsequent BCG treatment shifted the environment towards an anti-inflammatory environment through an increase of M2 and reduction of M1 macrophages, similar to mice with healthy microbiota. Immunohistochemistry of bladder tissue similarly reflected the FACs analysis findings.

Conclusions: BCG nonresponse was associated with bacteria that degrade PAH, a known carcinogen. Preliminary in vivo studies illustrated that urinary microbiota disruption alters the immune cell environment which shifts with BCG treatment, suggesting role of the microbiota in mediating BCG-induced response. Future work seeks to examine the role of urinary and gut microbiota on bladder cancer development and BCG-induced outcomes in mice with NMIBC.

Exploring the Landscape of Targetable Mutations in FGFR3 Wild-type Non-Muscle Invasive Bladder Cancer

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Introduction: Erdafitinib, an FGFR-inhibitor, is indicated in the treatment of select patients with metastatic bladder cancer. Although erdafitinib and other FGFR-inhibitors are currently being investigated in non-muscle invasive bladder cancer (NMIBC), many NMIBC tumors do not harbor FGFR alterations. The purpose of this study is to characterize the mutational landscape of NMIBC with a focus on FGFR3 wild-type (WT) tumors.

Methods: Patients with NMIBC who underwent tumor and normal sequencing were identified in a prospectively maintained genomics database. Demographic, clinical, and genomic data were abstracted. The OncoKB database was cross-referenced to identify potentially targetable mutations. The frequency of these mutations and others was determined.

Results: A total of 569 NMIBC specimens from 503 patients were identified. Median age was 68 years and median follow-up was 40 months. Most specimens were high-grade (HG) including HGTa (33.6%), HGT1 (45.6%), and CIS (10.5%). Potentially actionable gene mutations observed include KDM6A (38.0%), ARID1A (25.3%), PIK3CA (22.0%), ERCC2 (14.1%), and several in the RTK/RAS pathway [FGFR3 (38.3%), ERBB2 (12.3%), KRAS (6.1%), HRAS (3.2%)]. Of the larger cohort, 335 (59.0%) specimens were FGFR3-WT tumors. These were predominantly HG, including HGTa (25.6%), HGT1 (54.2%), and CIS (15.5%). Approximately 60% of patients in the FGFR3-WT group received intravesical BCG as initial therapy, with 18% undergoing upfront radical cystectomy. High-grade recurrences occurred in 40.6% of cases, of which 7.4% were muscle invasive. Potentially actionable mutations observed in the FGFR3-WT cohort include KDM6A (30.1%), ARID1A (27.4%), PIK3CA (19.3%), ERCC2 (17.8%), and multiple RTK/RAS pathway genes [ERBB2 (18.1%), KRAS (10.5%), and HRAS (5.4%)].

Conclusions: Potentially actionable mutations are frequently observed in NMIBC. Opportunities for preclinical investigation and umbrella trials should be explored to identify efficacious targeted therapies. Patients with FGFR3-WT tumors stand to benefit, as they represent a cohort of high-risk patients for which there are no well-established targeted therapies

Tumor-immune interactions and cisplatin resistance in localized muscle-invasive bladder cancer

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Introduction: Cisplatin-based chemotherapy followed by radical cystectomy remains the standard-of-care treatment for patients with localized muscle-invasive bladder cancer (MIBC). Neoadjuvant clinical trials studying the combination of cisplatin-based chemotherapy and immune checkpoint blockade (ICB) in MIBC reported encouraging pathologic complete response rates. However, the molecular mechanisms and cellular programs involved in response and resistance to cisplatin and ICB in MIBC remain incompletely characterized. Our goal is to define cellular transcriptional programs and chromatin accessible regions in cancer cells and infiltrating immune cells associated with response and resistance to neoadjuvant systemic therapy.

Methods: We performed single-cell Multiome ATAC and Gene expression sequencing (simultaneous identification of gene expression and open chromatin regions within the same cell) of tumors that had a complete response – "responders" – and tumors that had no response or progressed – "non-responders" – through neoadjuvant cisplatin-based chemotherapy (n=15). Single cell suspensions were prepared following the 10x Genomics protocol. Cell Ranger ARC was used for sequence alignment, peak calling, and generation of count matrices. Downstream single-cell gene expression analysis was performed with Scanpy and ATAC-seq with Seurat.

Results: We identified two distinct urothelial cell populations in "responders" vs "non-responders" based on single cell gene expression and genome wide chromatin accessibility regions. Cancer cells in "non-responders" preferentially express basal cell gene expression signatures. "Non-responders" were significantly enriched in suppressive tumor-associated macrophages (TAMs). Interestingly, SPP1+ TAMs known to have a pro-tumoral role and promote resistance to ICB were exclusively identified in "non-responders".

Conclusions: Single-cell gene expression and genome-wide chromatin accessibility regions identify heterogenous urothelial cell populations expressing basal cell markers in tumors resistant to neoadjuvant cisplatin-based chemotherapy. "Non-responders" are significantly enriched with TAMs implicated in resistance to ICB. Future studies will better define the implications of these findings in cancer therapeutics for patients with MIBC.

Comparative Analysis of very Reduced vs Full Dose BCG Treatment for Non-Muscle Invasive Bladder Cancer: A Contemporary Experience from Chile

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Introduction: Bacillus Calmette-Guérin (BCG) is the standard treatment for non-muscle invasive bladder cancer (NMIBC), but BCG shortages have led to exploration of reduced-dose regimens and shortened maintenance durations, with limited research on much lower BCG doses and outcomes. Oncological outcomes of patients with NMIBC comparing reduced vs full dose BCG treatment.

Methods: We performed a retrospective study of NMIBC patients treated with BCG between 2003 to 2022. We stratified patients by BCG dose received; reduced dose RD (1/4th dose) and full dose FD BCG. Univariate and multivariable Cox regression models were used to predict recurrence and overall survival. Kaplan-Meier method was used for oncological outcomes.

Results: Of total of 200 patients, 116 (58%) had RD and 84 (42%) FD BCG. Median follow-up was 57 months (IQR 29-100). Independent predictors of recurrence on multivariable analysis included BCG dose (FD HR 0.41, 95% CI 0.22-0.74; p=0.003), age, primary vs recurrent tumor at presentation, and total. BCG instillations. There were significant differences in RFS (p=0.0007) and HG-RFS (p=0.0006) but no significant differences in OS (p=0.10) between the groups.

Conclusions: A 1/4th dose of BCG treatment was associated with worse RFS and HG-RFS but not OS. Patients with reduced dose also had lower discontinuation treatment rates due to toxicity. Based on these findings, the risk of recurrence may outweigh the benefits of split dosing.

A Basality Score to differentiate urothelial bladder cancer genetic susceptibility patterns

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Introduction: Urothelial Bladder Cancer (UBC) is a complex disease with genetic and environmental factors participating and interacting in its development. GWAS have identified up to 45 variants associated with UBC. Suggestive evidences indicate that UBC is a heterogeneous disease, with RNA-based taxonomic subtypes having been characterized. However, the likely rare possibility of misclassification cannot be rule out when using clustering systems. Therefore, this study aims to build and apply a "basality" continuous score (BS) to identify UBC genetic susceptibility patterns.

Methods: Paraffin-embedded tumor tissue was obtained from 903 UBC patients included in the SBC/EPICURO study and used to assess immunohistochemical (IHC) protein expression of KRT5/6, KRT14, FOXA1, and GATA3. Their IHC quick-scores were used to assess a "basality" score. To find the one that discriminates best, four different scores were tried. Linear regression model was applied to analyze the association of the scores with the GWAS hits and their interaction with tobacco smoking. We extended the analysis by applying an ElasticNet penalized regression method considering 350.093 SNPs.

Results: Overall, the BSs were highly correlated with the previously assessed IHC molecular subtypes, with higher scores belonging to the BASQ-like subtype. We have observed variability in the genetic susceptibility patterns associated with the BSs. We identified GWAS hits loci that were differentially associated with the BSs. Interestingly, we identified tobacco smoking interaction for some of them.

Conclusions: In conclusion, this study provides evidence of distinct genetic profiles associated with a UBC basality continuum. Our results highlight the potential of using a continuous trait to avoid misclassification when stratifying UBC into molecular subtypes.

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Bladder cancer tumor evolution and metastasis modeled in PDXs

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Introduction: Different tumor characteristics impact the response to different therapies, however, only the primary tumors are available for evaluation in each patient, and it is not known to what extent the primary tumor molecular features change as tumor progress and form metastasis and can be used for therapeutic decisions.

Methods: Seven patient derived bladder cancer models representative of the main molecular subtypes, Bas/Sq, GU and Uro, were expanded in mice under different growth conditions (s.c, fat pad and under kidney capsule) to access genetic and phenotypic changes occurring over time and under different selective forces. Primary tumors are compared with tumors grown under different microenvironments, with spontaneous metastasis, and with metastasis from tumors cells injected into the blood stream. 120 samples collected over time across the different models were analyzed by RNA-seq, Array-CGH and exome sequencing as well as by IHC. In addition to events happening during tumor progression, the analysis of metastasis will allow the identification of the tumor subpopulations with the ability to invade, survive the blood stream and colonize a new environment, the metastatic niche.

Results:

Conclusions: Tumors grow and metastasize in a predictable way depending on the model. Gross appearance, histology and IHC markers expression is largely preserved over passages and molecular subtypes are stable. Mutations patterns are suggestive of a “punctuated equilibrium” where after the initial instability, the genomic makeup remains relatively stable. Analysis is ongoing to investigate changes in gene expression signatures, mutations over time and trace evolutionary trajectories.

