18th Meeting of the IBCN

Saturday, October 17th, 2020

Virtual Meeting

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	Introduction	
1500 CET	Welcome to IBCN Virtual Meeting	Goebell/Kamat

IBCN Speaker		
	Topic	**Nawroth/Dyrskjøt**
1510	The Ariadne's thread of FGFR3	Francois Radvanyi
1530	Discussion	

Abstract Session I		
	Translational Track	**Koti/Kim**
1540	Molecular profiling of post-pembrolizumab muscle-invasive bladder cancer (MIBC) reveals unique features that may inspire new seguential therapies in pathologically-nonresponders	Joep de Jong
1548	Recombinant BCG overexpressing STING agonist elicits trained immunity and improved antitumor efficacy in non-muscle invasive bladder cancer	Alok Singh
1556	Molecular Correlates of Cisplatin-based Chemotherapy Response in Muscle Invasive Bladder Cancer by Integrated Multi-omics Analysis	Ann Taber
1604	Improving Methods to Detect and Target Nucleotide Excision Repair (NER) Deficiency in Bladder Cancer	Kent Mouw
1612	Recurring urothelial carcinomas are clonal but incompatible with a direct relationship	Nour-al-dain Marzouka

	Open Poster Discussion
1620	Translational Track : Dyrskjøt, Malats, Weyerer Clinical Track: Schmitz-Dräger, Todenhöfer, Kukreja

1650	**5 minute break to transfer into breakout groups**

Industry Meets IBCN – Breakout 1655 – 17:55		
Partner	**Topic**	**Facilitators**
FerGene	Gene Therapy Innovations in NMIBC	Bivalacqua, Wachsmuth
BMS	Current and Future Treatment Landscape in Muscle Invasive and Locally Advanced Urothelial Carcinoma	Black, Maira-Arce
Merck	Checkpoint inhibitors in NMIBC and MIBC	Todenhöfer, Kapadia, Homet-Moreno
Janssen	Opening new therapeutic avenues along the disease continuum	Kamat, Spigelman

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Keynote		
	Topic	**Seiler/Todenhöfer**
1755	Improving Immune Checkpoint Inhibitor Therapy in Cancer	Dan Theodorescu
1825	Discussion	

Abstract Session II *(in parallel to Session III)*		
	Basic Research Track	**Real/Daugaard**
1835	Development and characterization of a novel autochthonous semi-spontaneous bladder cancer model by pathological evaluation, single cell sequencing and proteomic profiling	Iliana Kerzeli
1843	Genome-wide CRISPR screen reveals SLFN11 as a potent mediator of cisplatin sensitivity in muscle-invasive bladder cancer	Gunjan Kumar
1851	Identification of new driver and passenger mutations within APOBEC-induced hotspot mutations in bladder cancer	Ming-Jun Shi
1859	STAG2 and PPARg as drivers of luminal-type bladder cancer	Elenora Lapi
1907	Extraction-Free Comprehensive Transcriptome Sequencing of Carcinoma In-situ is Highly Feasible and Insightful in Non-Muscle Invasive Bladder Cancer	Noah Hahn

Abstract Session III *(in parallel to Session II)*		
	Clinical Track	**Kassouf/Gupta**
1835	Common deleterious germline variants shape the urothelial cancer genome	Bishoy Faltas
1843	Specific genetic susceptibility patterns of the urothelial bladder cancer taxonomic subtypes	Raquel Benítez Dorta
1851	Drug repurposing of bladder cancer driven by patients' proteomic signatures	Marika Mokou
1859	Results of a phase I/II single arm clinical trial assessing efficacy, safety and tolerability of the recombinant Bacillus Calmette Guérin (rBCG) VPM1002BC in patients with high-grade non muscle-invasive bladder cancer recurrence after BCG induction with or without BCG maintenance therapy – SAKK 06/14	Cyrill Rentsch
1907	Impact of UTUC on outcomes of non-muscle invasive bladder cancer treated with inravesical BCG	Kelly Bree

Wrap-Up	
1915	Awards Presentation – Schmitz-Dräger & Black Closing Remarks - Kamat & Goebell Invitation to 2021 Meeting - Kassouf

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Posters - Clinical Track		
	Batch#1	
1	Blue light cystoscopy for detection of invasive bladder tumor: Results from multi-institutional registry	Ahmadi
2	Restaging Transurethral Resection of HG Ta Bladder Tumors: A Risk-Adapted Approach	Hensley
3	Continuous bladder irrigation after transurethral resection of non-muscle invasive bladder cancer for prevention of tumour recurrence – a systematic review	Sengupta
4	Lytic effects of water on bladder cancer cell lines – implications for clinical use of water irrigation to reduce recurrence	Sengupta
5	Early Experience with Intravesical Gemcitabine-Docetaxel for BCG-Naïve Patients with High Grade Non-Muscle Invasive Bladder Cancer	Agarwal
6	Correlation between BMI, Diabetes Mellitus, and Outcomes in Patients Treated with BCG Immunotherapy for Non-Muscle Invasive Bladder Cancer	Brooks
7	Protective effect of BCG bladder instillations on pneumonia?	Vermeulen
	Batch#2	
8	Long-Term Outcomes and Costs Among BCG-treated High-Risk Non-Muscle Invasive Bladder Cancer Patients in an Equal Access Setting	Williams
9	Geographic Distribution of Racial Differences in Bladder Cancer Mortality in the United States: A Nationwide Population-Based Study	Williams
10	Comparing Costs of Radical Versus Partial Cystectomy for Patients Diagnosed with Localized Muscle-Invasive Bladder Cancer: Understanding the Value of Surgical Care	Williams
11	Use of Psychotropic Drugs Among Bladder Cancer Patients in the United States	Williams
12	Cabozantinib (CABO) plus durvalumab (DURVA) in patients (pts) with advanced urothelial carcinoma (UC) after platinum chemotherapy: safety and preliminary activity of the open-label, single-arm, phase 2 ARCADIA trial	Marandino
13	Age is associated with response to immune checkpoint blockade in advanced urothelial carcinoma	Beck
	Batch#3	
14	CtDNA as a predictor of outcome in patients treated with neoadjuvant atezolizumab in muscle invasive urothelial cancer	Powles
15	Role of CA 125, CA19-9 and CEA in predicting outcome following neoadjuvant chemotherapy in muscle invasive bladder cancer	Ahmadi
16	Measurable absolute basophil count is associated with progression to muscle invasive disease in patients with high-grade non-muscle invasive bladder cancer	Nykopp
17	Divergent immunobiological correlates of FDA-/EMA-approved PD-L1 assays and scoring algorithms in muscle-invasive bladder cancer	Eckstein
18	Predictive and Prognostic Performance of IHC3 Immunohistochemistry-based Molecular Subtyping in Muscle-Invasive Bladder Cancer	Hardy
19	Transition of ANXA10 expression is a useful diagnostic and prognostic marker in upper tract urothelial carcinoma	Hayashi

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Posters – Translational Track		
	Batch#4	
20	An evaluation of single-sample tumor subtype classification methods	Eriksson
21	Molecular subtyping and immune-gene signatures identify a subset of clinical T1 high-grade (cT1 HG) and cT2 bladder urothelial carcinoma (UC) as candidates for single-agent immune checkpoint inhibition (ICI)	Necchi
22	Heterogeneity-analysis of molecular subtypes of muscle-invasive bladder cancer and their precursor lesions in multiregion mapped whole-organ bladders	Weyerer
23	Regulation of PPARγ expression in luminal muscle invasive bladder cancer	Tortora
24	Proteins involved in mitochondrial biogenesis and dynamics are increased in bladder cancer and are inversely associated with tumor aggressiveness.	Cormio
25	An exploratory proteomic study delineating the local and systemic immune-oncologic profile of urinary bladder cancer patients	Lord
26	Genomic and transcriptomic characterization of metastatic urothelial carcinoma	Nakauma
	Batch#5	
27	Multiplex immunofluorescence to assess the tumor microenvironment in bladder cancer	Sweis
28	Efficacy of Urinary Mast Cell Activation Markers in Patients with Primary High-Grade Non-Muscle Invasive Bladder Cancer Treated with BCG Immunotherapy	Simsekoglu
29	Tumor Immune Microenvironment in Response to Radiotherapy vs BCG in a Murine Model of Bladder Cancer	Lombardo
30	Investigating sexual dimorphism in the tumour immune microenvironment of non-muscle invasive bladder cancer	Chenard
31	CDK4/6 inhibitors improve therapy of oncolytic adenovirus by manipulation of RB-E2F regulated transcription	Nawroth
32	Radiosensitisation of bladder cancer cells via short-chain fatty acids and/or other metabolites produced by the gut microbiota	Then
	Batch#6	
33	FBXW7 loss of function contributes to worse overall survival and is associated with accumulation of MYC in muscle invasive bladder cancer	Matsumoto
34	RBM10: The role of a splicing factor in urothelial homeostasis and tumorigenesis.	Maldonardo
35	Cytotoxic and genotoxic effects of epigenetic inhibitors on bladder cancer cells	Hoffmann
36	The potential for designing urothelial carcinomas using pluripotent stem cell-based systems	Melzer
37	Mutational signature modelling in vitro recapitulates bladder cancer initiation	Baker
38	Organotypic & in vitro monolayer modeling of urothelial carcinoma gives different cellular responses to proteinase activated receptor (PAR) agonism/antagonism	de Lima

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OPDIVO® (nivolumab)

OPDIVO® demonstrated efficacy in urothelial carcinoma patients after failure of prior platinum-containing therapy[1-3]

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(95% CI: 16.1–26.1, n=56/270)[2]
7% complete response rate
(n=18/270)[2]

8.6 months

8.6 months median OS
(95% CI: 6.1–11.3)[2]

Consistent safety profile
with that established across tumour types for OPDIVO® monotherapy[1-3]

* Phase II, single-arm study of OPDIVO® in 270 patients with metastatic or unresectable urothelial carcinoma who have progressed or recurred following treatment with a platinum agent. Patients received OPDIVO® 3 mg/kg intravenously every 2 weeks, with treatment continuing until progression, unacceptable toxicity or other protocol-defined reasons. The primary endpoint was objective response rate per blinded independent review committee (BIRC).[1-3]

CI – confidence interval; ORR – objective response rate; OS – overall survival

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For the German Summary of Product Characteristics, please click here.

References: 1. OPDIVO® Summary of Product Characteristics. Available at: https://www.ema.europa.eu/en/documents/product-information/opdivo-epar-product-information_en.pdf [Accessed September 2020]. **2.** Galsky MD, Saci A, Szabo PM et al. Nivolumab in Patients with Advanced Platinum-resistant Urothelial Carcinoma: Efficacy, Safety, and Biomarker Analyses with Extended Follow-up from CheckMate 275. Clin Cancer Res 2020; published online. DOI:10.1158/1078-0432.CCR-19-4162. **3.** Sharma P, Retz M, Siefker-Radtke A et al. Nivolumab in metastatic urothelial carcinoma after platinum therapy (CheckMate 275): a multicentre, single-arm, phase 2 trial. Lancet Oncol 2017;18(3):312-322.

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PODIUM PRESENTATIONS

Molecular profiling of post-pembrolizumab muscle-invasive bladder cancer (MIBC) reveals unique features that may inspire new seguential therapies in pathologically-nonresponders

Necchi A, de Jong JJ, Raggi D, Gallina A, Bandini M, Giannatempo P, Marandino L, Colecchia M, Briganti A, Montorsi F, Davicioni E, Seiler R, Black PC, Gibb EA

Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy

Introduction: In the PURE-01 study, a proportion of patients (pts) with muscle-invasive bladder cancer (MIBC; T2-4N0M0) had residual invasive disease (ypT2-4) at radical cystectomy (RC) after neoadjuvant pembrolizumab (pembro). The pembro-induced alterations in immunotherapy-resistant tumors remain largely unstudied. We aimed to investigate the biological characteristics of pembro-resistant tumors in comparison to neoadjuvant chemotherapy (NAC)-resistant tumors.

Methods: Gene expression profiling was performed on 26 RC samples from pts with ypT2-4 disease post-pembro, of which 22 had matched pre-pembro transurethral resection of the bladder tumor (TURBT) samples. Matched cases were analyzed by differential gene expression, hallmark signatures, and molecular subtyping (Decipher Bladder, TCGA, Consensus and Lund classifiers). Unsupervised consensus clustering (CC) was used to compare the 26 post-pembro samples with 133 post-NAC samples and to 21 samples collected from the former tumor bed of NAC-treated patients (scar tissue), all derived from RC specimens.

Results: Molecular subtyping of the pre- and post-pembro samples revealed significant 'subtype switching' with only 41% of samples having concordant subtypes using the Decipher Bladder classifier. The post-pembro samples did not cluster with scar tissues on clustering but were highly associated with immune-infiltrated cases from the post-NAC cohort. Two major groups of post-pembro tumors were identified, the first defined by markedly higher immune, stromal, angiogenesis and epithelial-to-mesenchymal signature scores and the second by higher tumor purity, KRAS and reactive oxygen species signature scores. Checkpoint inhibitor genes (CD274, PDCD1LG2) were consistently expressed in both groups.

Conclusions: This study expands our knowledge of pembro-resistant tumors finding the molecular characteristics of these tumors is strikingly different from NAC-resistant tumors. Two distinct clusters of tumors were identified post-pembro, neither of which were characterized as having a scar-like character. A larger cohort will be required to further understand the clinical implications of these findings.

Recombinant BCG overexpressing STING agonist elicits trained immunity and improved antitumor efficacy in non-muscle invasive bladder cancer

Singh AK[1], Praharaj M[1,4], Lombardo KA[2], Yoshida T[3], Matoso A[4], Baras AS[4], Zhao L[5], Prasad P[1], Srikrishna G[1], Powell JD[5], Kates M[2], McConkey D[2], Pardoll DM[5], Bishai WR[1],* Bivalacqua TJ[2]*

[1]Johns Hopkins University, School of Medicine, Department of Medicine, Center for Tuberculosis Research, Baltimore; [2]Johns Hopkins University, School of Medicine, Department of Urology, Baltimore; [3]Department of Urology, Hyogo Prefectural Nishinomiya Hospital, Japan; [4]Department of Pathology, The Johns Hopkins University, Baltimore; [5]The Bloomberg-Kimmel Institute for Cancer Immunotherapy at Johns Hopkins, Baltimore, USA

Introduction: BCG remains first-line therapy for non-muscle invasive bladder cancer (NIMBC) but its mechanism of action is not fully understood nor is it completely efficacious. We engineered a recombinant BCG (rBCG) that releases increased levels of STING agonist, c-di-AMP and compared effects of rBCG to wild type BCG (WT-BCG) by assessing antitumor efficacy and its ability to elicit trained immunity in NMIBC models.

Methods: Rat MNU-carcinogen model of NMIBC was used to compare antitumor efficacy of 6 weekly intravesical installations of WT-BCG versus rBCG by pathologic tumor grading of bladder tissue and qRT-PCR for Th1 cytokines. Primary human and murine macrophage and urothelial carcinoma cell lines were treated with WT-BCG and rBCG and cytokine response was compared using ELISAs while macrophage phenotypes were determined by flow cytometry. MB49 cells were injected into flanks of C57Bl/6J mice followed by intratumoral injection of WT-BCG and rBCG to compare infiltrating Tcells and Teffector responses by flow cytometry. An in vitro trained immunity model of primary human monocytes was used to evaluate epigenetic changes (H3K4Me3) on TNF-a and IL-6 gene promoters by ChIP-PCR.

