

# **International Bladder Cancer Network 16<sup>th</sup> Annual Meeting**

**- Abstract Book -**



**at the Intel Hotels Rotterdam Centre  
Rotterdam, The Netherlands**

**October 11-13, 2018**

**Accredited by:**

**Nederlandse Vereniging voor Urologie / Dutch Association of Urology  
Nederlandse Vereniging voor Medische Oncologie / Dutch Association of  
Medical Oncology**

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## **2018 IBCN Travel Award Winners:**

**Stephen Williams, MD**  
**University of Texas Medical Branch**  
**Galveston, Texas**

*“Radical Cystectomy Provides Improved Survival Outcomes and Decreased Costs Compared With Trimodal Therapy for Patients Diagnosed With Localized Muscle-Invasive Bladder Cancer”*

**David DeGraff, PhD**  
**Penn State Milton S. Hershey Medical Center**  
**Hershey, Pennsylvania**

*“PPAR $\gamma$  -mediated repression of TFAP2A Expression Identifies an Important Transcriptional Circuit in Basal-Squamous Bladder Cancer”*



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## Program 16<sup>th</sup> Meeting of the IBCN

October 11<sup>th</sup> – 13<sup>th</sup>, 2018, Rotterdam

### **THURSDAY, OCTOBER 11<sup>TH</sup>, 2018**

#### **19:00 Welcome Dinner (*Inntel Hotels Rotterdam Centre*)**



We will have the traditional “get-together” the evening prior to the meeting. This is an informal buffet dinner at the hotel at 7 pm.

### **FRIDAY, OCTOBER 12<sup>TH</sup>, 2018 (Mainport Meeting Rooms, Ground Floor, Inntel Hotels)**

(Breakfast in Down Under Restaurant **BEFORE** meeting)

|       | Introduction            |                    |
|-------|-------------------------|--------------------|
| 07:30 | Registration            |                    |
| 07:50 | Welcome to IBCN Meeting | Kamat/Goebell      |
| 08:00 | Welcome to Rotterdam    | Zwarthoff/Boormans |

| Abstract Session I |   |               |
|--------------------|---|---------------|
|                    | Genomic Subtyping   | Lars Dyrskj t |
| 08:10              | Consensus molecular classification of muscle-invasive bladder cancer  | Kamoun        |
| 08:20              | Novel immune basal subtypes to predict pathologic response to chemotherapy and survival benefit in MIBC       | Choi          |
| 08:30              | Combining DNA-Repair Gene Mutations and Molecular Subtyping for More Accurate Prediction of Outcome after NAC | Seiler        |
| 08:40              | Signature Immunohistochemical Classifier of Molecular Subtypes in Bladder Cancer                              | Guo           |
| 08:50              | A Genomic Classifier for Identifying a Neuroendocrine-like Bladder Cancer Subtype                             | Black         |
| 09:00              | Discussion  |               |

| Abstract Session II |   |                |
|---------------------|---|----------------|
|                     | Refining Subtyping  | David McConkey |
| 9:10                | Impact of chemotherapy on immune microenvironment   | Kim            |
| 9:20                | Connexin-32: a novel immunohistochemical marker of luminal MIBC that predicates a Ki67high subset driven by TGF R/pSMAD3 signalling | Hinley         |
| 9:30                | PPAR -mediated repression of TFAP2A Expression Identifies an Important Transcriptional Circuit in Basal-Squamous Bladder Cancer     | DeGraff        |
| 9:40                | Defining the proteomic landscape of non – muscle invasive bladder cancer  | Vlahou         |
| 9:50                | Dysregulation of EMT Drives the Progression to Clinically Aggressive Sarcomatoid Bladder Cancer                                     | Czerniak       |
| 10:00               | Discussion  |                |



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|                     |                                 |               |
|---------------------|---------------------------------|---------------|
| 10:10               | Introduction to Breakout Groups | Kamat/Goebell |
| Break 10:15 – 10:45 |                                 |               |

| Industry Meets IBCN – Breakout 10:45 – 12:45 |  |                         |
|--|--|-------------------------|
| Partner                                      | Topic  | Facilitator             |
| MDx Health                                   | Next Generation Epigenetic Biomarkers for Bladder Cancer | Zwarthoff/van Criekinge |
| BMS  | Next steps in early bladder cancer research              | Goebell/van der Heijden |

|                                   |
|-----------------------------------|
| Lunch & Poster View 12:45 – 14:00 |
|-----------------------------------|

