IBCN

INTERNATIONAL
BLADDER CANCER
NETWORK

Founded 1997

IBCN Annual Meeting - 2025
October 9th – October 11th
Hilton Munich City
Munich, Germany

A special thank you to our 2025 meeting industry sponsors:

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We would like to thank the following IBCN members for reviewing abstracts for the 2025 Annual Meeting:

Florestan Koll
Isabelle Bernard-Pierrot
Janet Kukreja
Jeremy Teoh
Michèle Hoffmann
Nagireddy Putluri
Rouf Banday
Timo Nykopp

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THURSDAY OCTOBER 9th, 2025

19:00 Welcome Reception - Hofbräukeller

The traditional IBCN welcome “get together”. Drinks will begin at 7 pm followed by a buffet dinner at 8 pm.

Hofbräukeller
Innere Wiener Straße 19,
81667 München, Germany

FRIDAY OCTOBER 10th, 2025

Breakfast on your own

Introduction

07:30	Registration Check-In	
08:00	Welcome to IBCN	Peter Black
08:05	Welcome to Munich	Roman Nawroth

Abstract Session I: Novel approaches to bladder cancer screening and treatment

Co-Chairs: Bernadett Szabados and Isabelle Bernard-Pierrot

08:15	Real World Evaluation of the GALEAS Bladder Urine Test	Douglas Ward
08:25	Reclassifying risk with a generalizable molecular classifier for aggressive tumors in low-grade bladder cancer	Ewan Gibb
08:35	ENLIGHTED phase 3 study: Interim Results of Efficacy and Safety of Padeliporfin Vascular Targeted Photodynamic therapy (VTP) in the treatment of Low-Grade Upper Tract Urothelial Cancer (LG UTUC)	Gautier Marcq
08:45	Preliminary results from LEGEND: a Phase 2 study of detalimogene voraplasmid, a novel, investigational, non-viral genetic medicine for high-risk non-muscle invasive bladder cancer (NMIBC)	Wassim Kassouf
08:55	Artificial Intelligence and Multiparametric MRI Predict Clinical Outcomes to Neoadjuvant Immunotherapy in Patients with Muscle Invasive Bladder Cancer (MIBC) Undergoing Radical Cystectomy	Andrea Necchi
09:05	Q&A	Co-Chairs

09:15 - 09:30 Break

Keynote Speaker I

09:30	Bytes to Bedside: Insights and opportunities offered by whole genome sequencing	Serena Nik-Zainal
10:00	Discussion	Sita Vermeulen
10:15	Breakout Session Overview	Peter Black

10:20 - 10:45 Break

10:45 – 12:45 Industry Meets IBCN – Breakout Sessions

Partner	Topic	Facilitator
CG Oncology	Cretostimogene in NMIBC: Trials, Translational Insights, and Future Directions	Peter Black, Colin Dinney, Vijay Kasturi
EnGene	Mainstreaming Non-Viral Gene Therapy in HR NMIBC	Marcq Gautier, Vignesh Packiam, Anthony Cheung, Amy Pott, Ron Cooper
ImmunityBio	Emerging strategies with Anktiva and re-combinant BCG for NMIBC	Wes Kassouf, Bobby Reddy
Johnson & Johnson	Intravesical Drug Releasing Systems in Bladder Cancer	Bernadett Szabados, Simran Gill
Pfizer	Charting the Future of Biomarkers in Bladder Cancer: Integration into Trial Design and Clinical Decision-Making	Julia Brinkmann, Tilman Todenhöfer, Seth Lerner

12:45 - 14:00 Lunch and Poster Viewing

IBCN Speaker

14:00	Predictive biomarkers: where do we stand?	David McConkey
14:30	Discussion	Roman Nawroth

Abstract Session II: -omic profiling of bladder cancer

Co-Chairs: Gillian Vandekerkhove and Rouf Banday

14:45	Genomic Landscape of Bladder Pure Squamous Cell Carcinoma Versus Urothelial Carcinoma of the Bladder	Saad Atiq
14:55	Spatial Profiling of the Tumor Microenvironment in Plasmacytoid Urothelial Carcinoma	Kathryn Gessner
15:05	Genomic characterization of upper urinary tract urothelial carcinoma and clonal evolution of intravesical recurrence	Alberto Nakauma-González
15:15	Tracking heterogeneity and the trajectory of neoplastic disease progression in bladder cancer subtypes using spatial transcriptomics and liquid biopsy	Subhajyoti De
15:25	Artificial Intelligence Predicts Molecular Subtypes and Outcomes in Muscle-Invasive Bladder Cancer from Whole Slide Images	Jacqueline Fontugne
15:35	Q&A	Co-Chairs
15:45	Closing words and poster session introduction	Roman Nawroth

16:00 - 18:00 Poster Session “Happy Hour”

20:00 IBCN Gala

Munich Residenz
Residenzstraße 1
80333 München, Germany

SATURDAY OCTOBER 11th, 2025

Breakfast on your own

IBCN – General Assembly

08:00	General Assembly Session	Members Only

Session I: Spatial Biology

Chair: Madhuri Koti

09:00	Multimarker exploration of the tumor microenvironment in patients with high-risk NMIBC using spatial proteomics	Trine Strandgaard
09:20	Spatial distribution of molecular features in tumors with divergent chemotherapy responses	Gottfrid Sjödahl
09:40	Geographies of resistance: How tumor niches silence immunity and fuel growth	Amir Horowitz (Virtual)

10:00 - 10:10 Break

Keynote Speaker II

10:10	New Insights Into BCG, the First Immunotherapy of Cancer	Michael Glickman
10:40	Discussion	Lars Dyrskjøt

Session II: Novel Clinical Trial Design and Updates

Chair: Roland Seiler

10:55	NMIBC clinical trials: landmark reports from 2025	Wassim Kassouf
11:15	Clinical trials in urothelial cancer: moving out of the platinum era	Michiel Van der Heijden
11:35	Challenging systems, pathways, and dogma: results of the BladderPath trial	Richard Bryan

11:55 - 12:05 Break

Abstract Session III: From tumor initiation to therapeutic discovery

Co-Chairs: Clémentine Le Magnen and Florestan Koll

12:05	The innate urothelial response to BK Polyomavirus infection drives interferon-mediated APOBEC3A mutational damage.	George Hatton
12:15	Integrated transcriptomic and spatial immunophenotyping reveals T cell exhaustion and B cell associated immunosuppressive niches in BCG-Unresponsive NMIBC	Kartik Sachdeva
12:25	Expanding the treatment Landscape of FGFR-Targeted Therapy in Bladder Cancer: Integrative Genomic and Functional Profiling of Patient-Derived Organoids	Vandana Solanki
12:35	Characterization of Growth Differentiation Factor 15 (GDF-15) as a Therapeutic Target in Urothelial Carcinoma	Florestan Koll
12:45	Phenotypic screening of bladder cancer cultures to find toxic natural products	Nagireddy Putluri
12:55	Q&A	Co-Chairs

13:00 - 13:50 Lunch and Poster Viewing

Session III: Mechanisms of Therapeutic Resistance

Chair: Kent Mouw

13:50	Exploring Pathways of Resistance to Immune-Mediated Cell Death in Bladder Cancer	Morgan Roberts
14:10	Mechanisms of resistance to antibody-drug conjugates	Niklas Klümper
14:30	The role of enhancer dysfunction in urothelial tumorigenesis	Yu Chen

Abstract Session IV:
Moving towards clinically tractable tumor classification

Co-Chairs: Jeffrey Damrauer and Vignesh Packiam

14:50	An Immunohistochemical Panel for Predicting Response to Neoadjuvant Chemotherapy in Muscle-Invasive Bladder Cancer	Ioana-Maria Mihai
15:00	Tumor mutational burden predicts the outcome of patients with muscle-invasive bladder cancer undergoing immune-checkpoint inhibitor-based neoadjuvant therapy	Chiara Mercinelli
15:10	Real-time single-sample Lund Taxonomy tumor classification in the prospective Swedish UROSCANSEQ study	Pontus Eriksson
15:20	Bulk and spatial transcriptional signatures of durable response from Durvalumab-based therapy in BCG-unresponsive (BCG-U) non-muscle invasive bladder cancer (NMIBC)	Roy Elias (Virtual) Noah Hahn
15:30	Leveraging Machine Learning to Identify Interpretable Histopathologic Features in Muscle-Invasive Bladder Cancer for Neo-Adjuvant Treatment Response Prediction	Nikolay Alabi
15:40	Q&A	Co-Chairs

Awards & Closing Remarks

15:45	Awards Presentation	Peter Black
15:55	Welcome to Toronto - IBCN 2026	Alexandre Zlotta
16:00	Adjourn	

Poster #	Abstract Title	Presenter
1	Genomic Landscape of Bladder Pure Squamous Cell Carcinoma Versus Urothelial Carcinoma of the Bladder	Atiq, Saad
2	Urinary TERT Promoter Mutations as a Robust Non-Invasive Biomarker for Predicting Bladder Cancer Recurrence and Progression	Avogbe, Patrice Hodonou
3	Concordance Between Tumor Genomic Profiling and ctDNA for Recurrence Risk in Muscle-Invasive Bladder Cancer	Aydogdu, Can
4	Urinary minimal residual disease testing identifies early recurrence and monitors BCG response in patients with high-risk non-muscle-invasive bladder cancer	Bahlburg, Henning
5	Cx32 status predicts mesenchymal acquisition in luminal MIBC with implications for subtyping	Baker, Jennifer
6	APOBEC-Driven CNV–SNV Conflict Shapes Tumor Immune Visibility	Banday, Rouf
7	Gene regulatory Network inference reveals p63 as a key protumoral master regulator in FGFR3-mutated luminal bladder cancers.	Bernard-Pierrot, Isabelle
8	Time Modeling of Mutational Signature of Bladder Cancer Development from Mucosal Field Effects on the Whole-Organ Scale	Czerniak, Bogdan
9	Impact of Molecular Subtyping and Immune Signatures on Pathological Response following Neoadjuvant Nivolumab, Gemcitabine and Cisplatin in Muscle-invasive Bladder Cancer	de Jong, Joep
10	Investigation of carcinogenic effects of polystyrene microplastic on human bladder cancer cells	Dewala, Sahab Ram
11	All-stage bladder cancer proteomics map tumor specifity and identify prognostic and predictive subtypes	Dressler, Franz
12	Disrupting the PDGFR_-driven progression signature leads to enhanced control of bladder cancer	El Ahanidi, Hajar
13	Hexaminolevulinate stimulates senescence and induces PD-L1 expression in muscle-invasive bladder cancer cell lines	Gakis, Georgios
14	Investigating Combination Therapies for Non-Muscle-Invasive Bladder Cancer Using Advanced 3D Human Cell-Based Preclinical Models	Gao, Jinhui
15	Outlining The Role of Hypoxia in Bladder Cancer Dedifferentiation and Progression	Garven, Andrew
16	Beyond Viral Mimicry: Transposable Element Expression Defines Prognostic Stress-Response Phenotypes in Bladder Cancer	Garven, Andrew
17	Vitamin D receptor regulates the invasive capacity of bladder cancer cells through interactions with AKT kinases	Günes, Cagatay
18	Pten loss promotes p53 loss of heterozygosity and immune escape in a novel urothelial organoid model with p53 missense mutations	Hamada, Akihiro
19	Long-Term Oncologic Outcomes and Extracellular Vesicle Signatures in Bladder Cancer	Handke, Analena
20	BEST-PATH: Scalable Research Training for Undergraduates in Bladder Cancer Pathology and Pathology Report Analysis	Harding, Allison
21	Characterization of the Heterogeneity of Antibody-Drug Conjugate Targets in Bladder Cancer on Single-Cell Level	Hauser, Lena Marie
22	Multi-level characterization of new patient-derived bladder cancer models	Herek, Paula
23	Augmenting PARP inhibitor efficacy in urothelial cancer cells by combination with a BET inhibitor	Hoffmann, Michèle J
24	Exploring Bladder Cancer Research Priorities - aligning science with patient need	Humayun-Zakaria, Nada
25	Spatial Assessment of FGFR3 Protein Expression and T-cell Infiltrations Reveals Distinct Distribution Patterns in Upper Tract Urothelial Carcinoma and Normal Urothelium	Iwata, Tomoko
26	Development of 3D bladder cancer organoid models to investigate the efficacy of cancer immunotherapies.	Jennings, Vicki
	Abstract Withdrawn	
28	Using the Computational Histologic Artificial Intelligence (CHAI) platform to predict FGFRm status in bladder cancer	Kiedrowski, Lesli
29	Investigating chronic inflammation induced accelerated immune aging and response to Bacillus Calmette Guérin immunotherapy in non-muscle invasive bladder cancer	Koti, Madhuri
30	Disparities in guideline-concordant care and outcomes in non-muscle invasive bladder cancer: The impact of age and socioeconomic marginalization in a universal healthcare system	Kwong, Jethro

Poster #	Abstract Title	Presenter
31	The role of event-free survival as a surrogate outcome in the BCG-naive and BCG-exposed NMIBC setting	Kwong, Jethro
32	Leveraging patient-derived organoids to identify novel therapeutic vulnerabilities in bladder cancer	Le Magnen, Clementine
33	Radiogenomic Analysis of Muscle-Invasive Bladder Cancer using CT-based Texture Analysis	Lerner, Seth
34	MNU carcinogen rat model of NMIBC: a robust platform for uncovering single-cell immune complexity following BCG immunotherapy	Lombardo, Kara
35	Exploring cisplatin-gemcitabine's impact on GLUT1 and KDM3A expression patterns in bladder cancer	Macedo Silva, Catarina
36	Microbiome-Based Predictors of Response to Immune Checkpoint Inhibitors in Urological Cancers	Magyar, Balázs
37	A universal molecular map of bladder cancer: from cell lines in vitro to tumors and back using co-regulatory networks	Marcq, Gautier
38	Biologically-informed machine learning identifies a new clinically-actionable MIBC subgroup with NRF2 overactivity	Mason, Andrew
39	Response Profiling of Enfortumab Vedotin and Sacituzumab Govitecan Using Patient-Derived Organoids: A Step Closer to Precision Oncology	Melzer, Michael Karl
40	The Genomic Landscape of Bladder Cancer: Insights from Copy Number Alteration Profiles	Moreno-Oya, Ane
41	Establishment of an innovative, human stem cell-based culture model to investigate molecular carcinogenesis of urothelial carcinoma	Morgenstern, Clara
42	Investigating Sex-Specific Transcriptional Differences in Urothelial Bladder Cancer	Myumyun, Ayse Nur
43	Genomic characterization of upper urinary tract urothelial carcinoma and clonal evolution of intravesical recurrence	Nakauma-González, Alberto
44	Adenoviral replication does not require progression into cellular S-phase	Nawroth, Roman
45	Enhancing Oncolytic Adenovirus Efficacy in Tumor Cells through Alpha-Fetoprotein Peptide-Mediated Targeting and Internalization	Nawroth, Roman
46	CDK4/6 inhibition initiates cell cycle arrest by nuclear translocation of RB and includes a multistep molecular response	Nawroth, Roman
47	Lymphatic sealing with titanium clips versus ultrasonic dissector during pelvic lymph node dissection at the time of radical cystectomy: A prospective, randomized trial	Nayak, Brusabhanu
48	Lynch Syndrome-Associated Urothelial Cancer: Distinct Genomic Landscape, Constrained Hypermutation, and Preliminary Results from the LS-URO Urine Tumor DNA Screening Study	Nikkola, Jussi
49	Implications of Gemcitabine-Cisplatin therapy on bladder cancer responsiveness to ADCs	Nössing, Christoph
50	Stromal Transcriptomic Signatures are Prognostic in High-Risk Non-Muscle Invasive Bladder Cancer treated with Intravesical Bacillus Calmette-Guerin	Packiam, Vignesh
51	Predicting Response to Intravesical Bacillus Calmette-Guerin Versus Gem/Doce for High-grade Non-muscle-invasive Bladder Cancer	Packiam, Vignesh
52	Non-invasive urine genomic profiling in patients with upper tract urothelial carcinoma	Pallauf, Maximilian
53	Targeting Compensatory DNA Repair Pathways to Restore ATR Inhibitor Sensitivity in Squamous Cell Carcinoma of the Bladder	Pannhausen, Julia
54	The Added Prognostic Value of the UROMOL Classification to the 2021 EAU Risk Stratification in Patients with Non-Muscle-Invasive Bladder Cancer in the COBLAnCE Cohort	Paper, Laura
55	Circulating immune-biomarkers of response to neoadjuvant Sacituzumab Govitecan (SG) alone and with pembrolizumab (pembro) in muscle-invasive bladder cancer (MIBC): secondary analyses from SURE-01 and SURE-02 trials	Pastorino, Giovanni Luigi
56	The Role of CCL2 in Bladder Cancer Progression and Metastasis: A Context-Dependent Effect	Pérez-Escavy, Mercedes
57	ABCG2-Mediated SN-38 Efflux Drives Payload-specific Resistance to Sacituzumab Govitecan in Urothelial Carcinoma	Prantl, Isabella
58	The IL6/JAK/STAT3 signaling axis is a therapeutic vulnerability in SMARCB1-deficient bladder cancer	Putluri, Nagireddy
59	Comprehensive Analysis of the Resistance Mechanisms against the Antibody Drug Conjugate Enfortumab Vedotin and its Cytotoxic Payload MMAE in Preclinical Urothelial Carcinoma Models	Rupp, Luis
60	Evaluating the roles of HAI-1 and ST14 in various cellular processes of bladder cancer cell lines	Saeed, Azad
61	Integrated multi-omics analysis of primary and PDX MIBC models suggests targetable molecular vulnerabilities	Saltzman, Alexander

Poster #	Abstract Title	Presenter
62	Discriminative capacity of the AUA/SUFU hematuria guideline 2025 update – How much progress?	Schmitz-Dräger, Bernd J.
63	Clinical applicability of an mRNA based urine test (Xpert® Bladder Cancer Detection*) for the detection of bladder cancer in patients with microhematuria	Schmitz-Dräger, Bernd J.
64	Final results of the prospective randomized UroFollow trial comparing marker-guided versus cystoscopy-based surveillance in patients with low/intermediate risk bladder cancer	Schmitz-Dräger, Bernd J.
65	Reduce length of hospital stay with the use of methylnaltrexone in radical cystectomy peri-operative; a retrospective study	St-Laurent, Marie-Pier
66	Evaluating the prognostic utility of circulating tumour DNA in metastatic urothelial cancer	Stephenson, Maria
67	Microbiome Immunogenicity in Bladder Cancer	Suleja, Agata
68	Clinical outcomes from 2020 EAU primary High-risk NMIBC patients treated with BCG by 2021 EAU risk classification	Tian, Ye
69	Physical activity before radical cystectomy – the PRACTICE trial: preliminary data	Volz, Yannic
70	Cost of care and oncologic outcomes associated with Blue Light Cystoscopy in an equal setting: results from the BRAVO Trial	Williams, Stephen
71	Quantitative Outcome-Based Grading for Whole Slide NMIBC Images	Yach, Evelyn
72	Differential KLF5 Isoform Usage in Non-Muscle Invasive Bladder Cancer (NMIBC)	Yuksek Tekben, Esra Hilal
73	SPP1 protein mediates resistance to neoadjuvant chemotherapy in non-urothelial-like MIBC subtypes	Zadoroznyj, Aymeric
74	Liquid Biopsy Guided Disitamab vedotin combined with toripalimab and radiotherapy for multimodal organ-sparing treatment of muscle invasive bladder cancer: a proof-of-concept study	Zhang, Ruiyun
75	Identifying Indolent Low Grade and Aggressive High Grade NMIBC: A Quantitative Grading Framework	Zhang, Taylor
76	Spatial Profiling of the Tumor Immune Microenvironment in Muscle-Invasive Bladder Cancer TREATED WITH Neoadjuvant Platinum Chemotherapy	Zheng, Nicolas

I B C N
INTERNATIONAL
BLADDER CANCER
NETWORK
Founded 1997

Arrival from Munich Airport (MUC) → Hilton Munich City Recommended: S-Bahn(fastest & easiest)

Travel time:approx. 35–40 minutes
Tickets: Buy at airport ticket machines or via the MVV app
(“Airport ticket” / full-network single ticket).

Directions:
At Munich Airport, follow signs to the S-Bahn platforms.
Take S8 towards the city center, Zentrum (S1makes a loop and takes longer).
Get off at Rosenheimer Platz (the station is directly beneath the Hilton).
Exit towards RosenheimerStraße
The Hilton Munich City (RosenheimerStr. 15) is just a short walk from the exit.

IBCN Welcome Reception (October 9th)

Drinks - 19:00, Dinner - 20:00

Hofbräukeller

InnereWiener Straße19, Haidhausen

Option A — By foot (pleasant if the weather is good)
Walk from the hotel east/southeast through the Haidhausen neighborhood to Wiener Platz.
Distance: ~1–2 km, time: ~15–25 minutes at a leisurely pace

Option B — Public transport
5 minute walk to tram stop Gasteig, take a tram to Wiener Platz.

IBCN Dinner (October 10th)

Drinks and Dinner - 20:00

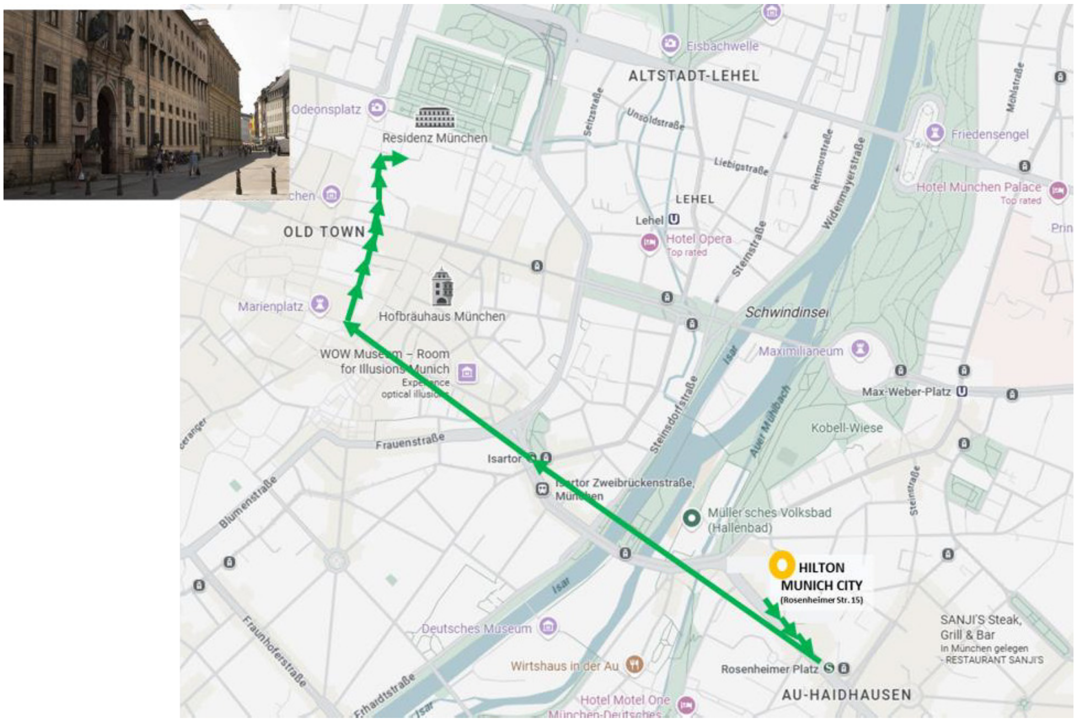
Munich Residenz–Max-Joseph-Saal

Residenzstraße1, Old Town

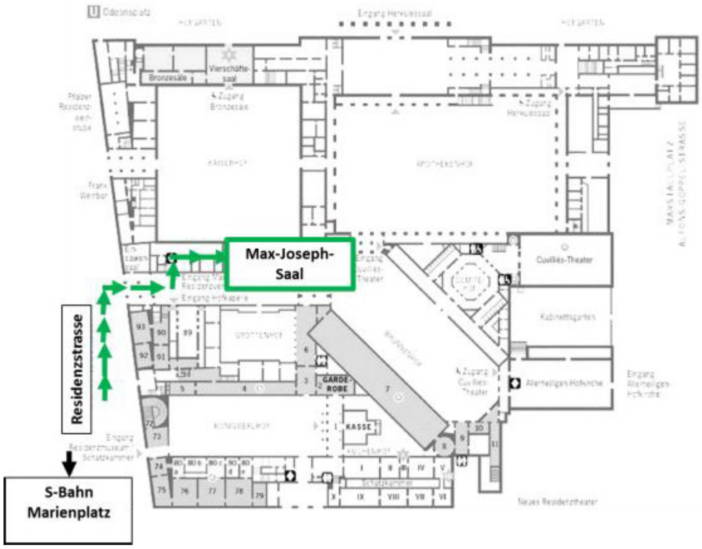
S-Bahn+ short walk

1. From RosenheimerPlatz, take any S-Bahntowards Marienplatz (2nd stop).

2. Walk from Marienplatzto the Residenz (~5–10 minutes), heading north towards Odeonsplatz.



3. From Residenzstraße, enter the Kapellenhof; on the right-hand side is the entrance to the Max-Joseph-Saal.



Abstracts selected for oral presentation

[order of presentation]

Real World Evaluation of the GALEAS Bladder Urine Test

Douglas Ward[1], Richard Bryan[1], Victoria Hewitt[2], Robert Hastings[2]

[1]University of Birmingham
[2]Nonacus Ltd

Background: The latest generation of urine tests for bladder cancer (BC) detection offer high sensitivity and specificity and may be incorporated into patient management in the near future. One such test, GALEAS Bladder, based on detecting BC-associated mutations in 23-genes by NGS is now undergoing service evaluation at multiple NHS Trusts in the UK. In parallel, the 3200 patient BC-Recon study is testing whether GALEAS Bladder could safely replace flexible cystoscopy in imaging negative haematuria patients.