Assessment of circulating tumor DNA status as a prognostic marker for adverse pathologic features and recurrence detection in patients with muscle invasive bladder cancer.

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Introduction: Though radical cystectomy (RC) is the gold standard treatment for patients with muscle invasive bladder cancer (MIBC), ~50% of patients recur. Here, we investigated the ability of circulating tumor DNA (ctDNA) as a prognostic biomarker to predict adverse pathologic features and disease recurrence in patients who underwent RC.

Methods: ctDNA analysis was retrospectively performed in a cohort of 74 MIBC patients with a median age of 69 years (range: 33-89 years) and 81.63% (60/74) male. In this cohort, patients were subjected to RC alone (RC group, N=57) or neoadjuvant chemotherapy (NAC) followed by RC (NAC+RC group, N=17). A total of 284 blood samples were collected with a median follow-up of 8.6 months (range: 0.8 - 38.7 months). ctDNA was analyzed prior to (N=72) and after RC (N=212) using a personalized, tumor-informed ctDNA assay (SignateraTM, mPCR - NGS assay); its association with clinical outcomes was assessed.

Results: Forty four patients had ctDNA samples in the presurgical setting, 19 (43%) of whom had a positive ctDNA. Positive ctDNA patients had a higher likelihood of advanced stage (III/IV vs I/II), node positive disease and a lower likelihood of pathologic downstaging, all of which were found to be statistically significant. Sixty four patients had ctDNA samples available within 12 weeks of RC or after adjuvant therapy, 19 (29%) of whom were positive. Recurrence free survival (RFS) was not reached for ctDNA negative patients and was 15.2 months for positive patients with a HR of 21 (2.6-163), $p<0.004$).

Conclusions: We report early results of presurgical (baseline) and post-RC ctDNA testing in patients with MIBC. ctDNA-positivity at both time points correlated with adverse pathologic features and worse recurrence free survival. Further clinical evaluation with a longer follow-up is warranted to fully validate the role of baseline ctDNA in upfront clinical disease management.

Prognostic value of inflammatory parameters in systemic therapy of urothelial carcinoma:Are early CRP dynamics exclusively immunotherapy-associated?

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Introduction: In metastatic renal cell and urothelial carcinoma (mUCC), it has already been shown that early CRP dynamics have a significant impact on progression-free (PFS) and overall survival (OS) of patients undergoing immunotherapy (IO). CRP flare responders, in which CRP levels doubled within the first month and then dropped below the baseline level after 3 months, have a particularly good response. Whether this phenomenon is exclusively IO-associated or also occurs in patients receiving chemotherapy (CTX) is still unclear. In addition to that, other routine inflammatory parameters at baseline were examined concerning potential prognostic value.

Methods: Survival data and laboratory parameters were retrospectively collected in 80 patients (40 patients under IO, 40 patients under CTX) from 2005 to 2021. CRP response was analyzed considering treatment regime. Furthermore, we investigated whether initially elevated inflammatory parameters affected PFS and OS. Survival data was analyzed by Kaplan-Meier curves, log-rank test, univariate and multivariate analysis using the Cox regression model.

Results: Median follow-up was 6 (range 0; 128) months. CRP flare responders were observed in both groups, 5 in the IO group and 9 in the CTX group. However, CRP kinetics had no significant effect on OS or PFS in either cohort. Subgroup comparison based on median inflammatory levels at baseline showed prolonged PFS for the IO group with a neutrophil/lymphocyte ratio < median ($p=0.029$), and with a myeloid cell/lymphocyte ratio < median ($p=0.043$). Elevated initial CRP and LDH levels correlated with worse outcome in OS and PFS in both treatment groups ($p\leq0.022$).

Conclusions: The CRP flare phenomenon can be observed in metastatic urothelial carcinoma patients independently of therapy regime. Thus, it is not an exclusive phenomenon of IO. Elevated LDH and CRP baseline levels are associated with an unfavorable patient survival

NRF2 activation promotes fitness disadvantage in normal urothelium and drives basal-like phenotype

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Introduction: NRF2 is a transcription factor that plays a key role in protecting cells from oxidative stress. NRF2 activating mutations occur at a frequency of 5.9% in muscle-invasive bladder cancer (MIBC). However, the role of NRF2 in MIBC has not been fully clarified. The aim of this study is to investigate how NRF2 affect the tumorigenesis process in bladder cancer.

Methods: We used genetically engineered mice (GEM) with a Cre inducible Nrf2 activating mutation (LSL-Nrf2-E79Q). To evaluate how NRF2 affect the tumorigenesis process in vivo, BBN was administrated to Upk3a-CreERT2; LSL-Nrf2-E79Q/+ (UN) mice after gene recombination with tamoxifen. We also established organoids from normal urothelium of Krt5-CreERT2; LSL-Nrf2-E79Q/+ (KN) mice for further exploration of the role of Nrf2. Additionally, we analyzed TCGA data to assess whether NRF2 would associate with molecular subtype in human MIBC.

Results: In BBN induced tumor model, UN mice did not differ in time to tumor development from control wild-type mice. Furthermore, UN tumors had lost the post-recombination allele of LSL-E79Q in genomic PCR. That means cells expressing Nrf2E79Q are selected against during the carcinogenesis process. In KN organoids, Nrf2E79Q activation enhanced relative mRNA expression of a number of basal related genes. In addition to that, vehicle organoids in differentiation media showed phenotypic differentiation, whereas those treated with tamoxifen did not demonstrate phenotypic signs of differentiation suggesting NRF2 activation promotes a basal-like phenotype. In TCGA data, NRF2 mutations are associated with a basal-like subtype and squamous differentiation in human MIBC.

Conclusions: These results suggested that Nrf2E79Q is tumor suppressive and promotes a fitness disadvantage in normal urothelium. Furthermore, Nrf2E79Q inhibits differentiation of KN organoids and associated with a basal subtype in human MIBC. Further investigation is needed to clarify the mechanism of NRF2 activation mutation affecting to the interaction of normal and cancer cells in bladder.

IHC-based molecular subtyping and phenotypic heterogeneity in bulk versus invasive front of pT1 bladder cancer

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Introduction: In MIBC, both luminal and non-luminal subtypes of cancer cells have been identified. Analyses of NMIBC indicate that nearly all tumors are luminal-like, but it is unclear if these co-exist heterogeneously with non-luminal subtypes in pT1. Here, we test if subtypes differ in the lamina propria invasive front and describe subtype intra-tumor heterogeneity (ITH) in full sections of pT1 tumors.

Methods: We identified 157 T1 tumors treated in Sweden between 2016-2019. 18 cases were excluded due to poor material or muscle invasive re-TURB. One selected tissue block per case containing pT1 disease was sectioned and stained with subtype-defining immunohistochemistry (IHC) for GATA3, KRT5, KRT20, CCND1, RB1, CD56, VIM, and H&E, dual desmin/pan-CK, and CD3. Areas larger than 1 mm2 with different subtypes confirmed by more than one IHC-marker constituted subtype ITH.

Results: Full section IHC classified 139 pT1 tumors according to the Lund Taxonomy as 86 (62%) Urothelial-like (Uro), 35 (25%) Genomically unstable (GU), 11 (8%) Basal/Squamous (Ba/Sq), 3 (2%) Neuroendocrine-like (Sc/NE), and one Mesenchymal-like (Mes). Three cases (2%) showed subtype ITH involving GU+Ba/Sq, GU+Mes, and GU+Sc/NE subtypes. Classification of only the lamina propria invasive front showed 88% concordance with full sections. Discordances included 8 Uro, GU at front; 4 GU, Uro at front; 3 Uro, Ba/Sq at front; 1 Sc/NE, Ba/Sq at front. Subtypes, ITH, and quantification of lymphocytes will be further analyzed in relation to recurrence, progression, receipt of BCG and cystectomy.

Conclusions: Non-luminal subtypes constituted only 11% of pT1 tumors increasing to 14% when classifying only the invasive front. This suggests that non-luminal subtypes are rare in T1 and mainly arise after invasion into muscularis propria. Subtype ITH of the cancer cells in pT1 tumors was infrequent. The rate of discordance between full sections and invasive front was low and did not involve any specific subtype.

CDK4/6 inhibitors induce a multistep molecular response in bladder cancer cells

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Introduction: CDK4/6 inhibitors have been approved for the treatment of breast cancer since 2015 and are largely dependent on the expression of the retinoblastoma protein (RB1). As monotherapy, their therapeutic success is rather limited which prompts the exploration of the underlying molecular mechanism that might result in identification of novel combination therapies.

Methods: Transcriptional alterations in T24 cells after 8,16,20,24 and 24 hours of CDK4/6 inhibition were analyzed by RNA sequencing. Expression vectors for RB1 have been cloned, lacking the last 42-137 aa, different internal binding sites for MDM2 and the NLS region. RB1 lacking its CDK phosphorylation sites was obtained by addgene. Inhibitors against CDK4/6 (Palbociclib, (PD), the proteasome (MG-132, epoxomicin), NAE (MLN4924), MDM2 (Idasanutlin), lysosome biogenesis (Eltrombopag, ML329), IPOα/β1 (Ivermectin) and siRNAs against MDM2, Gankyrin and Cullin-1 were designed and used. Protein expression was analyzed by western blotting, gene expression level by qPCR, cell viability by SRB assays, lysosome biogenesis by LysoTracker, cell cycle analysis by EdU-incorporation and FACS analysis and the nuclear and cytoplasmatic fraction was isolated. Synergism between PD and other compounds were calculated using the ZIP-Synergy Score.