| Industry Meets IBCN – Report from Breakout |            |                         |
|--|------------|-------------------------|
|  | Partner    | Kamat/Goebell           |
| 14:00                                      | MDx Health | Zwarthoff/van Criekinge |
| 14:15                                      | BMS        | Goebell/van der Heijden |

| Keynote |   |                  |
|---------|---|------------------|
|         | Topic   | Peter Black      |
| 14:30   | MIBC genomics in and beyond TCGA and the PanCancerAtlas | Gordon Robertson |
| 14:50   | Discussion  |                  |

|  |
|--|
| Health Break & Poster View 15:00 – 15:20 |
|--|

| \     | Topic Session – Tumor Heterogeneity  | William Kim |
|-------|--|-------------|
| 15:20 | Genomic and transcriptomic heterogeneity in bladder cancer - impact on clinical decision making? | Dyrskjot    |
| 15:35 | Influence of intra-tumor heterogeneity on the molecular classification of bladder cancer         | Sjödahl     |
| 15:50 | Spatial and temporal heterogeneity in bladder cancer with basal phenotype                        | Allory      |
| 16:05 | Panel Discussion   |             |

| Clinical Trial Concept |  |          |
|------------------------|--|----------|
| 16:20                  | Mismatch repair deficiency in upper tract and bladder urothelial carcinoma | Boormans |
| 16:30                  | Discussion   |          |

| Poster Session with Wine & Cheese |   |        |
|-----------------------------------|---|--------|
| 16:40                             | 45 second summary of each poster followed by poster viewing | Grivas |

| Ajdourn |         |               |
|---------|---------|---------------|
| 18:00   | Summary | Kamat/Goebell |

| IBCN Dinner |                              |  |
|-------------|------------------------------|--|
| 19:00       | Panorama Room, Inntel Hotels |  |



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**SATURDAY, OCT 13<sup>TH</sup>, 2018 (Mainport Meeting Rooms, Ground Floor, Intel Hotels)**

(Breakfast in Down Under Restaurant **BEFORE** meeting)

|      |                         |              |
|------|-------------------------|--------------|
| 8:00 | IBCN – General Assembly | Members Only |
|------|-------------------------|--------------|

| Keynote |  |                |
|---------|--|----------------|
|         | Topic  | Schmitz-Dräger |
| 9:00    | Insights in the role of chemokines in cancer metastasization | Marcus Thelen  |
| 9:20    | Discussion   |                |

| Abstract Session III |   |               |
|----------------------|---|---------------|
|                      | Novel Therapeutic Targets   | Roland Seiler |
| 9:30                 | Inhibition of a G9a/EZH2 network triggers an immune-mediated bladder cancer regression  | Paramio       |
| 9:40                 | RBM10: a new bladder tumor suppressor gene involved in alternative splicing   | Maldonado     |
| 9:50                 | Lentiviral Interferon: A Novel Method for Gene Therapy in Bladder Cancer  | Dinney        |
| 10:00                | Inhibitors of Metabolic Processes in Bladder Cancer Cells   | Agarwal       |
| 10:10                | Functional genomics with CRISPR/dCas9 and genetic screen identify multiple clinically actionably resistance mechanisms to CDK4/6 inhibition in bladder cancer | Tong          |
| 10:20                | Discussion  |               |

**Health Break & Poster View 10:30 – 11:00**

| Abstract Session IV |  |            |
|---------------------|--|------------|
|                     | Clinical   | Todenhöfer |
| 11:00               | Multispectral imaging enables multiparametric (mp) cystoscopy and transurethral resection of bladder cancer  | Bolenz     |
| 11:10               | Radical Cystectomy Provides Improved Survival Outcomes and Decreased Costs Compared With Trimodal Therapy for Patients Diagnosed With Localized Muscle-Invasive Bladder Cancer         | Williams   |
| 11:20               | Agreement between FDA/EMA approved PD-L1 assays in muscle-invasive bladder cancer with emphasis on therapy stratification for first-line treatment with Atezolizumab and Pembrolizumab | Eckstein   |
| 11:30               | PD-L1 expression according to five monoclonal antibodies in urothelial cell cancer: concordance and clinical implications  | Rijnders   |
| 11:40               | HRAS mutations in early-onset bladder cancer   | Stöhr      |
| 11:50               | Discussion   |            |



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| IBCN Speaker |   |                 |
|--------------|---|-----------------|
|              | Topic   | Roman Nawroth   |
| 12:00        | Bladder cancer and therapy from an immunologist's perspective | Molly Ingersoll |
| 12:20        | Discussion  |                 |