Methods: Urologists at 7 NHS Trusts were provided with GALEAS Bladder urine collection kits. Urine samples were posted to Nonacus Clinical Services, DNA extracted, sequenced, mutations called and the test results (positive/negative, mutations present and variant allele frequencies) returned to the urologists. We have completed an interim analysis of data from the first 431 patients.

Results: Out of 431 patients undergoing haematuria investigations 34 received a diagnosis of bladder cancer by the standard cystoscopy-based diagnostic pathway. Of these, 32 were GALEAS Bladder positive (94% overall sensitivity and 100% for high grade disease) and 353 out of 397 non-BC patients were GALEAS Bladder negative (89% specificity). The NPV was>99%. The most frequently detected mutations were in the TERT promoter, TP53, FGFR3, PIK3CA and ERCC2. The BC-Recon study is approaching full recruitment and results should be reported in 2026.

Conclusions: GALEAS Bladder is readily implementable in the NHS and has the potential to prevent tens of thousands of “unnecessary” flexible cystoscopies every year. The first independent prospective evaluation of GALEAS Bladder has resulted in higher sensitivity and specificity than originally published [1].

1. Ward, D., et al., Highly Sensitive and Specific Detection of Bladder Cancer via Targeted Ultra-deep Sequencing of Urinary DNA. European Urology Oncology 2023. 6(1): p. 67-75.

Reclassifying risk with a generalizable molecular classifier for aggressive tumors in low-grade bladder cancer

Tran Anh Thu Phung[1], Rachel Weng[1], Joep de Jong[2], Robert Bell[1], Lars Dyrskjøt[3], Ewan Gibb[1]

[1]Vancouver Prostate Centre
[2]Erasmus University Medical Center
[3]Aarhus University

Background: Low-grade, cTa (TaLG) NMIBC is often considered less aggressive, yet some patients experience reduced recurrence-free survival (RFS). However, every patient undergoes regular, invasive cystoscopic surveillance. Consensus clustering previously identified a clinically relevant subgroup of aggressive TaLG tumors - long non-coding RNA cluster 2 (LC2) - characterized by higher recurrence and progression rates. Here, we interrogated LC2 biology and developed a prognostic model to identify LC2 tumors in an independent cohort.

Methods: Quantile normalized RNA-sequencing (RNA-seq) data from UROMOL cohort ($n = 276$), with LC2 labels, was used for training and the Knowles et al. microarray cohort ($n = 72$) served for validation. Feature selection employed median absolute deviation (MAD) and nested cross-validation. A Lasso regression model was trained using 10-fold cross-validation. Performance was assessed using pathway comparisons, heatmap visualization and clinical relevance evaluated by Kaplan-Meier method and multivariable Cox regression.

Results: Biological characterization revealed that LC2 tumors exhibited higher proliferative potential and elevated FGFR3 pathway activity but reduced Sonic Hedgehog (SHH) signaling and immune activity ($p < 0.001$ for all). When our classifier was applied to the Knowles cohort, the model identified 7/72 tumors as LC2. Patients harboring LC2-TaLG NMIBC experienced significantly worse RFS compared to those with non-LC2 tumors (log-rank $p < 0.001$). On multivariable analysis, LC2 status remained an independent predictor of recurrence (HR = 4.63, 95% CI: 1.81–11.86, $p = 0.001$). Predicted LC2 tumors in Knowles mirrored the training cohort biology.

Conclusions: We trained a transcriptomic classification model to identify more aggressive TaLG NMIBC that might benefit the most from close monitoring, demonstrating reproducibility across RNA-seq and microarray platforms.

ENLIGHTED phase 3 study: Interim Results of Efficacy and Safety of Padeliporfin Vascular Targeted Photodynamic therapy (VTP) in the treatment of Low-Grade Upper Tract Urothelial Cancer (LG UTUC)

Gautier Marcq[1], Vitaly Margulis[2], Ronald P. Kaufman[3], Asaf Shvero[4], Neal D. Shore[5], Sarah P. Psutka[6], Edward M. Uchio[7], Hooman Djaladat8, Jay D. Raman[9], Ahmad Shabsigh[10], Stefen Rausch[11], Marc Colombel[12], Marcos Aller[13], Robert Grubb[14], Axelle Mroz[15], Genia Alpert[16], Juli Bravetti[17], Jonathan Coleman[18]

[1]Lille University Hospital, Lille, France
[2]UT Southwestern Medical Center, Dallas, TX
[3]Albany Medical College, Albany, NY
[4]Chaim Sheba Medical Center, Ramat Gan, Israel
[5]Carolina Urologic Research Center, Myrtle Beach, SC
[6]Department of Urology, University of Washington, Seattle, WA
[7]Department of Urology, University of California, Irvine Medical Center, Orange, CA
[8]Institute of Urology, USC Norris Cancer Center, Laton, CA
[9]Pennsylvania State University Hershey College of Medicine, Hershey, PA
[10]Ohio State University Comprehensive Cancer Center, Columbus, OH
[11]Department of Urology, Eberhard Karls University, Tübingen, Germany
[12]Hopital Edouard Herriot, Lyon, France
[13]Hospital Universitario de A Coruna, Coruna, Spain
[14]Medical University of South Carolina, Charleston, SC
[15]Steba France SAS, Versailles, France
[16]Steba/Impact Biotech, Ness Ziona, Israel
[17]Steba France SAS, Versailles, France
[18]Memorial Sloan Kettering Cancer Center, New York, NY

Background: Padeliporfin VTP has shown safety and activity in UTUC in a Phase 1 study (NCT03617003). It is a drug-device combination: IV padeliporfin plus an optical fiber laser delivering near-infrared light endoluminally to tumors. We present interim efficacy and safety results of padeliporfin VTP for LG UTUC from ENLIGHTED, a Phase 3 trial (NCT04620239).

Methods: Open-label Phase 3 trial in USA, EU, and Israel. Main criteria: ≤2 biopsy-proven LG UTUC with index tumor ≤15 mm (kidney) or ≤20 mm (ureter), and no high-grade cytology. VTP is done via retrograde endoscopy under anesthesia in low-light conditions; padeliporfin IV followed by optical fiber (20–40 mm diffuser) placement near lesion. Laser is activated for 10 min. Patients (Pts) receive Induction (ITP) and Maintenance Treatment Phases (MTP). ITP: 1–3 VTPs at 4-week intervals until CR or failure at Primary Response Evaluation (PRE). Primary Endpoint: CR at PRE (28 ± 3 days post last treatment) by endoscopy and negative cytology. Pts with CR move to MTP, evaluated every 3 mos, with VTP for recurrences up to 12 mos. After MTP, pts followed 48 mos for long-term outcomes. Target: 100 pts.

Results: Interim analysis per protocol at ~50% evaluable pts, cut-off 5 Nov 2024. CR 73%, Partial Response 13.5%, Recurrence 10.8%, Progression 2.7%, ORR 86.5%. Most common TEAEs (Grade 1–2, short duration): hematuria 14%, flank pain 10%, procedural pain 6.4%, dysuria 5.2%, UTI 5.2%, abdominal pain 4.7%, vomiting 4.7%, fatigue 4%, nausea 3.5%. Sixteen (9.2%) Grade 3 SAEs occurred. Grade 3 VTP-related events (renal colic, flank pain) resolved within 2 days.

Conclusions: Padeliporfin VTP shows efficacy and safety consistent with earlier data. ENLIGHTED enrollment continues, aiming to support approval of a novel organ-sparing treatment option with clinical benefit.

Preliminary results from LEGEND: a Phase 2 study of detalimogene voraplasmid, a novel, investigational, non-viral genetic medicine for high-risk non-muscle invasive bladder cancer (NMIBC)

Wassim Kassouf[1], Felix Guerrero-Ramos[2], Jen-Jane Liu[3], John A. Taylor[4], Shreyas Joshi[5], Ashish M. Kamat[6], Yair Lotan[7], Paras Shah[8], José Manuel De la Morena[9], Gautier Marcq[10], Vignesh T. Packiam[11], Sam S. Chang[12], Anne Schuckman[13], Katherine Chan[14], Ricardo Rendon[15]

[1]McGill University, Montreal, QC, Canada
[2]Hospital Universitario 12 de Octubre, Madrid, Spain
[3]Oregon Health & Science University, Portland, OR, USA
[4]University of Kansas Cancer Center, Kansas City, KS, USA
[5]Emory University School of Medicine, Atlanta, GA, USA
[6]University of Texas MD Anderson Cancer Center, Houston, TX, USA
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Background: Detalimogene voraplasmid (EG-70) is a novel, investigational, non-viral gene-based immunotherapy for high-risk NMIBC, including BCG-unresponsive disease. The Phase 1 portion of LEGEND (NCT04752722) demonstrated a promising safety profile and an overall complete response (CR) of 73% at any time. Phase 2 is ongoing; preliminary efficacy results of the pivotal Cohort 1 (BCG-unresponsive NMIBC with CIS) and safety for all cohorts are reported here.

Methods: Key eligibility criteria: ≥18 years; ECOG PS 0_2; high-risk NMIBC, ± resected coexisting papillary (Ta/T1) tumors, ineligible for/elected not to undergo cystectomy. Cohorts: BCG-unresponsive with CIS (pivotal Cohort 1); BCG-naïve with CIS (Cohort 2A); BCG-exposed with CIS (Cohort 2B); BCG-unresponsive with high-grade papillary disease without CIS (Cohort 3). Patients received four-doses, 50 mL intravesically at Weeks 1, 2, 5 & 6 of a 12-week cycle x 4 cycles. Primary endpoint: CR rate at Week 48; safety. Secondary endpoints: PFS; CR rate at Week 12, 24, 36 & 96, Percentage of patients with a durable CR at 12 Months.

Results: As of September 13, 2024, 21 patients were evaluable for safety and efficacy in Cohort 1. In the 42 safety-evaluable patients (all cohorts), treatment-related adverse events were observed in 20 (47.6%; all G1/2), most commonly (≥10%): dysuria (21.4%); bladder spasms (19.0%); pollakiuria (11.9%); fatigue (11.9%). Overall CR rate 71%; CR rate 67% at 3 Months and 47% at 6 Months.

Conclusions: Preliminary data from the pivotal Phase 2 portion of LEGEND suggest a promising safety/tolerability profile. Overall, 71% of patients achieved a CR, with 67% achieving a CR at 3 Months and 47% achieving a CR at 6 Months.

Artificial Intelligence and Multiparametric MRI Predict Clinical Outcomes to Neoadjuvant Immunotherapy in Patients with Muscle Invasive Bladder Cancer (MIBC) Undergoing Radical Cystectomy

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Background: PURE-01 pioneered the use of neoadjuvant pembrolizumab (pembro) before radical cystectomy (RC) in patients (pts) with MIBC. The present study aimed to predict pathological response using artificial intelligence (AI) and multiparametric (mp)MRI.

Methods: Pts had matched pre- and post-pembro MRIs, and tumors were segmented. The T2w signal intensities were standardized and used for radiomics (IBSI-compatible pyCERR software) and deep feature extractions (AI-BLADE toolbox using VGG19). For diffusion weighted (DW-) and dynamic contrast enhanced (DCE)-MRI data analysis, we also used AI-BLADE and performed monoexponential modeling and the extended Tofts pharmacokinetic model. The physiological biomarkers included apparent diffusion coefficient (ADC), volume transfer constant (Ktrans), extravascular extracellular space volume fraction (ve), and plasma blood volume fraction (vp). Associations were made with pathological major response (pMR, i.e., ypT<2) and pathological complete response (pCR, i.e., ypT0). Elastic Net and random forests were trained using 5-fold cross-validation. Models were evaluated with ROC curves.

Results: A total of 224 MRIs from 112 pts, 289 radiomic features, 4,096 deep features, ADC, Ktrans, ve, and vp were analyzed. For pMR, the best-performing model was post-pembro with an AUC of 0.96 (95% CI: 0.89–1.00) using radiomic shape feature (surface-to-volume ratio), post-DCE volume, and kurtosis of vp. A radiomics-only model with shape (flatness) and GLCM texture (Joint Avg StdDev) reached an AUC of 0.92 (95% CI: 0.81–1.00). Deep features alone also showed strong performance (AUC: 0.85; 95% CI: 0.69–1.00). For pCR, the best model was also post-pembro, based on radiomics alone (AUC: 0.86; 95% CI: 0.71–1.00). The deep-feature-only model had an AUC of 0.79 (95% CI: 0.59–0.98).

Conclusions: This is one of the first machine learning models using radiomics, deep features, and mpMRI to predict neoadjuvant immunotherapy response in MIBC.

Genomic Landscape of Bladder Pure Squamous Cell Carcinoma Versus Urothelial Carcinoma of the Bladder

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Background: Pure squamous cell carcinomas (SCC) account for 2-8% of bladder cancer cases and lack a standard of care. Treatments used in urothelial carcinoma of the bladder (UCB) are used for pure bladder SCC despite lower response rates. This study compared the prevalence of tumor genomic biomarkers in bladder SCC versus UCB.

Methods: A retrospective analysis was performed to identify patients with bladder SCC that underwent comprehensive molecular profiling using NextGen DNA sequencing (592-gene panel or WES) performed at Caris Life Sciences. A GU pathologist confirmed pure SCC cases. A UCB cohort was included for comparison. Biomarker prevalence was assessed using Chi-squared or Fisher's Exact tests with p-values adjusted for multiple comparisons.

Results: Of 8191 bladder cancer cases, 655 (8%) were coded as having components of SCC. Mixed histologies were excluded and 275 cases (3.4%) were reviewed by a GU pathologist. 169 (2.1%) cases of bladder pure SCC identified. 88 were females (51.1%) and 81 males (47.9%), with a median age of 70 (range 34-89). 4114 patients were coded as UCB. 1136 were female (27.6%) and 2978 were male (72.4%), with a median age of 72 (range 27-89). Pure SCC showed significantly higher mutation rates than UCB in PIK3CA (30.2% vs. 17.3%), CDKN2A (21.9% vs, 5.5%), SMAD4 (4.1% vs. 0.8%), FAT1 (19.8% vs. 4.7%), and PD-L1 IHC (56.7% vs. 40.2%). Pure SCC had significantly lower mutation rates than UCB in KDM6A (10.7% vs. 23.7%), RB1 (5% vs. 21%), and ARID1A (3.6% vs. 24.4%). TMB-H was numerically higher in UCB vs. SCC (39.1% vs. 27.3%, q-value 0.09). No significant differences were found in mutations of histology-agnostic biomarkers with FDA approvals.

Conclusions: Bladder pure SCC tumors demonstrated increased mutations in PIK3CA and CDKN2A, reflecting two potentially targetable pathways. About one-third of these tumors were TMB-H, indicating a population that may benefit from immune checkpoint inhibitor monotherapy or combination strategies.

Spatial Profiling of the Tumor Microenvironment in Plasmacytoid Urothelial Carcinoma

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Background: Plasmacytoid urothelial carcinoma (PUC) is an aggressive histologic subtype of bladder cancer that is frequently present with conventional urothelial carcinoma (CUC). Patients with PUC have worse response to cisplatin-based neoadjuvant chemotherapy, emphasizing the importance of therapeutic development. Understanding PUC tumor biology will provide insight into PUC-specific therapeutic vulnerabilities. PUC is characterized by the loss of E-cadherin by immunohistochemistry (IHC) and previous reports in other cancer types suggest a complex interplay between E-cadherin signaling, induction of epithelial-to-mesenchymal transition (EMT), resulting alterations in the tumor microenvironment (TME), and response to ICB. This study seeks to understand the TME composition in PUC and the interplay between cell types.

Methods: FFPE tissue samples from patients with urothelial carcinoma who underwent surgical intervention was obtained. Hematoxylin and eosin stains were reviewed by a GU pathologist to identify areas of histology consistent with CUC or PUC. IHC for E-cadherin was performed. Spatial transcriptomics was performed using the 10X Genomics Xenium In Situ assay. The expression of 477 genes were profiled in situ using a combination of the 10X Genomics Human Multi-Tissue and Cancer panel and a custom 100-gene panel, specifically curated to interrogate differences in bladder cancer specific genes. Xenium data analysis was performed using a combination of the 10X Genomics Xenium Explorer and R-based packages, including Seurat.

Results: Within areas of PUC histology, CDH1 (gene encoding E-cadherin) expression was absent, and genes associated with EMT signaling were upregulated. Relative changes in immune cells were characterized and PUC-specific cellular neighborhoods were identified, highlighting the impact of EMT signaling on the TME.

Conclusions: Leveraging spatial transcriptomics, this study maps the spatial organization of PUC and highlights potential therapeutic vulnerabilities.

Genomic characterization of upper urinary tract urothelial carcinoma and clonal evolution of intravesical recurrence

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Background: Upper urinary tract urothelial carcinoma (UTUC) is a rare cancer accounting for only 5-10% of all urothelial carcinomas. Patients with UTUC are at high risk of developing intravesical recurrences (IVR) within two years after surgery. The biology of IVR after surgery is poorly understood and urine markers for its detection are lacking. In this study, we characterized the genomic landscape of UTUC to reveal novel therapeutic approaches and investigated the molecular mechanism of development of IVR to identify diagnostic markers for IVR.

Methods: We performed targeted next-generation DNA-sequencing of 571 genes in a multi-institutional international cohort of 390 UTUC patients enrolled retrospectively (n=255) and prospectively (n=135) who received radical surgery. Clonality and evolution were assessed in 67 UTUC-IVR paired cases.

Results: High tumor mutational burden (TMB), prior urothelial carcinoma of urinary bladder, tumors localized in the ureter and FGFR3 mutations corresponded to IVR risk. We identified a novel genomic subtype with MTOR mutations mutually exclusive with FGFR3, TP53 and HRAS mutations, core genes to define previously described genomic subtypes of UTUC. The novel MTOR genomic subtype (13.1%) was exclusively present in patients from Northwestern Europe, which had reduced IVR risk, low TMB, no insertion/deletion mutations and lacked TERT promoter mutations. Clonal evolution of IVR occurred in 81% of cases via four evolutionary paths driven by selection on TERT, TP53, STAG2 and FGFR2/3 mutations. Furthermore, hotspot mutations in TERT, FGFR3 and HRAS were identified as potential markers for noninvasive surveillance by urine testing after surgery.

Conclusions: We identified molecular correlates of IVR with FGFR3 as a potential therapeutic target to reduce IVR risk. The clonal relationship between UTUC and IVR underscores the potential for patient-friendly noninvasive urine tests for surveillance after surgery.

Tracking heterogeneity and the trajectory of neoplastic disease progression in bladder cancer subtypes using spatial transcriptomics and liquid biopsy

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Background: Rare variant subtypes of urothelial carcinoma exhibit distinct prognoses and treatment responses compared to pure urothelial carcinoma; however, their underlying biological drivers remain poorly understood. We investigated their tumor microenvironment and cellular processes using spatial transcriptomics. Concurrently, we monitored tumor burden and disease progression via liquid biopsy from blood and urine in patients undergoing surgery.

Methods: Tumor, blood, and urine samples were collected from patients with urothelial carcinoma at the time of surgery under an ongoing prospective protocol. We developed a computational genomics framework to characterize tumor-stroma interactions at tumor margins and interiors, and to identify aggressive subclones via spatial transcriptomics. Cell-free DNA was profiled by whole genome sequencing, while cell-free RNA was analyzed using a CRISPR-Cas12a–based detection method to capture non-genetic alterations and gene expression changes.

Results: We analyzed 69 tumor and liquid biopsy samples including 58 with pure urothelial carcinoma (9 with carcinoma in situ) and 11 with variant histology (6 squamous, 2 micropapillary, 1 nested, 2 glandular and 1 neuroendocrine). Spatial transcriptomics profiling of a subset of the samples revealed significant subtype-specific differences in cell type composition, inter-cellular signaling, and cellular processes, which collectively suggested divergent mechanisms of microenvironment remodeling. Heterogeneity in the presence and architecture of tertiary lymphoid structures reflected differences in anti-tumor immunity. Cell-free DNA and RNA profiling further supported the dominance of aggressive subclones and provided a means to track tumor progression non-invasively during clinical management.

Conclusions: Our integrated genomics approach provides valuable insights into the progression trajectories of bladder cancer subtypes and facilitates the identification of aggressive malignancies.

Artificial Intelligence Predicts Molecular Subtypes and Outcomes in Muscle-Invasive Bladder Cancer from Whole Slide Images

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Background: Muscle-Invasive Bladder Cancer (MIBC) is a heterogeneous disease with distinct molecular subtypes influencing prognosis and treatment response. However, RNA-based profiling remains costly, time-consuming, and hampered by intra-tumoral heterogeneity. Our aim was to develop an Artificial Intelligence (AI) approach to infer molecular subtypes from routine histology slides and assess its prognostic value in patients treated with neoadjuvant chemotherapy (NAC).
Methods: We trained an AI model to predict the expression of 848 subtype-associated genes from whole-slide images of transurethral resection of bladder tumors, enabling spatial molecular subtyping at the tile level. The model was trained on 298 NAC-treated patients from the VESPER trial and evaluated on three external cohorts (COBLANCE, N=220; SAINT-LOUIS hospital, N=30; TCGA, N=311), encompassing diverse protocols and scanner types. Spatial transcriptomics (Visium HD) from three VESPER patients validated the spatial consistency of inferred gene expression profiles.
Results: Our approach achieved a ROC AUC of 0.89 ± 0.017 for subtype prediction, with over 90% of genes significantly predicted, showing that morphology reliably reflects transcriptomic deregulations. Predicted expression maps revealed spatially coherent patterns and identified intra-tumoral subtype heterogeneity. Notably, basal/squamous tumors inferred by the AI model were associated with worse progression-free survival (PFS) following NAC (log-rank $p = 0.0097$), consistent with RNA-based subtypes (log-rank $p = 0.001$).
Conclusions: This AI tool enables accurate and spatially resolved inference of gene expression and molecular subtypes in MIBC without direct sequencing. These findings could improve patient stratification in clinical practice and support the design of more targeted clinical trials.

The innate urothelial response to BK Polyomavirus infection drives interferon-mediated APOBEC3A mutational damage.

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Background: Growing epidemiological evidence from renal transplant recipients identifies BK Polyomavirus (BKPyV) as a risk factor for urothelial carcinoma (UCa). In support of this, we previously identified the capacity of BKPyV infection to induce antiviral APOBEC3 cytidine deaminase enzymes, which drive the major mutational burden in UCa. In the general population, it remains unclear how BKPyV could induce cancer-initiating APOBEC3 enzymes while remaining absent from the arising UCa. Here, we explore the innate urothelial response to BKPyV as a driver of APOBEC3 expression in human urothelium.

Methods: Mitotically quiescent, differentiated human urothelial cell cultures were infected with BKPyV. Expression of antiviral cytokines, JAK-STAT components, and APOBEC3A was analyzed via mRNA-seq, RT-qPCR, and immunoblotting. APOBEC3A localization was assessed by immunofluorescence microscopy. Nanopore sequencing was used to evaluate BKPyV-induced mutations.

Results: BKPyV infection of cultured urothelium induced a robust antiviral response as evidenced by the significant induction of IFNL1 and IFNK, enrichment of interferon-stimulated gene sets, phosphorylation of STAT2 and expression of the interferon-stimulated protein, IFIT1. Supporting interferon (IFN) signalling as a driver of APOBEC3A, exogenous treatment with TypeI/II/III IFNs all significantly induced APOBEC3A and blockade of IFN signalling during infection with JAK inhibitors prevented APOBEC3A-induction. Visualisation of BKPyV-induced APOBEC3A revealed the presence of APOBEC3A-positive virus-negative ‘bystander’ cells. BKPyV-infection led to the formation of APOBEC3 mutational signatures in normal urothelium, similar to those observed in UCa.

Conclusions: These data support a novel ‘bystander hypothesis’ for urothelial carcinogenesis in which BKPyV viral infection drives IFN-dependent APOBEC3A-mutagenesis in uninfected bystander cells that increase the risk of UCa development.

Integrated transcriptomic and spatial immunophenotyping reveals T cell exhaustion and B cell associated immunosuppressive niches in BCG-Unresponsive NMIBC

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Background: More than 50% of patients with non-muscle invasive bladder cancer (NMIBC) experience early recurrence or progression following treatment with Bacillus Calmette Guerin (BCG) immunotherapy. Our findings revealed that treatment naïve tumors exhibit increased immune exhaustion signatures. Aging murine model further showed a critical role of exhausted B cells in regulating anti-tumor effector T cell responses. This study was conducted to define atypical B cell associated local and systemic effector T cell responses in BCG treated patients with high-risk NMIBC.

Methods: Transcriptomic profiles from tumors with high-risk NMIBC were analyzed to determine the expression patterns of adaptive immune exhaustion signatures and their association with survival outcomes. Systemic profiles of isolated T cells, from patients undergoing BCG induction, were characterized using flow cytometry and single cell RNA-sequencing longitudinally. Single cell spatial sequencing and multiplex immunofluorescence was performed to investigate T cell functional states in the context of B cell neighborhoods.

Results: Increased B cell exhaustion associated genes in pre-BCG tumors were associated with shorter recurrence and progression-free survival. Further single cell analysis showed expansion of terminally differentiated effector subsets of both cytotoxic and helper T cells in patients with poor response to BCG therapy. Cellular neighbourhood analysis revealed a close association of exhausted B cell with regulatory T cell subsets in tumors from BCG non-responders.

Conclusions: These findings highlight a potential link between expansion of terminally differentiated T cells following BCG exposure in the induction phase and poor anti-tumor immune response. Spatial proximity of exhausted B cells underscores their potential immune suppressive microenvironment within treatment naïve tumors. Targeting these pathways in combination with BCG immunotherapy will improve therapeutic efficacy and outcomes in high-risk NMIBC patients.