Results: Compared to WT-BCG, intravesical installation of rBCG in MNU-rats resulted in significantly lower tumor involvement and a more potent induction of Th1 cytokines. rBCG treated primary macrophages showed increased IFN I, IL-6, TNF-a MCP-1 and IL-12 levels concomitant with increased M1 polarization shift. More pronounced IL-6, TNF-a and IL-1b levels were observed in several human and rodent urothelial carcinoma cells in response to rBCG. Intratumoral injection of rBCG caused increased tumor regression and stronger infiltration of IFN-g producing CD4+ T cells in MB49 tumors. rBCG elicited more potent epigenetic changes (H3K4Me3) on the TNF-a and IL-6 gene promoters following monocyte training.

Conclusions: Increased proinflammatory cytokines, potentiated IFN-g+CD4+ T cells, and increased macrophage reprogramming (increased M1 shift and increased epigenetic changes) contributes to enhanced antitumor efficacy of rBCG over WT-BCG in NMIBC tumor models. Recombinant rBCG may be a novel intravesical immunotherapy for patients with NMIBC.

Molecular Correlates of Cisplatin-based Chemotherapy Response in Muscle Invasive Bladder Cancer by Integrated Multi-omics Analysis

Taber A, Christensen E, Lamy P, Nordentoft I, Prip FF, Lindskrog CV, Birkenkamp-Demtröder K, Okholm TLH, Knudsen M, Pedersen JS, Steiniche T, Agerbæk M, Jensen JB, Dyrskjøt L

Department of Molecular Medicine (MOMA), Aarhus University Hospital, Denmark

Introduction: Overtreatment with cisplatin-based chemotherapy is a major issue in the management of muscle-invasive bladder cancer (MIBC), and currently none of the reported biomarkers for predicting response have been implemented in the clinic

Methods: Here we performed a comprehensive multi-omics analysis (genomics, transcriptomics, epigenomics and proteomics) of 300 MIBC patients treated with chemotherapy (neoadjuvant or first-line) to identify molecular changes associated with treatment response.

Results: DNA-based associations with response converged on genomic instability driven by a high number of chromosomal alterations, indels, mutations in a tri-nucleotide signature 5 context and/or BRCA2 mutations. Expression data identified the basal/squamous gene expression subtype to be associated with poor response. Immune cell infiltration and high PD-1 protein expression was also significantly associated with treatment response. We assigned patients to high and low genomic instability groups based on tri-nucleotide signature 5 mutations, indels, allelic imbalance and BRCA2 mutation status. Patients with high genomic instability had a response rate of 71% vs. 49% for patients with low genomic instability ($p = 0.007$). Through further integration of genomic and transcriptomic data, we identified a group of patients with a very high response rate (80%) characterized by high genomic instability and non-Ba/Sq gene expression subtype and a group of patients with a very low response rate (25%) characterized by low genomic instability and Ba/Sq gene expression subtype ($p<0.001$).

Conclusions: Our results highlight several molecular correlates of chemotherapy response and importantly, the integration of genomic instability and gene expression subtypes identified patient groups with vastly different response rates. This could pave the way for future patient selection following validation in prospective clinical trials.

Improving Methods to Detect and Target Nucleotide Excision Repair (NER) Deficiency in Bladder Cancer

Bekele R, Börcsök J, Sztupinszki Z, Gao SP, Diossy M, Samant AS, Dillon KM, Tisza V, Spisak S, Rusz O, Csabai I, Pappot H, Frazier Z, Konieczkowski D, Liu D, Vasani N, Rodrigues JA, Solit DB, Hoffman-Censits J, Plimack E, Rosenberg J, Lazaro JB, Taplin ME, Iyer G, Brunak S, Lozsa R, Van Allen EM, Szuts D, Szallasi Z, Mouw KW.

Dana-Farber Cancer Institute, Boston, USA

Introduction: Cisplatin-based chemotherapy is a first-line treatment for muscle-invasive and metastatic urothelial cancer, but only a subset of patients respond to therapy. Approximately 15% of urothelial tumors have a somatic missense mutation in the nucleotide excision repair (NER) gene ERCC2, which confers increased sensitivity to cisplatin-based chemotherapy. However, a significant subset of patients are ineligible to receive cisplatin-based therapy due to medical contraindications, and no NER-targeted approaches are available for platinum-ineligible or platinum-refractory ERCC2 mutant cases.

Methods: Using available WES/WGS datasets, we derive and validate a composite ERCC2 mutational signature in bladder cancer. In addition, we use in vitro gene editing techniques to create novel NER-deficient/proficient cell pairs and test the impact of NER loss on cellular DNA repair properties and therapeutic sensitivities.

Results: We identify a novel synthetic lethal relationship between tumor NER deficiency and sensitivity to irofulven, an abandoned anti-cancer agent previously shown to have only modest activity in Phase I/II trials in biomarker-unselected populations. Irofulven specifically targets cells with inactivation of the transcription-coupled NER (TC-NER) pathway and leads to robust responses in vitro and in vivo, including in models with acquired cisplatin resistance, while having minimal effect on cells with intact NER. In addition, we develop and validate a composite mutational signature of ERCC2 deficiency in bladder cancer that is strongly associated with cisplatin response in bladder cancer patients and is also associated with cisplatin and irofulven sensitivity in preclinical models. Although developed in ERCC2-mutant cases, the composite mutational signature is also associated with cisplatin response in cases that lack an ERCC2 mutation, and may therefore serve as an additional tool to identify patients likely to respond to NER-targeting agents including cisplatin and irofulven.

Conclusions: We define a composite ERCC2 mutational signature that is associated with cisplatin response, including in WT ERCC2 patients. The signature may be a useful tool to improve cisplatin response prediction in bladder cancer. We also identify a synthetic lethal relationship between tumor NER deficiency and sensitivity to irofulven which represents a potential targeted therapeutic strategy for cisplatin-ineligible or cisplatin-resistant bladder cancer patients with tumor NER deficiency.

Recurring urothelial carcinomas are clonal but incompatible with a direct relationship

Marzouka N, Lindgren D, Eriksson P, Sjödahl G, Bernardo C, Liedberg F, Axelson H, Höglund M.

Lund University, Sweden

Introduction: To describe the genetic relationship between the primary and recurrent bladder tumors and to identify their origins, different models such as the field cancerization, seeding, and intraepithelial migration have been proposed. The aim of this study was to investigate the genetic relationship in syn- and metachronous bladder tumors using different types of data, and to highlight supportive evidence for the proposed models.

Methods: The exophytic part of primary and recurrence urothelial tumors were collected by cold-cup biopsy from patients undergoing transurethral resection at the Lund University Hospital, Sweden, between 2001 and 2007. In total, 49 tumors from 18 patients and their 18 matched normal samples obtained from peripheral blood were collected and hybridized on HumanCNV370-Duo Genotyping BeadChips (Illumina Inc.). Gene expression data were generated using Illumina HumanHT-12 Expression BeadChip. Tumors were classified according the Lund classification system. For 27 tumor samples and matched normal blood samples from 10 patients, a custom target captured panel was used to sequence 1697 cancer genes.

Results: We used the fact that patients with non-muscle invasive bladder tumors show frequent local recurrences and often have multiple tumors to study re-initiation of tumor growth from the same urothelium. By extensive genomic analyses we show that tumors from the same patient are clonal. We show that gross genomic chromosomal aberrations may be present then absent in a recurrent tumor. By analyses of incompatible changes i.e., genomic alterations that cannot be reversed, we show that almost all tumors from a single patient may show such changes, thus the tumors cannot have originated from each other.

Conclusions: As recurring tumors share both genomic alterations and driver gene mutations these must have been present in the urothelium in periods with no overt tumor growth. The presence of similar mutational and CN profiles combined with incompatible events suggests that the primary and the recurrent tumors have a shared ancestry but are not originating from each other. This supports a model that includes a growing and evolving field of urothelial cells that occasionally, and locally, produce bursts of cellular growth leading to overt tumors.

Development and characterization of a novel autochthonous semi-spontaneous bladder cancer model by pathological evaluation, single cell sequencing and proteomic profiling

Kerzeli IK[1], Lord M[1], Stepanek I[1], Chourlia A[1], Larsson E[1], Elgendy R[3], Doroszko M[3], van Hooren L[3], Nelander S[3], Malmstrom PU[2], Dragomir A[3], Segersten U[1,2], Mangsbo SM[1]

[1] Department of Pharmaceutical Biosciences; [2] Department of Surgical Sciences;
[3] Department of Immunology, Genetics and Pathology, Uppsala University, Sweden

Introduction: There is scarce knowledge of the molecular events of recurrence and progression in non-muscle invasive bladder cancer (NMIBC). The heterogeneity of patients' clinical disease impacts the use of molecular tools and hampers therapeutic strategies. To improve the understanding of tumor development and progression in the native microenvironment, we induced and profiled an autochthonous bladder cancer model.

Methods: 0.05% OH-BBN was administrated in drinking water to Hgf-Cdk4(R24C) mice for 10 weeks. Histopathology evaluation of tissue, single cell RNA sequencing, and urine and serum proteomics by the Proximity Extension Assay were performed. Therapeutic studies (anti-PD1, CpG ODN) were performed at the NMIBC stage and on a transplanted novel cell line.

Results: Exposure of transgenic animals to OH-BBN for 10 weeks led to urothelial NMIBC that differed from exposed wt C57BL/6 mice, while females developed T1 tumors faster than males. Tumors were of Tis/T1 stage with squamous differentiation and within 10 weeks progressed to muscle invasive bladder cancer (MIBC). Single cell sequencing displayed three malignant or premalignant cell clusters with distinct molecular profiles trending towards the luminal or basal subtype and genomic instability. Carcinogen induced bladders presented a clear change in neutrophil, macrophage and fibroblast populations. Anti-PD1 monotherapy at NMIBC did not protect animals from progression, but a possible delay in tumor growth was seen in females. Sex differences in proteomic profiles of urine and serum were obvious, however indicators of myeloid cell recruitment to the tumor microenvironment were shared. Subcutaneous tumors of an outgrown Hgf-Cdk4(R24C) tumor derived cell line were significantly controlled using CpG ODN treatment only in female mice.

Conclusions: Hgf-Cdk4(R24C) mice develop NMIBC upon OH-BBN exposure and progress to MIBC. Female animals present more rapid tumor establishment than males. Single cell RNA sequencing revealed the coexistence of heterogeneous tumor cell populations. Sex specific differences appeared in tumor growth, proteomic profiling and therapeutic outcome and should be further explored.

Genome-wide CRISPR screen reveals SLFN11 as a potent mediator of cisplatin sensitivity in muscle-invasive bladder cancer

Kumar G, Ritch E, Oo HZ, Wang CK, Tortora D, Thaper D, Moskalev I, Wyatt A, Black PC, Daugaard M

University of British Columbia, Vancouver, Canada

Introduction: Bladder cancer is among the top ten most common cancer types in the world, with approximately 550,000 new cases annually. The highest burden of bladder cancer is currently on most developed communities across the world and an estimated 9,000 Canadians are diagnosed with bladder cancer each year. Cisplatin-based Neoadjuvant chemotherapy (NAC) followed by radical cystectomy in patients with muscle-invasive bladder cancer (MIBC) has been shown to improve five-year survival, and is therefore currently the first-line standard of care in patients. However, 60% of patients are inherently resistant to NAC at the time of cystectomy. While several mechanisms of cellular resistance to cisplatin have been proposed, the mechanisms presented thus far still do not offer and effective patient response prediction in the context of MIBC. There is therefore, an urgent and unmet need to determine clinically actionable mechanisms of cisplatin resistance.

Methods: In order to elucidate mechanisms of resistant to cisplatin, the study presented in this thesis takes advantage of a pooled genome-wide CRISPR knock-out library targeting 19,114 protein coding genes with 76,441 synthetic guide RNAs (sgRNAs) which allows for an unbiased screen. Upon completion of the screen the top hit was validated in vitro (CRISPR knockout cell lines), in vivo (mouse models) and in patient tumour tissue samples by immunohistochemistry.

Results: A full-scale screen revealed that several genes involved in the pro-apoptotic pathway (such as CASP8, BAX, and TNFSFR10A) and cell cycle regulation have the potential to confer resistance to cisplatin when knocked out. For this study however, we validated the top hit from our screen - Schlafen 11 (SLFN11) - and established that the complete loss of SLFN11 confers a cisplatin resistant phenotype in MIBC. We further established that SLFN11 is involved in the regulation of cell cycle progression upon cisplatin challenge and does so via interactions with Mediator of DNA Damage Checkpoint 1 (MDC1) protein.

Conclusions: Overall, the study presented here offers SLFN11 as a potential biomarker to aid in clinical decision making and to anticipate resistance to cisplatin-based NAC in MIBC. Furthermore, targeting SLFN11 associated pathways could allow for the development of combination therapies to be used in conjunction with cisplatin in the future.

Identification of new driver and passenger mutations within APOBEC-induced hotspot mutations in bladder cancer

Shi MJ*, Meng XY*, Fontugne J, Chen CL, Radvanyi F#, Bernard-Pierrot I#
*# contributed equally

Institut Curie, CNRS, UMR144, Molecular Oncology team, PSL Research University, Paris, France

Introduction: APOBEC-driven mutagenesis and functional positive selection of mutated genes may synergistically drive the higher frequency of some hotspot driver mutations compared to other mutations within the same gene, as we reported for FGFR3S249C. Only a few APOBEC-associated driver hotspot mutations have been identified in bladder cancer (BCa). Here, we systematically looked for and characterized APOBEC-associated hotspots in BCa.

Methods: We analysed 602 published exome-sequenced BCas, for part of which gene expression data were also available. APOBEC-associated hotspots were identified by motif-mapping, mutation-signature fitting and APOBEC-mediated mutagenesis comparison. Joint analysis of DNA hairpin stability and gene expression was performed to predict driver or passenger hotspots. Aryl hydrocarbon receptor (AhR) activity was calculated based on its target genes expression. Effects of AhR knockout/inhibition on BCa cell viability were analysed.

Results: We established a panel of 44 APOBEC-associated hotspot mutations in BCa, which accounted for about half of the hotspot mutations. Fourteen of them overlapped with the hotspots found in other cancer types with high APOBEC activity. They mostly occurred in the DNA lagging-strand templates and the loop of DNA hairpins. APOBEC-associated hotspots presented systematically a higher prevalence than the other mutations within each APOBEC-target gene, independently of their functional impact.A combined analysis of DNA loop stability and gene expression allowed to distinguish known passenger from known driver hotspot mutations in BCa, including loss-of-function mutations affecting tumour suppressor genes, and to predict new candidate drivers, such as AHR Q383H. We further characterized AHR Q383H as an activating driver mutation associated with high AhR activity in luminal tumours. High AhR activity was also found in tumours presenting amplifications of AHR and its co-receptor ARNT. We finally showed that BCa cells presenting those different genetic alterations were sensitive to AhR inhibition.

Conclusions: Our study identified novel potential drivers within APOBEC-associated hotspot mutations in BCa reinforcing the importance of APOBEC mutagenesis in BCa development. It could allow a better understanding of BCa biology and aetiology and have clinical implications such as AhR as a potential therapeutic target. Our results also challenge the dogma that all hotspot mutations are drivers and mostly gain-of-function mutations affecting oncogenes.