Results: The transcriptomics analysis revealed analysis significant differences between the 8 hour vs. subsequent time points on gene level and KEGG pathway enrichment analysis. At 8 hours, RB1 stability is regulated in a phosphorylation independent way and requires MDM2, Gankyrin, the E3 ubiquitin ligase complex SCF. In parallel and dependent on its NLS domain, RB1 is imported in the nucleus, a step that is essential for the induction of G1 arrest. Further cellular responses such as senescence, autophagy or apoptosis are initiated at subsequent steps.

Conclusions: Therapy response to CDK4/6 inhibitors can be divided in different phases which allow for better designed combination therapies

Proteogenomic characterization of bladder cancer reveals sensitivity to TRAIL-induced apoptosis in FGFR3-mutated tumors

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Introduction: Molecular understanding of muscle- and non-muscle-invasive bladder cancers (MIBC and NMIBC) are currently based primarily on transcriptomic and genomic analyses. Here, we integrated proteomic, genomic and transcriptomic data to gain insights into bladder cancer heterogeneity and identify underlying processes specific to tumor subgroups and therapeutic outcomes.

Methods: Proteomic data were obtained for 40 MIBC and 23 NMIBC for which transcriptomic and genomic data were already available. Proteomic groups from unsupervised analyses (uPGs) were characterized using clinico-pathological, proteomic, genomic, transcriptomic, and pathway enrichment analyses. Additional enrichment analyses were performed for FGFR3-mutated tumors. Treatments with recombinant TRAIL, SMAC mimetic (birinapant), pan-FGFR inhibitor (erdafitinib) and FGFR3 siRNA were conducted on four FGFR3-altered cell lines. Treatment effects on cell viability were evaluated and synergistic treatment effects were evaluated using the Zero Interaction Potency model.

Results: Five uPGs, covering both NMIBC and MIBC, were identified and bore coarse-grained similarity to transcriptomic subtypes underlying common features of these different entities. uPG-E was associated with the Ta pathway and enriched in FGFR3 mutations. Our analyses also highlighted an enrichment of proteins involved in apoptosis in FGFR3-mutated tumors, not captured through transcriptomics. Genetic and pharmacological inhibition demonstrated that FGFR3 activation regulates TRAIL-receptor expression and sensitizes cells to TRAIL-mediated apoptosis, further increased by combination with birinapant.

Conclusions: This proteogenomic study provides a comprehensive resource for investigating NMIBC and MIBC heterogeneity and highlights the potential of TRAIL-induced apoptosis as a treatment option for FGFR3-mutated bladder tumors, warranting clinical investigation

FBXW7 Loss-of-Function is associated with worse outcomes and leads to the accumulation of MYC in muscle-invasive bladder cancer

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Introduction: Muscle invasive bladder cancer (MIBC) has the third highest mutation rate of any solid tumour. FBXW7, which is involved in the proteasome degradation of oncogenic proteins including MYC, is one such frequently mutated (8.5%) gene in MIBC. In other cancers, loss of function mutations or decreased expression have oncogenic potential and are associated with poor prognosis. Here, we investigate the role of altered FBXW7 function and its relationship to oncogenic mechanisms in MIBC.

Methods: The MSK-IMPACT and TCGA2017-MIBC cohorts were queried for FBXW7 genomic alterations and mRNA expression levels in relation to clinical outcomes. FBXW7 was knocked out in basal (UM-UC3) and luminal (RT112) bladder cancer cell lines. Two FBXW7 hotspot-mutations (R479G and R505G) were introduced to explore their functional relevance. Phenotypic assays, downstream pathway analysis, and pharmacologic inhibition of the FBXW7-MYC axis were carried out on these cell lines.

Results: Low expression or genomically-altered FBXW7 associated with shorter patient survival and enriched MYC signalling pathways. In cell lines, FBXW7 knock out (KO) led to increased MYC and cell cycle mRNA expression（CCNE1, CCND1, CDK2, CDK4 and CDK6). Re-transfection of a wild-type FBXW7 coding plasmid in these KO cell lines normalized MYC and cell cycle gene expression. Transfection of R479G and R505G mutants in KO-UC3 retained the KO-induced phenotype and downstream effects compared to its wild-type rescued control.

Conclusions: We confirmed the enhanced sensitivity of FBXW7-deficient (mutated and KO) cell lines to MYC inhibit

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Predicting response to intravesical BCG in high risk non-muscle invasive bladder cancer using an artificial intelligence-powered pathology assay: outcomes from a multicenter cohort

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Introduction: High-risk non-muscle invasive bladder cancer (HR-NMIBC) is associated with increased risk of recurrence despite current treatments. The purpose of this study was to develop and validate a machine learning-based histologic biomarker that analyzes transurethral resection of bladder tumor (TURBT)-derived specimens to predict recurrence risk following intravesical BCG in HR-NMIBC.

Methods: Pre-BCG TURBT-derived histologic digital whole slide images (WSI) and clinical data were obtained for BCG naive HR-NMIBC cases diagnosed between 2007-2021 and treated with intravesical BCG across five tertiary-care centers. Data was censored at 24 months or date of last follow-up if sooner. WSI were analyzed through a pipeline incorporating tissue and nuclear segmentation followed by geometric feature extraction, which was the input for a multivariate Cox proportional hazards model to identify features correlated with recurrence-free survival (RFS). The final model was validated with internal-external cross validation via Kaplan Meier estimators and the Wald test. Patients were stratified into "high-recurrence" (upper 10%) and "low-recurrence" (bottom 90%) risk categories. Fixed effect meta-analysis was used to combine discrimination statistics across centers.

Results: A total of 344 patients (mean follow-up: 50 months) were included (TaHG: 41.6%, Tis only: 14.3%, T1: 44.1%; high-grade: 99.3%; CIS: 28.9%; Multifocality: 44.9%). The "high-recurrence" group had inferior RFS compared to the "low-recurrence" group (HR: 3.19, 95% CI: 1.95, 5.22, $p<0.001$). Kaplan-Meier analysis indicated that "high-recurrence" cases had a 3.62-fold, 2.56-fold and 2.41-fold increased risk of recurrence at 6, 12, and 18 months versus "low-recurrence" cases. BCG unresponsiveness was 2.3-fold higher in "high-recurrence" (53.5%) versus "low-recurrence" (23.6%) cases. The biomarker stratified recurrence risk independent of clinical features.

Conclusions: In this study, we developed an AI-based histologic biomarker that can identify cases that are at significantly higher risk of recurrence and BCG unresponsiveness. With further refinement, this algorithm could supplement existing clinical predictors to identify appropriate treatment pathways.

Multi-omic profiling of non-muscle invasive low-grade metastatic ta bladder tumors

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Introduction: Non-invasive, low-grade Ta bladder cancer (LG-Ta) is highly recurrent, but rarely progresses to muscle-invasive or metastatic disease. However, a small subset of LG-Ta patients experiences direct progression to distant metastasis without ever becoming muscle-invasive (mTa). We identified and molecularly profiled tumours from a cohort of mTa patients to understand their unique biology and identify potential therapeutic vulnerabilities.

Methods: Four mTa patients were included. For 3 out of 4 patients we included the diagnostic metastatic lession. We performed RNA-seq in 3 out of 4 patients (N=14 samples), and targeted-DNA sequencing in all 4 using a bladder-specific 60-gene panel (N=17 samples). For comparison, we included two non-metastatic Ta (n-mTa), and one metastatic (mUC) cohorts (PMIDs 29136510, 33863885, and 35086719 respectively).

Results: All patients had bulky LG-Ta primary bladder tumors that required multiple resections, and all metastasized to the lung. Targeted sequencing showed mutations or copy number alterations in 24/60 genes. Similar to other n-mTa cohorts, the most commonly mutated genes across tumours sequenced were FGFR3, KDM6A and KMT2D. However, mutations indicative of advanced muscle-invasive, or metastatic, disease (i.e. FBXW7, ATM, TERT, TSC1 and ERCC2) were also highly frequent. RNA-seq indicated 2 out of 3 patients had class 2b or class 3 NMIBC-subtype tumours (by the UROMOL classifier), suggestive of aggressive disease with high risk of progression. Sequencing of serial tumours showed high concordance between the primary and metastatic tissues both at the RNA and DNA level.

Conclusions: mTa tumours share profiles characteristic of both non-invasive and invasive disease, despite their LG-Ta histology. This suggests these tumors are molecularly aggressive and that using targeted therapeutic approaches may benefit some patients (e.g. rapalogs in tumours with TSC1-inactivating mutations). The scarcity of these patients warrants larger multi-centre multi-omic cohorts. Current integrative analysis with n-mTa and mUC for cross-cohort comparisons is ongoing.

Smoking Status is Underreported and Undermeasured in Bladder Cancer Drug Trials; Opportunities for Improvement in Clinical Trial Design

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Introduction: Cigarette smoking is the most common modifiable risk factor for the development of bladder. Though cigarette smoke contains many substances that can modulate the efficacy of anti-cancer drugs, a paucity of data exists on whether bladder cancer drug trials report smoking and include it as a control variable.

Methods: The National Cancer Institute (NCI) website was queried for bladder cancer (BC) drugs in the last 10 years and cross referenced for clinical trial (ClinicalTrials.gov). We examined clinical indication, FDA approval date, major clinical trial which facilitated FDA approval, journal in which the trial was published, total number of patients and number of smokers, if smoking status was reported and controlled for, and outcomes by smoking status. For comparison, we also assessed NCI endorsed lung cancer treatment drugs as this is also a smoking related malignancy and categorized as non-small cell lung cancer (NSCLC) and small-cell lung cancer (SCLC).