Expanding the treatment Landscape of FGFR-Targeted Therapy in Bladder Cancer: Integrative Genomic and Functional Profiling of Patient-Derived Organoids

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Background: Erdafitinib, an FGFR-targeted therapy, is EMA-approved for patients with susceptible FGFR3 alterations [FGFR-ALT] that activate the FGFR pathway. However, FGFR pathway activation can also occur in tumors lacking FGFR-ALT through mechanisms like increased FGFR protein expression. To address this, we developed the FGFR Predictive Response Signature (FGFR-PRS) to reflect FGFR pathway activation independent of FGFR-ALT, aiming to identify patients who might benefit from FGFR-targeted therapies. However, its clinical utility and validation in real-world patient samples have not yet established. This study aimed to address this gap by validating the FGFR-PRS using patient-derived bladder cancer organoids (PDOs).

Methods: FGFR3 mutation status (mutant [MT] vs wild-type [WT]) was determined for both patient tissue and PDOs using SNaPshot genotyping. PDOs were treated for 72 hours with serial concentrations of erdafitinib to generate dose-response curves. RNA sequencing was performed to calculate mRNA-based FGFR-PRS scores, classifying samples as PRS-high or PRS-low.

Results: Out of 30 PDOs, 6 harboured FGFR3 MT and 24 were WT. FGFR3-MT PDOs exhibited high sensitivity to erdafitinib (IC50: 4.5 nM to 200 nM). Notably, 21/24 FGFR3-WT PDOs were also sensitive (IC50: 4.5–47.4 nM), while 3 were resistant (IC50: 3000–4700 nM). FGFR-PRS was determined in 9 PDOs; both FGFR3-MT and WT-sensitive PDOs had high FGFR-PRS scores (0.02 to 0.45), whereas resistant FGFR3-WT PDOs had low FGFR-PRS scores (_0.3 to _0.6).

Conclusions: Our study demonstrates that erdafitinib sensitivity is not limited to PDOs with FGFR alterations but also includes FGFR-WT tumors with high FGFR-PRS scores. Conversely, FGFR-WT PDOs with low FGFR-PRS scores were significantly resistant to treatment. These findings suggest that the FGFR-PRS score can serve as a valuable predictive biomarker for FGFR-targeted therapy, potentially expanding patient selection beyond genetic alterations alone.

Characterization of Growth Differentiation Factor 15 (GDF-15) as a Therapeutic Target in Urothelial Carcinoma

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Background: Limited response rates to immune checkpoint inhibitors (ICI) pose a challenge in the treatment of metastatic urothelial carcinoma (mUC). The cytokine Growth Differentiation Factor 15 (GDF-15) is expressed in various tumor entities and can suppress anti-tumor immune responses. A clinical study demonstrated that treatment with an anti-GDF-15 antibody (visugromab) in combination with nivolumab can induce durable remissions in otherwise ICI-resistant mUC patients.

In this study, we analyzed GDF-15 expression in patients with UC and characterized its association with the tumor microenvironment (TME).

Methods: We assessed spatially resolved gene expression in tumor and stromal segments from 147 tumor samples of patients with muscle-invasive bladder cancer (MIBC) using the NanoString GeoMx Digital Spatial Profiling platform. In total, 305 segments were included in the analysis.

Results: Increased GDF-15 expression in tumor cells significantly correlated with the presence of lymph node metastases in MIBC patients after radical cystectomy ($p < 0.05$). Luminal molecular subtypes showed significantly higher GDF-15 expression compared to Ba/Sq, NE-like, and stroma-rich tumors. Elevated GDF-15 expression in tumors was associated with an immunosuppressive TME, characterized by reduced numbers of CD8_ T cells, dendritic cells, and NK cells, as well as increased numbers of fibroblasts.

Conclusion: The therapeutic target GDF-15 is highly expressed particularly in molecular luminal UC. Its higher expression is associated with an immunosuppressive TME. Further investigations are ongoing to assess the link between GDF-15 expression and response to ICI therapy.

Phenotypic screening of bladder cancer cultures to find toxic natural products

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Background: Bladder cancer accounts for 4 % of cancer diagnoses in the US. Current treatments primarily involve transurethral resection of bladder tumors (TURBT) and immunotherapy with Bacille Calmette-Guerin (BCG). Despite the efficacy of TURBT, issues with residual tumors persist. Our group sought to identify natural compounds that could be used as toxic agents against bladder cancer cells.

Methods: We utilize Cell Painting to phenotypically-screen a set of 1684 natural compounds against seven bladder cancer cell lines for toxicity. Using multiparametric high content analysis termed SPACe, we identified promising candidates that underscore the potential of flavonoids in targeting bladder cancer cells and elucidating their mechanisms of action.

Results: We discovered 42 natural product compounds that were toxic to at least three different bladder cancer cell lines. Some compounds such as xanthohumol show promise in reducing cancer cell viability by altering lipid metabolism. Others, like colchicine, halt mitosis. We further test the effectiveness of hit compounds in subsequent orthogonal 3D spheroid and chorioallantoic (egg) membrane model systems.

Conclusions: Overall, our studies emphasize the role of innovative multiparametric high throughput screening methods in drug discovery, and finding potential synergistic effects of combining flavonoids with existing therapies like BCG/Her2 for improved bladder cancer treatment outcomes.

An Immunohistochemical Panel for Predicting Response to Neoadjuvant Chemotherapy in Muscle-Invasive Bladder Cancer

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Background: This study aimed to develop a practical, low-cost immunohistochemical (IHC) panel that can be incorporated into routine pathology to predict response to neoadjuvant chemotherapy (NAC) in muscle-invasive bladder cancer (MIBC).
Methods: Tissue microarrays were created from 183 pre-treatment transurethral resection specimens (122 NAC-treated, 61 NAC-naïve) later managed by radical cystectomy. Pathologic response was defined as ypT≤1 N0 (responders) versus ypT≥2 or N+/M+ (non-responders). Fourteen IHC markers spanning differentiation, proliferation and progression (GATA3, CK20, UPKII, FOXA1, CK5/6, CK14, Ki-67, N-cadherin, p16, Cyclin D1, RB1, HER2, FGFR3) were scored by a genitourinary pathologist (H-score; RB1 wild-type vs altered; HER2 0–3+; Ki-67 % positive cells).
Results: NAC achieved a 39 % pathologic response, lengthening median overall survival to 37.0 months versus 14.0 months in NAC-naïve cases ($p < 0.001$) and delaying recurrence (31.0 vs 9.2 months, $p < 0.001$). Among NAC-treated tumours, responders had lower Cyclin D1 (median H-score 108 vs 151, $p = 0.026$) but higher UPKII (174 vs 126, $p = 0.022$) and p16 (179 vs 113, $p = 0.018$). RB1 alteration was enriched in non-responders (92.1 % vs 63.2 %, $p = 0.001$). Recurrence correlated with elevated CK14 (89.5 vs 43.8, $p = 0.030$) and altered RB1 (94.7 % vs 70.5 %, $p = 0.032$). Longer survival was linked to higher FOXA1 (163 vs 97.9, $p = 0.015$), whereas high CK14 trended toward poorer survival (96 vs 42.5, $p = 0.053$).
Conclusion: A streamlined panel of Cyclin D1, UPKII, p16, RB1, FOXA1 and CK14 stratifies NAC sensitivity and prognosis in MIBC using routinely available tissue. Ongoing work will test these markers in NAC-naïve tumours and validate them across external multi-institutional cohorts.

Tumor mutational burden (TMB) predicts the outcome of patients (pts) with muscle-invasive bladder cancer (MIBC) undergoing immune-checkpoint inhibitor (ICI)-based neoadjuvant therapy

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Background: ICIs have demonstrated efficacy as neoadjuvant therapy in MIBC, both as a monotherapy or in combination. The identification of outlier responders to ICI remains challenging in MIBC.

Methods: TMB values from baseline transurethral resection of bladder tumor (TURBT) samples of MIBC pts treated with neoadjuvant ICI across PURE-01 (NCT02736266), SURE-02 (NCT05535218), and NURE-Combo (NCT04876313) trials were analyzed as continuous values (optimal threshold analyzed with Youden's index) or at defined cutoffs towards pathological/clinical complete response (CR: yT0N0-x, at radical cystectomy [RC] or re-TURBT), event-free survival (EFS) and overall survival (OS). Logistic regression models were run for CR and Kaplan-Meier curves were used for EFS/OS by TMB.

Results: A total of 213 MIBC pts (85% male, median age 67 years) were included. 54% had clinical stage T2 and 46% T3-4. The median TMB was 10.5 mut/Mb and 90 pts (42%) had CR. The optimal TMB threshold for CR was 13.23 mut/Mb (Se:0.5; Sp:0.81) with a predicted probability (PP) of 43.4% (95%CI: 0.36-0.50). Incremental PP was seen with increasing TMB values. TMB ≥20 threshold (14% of pts), PP was 53.5% (95% CI: 0.43–0.63), with significant association with CR at multivariable analysis (OR: 3.41, 95%CI 1.48-8.39; AUC: 0.65). TMB ≥30 was the lowest threshold associated with a lower 95%CI limit>50% (PP 67.6%; 95%CI: 0.51–0.80). No significant effect by therapeutic regimen was observed. With a median follow-up of 63 months (IQR 25-77), 60m-EFS for TMB≥20 pts was 96.7% (90.4-100) vs 72.8% (65.6-80.7), p=0.03, and 60m-OS was 100% vs 78.8%(71.9-86.4), p=0.01.

Conclusions: This is one of the largest analyses assessing TMB as a predictive biomarker for neoadjuvant ICI-based therapy for pts with MIBC. TMB was shown to predict benefit from ICI and to identify exceptional responders, particularly when using a cutoff of at least 20 mut/Mb.

Real-time single-sample Lund Taxonomy tumor classification in the prospective Swedish UROSCANSEQ study

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Background: Conventional pathological assessment is insufficient to capture the complex biology of bladder cancer. Molecular characterization of tumor tissue will be essential for advancing precision medicine through improved patient stratification and treatment guidance. While transcriptomic profiling holds promise for prognostic and predictive applications, its reliability and practical utility in clinical settings have yet to be established.

Methods: Lund Taxonomy (LundTax) subtype classification was evaluated in prospectively RNA-sequenced consecutive transurethral biopsies between November 2018 and February 2022 in the translational/observational UROSCANSEQ study (ISRCTN15459149). To provide a comprehensive evaluation of each biopsy, additional indices of molecular tumor grade (based on the WHO1999 or WHO2022 system), proliferation, progression risk, and infiltration estimators for ten immune and three stromal cell types were developed and incorporated into the LundTax classifier.

Results: RNA-sequencing was successful in 706 of 784 biopsies (90%). The tumor stage distribution was 48% Ta, 27% T1 and 24% ≥T2 (1% Cis). Single-sample real-time LundTax classification recapitulated characteristic subtype-specific mRNA and protein expression patterns, with a distribution of 78% Uro, 13% GU, 5% BaSq, 1% Mes-like, and 1% ScNE. The classification was robust against RNA quality, preprocessing methods, and batch effects.

Conclusions: In the rapidly evolving treatment landscape of bladder cancer there is an unmet demand for robust molecular tumor characterization. We demonstrate that the LundTax single-sample classification system is robust and applicable in a real-time clinical situation where it provides an accurate and detailed readout of tumor subtype as well as molecular indices of tumor grade, proliferation, progression risk, and TME-composition estimates in individual tumor samples.

Bulk and spatial transcriptional signatures of durable response from Durvalumab-based therapy in BCG-unresponsive (BCG-U) non-muscle invasive bladder cancer (NMIBC).

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Background: Novel therapeutic strategies are needed for patients (pts) with BCG-U NMIBC.
Methods: We performed bulk and spatial RNA sequencing (RNAseq) on pre-treatment transurethral resection of bladder tumor (TURBT) samples from pts enrolled in the GU16–243 ADAPT-BLADDER Phase I study that investigated multiple Durvalumab-based regimens. Gene expression was compared between pts that did versus did not maintain a complete response of at least 12 months (12mCR) using differential expression analyses. Spatial RNAseq utilized the Visium CytAssist platform. Tumor and stromal regions were classified using k-means clustering (k=2) based on a tumor-specific gene panel (GATA3, KRT5, KRT19, TP63, CDH1, EPCAM).
Results: Of 28 pts enrolled, bulk RNAseq data was available for 16 pts: 7 12mCR (43.8%), 7 non-responder or CR < 12m (NR) (43.8%), and 2 non-evaluable (NE) (12.5%). We identified 446 differentially expressed genes (DEGs) in 12mCR pts (254 upregulated, 192 downregulated; FDR<0.01). Gene set enrichment analysis highlighted upregulation of TNFA, E2F, G2M, and interferon-gamma response pathways in 12mCR pts and myogenesis in NR pts. Spatial RNAseq data was available for 14 samples (6 12mCR, 6 NR, 2 NE), capturing 372 DEGs. Among these, 165 showed spatially distinct expression: 68 (41.2%) enriched in stromal and 97 (58.8%) in tumor compartments. Thirty genes were associated with 12mCR regardless of spatial compartment, 18 associated with 12mCR in only one spatial compartment (5 stroma, 13 tumor), and 91 associated with NR in only one spatial compartment (61 stroma, 30 tumor) ($p<0.05$).
Conclusions: Our study identifies bulk transcriptional signatures and spatial distribution of DEGs linked to durable response to Durvalumab-based therapy, representing one of the first prospective spatial analyses of durable response determinants to PD-(L)1 directed therapy in BCG-U NMIBC. These results highlight the potential of spatially informed biomarker and therapeutic target discovery.

Leveraging Machine Learning to Identify Interpretable Histopathologic Features in Muscle-Invasive Bladder Cancer for Neo-Adjuvant Treatment Response Prediction

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Introduction: Neoadjuvant chemotherapy (NAC) improves survival in muscle-invasive bladder cancer (MIBC), but only ~40% of patients achieve a pathologic response. The lack of predictive biomarkers limits treatment decisions. While histopathological features offer prognostic insights, their predictive value for NAC response remains unclear. This study develops an interpretable machine learning framework using histopathology images to identify biomarkers of NAC response.

Methods: Pre-treatment TURBT samples from 42 NAC-treated MIBC patients were analyzed. 122 whole slide images were encoded using histopathology foundation models. Increasingly complex models were tested via 5-fold cross-validation: a fully connected neural network (NN), a state-of-the-art multiple-instance learning model (MIL), and a multi-magnification MIL model (MMMIL). To enhance interpretability, high-attention regions were analyzed using HoverNet to extract cellular features (e.g., nuclear area, eccentricity, cell type proportions) and assess differences between responders and non-responders.

Results: MMMIL outperformed NN and MIL at both the slide (accuracy: 0.75 vs. 0.635 and 0.62) and patient level (accuracy: 0.77 vs. 0.67 and 0.65). Morphological analysis of high-attention patches showed non-responders had smaller nuclear size ($p < 0.001$) and greater eccentricity ($p < 0.001$), while responders showed increased solidity ($p < 0.0001$) and more inflammatory cells, including neutrophils ($p < 0.0001$). These findings suggest distinct microenvironmental features linked to response.

Conclusion: We present a deep learning histopathology model that identifies key tissue regions linked to NAC response in MIBC. Morphology within these regions reveals interpretable biomarkers that distinguish responders. This approach offers a strategy to support treatment decisions from pathology slides and advance precision oncology via explainable outputs. Next steps include model validation in a testing cohort (N=81).

Abstracts selected for poster presentation

[listed in alphabetical order by presenter]

Poster# 1

Genomic Landscape of Bladder Pure Squamous Cell Carcinoma Versus Urothelial Carcinoma of the Bladder

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Background: Pure squamous cell carcinomas (SCC) account for 2-8% of bladder cancer cases and lack a standard of care. Treatments used in urothelial carcinoma of the bladder (UCB) are used for pure bladder SCC despite lower response rates. This study compared the prevalence of tumor genomic biomarkers in bladder SCC versus UCB.

Methods: A retrospective analysis was performed to identify patients with bladder SCC that underwent comprehensive molecular profiling using NextGen DNA sequencing (592-gene panel or WES) performed at Caris Life Sciences. A GU pathologist confirmed pure SCC cases. A UCB cohort was included for comparison. Biomarker prevalence was assessed using Chi-squared or Fisher's Exact tests with p-values adjusted for multiple comparisons.

Results: Of 8191 bladder cancer cases, 655 (8%) were coded as having components of SCC. Mixed histologies were excluded and 275 cases (3.4%) were reviewed by a GU pathologist. 169 (2.1%) cases of bladder pure SCC identified. 88 were females (51.1%) and 81 males (47.9%), with a median age of 70 (range 34-89). 4114 patients were coded as UCB. 1136 were female (27.6%) and 2978 were male (72.4%), with a median age of 72 (range 27-89). Pure SCC showed significantly higher mutation rates than UCB in PIK3CA (30.2% vs. 17.3%), CDKN2A (21.9% vs, 5.5%), SMAD4 (4.1% vs. 0.8%), FAT1 (19.8% vs. 4.7%), and PD-L1 IHC (56.7% vs. 40.2%). Pure SCC had significantly lower mutation rates than UCB in KDM6A (10.7% vs. 23.7%), RB1 (5% vs. 21%), and ARID1A (3.6% vs. 24.4%). TMB-H was numerically higher in UCB vs. SCC (39.1% vs. 27.3%, q-value 0.09). No significant differences were found in mutations of histology-agnostic biomarkers with FDA approvals.

Conclusions: Bladder pure SCC tumors demonstrated increased mutations in PIK3CA and CDKN2A, reflecting two potentially targetable pathways. About one-third of these tumors were TMB-H, indicating a population that may benefit from immune checkpoint inhibitor monotherapy or combination strategies.

Poster# 2

Urinary TERT Promoter Mutations as a Robust Non-Invasive Biomarker for Predicting Bladder Cancer Recurrence and Progression

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Background: Cystoscopy remains the gold standard for diagnosis and surveillance of bladder cancer (BC), but it is invasive, costly and has limited sensitivity for low-grade tumors. While current commercial urine biomarkers are not recommended for routine BC management by urological societies, recent advances have identified promising candidate urine biomarkers, including urinary TERT promoter mutations (uTERTpm).

Methods: We assessed the clinical performance of uTERTpm as a non-invasive biomarker for predicting BC recurrence or progression using longitudinal urine samples (n=371 from 72 patients) post-TURBT in the DIAGURO cohort. ddPCR-quantified uTERTpm dominant mutation allele fractions (MAF) were analyzed via Cox models with time-varying covariates, adjusted for known risk factors. Subtype-specific analyses (MIBC/NMIBC) employed competing-risks models to evaluate recurrence/progression hazard ratios.

Results: The median post-TURBT follow-up time was approximately 3 years. A total of 57 total recurrence/progression events was observed. A 10% increase in uTERTpm MAF was associated with a 29% higher risk of recurrence or progression (HR=1.29, 95% CI: 1.10-1.51). In stratified analyses, the association was stronger for MIBC (HR=1.73, 95% CI: 1.22-2.43) compared to NMIBC (HR=1.32, 95% CI: 0.96-1.79).

Conclusions: uTERTpm is a highly accurate, non-invasive biomarker for monitoring BC recurrence, with strong prognostic value, particularly for predicting recurrence or progression to aggressive subtypes. Our results emphasize uTERTpm’s potential for earlier intervention and reduction of reliance on invasive cystoscopy. To confirm these promising results and support clinical adoption, larger, multicentre validation studies are essential to integrate uTERTpm into evidence-based clinical guidelines for BC surveillance.

Poster# 3

Concordance Between Tumor Genomic Profiling and ctDNA for Recurrence Risk in Muscle-Invasive Bladder Cancer

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Background: Detecting and monitoring recurrence in muscle-invasive bladder cancer (MIBC) remains challenging despite multimodal treatment. Combining tumor profiling with circulating tumor DNA (ctDNA) testing may help identify minimal residual disease and guide management. This study assessed the concordance of actionable mutations between tumor tissue and serial ctDNA samples and their association with recurrence in a real-world MIBC cohort.

Methods: This retrospective study included patients receiving curative-intent treatment for MIBC with NAC+RC, RC alone, or bladder-preserving approaches. Targeted multi-gene next-generation sequencing (NGS) was performed using the Altera platform, and serial ctDNA testing was conducted with the Signatera assay. Standard statistical tests were used.

Results: Between 2022 and 2025, 42 patients (median age 67.5 years) underwent curative treatment (48% NAC+RC, 19% RC, 33% bladder-preserving). Overall, 17% were concordant (ctDNA and tumor both with actionable mutations), 36% were tissue-unique, 24% ctDNA-unique, and 24% had no actionable mutations. The median number of non-VUS mutations was 7 (IQR 6-9), with TERT, TP53, ARID1A, and RB1 most common. No single mutation was significantly associated with recurrence or ctDNA positivity (all $p>0.05$), though YAP1 showed a trend for recurrence risk (OR=10.7, $p=0.09$) and CDKN1A for lower ctDNA positivity (OR=0, $p=0.07$). The recurrence model based on non-VUS mutation count showed moderate discrimination (AUC = 0.72).

Conclusion: In this real-world MIBC cohort, concordance between tumor profiling and ctDNA detection was limited. While no significant gene-level associations were found, trends suggest potential value for combined profiling. The low yield of actionable ctDNA findings suggests ctDNA alone may be insufficient for decision-making and should be interpreted alongside tumor profiling. Larger studies are needed to validate these findings.

Poster# 4

Urinary minimal residual disease testing identifies early recurrence and monitors BCG response in patients with high-risk non-muscle-invasive bladder cancer

Henning Bahlburg[1], Moritz Maas[1,2], Alberto Contreras-Sanz[1], Dalia Othman[1], Marie-Pier St-Laurent[1], Jussi Nikkola[1], Kevin G. Phillips[3], Barbara Hamlington[3], Shoujie Chai[3], Peter S. Lentz[3], Nicolai P. Lumbres[3], Vincent T. Bicocca[3], Tuomas Jalanko[4], Trevor G. Levin[3], Dirk Lange[1], Nicolas Zheng[1]

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Background: Intravesical Bacillus Calmette-Guerin (BCG) is the preferred treatment for high-risk non-muscle-invasive bladder cancer (NMIBC). However, many patients recur within the first year despite optimal therapy. Urine tumor DNA (utDNA) offers promise in stratifying therapy response to personalize care and is studied here in a multicenter cohort.

Methods: Urine from patients undergoing BCG for high-grade NMIBC was analyzed by UroAmp. Results were classified as minimal residual disease (MRD)-positive or -negative and assigned a genomic disease burden (GDB) according to a tumor naïve algorithm. A high specificity threshold was defined as GDB>50 post-BCG. Kaplan-Meier recurrence-free survival (RFS) analyses were conducted between groups.

Results: 56% (32/57) of patients were MRD-positive after resection and prior to BCG induction. These patients had an 18-month RFS of 53% compared to 84% for MRD-negative (HR=3.1, p=0.05). Patients who recurred had higher pre-BCG GDBs than those who did not (p=0.021). After BCG induction, 28% (12/43) of patients with negative surveillance evaluation were MRD-positive. MRD-positive patients had a 12-month RFS of 46% compared to 90% for MRD-negative (HR=6.8, p<0.005). Eight patients with post-BCG samples were positive according to the high-specificity GDB>50 threshold and had a 12-month RFS of 25% compared to 91% for negative patients (HR=9.9, p<0.005). Longitudinal analysis of pre- and post-BCG collection classified patients as molecular responders (n=35) or non-responders (n=12). Molecular responders had an 18-month RFS of 87% compared to 13% for non-responders (HR=12.5, p<0.005). utDNA testing identified 10 recurrences with a median lead-time of 3.7 months over standard-of-care, five of whom underwent radical cystectomy.

Conclusions: utDNA analysis reliably stratifies risk of early recurrence in patients with high-risk NMIBC receiving BCG and may enable more personalized treatment decision-making.

Poster# 5

Development of 3D bladder cancer organoid models to investigate the efficacy of cancer immunotherapies.

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Background: Bladder cancer remains a leading cause of cancer-related mortality, and current therapies are often limited by tumour heterogeneity and resistance to treatment. Recent advances in 3D organoid models provide an innovative platform to study cancer biology and evaluate therapeutic strategies.

Methods: Bladder cancer organ cultures were generated using surgical specimens of normal ureter cultured with 12 bladder cancer cell lines. Samples were cultured for four weeks, fixed and immunohistochemical staining of Ki67, GATA3 and FOXA1 was performed.

Results: Surgical specimens of normal ureters have successfully been cultured with 12 human bladder cancer cell lines to generate bladder cancer organoid cultures. These organoids were viable, variably proliferative (based on Ki67 labelling index), heterogeneous in differentiation (based on GATA3 and FOXA1 expression), generated complex tumour-stroma interactions and critically allowed the observation of invasive characteristics (including micro-vascular invasion) of the cell line.

Conclusions: Following the successful incorporation of tumour cells onto ureteric tissues, whole white blood cells will be next integrated into the bladder cancer organoids to further recapitulate the bladder cancer tumour microenvironment. Following the successful incorporation of immune cells, these models will then be used to assess the efficacy of various immunotherapies, such as Bacillus Calmette–Guérin vaccine, immune checkpoint inhibitors and oncolytic virus-based therapies. Immune cell phenotype and tumour growth will be monitored over time, providing valuable insights into immune modulation within the tumour microenvironment and how this determines tumour cell fate following therapy. This approach could lead to the identification of novel therapeutic combinations and biomarkers for treatment response, thus improving therapeutic options for bladder cancer patients.

Poster# 6

APOBEC-Driven CNV–SNV Conflict Shapes Tumor Immune Visibility

Dhanusha Yesudhas[1], Bilal Lone[1], Ecem Unal[1], Ayse Gokce Keskus[2], Arup Chakraborty[1], Salah Boudjadi[1], Jaimie Ryou[1], Sandeep Gurram[3], Andrea Apolo[1], Mikhail Kolmogorov[2], Rouf Banday[1]

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Background: Despite high tumor mutation burden (TMB), many tumors remain resistant to immune checkpoint blockade (ICB). This paradox is notable in APOBEC3-driven cancers, where abundant single-nucleotide variants (SNVs) do not reliably translate inflamed, immune-responsive tumors. Emerging evidence suggests that copy number variations (CNVs), including complex and extrachromosomal events, may suppress tumor immunogenicity. However, the interplay between mutational processes and immune escape is not well understood.