### Lunch & Poster View 12:30 – 13:30

| Controversies in Bladder Cancer  |            |              |
|--|------------|--------------|
|  | Debate     | Ashish Kamat |
| Urologists should be able to administer systemic immune checkpoint inhibitors        |            |              |
| 13:30  | Pro        | Goebell      |
| 13:40  | Con        | Stadler      |
| 13:50  | Discussion |              |
| Traditional histology will be replaced by molecular classification of bladder tumors |            |              |
| 14:00  | Pro        | McConkey     |
| 14:10  | Con        | Ahmadie      |
| 14:20  | Discussion |              |

| Abstract Session V |   |                      |
|--------------------|---|----------------------|
|                    | Biomarkers  | Ellen Zwarthoff      |
| 14:30              | Longitudinal Assessment of Multiplex Patient-Specific ctDNA Biomarkers in Bladder Cancer for Diagnosis, Surveillance, and Recurrence.                     | Birkenkamp-Demtröder |
| 14:40              | Association of circulating tumor (ct)-DNA genomic alterations (GA) with outcomes in metastatic urothelial carcinoma (mUC)                                 | Grivas               |
| 14:50              | Molecular Markers (FGFR3 mutation; p53 & Ki-67 expression) and Clinical Outcome of Radical Cystectomy for Bladder Cancer: A Multi-center, Multi-lab Study | van Rhijn            |
| 15:00              | STAG2 is a Biomarker for Prediction of Recurrence and Progression in Papillary Non-Muscle Invasive Bladder Cancer   | Waldman              |
| 15:10              | A non-invasive diagnostic urine assay to safely reduce the need for diagnostic cystoscopy in patients presenting with hematuria                           | de Jong              |
| 15:20              | Discussion  |                      |

### Wrap-Up & Closing Remarks & Awards Presentation

|       |                   |
|-------|-------------------|
| 15:30 | Kamat and Goebell |
|-------|-------------------|

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| Posters |   |                |
|---------|---|----------------|
| 1       | Long non coding RNA UCA1 as diagnostic and prognostic marker for bladder cancer.  | Borkowska      |
| 2       | Effect of the NAT2 Genetic Polymorphism on the p53 Mutagenic Spectrum in a Lebanese Urinary Bladder Cancer Cohort   | Dhaini         |
| 3       | Association of genetic variants with the occurrence of bladder cancer and adaptive selection in an arsenic-exposed population   | Fernandez      |
| 4       | The rs351855T allele of <i>FGFR4</i> is associated with invasive growth of urothelial bladder cancer  | Weyerer        |
| 5       | A comparison of pathologic and intermediate term oncologic outcomes following open and robotic radical cystectomy   | Kukreja        |
| 6       | Radical Cystectomy in the Neoadjuvant Immunotherapy Era   | Li             |
| 7       | Low awareness, adherence and practice but positive attitudes regarding lifestyle recommendations among bladder cancer patients  | Vreiling       |
| 8       | Characteristic mutational pattern and chromosomal arrangements in long-term cisplatin-treated urothelial cancer cell lines  | Schulz         |
| 9       | Viability of using adjusted tumour tissue instead of healthy tissue expression profiles for eQTL analysis   | Vermeulen      |
| 10      | Understanding muscle-invasive bladder cancer subtypes using <i>regulon</i> analysis   | Groeneveld     |
| 11      | Inhibition of Urothelial Carcinoma through Targeted Type I Interferon-Mediated Immune Activation  | Plote          |
| 12      | Concordance of PD-L1 expression in matched urothelial bladder cancer specimens  | de Jong        |
| 13      | Evolution of PD-1 and PD-L1 gene and protein expression in primary tumors and corresponding liver metastases of metastatic bladder cancer   | Eckstein       |
| 14      | Prognostic impact of tumor infiltrating lymphocytes and immune cell related gene expression after radical cystectomy in muscle-invasive bladder cancer  | Eckstein       |
| 15      | Combined Next Generation Sequencing and Flow Cytometry Analysis of an anti-PD-L1 Partial Responder over time: An exploration into mechanisms of PD-L1 activity and resistance                     | Kates          |
| 16      | Investigation of the role of inflammation in development of invasive bladder cancer   | Iwata          |
| 17      | BLASST-1 (Bladder Cancer Signal Seeking Trial), a phase II trial of neoadjuvant nivolumab with cisplatin and gemcitabine in muscle-invasive bladder cancer patients undergoing radical cystectomy | Gupta          |
| 18      | Influence of Chemotherapy on the relationship between urine and blood leukocytes in patients with muscle invasive bladder cancer.   | Rodriguez-Faba |
| 19      | Integration of BK polyomavirus in (micropapillary) urothelial carcinoma – a role for pathogenesis?  | Hartmann       |
| 20      | Grainyhead-like 3 (GRHL3) affects migration and invasion of bladder cancer cells in vitro   | Wezel          |
| 21      | Targeting a sub-group of luminal muscle-invasive bladder cancer with a cytochrome P450 activated pro-drug.  | Baker          |
| 22      | Targeting HER2 with T-DM1, an Antibody-cytotoxic Drug Conjugate, is effective in bladder cancer with HER2 IHC score 2+/3+   | Hayashi        |
| 23      | Molecular targeted therapy in bladder cancer: beyond tyrosine kinases   | Marqués        |
| 24      | Mitochondrial dysfunctions in bladder cancer: exploring their role as disease markers and potential therapeutic targets.  | Cormio         |