Results: Ten BC drugs were evaluated across 15 studies, 10 NSCLC drugs across 19 studies, and 4 SCLC drugs across 7 studies. A total of 5944 bladder, 8433 NSCLC and 2814 SCLC patients were included of which 1757 were smokers in BC, 4,447 in NSCLC, and 2407 in SCLC clinical trials. Smoking status was reported in 46.6% (n=7) and included as a control variable in 6.7% (n=1) of BC trials, compared to 85% (n=17) and 47.3% (n=9), in NSCLC and 100% (n=7) and 28.5% (n=7) for SCLC ($P<0.05$). Differences in outcomes stratified by smoking status were observed in 1 BC, 9 NSCLC, and 2 SCLC trials.

Conclusions: Bladder cancer drug trials infrequently report smoking and only a single study included smoking status as a control variable. The majority of lung cancer drug clinical trials control for smoking status. This study highlights an important deficiency in bladder cancer clinical trial design.

Development and external validation of an artificial intelligence-based prediction tool for tumour progression in non-muscle invasive bladder cancer: A retrospective multi-institutional cohort study

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Introduction: Several tools have been developed to predict the risk of tumour progression in non-muscle invasive bladder cancer (NMIBC). However, they do not reflect current practice and perform poorly. We aimed to develop and validate an artificial intelligence (AI)-based tool to better predict NMIBC progression.

Methods: The AI model, based on a random survival forest, was trained on NMIBC patients treated from Jan-2005 to Dec-2020 at the University Health Network, Canada (n=706). External validation was performed on patients treated from Jan-2005 to Mar-2022 at two community-based hospitals (Credit Valley Hospital, Canada, n=407; Mississauga Hospital, Canada, n=340), and a publicly available dataset of patients treated from Oct-2004 to Dec-2013 at Seoul National University, South Korea (n=198). Primary outcome was progression, defined as relapse of ≥pT2 disease. The AI model was compared against a Cox proportional hazards model using the same variables and the EAU risk groups.

Results: Overall, 1,651 patients were included, of which 230 had a prior history of NMIBC. During a median follow-up of 46 months (IQR 22-80), 197 patients developed tumour progression. Median time to progression was 12 months (IQR 5-32). In the training cohort, the AI model achieved a c-index of 0.79, compared to 0.77 and 0.74 for the Cox model and EAU risk groups, respectively (both p<0.01). In the external validation cohorts, the AI model achieved a c-index of 0.75-0.77 compared to 0.72-0.74 for the Cox model (p=0.01) and 0.64-0.74 for the EAU risk groups (p=0.02). At 10 years, the AI model demonstrated a higher net benefit compared to the other models for clinically relevant thresholds between 15-30%.

Conclusions: Our AI model performed favourably compared to contemporary prediction tools in both academic and community settings. Ongoing work is being conducted to evaluate the generalizability of our AI model in larger NMIBC cohorts.

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Circulating tumor DNA for characterization of ERBB2 as a predictive biomarker in metastatic urothelial carcinoma

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Introduction: ERBB2 encodes human epidermal growth factor receptor 2 (HER2) and is frequently altered in metastatic urothelial carcinoma (mUC). Prior clinical trials of HER2-targeted therapies in mUC have been largely unsuccessful, however recent HER2 antibody-drug conjugates show promise. Accurate biomarker-driven patient selection is critical to optimize the clinical benefit of HER2-targeted therapy.

Methods: We aim to evaluate the heterogeneity of ERBB2-altered tumors via plasma circulating tumor DNA (ctDNA) and reveal critical genomic variables modulating response to HER2-directed strategies in mUC. 411 plasma samples from 236 mUC patients were profiled with a custom targeted sequencing panel covering >50 genes frequently altered in mUC, including dense coverage flanking the ERBB2 locus and low-resolution genome-wide capture. Leukocyte DNA was profiled for all patients as a germline control.

Results: 76% of patients (179/236) had evidence for ctDNA in at least one plasma sample. Protein-altering ERBB2 mutations were identified in 14% of evaluable patients, with two-thirds at known oncogenic hotspots. In three patients, the oncogenic ERBB2 mutation was subclonal (cancer cell fraction <25%). Mutant-allele specific imbalance was observed with 3/13 clonal oncogenic mutations, all in tumors with >10 copies of ERBB2. ERBB2 copy gain was detected in 8% of patients overall, 9% when excluding low tumor fraction samples. Leveraging our custom panel, we resolved ploidy and ERBB2 locus amplification structure, revealing genomic breakpoints on chromosome 17 and cases of tandem duplication resulting in >50 gene copies. ERBB2 amplifications and oncogenic mutations identified in ctDNA were concordant across serial ctDNA and tissue samples.

Conclusions: Our study demonstrates the feasibility of a ctDNA-based approach for determining ERBB2 biomarker status in mUC. Ongoing work is comparing ctDNA results to mRNA and immunohistochemistry from tumor tissue. Moving forward, consideration of ERBB2 as a nuanced non-binary biomarker will be important for understanding response to HER2-targeted therapy

Investigating sex chromosomal influence on tumor progression and response to bacillus Calmette Guérin immunotherapy in the four-core genotype murine model of non-muscle invasive bladder cancer

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Introduction: The incidence of bladder cancer is significantly higher in males compared to females. Women generally present with advanced stage disease and experience shorter progression free survival following treatment with bacillus Calmette Guérin (BCG) immunotherapy. We previously demonstrated the association between increased intra-tumoral CD79a+ B cells pre-treatment tumors from patients with NMIBC who exhibit early recurrence and progression. B cells are critical to mucosal immunity, response to BCG and exhibit a sex and age dependent expansion and response to immunomodulatory treatments. This study was conducted with an aim to define the sex chromosomal influences on disease progression and response to BCG.

Methods: We used the Four Core Genotype (FCG) mouse model in which a male mouse lacking the testes determining Sry gene (XY-Chr3Sry+/XYM) is crossed with an XX female (XXF) mouse leading to the generation of offspring with four genotypes; two gonadal males (XYM, XXM) and two gonadal females (XXF and XYF). Aging mice were exposed to N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN) ad libitum to induce bladder carcinogenesis. Mice were treated with three intravesical doses of BCG. Systemic and bladder local immune profiling was performed at pre-and post-completion of BCG therapy.

Results: Hematoxylin and eosin staining of whole bladder sections revealed an overall higher immune cell infiltration and increased presence of tertiary lymphoid structures in the bladders of XXF mice compared to all other genotypes. Splenic and bone marrow immune profiles determined by multispectral flow cytometry revealed significant differences, specifically, in the total and atypical B cells (ABCs) post completion of BCG across the four genotypes.

Conclusions: Overall, these findings provide evidence for a sex associated role of ABCs in mediating response to BCG. Further investigations are warranted to understand the mechanisms underlying ABC associated poor outcomes in high-risk NMIBC patients who exhibit high intra-tumoral B cells in their pre-treatment tumors.

Predicting treatment outcome in muscle invasive bladder cancer using multimodal deep learning models

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Introduction: Treatment of non metastatic muscle invasive bladder cancer (MIBC) remains a challenge as only 40% of patients respond to the standard of care (Neoadjuvant chemotherapy + cystectomy). In this study we aimed at better characterizing groups of non-responding patients using deep learning multimodal models.

Methods: Digitized whole slide images (WSIs), transcriptomic and genomic data from 300 MIBC patients from TCGA were used to train predictive models of response to treatment. Performance as well as biological coherence (using interpretability of machine learning models) of the identified predictive features was evaluated for every model. Our histology based model consists of a two steps process : first H&E histology slides are mapped to feature vectors using a self-trained transformer architecture and then a multiple instance learning (MIL) architecture (CHOWDER; developed at Owkin) predicts the response based on those feature vectors.

Results: This architecture achieved 74.2% of area under the receiver operating characteristic (AUC) ± 5.3 of standard deviation (std). For RNAseq data, a baseline random forest classifier achieved 63.5% AUC ± 6.5 std. To leverage multimodality, we are using the MCAT architecture that uses dense co-attention mapping between WSIs and transcriptomic features . This architecture achieved 80.5% AUC ± 2.5%, showing the benefits of exploiting two different modalities of data.

Conclusions: In conclusion, using multimodal modes may not only lead to a more precise prediction of treatment response in bladder cancer, but may also enable a more complete understanding of the biology underlying non-response to standard of care. To the best of our knowledge, this is the first study to combine omics and histological features to predict response to treatment in MIBC patients.

Patterns of Smoking Cessation Strategies and Perceptions of E-Cigarette Harm Among Bladder Cancer Survivors: Findings from a Cross-Sectional Convenience Sample

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Introduction: Cigarette smoking is the leading preventable cause of bladder cancer. E-cigarette use has been proposed as a potential risk reduction strategy to promote smoking cessation. The use of e-cigarettes in this population is controversial due carcinogens and uncertain long-term risks. We sought to assess e-cigarette use and harm perception among bladder cancer patients

Methods: We performed a cross-sectional study on a convenience sample of patients with bladder cancer at UNC from 8/21 to 10/22. The survey instrument was sourced from questions included in the American Association for Cancer Research Cancer Patient Tobacco Use Assessment Task Force - Cancer Patient Tobacco Use Questionnaire (C-TUQ). This questionnaire included a comprehensive set of standardized tobacco use and cessation questions along with questions regarding e-cigarette harm perceptions.