Methods: We analyzed bladder tumors (n = 507) using short- and long-read whole-genome sequencing, bulk RNA-seq, and single-cell RNA-seq from both public and in-house datasets. Validation across more than 2,000 tumors from other cancer types was also performed. Structural variants, APOBEC signatures, and immune features were mapped using tools such as DRAGEN, JaBbA, CoRAL, SigProfiler, and SingleR. Associations were evaluated using machine learning and regression modeling. Functional validation involved gene perturbations and drug-based modulation, combined with RNA-seq, Western blotting, mass spectrometry, and high-resolution imaging.

Results: We identified recurrent amplifications on Chromosomes 1 and 3 as top signals associated with APOBEC3 mutational signatures (SBS2/13) and SNV-driven neoantigen burden. Breakpoints were enriched for APOBEC-target motifs in long-read data, implicating APOBEC in CNV formation—confirmed via high-throughput deamination assays. Co-amplified genes suppressed STAT1 signaling, T cell infiltration, and interferon responses. Perturbation of key genes or targets altered JAK–STAT signaling and T cell migration. Tumors with more cells expressing select co-amplified genes showed reduced ICB responsiveness.

Conclusions: APOBEC3-high tumors exhibit a genomic tug-of-war where CNVs undermine SNV-driven immune visibility. This mechanism of immune escape highlights novel biomarkers and therapeutic targets for high-TMB cancers.

Poster# 7

Gene regulatory Network inference reveals p63 as a key protumoral master regulator in FGFR3-mutated luminal bladder cancers.

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Background: Activating FGFR3 mutations are among the most frequent alterations in bladder cancer, occurring in over 65% of NMIBCs and ~20% of MIBCs, particularly in luminal subtypes. FGFR3-mutant tumors show oncogenic dependency on FGFR3 signaling. Using a genetically engineered mouse (GEM) model, we previously demonstrated that FGFR3 mutations are sufficient to drive bladder tumorigenesis. However, the gene regulatory network (GRN) downstream of FGFR3 remains poorly characterized, limiting strategies to overcome resistance to FGFR inhibitors.

Methods: We inferred an FGFR3-driven GRN using a bioinformatics pipeline that integrates transcriptomic data from bladder cancer cell lines, FGFR3-mutant tumors, and in vitro/in vivo perturbation models (including GEM-derived tumors). We identified FGFR3-regulated transcription factors (TFs) and co-factors, and prioritized essential regulators through CRISPR-Cas9 viability screen public data. Further functional validation included TP63 knockdown, RNA-seq, p63 ChIP-seq, and single-cell RNA-seq.

Results: We identified p63 (TP63) as a key FGFR3-regulated TF. FGFR3 activation led to p63 upregulation in both patient-derived xenografts and cell lines. Single-cell RNA-seq revealed heterogeneous p63 activation associated with basal differentiation within luminal tumors. TP63 knockdown reduced tumor growth, proliferation, and migration in FGFR3-dependent models. Transcriptomic and ChIP-seq analyses confirmed that p63 directly regulates oncogenic programs and is engaged in a positive feedback loop with FGFR3.

Conclusions: Our study defines the FGFR3-regulated GRN in bladder cancer and identifies p63 as a central pro-tumorigenic regulator in FGFR3-mutated tumors despite their luminal differentiation. These findings provide insight into FGFR3-driven tumor biology and offer new avenues to address resistance to FGFR-targeted therapies.

Poster# 8

Time Modeling of Mutational Signature of Bladder Cancer Development from Mucosal Field Effects on the Whole-Organ Scale

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Background: The mechanisms that initiate carcinogenesis involve normal appearing mucosa referred to as field effects. Understanding of these mechanisms is not possible unless they are analyzed in the geographic context of the entire organ.
Methods: We performed whole-organ mapping combined with mutational analysis on nine cystectomies with invasive cancer comprising of 433 mucosal samples corresponding to microscopically normal urothelium (NU), low-grade intraurothelial neoplasia (LGIN; n=243), high-grade intraurothelial neoplasia (HGIN; n=90), and invasive carcinoma (UC; n=100). DNA sequencing was performed to an average depth of 300-500x.
Results: We identified 16562 ± 22028 non-silent mutations per cystectomy. The number of non-silent mutations varied from 2166 to 57794 in each cystectomy. In two maps the hypermutator phenotypes with 48434 and 57794 mutations was identified. The analysis of mutational landscape identified three types of mutations based on their geographic distribution and variant allele frequency (VAF) referred to as _, _, and _. Time modeling revealed that carcinogenesis spans 30 years and can be divided into dormant and progressive phases. The _ mutations developed in the dormant phase and were confined to small areas of bladder mucosa. The progressive phase lasted five years and was signified by the advent of clonally expanding _ mutations, but it was driven by _ mutations which dominated the mucosal field and developed during the last 2-3 years of disease progression. Phylogenetic analysis revealed multiple waves of clonal expansion corresponding to successive cell populations following linear or branching progression. The development of HGIN was preceded by multiple waves of clonal expansion in microscopically normal appearing bladder mucosa.
Conclusions: This study provides a comprehensive characterization of the mutational signature of bladder cancer evolution and identified three types of consecutive mutational waves.

Poster# 9

Impact of Molecular Subtyping and Immune Signatures on Pathological Response following Neoadjuvant Nivolumab, Gemcitabine and Cisplatin in Muscle-invasive Bladder Cancer

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Background: The BLASST-1 trial (NCT03294304) evaluated the use of neoadjuvant nivolumab, gemcitabine and cisplatin and radical cystectomy in muscle invasive bladder cancer (MIBC). Here, we evaluated the ability of transcriptomic signatures to predict pathological outcomes among patients enrolled in the BLASST-1 trial.

Methods: Pathological response rate (PaR, ≤ypT1N0) and pathological complete response (PCR, ypT0N0) at RC were examined as endpoints. TURBT specimens were analyzed with the Decipher Bladder Genomic Subtyping Classifier (GSC), a clinical-grade transcriptome-wide assay (Veracyte, San Diego, CA) and 194 previously reported transcriptomic signatures were retrieved from Decipher Genomic Resource for Intelligent Discovery (GRID, v3.1) database. Multivariable logistic regression analyses for pathologic responses were adjusted for patient age and sex.

Results: Transcriptome data were available for 37/43 (86%); median age was 65 (IQR, 58-70) and 41% were female and 89% were cT2N0. Higher tertiary lymphoid structure (TLS) (OR 3.6, $p<0.03$) and mismatch repair (MMR) (OR 2.9, $p<0.04$) were more likely to achieve PaR (n=24), whereas higher scores for TGF-β signaling (OR 0.03, $p<0.005$), immune exclusion (OR 0.37, $p<0.03$) and cancer associated fibroblasts (CAF) (OR 0.41, $p<0.03$) were associated with no pathological response. Multiple signatures relating to T-cell activity were associated with higher PCR rates (n=13) including TAP1 (OR 3.9, $p<0.005$), TAP2 (OR 3.7, $p<0.007$), cytotoxic T lymphocytes (OR 7.04, $p<0.02$) and CD8+ T-cells (OR 4.7, $p<0.02$) in contrast to M2 to M1 macrophage ratio (OR 0.37, $p<0.02$) and myeloid derived suppressor cells (OR 0.42, $p<0.05$). Signatures associated with pathological responses were elevated in GSC non-luminal subtype (78%) and accordingly 12 out of 13 patients achieving PCR were non-luminal subtype tumors.

Conclusions: These data support molecular biomarkers as a potential tool for personalizing neoadjuvant therapy selection.

Poster# 10

Investigation of carcinogenic effects of polystyrene microplastic on human bladder cancer cells

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Background: In our increasingly industrialized world, Humans are increasingly exposed to microplastics (MPs) and nano plastics (NPs) through ingestion, inhalation, and skin contact. These particles may affect organs such as the brain, liver, kidneys, and bladder by triggering inflammation, altering immune responses, and disrupting the urothelial barrier—a risk factor for bladder cancer and interstitial cystitis.

Methods: We investigated the uptake of 0.5_µm and 0.03_µm polystyrene microplastics by human bladder cancer cells (SW-780 and UMUC-3). Both cell lines showed altered proliferation compared to controls. SW-780 cells responded significantly to 8,000 and 16,000 and 32,000 p/mL, while 64,000 p/mL was toxic. UMUC-3 proliferation increased at all concentrations after 50 hours.

Results: Wound closure was significantly reduced (13_±_6_µm vs. 17_±_6_µm in controls). Live-cell imaging showed strong MP attachment to the cell surface, while confocal imaging confirmed uptake, cytoplasmic accumulation, and nuclear translocation. Both PS particle sizes were efficiently internalized.

Conclusion: Our findings suggest that PS microplastics influence bladder cancer proliferation and migration, hallmarks of cancer progression. The presence of microplastics in the cytoplasm likely alters gene expression or cellular signaling pathways, warranting further investigation. Given the rising plastic pollution, microplastics may be emerging risk factors for bladder cancer, warranting thorough toxicological and epidemiological studies.

Poster# 11

All-stage bladder cancer proteomics map tumor specifity and identify prognostic and predictive subtypes

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Background: Most molecular classifications of urothelial bladder cancer (UC) focus on muscle-invasive UC and are transcriptome-based, relating only indirectly to the therapeutically relevant protein level. Additionally, the introduction of ADCs requires quantitative, proteome-level data about the tumor specificity of their target proteins.
Methods: We performed deep proteomic profiling of a comprehensive cohort by optimized tandem liquid chromatography-coupled tandem mass spectrometry. Data acquisition was validated by immunoblotting, immunohistochemistry and bioinformatic reclassification within existing, filtered transcriptomic data. Protein profiles were individualized and separately evaluated with drug repurposing libraries. Cell viability assays were performed for a panel of twelve UC cell lines to validate these predictions in vitro.
Results: We analyzed 434 samples with 242 tumors and 192 paired normal mucosae, covering all stages of UC for almost 10k proteins. Five distinct and robust proteomic subtypes were identified and validated internally and externally, showing relevant survival stratification. These subtypes were independent from histopathological information. Tumor specificity of all proteins was highly heterogeneous across stages and subtypes. The ADC target NECTIN4, e.g., was overexpressed mainly in non-muscle-invasive UC. Drug repurposing revealed several new candidate drugs, each specific to different proteomic subtypes. In vitro data confirmed drug prediction by subtype for the majority of these drugs.
Conclusions: Proteomic subtypes add independent prognostic information and carry predictive value for several newly identified adjuvant drug candidates. The actual tumor specificity of biomarkers and ADC targets is highly dependent on stage and subtype and calls for individualized patient-specific predictive testing. The comprehensive dataset is available online at cancerproteins.org.

Poster# 12

Disrupting the PDGFRb-driven progression signature leads to enhanced control of bladder cancer

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Background: Bladder cancer (BC) is a significant global health challenge, with high recurrence rates in non-muscle invasive BC (NMIBC) and poor survival in muscle-invasive BC (MIBC). Current immunotherapies are only effective in a subset of patients, emphasizing the need for improved biomarkers and therapeutic targets. Here, we aimed to identify key drivers of BC progression with the potential to enhance existing treatments.

Methods: Using Nanostring nCounter technology, we profiled primary and recurrent tumor biopsies from 12 patients within a prospective cohort of 140 BC patients. This analysis identified a 176-gene signature associated with disease progression. Among those genes, PDGFRb (platelet-derived growth factor receptor beta) emerged as a top candidate. PDGFRb upregulation in MIBC was validated in larger cohort of patients and correlated with poor overall and progression-free survival across several BC datasets.

Results: CRISPR-Cas9-mediated PDGFRb knockout (KO) in human and murine BC cell lines impaired tumor cell migration and reduced expression of epithelial-to-mesenchymal transition genes, as assessed by mRNA sequencing. In vivo, intravesical instillation of PDGFRb KO BC cells in mice resulted in improved survival, better tumor control, reduced metastasis, and a repolarized tumor microenvironment, characterized by increased infiltration of CD8 T cells and B cells, as well as an increase of anti-tumoral macrophages along a reduction of pro-tumoral macrophages, compared to animals instilled with the wild-type BC cells.

Conclusions: Our findings position PDGFRb as a central regulator of BC aggressiveness and immune evasion. Targeting PDGFRb, alone or combined with immune checkpoint blockade, offers a promising strategy to improve patient outcomes.

Poster# 13

Hexaminolevulinate stimulates senescence and induces PD-L1 expression in muscle-invasive bladder cancer cell lines

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Background: Hexaminolevulinate (HAL) is a diagnostic drug used in conjunction with blue-light cystoscopy to enhance the detection of bladder cancer. Our study aimed to further explore the potential therapeutic molecular effect of HAL in bladder cancer.

Methods: Spheroids derived from human MIBC: T24 and J82 cell lines were cultivated in Cero 3D incubator. Spheroids were treated with HAL for 1h followed by exposing them to white or blue light. Controls spheroids were prepared equally but were no exposed to any light source. Functional assay were performed and expression of Ki-67, BAX, BCL-2, HO-1, P-21, and PD-L1 were analyzed.

Results: We found that J82, but not T24, spheroids treated with 8 mM HAL exhibited a significantly decreased ATP production independently of light exposure. In J82 spheroids, caspase assay revealed significantly reduced but in T24 spheroids increased activity. HAL induced cytotoxicity on the first day of exposure. J82 spheroids demonstrated a constant Ki-67 level, while T24 spheroids reduced one. The intrinsic pathway of apoptosis remained balanced under all experimental conditions. Heme oxygenase 1 expression was increased by HAL exposure, whereas light exposure reduced its level in J82 but not in T24 cell lines. P21 gene expression was upregulated after HAL and light exposure, indicating increased cell senescence. Furthermore, PD-L1 expression was upregulated after HAL administration on both J82 and T24 spheroids.

Conclusion: Our findings indicate that HAL alters the behavior of human MIBC spheroids independently from light exposure and reduces cell proliferation. We observed increased p21 and PD-L1 expression at the end of the observation period. This finding may present a therapeutic opportunity, such as checkpoint inhibitors in the management of bladder cancer.

Poster# 14

Investigating Combination Therapies for Non-Muscle-Invasive Bladder Cancer Using Advanced 3D Human Cell-Based Preclinical Models

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Background: Bladder cancer is the 10th most common cancer worldwide, with non-muscle-invasive bladder cancer (NMIBC) accounting for 75–80% of new cases. Despite standard treatments—transurethral resection, intravesical BCG, and mitomycin C (MMC)—NMIBC is associated with high recurrence rates (60–70%) and limited options following BCG failure. A major barrier to therapeutic progress is the lack of reliable preclinical models that accurately reflect the human bladder environment. To address this, we developed the 3D-UHU-TU model—a physiologically relevant platform in which bladder cancer spheroids are integrated into a differentiated human urothelium cultured in 100% urine. This model enables assessment of both anti-tumour activity and toxicity to normal tissue. As a next step toward personalised therapy, we are incorporating patient-derived bladder cancer organoids into this system. BRD4 is overexpressed in bladder cancer and associated with poor prognosis. We hypothesised that combining MMC with the BRD4 inhibitor JQ1 would yield enhanced therapeutic efficacy over monotherapy.

Methods: Bladder cancer cell lines (RT112, T24) were tested in 2D, 3D spheroids, and the 3D-UHU-TU model. Cell viability and death were assessed using proliferation assays, flow cytometry, and confocal imaging. Patient-derived organoids were optimised to embed with the healthy urothelium.

Results: MMC + JQ1 showed synergistic effects, significantly reducing cancer cell viability and increasing apoptosis across models. In the 3D-UHU-TU system, treatment induced tumour-specific changes while preserving healthy urothelial integrity, allowing assessment of efficacy and safety.

Conclusion: Combined MMC and JQ1 treatment enhances anti-cancer effects in bladder cancer models. The 3D-UHU-TU platform, alongside patient-derived organoid integration, offers a robust, human-relevant system for preclinical drug testing and personalised medicine development.

Poster# 15

Outlining The Role of Hypoxia in Bladder Cancer Dedifferentiation and Progression

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Background: Bladder cancer (BC) arises from the urothelium, comprising basal progenitor cells and their differentiated luminal progeny. Early-stage non-muscle invasive BC (NMIBC) typically exhibits a luminal phenotype and are often responsive to therapy. In contrast, the aggressive basal subtype emerges predominantly in muscle-invasive BC (MIBC), with greater metastatic potential and worse clinical outcomes. This study investigates mechanisms driving the transition from luminal NMIBC to basal MIBC.
Methods: To investigate transcriptional differences between subtypes, we analyzed RNA-seq data from 419 MIBC tumours in TCGA-BLCA and flow-sorted SW780 cells, comparing luminal (GATA3_) and basal (CK5_) populations using differential expression and pathway enrichment analyses. Single-cell trajectory analysis reconstructed lineage relationships and traced the emergence of basal phenotypes from luminal precursors. Oxidative stress was modeled by culturing SW780 cells under hypoxic conditions, followed by quantification of differentiation markers, hypoxia-associated genes, and cellular stress indicators via qPCR.
Results: Oxidative stress pathways, including hypoxia and cellular stress responses, were upregulated in basal versus luminal samples from TCGA-BLCA and SW780 cells. Single-cell analysis showed luminal-to-basal transitions marked by transcriptional changes linked to stress and dedifferentiation. Hypoxic culture of SW780 cells increased HIF1_, LOX, and eIF2_, while inducing a basal phenotype (higher KRT5, lower GATA3).
Conclusion: Lineage plasticity, driven in part by hypoxia and oxidative stress, enables luminal BC cells to acquire basal-like features, promoting progression to MIBC. Elucidating mechanisms of luminal-to-basal transition provide insights into the development of lethal BC, supporting earlier detection of high-risk cases and guiding the development of novel therapies.

Poster# 16

Beyond Viral Mimicry: Transposable Element Expression Defines Prognostic Stress-Response Phenotypes in Bladder Cancer

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Background: Transposable elements (TEs) are mobile genetic sequences derived from endogenous retroviruses, comprising ~45% of the human genome. Bladder cancer (BC) exhibits the second highest level of TE transcription across all tumor types, driven by pronounced epigenetic instability. While TE expression has been linked to antiviral immune activation in other cancers, its immunologic consequences in BC remain poorly defined. As immunotherapy is important in BC, we investigated the relationship between TE expression, immune response, and patient outcomes using transcriptomic and immunohistochemical profiling.

Methods: TE expression was quantified using REdiscoverTE across three RNA-seq cohorts: TCGA-BLCA (MIBC, n=412), UROMOL (NMIBC, n=535), and PURE-01 (MIBC, n=82). A 16-TE prognostic signature was identified using LASSO-Cox regression and used to define two TE phenotypes, validated via a random forest classifier. Associations with survival, pathway activity (GSVA, GSEA), immune composition (EPIC deconvolution), DNA methylation (Illumina 450K arrays), and transcription factor binding (HOMER motif enrichment) were evaluated.

Results: TE-defined molecular phenotyping outperformed clinical features in predicting overall survival in both NMIBC and MIBC. The TE-Resilient phenotype (high TE expression) was associated with favorable outcomes and activation of stress adaptation pathways. The TE-Exhausted phenotype (low TE expression) showed poor prognosis, inflammation, and T cell exhaustion. Despite high TE expression, there was no evidence of viral mimicry activation; instead, TE levels defined distinct transcriptional stress-response programs with prognostic relevance.

Conclusion: TE expression defines clinically relevant stress-response phenotypes in bladder cancer, offering a novel and robust biomarker for patient stratification independent of antiviral immunity.

Poster# 17

Vitamin D receptor regulates the invasive capacity of bladder cancer cells through interactions with AKT kinases

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Background: Beyond its role in calcium and phosphate metabolism, vitamin D has been shown to exhibit an anti-cancer potential through binding to the vitamin D receptor (VDR). VDR expression is frequently dysregulated in bladder cancer (BC), and its expression has been correlated with clinicopathological parameters and patient survival. However, the molecular mechanisms by which VDR exerts antitumor effects in BC remain elusive.

Methods: A genome-wide RNAi screening revealed an anti-migratory/anti-invasive role of VDR. VDR levels were analyzed in cells derived from normal ureter epithelium as well as in non-invasive and invasive BC cell lines. Immunohistochemistry staining was applied to evaluate VDR levels in human and murine BC. Knockdown and overexpression experiments were performed to investigate the impact of VDR on the invasive and migratory capacities of BC cells in combination with Boyden chamber and ex vivo porcine bladder invasion assay. Immunoprecipitation and Western blot approaches were applied to investigate molecular pathways.

Results: We show that VDR mRNA and protein levels are higher in non-cancer cells compared to cancer cells. Moreover, VDR mRNA and protein levels were higher in non/low-invasive BC cell lines compared to highly invasive BC cell lines. Importantly, VDR levels were lower in human and murine BC compared to normal tissues. Functional experiments show that VDR impairs the invasive and migratory capacities of BC cells. Elucidating the molecular mechanism revealed that VDR inhibits Akt/PKB signaling pathways by directly binding AKT1-3, suggesting that VDR inhibits AKT-phosphorylation by physical interactions.

Conclusions: The present results indicate that VDR inhibits the progression of BC and regulates BC cell invasion by inhibition of AKT pathway activation through physical interactions with AKT proteins.

Poster# 18

Pten loss promotes p53 loss of heterozygosity and immune escape in a novel urothelial organoid model with p53 missense mutations

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Background: Missense mutations in p53 account for over 60% of genetic alterations in bladder cancer, whereas homozygous deletions (HOM) are observed in fewer than 5% of cases. However, most existing mouse models lack Trp53 entirely, limiting the chance to investigate the specific roles of Trp53 missense mutations over HOM.
Methods: To address this gap, we have been working on establishing organoids derived from the mouse bladder urothelium harboring a Trp53 missense mutation, using genetically engineered mouse in which Cas9 and target gene mutation are specifically expressed in Krt5-expressing cells by Cre-LoxP system.
Results: Organoids derived from Krt5-expressing mouse urothelium (K5-mUrorganoids) demonstrated the crucial role of Pten loss in driving loss of wild-type allele of Trp53 (resulting in Trp53R172H/LOH), which conferred tumorigenic ability to K5-mUrorganoids in athymic mice. These tumors recapitulated the histological and genetic features of the human basal-squamous subtype of bladder cancer. Both Trp53R172H/_; Pten_/_ and Trp53_/_; Pten_/_ organoids formed tumors in athymic mice, however only Trp53R172H/_; Pten_/_ organoids were able to form tumors in immunocompetent mice. The absence of wild-type Trp53 led to increased proliferative signaling, while the presence of mutant Trp53 allele resulted in an immune-excluded microenvironment. Cytokine profiling revealed reduced secretion of cytokines that mediate inflammation and CD8+ T cell activation in organoids expressing mutant Trp53, compared to Trp53-null organoids.
Conclusions: This study highlights the functional role of p53 mutant LOH in bladder cancer, contributing to key cancer hallmarks like sustaining proliferation and evading immune destruction. Furthermore, it introduces a novel immunocompetent mouse model of urothelial carcinoma harboring p53 mutations as a valuable tool for future cancer immunology research.

Poster# 19

Long-Term Oncologic Outcomes and Extracellular Vesicle Signatures in Bladder Cancer

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Background: Anesthetic agents may influence tumor biology via extracellular vesicles (EVs) and tumor-related microRNAs (miRNAs). Prior data showed increased miR-451a levels under propofol anesthesia in bladder cancer surgery [1]. This follow-up study assesses the impact of anesthetic technique on long-term outcomes.

Methods: Patients from the original prospective, randomized trial (Propofol (P) vs. Sevoflurane (S) anesthesia) were followed longitudinally. Clinical endpoints included overall survival (OS) and progression-free survival (PFS). Plasma samples collected perioperatively were reanalyzed to correlate EV concentration and miRNA profiles with long-term outcomes. Restricted mean survival time analysis at the 2-year landmark was used to assess PFS and OS, respectively.

Results: Plasma EV size during (P: 122.4 nm, SD 8.7 nm; S: 109.8, SD 11.7nm, p= 0.002) and at the end of surgery (P: 117.3 nm, SD 10.2 nm; S: 107.1, SD 10.3nm, p= 0.015) was significantly larger in the subgroup of patients undergoing propofol-based anesthesia. Additionally the concentration of EV in the region of interest (50 to 150 nm) was considerably higher in the P-group (9.2 x1011 particles/ml) than in the S-group (4.4 x1011 particles/ml, p=0.014). Similarly, the plasma concentration of EV was higher throughout the surgery in patients who had previously undergone neoadjuvant chemotherapy (NAC) (4.6 x1011 vs. 2.13 x1011 particles/ml, p=0.008). Regarding PFS and OS, no differences were observed based on EV size or concentration. However, the OS was significantly longer in patients who had undergone NAC (23.2 months vs. 19.9 months, p = 0.042).

Conclusions: Propofol anesthesia and prior NAC are associated with distinct EV profiles during bladder cancer surgery. Although EV traits did not correlate with survival, longer OS in NAC patients and sustained miR-451a expression under propofol suggest possible biological relevance.

[1] Gluth L, et al. Cells 2023;12:2503.

Poster# 20

BEST-PATH: Scalable Research Training for Undergraduates in Bladder Cancer Pathology and Pathology Report Analysis

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Background: Health science laboratories train undergraduate researchers through shadowing graduate students. This traditional approach overburdens graduate stu-dents and laboratories while limiting research opportunities for undergraduates. Lack of standardized approaches to undergraduate training also creates inconsistent knowledge and skill development, making training less transferable among laborato-ries. The significant amount of independent learning that accompanies shadowing of-ten causes anxiety, confusion and disorganization. Graduate-student mentors are of-ten not prepared for the teaching role and are simultaneously managing the demands of thesis projects. Shadowing demands significant time and effort from laboratories to train students with varying levels of commitment. As a result, limited experiential learn-ing opportunities are available, deterring eager students from joining laboratories and contributing to oncology research. This study addresses current problems in laboratory training using an e-learning ap-proach developed with pedagogical research called BEST-PATH (Bladder cancer Standardized Training in Pathology, Anatomy, Treatment and Histology). As an alter-native approach for training undergraduate researchers in bladder oncology, BEST-PATH is proposed to improve student performance and satisfaction while eliminating training-associated burden on undergraduate students, graduate students, and la-boratories.