**FRIDAY OCTOBER 12, 2018**

# Consensus molecular classification of muscle-invasive bladder cancer

International consortium for MIBC molecular classification\*

## Abstract

Over the last decade, molecular subtyping efforts from several teams have led to distinct or partially overlapping molecular classifications of Muscle Invasive Bladder Cancer (MIBC). Recent studies suggest that molecular subtyping on the basis of these classifications could be clinically useful to predict response to chemotherapy and/or immunotherapy. Therefore, reaching a consensus on the molecular classification of MIBC has become crucial for further clinical applications and development.

We report a joint and comprehensive effort from several teams to reach a consensus on MIBC molecular classification. 1617 MIBC transcriptomes were assigned molecular subtypes according to six published independent classification systems. A network-based approach was used to analyse the relationships between the six systems and construct a consensus classification accordingly. A 6-class consensus system was revealed, successfully reconciling the distinct structures from each input molecular classification.

\*Countries and teams involved, alphabetical order:

### **Brazil:**

- Bioinformatics and Systems Biology Laboratory, Federal University of Paraná Polytechnic Center, Curitiba, Brazil (**Mauro AA Castro, Clarice Groeneveld**)

### **Canada:**

- Canada's Michael Smith Genome Sciences Center, BC Cancer Agency, Vancouver, Canada (**A. Gordon Robertson**)
- Department of Surgery, Division of Urology, University of Toronto, Mount Sinai Hospital and University Health Network, Toronto, ON, Canada (**Alexandre Zlotta**)
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- Johns Hopkins Greenberg Bladder Cancer Institute and Brady Urological Institute, Johns Hopkins University, Baltimore, MD, USA (**Woonyoung Choi, David J McConkey**)
- Scott Department of Urology, Dan L. Duncan Cancer Center, Baylor College of Medicine, Houston, TX, USA (**Seth Lerner**)
- The Cancer Genome Atlas Research Network, USA

## **Novel immune basal subtypes to predict pathologic response to chemotherapy and survival benefit in muscle invasive bladder cancer**

Woonyoung Choi, Debashish Sundi, I-ling Lee, Arlene Siefker-Radtke, Colin Dinney, Seungchan Kim, David J. McConkey

Johns Hopkins school of Medicine

Using gene expression profiling, we reported molecular subtypes that were enriched with clinically actionable features in muscle invasive bladder cancer (MIBC). To further explore the granular features of the subtypes, we extended our 3 subtypes (basal, luminal and p53-like) into 5 subtypes in TCGA MIBC dataset. Since activated PPAR $\gamma$  and FGFR3 mutation can be distinctive therapeutic targets in luminal tumors, luminal subtype was divided into two novel luminal subtypes using FGFR3 signature – luminal-papillary and LP. Using immune infiltration markers that are known as prognostic values in multiples solid tumors, two novel immune subtypes within basal MIBC were identified – basal immune enriched (BIE) and basal immune suppressed (BIS). Further investigation of immunogenomics signatures in each subtype shows BIE subtype was enriched with tumor infiltrating lymphocytes and IFN $\gamma$  signature compared to other subtypes. In addition, BIE had significantly improved survival outcomes while BIS still remained the subtype with the worst survival outcomes. Since immune activation has been associated with the response to chemotherapy, the potential significance of novel immune subtypes was further explored using a meta-dataset consisting of 148 TUR specimens from patients who received neoadjuvant chemotherapy (NAC) followed by radical cystectomy. In this cohort, chemotherapy was active as measured by an overall pathological downstaging rate ( $\leq$ pT1 at cystectomy) of 47% (70/148). Based on pathological downstaging rate, BIE was the most responsive to chemotherapy (response rate; 80%,  $P < 0.001$ ) while BIS and p53-like tumors were resistant to chemotherapy (response rate; 24% and 26%, respectively,  $P < 0.001$ ). In addition, BIE patients had the best survival outcomes (median OS: 211 months,  $P = 0.05$ ) among subtypes. Our results demonstrate that we have first discovered the association between molecular subtype and pathological response to NAC and suggest that it is possible to prospectively identify the patients who most likely will benefit from NAC.