Results: A total of 104 survey respondents were included, mean age was 72 years, 27% were female, and the majority had muscle invasive disease (55%). 20% (n=21) of respondents were current smokers (median pack years 40) while 51% (n=51) were former smokers (median pack years 20). 5% (n=5) quit at the time of diagnosis. Pharmacotherapy as a smoking cessation aid included nicotine patches (25%), nicotine gum (21%), e-cigarettes (8%), nicotine lozenges (8%), and Varenicline/Wellbutrin (4%). Respondents who smoked more commonly perceived e-cigarettes are not harmful to your health (11%), compared to former (4%) and non-smokers (4%) (P=.048). However, all 3 groups perceived e-cigarettes to be as addictive as traditional cigarettes (62%, 68%, 78%, P=.34). Many (43%) of patients who smoked would consider switching to e-cigarettes as a smoking cessation aid.

Conclusions: Bladder cancer survivors who smoke more commonly perceive e-cigarettes are not harmful to your health and many would be willing to switch to e-cigarettes to try to quit smoking. FDA approved pharmacotherapies are infrequently used as a cessation aid highlighting an implementation gap.

Longitudinal personalized urinary tumor DNA analysis in muscle invasive bladder cancer from the neoadjuvant immunotherapy trial RJBLC-I2N003

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Introduction: RJBLC-I2N003 is an investigator-initiated study to evaluate the clinical activity and predictive biomarkers for neoadjuvant immunotherapy with toripalimab (anti-PD-1) in muscle invasive bladder cancer (MIBC).

Methods: Twenty patients with pathologically confirmed MIBC were enrolled and received toripalimab (3 mg/kg Q2W, 4 cycles) before radical cystectomy. The safety and efficacy of neoadjuvant toripalimab were assessed. Serial urinary cell-free DNA (ucfDNA) and blood cell-free DNA (bcfDNA) were obtained at baseline and after each cycle of toripalimab treatment. Personalized minimal residual disease (MRD) assays and low-pass whole genome sequencing (LP-WGS) were applied to analyze liquid biopsy samples.

Results: Eighteen patients (90%) completed all 4 cycles of neoadjuvant treatment. Grade 3-4 immune-related adverse events occurred in two patients (10%). Eight patients (40%) achieved a pathological complete response (pCR). Thirteen patients (65%) had no remaining invasive disease (pCR or pTisN0/pTaN0). Pre-treatment somatic variants and copy number abnormalities were prevalently detected in ucfDNA as compared to bcfDNA. On-treatment urinary tumor DNA (utDNA) clearance was associated with objective responses. Preliminary concordance was observed between molecular and pathological MRD status.

Conclusions: These findings suggest that neoadjuvant administration of PD-1 blockade followed by surgical resection represents a feasible and efficacious approach to treat MIBC. The exploratory biomarker assessment demonstrates the potential utility of longitudinal personalized utDNA analysis to complement existing trial endpoints.

Novel and non-invasive tool for immunotherapy-response prediction in non-muscle invasive bladder cancer patients based on miRNAs and cytokine detection in urine

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Introduction: Bladder cancer (BC) represents a clinical and social challenge due to its high incidence and recurrence rates, as well as the scarce advances in effective disease management. For non-muscle-invasive BC, the best therapeutic alternative has proven to be intravesical immunotherapy based on BCG instillation. This provokes severe inflammation that, in responder patients, elicits an anti-tumor immune response. However, in clinical practice, there is no way to predict which patients will benefit from this therapy and about 50% suffer inflammatory reactions without the expected clinical outcome. Liquid biopsy, and especially urine-based methods, represents the best non-invasive approach to personalize therapy for BC patients. Here, we present the selection and urine evaluation of microRNAs and cytokines with good predictive capability for BCG immunotherapy response, alone or in combination with anti-PD-L1 therapy.

Methods: Ncounter miRNA expression panel was used to identify DE miRNAs from primary tumor of responder (R) and non-responder (NR) patients and miRNA-specific RT-qPCR was performed to validate it in urine samples. Identification and validation of cytokines with predictive capacity in urine was performed by LEGENDplex and ELISA analysis, respectively.

Results: We identified 26 DE miRNAs able to distinguish tumor samples from R vs NR patients. Some selected miRNAs were evaluated in urine samples and finally we selected 7 miRNAs that together efficiently discriminate between R and NR patients with an ROC curve with an AUC=1 (P = 0.0004) with a specificity and sensitivity of 100%. Moreover, we have identified 3 cytokines with predictive capacity in urine samples with AUC > 0.85-0.9. Evaluation of these biomarkers in a small validation cohort validates this excellent prognostic result.

Conclusions: Dysregulation of urine miRNA and cytokine expression allows discrimination between R and NR BC patients to immunotherapy prior to treatment, becoming the first accurate urine-based tool for this urgent unmet clinical need.

Establishment of the Multiplex IHC panel combined with FGFR3 RNAScope for exploration of tumor immune microenvironment in upper tract urothelial carcinoma.

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Introduction: The T-cell depleted tumour phenotype compromises the response of immune checkpoint inhibitors (ICIs). FGFR3, a tyrosine kinase receptor for fibroblast growth factors, is enriched in upper tract urothelial carcinoma (UTUC) and its mutations was shown to be associated with immune-cold tumour microenvironment. Functional suppression of T-cell infiltration into tumour by FGFR3 could mean that FGFR3 inhibition may de-suppress this immune phenotype, potentially improving the ICI response. The aim of this study was to develop an exploratory tool to examine the spatial effects of FGFR3 in the tumour immune microenvironment of UTUC.

Methods: UTUC diagnostic FFPE specimens (n=24) were obtained from NHS Greater Glasgow & Clyde Biorepository under approval. Chromogenic Immunohistochemistry (IHC) and multiplex immunofluorescence (mIF) was performed using ImmPRESS Polymer reagents (Vector Laboratories) with DAB or Opal-TSA (Akoya Biosciences), respectively. FGFR3 mRNA was detected using RNAScope (ACDBio) using Opal-TSA. QuPath was used for quantitative image analysis. SPSS was used for statistical evaluation.

Results: The UTUC cohort consisted of both males and females, with a median age of 70 years. Samples were mostly at lower stages (T0 and T1) and higher grades (G2 and G3). FGFR3 stained by chromogenic IHC and IF was confirmed to be similar. An Opal mIF panel with FGFR3, CD3, CD8, PD-1, and PanCK, as well as a panel of FGFR3 RNAscope with mIF with FGFR3 and PanCK was optimized for UTUC FFPE tissues. The mIF panel enabled simultaneous detection and quantification of T cells alongside FGFR3 protein expression. FGFR3 RNAscope immediately followed by mIF with FGFR3 and PanCK antibodies enabled detection and quantification of both FGFR3 mRNA and proteins in the same section.

Conclusions: The mIF and RNAscope workflow was successfully established to evaluate FGFR3 mRNA and protein expression with selected populations of T cells in UTUC tissues

Unraveling circulating T follicular helper cells profiles in non-muscle invasive bladder cancer.

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Introduction: Increased density of B cells within pre-treatment tumors significantly associates with unfavorable clinical outcomes in patients diagnosed with non-muscle invasive bladder cancer (NMIBC). Specifically, we have identified a specialized subset of B cells called 'atypical B cells (ABCs)' located within tertiary lymphoid structures associated with NMIBC tumors. Considering the critical role played by T follicular helper (TFH) cells in regulating B cell responses at mucosal surfaces, we hypothesize that circulating and local profiles of cTFH cells could offer insights into the exhaustion levels of B cells, which are intricately linked to tumor stage and treatment response.

Methods: We investigated the relationship between TFH-associated transcript abundance and clinical outcomes in NMIBC patients. Analysis of bulk-RNAseq data from 535 pre-treatment tumor samples (UROMOL) was conducted. Profiles of cTFH cells and ABCs were evaluated using multi-parametric flow cytometry in patients undergoing transurethral bladder tumor resection (TURBT) for NMIBC, at the Kingston Health Sciences Center. were assessed In the N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN) carcinogen murine model of bladder cancer, profiles of TFH cells and ABCs were evaluated.

Results: RNAseq analysis revealed sex differences possibly linked to B and T cell interaction. Preliminary results show an inverse correlation between cTFH and circulating ABCs in patients undergoing TURBT (n=25). Similar correlations were observed during cancer progression in BBN-exposed aging female and male mice.

Conclusions: Preliminary findings suggest a potential link between cTFH and ABCs in NMIBC progression. Further research is needed to establish the precise roles of cTFH cell's in tumor progression and treatment response. Findings will potentially lead to understanding the mechanisms underlying B cell exhaustion in high-risk NMIBC and the development of immune monitoring-based biomarkers and therapeutic targets within the cTFH pathways.

The 'normal' bladder: A comprehensive investigation into the immune landscape and microbiome in normal bladder.

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Introduction: Little is known about the immune microenvironment and microbiome (ecosystem of bacteria that reside within cells) of normal adult bladder. Existing, limited knowledge is outdated and derived mainly from rudimentary, single-cell staining, immunohistochemical studies (IHC) of bladder tissue. Normal adjacent tissue from radical cystectomy specimens traditionally represents 'normal' bladder, but may be inaccurate given the surrounding, active malignant process. Examination of healthy, normal bladder tissue is essential to gain insights into the immune and microbial dysfunction in cancer. This will indicate key immunomodulatory interventions that could result in improved outcomes for bladder cancer patients.We are collaborating with the National Disease Research Interchange (NDRI, USA) to procure tissue through an institutionally approved, ethical process for this research. Tissue is being collected from consented patients post-mortem, with post-mortem interval of <12 hours, and no prior history of urological disease.