Methods: A randomized controlled trial compared traditional shadowing to BEST-PATH training. Mixed method analysis concluded student assessment scores, satisfac-tion, and self-perceived understanding of concepts were greater among students who completed BEST-PATH.

Results: A comparison of post-test scores showed significantly greater performance among the BEST-PATH cohort ($p = 0.040$).

Conclusions: The BEST-PATH training structure was confirmed as an effective and easily instituted method of training undergraduate pathology and oncology research-ers, scalable to a variety of laboratories.

Poster# 21

Characterization of the Heterogeneity of Antibody-Drug Conjugate Targets in Bladder Cancer on Single-Cell Level

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Background: Antibody-drug conjugates (ADCs), such as Enfortumab vedotin (EV), have shown promising results in treating bladder cancer (BC), but resistance mechanisms remain largely unknown. The heterogeneity of BC regarding ADC-target expression is speculated to contribute to ADC resistance. Therefore, understanding this heterogeneity is crucial for developing new therapies to overcome ADC treatment failures.

Methods: Publicly available scRNA-seq data from muscle-invasive BC patients ranging across different histomorphological subtypes was used to characterize NECTIN-4 expression on a single-cell level. Through CNV analysis identified tumor cells were added into pseudobulks for differential gene expression analysis and gene-set enrichment analysis (GSEA). The in-house-generated, patient-derived cell model VUC26 was sorted based on NECTIN-4 surface expression and tested for their sensitivity to EV and 772 compounds in a high-throughput drug screen.

Results: In line with our hypothesis, NECTIN-4 is expressed at variable levels in muscle-invasive BC. Selected pathways from GSEA point towards a stem cell signature and an epithelial-to-mesenchymal transition in NECTIN-4 negative cells (VUC26 N4-). VUC26 N4- showed a 548-fold increase in resistance to EV (120h) compared to VUC26 N4+. Selected microtubule inhibitors, such as the EV payload MMAE, are more active in VUC26 N4- than in VUC26 N4+. This indicates that the decreased sensitivity to EV seems to be mediated by the lack of NECTIN-4, since VUC26 N4- are markedly more sensitive to the EV payload MMAE than VUC26 N4+.

Conclusions: NECTIN-4 exhibits intra- and inter-tumor heterogeneity in bladder cancer at both RNA and protein level, influencing sensitivity to EV and specific transcriptional signatures. Additionally, we aim to associate NECTIN-4 expression levels with distinct molecular cell profiles and identify transcription factors involved in NECTIN-4 expression.

Poster# 22

Multi-level characterization of new patient-derived bladder cancer models

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Background: Despite the recent advancements in treating BC, a substantial proportion of patients experiences treatment failure. High heterogeneity of BC hampers the development of successful treatments. Furthermore, there is a lack of preclinical models faithfully recapitulating BC biology. The main objective of this study is to generate patient-derived BC models, with the purpose of providing a novel platform for compound testing and identifying factors responsible for drug resistance and treatment failure.

Methods: Tumor pieces corresponding to muscle-invasive BC were brought into culture under conditions best suited for the outgrowth of malignant cells. Models from more than 50 patients have been successfully generated and subjected to Whole Exome Sequencing (WES) and bulk RNA sequencing (RNA-seq). A high-throughput, 1000-compound, drug screen was performed to assess the pharmacological profiles to be further integrated with molecular and clinical information.

Results: RNA-seq data groups the models into 3 different clusters based on gene-expression signatures. Based on molecular features, we can assign the models to defined molecular consensus classes. WES analysis shows copy number alterations and gene mutations frequently seen in BC. Drug-screen data reveals both pan-active and selectively-active compounds. There is a correlation between molecular and histological subtypes with responses to certain compound classes. Further contextualization of all levels of information obtained by molecular and pharmacological profiling will be done using bioinformatic approaches.

Conclusions: The integrated multi-omic analysis of BC cell lines offers valuable insights into the molecular landscape of BC and identifies potential avenues for personalized therapeutic interventions. These preclinical models are an invaluable tool for compound testing in a defined histological and molecular background and serve as an important starting point for investigating factors of BC aggressiveness and targetable vulnerabilities.

Poster# 23

Augmenting PARP inhibitor efficacy in urothelial cancer cells by combination with a BET inhibitor

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Background: Clinical trials on PARP inhibitors (PARPi) for urothelial carcinoma (UC) reported poor results, particularly in cisplatin pre-treated UC. PARPi are effective in BRCA1/2 mutated cancers (BRCAness), which are rare in UC. We reported on pharmaceutically induced BRCAness in UC cells (UCCs) by bromodomain and extra-terminal motif inhibitors (BETi). Here, we investigated the synergistic effects of combined treatment with PARPi Talazoparib (Tala) and BETi Birabresib (Bira) in UCCs and their cisplatin-resistant sublines (LTTs) and patient-derived organoids (PDOs).
Methods: Dose response and synergism was analysed in T24 and J82, their LTTs, PDOs from cisplatin-responders/ non-responders and benign HBLAK cells using the Combenefit tool. Low dose combination treatment was analysed for cellular effects.
Results: IC50 of UCCs were in a low µM range. LTTs were more sensitive to Bira. Benign HBLAK cells had similar IC50 compared to UCCs with the exception of J82 being more resistant to Bira (IC50 J82: 20.0 µM; T24: 0.3 µM; HBLAK: 0.2 µM) and RT112 being more resistant to Tala (IC50 RT112: 10.0 µM; T24: 0.2 µM; HBLAK: 0.3 µM). However, strong synergism was achieved in all UCCs by the combination, allowing a dose reduction (0.5x IC50). The combination caused DNA damage, while impairing homologous recombination repair. Cell cycle arrest and apoptosis were induced, long-term proliferation was strongly reduced. Cisplatin-sensitive and resistant PDOs had similar IC50 values for Bira compared to UCCs. Though PDOs responded poorly to Tala treatment (IC50 > 50 µM), the combination again achieved synergistic effects with reduced dosages. Synergy mechanisms were identified by RNA Seq.
Conclusions: The combination of BETi and PARPi had strong synergistic effects on all investigated models, including cisplatin pre-treated and those that were resistant to mono-treatment. Thus, we suggest to improve PARPi efficacy in a wide range of UC patients by combined treatment with BETi.

Poster# 24

Exploring Bladder Cancer Research Priorities - aligning science with patient need

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Background: Bladder cancer is a significant global health concern predominantly affecting individuals over 55; its burden extends beyond clinical outcomes to deeply impact patient quality of life. Recognizing the need to streamline research efforts, a recent priority-setting initiative aimed to identify the most urgent and impactful research questions in bladder cancer, incorporating perspectives from patients, clinicians, and researchers.

Methods: Using a structured consensus approach adapted from Bessa et al, ten top-priority research questions were identified. These focused on improving diagnostic strategies, reducing reliance on invasive procedures, and enhancing patient-specific treatment through molecular and genomic markers. Through live polling and discussion, delegates were invited to reflect on how these research priorities can guide future studies and clinical translation.

Results: Central themes included the potential of urinary and blood-based biomarkers to reduce reliance on invasive procedures like cystoscopy, especially in the surveillance of high-risk non-muscle invasive bladder cancer (NMIBC). Additional priorities addressed the optimization of post-treatment surveillance strategies for muscle-invasive bladder cancer (MIBC), the integration of predictive biomarkers into treatment planning, and the role of genomic profiling in risk stratification and therapy response prediction. Emerging interest in combining immune checkpoint inhibitors with chemotherapy in the neoadjuvant setting for advanced bladder cancer was also explored.

Conclusions: The study not only guides future research funding and policy but also invites continued collaboration to translate these priorities into real-world clinical benefits. This approach underscores a transformative step in optimizing bladder cancer care through research that is both impactful and patient-centered.

Poster# 25

Spatial Assessment of FGFR3 Protein Expression and T-cell Infiltrations Reveals Distinct Distribution Patterns in Upper Tract Urothelial Carcinoma and Normal Urothelium

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Background: Fibroblast growth factor receptor 3 (FGFR3) mutation is enriched in upper tract urothelial carcinoma (UTUC) and was shown to be associated with immune-cold tumour microenvironment. The T-cell depleted phenotype is a barrier to an effective treatment by immune checkpoint inhibitors. FGFR3 inhibition could be potentially useful in repopulating T cells in the tumour. However, heterogenous expression of FGFR3 in tumour tissues may influence treatment response. The relationship of FGFR3 expression and immune cells in the normal urothelium is largely unknown. In this study, we have characterised the spatial gradient of FGFR3 protein expression and tumour immune microenvironment in UT urothelial tumours and in the adjacent normal urothelium.

Methods: Diagnostic FFPE specimens (n=24) were obtained from NHS GG&C Biorepository, UK. Histopathology was reviewed by pathologists (KLWM, JMS). Multiplex immunofluorescence (mIF) panel with FGFR3 (B9), CD3, CD8, PD-1, PanCK was performed using ImmPRESS Polymer reagents (Vector) with Opal-TSA (Akoya Biosciences). QuPath was used for quantitative image analysis.

Results: A high level of heterogeneity was observed in both FGFR3 expression and T-cell infiltration in UT tumours. Two distinct subgroups were identified based on the patterns of T-cell density along FGFR3 expression gradient, negative, low and high. A lower level of T cell infiltration was observed when FGFR3 expression was higher in the immune-cold subgroup and muscle-invasive cases. The normal urothelium adjacent to tumour in the same cohort exhibited a lower FGFR3 expression and higher T cell infiltration than tumours, with T cell infiltration positively correlating to the FGFR3 expression gradient.

Conclusions: The contrasting pattern of T-cell infiltration along FGFR3 expression gradient in UT tumours and the normal urothelium implicate that the effects of FGFR3 on immune cells may differentiate once expressed in tumours.

Poster# 26

Development of 3D bladder cancer organoid models to investigate the efficacy of cancer immunotherapies.

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Background: Bladder cancer remains a leading cause of cancer-related mortality, and current therapies are often limited by tumour heterogeneity and resistance to treatment. Recent advances in 3D organoid models provide an innovative platform to study cancer biology and evaluate therapeutic strategies.

Methods: Bladder cancer organ cultures were generated using surgical specimens of normal ureter cultured with 12 bladder cancer cell lines. Samples were cultured for four weeks, fixed and immunohistochemical staining of Ki67, GATA3 and FOXA1 was performed.

Results: Surgical specimens of normal ureters have successfully been cultured with 12 human bladder cancer cell lines to generate bladder cancer organoid cultures. These organoids were viable, variably proliferative (based on Ki67 labelling index), heterogeneous in differentiation (based on GATA3 and FOXA1 expression), generated complex tumour-stroma interactions and critically allowed the observation of invasive characteristics (including micro-vascular invasion) of the cell line.

Conclusions: Following the successful incorporation of tumour cells onto ureteric tissues, whole white blood cells will be next integrated into the bladder cancer organoids to further recapitulate the bladder cancer tumour microenvironment. Following the successful incorporation of immune cells, these models will then be used to assess the efficacy of various immunotherapies, such as Bacillus Calmette–Guérin vaccine, immune checkpoint inhibitors and oncolytic virus-based therapies. Immune cell phenotype and tumour growth will be monitored over time, providing valuable insights into immune modulation within the tumour microenvironment and how this determines tumour cell fate following therapy. This approach could lead to the identification of novel therapeutic combinations and biomarkers for treatment response, thus improving therapeutic options for bladder cancer patients.

Poster# 28

Using the Computational Histologic Artificial Intelligence (CHAI) platform to predict FGFRm status in bladder cancer

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Background: Assessment of mutation status in tumors is vital for anti-FGFR targeted therapy selection in bladder cancer. A tool that uses existing histologic diagnostic slides could reduce cost with fast turnaround time. We sought to determine if the CHAI platform, which uses deep learning to quantify features from whole slide images (WSI) of H&E specimens, could predict FGFR mutation status.

Methods: Bladder cancer cases with available H&E digitized WSIs and FGFR2/3 tumor genomic calls from The Cancer Genome Atlas (TCGA) were analyzed using the previously described CHAI assay using a 70-30% random train-test split. The model was trained to identify histologic features associated with FGFR2/3 status using random forest classifiers. Thresholds were negative predictive value (NPV) optimized for a rule-out test designed to identify cases where traditional genomic testing would not identify FGFR2/3 mutations. Metrics were calculated on the test set.

Results: 380 cases were analyzed (266 train; 114 test). 59 (15.5%) patients had characterized deleterious FGFR2/3 mutations (FGFRm) with 45 (16.9%) in the training set and 14 (12.2%) in the test set. The model had an area under the curve of 0.89 (95% CI 0.86, 0.91) for FGFRm status. A threshold optimizing for a rule-out approach identified 50 (43.9%) patients as FGFR2/3 mutation-negative, with 98% NPV and 92.8% sensitivity.

Conclusions: A deep learning-powered assay quantifying histologic features on H&E pathology slides can predict FGFRm status using bladder cancer diagnostic specimens without the need for consumptive genomic testing. A rule-out test demonstrated high NPV and sensitivity, allowing for targeted identification of the subset of patients in need of confirmatory testing; this can reduce costs and testing time, and conserve precious tissue specimens. This histologic biomarker could facilitate rapid and efficient access to personalized treatment by informing FGFRm status in patients with bladder cancer.

Poster# 29

Investigating chronic inflammation induced accelerated immune aging and response to Bacillus Calmette Guérin immunotherapy in non-muscle invasive bladder cancer

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Background: Chronic inflammation, accompanied by aging and co-morbidities such as autoimmunity, are factors associated with increased incidence and poor response to BCG in high-risk non-muscle invasive bladder cancer (NMIBC). Treatment with BCG is known to reduce the development and progression of autoimmunity. In contrast, BCG-treated patients with NMIBC with a history of pre-existing autoimmunity often experience poor outcomes. Shared mechanisms of mucosal immune dysregulation exist in the context of carcinogenesis, chronological aging, and autoimmunity. Our prior work identified aging associated pre-BCG systemic and mucosal immune exhaustion as a driver of poor outcomes. This study aims to delineate the mechanisms of chronic inflammation and autoimmunity associated accelerated immune aging and response to BCG in NMIBC.

Methods: Evaluation of immune cell infiltration patterns in tumors from patients with pre-existing autoimmune conditions, treated with BCG for high-risk NMIBC, was performed. A murine model of spontaneous autoimmunity was exposed to BBN carcinogen and treated using intravesical BCG in combination with chemotherapy followed by local and systemic immune profiling.

Results: Despite a younger age, mice exhibited a shorter time to tumor induction and tertiary lymphoid structure formation in the bladder mucosa. Similar profiles were noted in patients with an underlying autoimmune disease. Cancer progression occurred after three intravesical doses in mice treated with BCG alone. Systemic immune profiling showed increase in splenic exhausted B cells and M2-like macrophages following repeated BCG treatment.

Conclusions: This study provides the first evidence demonstrating accelerated immune aging associated mucosal exhaustion as a factor underlying poor response to BCG in patients with pre-existing autoimmune conditions. Findings from this study will lead to development of early stratification and intervention approaches for improving response rates to BCG in patients with high-risk NMIBC.

Poster# 30

Disparities in guideline-concordant care and outcomes in non-muscle invasive bladder cancer: The impact of age and socioeconomic marginalization in a universal healthcare system

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Background: The impact of socioeconomic disparities and marginalization on outcomes in NMIBC patients is poorly understood. Recent US data from SEER suggest that individuals with low household income experience worse survival. However, the extent to which these disparities affect outcomes within publicly funded healthcare systems, such as in Canada, remains unclear.
Methods: The study cohort comprised of NMIBC patients treated between 2005-2023 across 16 Canadian academic and community institutions through the Canadian Bladder Cancer Information System. We examined the association between age (per 10 years), sex, socioeconomic marginalization, and ancestry on recurrence, progression, and cancer-specific mortality. Socioeconomic marginalization was based on the Canadian Marginalization Index, characterizing health inequality based on four validated dimensions: age and labour force, household and dwellings, racialized and newcomer populations, and material resources. Cumulative incidence curves were estimated for time to recurrence, progression, or cancer-specific mortality, with all-cause mortality or non-cancer-specific mortality as the competing risk. We also examined the association between socioeconomic and demographic factors on receipt of repeat TURBT and adequate BCG, after adjusting for clinicopathological risk factors.
Results: In 6915 patients, after adjusting for multiple comparisons, no clinically meaningful differences in recurrence, progression, or cancer-specific mortality were observed across each marginalization dimension (all $p>0.05$). While increasing age was associated with lower odds of receiving a repeat TURBT (OR 0.81, 95% CI 0.75-0.89) or adequate BCG (OR 0.87, 95% CI 0.81-0.93), no such associations were observed across any of the marginalization dimensions (all $p>0.05$).
Conclusions: Reassuringly, we did not find an association between marginalization and NMIBC clinical outcomes, which may reflect the benefits of a universal healthcare system.

Poster# 31

The role of event-free survival as a surrogate outcome in the BCG-naive and BCG-exposed NMIBC setting

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Background: Event free survival (EFS) in non-muscle invasive bladder cancer (NMIBC) is an endpoint defined as the time to the earliest occurrence of high grade disease, persistence of carcinoma-in-situ (CIS), T1 disease, progression (to muscle invasion or metastases), or death from any cause. In BCG-unresponsive NMIBC, it is an FDA-approved endpoint, however its relevance in BCG-naïve, BCG-exposed, or in patients who did not receive BCG remains unknown. Its surrogacy for cancer-specific survival (CSS) and overall survival (OS) has also not been clearly established. EFS is an endpoint of the randomized Phase III Trial of intravesical BCG versus intravesical gemcitabine and docetaxel in BCG-naïve high grade NMIBC (BRIDGE trial) but the results are still pending.

Methods: The study cohort included 3324 patients from an NMIBC database, treated at the University Health Network, Sinai Health System, and Trillium Health Partners in Toronto, Canada from 2005 to 2022. We used Kaplan Meier analyses and Cox proportional hazards to assess whether EFS was predictive of both CSS and OS.

Results: In the entire cohort, a longer time to high-risk recurrence was associated with a better CSS (HR 0.58, 95% CI 0.54-0.63) and OS (HR 0.78, 95% CI 0.77-0.80). Similarly, among BCG-naive patients (n=1521), a longer time to high-risk recurrence was also associated with improved CSS (HR 0.40, 95% CI 0.34-0.47) and OS (HR 0.70, 95% CI 0.68-0.73).

Conclusions: EFS can be considered as a surrogate endpoint for CSS and OS in NMIBC patients in general and not only in BCG-unresponsive disease. As our results were observed in a single Canadian city, additional studies in other countries and settings should confirm our observations.

Poster# 32

Leveraging patient-derived organoids to identify novel therapeutic vulnerabilities in bladder cancer

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Background: Effective biomarkers and new targets are needed to enable personalized medicine and increase treatment efficacy in bladder cancer (BC). Here, we aim at combining molecular analyses and functional drug screening to identify BC subtype- and patient-specific therapeutic vulnerabilities.
Methods: BC patient samples from biopsies or resections were used to generate patient-derived organoids (PDOs). PDOs and matched tumor tissues were characterized using H&E, IHC, immunofluorescence, whole exome sequencing, scRNA-seq, and single-cell multiplexed protein analysis in situ. Over 2,000 compounds were screened on PDOs at 1 µM; those reducing viability by >50% (via ATP-based luminescence and morphology analysis) were considered hits. Hit compounds were further validated using dose-response screens across a 10 pM–10 µM range.
Results: Out of 129 collected samples, 60 grew as short-term cultures (47%) and 16 samples were established as long-term PDO lines (12%), which emulated relevant molecular and histological subtypes of BC. Single-cell transcriptomic and phenotypic analysis allowed a deeper dive into PDO cellular heterogeneity, highlighting model- and subtype-specific features. High-throughput drug screen was performed in six PDO lines, which exhibited distinct levels of sensitivity to specific drugs, correlating with PDO aggressiveness. Out of 2’016 compounds tested, 148 hits were identified (7.3%), uncovering vulnerabilities towards compounds targeting the DNA synthesis, microtubules, histone deacetylases, and tyrosine kinases, as well as antimicrobial agents. A subset of these hit candidates was further validated in dose response analyses, allowing to define a newly-identified “drug repurposing” panel, which is currently being prospectively tested in organoids freshly derived from patients’ samples.
Conclusions: We anticipate to identify repurposed compounds that are preferentially active on certain BC subtypes and molecular predictors of drug response.

Poster# 33

Radiogenomic Analysis of Muscle-Invasive Bladder Cancer using CT-based Texture Analysis

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Background: Urothelial bladder cancer exhibits substantial pathological, molecular, and clinical heterogeneity. Genomic and transcriptomic profiling of muscle-invasive bladder cancer (MIBC) has identified recurrent alterations that inform classification and therapeutic targets. However, evolving mutational landscapes and the infrastructure required for testing limit routine clinical applicability. Computed tomography (CT), used routinely for staging and surveillance, may offer a noninvasive method to infer tumor biology. Radiomics—the extraction of quantitative imaging features—may help link phenotypic imaging signatures to underlying molecular alterations.

Methods: We integrated CT imaging data from The Cancer Imaging Archive with genomic data from The Cancer Genome Atlas for 89 patients with biopsy-confirmed MIBC. An in-house CT-based radiomics pipeline computed 488 texture features capturing brightness distribution, pixel relationships, and structural patterns. Three machine learning classifiers—Random Forest, Extreme Gradient Boosting, and Elastic Net—were trained on radiomics features and evaluated using 10-fold cross-validation with area under the receiver operating characteristic curve (AUC) as the performance metric.

Results: Of 15 recurrent DNA mutations with >10% prevalence in the study population, EP300, FGFR3, and ARID1A were most reliably predicted (AUC = 0.77, 0.76, and 0.75 respectively). Models also identified tumors with high tumor mutational burden (AUC = 0.61), poor-prognosis mRNA signatures (AUCs = 0.73, 0.65), and elevated expression of key cell cycle and apoptosis regulators CDKN1A (AUC = 0.76) and CASP3 (AUC = 0.71). The luminal infiltrated subtype was discriminated from others with an AUC of 0.69.

Conclusions: Our study demonstrates that CT-derived radiomics features can capture biologically and clinically relevant information in MIBC. Radiomics may serve as a scalable, noninvasive adjunct to molecular profiling.

Poster# 34

MNU carcinogen rat model of NMIBC: a robust platform for uncovering single-cell immune complexity following BCG immunotherapy

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Background: We developed a recombinant BCG (BCG-STING) that over expresses cyclic-di-AMP, a STING agonist, which strongly induces STING-mediated type I interferons. To further understand the mechanism of BCG-STING induced immune response in a relevant, immunocompetent model, we characterized the MNU carcinogen rat model of bladder cancer and analyzed single-cell immune cell complexities comparing MNU-induced tumors treated with wild-type (WT) BCG or BCG-STING.
Methods: MNU instillations were administered to induce bladder tumors in rats. Whole bladders with stage T1 disease were processed for bulk RNA sequencing and subtyped based on current molecular classifiers. Basal and luminal protein marker expression was validated by immunohistochemistry (IHC). Tumor infiltrating immune cells were inferred from gene expression data and T-cell infiltration was validated by IHC. Next, rats with MNU-induced tumors were treated once per week with either WT-BCG or BCG-STING for a total of 6 instillations and whole bladders were harvested for single-cell RNA sequencing.
Results: The MNU carcinogen rat model of NMIBC retains protein expression of both luminal and basal makers, while gene expression data resulted in clustering of these tumors with human and murine luminal papillary tumors. Immune cell inference showed high immune infiltration similar to human and murine basal/squamous tumors. BCG-STING induced greater induction of immune cells globally, and specifically induced B-memory, T-regs, CD8 NK cells and Th1 cells within the lymphoid compartment and myeloid derived suppresorc cells (MDSCs), tumor associated macrophages (TAMs) and dendritic cells within the myeloid compartment.
Conclusions: Mechanisms of superior efficacy of BCG-STING in the MNU rat model of NMIBC are driven primarily by Th1 and B-cell responses. Targeting elevated MDSCs and TAMs along with BCG-STING may further improve efficacy and highlights potential targets for novel combination therapies.

Poster# 35

Exploring cisplatin-gemcitabine's impact on GLUT1 and KDM3A expression patterns in bladder cancer

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Background: The consistent pattern of bladder cancer (BlCa) patients experiencing recurring episodes poses a significant clinical challenge. Currently, BlCa treatments are primarily based on broad categories. Given that, the main goal of the present work is to identify novel molecular markers that can inform tailored treatment strategies in BlCa, towards precision medicine.

Methods: In a discovery setting, BlCa tissue slices were cultured ex vivo and treated for 48 hours with either vehicle or standard chemotherapy. Subsequently, tissue was sent for spatial transcriptomic (ST) analysis. Ultimately, a retrospective cohort of fifty-six BlCa patients treated with neoadjuvant chemotherapy (NAC) was used for validation of ST findings.

Results: Spatially, chemotherapy-treated BlCa tissue was dominated by distinct cell subpopulations. Interestingly, the most prevalent cluster was driven by Hypoxia. Among the most significant genes supporting hypoxia in this cluster, GLUT1 (SLC2A1) and KDM3A, stood out as promising makers. Immunostaining validation revealed that GLUT1 was significantly upregulated, while only a trend for KDM3A, in other ex vivo tissues after cisplatin+gemcitabine treatment. KDM3A co-localized with GLUT1 in some of the cases. Retrospectively, for the purpose of clinical translational validation, higher GLUT1 (3+) was associated with incomplete response to NAC. Moreover, increased GLUT1 after NAC has the potential to identify patients who will need closer post-surgical surveillance, due to the higher recurrence risk. Unlike GLUT1, KDM3A does not appear to be a predictive biomarker for treatment response. However, its potential involvement in mechanisms of chemotherapy resistance needs to be further investigated.