# Combining DNA-Repair Gene Mutations and Molecular Subtyping for More Accurate Prediction of Outcome after Neoadjuvant Chemotherapy for Bladder Cancer

Roland Seiler<sup>1,2</sup>, José Batista da Costa<sup>1</sup>, Kenichiro Ikeda<sup>1</sup>, Joshua Zhou<sup>1</sup>, Ewan A. Gibb<sup>3</sup>, Elai Davicioni<sup>3</sup>, Brian Winters<sup>4</sup>, Stanislav Volik<sup>1</sup>, Jonathan Wright<sup>4</sup>, Matthew Sommerland<sup>5</sup>, James Douglas<sup>5</sup>, Colin Collins<sup>1</sup> & Peter C. Black<sup>1</sup>

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<sup>5</sup> Department of Urology, University Hospital of Southampton, Hampshire, UK

**Introduction:** There remains a critical need to identify markers to predict response to cisplatin-based neoadjuvant chemotherapy (NAC) for muscle invasive bladder cancer (MIBC) so that we can avoid NAC and proceed to timely surgery in likely non-responders. Recent publications have suggested that mutations in DNA damage repair genes (DDRG) and molecular subtyping of bladder cancer are predictive of outcome after NAC. Here we aimed to combine these markers in a multicenter cohort of patients to determine the interaction and complementary value of both biomarkers.

**Methods:** Whole exome sequencing and transcriptome microarray analysis were performed on pre-NAC MIBC samples. Any mutation in ERCC2, FANCC, RB1 or ATM that was predicted to be deleterious was considered a DDRG mutation. Molecular subtype was assigned with previously reported single patient classifier. Response to NAC was defined as the absence of MIBC in the cystectomy sample.

**Results:** Our cohort of 106 patients with a median follow-up of 2.3 years included 69 (65%) non-responders (23% with DDRG mutation) and 37 (35%) responders (57% with DDRG mutation). The 3 year OS was 77.8% and 50.8% for patients with and without a DDRG mutation, respectively ( $p=0.0083$ ). With the caveats of small sample size in each subtype, OS could be further sub-stratified by DDRG mutation status in basal tumors but had no impact on outcome in the other subtypes.

**Conclusions:** Both DDRG mutations and molecular subtypes predict survival after NAC for MIBC. This is the largest NAC cohort to date with DDRG mutation results, and the first to incorporate also subtyping. Both markers appear to provide complementary predictive information.

## Signature Immunohistochemical Classifier of Molecular Subtypes in Bladder Cancer

Charles Guo, Jolanta Bondaruk, Tadeusz Majewski, Li Zhang, Ashish Kamat, Colin Dinney, David McConkey, Bogdan Czerniak

The University of Texas MD Anderson Cancer Center

**Objectives:** Recent studies have revealed that bladder cancer can be divided into two molecular subtypes referred to as luminal and basal with distinct clinical behaviors and sensitivities to chemotherapy. We aimed to identify signature immunohistochemical markers that would permit simple and cost-effective classification of the disease in routine clinical practice.

**Materials and Methods:** We analyzed genomic expression profiles of formalin-fixed paraffin-embedded bladder cancer samples (n=74) to validate the expression signatures of luminal and basal subtypes and relate them to clinical data. We also performed immunohistochemistry on the matched tumor samples to identify immunohistochemical markers that permitted the molecular classification of bladder cancer.