Methods: NDRI-sourced formalin-fixed paraffin-embedded (FFPE) 'normal' bladder tissue was collected from 10 patients (5 males: 5 females). RNA and DNA was extracted from 10µm paraffin curls for bulk profiling (Nanostring® IO360) and metagenomic sequencing. State-of-the-art PhenoImager HT software was used to image tissue stained with 8-plex IHC.

Results: Data will be presented on metagenomic sequencing from normal bladder. Through multiplex IHC and bulk profiling, we will present key features of the immune landscape and spatial relationships between immune cells in normal bladder. For both, a comparison of the results from males and females will be presented.

Conclusions: Through use of spatial biology technologies and multi-omics approaches this analysis will be the first to comprehensively profile the microbiome of normal bladder tissue and the corresponding immune microenvironment. We will be able to address whether any gender differences exist in normal bladder tissue and whether this knowledge can be used to aid the evolving fields of disease prevention and immunotherapy in bladder cancer.

Tumor-spatial and immune features are associated with response to neoadjuvant chemo-immunotherapy for muscle-invasive bladder cancer

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Introduction: Neoadjuvant chemotherapy (NAC) using platinum-based drugs is standard treatment for muscle-invasive bladder cancer (MIBC). Achieving pathological response to NAC is associated with improved survival, but strategies to enhance response rates are needed. Incorporating immune checkpoint inhibitors (ICI) has shown promise, and understanding tumor microenvironment spatial characteristics could aid in predicting NAC-ICI response. We analyzed data from two NAC-ICI trials (LCCC1520 and BLASST-1) to identify spatial, molecular, and cellular features linked to treatment and outcomes.

Methods: Using proteomic digital spatial profiling (DSP), we examined bladder tumor samples from 18 responders and 18 non-responders in LCCC1520 and BLASST-1. DSP involved 52 protein markers related to immune cell profiling, immunotherapy drug targets, and immune activation status. Additional molecular and cellular features were analyzed in LCCC1520, including mutational frequencies, tumor antigens, immune checkpoint gene expression, and immune populations.

Results: DSP marker expression showed high intratumoral heterogeneity, but specific markers including PD-L1, Ki-67, HLA-DR, and HER2 were associated with response. An elastic net model incorporating DSP markers and regions outperformed an ROI-agnostic model in predicting response. Treatment induced changes in mutational profiles, immune gene signatures, and tumor subtypes in LCCC1520. Response correlated with increased plasma IL-9 from pre-treatment to pre-Cycle 2. Tumors exhibited diverse predicted antigen landscapes associated with various causes. Higher pre-treatment tumor PD-L1 and TIGIT RNA expression were associated with complete response. IL-8 signature and Stroma-rich subtype were linked to improved response rates to NAC-ICI.

Conclusions: Despite intratumoral heterogeneity, specific DSP markers showed associations with response to NAC-ICI in a spatial context. We identified pre- and post-treatment features that changed with NAC-ICI or were linked to response in MIBC. Immune-related features can define patients more likely to respond to NAC-ICI and benefit from combining NAC with ICI. This study supports further exploration of tumor-spatial and immune biomarkers to predict MIBC NAC-ICI response.

Investigating BCG response associated intra-tumoral profiles of CD79a+ B cells, CD103+ tissue resident memory T cells and tertiary lymphoid structures IN non-muscle InVASIVE BLADDER CANCER

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Introduction: Recurrence post treatment with intravesical bacillus Calmette-Guérin (BCG) occurs in over 50% of patients and is remains the major barrier in management of non-muscle invasive bladder cancer (NMIBC). The pre-treatment tumor immune microenvironment is a critical determinant of therapeutic outcomes. We evaluated immune cell infiltration patterns and presence of tertiary lymphoid structures (TLSs) in pre-treatment tumors from female and male patients with NMIBC.

Methods: Spatial immunophenotyping of pre-treatment tumors from 173 patients (n=34 females and 139 males) treated with BCG immunotherapy at the Kingston Health Sciences Center, was performed using multiplex immunofluorescence assay. The density and spatial distribution of CD79a+ B cells, CD103+ tissue resident memory T cells, Ki67+CD8+ cytotoxic T cells, CD163+ M2 macrophages including the checkpoints PD-1 and PD-L1 was determined. Hematoxylin and eosin-stained whole sections from the immunophenotyped tumors were evaluated for the presence of TLSs. Immune cell abundance was analyzed using R version 4.1.2. Survival and multivariable analyses were performed using survminer, survival, and finalfit packages. Differences in immune cell density between response groups were tested using the Mann-Whitney U test.

Results: Female patients treated with BCG exhibited worse RFS, and progression free survival (PFS) compared to their male counterparts. Tumors from BCG refractory patients exhibited higher density of both stromal B cells and M2 like macrophages compared to BCG responders. Notably, high stromal CD79a+ B cell density was significantly associated with higher tumor stage and shorter PFS in a multivariable cox proportional hazards model that considers gender, age, AUA risk score, and BCG treatment. High densities of CD8+ cytotoxic T cells, as well as CD103+ tissue resident memory T cells in either compartment associated with poor RFS.

Conclusions: Findings suggest a potential role of CD79a+ B cells, tissue resident memory T cells and TLSs in mediating poor response to BCG immunotherapy.
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Analysis of the Tumor Microenvironment and PD-L1 Expression Reveals Myofibroblasts as a Prognostic Biomarker in Non-Invasive Bladder Cancer

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Introduction: Non-muscle invasive bladder cancer (NMIBC) tumor microenvironment (TME) study has been neglected compared to other stages of this disease and other types of tumors. While anti-PD-L1 drugs are rising as a treatment option in patients with high risk NMIBC, we still lack a good characterization of the expression of this target in NMIBC tumors. In this work we evaluated NMIBC PD-L1 expression and TME to determine its influence in tumor grading and patient prognosis.

Methods: We have collected samples of tumor and non-pathological tissue from 66 patients with NMIBC and analyzed cell populations and PD-L1 expression using flow cytometry. Relevant results were validated by histology studies. We generated gene signatures from single cell-RNAseq data for the cell subsets of interest, and challenged clinical data from over 500 patients. Cox regression and multivariate analysis was used to test the prognostic value of gene signatures.

Results: We generated a reference map of PD-L1 expression and relative proportion of the cell types that comprise 85% of NMIBC tumors. We show that increased PD-L1 expression in tumors comes from tumor stroma rather than cancer cells, with macrophages displaying the highest percentage of PD-L1+ cells. Using computational tools, we found that myofibroblasts and M2-like macrophages are enriched in high grade NMIBC tumors. We used immunofluorescence to show the distribution of these cell types. By applying gene signatures to the largest transcriptomic data set in NMIBC, we confirmed enrichment of myofibroblasts in aggressive NMIBC. Univariate and multivariate analysis demonstrates the prognostic value of the myofibroblast signature.

Conclusions: We provide information on PD-L1 expression which might be key to determine patient's response to check-point inhibitors in future studies. The finding of myofibroblasts as a predictive factor is of special importance given that, to date, there are no reliable tools to determine patient's recurrence risk after treatment.

Investigating the role of atypical B cells in response to bcg immunotherapy in a murine model of non-muscle invasive bladder cancer.

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Introduction: Non-muscle invasive bladder cancer (NMIBC) accounts for 75% of diagnosed bladder cancer (BC) cases worldwide. The standard treatment for intermediate and high-risk NMIBC is intravesical bacillus Calmette-Guerin (BCG) immunotherapy, but over 50% of patients experience early recurrence or progression despite its proven efficacy. Our previous findings demonstrated that increased intra-tumoral infiltration of B cells in pre-treatment tumors associates with poor outcomes following BCG therapy in patients with NMIBC. We hypothesized that a subset of anergic B cells called atypical B cells (ABCs), which expands during chronic inflammation, biological aging, repetitive immunization, and/or autoimmunity in a sex-differential manner, is recruited to the bladder mucosa during repeated BCG instillations in the induction phase of treatment. This exhausted population of B cells dampens the local anti-tumor immune responses and tumor progression.

Methods: The N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN) carcinogen-induced murine model of BC was used. Aging female and male mice exposed to BBN were treated with intravesical BCG immunotherapy with or without B cell depletion. Systemic and local immune profiling was performed using multispectral flow cytometry and multiplex immunofluorescence. Plasma cytokine levels were measured using multiplex cytokine profiling.

Results: Increased infiltration of B cells and expansion of ABCs systemically and locally (bladder) was observed after repeated BCG instillations. In vivo, depletion of B cells during BCG treatment showed enhanced recovery of the urothelium with high myeloid cell infiltration, with a pronounced effect in aging female mice. Alterations in the frequency of T cell subsets and elevated levels of plasma Th1 and Th2 cytokines were also observed.

Conclusions: Novel findings from this study demonstrate the potential role of ABCs in mediating poor response to BCG. Therapeutic targeting of ABC-associated targets could be a novel approach for the treatment of NMIBC patients such that response rates can be improved in both male and female patients

The second generation histone deacetylase inhibitor quisinostat strongly synergises with cisplatin and the PARP inhibitor talazoparib in cisplatin resistant and naïve bladder cancer cells

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Introduction: Chemoresistance limits prognosis of patients with muscle-invasive bladder cancer (MIBC), underpinning the need for new therapies. Overcoming chemoresistance by epigenetic priming represents an interesting approach. We found that quisinostat, a second generation HDAC inhibitor (HDACi) with improved pharmacodynamics profile, had moderate antineoplastic effects on BC and normal cells. In this study, we evaluated the sensitising effect of quisinostat to cisplatin and the PARP inhibitor talazoparib in BC cells and their cisplatin resistant sublines (LTTs).