Conclusion: Altogether, these findings indicate a possible link between GLUT1 and poor prognosis in BlCa patients. Identifying non-responders to NAC early could guide clinical decisions towards alternative treatments.

Poster# 36

Microbiome-Based Predictors of Response to Immune Checkpoint Inhibitors in Urological Cancers

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Background: Immune checkpoint blockade (ICB) has improved outcomes in urothelial and renal cell carcinoma (UC, RCC), yet response rates remain heterogeneous. The gut microbiome is increasingly recognized as a modulator of ICB efficacy, suggesting it may hold predictive value.
Methods: We prospectively collected pre-treatment stool samples from 30 patients (19 UC, 11 RCC) treated with ICB between 2020–2024 at two oncology centers. Shotgun metagenome sequencing was performed. Discriminative taxa and metabolic pathways most strongly associated with clinical endpoints were identified using LEfSe analysis separately in UC and RCC. In parallel, literature-based taxa were selected through manual curation. Taxonomical and metabolic data as well as antibiotic (AB) use were correlated with overall survival (OS), progression-free survival (PFS), overall response rate (ORR), and disease control rate (DCR) endpoints.
Results: Among literature-derived taxa, Akkermansia muciniphila, the Blautia genus, and the Mycoplasmatota phylum were validated as negative predictive markers for ICB efficacy in both the UC-only and combined UC+RCC cohorts. From the LEfSe analysis Bacteroides stercoris and the Butyricimonas genus emerged as most promising, with the former acting as a negative and the latter as a positive predictor. In both cohorts, three isoforms of the arginine biosynthesis pathway showed consistent correlation with multiple endpoints as markers of non-response. AB administration within 3 months prior to ICB significantly reduced OS, PFS, and DCR, an effect not observed with wider or narrower time windows.
Conclusions: Our data validate known microbial biomarkers and discover new taxa and pathways potentially predictive of ICB efficacy while highlighting AB use within 3 months of ICB treatment as a negative predictor of outcome. These findings support the integration of taxonomic and functional microbiome profiling into personalized immunotherapy strategies.

Poster# 37

A universal molecular map of bladder cancer: from cell lines in vitro to tumors and back using co-regulatory networks

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Background: Non–muscle-invasive (NMIBC) and muscle-invasive (MIBC) bladder cancer (BC) transcriptomic data have yielded important insights, but remain fragmented and technically complex. Existing molecular classifications have improved understanding but lack a unified framework that integrates both stages. A centralized, systems-level platform incorporating regulatory programs is needed for integrative modeling and translational application.

Methods: We developed BLCA-cRegMap (https://blca.cregmap.com), a web resource offering a co-regulatory network view of BC heterogeneity and progression. Using cell lines DepMap transcriptomes, we inferred BLCA-CoRegNet—491 transcription factors (TFs)/co-TFs and 6,374 targets—via the hLICORN algorithm. This network was projected onto a curated meta-cohort of 2,745 tumor transcriptomes to generate BLCA-CoRegMap, a causal regulatory manifold enabling: (1) signal enhancement and noise reduction; (2) dataset integration without batch effects; and (3) alignment of tumor and cell-line profiles. CoRegQuery allows users to visualize, annotate, and project external data onto these references.

Results: Nine molecular subtypes were identified out of 2,745 tumors (BLCA-CoRegMap): luminal (Lum1/Papillary: 21%, Lum2/OXPHOS: 15%, Lum3/GU: 14%, Lum4/ECM: 12%), basal/squamous (Mixed/Sq: 8%, Ba/Sq: 13%), EMT-Stroma (Stroma-rich: 12%, EMT-ClaudinLow: 2%), and neuroendocrine (NE-like: 2%). These spanned NMIBC and MIBC stages, challenging traditional dichotomies. Subtypes showed distinct prognoses in both NMIBC and MIBC, with novel regulatory programs validated via large-scale CRISPR-Cas9 screens. Lum2, Lum4, Stroma-rich, and NE-like subtypes lacked adequate cell line representation.

Conclusions: We propose a unified molecular classification bridging NMIBC and MIBC, providing a foundation for improved biological insight, prognostic stratification, and translational research. Prospective validation and clinical implementation are needed.

Poster# 38

Biologically-informed machine learning identifies a new clinically-actionable MIBC subgroup with NRF2 overactivity

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Background: Muscle-invasive bladder cancer (MIBC) is a diverse disease where gene expression profiling has failed to provide clinically-actionable subgroups. Existing classifiers built on tumour expression data subdivide MIBC across the continuum of differentiation and immune status, confounded by variable sampling of the tumour microenvironment. It is essential we refine and develop our computational approaches to find distinct and treatable patient groups.

Methods: To focus on tissue-specific urothelial biology, we generated co-expression networks from histologically normal bladder (n=88), selecting genes commonly expressed across three differentiation states. Transcription factors (TFs) were prioritised in the network, with 98 TFs consistently emerging as highly-interconnected hub genes.

Results: Stratification of The Cancer Genome Atlas (TCGA) MIBC cohort using these 98 TFs revealed a novel subgroup of 20 basal tumours characterised by the detoxification and glutaminolysis activity of NRF2 (NFE2L2), with very poor outcomes (15% 2-year survival). These tumours were highly invasive, immune exclusionary, and had signatures of PI3K, MTOR and RA signalling. NRF2 overactivity renders these tumours resistant to standard MIBC interventions, but provides an opportunity for repurposing NRF2 inhibitors used in lung squamous carcinoma (LUSC). Intriguingly, while 11 patients in our subgroup had canonical activating NRF2/KEAP1 mutations, all MIBCs with driver NFE2L2 mutations in the ETGE region were excluded from our subgroup. This highlights the importance of our expression-based classifier, as re-analysis of NFE2L2-mutated LUSC trial data showed that ETGE-mutated tumours did not respond to NRF2 inhibition. Our classifier provides a pan-cancer strategy to target NRF2 inhibition.

Conclusions: We have demonstrated how a network representation of gene regulation in healthy urothelium can biologically-inform the identification of a new clinically-actionable MIBC subgroup.

Poster# 39

Response Profiling of Enfortumab Vedotin and Sacituzumab Govitecan Using Patient-Derived Organoids: A Step Closer to Precision Oncology

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Background: Antibody-drug conjugates (ADC) such as Enfortumab Vedotin (EV) or Sacituzumab Govitecan (SG) enable targeted tumor therapy by binding to specific molecules - Nectin-4 and Trop-2, respectively – that are frequently overexpressed in urothelial carcinoma. While patient-derived organoids (PDOs) have shown promise in predicting responses to conventional chemotherapy, little is known about their potential to model responses to ADCs such as EV or SG.

Methods: Patient-derived organoids (PDOs) were established from freshly resected urothelial carcinoma samples and propagated as part of an institutional organoid biobank. To assess target expression, Nectin-4 and Trop-2 levels were analyzed in both primary tumor tissues and corresponding PDOs via immunohistochemistry and immunofluorescence. A drug-response assay with serial dilutions of EV was performed to evaluate cytotoxic effects. Cell viability was quantified using the CellTiter-Glo 3D luminescent assay and monitored by live-cell imaging.

Results: First, the presence of target molecules for EV and SG were analyzed. Similar to primary tumors, Nectin-4 and Trop-2 expression were detected in PDOs, fulfilling the key prerequisite for the mechanism of action of EV and SG. Upon treatment with EV and SG, PDOs exhibited a clear dose-dependent reduction in viability. Notably, the degree of sensitivity to EV and SG varied across PDO samples, suggesting heterogeneity between the PDO lines and individual patients.

Conclusions: In summary, our results show that PDOs express both Nectin-4 and Trop-2, reflecting their phenotypic similarity to the primary tumor. Exposure to EV and SG led to a dose-dependent cytotoxic response with different sensitivity levels across PDO lines. These findings support the use of organoids as a potential in vitro model for ADC response profiling with possible application in treatment response prediction.

Poster# 40

The Genomic Landscape of Bladder Cancer: Insights from Copy Number Alteration Profiles

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Background: Urothelial bladder cancer (UBC) is a complex disease influenced by genetic and environmental factors. Copy number alterations (CNAs) play a crucial role in tumour development and progression, with recurrent CNAs identified in both non-muscle-invasive bladder cancers (NMIBC) and muscle-invasive bladder cancers (MIBC). This study aims to characterise the CNA landscape in bladder cancer, focusing on risk groups and prognosis.

Methods: Shallow whole-genome sequencing was performed on 152 patients from the EPICURO/SBC study. Global CNAs were analysed by comparing their number and size across risk groups. A Bayesian generalised linear regression estimated the variance among risk groups explained by CNAs, identifying the top alterations. The association between CNAs and NMIBC prognosis was assessed. An unsupervised hierarchical clustering of principal components was conducted, characterising clusters based on clinicopathological features, mutations, immunohistochemical molecular subtypes, and copy number signatures.

Results: Recurrent alterations included gains in chromosomes 8q and 1q and losses in 8p, 9p, 11p, and 11q. NMIBC low-risk patients had fewer CNAs compared to NMIBC high-risk and MIBC patients, with TaG1 tumours showing fewer CNAs than TaG2 tumours. CNAs accounted for a substantial variance among higher-risk groups and were more frequent in NMIBC cases that progressed; however, no association was found with recurrence. Three CNA clusters were identified: one enriched in gains (12.8%), one in losses (9.6%), and one with few alterations (77.6%). These clusters correlated with BC risk groups and with prognosis. The cluster with fewer alterations was associated with FGFR3 mutations and the Luminal subtype.

Conclusions: In conclusion, this study provides evidence of a BC subtype with few CNAs associated with a better prognosis, alongside subtypes with several alterations, poorer outcomes, and enrichment for Basal/Squamous and Mixed subtypes.

Poster# 41

Establishment of an innovative, human stem cell-based culture model to investigate molecular carcinogenesis of urothelial carcinoma

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Background: Malignant tumors of the urinary bladder will result in stagnating mortality rates in future decades due to high molecular variance among tumor entities and a complex tumor. As current bladder carcinoma models only reflect the end-stage disease, thereby neglecting tumor heterogeneity, there is a demand for new models. In this respect, human pluripotent stem (hPSCs) offer a unique alternative as they can be differentiated towards almost any cell type. Besides, they are easy to modify (e.g. CRISPR-based).

Methods: A fluorescence reporter modified hPSC line was generated to visualize the expression of the urothelial markers KRT7 and UPK2 upon differentiation. As the bladder emerges from the hindgut, different hindgut inducing media were tested. Moreover, several cytokines and treatment periods were examined. The differentiation efficiency was assessed via qPCR, FACS analysis and immunofluorescence staining of relevant urothelial markers. On top, different cell ratios were tested to form 3D spheroids that were further cultured on a basement membrane matrix to investigate the impact of the culture format on the differentiation outcome.

Results: The data gained so far already give promising results as differentiation of the hPSC reporter line demonstrated a strong expression of mVenus. Moreover, a robust expression of urothelial identity markers such as GATA3, CK5, CK20 or UPK2 was validated on RNA and protein level. In this regard, an optimal cell ratio of 1000 cells per AggreWell was assessed for the formation of 3D spheroids.

Conclusion: In summary, further optimizations are pending, including the use of additional cytokines, to increase the efficiency of the protocol. The differentiation of stem cells offers numerous advantages, as they are easy to modify, genetically clean and human. Therefore, this new strategy provides an innovative model to investigate the early onset of urothelial carcinoma, thereby overcoming the limitations of present models.

Poster# 42

Investigating Sex-Specific Transcriptional Differences in Urothelial Bladder Cancer

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Background: The incidence of bladder cancer is 3-4 times higher in men than in women. While smoking, occupational exposures, hormonal differences, X chromosome inactivation, and anatomical variations contribute, they do not fully explain the higher prevalence in males. The study aim is that to identify molecular mechanisms that contribute to sex-based disparities, with a particular focus on the early stages of bladder cancer.

Methods: Genome-wide transcriptome analysis was conducted using the Decipher Bladder GSC assay (Veracyte, Inc San, Diego) on primary NMIBCs from 657 patients (151 female and 506 male). Differential gene expression, gene set enrichment and tumour microenvironment analyses were performed.

Results: We identified 84 differentially expressed genes (adjusted p value<0.05) including 19 X-chromosome, 37 autosomal, and 28 Y-chromosome genes. Among these, 3 X-chromosome genes, 5 autosomal genes, and all 28 Y-chromosome genes were up-regulated in males while 16 X-chromosome and 32 autosomal genes were up-regulated in females. A total of 26 cancer hallmark pathways were significantly enriched in females, while no hallmark pathways were significantly enriched in males. The most enriched pathways in females were IL6 JAK STAT3 signalling, interferon alpha response, interferon gamma response, and TNFA signalling via NFKB (p_<_0.01).CD4+ T cells, CD8+ T cells and monocyte levels were higher in female NMIBCs. Levels of M1 and M2 macrophages were also higher in females, with M2 macrophages more abundant than M1 in both sexes.

Conclusions: Gene expression analyses reveal sex-specific molecular and immunological differences in NMIBC. Female patients exhibit stronger immune activation, characterised by enrichment of key immune pathways and higher infiltration of immune cells within the tumour microenvironment.

Poster# 43

Genomic characterization of upper urinary tract urothelial carcinoma and clonal evolution of intravesical recurrence

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Background: Upper urinary tract urothelial carcinoma (UTUC) is a rare cancer accounting for only 5-10% of all urothelial carcinomas. Patients with UTUC are at high risk of developing intravesical recurrences (IVR) within two years after surgery. The biology of IVR after surgery is poorly understood and urine markers for its detection are lacking. In this study, we characterized the genomic landscape of UTUC to reveal novel therapeutic approaches and investigated the molecular mechanism of development of IVR to identify diagnostic markers for IVR.

Methods: We performed targeted next-generation DNA-sequencing of 571 genes in a multi-institutional international cohort of 390 UTUC patients enrolled retrospectively (n=255) and prospectively (n=135) who received radical surgery. Clonality and evolution were assessed in 67 UTUC-IVR paired cases.

Results: High tumor mutational burden (TMB), prior urothelial carcinoma of urinary bladder, tumors localized in the ureter and FGFR3 mutations corresponded to IVR risk. We identified a novel genomic subtype with MTOR mutations mutually exclusive with FGFR3, TP53 and HRAS mutations, core genes to define previously described genomic subtypes of UTUC. The novel MTOR genomic subtype (13.1%) was exclusively present in patients from Northwestern Europe, which had reduced IVR risk, low TMB, no insertion/deletion mutations and lacked TERT promoter mutations. Clonal evolution of IVR occurred in 81% of cases via four evolutionary paths driven by selection on TERT, TP53, STAG2 and FGFR2/3 mutations. Furthermore, hotspot mutations in TERT, FGFR3 and HRAS were identified as potential markers for noninvasive surveillance by urine testing after surgery.

Conclusions: We identified molecular correlates of IVR with FGFR3 as a potential therapeutic target to reduce IVR risk. The clonal relationship between UTUC and IVR underscores the potential for patient-friendly noninvasive urine tests for surveillance after surgery.

Poster# 44

Adenoviral replication does not require progression into cellular S-phase

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Background: Our group developed previously a novel combination therapy consisting of CDK4/6 inhibitors (CDK4/6is) and the oncolytic Adenovirus XVir-N-31 that improves the therapeutic potency. In the current model, adenoviral replication depends on cell cycle progression to S Phase that is suppressed by CDK4/6is. We investigated correlation of cell cycle progression and E2F activity after adenoviral infection and examined effects on replication.

Method: Three novel adenoviruses were designed that interfere with E2F1-3 activity, sequester E2F and express reporter constructs. Other viruses used were Ad5, Xir-N-31 and dl520. E2F expression was manipulated by siRNAs and as a CDK4/6i we used Palbociclib (PD). Cell cycle progression was analysis by EdU/BrdU incorporation, APC staining and FACS analysis. Life cell imaging in cells expressing the FUCCI system and azurite expressing adenoviruses allowed to specifically follow cell cycle stages until cell lysis. For differentiation of cellular and viral DNA, cells were labeled with EdU to precipitate de novo synthesized DNA which was analyzed by qPCR. Protein level were analyzed by Western Blot, cell survival by SRB staining and viral replication by qPCR. Experiments were performed in T24, UMUC-3 and 5637 bladder cancer cell lines.

Results: Following viral life cycle in living cells, host cell progression in S-phase is not an essential event for viral replication. Adenoviral infection resulted in an increased S-phase population that shifts to the lower right part of the S-phase gate in the FACS plot. We demonstrate that this de novo DNA signal originates from only novel adenoviral genomes. Interference with E2F availability or activity improved viral replication dramatically.

Conclusion: Adenoviral infection and replication does not require cell cycle progression. Inactivation or ablation of E2Fs improves viral replication and therapy response.

Poster# 45

Enhancing Oncolytic Adenovirus Efficacy in Tumor Cells through Alpha-Fetoprotein Peptide-Mediated Targeting and Internalization

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Background: The use of oncolytic adenoviruses is a novel therapy recently receiving fast track and breakthrough therapy status by the FDA for the treatment of NMIBC. We aimed to enhance cancer cell specific infectivity and internalization capabilities of oncolytic adenoviruses in tumor cells using the receptors for the alpha-fetoprotein (AFP), which is a cancer biomarker, as target. This study aims to improve selectivity of the oncolytic virus XVir-N-31 that infect integrin expressing cells by a RGD motive in the fiber protein.

Methods: Based on the oncolytic adenovirus XVir-N-31, the RGD motive in the HI loop of the fiber knob domain was replaced with an AFP-peptide. UMUC-3 bladder cancer and MRC-5 lung fibroblast were infected to assess receptor-specific internalization and oncolytic effects. Post-infection, non-internalized viruses were removed by trypsinization. Gene expression was analyzed via qPCR, cell viability was evaluated using SRB assays, and viral particle formation and release were quantified using a hexon based titer test.

Results: Alteration of adenoviral tropism through the substitution of the RGD motif with AFP-peptide enhanced viral internalization in tumor cells and reduced it in fibroblast cells upon infection. RECAF specificity was demonstrated by using AFP conditioned medium as a competitor. In tumor cells, the addition of the AFP peptide led to increased viral replication, higher viral titers, and reduced cell viability. Conversely, these effects were diminished in fibroblasts as expected.

Conclusion: Substituting the RGD motiv with an AFP-peptide in XVir-N-31 enhanced specific oncolytic efficacy highlighting the still enormous potential in improving the design of oncolytic viruses.

Poster# 46

CDK4/6 inhibition initiates cell cycle arrest by nuclear translocation of RB and includes a multistep molecular response

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Background: CDK4/6 inhibitors (CDKi) are the standard of care in combination with hormone ablation for treating metastatic breast cancer. However, as monotherapy efficacy in most clinical trials was limited. Understanding the molecular mechanisms underlying the therapeutic response may lead to development of an improved treatment design.

Methods: Next generation sequencing was applied to T24 cells w/o CDK4/6i in a time kinetics comprising 48 hours. Inhibitors targeting CDK4/6 (Palbociclib, PD), the proteasome (MG-132, Epoxomicin), NAE (MLN4924), lysosome biogenesis (Eltrombopag, ML329), KPNB1 (Importazole), Exportin-1 (LMB), and siRNAs against Gankyrin, Cullin-1, and KPNA/B were utilized. Protein expression was analyzed by Western blotting, gene expression level by qPCR, cell viability by SRB assays, lysosome biogenesis by LysoTracker and cell cycle analysis by EdU incorporation/FACS analysis. Subcellular localization of proteins was studied using immunofluorescence. Synergistic interactions were calculated using the ZIP-Synergy model.

Results: The response mechanism to CDKi follows a multi-step process. First, induction of G1 arrest is initiated by translocating RB into the nucleus through activation of importin _/_. In parallel, RB is proteolyzed in the cytoplasm, a process regulated by gankyrin and the SCF complex. At 16-24 hours, cells either express the MiT/TFE protein family, resulting in lysosomal biogenesis inducing senescence and apoptosis, which is essential to maintain this response. Alternatively, signaling pathways are activated that cause resistance.

Conclusions: Our data reveal molecular mechanisms to CDKi that induce therapy response, and maintenance or resistance. This might allow identification of predictive markers and development of novel combination therapies.

Poster# 47

Lymphatic sealing with titanium clips versus ultrasonic dissector during pelvic lymph node dissection at the time of radical cystectomy: A prospective, randomized trial

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Background: Lymphocele is the most common complication after pelvic lymph node dissection (PLND). Meticulous ligation of the lymphatic channels can significantly decrease lymph formation during PLND. We aimed to compare the incidence of lymphorrhea and lymphoceles in patients undergoing PLND during radical cystectomy using harmonic versus titanium clips.

Methods: We randomly assigned 82 patients aged ≥18 years undergoing open radical cystectomy with standard PLND to undergo lymphatic ligation with either harmonic (N = 42) or titanium clips (N = 40). Patients with preoperative ascites were excluded. The primary outcome was to compare the postoperative drain output and incidence of lymphocele in both groups. Secondary outcome was to compare the postoperative hospital stay and morbidity in both groups.

Results: The mean operative time was similar between the clip vs harmonic group (5.5 hrs vs 5.08 hrs, P value = 0.09). The mean drain volume and total drain volume were statistically non-significant between the 2 groups (130.8 ml vs 132.9 ml, P value = 0.7; 550 ml vs 645 ml, P value 0.77). The median day of drain removal also remained statistically not significant (5 days vs 5 days, P value = 0.69). There were 6 lymphoceles in the harmonic group of which 3 were symptomatic and all required drainage. 3 lymphoceles were present in the clip group and none of them were symptomatic. The mean lymphadenectomy time and length of postoperative hospital stay were also found to be statistically non-significant between the 2 groups (35.8 min vs 36 min, P value 0.8; 10 days vs 11.1 days, P value 0.3).

Conclusion: Sealing of lymphatic vessels appears equally effective and safe using harmonic or titanium clips during PLND in open radical cystectomy with regard to postoperative drain volume and postoperative morbidity. The overall incidence of lymphoceles and symptomatic lymphoceles appeared to be higher in the harmonic group but did not reach statistical significance.

Poster# 48

Lynch Syndrome-Associated Urothelial Cancer: Distinct Genomic Landscape, Constrained Hypermutation, and Preliminary Results from the LS-URO Urine Tumor DNA Screening Study

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Background: Lynch syndrome (LS) is a hereditary cancer predisposition syndrome caused by germline mutations in DNA mismatch repair (MMR) genes, conferring a 10–25% lifetime risk of urothelial cancer (UC), particularly in the upper urinary tract. Currently, there are no evidence-based screening methods for LS-associated urothelial cancer (LS-UC). We investigated the somatic mutational landscape of LS-UC and initiated an interventional, multicenter screening study in Finland and Canada using urine tumor DNA (utDNA) for early detection of LS-UC (LS-URO; NCT06218433).
Methods: We re-classified and analyzed DNA from 41 biobanked formalin-fixed, paraffin-embedded (FFPE) tumor samples and 3 urine DNA samples from 34 LS-UC patients. Immunohistochemical staining was performed to assess MMR protein expression. The LS-URO study utilizes in-mail urine collection with high-sensitivity utDNA analysis (UroScout) to screen LS carriers, with 184 patients recruited to date.
Results: TERT promoter mutations, present in 83% of sporadic UC, were nearly absent (5%) in LS-UC ($p < 0.00001$). LS-UC exhibited a constrained mutation landscape driven by 5-methylcytosine deamination (CG>TG) and microsatellite instability, with highly frequent mutations in ARID1A (82%), FGFR3 (80%), and KMT2D (78%). We propose that the scarcity of TERT promoter mutations results from an inability to generate the GABP binding motif (5’-GGAA) through CG>TG transitions or microsatellite instability. Preliminary results from LS-UC screening using non-invasive utDNA analysis demonstrates feasibility and high patient uptake, with utDNA-positive individuals undergoing clinical evaluation.
Conclusions: LS-UC represents a genomically distinct disease characterized by constrained hypermutation and near-universal absence of TERT promoter mutations. Preliminary data from the LS-URO screening support the potential of utDNA-based non-invasive screening for LS carriers.

Poster# 49

Implications of Gemcitabine-Cisplatin therapy on bladder cancer responsiveness to ADCs

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Background: For decades, cisplatin combined with gemcitabine has been the standard of care for locally advanced and metastatic bladder cancer (BC). With the advent of new treatment options, we want to investigate the implications of prior Gemcitabine-Cisplatin therapy on the responsiveness to ADCs (Antibody-drug-conjugates).

Methods: We established a panel of patient-derived bladder cancer cell lines with acquired drug resistance by exposing sensitive cells to increasing concentrations of Gemcitabine-Cisplatin. In this panel of parental and resistant cell line pairs we assessed the correlation of the protein expression levels of target proteins (NECTIN-4, HER2 and TROP2) to the treatment sensitivity with the corresponding ADCs. Moreover, with the use of bulk RNA-sequencing we will also elucidate transcriptional signatures associated with chemoresistance. Lastly, we will investigate expression levels of ADC target proteins in a patient cohort with chemotherapy unresponsive tumours by analysing tissue samples pre- and post-chemotherapy and from metastatic lymph nodes.

Results: Expression levels of ADCs target proteins were heterogeneous in the panel of parental and resistant cell line pairs. While TROP2 expression was a poor marker for Sacituzimab Govitecan (SG) treatment efficacy, cells with high HER2 expression levels were sensitive to Trastuzumab deruxtecan and Disitamab Vedotin. Nevertheless, Nectin-4 expression was the only ADCs target protein, that was consistently downregulated in the majority of chemoresitant cell lines and its expression levels correlated with sensitivity to Enfortumab Vedotin (EV) treatment. We could validate this finding in a patient cohort.

Conclusion: Our results showed that Gemcitabine-Cisplatin therapy renders BC cells cross-resistant to EV treatment. This finding might have implications for second-line treatment options of BC patients that progress after neoadjuvant chemotherapy.