STAG2 and PPARg as drivers of luminal-type bladder cancer

Lapi E, Kalisz M, Martínez de Villareal J, Santos C, Sjödahl G, Dyrskjøt L, Höglund M, Losada A and Real FX

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Introduction: Several groups, including ours, have identified STAG2 as a new bladder tumor suppressor gene. STAG2 is part of cohesin, a complex involved in chromosome segregation, DNA repair and chromatin organization. A role for STAG2 in hematopoietic stem cell self-renewal and differentiation has been shown. The mechanisms through which STAG2 contributes to UBC have not been elucidated, but we proposed that they involve processes different from altered chromosome segregation. STAG2 inactivation is associated with a class of differentiated, luminal tumours significantly enriched in the PPARg pathway, a driver of urothelial differentiation. We have set out to determine whether STAG2 inactivation alters stemness and differentiation balance in UBC.

Methods: We have established 2D cultures of primary murine urothelial cells (NU-1), applied integrated ChIPseq and RNAseq data, and generated organoids from conditional Stag2 knockout mice.

Results: - Stag2 loss of expression is significantly associated with the luminal subtype, both in NMIBC and MIBC - Spontaneously immortalized NU-1 cells undergo urothelial differentiation upon PPARg activation and EGFR inhibition - In differentiated NU-1 cells, STAG2 localizes predominantly at promoters and enhancers and is important for the transcriptional regulation of the associated genes - ChIPseq and co-IP data indicate that PPARg and STAG2 - but not STAG1 - are present in the same complex and cooperate in the regulation of tissue-specific transcription - Stag2 knock out urothelial cells show higher clonogenic potential - Concomitant STAG2 loss and urothelial damage lead to hyperplasia, suggesting a role in homeostatic regeneration

Conclusions: STAG2 loss of expression is associated with luminal tumors. Our genomic and biochemical data suggest that STAG2 cooperates with PPARg in regulating urothelial differentiation and its loss alters gene expression, priming cells to proliferate. ATAC-seq experiments will shed light on the effects of Stag2 inactivation on genome accessibility and regulation of gene expression in proliferating and differentiated urothelial cells. Organoids and 2D cultures derived from conditional Stag2 knockout mice provide a novel platform for functional analyses and suggest a possible role of Stag2 in stem cell homeostasis.

Extraction-Free Comprehensive Transcriptome Sequencing of Carcinoma In-situ is Highly Feasible and Insightful in Non-Muscle Invasive Bladder Cancer

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Introduction: Most carcinoma in-situ (CIS) molecular investigations to date have studied CIS in the context of concurrent papillary (Pap) tumors (conPap). A critical gap remains in our understanding of unique CIS biology. We investigated the feasibility of obtaining high-quality transcriptome data from CIS specimens utilizing the novel extraction-free EdgeSeq Precision Immuno-Oncology Panel (PIP) platform.

Methods: Formalin-fixed paraffin embedded CIS, CIS with conPap, and pure Pap tumors were identified. Tumor cells were macro-dissected from two unstained 5-micron thick slides and analyzed on the PIP panel comprised of 1,410 immunomodulatory genes. Principle components analyses (PCA) were performed to identify distinct cohorts. The Bioconductor DEseq2 package was used to identify differential gene expression between CIS +/- conPap and pure Pap tumors. Functional and pathway analyses were performed using Ingenuity Pathway Analysis software. Secondary analyses assessed any confounding effects of relevant patient clinical features.

Results: 135 specimens were identified including: 51 CIS (32 CIS, 19 CIS + concurrent high grade (HG) Ta-T2), 55 HG Pap (21 HGTa, 21 HGT1, 12 HGT2), and 29 low grade (LG) Pap (28 LGTa, 1 LGT1) tumors. 85 patients (63.4%) were BCG-naïve. PIP data from 117 (87.3%) specimens, including 43 of 51 (84.3%) CIS specimens passed all QC measures and were included in analyses. PCA distinguished CIS from pure Pap specimens. Compared to HG Pap, CIS +/- conPap specimens demonstrated increased expression of EMT pathway genes including SNAI1/2, ZEB1, CDH1, GATA3, TWIST2, EPCAM ($p<0.001$, FDR<0.005). In evaluable patients who received subsequent BCG therapy (n=26), increased expression of antigen presentation and B-cell function genes (CCL19, CCL22, LTB, CD52, CCL17, FCMR, MS4A1) were associated with a recurrence-free survival of 12 months or more ($p < 0.001$, FDR<0.1).

Conclusions: Comprehensive transcriptome profiling of archived CIS specimens is highly feasible including pure CIS samples. CIS tumors and tumors from durable responders to BCG therapy are characterized by unique gene expression signatures. Validation of these findings in prospective clinical trials is ongoing.

Common deleterious germline variants shape the urothelial cancer genome

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Introduction: The prevalence and biological consequences of deleterious germline variants (DGVs) in urothelial cancer (UC) are unknown.

Methods: We performed whole-exome sequencing (WES) of 158 tumors and corresponding germline DNA from 80 UC patients at Weill-Cornell Medicine (WCM). We developed a novel computational framework (DGVar) to detect DGVs from germline WES data and predict their biological functions. We used strict criteria to identify truncating variants in 1604 tumor suppressor genes (TSGs) from germline WES data. We assessed germline-somatic interactions over the lifetime of each tumor. We confirmed our findings by applying DGVar to germline WES from 398 patients from the Cancer Genome Atlas (TCGA). We performed extensive validation of our results in other UC cohorts and whole-exome sequencing data from more than 13,000 non-cancer subjects.

Results: DGVs were identified in 45/80 patients (56%) of the WCM UC cohort and 315 DGVs in 48% (190/398) of patients in the TCGA UC cohort. DGVs were significantly more common in UC patients of WCM and TCGA compared to 2,504 subjects from the 1000 genome project (1KGP) population ($p<0.0001$). Similarly, our DGVs were more common in WCM and TCGA UC compared to 11,209 adult parents of European ancestry from the SPARK study of autism. KLK6, POLQ, and ITGA7 variants were the most common DGVs in WCM (15%) and TCGA UC (5%) cohorts. DGVs in the DNA repair pathway genes, including BRCA2, FANCA, POLQ, and XPA were identified in 7.5% of patients. Select DGVs were validated by Sanger sequencing of germline DNA including a novel XPA Leu200* variant, which eliminated the DNA-binding domain. DGVar identified 60 DGVs which are not detectable using currently used germline clinical targeted sequencing panels. Functional modeling showed that 96% of DGVs clustered with somatic variants within 15 Angstroms in genes with known crystal structures. Consistent with the pathogenic role of identified DGVs, we discovered frequent bi-allelic inactivation events arising from somatic losses of wild-type alleles. Furthermore, we observed deepening loss of heterozygosity of DGV genes in serial tumor samples as UC progressed from primary to metastatic sites. Finally, we identified extensive intra-patient heterogeneity in germline-somatic variant interactions and characterized their biological consequences.

Conclusions: We show that close half of UC patients harbor DGVs, which potentially play a critical role in UC initiation and progression. Our results redefine the landscape of germline variants in urothelial cancer and provide a pivotal new understanding of their unique role in the biology of the disease.

Specific genetic susceptibility patterns of the urothelial bladder cancer taxonomic subtypes

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Introduction: Urothelial bladder cancer (UBC) is a complex disease with diverse genetic and environmental factors participating and interacting in its development. There are evidences indicating that UBC is a heterogeneous disease, with molecular subtypes having been characterized. We aimed to identify the UBC genetic susceptibility patterns according to the UBC taxonomic subtypes.

Methods: Subjects came from the SBC/EPICURO case-control study. Paraffin embedded was used to assess immunohistochemical protein expression of KRT5/6, KRT14, FOXA1, and GATA3. Multiple imputation through chained equations was used to replace missing values. The marker quick-scores were measured and used in an unsupervised hierarchical cluster to define the UBC subtypes. Germline DNA was extracted from leukocytes and genotyped with Illumina HumanHap1M array. We analyzed the association between subtypes and genetic variants by applying multinomial logistic ENET with 350,084 SNPs that passed quality control filters. The SNPs specifically associated with each subtype were annotated as protein coding genes and GO biological processes using the FUMA GWAS platform.

Results: Three significant clusters of cases were identified: BASQ-like (8%), Luminal-like (64%) and Mixed (28%). ENET selected 2,888 SNPs differentially associated with the UBC subtypes: 274 SNPs associated with BASQ-like tumors and mainly annotated to GO Lipid Hydroxylation processes; 1654 SNPs associated with Luminal-like tumors and annotated to 24 different GO biological processes, including a phenol containing compound metabolic process involved in tobacco compounds metabolism; 960 SNPs associated with Mixed tumors and annotated to GO cornification processes.

Conclusions: Our results point to a specific genetic susceptibility background for each UBC subtypes. Artificial Intelligence approaches (ENET) has helped in delineating and better understanding of bladder cancer etiology.

Drug repurposing of bladder cancer driven by patients' proteomic signatures

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Introduction: Molecular signatures may present sources of druggable targets not adequately explored yet. The objective of this study was the identification of known de-risked potential therapeutic compounds (drug repurposing) for the treatment of high-risk NMIBC and MIBC, based on high resolution proteomics profiles.

Methods: The next generation Connectivity Map (CMap) was employed for drug repurposing using as input proteomic signatures from patients with MIBC, and NMIBC of low (NPS3) and high aggressive molecular profile (NPS1) from our recently published datasets (Stroggilos et al. Int J Cancer.2020;146(1):281). The retrieved candidate drugs were ranked according to the disease–drug connectivity score. The impact of selected drugs was investigated in vitro in a panel of multi-origin BC cell lines including benign (HBLAK), non–muscle invasive (BFTC-905, SW1710) and invasive (T24, T24M, VmCub1, 253J, HT-1376) cells. The impact on cell proliferation (MTS), colony formation (matrigel), and apoptosis (Annexin V) was assessed.

Results: Among the top ranked compounds reversing the molecular signature of NMIBC from high to low risk subtype were mTOR inhibitors, tubulin inhibitors, caspase activators and RAF inhibitors. WYE-354, an ATP-competitive inhibitor of mTOR, had the highest connectivity score. WYE-354 was also ranked among the top candidate drugs when using as input proteins with consistent changes at the mRNA level in NMIBC progressors versus non-progressors from earlier published studies or proteomics changes in MIBC versus NMIBC. In vitro administration of WYE-354 resulted in a significant reduction in the proliferation rate of all the tested BC cell lines in a concentration-dependent manner and also impaired colony growth by significantly decreasing the size of the colonies. However, no significant effect on BC cell apoptosis could be observed.

Conclusions: In this study, we describe a promising pipeline for identifying repurposed drugs of potential therapeutic impact for aggressive NMIBC, based on patients' proteomic signatures. Deciphering the molecular mechanism of the impact of WYE-354 based on the molecular characteristics of the tested cell lines is ongoing. Evaluation of further compounds or combinations predicted by the CMap analysis is also planned.

Results of a phase I/II single arm clinical trial assessing efficacy, safety and tolerability of the recombinant Bacillus Calmette Guérin VPM1002BC in patients with non-muscle invasive bladder cancer recurrence after BCG induction with or without BCG maintenance therapy – SAKK 06/14

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Introduction: VPM1002BC is a genetically modified *Mycobacterium bovis* Bacillus Calmette Guérin (BCG) strain with potentially improved immunogenicity and attenuation. Here, we report on efficacy, safety and tolerability of intravesical VPM1002BC for the treatment of non-muscle invasive bladder cancer (NMIBC).
Methods: We designed a phase I/II single arm trial (NCT02371447). Patients were eligible with BCG failure (2008 EAU definition) and intermediate to high risk for NMIBC progression after conventional BCG therapy. The primary endpoint of the phase II part was defined as recurrence free survival (RFS) in the bladder 60 weeks after the first VPM1002BC instillation. From September 2015 to April 2018, a total of 40 patients (6 from phase I and 34 from phase II) were included into the trial. Patients were scheduled for a standard treatment of 6 weekly instillations with VPM1002BC followed by a maintenance regimen of 1 year.
Results: The study population consisted of 4 female and 36 male patients (median age 72 years). Previous maintenance BCG therapy was conducted in 14/40 patients. All recurrent tumours were high grade (2004 WHO definition) and 27 (67.5%) patients presented with carcinoma in situ (CIS). RFS in the bladder at 60 weeks after the first instillation was 49.3 % [95% CI 32.1%, 64.4%]. At the same time, progression in stage, grade or new occurrence of CIS had occurred in 12 (30%) patients, 3 of which had progression to muscle-invasive disease. Two patients died from bladder cancer (> 60 weeks after start of therapy). Over the whole course of therapy, treatment related grade 1, 2 and 3 AEs were observed in 15%, 52.5%, and 5% of the patients, respectively. No adverse events (AEs) grade ≥4 occurred. Two (5%) out of the 40 patients did not tolerate ≥ 5 instillations during induction. Fifteen patients (37.5%) received all scheduled instillations.
Conclusion: One year after start of treatment, therapy with VPM100BC resulted in freedom from NMIBC recurrence in the bladder in almost half of the patients with recurrence after previous BCG exposure. The treatment is safe and well tolerated.

Impact of UTUC on Outcomes of Non-Muscle Invasive Bladder Cancer Treated with Intravesical Bacillus Calmette-Guerin

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Introduction: There is a paucity of information regarding impact of upper tract urothelial carcinoma (UTUC) on the patterns of BCG response in patients with NMIBC treated with BCG. We present the clinicopathological characteristics of patients with a history of NMIBC and UTUC treated with intravesical BCG and evaluate the impact of UTUC on BCG response and progression.

Methods: An IRB approved review of patients with NMIBC patients treated with at least induction BCG at our institution between 2000 and 2018 was performed. Patients were then stratified by (1) presence of UTUC and (2) time of UTUC onset (prior to NMIBC diagnosis vs synchronous or metachronous disease). Outcomes were calculated from date of index transurethral resection.

Results: Of 577 patients who received BCG induction for NMIBC, 63 (11%) patients were diagnosed with UTUC. Of these, 36 patients had a history of UTUC prior to NMIBC (median 344 days prior, IQR 211 – 821 days), while 27 developed UTUC after diagnosis of NMIBC (8 synchronous and 19 metachronous, median 350 days after, IQR 25-833 days). There was no difference between the UTUC and no UTUC groups, except that the UTUC group had more multifocal bladder tumors (71% vs 50%, P=0.002) and prostatic urethral involvement (12% vs 5%, P=0.023). The UTUC group had more non-responders to BCG with respect to recurrence (60% vs. 36%, P=0.0002), any stage/grade progression (25% vs 11%, P=0.005), and progression to muscle invasive or metastatic disease (18% vs 8%, P=0.016). There was no significant difference in rates of recurrence or progression based on timing of UTUC with respect to bladder tumor, albeit this analysis is limited by small numbers.

Conclusions: Presence of UTUC – whether prior to or after diagnosis of bladder cancer - was associated with an almost 2-fold increased recurrence and progression rate following BCG. This should be factored in when counselling patients and designing cohorts for clinical trials.

POSTERS

Blue light cystoscopy for detection of invasive bladder tumor: Results from multi-institutional registry

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Introduction: The role of blue light cystoscopy in increasing detection and decreasing recurrence and possibly progression rates in non-muscle invasive bladder cancer compared to white light cystoscopy is well supported in the literature. Here we evaluated the role of blue light cystoscopy in detecting invasive tumors that were not visible on white light cystoscopy.