Methods: BC and LTT cells with different degrees of resistance to cisplatin were used: J82LTT (moderately resistant), T24LTT (resistant) and RT112LTT (highly resistant). Dose-response curve analysis was performed for mono-treatment with quisinostat, cisplatin, talazoparib and the combinations at fixed dose ratio after 72h. Synergism was assessed using Chou-Talalay method. Effects of the combinations at low dose ratios (0.5xIC50) on cell cycle, apoptosis induction, DNA damage and long term proliferation were analysed by FACS, caspase 3/7 activity, western blots and clonogenicity assay, respectively. RNA sequencing was performed.

Results: Quisinostat synergised with cisplatin and talazoparib in BC lines. Quisinostat led to a decrease in cisplatin IC50 in J82LTT (3.2 x) and T24LTT (2x), indicating re-sensitisation. In J82LTT and T24LTT, both cisplatin and talazoparib synergised with quisinostat at low dose ratio decreasing cell viability significantly. Both low dose combinations increased caspase 3/7 activity, PARP cleavage and γH2AX levels. Long term proliferation ability was markedly decreased, and cell cycle was disrupted. Normal control cells tolerated both low dose combinations. Transcriptome analysis revealed that genes involved in apoptosis, negative regulation of cell proliferation, nuclear division and regulation of transcription were altered.

Conclusions: Quisinostat sensitises chemo-naïve and cisplatin resistant BC cells to cisplatin and talazoparib. Synergy mechanisms involve caspase-dependent apoptosis and cell cycle disruption. In vivo experiments will further characterise these promising new therapeutic options for MIBC.

Development and initial evaluation of infigratinib-eluting seeds for localized treatment of non-muscle invasive bladder cancer (NMIBC)

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Introduction: Repeated resections make non-muscle invasive bladder cancer (NMIBC) a challenge for patients and healthcare systems. Intravesical therapy has been limited to chemotherapeutic agents and BCG, ignoring the high rate of activating FGFR3 mutations in NMIBC. The use of systemic FGFR inhibitors (FGFRi) in NMIBC is limited due to the high rate of adverse effects. Therefore, our aim was to develop a novel approach for sustained intravesical delivery using titanium seeds coated with the FGFRi infigratinib (Infb).

Methods: Titanium seeds were coated with Infb using a standardized coating template (PMID 34328312). Liquid chromatography was used to evaluate the seeds' ability to release Infb in cell-culture media over time. The antiproliferative efficacy of seed-released Infb was compared with freshly prepared Infb solution in vitro in FGFRi-sensitive (RT112) and FGFRi-resistant (UM-UC13) bladder cancer cell lines. In vivo efficacy and release tests in mouse models including 4 treatment arms (negative control without treatment, positive control with oral gavage of Infb, seeds without Infb coating and Infb-coated seeds) are currently ongoing.

Results: Seeds with an adhesive Infb-containing coating were successfully developed after testing multiple coating solutions. Continuous release of Infb over a period of 15 days was observed, resulting in cumulative doses of up to 12 µM. Seed-released Infb showed inhibition of RT112 growth comparable to 10 µM freshly prepared solution. Incubation with media from coated seeds without integrated Infb-coating showed no growth reduction. Results of in vivo experiments are pending.

Conclusions: Current results demonstrate the successful release of biologically active Infb from coated seeds in antiproliferative doses in vitro. The transferability of these results regarding growth inhibition and Infb release into blood and locally in tissue is currently under evaluation. Assuming positive results, this could broaden the therapeutic landscape of NMIBC by providing local applicability of FGFRi.

Combined positive score (CPS) and PD-L1 status in patients with high-risk non-muscle invasive bladder cancer are influenced by an intravesical therapy with Bacillus Calmette-Guérin (BCG)

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Introduction: The standard therapy for high-risk non-muscle-invasive bladder cancer (HR NMIBC) is intravesical BCG therapy. However, immune checkpoint inhibitors (ICI) are emerging as therapeutic option also at this disease stage. In metastatic urothelial cancer (mUC), the PD-L1 status is a predictive marker for response to ICI, broad evidence of this feature also in NMIBC is pending. Our study aimed to evaluate the variability of the PD-L1 status in a cohort of HR NMIBC patients depending on an eventual received immunogenic BCG therapy.

Methods: Using a monocentric retrospective approach, we identified HR NMIBC patients with an additional TURBT sample available during follow-up. 126 tissue samples (63 patients, 38x BCG, 25x BCG-naive) were evaluated for combined positive score (CPS) and PD-L1 status and correlated with clinicopathological parameters. The prognostic significance of the initial PD-L1 status and its dynamics were evaluated using Kaplan-Meier (KM) analyses and uni-/multivariate Cox-Proportional-Hazard analyses for overall, recurrence-free, and progression-free survival (OS, RFS, PFS).

Results: Patients receiving BCG showed a significant increase in mean CPS after BCG (4.03 vs. 12.00, p=0.0074), which was not apparent in BCG-naive patients (2.16 vs. 3.37, p=0.7766). This correlated with an increase in PD-L1 positive patients from 5 to 11 (BCG) vs. consistent 2 PD-L1 positive patients (BCG-naive). KM analyses and univariate Cox-Proportional-Hazard analyses revealed no prognostic significance for OS, PFS and RFS, multivariate analysis showed a positive prognostic significance for RFS (p=0.024) and a trend towards a positive prognostic significance for PFS (p=0.053) when the PD-L1 status changed from negative to positive in the overall cohort.

Conclusions: CPS and associated PD-L1 status demonstrated affectability by intravesical BCG therapy. Although its prognostic value is limited, this affectability might have implications for the therapeutic sequence in NMIBC if ongoing clinical trials conform a predictive value of the PD-L1 status also for NMIBC.

DOT1L regulates the expression of key driver genes in luminal muscle invasive bladder cancer

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Introduction: Bladder neoplasms are classified into Non-muscle invasive bladder cancer (NMIBC) and Muscle invasive bladder cancer (MIBC). While NMIBCs are low grade tumors, MIBCs are high grade tumors that tend to metastasize. MIBCs are further grouped into different subtypes defined by specific gene expression signatures. Of these, luminal tumors are characterized by high expression of GATA3, FOXA1, and PPARγ that are drivers of this subtype. High PPARγ expression is a prominent feature of luminal MIBC, however, the mechanisms of PPARγ regulation are unclear and need further examination to understand lineage commitment and enable new therapeutic strategies.

Methods: To identify the regulators of PPARγ, we conducted a genome-wide CRISPR knockout screen in luminal MIBC cells, using a cell-based fluorescence reporter system that reflected endogenous PPARγ expression. Out of the highest-ranking hits identified in the screen, DOT1L (disruptor of telomeric silencing 1-like) was picked up as a non-essential gene to study the regulatory activity of H3K79 methylation catalyzed specifically by DOT1L.

Results: Here, we report the role of DOT1L in regulation of PPARγ and target genes in driving the luminal subtype and the inhibition of H3K79 methylation using DOT1L inhibitors resulting in a decrease in cell survival in vitro.

Conclusions: We find that H3K79 methylation catalyzed by DOT1L is required for maintaining a luminal MIBC phenotype.

State tobacco control policies: Opportunities for smoking cessation in urologic health

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Introduction: Cigarette smoking is the strongest modifiable risk factor for the development of bladder cancer (BCa). Urologists may have limited knowledge of tobacco taxation efforts that impact smoking cessation among BCa patients. We assessed state cigarette tax, cost per pack of cigarettes, and smoking cessation rates in states with low, intermediate, and high tobacco costs.

Methods: The American Lung Association State of Tobacco Control was used to identify states with high (Washington DC [DC]), intermediate (Washington [WA]), and low (North Carolina [NC]) tobacco costs. Populated weighted BCa incidence per state was obtained from CDC US Cancer Statistics. These 3 states were then cross referenced to the CDC State Tobacco Activities Tracking and Evaluation (STATE) system to determine state cigarette tax and cost per pack of cigarettes from 1970 to 2019. This study used the NCI-sponsored Tobacco Use Supplement to the Current Population Survey to determine longitudinal smoking cessation rates stratified by state. Smoking cessation was defined as the percent of former smokers among ever smokers. Linear regression was used to determine best fit values with 95% confidence intervals.

Results: DC (BCa 4.3/100k) had the highest cost per pack of cigarettes ($10.42), followed by WA (BCa 19.6/100k ($8.57) and NC (BCa 18.3/100k) ($5.36). DC cigarette tax was $4.94 (increase over time, or slope 0.07, 95% Cl 0.06-0.08), WA was $3.03 (slope 0.07, 95% Cl 0.06-0.08) and NC was $0.45 (slope 0.01, 95% Cl 0.008--0.01). Smoking cessation rates were lowest in NC at 53.3%, compared to both states with higher cigarette cost and taxes (DC 57%, WA 61%).