**Results:** Genomic expression analysis showed that the bladder cancers in our cohort were divided into two distinct molecular subtypes, luminal (n=57) and basal (n=17). Luminal tumors were characterized by the expression signature similar to the intermediate/superficial layers of normal urothelium, including overexpression of KRT7, KRT8, KRT18, KRT20, and GATA3 genes. Additionally, they showed the upregulation of E-Cadherin, HER2/3, Rab-25, Src and PPAR $\gamma$ -target genes. Basal tumors were characterized by the expression signature similar to the basal layer of normal urothelium, including overexpression of KRT 1, KRT5, KRT 6, KRT14, and KRT16. Additionally, they showed the upregulation of CD49, Cyclin B1, EGFR, and p63-target genes. Survival analyses showed that the muscle-invasive basal bladder cancers were more aggressive when compared to luminal cancers. The immunohistochemical expressions of only two markers, luminal (GATA3) and basal (KRT5/6), were sufficient to identify the molecular subtypes of bladder cancer with over 90% accuracy.

**Conclusions:** Basal and luminal molecular subtypes of bladder cancer have distinct clinical behaviors and sensitivities to chemotherapy, and a simple two-marker immunohistochemical classifier can be used for prognostic and therapeutic stratification.

## A Genomic Classifier for Identifying a Neuroendocrine-like Bladder Cancer Subtype

Jose Batista da Costa<sup>1</sup>, Jonathan Wright<sup>11</sup>, Marc A Dall'Era<sup>2</sup>, Trinity Bivalacqua<sup>3</sup>, Roland Seiler<sup>4</sup>, Yang Liu<sup>5</sup>, Ewan A. Gibb<sup>5</sup>, Qiqi Wang<sup>5</sup>, Nicholas Erho<sup>5</sup>, Mohammed Alshalalfa<sup>5</sup>, Elai Davicioni<sup>5</sup>, Badrinath R. Konety<sup>12</sup>, Paari Murugan<sup>12</sup>, Jason A Efstathiou<sup>6</sup>, James Douglas<sup>7</sup>, Joost L. Boormans<sup>8</sup>, Michiel S. Van der Heijden<sup>9</sup>, Yair Lotan<sup>10</sup>, Peter C. Black<sup>1</sup>

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**Introduction:** Neuroendocrine bladder carcinoma (NEBC) is a rare and aggressive variant. Molecular subtyping studies have found that 5-10% of muscle invasive bladder tumors have transcriptomic patterns consistent with NEBC in the absence of neuroendocrine (NE) histology. Identifying NE variants may have prognostic implications and modify treatment recommendations. In this study, we present a genomic classifier trained to identify NE-like tumors.

**Methods:** Using unsupervised clustering of transcriptome-wide expression profiles, we generated a series of clustering solutions, evaluating the resultant model on a testing cohort (n=225). A GLMnet model was used to train the classifier to identify NE-like tumors in a training cohort (n=173). The classifier was applied to 4 validation cohorts (n=1030). Uni- and multi-variable survival analyses were used to characterize the clinical outcomes of the NE-like tumors.

**Results:** In the training set, hierarchical clustering using a panel of 84 genes showed a cluster of 17 patients (9.8%) with highly heterogeneous expression of NE markers but no expression of basal or luminal markers. This biological profile was consistently seen across the 4 validation cohorts. These patients had significantly worse 1 year progression free survival (65% vs 82% for NE-like vs overall; p=0.046). After adjusting for various clinical and pathological factors, patients with NE-like tumors had a 6.40 increased risk of all-cause mortality (p=0.001).

**Conclusions:** We have developed a single patient classifier that identifies a particularly high-risk group of NE-like tumors that may need treatment intensification, alternative chemotherapy or clinical trials. Further validation will be required to assess potential clinical utility.

## **Impact of chemotherapy on immune microenvironment**

William Y. Kim

University of North Carolina at Chapel Hill

In patients with bladder cancer, programmed death-ligand-1 (PD-L1) and programmed death-1 (PD-1) inhibitors have been shown to be effective in around twenty percent of patients, and there is evidence indicating that the level of immune infiltration and immune suppression within the tumor microenvironment correlates with response to these treatments. We have previously shown that there are intrinsic subtypes of bladder cancer, with the basal subtype characterized by high levels of immune infiltration, and the luminal subtype characterized by immune exclusion. Here we show that treatment with current standard of care chemotherapeutic agents has a subtype specific effect on the tumor microenvironment and that different chemotherapeutic regimens alter the immune microenvironment differently. Cisplatin-based chemotherapeutic treatment of luminal tumors induces a mesenchymal phenotype and immune infiltration. Two of the most widely used standard of care chemotherapeutic regimens are Cisplatin-Gemcitabine (GemCis) and Methotrexate-Vinblastine-Doxorubicin-Cisplatin (MVAC), and we show that MVAC treatment induces significant immune infiltration within the luminal subtype while GemCis treatment does not, indicating there are treatment specific effects on the immune microenvironment. Furthermore, using subtype-specific mouse models of bladder cancer previously developed by our lab, we demonstrate that GemCis and MVAC have differing effects on the immune microenvironment. These results indicate that differing chemotherapeutic regimens have differential effects on the tumor microenvironment which could potentially be used to increase the efficacy of immune checkpoint inhibitors.