Methods: Using the multi-institutional Cysview registry database, patients who had at least one white light negative/blue light positive lesion with invasive pathology (≥T1) as highest stage tumor were identified. All white light negative/blue light positive lesions and all invasive tumors in the database were used as denominators. Relevant baseline and outcome data were collected.

Results: A total of 3514 lesions from 1257 unique patients were evaluated. Of all the lesions in the database, 818 (23.2%) lesions were white light negative/blue light positive of which, 7% (55 lesions from 47 unique patients) were invasive. When all the invasive lesions in the database were considered (total of 494 lesions), 11% were white light negative/blue light positive. Of patients with complete follow up in the database (32), 22 (68%) patients underwent radical cystectomy and 11/22 (50%) showed pathologic upstaging including 4/22 (18%) patients with node positive disease.

Conclusions: A considerable proportion of invasive lesions are only detectable by blue light cystoscopy and rate of pathologic upstaging is significant. Our findings suggest an additional benefit of blue light cystoscopy in detection of invasive bladder tumors that has implications for treatment approach.

Restaging Transurethral Resection of HG Ta Bladder Tumors: A Risk-Adapted Approach

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Introduction: The AUA and EAU Guidelines recommend patients with high grade (HG) Ta bladder tumors undergo restaging transurethral resection (reTUR) if categorized as 'High Risk' or in the absence of muscle in the specimen, respectively. These statements were generated from a paucity of data. We investigated the role of reTUR in BCG response to create a risk-adapted approach to the management of HG Ta lesions.

Methods: Review of patients with HG Ta bladder cancer at index transurethral resection (TUR) who received induction BCG at our institution from 2000-2018 was performed. Patients were stratified by (1) presence of residual disease on reTUR and (2) use of AUA Guideline-driven reTUR.

Results: Of the 209 patients with primary HG Ta bladder cancer who received BCG, 95 underwent reTUR which identified residual disease in 42 patients (44%). Factors associated with residual disease on reTUR included tumor multifocality (67% in residual disease group vs. 38% in no residual disease, P=0.004) and carcinoma in situ (CIS, 47% vs. 15%, P=0.001). Index TUR performed at our institution and use of peri-operative chemotherapy were both associated with no residual disease on reTUR (14% vs. 34%, P=0.024; 5% vs. 25%, P=0.009, respectively). Higher EORTC nomogram scores and AUA risk stratification were associated with residual disease on reTUR, but the CUETO risk score was not predictive. When comparing patients categorized as AUA Hight Risk with HG Ta tumors, those who underwent guideline-based reTUR (n=66) had similar outcomes compared to those who did not (n=75). When evaluating for BCG response, we found no difference in recurrence-free survival or progression-free survival between patients who underwent reTUR vs. those who did not, nor between those who had residual disease at reTUR vs. those who did not.

Conclusions: We identified risk factors predicting presence of residual disease at reTUR for HG Ta lesions. However, neither the presence of residual disease on reTUR nor the omission of reTUR in High Risk lesions resulted in adverse response to BCG. We propose that routine reTUR in HGTa patients may be omitted for the sake of cost and morbidity savings without detriment to oncologic outcome in selected patients receiving adequate induction BCG.

Continuous Bladder Irrigation after Transurethral Resection of Non-Muscle Invasive Bladder Cancer for Prevention of Tumour Recurrence – A Systematic Review

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Introduction: Non-muscle invasive bladder cancer (NMIBC) has a high recurrence rate despite transurethral resection of bladder tumour (TURBT) for clearance of macroscopic disease, partly as a result of seeding from exfoliated malignant cells. The immediate instillation of intravesical chemotherapy (IC) can reduce recurrence and is guideline-recommended but has low usage. It is postulated that continuous bladder irrigation (CBI) immediately post TURBT can itself prevent re-implantation and reduce recurrence, which may provide a simple, cheap and practical alternative to IC. We undertook a systematic review of the literature to assess the effect of CBI on NMIBC recurrence.

Methods: Following PRISMA guidelines, relevant publications were identified from an online search of the literature using databases including Ovid Medline and EMBASE between 1980 to 2018. All published prospective randomized controlled trials (RCTs) which compared CBI post-TURBT to a control group were included. The primary endpoint of the study was recurrence

Results: Our search yielded 514 studies of which six met our inclusion criteria. Two studies (935 participants) comparing CBI to no CBI showed a reduction in recurrence at 2 years. Four publications from 3 trials (331 participants) compared CBI to IC, showing comparable recurrence rates at 1 year (OR 1.29, 95% confidence interval 0.78 to 2.13) but a lower risk of adverse events (6-34% vs 27-48%).

Conclusions: CBI post TURBT appears to yield one-year recurrence rates of NMIBC comparable to immediate IC. However, existing studies are small and of heterogenous design, precluding definitive conclusions. Further trials are required to determine if CBI can be implemented routinely to reduce NMIBC recurrence, as well as the optimal irrigant, volume and duration.

Lytic Effects of Water on Bladder Cancer Cell Lines – Implications for Clinical Use of Water Irrigation to Reduce Recurrence

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Introduction: Recurrence of bladder cancer (BC) following initial management with transurethral resection of bladder tumour (TURBT) is observed in 15-60% of patients. Intraoperative tumour spillage can be a concern during TURBT, given that it can lead to tumour cell re-implantation and local recurrence. Immediate post-TURBT instillation of chemotherapy is effective at reducing recurrence, but remains underutilised. There is evidence that mechanically washing out free-floating tumour cells with bladder irrigation may be a comparable but less expensive or toxic alternative. Irrigating with sterile water may be more effective compared to iso-osmotic irrigants (such as saline) by virtue of causing additional osmotic cytolysis, but this has not been well characterised. This in vitro study aimed to ascertain the time-course of osmotic effects of water on BC cell lines to guide its meaningful clinical usage.

Methods: In-vitro studies were conducted on HT1197 and HT1376 BC cell lines, red blood cells, and white blood cells. Each cell line was exposed to either water, 0.9% saline or 1.5% glycine. Cell counts (in triplicate) were performed at 10, 20, and 40 minutes, and then hourly for up to 5 hours using a haemocytometer. Cell viability was determined using Trypan blue.

Results: In both BC cell lines, saline and glycine caused respectively ~50% and 65% reduction in the number of viable cells after a minimum of 1 hour of exposure ($p<0.0001$ vs time 0). Conversely, the effect of water was rapid, inducing 100% cell lysis in under 20 minutes ($p = 0.0001$ vs time 0), while saline and glycine had no measurable effect in this timeframe ($p = 0.9255$ and 0.0662 vs time 0, respectively). For red blood cells and white blood cells, water also precipitated cell lysis within 10 minutes, whilst saline and glycine had minimal effect.

Conclusions: These results suggest that water has a rapid osmolytic effect upon BC cells. If confirmed using ex vivo samples from patients, this suggests that a short period of irrigation with water may potentially be effective in reducing BC recurrence.

Early Experience with Intravesical Gemcitabine-Docetaxel for BCG-Naïve Patients with High Grade Non-Muscle Invasive Bladder Cancer

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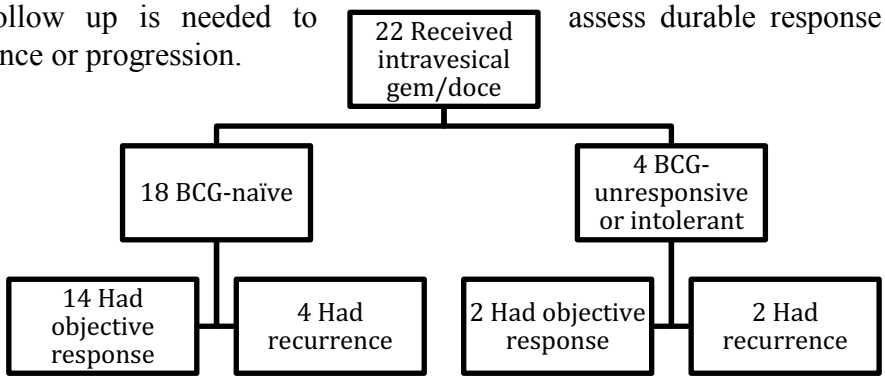
Introduction: Intravesical BCG remains a first-line treatment for patients with intermediate/high risk non-muscle invasive bladder cancer (NMIBC) desiring bladder preservation. In the era of BCG shortage, alternative therapies such as sequential dual intravesical gemcitabine and docetaxel (gem/doce) have been described. We report short term outcomes of patients with BCG-Naïve NMIBC treated with gem/doce in lieu of BCG.

Methods: Patients with high grade NMIBC who had not previously received BCG underwent visually complete TURBT followed by 6 weekly instillations of gemcitabine and docetaxel. At each instillation, patients received 1g of gemcitabine followed by 40mg of docetaxel each for 1-hour dwell. Primary outcome measure was 6-month cystoscopic response rate. Secondary outcome measures include 3-month cystoscopic response rate and progression to MIBC.

Results: 18 BCG-naïve subjects received intravesical gem/doce and completed follow up cystoscopy. Initial staging was Ta in 10, T1 in 4, and CIS in 4. At 6-month cystoscopy, 14/18 patients (78%) were visually free of recurrence. Of the 4 patients with CIS at initial staging, 3 (75%) were visually free of recurrence at 6-month cystoscopy and had benign urine cytology. On ensuing TURBT, there were 2 histologic recurrences. One patient had progression from CIS with Ta disease to CIS with T1 disease and one had persistent T1 disease. No patient progressed to muscle invasion during follow up. There were no further instances of tumor recurrence or progression between 3 and 6 months. All patients started on gem/doce (n=18) completed induction without dose reduction or missed instillation due to adverse effects.

Conclusions: Sequential adjuvant gemcitabine/docetaxel intravesical therapy after TURBT shows promising 6-month response rates for BCG-naïve high grade NMIBC. Longer follow up is needed to assess durable response or effects on recurrence or progression.



Correlation between BMI, Diabetes Mellitus, and Outcomes in Patients Treated with BCG Immunotherapy for Non-Muscle Invasive Bladder Cancer

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Introduction: The association of body mass index (BMI) and diabetes mellitus (DM) with clinical outcomes for patients with solid malignancies including bladder cancer demonstrates disparate and inconsistent effects. We explored this relationship in 582 patients who completed at least an induction course of BCG therapy (iBCG).

Methods: All NMIBC patients treated with iBCG (≥5/6 instillations) from 2006 -2018 were reviewed. BMI was calculated from measured weight and height at the initial clinic visit. Demographic, cancer-specific, and information regarding potential confounding variables including aspirin, statin, metformin, and beta-blocker prescriptions were collected. Univariate and multivariate Cox models were used to analyze recurrence-free (RFS), progression (to MIBC or metastasis) free (PRS), cancer-specific (CSS) and overall (OS) survival. Survival was from the start date of iBCG.

Results: Mean age (582 patients) was 67 yrs. 89% of patients were high grade and 95% of patients received additional BCG after induction. 24% of patients were normal or underweight (<1% of overall patients were underweight) while 76% of patients were obese (51%) or overweight (49%). 18% of patients had DM. Median follow-up was 56 months. Recurrences occurred in 35%, progression in 10%, and cystectomy in 15% of patients. Overall 10 yr CSS was 93% and OS was 63%; BMI ≥25 was significantly associated with higher PFS (64% vs 43% at 10 years), CSS (10 yr: 96% vs 86%), and OS (10 yr: 68% vs 49%). DM was associated with significantly worse RFS (multivariate HR 1.6, 95% CI 1.2-2.1). The impact of BMI ≥ 25 on improved outcomes was maintained on multivariate analysis where the hazard ratio (95% CI) for CSS, OS, and PFS was 0.31 (.011-0.85), 0.49 (0.33-0.74), and 0.58 (0.39-0.85), respectively.

Conclusions: In this contemporary cohort of patients adjusting for known confounders of the metabolic syndrome, overweight and obese patients exhibited significantly improved OS, CSS, and PFS when receiving BCG therapy while those patients with DM experienced worse RFS. These findings support translational research to identify the underlying biologic driver of this interaction.

Protective effect of BCG bladder instillations on pneumonia?

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Introduction: BCG vaccination for tuberculosis is associated with a reduced risk of pneumonia and influenza due to induction of a long-term improved innate immune response termed trained immunity. Its potential protective effect against COVID-19 is currently evaluated in trials. Instillation of BCG in the bladder may also induce a trained immunity phenotype and protection from respiratory infections (RIs). Here, we are the first to present preliminary empirical data on BCG bladder instillations and RIs.
Methods: Non-muscle invasive bladder cancer (NMIBC) patients with date of diagnosis between May 2014 and April 2020 who are participating in BlaZIB or UroLife, two ongoing observational studies, and their spouse/life partner were invited in May 2020 to fill out a questionnaire on bladder instillations, RIs between November 2018 and May 2020, and COVID-19. Clinical and treatment characteristics were extracted from the Netherlands Cancer Registry.
Results: Data on 383 BCG-treated and 211 non-BCG treated NMIBC patients were analyzed. A reduced frequency of all RIs was found in the BCG group, also after adjustment for potential confounders (age, sex, smoking, flu vaccination, lung disease). Unexpectedly, stronger associations were not observed after limiting the BCG group to those 67 patients who were considered BCG exposed (i.e. between 6th induction instillation and <1 year after final instillation) during the whole outcome assessment period, but small numbers prevent meaningful conclusions.

	BCG (n=383)	non-BCG (n=211)	adjusted Odds Ratio (95% CI)	BCG (n=67)
Pneumonia (%)	21 (5.5)	22 (10.4)	0.50 (0.26-0.95)	5 (7.5)
Bronchitis (%)	11 (2.9)	8 (3.8)	0.87 (0.34-2.27)	4 (6.0)
Laryngitis (%)	21 (5.5)	13 (6.2)	0.92 (0.44-1.92)	5 (7.5)
Flu (%)	72 (18.8)	46 (21.8)	0.82 (0.54-1.26)	16 (23.9)
Cold (%)	214 (55.9)	126 (59.7)	0.91 (0.64-1.29)	34 (50.7)
COVID-19 (%)	2 (0.5)	1 (0.5)	-	0 (0)

Conclusions: Our preliminary results suggest a reduced risk of pneumonia and cold by BCG instillations but are inconsistent for other RIs. At this stage, the results cannot support nor contradict the observations in the field of BCG vaccination. We are currently optimizing the data analyses, extending our research into death due to RIs, and studying direct measures of BCG-induced trained immunity in biospecimens of NMIBC patients.