Poster# 50

Stromal Transcriptomic Signatures are Prognostic in High-Risk Non-Muscle Invasive Bladder Cancer treated with Intravesical Bacillus Calmette-Guerin

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Background: There are several proposed molecular biomarkers to predict response to intravesical Bacillus Calmette-Guerin (BCG) for high-risk non-muscle invasive bladder cancer (HR-NMIBC), although external validation is lacking. Therefore, we evaluated transcriptomic signatures and subtypes on an independent HR-NMIBC cohort.

Methods: We assessed a cohort of treatment-naïve HR-NMIBC treated with BCG. Archived TURBT specimens were analyzed with the Decipher Bladder Genomic Subtyping Classifier (GSC), a clinical-grade transcriptome-wide assay (Veracyte, San Diego, CA). Molecular subtypes using the GSC, consensus-MIBC and UROMOL21 models, and 194 locked transcriptomic signatures in GRID (v3.1) were examined. Cox-regression and Kaplan-Meier analyses were used to evaluate associations between signatures and outcomes including high-grade recurrence-free survival (HG-RFS), adjusting for age, sex, treatment year and CIS.

Results: Of 92 patients, there were 31 Ta, 59 T1, 2 pure CIS, and 25 with any CIS. Median follow-up was 49 months and 33 patients had HG disease recurrence. In total, 85% of patients were classified as GSC luminal, 73% as Consensus luminal papillary subtypes, and 50% UROMOL Class 3. UROMOL Class 1 (14%) had 92% HG-RFS at 3-years (vs 61% in others). Tumor microenvironment signature analysis revealed a stromal gene signature (Efstathiou2018) associated with worse HG-RFS ($p<0.01$), PFS ($p=0.012$) and OS ($p=0.03$). Stromal gene signature scores were higher in GSC or Consensus non-luminal, and UROMOL21 Class 2b tumors. Several angiogenesis signatures were associated with progression to MIBC (Uhlik2016 $p<0.03$ & Masiero2013 $p<0.02$).

Conclusions: This extensive transcriptomic analysis of BCG-treated HR-NMIBC showed predominately luminal molecular subtype and substantial variation in other tumor biology scores. Notably, high stromal scores were associated with inferior BCG response. Improved NMIBC subtyping models are needed to better predict outcomes.

Poster# 51

Predicting Response to Intravesical Bacillus Calmette-Guerin Versus Gem/Doce for High-grade Non-muscle-invasive Bladder Cancer

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Background: Intravesical bacillus Calmette-Guerin (BCG) is standard first-line therapy for high-grade (HG) non-muscle invasive bladder cancer (NMIBC). Recently, sequential intravesical gemcitabine and docetaxel (Gem/Doce) has emerged as a promising alternative. A biomarker to select the optimal treatment regimen could facilitate clinical decision-making. The Computational Histologic Artificial Intelligence (CHAI) platform was previously used to develop an artificial intelligence-powered histologic assay (CHAI biomarker) to predict outcomes following BCG. We assessed the predictive ability of the CHAI biomarker among patients with treatment-naive HG-NMIBC who received intravesical BCG or Gem/Doce.

Methods: 253 patients with treatment-naïve HG-NMIBC from weretreated with BCG or Gem-Doce based on BCG availability. H&E tissue specimens from pre-treatment TURBT were analyzed with the CHAI platform to determine biomarker status. Multivariable CPH regression was performed to compare HG recurrence-free survival (RFS) and the Kaplan-Meier method was used to estimate HG-RFS in BCG- and Gem/Doce-treated cases stratified by biomarker status. Likelihood ratio tests were used to test biomarker-treatment interactions.

Results: Among patients with presence of the CHAI biomarker, those treated with BCG had 24-month HG-RFS of 56% (95% CI [43-73%]) and those treated with Gem/Doce had HG-RFS of 90% (HR 5.4, p=0.007). Among patients without the CHAI biomarker, patients treated with BCG or Gem/Doce had no significant difference in HG-RFS (HR 1.3, p=0.5). The interaction term between the CHAI biomarker and treatment type was significant (p=0.03) indicating an association between the biomarker and the clinical outcome that is dependent on the treatment received.

Conclusions: This data demonstrates that the CHAI biomarker predicts which HG-NMIBC patients are less likely to benefit from BCG and may experience improved clinical benefit from alternative treatments including Gem/Doce.

Poster# 52

Non-invasive urine genomic profiling in patients with upper tract urothelial carcinoma

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Background: The diagnosis and monitoring of patients with upper tract urothelial carcinoma (UTUC) remains a clinical challenge. Non-invasive testing capable of detecting and staging UTUC has the potential to transform clinical practice. In this retrospective study, we evaluated the performance of urine- and plasma-based genomic profiling in patients treated for UTUC.

Methods: 55 urine samples were collected from 41 patients who underwent surgical extirpation for UTUC. Matched plasma and tumor (FFPE) were analyzed when available. Comprehensive genomic profiling (CGP) was performed using UroAmp (Convergent Genomics), an assay previously validated for urothelial carcinoma of the bladder. The primary analysis was disease classification compared to pathology. Secondary analyses were mutation comparison by stage, and cytology classification compared to pathology.

Results: Stage distribution was 20% pTa, 17% pT1, 15% pT2, 31% pT3, 5% pT4, 10% pTis, and 2% pT0. All tumors were high grade. Urine CGP correctly classified 51/55 (93%) of UTUC patients. The pT0 sample was correctly classified as negative. Using urine-informed mutations, matched plasma was positive in 6/29 samples (21%), including 4 pT3 samples (50%). Using tumor-informed mutations, plasma was positive in 3/18 samples (17%), including 2 pT3 samples (50%). Comparison of urine CGP in muscle-invasive (n = 21) and non-muscle-invasive UTUC (n = 19) revealed a significant enrichment of TP53 mutations in muscle-invasive disease (OR 7, P = 0.01) . Matched cytology was available for 37 urine samples, returned conclusive results in 27 (73%), and correctly classified 18 (67%). Urine CGP correctly classified 100% of atypical cytology results (n = 10).

Conclusions: Urine CGP identifies mutations associated with UTUC and may support noninvasive staging when paired with plasma samples. Furthermore, urine CGP may provide diagnostic and prognostic information for the management of UTUC.

Poster# 53

Targeting Compensatory DNA Repair Pathways to Restore ATR Inhibitor Sensitivity in Squamous Cell Carcinoma of the Bladder

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Background: Previously we demonstrated that ATR inhibitor (ATRi) enhanced radiotherapy efficiency in TP53-deficient bladder cancers (BLCAs), including squamous cell carcinoma (SCC). Now, we investigated downstream effects of ATR dysfunction and long-term ATRi treatment to identify targetable bypass mechanisms for synthetic lethality-based approaches.

Methods: Patient-derived ATRi-adapted SCC models (p-SCC^ATRi) were established by long-term Ceralasertib exposure. PBMC co-culturing was performed for studying cellular crosstalk. Drug sensitivity was evaluated by XTT (IC50) and clonogenic survival assays (+/- radiation). RNA-seq, kinase arrays, qPCR and western blot analyses characterized compensatory mechanisms. DNA damage (_H2AX), cell cycle (FACS) and tumor growth (in ovo CAM assay) were assessed with RAD51 inhibitor (RAD51i) B02. Apoptosis was measured by immunohistochemistry (cleaved Caspase-3 staining).

Results: Transcriptomic profiling of ATRi-adapted cells revealed enrichment of cell cycle pathways (E2F targets, G2/M checkpoint), chromatin remodeling, DNA repair networks, along with activation of homologous recombination (HR) repair pathways, notably RAD51, BRCA1 and BRCA2. Inflammatory response and interferon-_ signaling were modulated, also confirmed by kinome activity screening, and linked to impaired cell growth in the presence of PBMCs. RAD51i treatment reversed ATRi resistance (_IC50 by 40–80%), increasing genomic instability (2.2-fold _H2AX foci/cell) and G2/M arrest (2.4-fold). In CAM xenografts, RAD51i suppressed tumor growth by up to 37%.

Conclusions: In bladder cancer, ATR dysfunction altered genomic stability of SCC cells and crosstalk with immune cells. Moreover, identifying HR as a key compensatory mechanism facilitating ATRi resistance suggests a synthetic lethality relationship between both. Thus, targeting RAD51 and/or ATR could be promising novel therapeutic strategies especially for ATR mutated or HR-deficient BLCAs, including SCC.

Poster# 54

The Added Prognostic Value of the UROMOL Classification to the 2021 EAU Risk Stratification in Patients with Non-Muscle-Invasive Bladder Cancer in the COBLAnCE Cohort

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Background: The 2021 risk stratification (RS) of the European Association of Urology (EAU) for non-muscle-invasive bladder cancer aims to identify patients (pts) at high risk of progression, but its predictions are not always accurate. The UROMOL classification (UC), based on molecular signatures, distinguishes four subtypes with different biological and prognostic profiles. We aimed to assess the added prognostic value of combining the UC and the 2021 EAU RS.

Methods: We used data from the French national prospective cohort COBLAnCE on bladder cancer, focusing on pts with stage Ta/T1 tumors who underwent Transurethral Removal of Bladder Tumour as their inclusion surgery and with available RNAseq data (n=657). The primary endpoint was time to first progression. Bladder cancer-related deaths were considered as events, while pts without progression at last follow-up or those who died from other causes before progression were censored. Cox proportional hazards models including EAU RS and UC were used, while grouping UC 2a/2b versus 1/3. Discrimination of models with and without UC was assessed. The time-dependent area under the ROC curve (AUROC) was calculated at 1 and 5 years. For each EAU risk group, Cox models were built, including UC to evaluate its ability to identify higher-risk pts within subgroups.

Results: A total of 58 events were observed over a median follow-up of 5.8 years. UC 2a/2b (n=270) was significantly associated with progression when added to the EAU RS (hazard ratio (HR)=2.6[1.3–5.5], p=0.01) compared to classes 1/3 (n=387). The AUROC improved from 0.76[0.71-0.8] to 0.77[0.72-0.81] at 1 year and from 0.8[0.78-0.83] to 0.82[0.8-0.85] at 5 years. Subgroup analysis suggested an increased progression risk for UC 2a/2b (n=143) in high-risk pts (n=202) (HR=3.92[1.17-13.10], p=0.01).

Conclusions: Our findings suggest that UC is a prognostic biomarker associated with progression, and could help better characterize pts within the high-risk subgroup.

Poster# 55

Circulating immune-biomarkers of response to neoadjuvant Sacituzumab Govitecan (SG) alone and with pembrolizumab (pembro) in muscle-invasive bladder cancer (MIBC): secondary analyses from SURE-01 and SURE-02 trials

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Background: Antibody drug conjugates and immunotherapy are promising novel neoadjuvant therapies (NAT) in patients (pts) with MIBC who refuse or are ineligible for cisplatin-based chemotherapy. SURE-01 (NCT05226117) and SURE-02 (NCT05535218) trials enrolled pts with MIBC to receive 4 cycles of neoadjuvant SG alone or with pembrolizumab, respectively. Currently, no biomarkers of response to either strategies have been identified. Interim efficacy and tumor biomarkers findings have been reported. Herein we report results on circulating immune-biomarkers.
Methods: Fresh peripheral blood (PB) samples were collected at three time-points before surgery: at baseline (T0), after the first cycle (T1) and at the end of NAT (T2). Multiparametric flow cytometry evaluated circulating immune cells changes during treatment as previously reported (PMID: 39241203). T-test or Wilcoxon-Mann-Whitney test analyzed associations with pathological response.
Results: As of June 2025, 69 pts (33 in SURE-01; 36 in SURE-02) were assessable. In SURE-02, major responders (Rs; ypT≤1N0-X) exhibited a lower percentage (%) of polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs, CD11b+CD33dimHLADR-CD15+CD14-; median % 52.1 vs 67.9, p=0.010) and natural killer cells (NKs; 11.7% vs 19.4%, p=0.040) compared to non-responders (non-Rs) at T0. These differences were nonsignificant at subsequent time points. In SG monotherapy (SURE-01), Rs do not associate with any immune population at T0. However, non-Rs vs Rs show increased early-stage MDSCs (e-MDSCs; CD11b+CD33+HLADR-CD15-CD14-) from T0 to T1 and lower % of classical (10.9% vs 3.2%; p=0.044) and intermediate monocytes (2.16% vs 0.39%, p=0.045) at T2.
Conclusions: Pre-treatment immune signatures in PB may associate with pathological response to SG + pembro, primarily as a pembrolizumab-related effect. However, modulation of e-MDSCs and monocytes emerged to be a putative SG-induced effect. Updated findings will be presented.

Poster# 56

The Role of CCL2 in Bladder Cancer Progression and Metastasis: A Context-Dependent Effect

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Background: Bladder cancer (BC) remains a complex disease that demands more effective therapeutic strategies. The CCL2-CCR2 axis has been implicated in tumor progression and metastasis. However, previous studies have produced inconsistent findings, and it remains unclear whether the cellular source of CCL2 plays a critical role in tumor development.

Methods: We used mouse models of BC with two (Pten; Trp53) or four (Pten; Trp53; Rb1; Rbl1) floxed tumor suppressor genes. Aggressive tumors representative of human BC were induced via intravesical administration of adenovirus expressing Cre recombinase. Using these models and tumor-derived cells, we generated new Ccl2 knockout (KO) mouse and cellular models via CRISPR/Cas9, used for both adenovirus-mediated tumor induction and orthotopic injection. Tumor growth, metastasis, and histopathology were evaluated. The tumor microenvironment (TME) was assessed by spectral cytometry. Additionally, transcriptomic data from human samples in the TCGA cohort were analyzed. Ongoing experiments include a time course of orthotopic WT and KO tumors to assess temporal effects, from which macrophages have been isolated for coimplantation studies to examine their role in tumor progression.

Results: Loss of Ccl2 in induced tumors led to increased tumor incidence, greater metastatic dissemination, and more differentiated tumors. Spectral cytometry confirmed an immunosuppressive TME in Ccl2-KO models. In orthotopic models, Ccl2 loss in tumor cells enhanced tumor growth and peritoneal spread, also associated with an immunosuppressive TME. Preliminary coimplantation experiments suggest that macrophages from KO tumors promote greater tumor growth than those from WT tumors. Human data confirmed a correlation between CCL2 expression, inflammation, and tumor differentiation.

Conclusions: Our findings highlight that the cellular origin of CCL2 plays a pivotal role in BC progression and metastasis, likely by shaping distinct tumor microenvironment.

Poster# 57

ABCG2-Mediated SN-38 Efflux Drives Payload-specific Resistance to Sacituzumab Govitecan in Urothelial Carcinoma

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Background: Sacituzumab govitecan (SG) is a TROP2-directed antibody-drug conjugate (ADC), coupled to the Topoisomerase I inhibitor SN-38. SG holds great promise in urothelial carcinoma (UC) and initially received FDA accelerated approval after platinum-based chemotherapy and immune checkpoint inhibitors (TROPHY-U-01). However, SG was withdrawn from this indication, as the phase III TROPiCS-04 trial did not demonstrate statistically significant survival benefits compared to standard chemotherapy despite higher overall response rates. This underlines the need for a refined understanding of molecular responsiveness and resistance determinants of UC towards SG.

Methods: SG- and SN-38-resistant sublines were generated by constant exposure to the respective compounds in vitro. These resistant derivates were molecularly characterized. Western blot analysis was used to assess changes in TROP2 and ABCG2 expression. Cell surface expression of these proteins was evaluated using flow cytometry and fluorescence microscopy. Furthermore, combination assays, where parental cells and the resistant sublines were treated with SG/SN-38 and co-exposed to selective ABCG2 inhibitors were performed. Finally, comprehensive in silico datasets were analyzed to support the in vitro data.

Results: TROP2 deregulation does not play a major role in SG resistance development. Insensitivity of resistant sublines might rather be dominated by mechanisms affecting SN-38. Acquired SG resistance of UC models was mediated by ABCG2-dependet SN-38 efflux, which could be completely reverted by specific ABCG2 inhibitors.

Conclusions: These results highlight ABCG2 as a molecular key factor in SG-resistance in UC. Our data suggest selective dual-conjugation of ADCs with ABC transporter inhibitors to mitigate systemic toxicity via specific delivery and release in the malignant tissue, while in parallel counteracting drug resistance development.

Poster# 58

The IL6/JAK/STAT3 signaling axis is a therapeutic vulnerability in SMARCB1-deficient bladder cancer

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Background: Bladder cancer is the fourth most common cancer among men and the ninth most common overall, accounting for approximately 150,000 deaths worldwide each year. Despite advancements in systemic therapies—including targeted therapies and antibody-drug conjugates—a substantial proportion of bladder cancer (BLCA) patients (30%–70%) exhibit resistance to these treatments. To overcome this challenge, novel therapeutic strategies based on a deeper understanding of the underlying tumor biology are urgently needed. Genomic analyses of bladder cancer patients have revealed that over 60% of cases harbor inactivating alterations in components of the SWItch/Sucrose Non-Fermentable (SWI/SNF) chromatin remodeling complex. SMARCB1, a key subunit of this complex, is critical for its chromatin remodeling function.

Methods: To investigate the role of SMARCB1 loss in promoting tumor growth and metastasis, we employed CRISPR/Cas9 to knock out (KO) SMARCB1 in the human T24 bladder cancer cell line. To validate the specificity of the knockout phenotype, we generated a SMARCB1 rescue cell line by re-expressing full-length SMARCB1 in the KO background.

Results: In xenograft models, SMARCB1 knockout cells demonstrated enhanced tumor growth and organ-specific metastasis compared to controls. Notably, this phenotype was reversed upon SMARCB1 rescue. Transcriptomic profiling of xenografts derived from SMARCB1-modulated cells revealed enrichment of Hallmark pathways related to hypoxia and interferon-gamma response. We hypothesize that SMARCB1 regulates the tumor microenvironment in SMARCB1-deficient tumors.

Conclusions: In conclusion, our findings provide preclinical evidence supporting the development of targeted therapies for patients with metastatic bladder cancer characterized by SMARCB1 deficiency, offering a promising avenue for future therapeutic strategies.

Poster# 59

Comprehensive Analysis of the Resistance Mechanisms against the Antibody Drug Conjugate Enfortumab Vedotin and its Cytotoxic Payload MMAE in Preclinical Urothelial Carcinoma Models

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Background: In recent years, targeted therapies in the form of Antibody Drug Conju-gates (ADC) have gained attention in Urothelial Carcinoma (UC). Enfortumab Vedotin (EV), targeting Nectin-4 (N-4), has significantly shifted the treatment paradigm. Alt-hough the combination trial Keynote A39 led to remarkable results, resistance remains inevitable. This project focuses on understanding the molecular and mechanistic basis of resistance to EV and its payload MMAE. Three patient-derived Vienna Urothelial Carcinoma (VUC) models were selected to generate resistant subpopulations to either the ADC or MMAE, to elucidate resistance mechanisms.
Methods: Multidose in vitro cytotoxicity assays will determine sensitivity and confirm resistance. Western blot and flow cytometry will assess N-4 expression and surface localization. CNV and RNA sequencing will characterize models and reveal resistance mechanisms.
Results: Three VUC models were selected based on high intrinsic EV/MMAE sensi-tivity and N-4 expression. Two models have already been rendered resistant, with con-firmed resistance to EV and MMAE. N-4 expression was assessed with Flow Cytome-try and Western blot, revealing a significant decrease in one model, while another model showed even an increase in N-4 in resistant models. These data indicate the necessity for further in-depth dissection of N-4-associated versus payload-specific mechanisms driving EV resistance. Additionally, in one model ABCB1, a multidrug ef-flux pump, was upregulated in both EV- and MMAE-resistant sublines, indicating -at least in this model- payload efflux as a predominant mechanism. Conversely, no ABCB1 upregulation was observed in the EV-resistant line of another model, pointing to a distinct resistance mechanism unrelated to MMAE, which needs to be further en-lightened.
Conclusion: These complementary approaches aim to unravel the molecular basis of acquired EV resistance in UC, ultimately guiding improved therapeutic or combination strategies.

Poster# 60

Evaluating the roles of HAI-1 and ST14 in various cellular processes of bladder cancer cell lines

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Background: HAI-1 (SPINT 1) and matriptase (ST14) are a membrane-associated protease-regulator pair essential for sustaining epithelial homeostasis and are involved with cancer progression. However, their precise roles in key biological processes within bladder cancer (BC) cells remain unclear. This study aims to elucidate their roles in bladder carcinogenesis.

Methods: Bladder cancer cell lines (T24, VM-CUB-1, and 5637) were utilised to investigate expression of HAI-1 and ST14. The two lines with the highest levels of expression were subsequently transfected with siRNA directed at SPINT 1 and ST14 for use in several phenotypic experiments, including clonogenic, wound scratch, invasion, and cell cycle assays.

Results: Gene and protein expression levels were lowest in T24, intermediate in VM-CUB-1, and highest in 5637. Knockdown efficacy was evaluated in both VM-CUB-1 and 5637: approximately 70% knockdown was achieved as determined by qPCR and Western blotting. HAI-1 and matriptase knockdown did not affect cellular migration. In clonogenic experiments, HAI-1 knockdown elevated the number of colonies by 40-50% in both cell lines ($p<0.05$), and also increased colony size. Knockdown of matriptase resulted in a decrease in colony numbers by approximately 40% in both cell lines ($p<0.05$). In invasion assays, HAI-1 knockdowns significantly increased the number of invading cells by 50-75% ($P<0.001$). Conversely, matriptase knockdown decreased the number of invading cells by 60% ($p<0.001$) in VM-CUB-1 and 20% in 5637 ($P<0.001$). In cell cycle assays, HAI-1 knockdown resulted in a 35% rise in cells in G2 phase in VM-CUB-1 and a 23% increase in 5637, whereas matriptase knockdown led to a drop of 17% in VM-CUB-1 and 18% in 5637.

Conclusions: Decreased expression of HAI-1 is associated with increased cellular proliferation, invasion, and division. Conversely, knockdown of matriptase results in a reduction of these cellular events.

Poster# 61

Integrated multi-omics analysis of primary and PDX MIBC models suggests targetable molecular vulnerabilities

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Background: Muscle-invasive bladder cancer (MIBC) is a heterogeneous disease with multiple TCGA-classified molecular subtypes. To better understand MIBC molecular hallmarks across subtypes, we performed comprehensive multi-omic profiling of eight distinct MIBC PDX models and their corresponding patient tumors.

Methods: We established eight primary tumors as murine PDX models and performed multi-omic profiling, including whole-exome sequencing, copy number analysis, RNA sequencing, global and phosphoproteomics, reverse-phase protein array, and metabolomics.

Results: TCGA-informed subtyping identified luminal, luminal papillary, luminal-infiltrated, basal, and neuronal subtypes in our cohort. Classifier-based subtype calls were largely concordant across omics platforms, with minor differences between some tumors and their matched PDXs. Multiple models harbor deleterious TP53 mutations, consistent with loss of tumor suppressor function. Beyond mutational burden, many models exhibit large-scale copy number alterations, including the co-amplification of RAF1 and YAP1 in one case, accompanied with increases in transcript and protein levels across multiple omics platforms. Several models demonstrated high expression of HLA class I and II molecules, including immunoinhibitory proteins HLA-E and –F. One in particular exhibited a pronounced hot immune phenotype, with CD274 (PD-L1) amplification, along with high expression of CD40, TNFRSF9 (4-1BB), and CD276 (B7-H3). This model also showed engagement of the alternative NF-kB pathway.

Conclusions: These results underscore the diversity of MIBC and support testing model-specific therapeutics. The observed co-amplification of YAP1 and RAF1 likely reflects convergent activation of proliferative and survival signaling pathways, nominating this model for combined YAP-RAF inhibition. In parallel, high expression of HLA molecules and immune checkpoint regulators, including CD274 (PD-L1), nominate several for immunotherapy.

Poster# 62

Discriminative capacity of the AUA/SUFU hematuria guideline 2025 update – How much progress?

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Background: The 2020 AUA/SUFU guideline is the current standard regarding man-agement of patients with microhematuria (mH). To enhance risk stratification, a recent update (2025 update) was introduced, primarily reflecting revisions to age-related cut-off values. This study was designed to assess the discriminative capacity of the previ-ous and current recommendation.
Methods: In this simulation, 716 patients with a newly diagnosed mH and no previous urothelial cancer (UCa) were derived from an institutional database. All patients un-derwent urological work-up including patient history incl. smoking status, urological examination, cystoscopy and upper tract imaging (ultrasound and advanced imaging if indicated). Patients with positive findings were referred for transurethral resection. Risk stratification was performed along the criteria of the 2020 AUA/SUFU guideline and the 2025 update.
Results: A histologically confirmed UCa was found in 57/716 patients (8%). Applying the 2020 AUA/SUFU guideline, 604/716 patients (84.2%) with 54/57 UCa (94.7%) and for the 2025 update 513/716 patients (71.5%) with 51/57 UCa (89,5%) were consid-ered intermediate(IR)/high risk(HR). UCa prevalence for the 2020 AUA/SUFU guide-line and the 2025 update was 8.9% vs. 9.9% in the IR/HR groups vs. 2.7% and 3% in low risk (LR) patients, respectively. 3.4% (AUA 2020) and 6.9% (2025 update) high risk tumors occurred in LR patients. Assessment of 11.2 patients (AUA 2020) and 10 pa-tients (2025 update) was required to diagnose one UCa case.
Conclusions: The changes of age cut-offs for females and of erythrocyte counts in the 2025 update considerably increases the fraction of LR patients saved from invasive examinations and decreases the number of patients requiring assessment. Neverthe-less, 75% of all patients would have to undergo cystoscopy including 65% with a neg-ative result. Further improvement is expected by implementing molecular urine mark-ers into risk assessment in mH.