## **Connexin-32: a novel immunohistochemical marker of luminal MIBC that predicates a Ki67high subset driven by TGFβR/pSMAD3 signalling**

Jennifer Hinley<sup>1</sup>, Simon C. Baker<sup>1</sup>, Andrew Mason<sup>1</sup>, Peter Rubenwolf<sup>2</sup>, Wolfgang Otto<sup>3</sup>, Maximilian Burger<sup>3</sup>, Jens Stahlschmidt<sup>4</sup>, Jennifer Southgate<sup>1</sup>

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**Aims.** Intercellular communication through connexin gap junctions is integral to urothelial tissue homeostasis. Following malignant transformation, dysregulation of connexin channels can influence cell-cell communication, resulting in enhanced tumour aggressiveness. This study aimed to examine the role of differentiation-associated connexin-32 (Cx32) in urothelial malignancy.

**Experimental Approach.** In vitro studies of normal human urothelial (NHU) cells with engineered Cx32 were employed to evaluate intercellular signalling-dependent effects on migration and proliferation, using immunocytochemistry and wound-healing assays. RNAseq was performed to examine the influence of Cx32 modification on the urothelial transcriptome. Cx32 distribution and associations with GATA3, Cytokeratin 5/6, FOXA1, Ki67 and pSMAD3 were assessed by semi-quantitative immunohistochemistry of 55 muscle-invasive bladder cancers (MIBCs) arrayed from central and invasive zones.

**Results.** Immunohistological classification of MIBC revealed that Cx32 expression was significantly allied to the PPARγ-driven GATA3<sup>high</sup>/FOXA1<sup>high</sup> (luminal) subset of tumours (P<0.01). Cx32 cell-cell communication inhibited pSMAD3 signalling and regeneration of differentiated NHU cultures. Overexpression of dominant negative Cx32 to ablate channel function resulted in significant upregulation of genes associated with epithelial migration, including downstream canonical TGFβR target genes involved in collagen remodelling. Aberrant non-membrane localised Cx32 expression was significantly linked to a pSMAD3<sup>high</sup>/Ki67<sup>high</sup> subset of MIBC (p<0.001).

**Conclusions.** Three functional MIBC subsets are distinguished immunohistochemically by Cx32, which in normal urothelial cells acts to dampen migratory and proliferative regenerative behaviour. Our findings have identified a specific novel subset of MIBC comprising 50% of luminal tumours and predicted to respond to TGFβR targeted therapy.

## PPAR $\gamma$ -mediated repression of TFAP2A Expression Identifies an Important Transcriptional Circuit in Basal-Squamous Bladder Cancer

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Bladder Cancer is the second most common urologic malignancy, and advanced disease poses a direct threat to the life of the patient. Recent identification of specific gene expression subtypes of bladder cancer provide an opportunity to identify high risk patients, and potentially tailor disease management. In addition to being transcriptionally regulated, previous studies suggest molecular subtype is “plastic” and can change from a Luminal to Basal-Squamous signature during tumor progression. However, the transcriptional drivers of Basal-Squamous bladder cancer, as well as the degree to which they contribute to the aggressive phenotype typical of this subtype is unknown. Because of the important role of Peroxisome Proliferator Activated Receptor Gamma (PPAR $\gamma$ ) in regulating Luminal-specific gene expression in bladder cancer, we hypothesized that inactivation of PPAR $\gamma$  signaling during tumor progression results in increased expression of transcription factors (TFs) specific to the Basal-Squamous subtype. To identify PPAR $\gamma$ -repressed TFs that promote a Basal-Squamous signature, we initiated a pharmacologic and RNA-seq-based screen following treatment of UMUC1, SW780 and 5637 cell lines with the PPAR $\gamma$  agonist rosiglitazone (TZD). Hierarchical clustering of RNA-seq data identified a number of TFs regulated by PPAR $\gamma$  activation. Several of these transcription factors are implicated in urothelial and squamous differentiation. One PPAR $\gamma$ -repressed TF implicated in skin development and SqD identified by this screen was Transcription Factor Activating Protein 2 alpha (TFAP2A). Computational analysis of publically available data, as well as immunohistochemistry of our in-house tissue cohort shows TFAP2A and its paralog TFAP2C are significantly overexpressed in Basal-Squamous bladder cancer and in areas of SqD in cystectomy samples. In addition, we show TFAP2A and TFAP2C overexpression are additionally associated with adverse oncologic outcomes. Q-RT-PCR and western blotting analysis also confirmed the ability of PPAR $\gamma$  activation to repress TFAP2A, and PPAR $\gamma$  antagonist studies indicate a requirement of a functional receptor for TZD-induced TFAP2A repression. Additional *in vitro* experimentation and *in vivo* tissue recombination experiments show TFAP2A and TFAP2C regulate bladder cancer cell migration, invasion and promote tumor growth, attributes typically associated with the Basal-Squamous subtype. Taken in light of previous studies, these findings definitively identify PPAR $\gamma$  as a master regulator of bladder cancer cell fate, and further identify TFAP2A as a PPAR $\gamma$ -repressed transcriptional driver of Basal-Squamous bladder cancer. These results further suggest that PPAR $\gamma$  inactivation, as well as TFAP2A and TFAP2C overexpression cooperate with other TFs and epigenetic changes to promote development of Basal-squamous disease during progression.