Long-Term Outcomes and Costs Among BCG-treated High-Risk Non-Muscle Invasive Bladder Cancer Patients in an Equal Access Setting

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Introduction: Management of high-risk non-muscle invasive bladder cancer (NMIBC) represents a clinical challenge due to high failure rates despite prior bacillus Calmette-Guérin (BCG) therapy. We describe real-world patient characteristics, long-term outcomes, as well as the economic burden in a high-risk NMIBC population.
Methods: We retrospectively identified a random sample of 412 high-risk NMIBC patients who received ≥ 1 dose of BCG within Veterans Affairs (VA) centers from Jan 1, 2000, to Dec 31, 2016. HR NMIBC was defined as high-grade Ta (TaHG), T1, and/or carcinoma-in-situ (CIS). Adequate BCG induction included at least 5 of 6 instillations, and adequate BCG therapy was at least 7 instillations. We used the Kaplan-Meier method to estimate outcomes including event-free survival. All-cause expenditures were summarized as medians with corresponding interquartile ranges (IQR) and adjusted to 2019 USD.
Results: The total follow-up was 2,694 person-years. At high-risk NMIBC diagnosis, 69 (17%) patients had CIS +/- T1 or TaHG, and 341 (83%) had TaHG or T1, no CIS. A total of 392 (95%) patients received adequate BCG induction and 152 (37%) patients received adequate BCG therapy. Recurrence and progression were observed in 61 (32%) and 71 (17%) patients, respectively. There were 166 deaths during follow-up, of which 27 (7%) patients died from bladder cancer. Total median costs at 1, 2 and 5-year were $29,459 ($14,991-$52,060), $55,267 ($28,667-$99,846), and $117,361 ($59,680-$211,298), respectively. Patients which progressed had significantly higher costs (5-yr, $232,729 vs. $94,879, p<0.001) with outpatient care, pharmacy, and surgery related costs contributed largely to the higher costs associated with disease progression. From initial BCG dose to end of follow-up among patients that underwent radical cystectomy, the median all-cause expenditure per patient was $366,857 (278,462 – 668,378).
Conclusions: In an equal access setting, the vast majority of BCG-treated patients with high-risk NMIBC received adequate BCG induction; however, less than 50% of patients received adequate maintenance therapy. Patients with CIS had increased risk of progression which was associated with significantly increased costs up to 5-yrs after diagnosis. These findings associated with $366,857 per patient that underwent subsequent radical cystectomy further highlight the considerable economic burden of managing BCG treated high-risk NMIBC.

Geographic Distribution of Racial Differences in Bladder Cancer Mortality in the United States: A Nationwide Population-Based Study

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Introduction: African-Americans (AAs) have up to two times increased risk of bladder cancer death than Caucasians. Bladder cancer mortality increases exponentially once it invades the muscle. Geographic heterogeneity in bladder cancer mortality according to race remains to be determined. The purpose of this study was to determine the geographic distribution of muscle-invasive bladder cancer (MIBC) mortality according to race.

Methods: Analysis of Surveillance, Epidemiology, and End Results (SEER)-Medicare data for 6,044 patients aged 66-85 diagnosed with clinical stage T2-T4 N0M0 bladder cancer from January 1, 2002 to December 31, 2011. Fine and Gray competing risks regression models, including an unadjusted model and an adjusted model with an interaction term between race and registry, were used to assess the association of race with bladder cancer-specific mortality (BCSM) according to tumor registry.

Results: Out of 6,044 patients, 5,408 (89.5%) were Caucasian, 352 (5.82%) were non-Hispanic AA, 85 (1.4%) were Hispanic, and 199 (3.29%) were other. Of the 18 registries, AAs with bladder cancer were largely concentrated in Louisiana (19%), New Jersey (17.9%) and Georgia (17.6%). New Jersey was the only registry where AAs had increased risk of BCSM than Caucasians and only for stage T2 disease: (AHR, 1.74; 95% CI 1.22-2.47, P=0.002). AAs in New Jersey had worse BCSM than Caucasians regardless of management: no curative treatment (AHR, 1.61; 95% CI 1.09-2.37, P=0.0168), radical cystectomy (AHR, 2.05; 95% CI 1.26-3.35, P=0.0039), and trimodal therapy (AHR, 1.55; 95% CI 1.03-2.35, P=0.0367).

Conclusions: We observed geographic variation in death from bladder cancer which impacted one registry which had one of the largest population of AAs. These findings support further investigation into the social determinants of race (i.e. socioeconomic status and distance to health care facility) which may drive these results.

Comparing Costs of Radical Versus Partial Cystectomy for Patients Diagnosed with Localized Muscle-Invasive Bladder Cancer: Understanding the Value of Surgical Care

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Introduction: Prior studies noted substantial costs associated with radical cystectomy, however, they lack surgical comparison to a less costly partial cystectomy. We compared costs associated with radical versus partial cystectomy.

Methods: A total of 2,305 patients aged 66-85 years diagnosed with clinical stage T2-4a muscle-invasive bladder cancer from January 1, 2002 through December 31, 2011 were included. Total Medicare costs within one year of diagnosis following radical versus partial cystectomy were compared using inverse probability of treatment-weighted propensity score models. Cox regression and competing risks analysis were used to determine overall and cancer-specific survival, respectively.

Results: Median total costs were not significantly different for radical than partial cystectomy in 90 days ($73,907 vs. $65,721; median difference $16,796, 95% CI $10,038 to $23,558), 180 days ($113,288 vs. $82,840; median difference $36,369, 95% CI $25,744 to $47,392), and 365 days ($143,831 vs. $107,359; median difference $34,628, 95% CI $17,819 to $53,558), respectively. Hospitalization, surgery, pathology/laboratory, pharmacy, and skilled nursing facility costs contributed largely to costs associated with either treatment. Patients who underwent partial cystectomy had similar overall survival but had worse cancer-specific survival (Hazard Ratio (HR) 1.45, 95% Confidence Interval (CI), 1.34-1.58, $p<0.001$) than patients who underwent radical cystectomy.

Conclusions: While treatments for bladder cancer are associated with substantial costs, we showed radical cystectomy had similar total costs when compared to partial cystectomy among patients with muscle-invasive bladder cancer. However, partial cystectomy resulted in worse cancer-specific survival further supporting radical cystectomy as a high-value surgical procedure for muscle-invasive bladder cancer.

Use of Psychotropic Drugs Among Bladder Cancer Patients in the United States

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Introduction: Bladder cancer patients are at increased risk of physical and emotional distress, however, prescription utilization patterns largely remain to be elucidated. Our objective was to comprehensively assess prescription patterns and predictors in bladder cancer patients.

Methods: A total of 10,516 patients diagnosed with clinical stage T1-T4a, N0, M0 bladder urothelial carcinoma from January 1, 2008, to December 31, 2012 from the Surveillance, Epidemiology, and End Results (SEER)-Medicare were analyzed. We used multivariable analysis to determine predictors associated with psychotropic prescription rates. Medication Possession Ratio (MPR) was used as an index to measure adherence in intervals of 3-months, 6-months, 1-year, and 2-years. Evaluation of psychotropic prescribing patterns and adherence across different drugs and demographic factors.

Results: Of the 10,516 patients, 5,621 (53%) were prescribed psychotropic drugs following cancer diagnosis. Overall, 3,972 (38%) patients had previous psychotropic prescriptions prior to cancer diagnosis, and these patients were much more likely to receive a post-cancer diagnosis prescription. Prescription rates for psychotropic medications were higher among patients with higher stage bladder cancer ($P < .001$). GABA modulators/stimulators and serotonin reuptake inhibitors/stimulators were the highest prescribed psychotropic drugs in 21% of all patients. Adherence for all drugs was 32% at 3-months and continued to decrease over time.

Conclusions: Over half of bladder cancer patients received psychotropic prescriptions within two years of their cancer diagnosis. Given the chronicity of psychiatric disorders with observed significantly low adherence to medications warrants an emphasis on prolonged patient monitoring and further investigation.

Cabozantinib (CABO) plus durvalumab (DURVA) in patients (pts) with advanced urothelial carcinoma (UC) after platinum chemotherapy: safety and preliminary activity of the open-label, single-arm, phase 2 ARCADIA trial

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Introduction: Both DURVA and CABO, an inhibitor of MET, AXL, and VEGFR, have shown single-agent activity in pts with UC. ARCADIA is a phase 2 study evaluating the combination of CABO with DURVA in pts with advanced UC or non-UC histology (NCT03824691). Herein we report the results of the interim safety analysis and the preliminary activity.

Methods: Pts receive CABO 40 mg daily, orally, and are administered DURVA 1500 mg, intravenously, q28 days, until disease progression (PD, by RECIST 1.1) or onset of unacceptable toxicity. Key inclusion criteria are: ECOG-PS 0-1, UC and non-UC histology, failure of 1 or 2 platinum-based regimen for metastatic disease. Response is evaluated by RECIST criteria v.1.1 q2 cycles by CT and PET/CT scans. The primary endpoint of the study is OS. Other endpoints include safety (CTCAE v.4.03), objective response-rate (ORR), duration of response, progression-free survival. PD-L1 expression is assessed using with the Ventana SP142 assay. Next-generation sequencing tests (FoundationOne) on pre-therapy tumor samples is also performed

Results: As of May 20, 2020, 14 pts were enrolled with a median follow-up of 5 mo (range 1-8). Median age was 66 yrs (range 44, 74), 21% were male, and 60% had ECOG PS 1. 2 pts had pure neuroendocrine (NE) histology. Four pts (28%) had received 2 prior systemic anticancer therapies. Median tumor mutational burden (TMB) was 6 mut/Mb. Treatment-related AEs (TRAEs) occurred in 9 pts (64.3%), including 2 (14.3%) Grade 3 TRAEs, within the first 2 cycles. 3 pts (21.4%) discontinued CABO due to toxicity, none DURVA. The most common of any grade AEs were increased transaminases (35.7%) asthenia and diarrhea (28.6%), and hypertension (21.4%). In 10 response-evaluable pts, partial response (PR) was obtained in 2 (20%), ongoing at 6+ mo in one case harboring a driver RET (F116L) mutation. 0/2 NE tumors responded.

Conclusions: CABO in combination with DURVA demonstrated encouraging clinical activity in pts with advanced UC with an acceptable safety profile. More mature results according to CABO-response biomarkers and histology will be presented.

Age is associated with response to immune checkpoint blockade in advanced urothelial carcinoma

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Introduction: Urothelial cancer patients treated with immune checkpoint inhibitor (ICI) therapy have varied response and survival. It has been shown that older age is associated with ICI response and survival in melanoma but a prior analysis of the Keynote-052 trial did not demonstrate significant differences in response or survival by age. Age and other biomarkers could help predict ICI response and survival to inform decisions about patient selection for ICI treatment.

Methods: The association of age with response and survival was analyzed in a set of 347 urothelial cancer patients treated with ICI from the IMvigor210 study (Mariathasan 2018). Data were divided into discovery (2/3) and validation (1/3) sets. From clinical metadata, elastic net modeling was used to assess the predictive value of age for response and survival.

Results: In urothelial cancer patients treated with ICI, the optimal age cut-point for analyses of both survival and response was calculated to be 72 years. Patients over 72 years old have significantly higher rates of response ($p = 0.024$) and survival ($p = 0.043$). In univariate analysis with age as a continuous variable, age is significantly correlated with response ($p = 0.032$) but not survival ($p = 0.066$). In multivariable analysis encompassing clinical metadata, age is selected by the final elastic net model of response ($\beta age = 0.0184$ [95% CI 0.0154,0.0213], AUC_model = 0.788, p_AUC = 0.0145). A model built using all clinical metadata including age did not show significantly improved fit in prediction of response compared to a model built with age omitted as a predictor variable (likelihood ratio test: $p = 0.538$). In multivariable analysis encompassing clinical metadata, age is not selected by the final elastic net model of survival (c-index_model = 0.658, p_c-index = 0.00592). Common predictive biomarkers of response, including tumor mutational burden, IFNG expression, and F-TBRS score (Mariathasan 2018) are not correlated with age, suggesting age's association with clinical benefit is independent of these features.

Conclusions: Among urothelial cancer patients treated with ICI, older patients have better response and survival. Age helps predict response to ICI, and age may be an important feature in a model of response from clinical metadata. We propose that age could serve as an important predictor of ICI response to better inform treatment selection in UC.

CtDNA as a predictor of outcome in patients treated with neoadjuvant atezolizumab in muscle invasive urothelial cancer

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Background: To explore the clinical utility of circulating tumor DNA (ctDNA) with neoadjuvant atezolizumab and radical cystectomy (RC) in muscle invasive urothelial bladder cancer (MIUC).

Methods: Forty patients from a phase II of neoadjuvant atezolizumab (2 cycles) and RC study in MIUC were included (NCT02662309). Whole-exome sequencing was performed on tumor and matched normal DNA to identify tumor-specific mutations and design a personalized multiplexed PCR Next Generation Sequencing (NGS) assay (bespoke, Signatera™) for ctDNA detection in plasma. Samples were taken at baseline, post-atezolizumab but pre-surgery, and 1-6 months post-surgery. Response (pathological complete response (pCR) and major pathological response (mPR)) and recurrence free survival (RFS) were correlated with ctDNA status (Pos/Neg) and level. Responders are pCR and mPR patients, whereas non-responders are SD and relapse patients.

Results: The pCR rate and 2 years RFS was 21.4% and 73.5%, respectively. ctDNA positivity at baseline, post-neoadjuvant pre-surgery (PreCx), and post-surgery (PostCx) were 25/40 (63%), 14/30 (47%), and 5/36 (14%), respectively. ctDNA status was highly prognostic at all timepoints, where PostCx had an HR of 78.11 ($p<0.001$), and zero events were observed for ctDNA negative patients at baseline and PreCx. An association between the change in ctDNA during neoadjuvant atezolizumab treatment and patient outcome was observed (mean of -90% ctDNA change in responders versus +27% in non-responders ($p=0.04$)). Patients who were PDL1 positive at baseline were more likely to be ctDNA positive at baseline ($p=0.01$), which was not the case for post-treatment time points. For patients who

were ctDNA positive at baseline, PDL1-positive patients had better response rates than PDL1-negative (35% vs. 0%), as well as superior RFS (HR=0.11, p=0.0085). Pre-treatment ctDNA positivity was enriched in SCCL (Lund) and basal squamous (TCGA) patients. Tumors from ctDNA positive patients at baseline were enriched for immune signatures and the epithelial to mesenchymal transition (EMT) signature.

Conclusion: ctDNA dynamics across timepoints correlates with clinical outcomes, including response rates and RFS in patients treated with neoadjuvant atezolizumab in MIUC. Immune biomarkers may be relevant in these responses. ctDNA may help the development of personalized therapy in this setting in the future.

Role of CA 125, CA19-9 and CEA in predicting outcome following neoadjuvant chemotherapy in muscle invasive bladder cancer

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Introduction: We have previously shown the prognostic value of three tumor markers (TMs) including Carbohydrate Antigen 125 (CA-125), Carbohydrate Antigen 19-9 (CA 19-9) and Carcinoembryonic Antigen (CEA) in muscle invasive bladder cancer (MIBC). Current report presents an update on TM levels before and after neoadjuvant chemotherapy (NAC) and their association with oncological outcomes.

Methods: Serum levels of three TMs were prospectively measured in patients with MIBC who underwent NAC between 2011 and 2019. Rate of pathological upstaging (Path-U) and recurrence-free (RFS) was compared between patients with: (1) Elevated versus normal pre-NAC TM (2) Elevated versus normal post-NAC TM, and (3) Elevated pre-NAC TMs with normalized post-NAC TMs (TM responders) versus persistently elevated post-NAC TMs (TM non responders).

Results: Of a total of 199 patients, 63 patients had both pre- and post-NAC TMs. 33/63 (52%) patients had elevated pre-NAC TM of whom, 15/33 (45%) were TM responders. Patients with elevated pre-NAC TM had significantly higher rate of Path-U compared to those with normal pre-NAC TM (62% vs. 22.5%, respectively; $P<0.001$). There was no significant difference in Path-U in the other two comparison groups. Patients with elevated pre- and post-NAC TM had significantly lower RFS. Compared to TM responders, TM non responders had significantly higher rate of recurrence (70% vs 34%) and shorter median time to recurrence (4.2 months vs 13.5 months) ($P=0.03$). In six patients with recurrence who had complete post cystectomy TM, TM recurrence preceded clinical recurrence by median of 1.2 months (IQR 0.8 – 2.4 months).