Poster# 63

Clinical applicability of an mRNA based urine test (Xpert® Bladder Cancer Detection*) for the detection of bladder cancer in patients with microhematuria

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Background: Non-visible hematuria (nvH) is a frequent condition in the general population. However, although established as a risk factor of urothelial cancer (UC), the prevalence of UC in nvH patients is low. The aim of this meta-analysis was to test options to reduce unnecessary examinations using the Xpert® Bladder Cancer Detection (Xpert BC-D) urine test to stratify nvH patients into high risk and low risk of being diagnosed with UC.
Methods: We conducted an individual patient data meta-analysis comprising 3 prospective cohort studies in patients with nvH receiving an Xpert BC-D test. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were calculated for the overall detection of UC and for high grade UC. The Random Forest algorithm was used to develop a clinical predictive model for the presence of bladder cancer. Missing data were imputed using multiple imputation method. Decision curve analysis was performed to evaluate the clinical utility of the model.
Results: Of 742 patients available for analysis, a UC was confirmed in 47 cases (6.3%). Xpert BC-D had a sensitivity of 85.1% and a specificity of 83.5% for the overall detection of UC; PPV and NPV were 25.8% and 98.8%. For high grade UC, sensitivity, specificity, PPV, and NPV were 96.7%, 82.3%, 18.7% and 99.8%, respectively. On decision curve analysis, the addition of Xpert BC-D to the standard prediction model showed an increase in net benefit across all threshold probabilities. Starting at a threshold probability of 2% for having bladder cancer, the Xpert showed a reduction in 0.32 unnecessary cystoscopies per patient and 0.66 in the case of high grade tumors, without missing UCs.
Conclusion: The Xpert BC-D showed superior performance in the detection of UC compared to urine cytology, and a significant reduction in unnecessary cystoscopies. The test could be used as a first-line test in patients with nvH to identify patients at high risk of UC.

Poster# 64

Final results of the prospective randomized UroFollow trial comparing marker-guided versus cystoscopy-based surveillance in patients with low/intermediate risk bladder cancer

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Background: A growing body of evidence suggests that current follow-up of non muscle-invasive bladder cancer (NMIBC) yields considerable overdiagnosis. The goal of the UroFollow trial was to explore the efficacy and safety of a marker-based follow-up in patients with low/intermediate risk NMIBC.

Methods: Patients with Ta low and high grade (G1-2) NMIBC were prospectively randomized to either standard of care (SOC) or 6-monthly marker-based follow-up (algorithm comprising UroVysion and NMP22 assays, urine cytology and ultrasound). After a negative 3-month cystoscopy (WLC), only patients with a positive algorithm underwent WLC in the marker arm (MA). An end-of-study WLC was recommended at 3 years to all patients without recurrence. Several innovative urine markers were also examined.

Results: In total, 214 patients were randomized to the SOC (n=109) and MA (n=105). No significant differences regarding baseline tumor characteristics were observed. Median follow-up was 2.4 years. Overall, 59 tumor recurrences were diagnosed in 24.8% vs. 25.0% patients in the SOC and MA. Sensitivity was 96.5% vs. 81.5% (p= 0.096) with 1 and 5 Ta LG tumors being overlooked in the SOC and MA, respectively. No tumor progressing in stage or grade was missed. The number of WLCs was 589 in SOC and 148 in the MA (p < .001). Among 5 other markers (ADX-Bladder, CellDetect, Bladder EpiCheck, UBC rapid, and Xpert® Bladder Cancer Monitor (BC-M), Bladder EpiCheck and Xpert BC-M showed similar performance to the algorithm.

Conclusions: UroFollow is the first randomized trial including urine markers into clinical decision making in surveillance of patients with low/intermediate risk NMIBC. With the restrictions of a small-sized trial, we conclude that 6-monthly marker-guided follow-up after a negative 3 month WLC is safe in this cohort. Results of modern urine markers suggest the potential for use in marker-guided surveillance.

Poster# 65

Reduce length of hospital stay with the use of methylnaltrexone in radical cystectomy peri-operative; a retrospective study

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Background: Post-operative ileus (POI) is a frequent complication after radical cystectomy (RC) and contributes to extended hospitalization. Alvimopan, a peripherally acting opioid antagonist (PAMORA), reduces length of hospital stay (LOS) and improves bowel recovery after RC but is unavailable in Canada. This study investigated the impact of adding methylnaltrexone (MNTX), a PAMORA available in Canada, to the enhanced recovery after surgery (ERAS) protocol on LOS and POI.

Methods: This single-center retrospective review included patients undergoing RC for bladder cancer (October 2021–August 2024). MNTX (12 mg SC pre-incision, then daily for up to 6 days post-surgery) was added to the ERAS protocol in May 2023. Patients undergoing additional surgeries (e.g., bowel resection) or taking preoperative narcotics were excluded. The primary outcome was LOS, with secondary outcomes including time to flatus, time to bowel movement, inability to tolerate oral diet by day 7, nasogastric tube placement, POI, and 30-day emergency visits. Multivariable regression analyses adjusted for covariates (age, sex, BMI, surgeon and preop narcotics.

Results: Among 295 cases, 220 patients were included (96 MNTX, 124 controls). Median age was 71 [IQR 66–77], 79% were male, and 88% had an epidural. LOS was significantly shorter in the MNTX group (median 6 days [5, 7]) compared to controls (median 7 days [6, 8]; $p < 0.001$). There was no difference in POI (17.9% vs. 21.0%; $p = 0.7$) or other secondary outcomes (all $p > 0.05$), and 30-days complication rates were comparable. On multivariable quantile regression, patients in the MNTX group had a significantly shorter LOS of 0.86 days (95% CI -1.13 – -0.55).

Conclusions: The addition of MNTX to our standardized ERAS protocol significantly reduced LOS by one day. Given its safe profile and modest cost relative to hospitalization expenses, MNTX may offer a cost-effective adjunct to ERAS protocol and resource utilization following RC.

Poster# 66

Evaluating the prognostic utility of circulating tumour DNA in metastatic urothelial cancer

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Background: Metastatic urothelial cancer (mUC) is an aggressive disease with diverse clinical outcomes. Recent studies have suggested that the fraction of circulating DNA in the blood plasma that is tumour-derived (i.e. ctDNA%) carries prognostic information in mUC. However, there is ambiguity as to the relation between precise ctDNA levels and patient prognosis, and the relationship between ctDNA% and clinical disease characteristics is under-characterized.

Methods: We evaluated the prognostic relevance of pretreatment ctDNA%, and associations between ctDNA% and patient clinical characteristics. For this interim analysis (accrual is ongoing), our dataset consists of 150 plasma cell-free DNA samples from 138 mUC patients. Blood was collected within 31 days prior to initiation of first (n=119) or second line (n=31) therapy. Samples underwent deep targeted DNA sequencing and ctDNA% was determined via somatic mutations and copy number alterations.

Results: ctDNA% was significantly correlated with serum lactate dehydrogenase (LDH, R=0.68, p=6.04e-18) and presence of liver metastases (22% vs 3.3%, p=5.3e-7). In multivariate analyses, ctDNA% was independently prognostic when incorporating liver metastases, but not LDH. Higher ctDNA% may also associate with worse ECOG performance status (2+ vs 0-1; 15.6% vs 4.5%, p=0.30). ctDNA% was not correlated with age at metastatic diagnosis (R=-0.22), sex (p=0.38), other metastatic sites (lung p=0.56, bone p=0.24, lymph node p=0.23), kidney function (i.e. glomerular filtration rate, R=0.07), nor other serum factors (hemoglobin R=0.03, neutrophil to lymphocyte ratio R=0.1). Higher ctDNA% was associated with shorter overall survival (OS). Patients with ctDNA% within the first quartile had the highest median OS (< 1.3%, 19.5 months), compared to patients within the second quartile (1.3-8.4%, 13.3 months), within the third (8.4-28%, 9.5 months), and within the fourth (>28%, 9.6 months).

Conclusion: These results support ctDNA% as a robust prognostic factor in mUC.

Poster# 67

Microbiome Immunogenicity in Bladder Cancer

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Background: With the increased interest in the characterizing the human microbiome, some species have been shown to be associated with the occurrence of bladder cancer (BC) and response to therapies. However, current studies are limited by variations in sampling, and none have examined the immune response to microbiota in BC. Our aim is to assess the immune response against microbiota in BC patients treated with different modalities.

Methods: The study included baseline (B) and follow-up (FU) serum samples of bladder cancer patients and healthy controls, used to profile antibody repertoires against 344,000 microbial antigens using high-throughput phage-display immunoprecipitation sequencing (PhIP-Seq). The antibody response against microbiota was compared between healthy individuals and bladder cancer patients treated with surgery only, chemotherapy, or immune checkpoint inhibitors (ICIs) along with their oncological outcomes.

Results: Our study included 329 BC and 40 healthy serum samples. The median age of the BC cohort was 67 years, and 62 years for the controls. A median of 546 peptides were enriched in each sample. The median interval between B and FU samples was 76 days. In controls, a more prevalent response against Bacteroides, Clostridioides and E. Coli was observed. Among patients treated with ICIs, responders showed a more prevalent response against Streptoccocus, Staphylococcus and Clostridioides. Moreover in patients BCG failure, there was a more prevalent response against Lactobacillus comparing with patients without BCG exposure.

Conclusions: We observed differences in antibody response patterns against microbiota when comparing BC versus healthy individuals, as well as comparing different outcomes, with a general tendency towards broader repertoire diversity in healthy individuals and favourable outcomes. This suggests that generally impaired anti-microbiome B cell responses might represent a proxy for increased BC risk or negative predictive marker.

Poster# 68

Clinical outcomes from 2020 EAU primary High-risk NMIBC patients treated with BCG by 2021 EAU risk classification

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Background: Post-operative intravesical BCG is the standard regimen for most EAU high-risk non-muscle-invasive bladder cancers (NMIBC). The 2021 EAU risk groups were derived from cases without BCG treatment. We aimed to validate the 2021 risk groups for recurrence and progression among primary BCG-treated NMIBC patients.
Methods: 212 BCG-treated cases were identified from the Bladder Cancer Prognosis Programme, and BCG exposure was defined by the International Bladder Cancer Group. Patients were annotated as intermediate (IR), high (HR) or very high-risk (VHR) according to EAU 2021. Kaplan-Meier curves of recurrence and progression were plotted to present survival. The Log-Rank statistic was used to test differences in survival and predictive accuracy quantified with Harrell's concordance index (C index).
Results: For patients receiving at least induction BCG, there were no differences in PFS for the HR (sub-hazard ratio (sHR), 1.08; 95%CI, 0.24-4.74; p=0.92) and VHR (sHR, 1.94; 95%CI, 0.43-8.77; p=0.39) patients compared to IR (p = 0.30, C index, 0.57). We found a statistically significant difference (p=0.03) with RFS. However, the sHR analysis was comparable to that of PFS (C index, 0.56) (HR: sHR, 1.23; 95%CI, 0.48-3.12; p=0.67, VHR: sHR, 2.20; 95%CI, 0.85-5.66; p=0.10). In patients with adequate BCG there was no statistical difference on PFS for HR (sHR, 1.00; 95%CI, 0.12-8.14; p=0.99) and VHR (sHR, 1.25; 95%CI, 0.14-11.19; p=0.84 compared to IR (p=0.94, C index, 0.51). RFS for IR and HR (sHR, 1.88; 95%CI, 0.45-7.91; p=0.39) or VHR (sHR, 2.97; 95%CI, 0.69-12.80; p=0.15) were not statistically different (p=0.17, C index, 0.56) but a trend towards poorer outcomes in VHR was observed.
Conclusions: The 2021 EAU risk classification appears to be predictive for RFS in our cohort. However, in terms of PFS the risk groups are not discriminatory, although this study's power is limited by a small IR group. More studies are needed to inform radical cystectomy decisions

Poster# 69

Physical activity before radical cystectomy – the PRACTICE trial: preliminary data

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Background: Radical cystectomy (RC) is linked to high morbidity and prolonged recovery. Prehabilitation through structured physical activity may improve outcomes but is understudied in RC. This randomized trial evaluates the effect of preoperative physical activity on perioperative outcomes.
Methods: This bi-center, prospective, single-blinded trial began enrolling patients in November 2024. Patients undergoing RC are randomized to intervention (daily step goal: 8,000–10,000) or a control group (no step target). All participants wear activity trackers to monitor movement and energy expenditure. Physical fitness (chair-rising, handgrip, six-minute walk) and quality of life are assessed at multiple time points up to 12 months postoperatively. Clinical-Trials number: NCT06806059.
Results: Nineteen patients were enrolled; data are currently available for 17 (n=10 intervention, n=7 control). Median age was 71 in control and 61 years in the intervention group (p=0.64). In the intervention group, 8/10 reached ≥8,000 steps/day; 3/8 reached 10,000. Median daily steps were significantly higher in the intervention vs. control group (9,099 vs. 3,250; p=0.04).

No significant differences were found at baseline or in preoperative functional tests. However, one week post-op, the intervention group showed significantly better results in the six-minute walk (404_m vs. 274_m; p=0.03) and chair-rising test (10.7_s vs. 16.3_s; p=0.03). At discharge, these differences were no longer significant. No relevant differences were seen in hospital stay, transfusions, major complications (Clavien-Dindo ≥3), embolic events, or infections. Notably, bowel movements occurred significantly earlier in the intervention group (p=0.012).
Conclusions: Preliminary data show that a daily step goal is feasible and achieved by most patients. Our findings suggest improved postoperative mobility and faster gastrointestinal recovery, without significant differences in complications or transfusion.

Poster# 70

Cost of care and oncologic outcomes associated with blue light cystoscopy in an equal access setting: results from the BRAVO study

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Background: Bladder cancer ranks as one of the costliest malignancies in the United States. This study aimed to compare the healthcare costs and oncologic outcomes as-sociated with blue light cystoscopy (BLC) versus white light cystoscopy (WLC), in non-muscle invasive bladder cancer (NMIBC).

Methods: Patients diagnosed with NMIBC between 1997 and 2021 and underwent BLC or WLC-only in the Veterans Affairs (VA) healthcare system were identified. Pro-pensity-score matching accounting for demographic and clinical variables was used. The primary objective was determining the difference in total healthcare costs over 1, 2, and 5-year intervals. Cost effectiveness analysis was conducted by incorporating cost associated with NMIBC recurrence from SEER data and literature.

Results: Among 622 matched patients with available cost data (311 BLC and 311 WLC), median age was 71 years, 98% were male, and 10% were African American. The study population was predominantly high-risk NMIBC (61%). BLC vs. WLC pa-tients were more likely to receive intravesical BCG (61 vs 43%; $p<0.01$) and intravesi-cal chemotherapy (49% and 28%, $p<0.01$), respectively. Total PPPY costs were higher in the BLC group ($27,054 vs $20,005; $p<0.01$), with outpatient costs being the main driver ($23,609 vs $16,880; $p<0.01$). No difference in inpatient costs were noted. BLC use was associated with decreased risk of recurrence (HR 0.62, 95% CI 0.45 – 0.86). Cost effectiveness analysis showed estimated recurrence cost offset between $17,000-$30,000 per patient, with a NNT of 10 patients. This potentially narrows the cost gap between BLC and WLC to as little as $4,429 per patient over five years.

Conclusions: In a real-world equal-access setting, while BLC exposure was associat-ed with increased healthcare costs per patient, we noted significantly reduced risk of recurrence and estimated recurrence cost offset with BLC.

Poster# 71

Quantitative Outcome-Based Grading for Whole Slide NMIBC Images

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Background: Patients with non-muscle invasive bladder cancer (NMIBC) endure frequent surveillance and treatment, contributing to significant burden and cost for themselves and healthcare systems. Treatment and care are titrated to each patient's needs using the American Urological Association (AUA) risk score which is heavily influenced by cancer grade. Pathologists grade bladder cancer by evaluating prognostically relevant features across whole slide images (WSIs). However, current grading practices are subjective; undermining reproducibility and prognostic power. To assist pathologists, we are developing automated image analysis tools to objectively quantify histologic features in digital WSIs and build models that relate them to patient outcomes.

Methods: Using a cohort of patients with stage Ta NMIBC, 163 WSIs were processed using imaging software (Visiopharm, Hørsholm, Denmark). A U-Net deep learning algorithm was trained to automatically isolate regions of interest (ROI) enriched for cancer cells. Within these ROIs, we detected cancer cell nuclei and mitotic figures, and computed the mitotic index alongside 11 additional nuclear features. Features were used to train a random survival forest (RSF) to predict patient recurrence and compared to a cox proportional hazard (CPH) model built using AUA risk scores.

Results: On a hold-out ROI test set, mitotic figures were identified with ≥80% accuracy, frequently identifying mitotic figures that manual feature annotation missed. RSF models using histological features to predict recurrence free survival achieved a C-index of 0.70 (95% CI: 0.66-0.70) outperforming the AUA risk score (C-index of 0.58; 95% CI: 0.43-0.71).

Conclusions: This work demonstrates superior prognostic performance of quantitative grading and a WSI pipeline that advances clinical implementation. Future efforts will focus on external validation and development of progression models that improve clinical decision-making.

Poster# 72

Differential KLF5 Isoform Usage in Non-Muscle Invasive Bladder Cancer (NMIBC)

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Background: This pilot study explored isoform switching between normal bladder tissue and NMIBC samples, a phenomenon not yet investigated in detail within this context. Isoform switching, which leads to altered protein functions and disease phenotypes, has been implicated in various cancers.
Methods: We used IsoformSwitchAnalzyeR(1.18.0) and 3D RNA-seq(v1.0) tools to detection of isoform switching between full RNA-sequencing of 9 normal bladder tissue samples and 65 NMIBC samples.These samples were collected with ethical approval from the Bladder Cancer Prognosis Programme.
Results: IsoformSwitchAnalzyeR identified 21 genes with significant isoform switchingevents, including KLF5, which was also identified by the 3D analysis. Our analysis showed significant isoform switching of the KLF5 gene between normal and NMIBC samples, while there was no difference in gene expression. KLF5 is a transcription factor known to regulate cell proliferation, differentiation, and angiogenesis in various cancers, including bladder cancer.Specifically, the isoform ENST00000539231 exhibited a highly significant increase in isoform usage in NMIBC compared to normal tissue.While gene expression levels of KLF5 were not significantly different between normal and NMIBC conditions, the substantial alteration in isoform usage of ENST00000539231 suggests a critical role for this specific isoform in NMIBC pathogenesis.
Conclusions: These findings suggest that KLF5 isoform switching, particularly the increased usage of ENST00000539231, could be an early event in NMIBC pathogenesis. Given KLF5's role in promoting cancer cell proliferation and angiogenesis, this altered isoform usage may contribute to NMIBC development. Future research will focus on understanding the functional implications of this specific KLF5 isoform using larger datasets and validating its potential as a diagnostic biomarker through in vitro wet-lab experiments. Further analysis of KLF5 downstream target expression is ongoing.

Poster# 73

SPP1 protein mediates resistance to neoadjuvant chemotherapy in non-urothelial-like MIBC subtypes

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Background: Cisplatin-based neoadjuvant chemotherapy is standard treatment for muscle-invasive bladder cancer (MIBC). However, survival benefits are modest and new tools to predict response to treatment are needed. In a previous subtype-stratified analysis of a Swedish patient cohort, we found that high SPP1 RNA expression is associated with chemotherapy resistance in non-urothelial-like MIBC subtypes. Here, we aim to identify the cells that express SPP1 and to shed light on mechanisms of SPP1-mediated chemoresistance in bladder cancer.
Methods: Single-cell RNA-seq data and multiplex immunofluorescence analysis on tissue microarrays were used to identify the SPP1 expressing cells. Protein expression was correlated with pathological response stratified by Lund taxonomy subtype. Cell lines and organoids of different subtypes were subjected to cisplatin treatment and SPP1 modulation, e.g. knock-down, overexpression and co-culture with SPP1+ macrophages. The role of SPP1 receptors, CD44/integrins, were analyzed by inhibiting or blocking the receptor-ligand interactions. The mechanisms involved in SPP1-mediated treatment resistance will be analyzed by RNA-Seq.
Results: SPP1 protein is expressed by cancer cells or by CD68+ macrophages. Both sources show a similar subtype-dependent effect on treatment resistance ($p= 0.0068$, $p=0.0004$ respectively). Exogenous or overexpressed SPP1 protected basal/squamous but not urothelial-like cells from cisplatin induced cell-death in vitro. This effect was abrogated by blocking the binding to integrins but not CD44. The resistance mechanisms involved and the role of macrophage produced SPP1 are currently under investigation.
Conclusion: Presence of macrophage-, or cancer cell- derived SPP1 can increase chemoresistance in non-urothelial-like MIBC subtypes, possibly by interacting integrins. High pre-operative SPP1 protein levels combined with subtyping may identify tumors that are unlikely to respond to peri-operative chemotherapy.

Poster# 74

Liquid Biopsy Guided Disitamab vedotin combined with toripalimab and radiotherapy for multimodal organ-sparing treatment of muscle invasive bladder cancer: a proof-of-concept study

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Background: Although trimodal therapy is currently the standard organ-sparing approach for muscle-invasive bladder cancer (MIBC), its clinical benefit is limited, and noninvasive biomarkers to guide dynamic decision-making are lacking.

Methods: We conducted a proof-of-concept study evaluating disitamab vedotin (RC48, a HER2-targeted antibody-drug conjugate) combined with toripalimab (JS001, anti-PD-1) and radiotherapy for bladder preservation in six patients with localized HER2-positive MIBC (ClinicalTrials.gov identifier: NCT05979740). Longitudinal liquid biopsy analyses of circulating tumor DNA (ctDNA) and urinary tumor DNA (utDNA) were performed using the PredicineCARE assay to monitor therapeutic response.

Results: The combination therapy was overall tolerable, with no grade 4 treatment-related adverse events or deaths. Five patients (83.3%) achieved a complete response and remained recurrence-free. utDNA testing demonstrated high accuracy in monitoring therapeutic effectiveness and enabled early detection of tumor relapse, whereas ctDNA was largely undetectable in blood samples.

Conclusion: These findings establish the feasibility, efficacy, and potential biomarker utility of this novel bladder-preserving regimen, paving the way for a paradigm shift in MIBC management.

Poster# 75

Identifying Indolent Low Grade and Aggressive High Grade NMIBC: A Quantitative Grading Framework

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Background: Non-muscle invasive bladder cancer (NMIBC) is graded using two sys-tems: WHO 1973 (Grade 1, 2, 3) and WHO 2004 (Low Grade [LG], High Grade [HG]). The WHO 1973 system lacks clear criteria, causing frequent disagreement among pathologists. The WHO 2004 system improves consistency by collapsing grades into two tiers, but fails to identify the most aggressive tumors by excluding Grade 3. Hybrid systems have aimed to improve risk stratification within the HG category but often over-look the clinical and biological distinctiveness of the LG group. We designed two com-plementary approaches to define quantitative boundaries for NMIBC grading.

1. An interquartile range (IQR)–based outlier detector to capture rare, atypical Grade 3 nuclear traits linked to worse prognosis.
2. Recurrence–based analysis to identify a subset of slowest–recurring LG tumors with highly uniform nuclear morphology.

Methods: Image analysis software (Visiopharm) extracted 11 quantitative nuclear measurements from photomicrographs sampled from 255 stage Ta, BCG–naïve NMIBC patients. An IQR–based outlier detection was trained using a reference cohort of 5 Grade 2–HG samples. The detector was applied to the remaining samples to compute their percentage of feature outliers. The merged LG category was compared by each feature's mean and standard deviation (SD) to characterize low–risk nuclear morphology. Kaplan-Meier analysis assessed recurrence–free survival.

Results: Percentage of outliers for larger nuclear diameter best identified rapidly re-curring cancers ($p=0.024$), carrying greater prognostic power than employing mean or SD (both n.s.). Mitotic activity and variability in nuclear shape below the 50th percentile defined indolent LG cancers ($p<0.001$).

Conclusions: These methods enhance grading standardization, reduce interobserver variability, and help identify the most slowly and rapidly recurring cancers. The findings support subdividing distinct subgroups within LG and HG cancers.

Poster# 76

Spatial Profiling of the Tumor Immune Microenvironment in Muscle-Invasive Bladder Cancer TREATED WITH Neoadjuvant Platinum Chemotherapy

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Background: Neoadjuvant chemotherapy (NAC) followed by radical cystectomy (RC) is standard for muscle-invasive bladder cancer (MIBC), yet only ~40% of patients respond. The tumor immune microenvironment (TIME) influences therapy response, but prior studies lack RC-alone cohorts, limiting insight into predictive vs. prognostic biomarkers. We analyze TIME spatial features to identify immune correlates of response to NAC, pembrolizumab (IO), and clinical outcomes.
Methods: Pre-treatment transurethral resection (TUR) specimens from 42 NAC+RC, 90 IO+RC, and 61 RC only patients were analyzed, alongside matched residual tumors (≥pT1N0-3M0-1) from 13, 9, and 32 patients, respectively. Tissue microarrays underwent multiplex immunofluorescence with three panels (B&T cell, adaptive resistance, and myeloid panels). Digital analysis and spatial modeling assessed first nearest-neighbor distances, cellular triads, and immune-tumour dynamics patterns.
Results: TIME spatial structure in TUR specimens was linked to treatment response. Increased heterogeneity surrounding CD4 cells was associated NAC (OR=1.11, 95% CI: 0.8–1.80, p=0.02) and IO response (OR=2.08, 95% CI: 1.30–3.40, p=0.001). Closer CD4-tumor proximity also predicted better response (NAC: OR=0.65, 95% CI: 0.45–0.92, p=0.02; IO: OR=0.60, 95% CI: 0.42–0.86, p=0.004). In the IO-cohort, CD8-associated cell type heterogeneity (OR=1.58, 95% CI: 1.35–1.96, p=0.03) and reduced CD8–tumor distance (OR=0.58, 95% CI: 0.35–0.96, p=0.03) correlated with improved 2-year survival. Post-treatment RC specimens from resistant tumors showed increased stromal CD4 density vs. matched TUR samples (NAC: p=0.002; IO: p=0.04).
Conclusion: Spatial immune metrics correlate with NAC and IO response, and survival in MIBC. Ongoing refinement of the models, inclusion of a NAC+IO (BLASST-1) cohort and external validation aim to support biomarker-guided personalized treatment strategies.