Conclusions: The highest smoking cessation rates were observed in states with higher cost per cigarette pack and higher tobacco tax rates. These findings can inform effective tobacco control measures which impact patients with bladder cancer who smoke

Combining Antibody-Drug Conjugates with Radiation in Preclinical Bladder Cancer Models

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Introduction: Antibody-drug conjugates (ADCs) are a novel class of therapeutics that combine a tumor cell targeting antibody with a cytotoxic payload. Two ADCs are currently approved in the US for treatment of advanced bladder cancer: enfortumab vedotin (EV) and sacituzumab govitecan (SG). Radiation (RT) plays a central role in trimodality therapy (TMT), a curative treatment approach for muscle-invasive bladder cancer (MIBC). However, the biological activity of ADCs combined with radiation (RT) in preclinical MIBC models has not been reported.

Methods: We use a molecularly annotated panel of human MIBC cell lines and patient-derived models to test the impact of RT on expression of ADC targets and define the combined activity of EV and SG with RT. We also test the in vivo activity of combining ADCs with image-guided, fractionated RT in bladder cancer xenograft models.

Results: Radiation has variable impact on expression levels of nectin-4 and trop-2, the targets of EV and SG, respectively, as determined by immunoblot, immunofluorescence microscopy, and immunohistochemistry (IHC). Whereas dose-dependent increases in nectin-4 and/or trop-2 levels were observed in some models, no significant changes were observed in other models. EV and SG showed additive or synergistic cell killing when combined with RT across preclinical models in vitro. Similarly, combining EV or SG with fractionated RT was well-tolerated, improved tumor control, and prolonged survival in bladder cancer xenograft models compared to either agent alone. Finally, we find that bladder tumors with acquired radiation resistance show variable decrease in expression of nectin-4 and/or trop-2.

Conclusions: ADCs demonstrate combination activity with RT across a panel of molecularly diverse bladder cancer preclinical models. These studies provide preclinical data supporting clinical trials to investigate the safety and efficacy of combining ADCs with RT as a bladder-preserving therapy for MIBC

The prognostic value of HER expression in the era of HER targeted therapies in metastatic urothelial carcinoma

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Introduction: HER -targeting antibody drug conjugates have shown promising results in the treatment of metastatic urothelial carcinoma. The prognostic value of HER overexpression is currently unknown.

Methods: Samples from a phase III trial (LAMB, NCT00949455) which compared maintenance lapatinib versus placebo after completion of first-line, platinum-based chemotherapy in patients with HER 1/2–positive metastatic urothelial carcinoma were collected. Corresponding baseline and follow-up data included patients enrolled onto study and those who screen failed. HER 1-4 expression was assessed, by a single pathologist, independently and blinded. Samples were grouped according to HER expression (negative vs positive) and correlated with baseline tumor characteristics and survival.

Results: Of the 446 screened samples in the LAMB study, 39% (175/446), 14% (60/446) and 15% (66/446) were HER-1 positive, HER-2 positive and HER negative, respectively. Furthermore, a subset of samples (n=79) were also analyzed for HER 3-4 expression. 54% (43/79), 68% (54/79) had high HER-3 membranous and cytoplasmic expression. Similarly, 52% (41/79) and 85% (67/79) showed high HER-4 membranous and cytoplasmic expression. There was no correlation between HER expression and tumor stage at diagnosis or subsequent treatment with lapatinib. HER1 and/or HER2 protein expression were correlated with outcome. Neither proteins were prognostic for progression free survival (PFS) (p=0.37) or overall survival (p=0.35). Similarly, there were no significant difference in the OS in terms of the HER3/4 status (p=0.35) or PFS (p=0.37) from completion of chemotherapy. Next, gene expression for EGFR, ERBB2, ERBB3, and ERBB4 were analyzed. Low ERRB2 gene expression corresponded with a significantly better PFS (median 6.7 months vs 3.9months, p=0.04).

Conclusions: HER expression using immunohistochemistry was not prognostic of outcome in metastatic urothelial carcinoma, however low ERRB2 gene expression corresponded to a significantly survival. Further exploration of HER as a therapeutic target is necessary.

Missense tumor mutations in the MTAP gene are only present in bladder cancer patients who are active smokers

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Introduction: Recent genome wide association study (GWAS) demonstrates that smoking status and genetic variants at 9p21.3 increase the risk of bladder cancer. Important genes located at the locus 9p21 include MTAP and CDKN2A. We postulated that bladder cancer patients who are active smokers might have tumor mutations in these specific genes more often. Therefore, we evaluated the presence of tumor mutations in these specific genes in bladder cancer for smokers and non-smokers using publicly available data from the Cancer Genome Atlas Project.

Methods: We queried the available bladder cancer studies in cbioportal.org and identified 3 studies that had tumor mutational data and characterized patient smoking status as active (current), former, and never. This provided a total of 1839 samples.

Results: We found no significant survival difference in active and former smokers, however, there was a clear survival difference between never smokers and any history of smoking. The frequency of tumor mutations in the MTAP gene were 50% in active smokers but only 15% in former smokers and 23% in never smokers. Of note, all of the MTAP mutations seen in active smokers were missense mutations leading to MTAP-deficient tumors. Interestingly, there was no significant difference in the frequency of CDKN2A mutations.

Conclusions: MTAP missense mutations strongly correlate with an active smoking history based on data from the Cancer Genome Atlas Project. GWAS data also demonstrate a strong association with smoking and genetic variants at the MTAP gene and the risk of developing bladder cancer. These findings dovetail with the recent finding that MTAP-deficient bladder cancers are more aggressive than MTAP-wild type bladder cancers. Future work may entail studying the loss of MTAP function and the restitution of MTAP function in bladder cancer tumor models to study the potential effect of smoking.

Round Ligament Vaginal Colpopexy for Prevention of Post Anterior Exenteration Pelvic Organ Prolapse

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Introduction: For female patients with muscle-invasive bladder cancer, organ-sparing surgery may not be feasible, leading to concomitant hysterectomy with anterior vaginectomy during radical cystectomy. In doing so, the loss of structural tissues leads to the absence of support for the vagina and vulvar region of the patient increasing the risk of pelvic organ prolapse (POP). In this population, the risk of POP following radical surgery for bladder cancer is thought to be as high as 6%.

Methods: At our institution, we began performing round ligament colpopexy at the time of anterior exenteration either through a robotic or open approach. Round ligaments were preserved bilaterally and at the time of vaginal reconstruction were incorporated at the lateral apex of the vaginal closure bilaterally. An IRB-approved retrospective analysis was then performed, which identified 31 patients between 2020-2023 who underwent anterior exenteration with colpopexy which were then used in our initial analysis. Age in this cohort was between 36-83.

Results: A total of 31 patients were identified who underwent anterior exenteration with round ligament colpopexy. Of these, 1 patient (3%) developed prolapse that was subsequently managed conservatively with the use of a pessary device. No adverse events related to the incorporation of round ligament colpopexy were identified in this population.

Conclusions: Prolapse following female anterior exenteration is a known risk, with prior series demonstrating an approximately 6% risk. Here we present a novel technique that appears to be both safe, effective, and does not increase operative time significantly. Further study of this technique in a larger population is necessary to confirm a reduced incidence of symptomatic pelvic organ prolapse moving forward in order to be used more widely.

Cisplatin (cis)- or carboplatin (carbo)-based chemotherapy plus pembrolizumab (pembro) in advanced urothelial cancer (aUC): Exploratory analysis from the phase 3 KEYNOTE-361 study

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Introduction: KEYNOTE-361 (NCT02853305) evaluated first-line pembro±platinum-based chemotherapy versus chemotherapy in aUC, and primary end points of PFS (HR, 0.78; 95% CI, 0.65-0.93) or OS (0.86; 0.72-1.02) were not met. Exploratory analysis of efficacy by platinum agent (cis or carbo) is reported.

Methods: Patients with previously untreated aUC were randomly allocated 1:1:1 to receive pembro 200 mg IV Q3W for ≤35 cycles ± chemotherapy (gemcitabine [gem] + cis or carbo) or chemotherapy alone (data for pembro alone not reported). This analysis evaluated PFS and ORR per RECIST v1.1 by BICR and OS for pembro+cis/gem versus cis/gem and pembro+carbo/gem versus carbo/gem.

Results: Of 1010 patients enrolled, 351 and 352 received pembro+chemotherapy (cis/gem/pembro, n=156; carbo/gem/pembro, n=195) and chemotherapy (cis/gem, n=156; carbo/gem, n=196), respectively. Median follow-up was 31.3 months (range, 22.1-41.6). For cis/gem/pembro versus cis/gem, median PFS was 8.5 versus 7.1 months (HR, 0.67; 95% CI, 0.51-0.89) and median OS was 20.1 versus 16.4 months (0.88; 0.67-1.15). ORR was 64.1% versus 48.7%, respectively. For carbo/gem/pembro versus carbo/gem, median PFS was 8.0 versus 6.7 months (HR, 0.86; 95% CI, 0.68-1.09) and median OS was 15.5 versus 12.3 months (0.84; 0.67-1.06). ORR was 47.2% versus 41.8%, respectively. A total of 11/156 patients (7.1%) assigned to cis/gem/pembro, 87/156 (55.8%) to cis/gem, 12/195 (6.2%) to carbo/gem/pembro, and 82/196 (41.8%) to carbo/gem received subsequent anti–PD-1/L1 therapy after discontinuing treatment; 22/156 (14.1%) and 11/196 (5.6%) assigned to cis/gem and carbo/gem, respectively, started anti–PD-1/L1 therapy after treatment discontinuation without progression.

Conclusions: Primary end points of PFS and OS in the ITT were not met. This exploratory subset analysis suggests modest and similar results across all efficacy end points in the cis/gem/pembro cohort relative to cis/gem and carbo/gem/pembro cohort relative to carbo/gem. OS results may have been influenced by active subsequent therapies in the control arm, which are widely available.