Conclusions: Elevated TM prior to NAC is associated with pathologic upstaging. TM elevation pre- or post-NAC predicts a worse outcome. Post-cystectomy TM might play a role in earlier detection of recurrence.

Measurable Absolute Basophil Count is Associated with Progression to Muscle Invasive Disease in Patients with High-Grade Non-Muscle Invasive Bladder Cancer

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Introduction: Intravesical bacillus Calmette-Guérin (BCG) is the standard adjuvant treatment for high-grade non-muscle invasive bladder cancer (NMIBC) after transurethral resection of the bladder tumor (TURBT). Despite this standard of care - 40% of patients experience a recurrence and - 15% progress to muscle invasive disease. Tumors with Th1-polarized lymphocytic infiltrate in the tumor immune microenvironment may have more favorable response to BCG compared to Th2- polarized tumors. Since basophils have a promoting role in Th2-polarization, we explored the association of blood basophils on the outcome of patients with high-grade NMIBC receiving BCG instillations.

Methods: Clinical data and pre-operative complete blood count (CBC) results from patients with high-grade NMIBC (Ta, CIS and T1) who received BCG instillations at the Vancouver Prostate Centre (VPC) during years 2011 – 2018 were collected. Absolute basophil count (ABC) was correlated with response to BCG, disease recurrence and progression to muscle invasive disease.

Results: A total of 174 patients with primary high grade NMIBC treated with BCG at VPC were identified. Pre-operative complete blood count (CBC) data were available from 161 patients. After the initiation of BCG therapy 40% of patients developed recurrent tumors and 13% progressed to muscle invasive disease. ABC was measurable (□ 0.1 x 103 cells /µl) in the blood of 33 (19%) patients of whom 19 (58%) recurred. A total of 95% of recurrent tumors with measurable ABC were considered BCG-unresponsive. In Kaplan-Meier analysis measurable ABC was associated with tumor recurrence ($p = 0.024$) and progression to muscle invasive disease ($p = 0.00015$). In multivariate cox-regression analysis measurable ABC was considered an independent prognostic factor for progression to muscle invasive disease (HR 2.9, 95% CI:1.038 – 8.535, $p = 0.042$).

Conclusions: Measurable blood absolute basophil count may have a prognostic impact for patients with high-grade NMIBC. Further studies with larger patient cohorts are needed to confirm these preliminary results.

Divergent immunobiological correlates of FDA-/EMA-approved PD-L1 assays and scoring algorithms in muscle-invasive bladder cancer

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Introduction: Immune checkpoint inhibition gained significant impact in therapeutic handling of advanced bladder cancer. PD-L1 testing is currently required for first-line immune therapy in platinum-ineligible patients and bases on complex scoring algorithms. We aimed to decipher the composition of distinct scoring algorithms and their associations with immune biological determinants.

Methods: 193 consecutively collected MIBC patients were assessed with four approved PD-L1 assays for all currently established scoring algorithms (IC-Score [companion drug: atezolizumab], CPS [companion drug: pembrolizumab], ICarea/TC-score [companion drug: Durvalumab]). Results were correlated with a combined immune infiltration and immune checkpoint signature consisting of multiple immune cell populations (CD3, CD8, FOXP3, GZMB, CD56, CD68) and other immune checkpoint proteins (LAG3, CTLA4, PD-1). Findings were correlated with clinico-pathological data to elucidate the biological implications of different PD-L1 scoring algorithms.

Results: Of the three scoring algorithms exclusively the IC-score identified patient subsets with different survival rates between PD-L1 negative and positive subgroups (improved survival for PD-L1 positive subset; HR=0.44, P=0.0004) which predominantly detects patients with high PD-L1 expression on immune cells, while the CPS predominantly covers PD-L1 tumor cell positive cases. The ICarea/TC score equally identified both populations. Deeper analysis of the underlying immune biology revelead that high aberrant PD-L1 tumor cell expression associated with worse survival while PD-L1 immune cell expression is highly associated with improved survival. Compared to predominantly PD-L1 tumor cell positive tumors, general immune infiltration is higher in cases with strong PD-L1 expression on immune cells (P=0.0037).

Conclusions: PD-L1 scoring algorithms identify different patient populations with either predominant immune cell (IC-Score [atezolizumab]) or tumor cell expression (CPS [pembrolizumab]), while the ICarea/TC-score (durvalumab) sufficiently covers both populations. High expression of PD-L1 on tumor cells associated with worse survival indicating high predictive potential for PD-L1 inhibition while high expression on immune cells is a baseline characteristic for improved patient survival (positive prognostic value).

Predictive and Prognostic Performance of IHC3 Immunohistochemistry-based Molecular Subtyping in Muscle-Invasive Bladder Cancer

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Introduction: Luminal/basal molecular subtyping in muscle-invasive bladder cancer (MIBC) has shown important relationships to prognosis and chemosensitivity, where tumours of the basal subtype have been reported to be more aggressive, yet more sensitive to cytotoxic therapy. Despite this, molecular subtyping of MIBCs has yet to play a role in clinical treatment selection, largely limited by the complexity and artifacts associated with transcriptomic profiling methods. These shortcomings have in part been addressed by a group form Lund University who validated their subtyping using immunohistochemistry (IHC) to identify 3 key intrinsic subtypes of MIBC: Urothelial-like (URO), Genomically Unstable (GU) and Basal/Squamous cell carcinoma like (SCCL). We aimed to assess the ability of a simplified IHC-based algorithm, termed "IHC3" to identify key intrinsic molecular subtypes and explore the relationships of this classification to prognosis and treatment outcomes.

Methods: 3 tissue microarrays (TMAs) were constructed from (n=133) individual samples from trans-urethral resection of bladder tumour (TURBT) and cystectomy specimens from 87 patients. TMAs were stained using clinical grade IHC assays for GATA3, KRT5 and p16. Scoring was conducted with visual and digital image analysis using percentage positive tumour cells, staining intensity and spatial localization.

Results: IHC3 identified 3 subtypes of MIBC with prognostic differences. Whether given before (NACT) or after surgery (ACT) no significant association between subtype and survival benefit from chemotherapy treatment. Nevertheless, because of their inferior prognosis, Basal/SCCL and GU subtypes might derive the most benefit from an effective systemic therapy.

Conclusions: Using just three antibodies, IHC3 identified key molecular subtypes with prognostic significance, where GU and Basal subtypes showed poor prognosis compared to URO, which showed good prognosis. Application of IHC3 to an expanded cohort of 500+ patients will allow the extension of this work to further explore the predictive significance of IHC3 subtypes and confirm their potential clinical utility.

Transition of ANXA10 expression is a useful diagnostic and prognostic marker in upper tract urothelial carcinoma

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Introduction: Little is known about the diagnostic and prognostic markers of upper urinary tract urothelial cancer (UTUC) because of its rarity. ANXA10, a calcium dependent phospholipid binding protein, is differentially expressed in several cancers. It has been reported that ANXA10 expression is a urinary mRNA biomarker for detection of bladder urothelial cancer (BUC), and loss of ANXA10 expression is associated with BUC progression. To clarify the significance of ANXA10 in UTUC, we studied ANXA10 expression with immunohistochemistry (IHC).

Methods: We analyzed ANXA10 by IHC in 117 respective cases of UTUC treated by radical nephroureterectomy without neoadjuvant chemotherapy. ANXA10 positivity (ANXA10+) was defined as >10 % of nuclear staining.

Results: ANXA10 expression was weak in normal urothelium of upper tract and was positive in 39/117 (33%) of UTUC. ANXA10+ was more frequently in tumors with pure UC (36%, $p<0.05$), papillary morphology (50%, $p<0.01$), histological grade 1/2 (G1/2: 57%, $p<0.01$) and pTa/is/1 stage (55%, $p<0.01$) than in those with histological variants (0%), nodular morphology (9%), G3 (16%) and pT2/3/4 (13%), respectively. Patients with ANXA10+ showed better cancer specific survival (CSS) than those with ANXA10- ($P<0.05$). With IHC, ANXA10+ was detected more in TP53 negative cases (44%, $P<0.01$) and Uroplakin 3 (UPK3) positive cases (44%, $p= 0.089$) than in TP53 positive cases (6%) and UPK3 negative cases (28%), respectively. In TCGA dataset of MIBC, higher ANXA10 expression was correlated with papillary morphology, lower grade/stage, luminal papillary subtype and wild type of TP53.

Conclusions: ANXA10 expression increases during carcinogenesis, and is observed more frequently in papillary UC with lower grade and stage. However, its expression decreases during cancer progression. Transition of ANXA10 expression in UTUC mimicking BC is clinically useful for decision-making.

An evaluation of single-sample tumor subtype classification methods

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Introduction: Large efforts have been made to divide bladder cancer into prognostic or predictive subtypes by unsupervised or semi-supervised clustering of mRNA data. To validate and make use of subtypes, classifiers must be constructed that accurately predicts subtypes in new samples. Variations of nearest centroid classification is commonly used, where each class is represented as a vector of mean fold changes of selected genes. The similarity to these vectors is then used to predict the class of a new sample. Using relative fold change values means that classification becomes related to both the composition of the training cohort and the cohort the new sample originates from. Without thorough data preprocessing, this can result in major classification errors. Optimally, we want to classify a new sample in isolation in a manner robust to preprocessing, sample purity, and mRNA measurement technology.

Methods: We evaluated single sample classification methods based on using sets of gene-pair rules (e.g. Gene A>Gene B indicates Subtype X), using the R packages AIMS and SwitchBox, and a custom iterative RandomForest (RF) approach, as well as the single sample centroid approach used by Kamoun et. el. These were tested on Lund, TCGA, and IMvigor210 data.

Results: Rule-based and centroid based single sample classifiers were able to achieve similar accuracy in predicting LundTaxonomy subtypes in the datasets. The single sample centroid classifier based on raw expression values suffered from poor separation between predictions (delta between best and second-best prediction), which becomes worse as more genes were included. Rule-based methods, particularly RF, had much more distinct subtype calls. The custom RF approach outperformed the published AIMS and SwitchBox methods.

Conclusions: Each single sample classifier method had strengths and limitations. The RF based approach emerged as a promising new method, being robust even when using very few genes, while also allowing for evaluation of class stability and putative incorrect reference labels during training. The main challenge is how to select which gene-rules to use in the classifier. An R package ("multiclassPairs") for building SwitchBox and RandomForest classifier models is currently being prepared.

Molecular subtyping and immune-gene signatures identify a subset of clinical T1 high-grade (cT1 HG) and cT2 bladder urothelial carcinoma (UC) as candidates for single-agent immune checkpoint inhibition (ICI)

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Introduction: We aimed to determine whether a similar ICI-based approach could be used by considering selected cT1 HG and cT2 bladder UC as optimal candidates for single-agent ICI.

Methods: Data from transurethral resection of the bladder tumor (TURBT) specimens from 2 studies was evaluated. The MOL cohort included 206 patients (pts) with HG cT1N0M0 (N=87) or cT2N0M0 (N=119) bladder UC who underwent radical cystectomy (RC) without any neoadjuvant therapy. The PURE-01 cohort (N=102), was used as ICI-treated UC reference. Specimen collection and sample processing were conducted using a clinical-grade whole-transcriptome assay (Decipher® assay). Immune-signatures scores (ISS) and molecular subtyping were evaluated. Kaplan-Meier curves and log-rank tests were used for exploratory analyses of the outcomes in relation to the molecular signatures and ISS.

Results: In the MOL cohort, luminal tumors (LT) were less frequently upstaged at RC vs non-LT (p=0.02). However, organ-confined (OC; pT□2; N=75) LT and non-OC (NOC; pT□3; N=24) LT at RC had statistically similar ISS, assessed with immune190 (0.09 vs 0.09), IFN-a (0.003 vs 0.26), IFN-g (-0.19 vs -0.17) and inflammation score (-0.1 vs -0.1). The remaining OC (N=57) and NOC (N=49) tumors were classified as basal (26/25), luminal infiltrated (19/11), claudin-low (11/8) and neuroendocrine-like (NE-like; 1/5). Except for the NE-like cases, the remaining OC and NOC non-LT showed higher ISS, with particularly high-scores for the claudin-low tumors. Progression-free survival outcomes for LT vs non-LT were favorable, but non-significant (p=0.2). In the MOL cohort, overall survival was inferior for Immune190-high vs low tumors (median split, p=0.042).

Conclusions: In further analyzing the MOL cohort we identified a population of cT1-T2N0M0 tumors which shared molecular features with tumors included in PURE-01. These profiles were consistent regardless of whether the tumor was ultimately identified as OC or NOC, suggesting that treatment with ICI could be proposed to more selected cT1 HG. Clinical trials will be required to confirm the clinical utility of these observations.

Heterogeneity-analysis of molecular subtypes of muscle-invasive bladder cancer and their precursor lesions in multiregion mapped whole-organ bladders

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Introduction: In the recent years, molecular subtypes of urothelial bladder cancer have been described which seem to have a predictive effect for the efficacy of chemotherapy regimens and therefore are now under clinical investigation. Due to this clinical implication and possible decision guidance, the aim of our study is to characterize in which extent precursor lesions and tumors exhibit a heterogeneity of molecular subtypes, which possibly could influence response to chemotherapy regimens.

Methods: 23 positions of three whole-organ mapped bladder specimens were histomorphologically reviewed for classifying location of normal urothelium, precursor lesions and different areas of the tumor. Immunohistochemical analysis of luminal (CK20, GATA3, FOXA1) and basal markers (CD44, CK14 and CK5/6) was carried out in selected precursor lesions and tumors to map the distribution of luminal and basal subtypes across the different positions/lesions.

Results: Out of three analyzed multiregion mapped bladder tumors, one showed a heterogenous luminal and double-negative molecular subtype in multiple tumor positions as well as a mixed morphology including conventional urothelial and neuroendocrine areas. Moreover, among the second analyzed bladder tumor, all tumor positions showed a high expression of basal markers whereas the included Carcinoma in situ demonstrated a luminal subtype with almost total absence of basal markers. The third analyzed mapped bladder tumor demonstrated a homogenous luminal molecular subtype in all precursor and tumorous lesions.

Conclusions: This first analysis of three multiregion mapped bladder tumors shows divergent results of subtype distribution: Heterogeneity of subtypes can be observed, but other tumors and associated precursor lesions show a homogenous distribution of subtypes. To further elucidate how often subtype heterogeneity occurs in whole multiregion mapped bladder tumors, data of further bladder specimens will be presented at the meeting.

Regulation of PPARγ expression in luminal muscle invasive bladder cancer

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Introduction: Peroxisome proliferator-activated receptor gamma (PPARγ) is a ligand-dependent transcription factor belonging to the type II nuclear receptor family. PPARγ overexpression and activation are important hallmarks of the luminal subtype of muscle invasive bladder cancer (MIBC), where PPARγ has been ascribed an oncogenic role and has been associated with immune exclusion. There is therefore an important need to better understand the mechanisms that regulate PPARγ.

Methods: A high throughput genome-wide CRISPR knock-out screen in a luminal MIBC cell line was performed to identify endogenous regulators of PPARγ expression. Using CRISPR knock-in technology, the cells were engineered to express GFP and PPARγ proportionally. The top candidates were then individually validated by RNAi and CRISPR gene knockout for their ability to alter PPARγ mRNA and protein levels.

Results: The screen highlighted 47 potential regulators of PPARγ expression with a false discovery rate below 1%. These can be grouped in functional clusters of transcription factors and chromatin remodeling enzymes currently known for orchestrating cellular oxidative stress response, detoxification from xenobiotic chemicals or general gene transcription regulation.

Conclusions: In this study we developed a powerful screening tool for the characterization of novel factors involved in the expression of key bladder cancer players. In particular, our results revealed the intricated regulatory mechanisms of PPARγ expression exploited by luminal MIBC, thus highlighting the importance of fine-tuning of this nuclear receptor in the biology of the disease.

Proteins involved in mitochondrial biogenesis and dynamics are increased in bladder cancer and are inversely associated with tumor aggressiveness.

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Introduction: Bladder cancer (BC) is a common malignancy but its potential correlation with mitochondrial function is poorly known. This study aimed to investigate whether mitochondrial alterations are present in BC and whether they correlate with tumor aggressiveness.

Methods: Samples of bladder cancer and of healthy-looking bladder wall (control tissue) were obtained from patients scheduled for transurethral resection of bladder tumor or radical cystectomy. The expression levels of proteins involved in mitochondrial biogenesis, dynamics and proteolysis and the mtDNA content were assessed by western blotting analysis and real time PCR, respectively.

Results: Compared to control tissue, the neoplastic tissue displayed greater expression levels of proteins involved in mitochondrial biogenesis, dynamics and proteolysis suggesting an increase of mitochondrial biogenesis. Interestingly, when stratifying for tumor aggressiveness, many of these analyzed proteins, mtDNA content decreased in patients with High Aggressive Potential (HAP) (high-grade T2/T3/T4) disease than in those with Low Aggressive Potential (LAP) (low-grade Ta) disease.

Conclusions: Overall, findings suggest that oxidative metabolism tends to reduce in HAP tumors. A deeper understanding of mitochondrial metabolism could provide opportunities for novel therapeutic strategies.

An exploratory proteomic study delineating the local and systemic immune-oncologic profile of urinary bladder cancer patients

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Introduction: Cancer of the urinary bladder (UBC) primarily derives from the urothelium, encompassing the inner surface of the bladder. Tumors infiltrating the detrusor muscle are categorized as muscle-invasive bladder cancer (MIBC) and constitutes 20-25% of all newly diagnosed UBC. These tumors are more likely to spread to peripheral organs and lymph nodes, compared to the more prevalent (75-80%) non-muscle invasive bladder cancer subtype (NMIBC). To this end, cystoscopy and urine cytology are the current standards for diagnosing and surveying UBC, which provides limited information regarding their immunological profile.

Methods: We performed a targeted human protein biomarker analysis from collected plasma and urine from a well-categorized UBC patient cohort. In short, 92 immuno-oncology related proteins were investigated by a novel Proximity Extension Assay multiplex technology (Olink).

Results: The assayed proteins depicted a heterogenic immune profile. Neither PCA or unsupervised hierarchical clustering identified distinct groups associated with clinical features. However, a direct comparison between non-invasive and invasive cases resulted in 4 differentially expressed proteins ($p<0.05$). MIBC patients had lower urine levels of the TNF-receptor superfamily members CD27 and CD40, and a systemic increase of matrix metallopeptidase 7 (MMP7) and C-C motif chemokine ligand 23 (CCL23). We further developed and trained a random forest machine learning classifier on our plasma samples. It consistently ranked CCL23 and MMP7 as the most important markers for invasiveness. When extended to survival analysis, we moreover found MMP levels to best predict a dismal survival status with MMP12 being the highest ranked variable. A finding supported by MMP12 being the most significant protein in individual cox-regression survival analyses.

Conclusions: This study highlights the heterogenic nature of the immunogenic landscape in UBC and its potential role in immunotherapy treatment responses. Our findings indicate that systemic MMP levels should be further explored as a potential liquid biopsy marker for patient stratification purposes. The role of MMPs in UBC may be linked to an invasive tumor characteristic, as shown by previous UBC studies where high levels of MMPs correlated to reduced overall survival.

Genomic and Transcriptomic Characterization of Metastatic Urothelial Carcinoma

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Introduction: Standard treatment for patients with metastatic urothelial carcinoma (mUC) consists of platinum-based chemotherapy, and recently systemic immunotherapy has become available. Nevertheless, the overall survival of mUC patients remains poor and new therapies are needed. To identify novel targets for therapy, large-scale sequencing efforts are mandatory. The Cancer Genome Atlas (TCGA) initiative substantially improved our knowledge on the genomic and transcriptomic characteristics of primary UC, however, our knowledge on mUC is still scarce.

Methods: We performed whole genome DNA sequencing (WGS) on 116 biopsies of mUC, and mRNA sequencing (RNAseq) on 90 matched biopsies. We applied genomic alteration analysis on the WGS data and used consensus clustering on the RNAseq data.

Results: WGS analysis showed that TP53 mutations were enriched in mUC compared to primary UC. APOBEC mutagenesis was detected in 91% of the samples and correlated with APOBEC expression. Two major mutational processes covering 92% of the samples were identified in mUC and validated in TCGA data. Tumors with high APOBEC mutagenesis had high ploidy, more somatic mutations and more copy number aberrations. Furthermore, five molecular subtypes were identified by RNAseq analysis. Two subtypes (40%) resembled the luminal subtypes identified in primary UC, the other subtypes had a stroma rich (24%), basal/squamous (23%) or unspecific phenotype (12%). The subtypes were different in gene expression, genomic alterations, pathway activity and immune cell infiltration. These differences suggest that these subtypes are clinically relevant. The luminal subtypes showed high frequency of FGFR3 and PPARG alterations as well as high expression of these genes and may be the group benefitting most from FGFR and PPARG inhibitors. The basal/squamous subtype had high levels of CD274, the gene that encodes PD-L1 for which checkpoint inhibitors could be effective. The stroma rich subtype seems highly PDGFRA driven and therefore PDGFR inhibitors may be the way to improve treatment in this group.

Conclusions: Using WGS and RNAseq analyses, we described a comprehensive overview of the molecular landscape of mUC that can serve as a framework for further research on personalizing systemic treatment for mUC patients.

Multiplex immunofluorescence to assess the tumor microenvironment in bladder cancer

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Introduction: A T cell-inflamed tumor microenvironment (TME) is linked to improved prognosis and response to immunotherapy in bladder cancer. Batf3+ dendritic cells (DCs) have been observed in murine models to be critical for both priming an immune response and recruiting effector CD8+ T cells to the TME. However, the role of Batf3+ DCs in human bladder cancers, especially in relation to effector CD8+ T cells, remains unknown.

Methods: We performed multiplex immunofluorescence imaging on 47 surgically resected muscle invasive bladder cancer specimens to evaluate the population of tumor infiltrating immune cells (TIICs) marked by BATF3, CD8, FoxP3, PD-1, and PD-L1. The relationship of TIICs to a previously described immune gene signature based on RNA sequencing and survival outcomes were investigated.

Results: The proportion of T cell-inflamed tumors determined by RNA sequencing was higher in groups with above median TIICs versus below except for PD-1 (70.8% vs 21.7% for CD8 [P = 0.003], 66.7% vs 26.1% for Batf3 [P = 0.010], and 58.3% vs 34.8% for PD-1 [P = 0.266], 66.7% vs 26.1% for FOXP3 [P = 0.010], and 62.5% vs 30.4% for PD-L1 [P = 0.056], respectively). Analyzing Batf3 and CD8 jointly identified the group with the highest proportion of T cell-inflamed tumors (86.7% for CD8highBatf3high vs 34.8% for others [P = 0.001]) and showed a stronger association than with CD8 alone. In a spatial analysis using K function between CD8+ cells and Batf3+ cells, the median area under the curves of the cases were significantly higher than that of a hypothetically calculated random distribution curve, indicating that CD8+ cells clustered in proximity to Batf3+ cells. In survival analysis, CD8highBatfhigh population showed a significantly better RFS and OS than others (HR 0.307 [P = 0.019] for RFS, HR 0.361 [P = 0.044] for OS), which was more differentiating CD8 status alone (HR 0.393 [P = 0.021] for RFS, HR 0.436 [P = 0.045] for OS).

Conclusions: Most of the investigated immune cell types correlated with a T cell-inflamed TME by gene expression profiling. CD8highBatf3high tumors were most associated with a T cell-inflamed TME and showed better survival outcomes. Further analyses to assess associations between TME and immunotherapy response are ongoing.

Efficacy of Urinary Mast Cell Activation Markers in Patients with Primary High-Grade Non-Muscle Invasive Bladder Cancer Treated with BCG Immunotherapy

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Introduction: Mast cells play critical roles in cancer pathway. We aimed to prospectively investigate the urinary mast cell activation markers in patients with non-muscle invasive bladder cancer (NMIBC) treated with Bacillus Calmette-Guérin (BCG).

Methods: Nineteen patients who were received immunotherapy due to NMIBC and 19 healthy participants were enrolled. Urine samples were collected to assay N-methylhistamine, histamine and tryptase levels immediately before the first BCG instillation, immediately after the third and sixth instillations, and four weeks after the sixth instillation in patients with NMIBC and at a single visit in healthy participants. Cystoscopy were performed on the patient with NMIBC at three-month intervals for two years. The changes in urinary markers due to BCC response, BCG instillation, and presence of NMIBC were assessed.

Results: The average age was 56.1 ± 10.5 years. Fourteen patients had high-grade Ta tumors, and 5 had high-grade T1 tumors. While 12 patients responded, 6 presented with recurrence and 1 with progression. There was no correlation between mast cell activation markers and BCG response. The N-methylhistamine and histamine levels were increased significantly with the onset of immunotherapy and N-methylhistamine levels were decreased significantly when immunotherapy was terminated. Pre-BCG estimated marginal means of N-methylhistamine were significantly higher in patients with NMIBC than healthy participants.

Conclusions: This is the first study to determine that urine N-methylhistamine levels increase with BCG instillation and NMIBC presence. However, urinary mast cell activation markers were not found to significantly predict the patients' response to immunotherapy. Further studies are required to ascertain the clinical implications of these finding.

Tumor Immune Microenvironment in Response to Radiotherapy vs BCG in a Murine Model of Bladder Cancer

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Background: The role of radiotherapy combined with immunotherapy in patients with non-muscle invasive bladder cancer (NMIBC) is currently being investigated. Radiation induced DNA damage triggering immunogenic tumor cell death and consequent immune responses in bladder tumor microenvironment is not fully understood. We compared local immune response following radiotherapy with BCG-induced immune response using a BBN murine model of urothelial carcinoma (UC).

Methods: Female C57BL/6 control(no tumor, n=15) or BBN exposed mice(tumor, n=13) received radiotherapy(single external beam dose,15 Gy,n=10), intravesical BCG instillation (3x100µL of $1x10^8$CFU,n=9) or saline treatment (n=9). Tumor involvement was assessed by pathologic grading of bladder tissue and immune responses quantified using Nanostring Myeloid Innate Immunity Panel. Infiltrating Tcells were evaluated by CD4, CD8 and FOXP3 immunohistochemistry (IHC) and tissue macrophages quantified as M1 or M2 phenotype based on CD86 and CD206 IHC.

Results: No tumors were seen in BBN+BCG bladder tissues compared to BBN+saline or BBN+radiotherapy. Intravesical BCG instillation in BBN mice elevated cytosolic DNA sensing pathway as seen by increased mRNA levels of *tmem173*(STING) that was consistent with elevated levels of downstream cytokines *tnfa*, *infg*, *il6* and chemokine *cxcl10*. mRNA expression of 3'exonuclease *trex1*, that degrades 3' ends of nicked DNA during immunogenic cell death, was elevated in BBN+BCG as well as BBN+radiation samples. BBN+BCG samples had increased CD8/FOXP3 and CD4/FOXP3 ratios assessed by IHC and increased M1 macrophage shift validated by mRNA expression and IHC.

Conclusion: BCG instillation caused a robust proinflammatory immune response compared to radiation in murine UC possibly via STING-dependent induction of proinflammatory cytokines and chemokines concomitant with shift toward M1 macrophage polarization and increased Teff/Treg ratios. Elevated *trex1* may degrade DNA damage as a direct response to radiation thereby inhibiting STING activation and cytokine response in radiated samples. These findings highlight the need to uncover mechanisms of radiation induced immune response in NMIBC to optimize utilization of immunotherapy with radiotherapy.

Investigating sexual dimorphism in the tumour immune microenvironment of non-muscle invasive bladder cancer

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Introduction: The incidence of urothelial carcinoma of the bladder is four times higher in men compared to women. However, women with bladder cancer present with more aggressive disease and do not respond as well to Bacille Calmette-Guérin immunotherapy, the gold-standard treatment for non-muscle invasive bladder cancer (NMIBC). Based on the established evidence on sexual dimorphism in physiologic immune responses in bladder mucosa, we hypothesized that sex associated bladder immune physiology may influence the tumour immune microenvironment (TIME) and clinical outcomes.

Methods: We interrogated the expression patterns of genes associated with immune cell phenotypes (T cells, B cells and macrophages) and immune checkpoint pathways using tumour whole transcriptome profiles from male (n=376) and female (n=109) NMIBC patients. In an independent cohort of 390 tumours (n=85 females and n=305 males) on a tissue microarray, we conducted multiplexed immunofluorescence to evaluate the density and spatial distribution of CD163+ M2 tumour associated macrophages, CD3+CD8- (T helper), CD8+ (T cytotoxic), CD79a+ (B), CD103+ (T resident) cells and the immune checkpoint proteins PD-1 and PD-L1.

Results: High-grade tumours from female patients exhibited significantly increased expression of CD274 (PDL1), CTLA4 and IDO1 immune checkpoint genes. Significantly higher infiltration of CD163+ M2 macrophages in the epithelial and stromal compartments was observed in high-grade tumours from females. Significantly increased PD-L1 protein expression was also observed in the epithelial compartment of high-grade tumours from females compared to those from males. Higher infiltration of CD163+ M2 macrophages and CD79a+ B cells significantly associated with shorter recurrence-free survival in high-grade tumours irrespective of patient sex.

Conclusions: Findings from this study provide the first evidence supporting sexual dimorphism in the TIME of NMIBC. Future mechanistic studies are warranted to determine the potential roles of M2 tumour associated macrophages in promoting an aggressive disease behaviour and inferior clinical outcomes, specifically in female patients.

CDK4/6 inhibitors improve therapy of oncolytic adenovirus by manipulation of RB-E2F regulated transcription

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Introduction: Effective viral replication is the key in oncolytic virotherapy not only to induce the lytic potential of the virus but also in triggering a systemic immune response against the tumor. We investigated CDK4/6 inhibitors and their effects on the replication of the oncolytic adenovirus XVir-N-31.

Methods: Bladder cancer cell lines (UMUC3, T24, RT112, 253J, 647V, 639V) were treated with the oncolytic adenovirus XVir-N-31 and/or small molecule inhibitors against Checkpoint kinase 1 (Chk1) (UCN-01, AZD7762) or CDK4/6 (PD-0332991, LEE011, LY-2835219) (CDK4/6i). Cells were infected with viral titers that do not result in cell death were combined with the relative IC 50 of targeted therapies. Cell viability was assessed using a SRB assay and biochemical effects were examined by immunoblotting against E1A, E2A, Hexon, E1B55k, RB, p107, p130, p21, p27, DP1, E2F1-5, Myc, cyclin D1 and E2, ERK and AKT. Virus replication and titer was determined by using qPCR and a titer test on Hek293 cells. siRNAs were used to interfere with E2F1-3 and RB expression level. Effect of E2F binding was examined by cloning different viral vectors modified in E2F binding sites or also E2F trapping mutants.

Results: Only the combination of CDK4/6i and XVir-N-31 induced virus specific cell death of 70-90% compared to monotherapy with the inhibitors used. Rb negative cell lines, resistant to PD-0332991, did not show additional effects upon combination treatment. Previously described effects of UCN-01 are due to its ability to also target CDK4/6 but specific CHK-1 inhibition had no effects on virus induced cell death and replication. An increase of 5-10 fold in replication was observed upon use of CDK4/6 inhibitors that decrease the level of RB/E2F1. This modulation induces an earlier and increased transcription of viral genes and was confirmed with viral mutant constructs.

Conclusions: Oncolytic adenovirotherapy can be improved in combination with specific CDK4/6 inhibitors by reducing the level of RB/E2F protein complex during early adenoviral life cycle.

Radiosensitisation of bladder cancer cells via short-chain fatty acids and/or other metabolites produced by the gut microbiota

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Introduction: New non-toxic radiosensitisers are needed in the treatment of muscle-invasive bladder cancer because elderly patients are very vulnerable to chemotherapy-related toxicity of currently available radiosensitisers. Our previous study showed that high fibre diets sensitised RT112 xenografts to irradiation by modifying the gut microbiome and this phenotype was positively correlated with B. acidifaciens abundance. Short chain fatty acids (SCFAs) are major products of fibre fermentation by the gut microbiota. Therefore, we hypothesise that B. acidifaciens may radiosensitise tumours via secretion of SCFAs and/or other metabolites.

Methods: We treated the RT112 human bladder cancer cell line with SCFAs to determine histone acetylation levels by Western Blot and radiosensitivity by clonogenic assay. We also used a cell viability assay to validate the cytotoxic effects of SCFAs and bacterial supernatants on cancer cells.

Results: We showed that all three SCFAs increased histone acetylation (10 mM acetate $p=0.014$, 10 mM propionate $p=0.004$, 10 mM butyrate $p<0.001$) of RT112 bladder cancer cells, in a dose-dependent manner. All SCFAs tended to increase radiosensitivity in the clonogenic assay, with butyrate having a statistically significant effect ($p=0.002$ for butyrate at 8Gy). In a cell viability assay, we saw that the combination of SCFAs in a physiological ratio conferred a stronger phenotype than single SCFAs, in a time-dependent manner. To validate the anti-tumoural effects of B. acidifaciens, we treated the bladder tumour cells with bacterial supernatants of B. acidifaciens and its cross-feeding with F. prausnitzii, and compared the effects to Bifidobacterium (acetate-producer) and F. prausnitzii (butyrate-producer). Bacterial supernatants of B. acidifaciens and its cross-feeding with F. prausnitzii significantly increased the cytotoxic response of bladder tumour cells compared to the other supernatants.

Conclusions: In conclusion, our in vitro experiments in RT112 human bladder cancer cells suggest a role for short-chain fatty acids and/or other metabolites generated by the microbiota in radiosensitisation of tumour cells.

FBXW7 loss of function contributes to worse overall survival and is associated with accumulation of MYC in muscle invasive bladder cancer

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Introduction: With a five-year overall survival of approximately 50%, there is an important clinical need to understand the molecular behavior of muscle-invasive bladder cancer (MIBC). FBXW7, a subunit of an E3-ubiquitin ligase complex, is a tumour suppressor gene. Mutations in FBWX7 have been described in an expanding number of human neoplasms, including 10% of MIBC, making it one of the most commonly mutated genes in MIBC. However, it has received little attention in MIBC up to now. We aimed to assess the role of FBXW7 as a tumour suppressor in MIBC.

Methods: We studied clinical implications of FBXW7 mutation and expression using The Cancer Genome Atlas (TCGA) database (N=405). FBXW7 was silenced in human bladder cancer cell lines with Crispr-Cas9 in order to assess the functional significance of modulating FBXW7 in vitro.

Results: Low FBXW7 mRNA expression significantly correlated with worse overall survival (OS) in TCGA, and Cox regression analysis demonstrated that it was an independent predictor of OS. FBXW7 mRNA expression was low in the luminal-infiltrated, luminal and basal/squamous subtypes compared to the luminal-papillary subtype. Furthermore, deletion and putative mutations of FBXW7 correlated with worse OS in TCGA. GSEA analysis showed that MYC targets were enriched by low FBXW7 expression or the deletion/putative mutations. Consistent with this, upregulation of MYC protein was detected in FBXW7 knock out cells, and this was associated with the upregulation of numerous cell cycle genes.

Conclusions: FBXW7 loss of function was significantly correlated with worse OS. Preliminary analysis suggests that this may be related to MYC accumulation and downstream cell cycle gene upregulation. MYC, which is a substrate for FBXW7, could potentially be targeted to attenuate MIBC growth.

RBM10: The role of a splicing factor in urothelial homeostasis and tumorigenesis.

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Introduction: Bladder cancer (BC) represents a high economic burden for the health systems since patients frequently relapse. Most BC are urothelial tumors; these are clinically and molecularly heterogeneous and are characterized by a high mutational burden, including mutations in several splicing factor genes. One of the genes is recurrently, although at low frequency (2-5%), mutated in BC is *RBM10*. This gene maps to the X chromosome and encodes an RNA-binding protein involved in alternative splicing. Germline RBM10 mutations cause TARP syndrome, causing pleiotropic effects and early death. The majority of *RBM10* somatic mutations lead to a premature stop codon and loss of protein expression, supporting the notion that it is a tumor suppressor gene. *RBM10* mutations occur in both non muscle-invasive and muscle-invasive tumors. *RBM10* mutations have also been reported in lung (7-9%), pancreatic, and colorectal cancers.

Methods: In order to reveal the molecular mechanisms associated to RBM10 loss in development and cancer, we have established an *Rbm10* constitutive and conditional knockout mouse models, also generating urothelial organoids from these mice, and a collection of *RBM10*-mutant and -wt human BC patient derived organoids in collaboration with M. Shen laboratory.

Results: Upon germline inactivation, TARP syndrome is partially recapitulated in knockout mice, although some mutant mice survive into adulthood. Nevertheless, tissue-wide *Rbm10* inactivation in young adult mice is well tolerated. Upon *Rbm10* conditional inactivation in urothelial organoids, significant upregulation of luminal signatures along with downregulation of transcription related signatures is observed by RNA-Seq analysis. We are currently determining the role of RBM10 on growth factor dependency, cell proliferation, differentiation and splicing patterns in patient-derived *RBM10*-KO and *RBM10*-reconstituted organoids.

Conclusions: Our data suggest that RBM10 inactivation promotes a luminal-like phenotype in normal mouse urothelial organoids. BC patient derived organoids will provide an improved understanding of the molecular and cellular mechanisms through which RBM10 contributes to bladder cancer.

Cytotoxic and genotoxic effects of epigenetic inhibitors on bladder cancer cells

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Introduction: Urothelial carcinoma (UC) are characterized by frequent mutations and incorrect expression of epigenetic regulator proteins. Thus epigenetic inhibitors (Epi-I) may be suitable as new therapeutic approaches or supplements to standard therapy. Previous work demonstrated additional cytotoxic effects of Epi-I which may result from disturbed DNA synthesis and DNA damage response (DDR). These were systematically investigated to identify options for novel combination therapies.

Methods: UC cell lines VMCUB-1 and UM-UC-3 were treated with GSK126 (EZH2), GSK-J4 (inhibitor of histone demethylases such as UTX and JMJD3), PLX51107 (bromodomain protein (BET) inhibitor) and the class I HDAC inhibitor romidepsin. In addition to the effects on short and long-term proliferation, effects on cell cycle and apoptosis induction were investigated using flow cytometry and Western blot analyses. Induction of DNA damage and DDR were analysed using immunocytochemistry and Western blot. Synergy of combination treatment was evaluated by the Chou-Talalay method. RNA of UCCs treated with romidepsin and PLX51107 was subjected to RNA sequencing. Normal HBLAK control cells were used as a control.

Results: All Epi-I reduced cell viability and colony formation in both cell lines. Cells responded most weakly to GSK126, while PLX51107 had most significant effects on apoptosis induction and regulation. The apparently aneugenic mode of action of GSKJ4 induced mitotic disorders recognizable by accumulation of micronuclei, "lagging chromosomes" and chromosome bridges and altered expression of DDR markers. Although the clastogenic mode of action of romidepsin and PLX51107 resulted in accumulation of DNA double-strand breaks, no activation of checkpoint kinases was found. On the contrary, expression of CHK1 and factors of homologous recombination was downregulated. GSK126 was cytotoxic without induction of DNA damage. Combination treatments of PLX51107 and inhibitors of DDR or chemotherapeutic compounds were highly synergistic with tolerable toxicity in benign control cells.

Conclusions: The genotoxic mode of action of Epi-I, particularly of HDAC and BET proteins, enables new approaches for a combination therapy with inhibitors of DNA repair mechanisms or standard chemotherapy.

The potential for designing urothelial carcinomas using pluripotent stem cell-based systems

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Introduction: Despite a variety of therapeutic options for urothelial carcinoma of the bladder (UCB) in the early stages, the efficacy of treatment options in metastatic disease remains modest. Investigating the influence of different mutational patterns during the complex process of carcinogenesis may enable the development of innovative therapeutic agents. The differentiation of human pluripotent stem cells (hPSCs) into mature bladder urothelial cells offers a distinct platform to study embryonic development and disease modelling in the human urothelium. Given the precisely defined genetic background of those cells, it becomes evident that hPSCs-derived urothelium provides a valuable tool to study the processes during early carcinogenesis upon defined oncogenic stimuli.

Methods: Differentiation of hPSCs into urothelial cells was performed by mimicking different stages of embryonic development with the induction of definitive endoderm, a hindgut differentiation step and finally, the induction of mature urothelium. To study the influence of different genes on the carcinogenesis of UCB, knockouts of TP53, BRCA2 and ATM were generated by CRISPR-Cas9 mediated gene editing.

Results: A protocol for the differentiation of urothelial cells from hPSCs was established by reaching different milestones of embryonic development (1. definitive endoderm, 2. hindgut, 3. urothelium). The induction of urothelium was confirmed by upregulation of uroplakins (UPK2, UPK3a) in the differentiated cells. Functional relevance of the knockouts was confirmed by reduced gene and protein expression (BRCA2, TP53), the presence of truncated proteins (ATM and BRCA2) and enhanced sensitivity to genotoxic stress.

Conclusions: An efficient generation of urothelial cells from hPSCs is feasible. The functional relevance of different gene knockouts underlines the validity of the gene-editing strategy. In future studies, the significance of exogenous and endogenous carcinogenic stimuli in the context of distinct mutational signatures during early carcinogenesis of UCB can be investigated in this defined genetic background.

Mutational signature modelling in vitro recapitulates bladder cancer initiation

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Introduction: Smoking is the best established risk factor for bladder cancer and direct mutagenesis by smoke carcinogens on the cellular genome can be described in clonal populations as mutational signatures. A mutational signature summarises a lifetime of DNA-damage (caused by interaction between mutagenic processes, tissue-specific gene transcription, and the DNA-repair machinery) as mutational classes in the context of their local genomic chemistry. Our aim was to establish a novel experimental model for understanding carcinogenic initiation in normal human urothelium.

Methods: Finite normal human urothelial cell cultures were established in vitro and differentiated to form functional barrier epithelia capable of activating the smoke-derived procarcinogen benzo[a]pyrene (BaP), via CYP1A1, into mutagenic metabolites. New in vitro approaches were developed allowing chronic BaP-exposure, subsequent clonal expansion of NHU cells for whole genome DNAseq and mutational signature derivation. This approach retains tissue heterogeneity during the carcinogenic exposure, modelling the early selection pressures in urothelium that lead to clonal expansion and cancer.

Results: The single-base (SBS) and double-base signatures (DBS) derived from BaP-exposed urothelial tissues were dominated by C>A and CC>AA transversions, respectively. The InDel (ID) signature described cytosine deletions in homopolymer regions. Comparison to the Catalogue of Somatic Mutations in Cancer (COSMIC) using the Signal pipeline showed homology with SBS4, DBS2 and ID3, respectively; which have been detected by others in bladder cancer patients. Mutation enrichment in specific genes (eg KMT2D and CDKN1A) mirrored in situ observations by others; effectively modelling the competitive advantage mutations convey in the carcinogenic tissue environment.

Conclusions: BaP is detected in urine, metabolically activated by urothelial CYP1A1, and therefore may be a bladder mutagen. However, C>A transversions contribute a minor fraction of all mutations recorded in bladder tumours. Widespread DNA damage caused by chronic BaP exposure did not trigger any of the APOBEC-like mutational processes (SBS2/13) that dominate bladder tumour genomes, indicating a missing factor that the new model system is well positioned to identify.

Organotypic & in vitro monolayer modeling of urothelial carcinoma gives different cellular responses to proteinase activated receptor (PAR) agonism/antagonism

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Introduction: We hypothesized that microenvironment proteases generated by urothelial carcinoma (UC) cells dictate cell invasion & metastasis by signaling via Proteinase-Activated Receptors (PARs) and evaluated this hypothesis in UC cell lines in in vitro monolayer & organotypic 3D cultures

Methods: UC cell lines: RT4, SW780, T24, 5637 In vitro: PAR1/2 function was assessed via calcium signalling assays. 2 methods detected UC-secreted proteases that cleave PAR1/2: 1) N-luciferase tag fused to PAR1/2 in a non-UC cell line released upon cleavage (paracrine proteinase activity); 2) N-terminal mCherry/RFP;C-terminal eYFP-tagged PAR construct transfected into UC cells to visualize receptor autocrine cleavage (intact receptor=yellow; cleaved receptor=green). The PAR1/2 effect on UC cell migration & invasion was assessed in monolayer wound healing assays with & without PAR1/2 agonism. Organotypic Model: UC cells were cultured in a bladder-derived fibroblast-containing 3D collagen biomatrix model that maintains epithelial phenotype & supports the growth of UC cell lines. UC cells were evaluated for proliferation, apoptosis, differentiation, & invasion in this model with/without PAR activation/inhibition.

Results: UC cell lines express PAR1/2 & secrete proteases & protease inhibitors that induce &/or affect signaling. PAR1/2 activating peptides stimulated migration & invasion of UC cells in monolayer cultures with inhibition by a PAR1 antagonist. In the organotypic setting neither PAR1 or 2 agonism caused significant proliferation, apoptosis, or invasion. Uniquely in the organotypic model we observed induction of differentiation in non-invasive cells (SW780 & RT4) & increased cytokeratin CK5-6 expression switching in the cell lines induced by PAR1 agonism & antagonism.

Conclusions: Functional PAR1/2 are expressed by UC cells. Activation of these receptors increases invasion & migration in monolayer cultures, but not in organotypic cultures. Our data illustrate significant differences between monolayer & organotypic cultures & suggest PAR1 impacts cytokeratin expression & UC cell differentiation.