## Defining the proteomic landscape of non – muscle invasive bladder cancer

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The implementation of DNA/ RNA sequencing approaches has resulted in the determination of bladder cancer (BC) subtypes of differential prognosis. Nevertheless, discrepancies between the current molecular, pathological and cell phenotypes exist. The objective of our work is to establish a proteomic definition of BC subtypes aiming to fill existing gaps and define optimal patient management schemes. We analysed 98 fresh-frozen tissue samples from non-muscle invasive BC (58 pTa and 40 pT1) by high resolution proteomics (LC-MS/MS). Limiting to peptides identified with high confidence at a frequency threshold of at least 20%, 1309 proteins could be assessed. Clustering (k-means) indicated stability for k = 3 classes. These differed in size (class 1: 17; class 2: 42 and class 3: 39 members), and stage and grade: (class 1: mostly pT1, grade III; class 2: representation of all grades-stages, class 3: mainly pTa-low grade tumors; Fisher's exact  $p < 0.001$ ). Basal, cancer stem cell and inflammation markers were up-regulated in class 1, whereas luminal markers were found increased in class 3 cancers. Class 2 exhibited a mesenchymal phenotype. Further molecular characterization combined with pathway and geneset functional assessment suggested a high risk proteome signature for class 1 and best prognosis for class 3. To validate this observation, 19 muscle invasive tumors (MIBC, pT2+) were also analyzed by proteomics. Principal component analysis indicated higher proximity of class 1 and, conversely, greater distance of class 3, to the proteome observed for MIBC, while classification of the 19 MIBC to the three consensus subclasses resulted in assignments of MI tumors mainly to classes 1 and 2. Comparative analysis with Hedegaard, J. et al. (2016) NMIBC data revealed similarities between the proteomic- and transcriptomic-based subtypes. Collectively, this study indicates the existence of distinct protein-based subtypes of NMIBC placing them in the context of current stratification schemes.

## **Dysregulation of EMT Drives the Progression to Clinically Aggressive Sarcomatoid Bladder Cancer**

Charles C. Guo, Tadeusz Majewski, Li Zhang, Hui Yao, Jolanta Bondaruk, June Goo Lee, Sangkyou Lee, David Cogdell, Peng Wei, Colin Dinney, Keith Baggerly, David McConkey, and Bogdan Czerniak

The University of Texas MD Anderson Cancer Center

**Objective:** We report on the genome-wide characterization of the sarcomatoid variant of bladder cancer (SARC) including its miRNA, gene expression, and whole-exome mutational profiles, which identified unique molecular features associated with its aggressive nature that may be relevant for the early detection and treatment of this highly lethal variant of bladder cancer.

**Material and Methods:** A comprehensive genomic analysis was performed on 28 cases of SARCs and 84 cases of conventional UCs, with the TCGA cohort of 408 muscle-invasive bladder cancers serving as the reference cohort.

**Results:** SARCs showed a distinct mutational landscape with enrichment of TP53, RB1, and PIK3CA mutations. They were related to the basal molecular subtype of conventional UCs and could be divided into “epithelial” and more clinically aggressive “mesenchymal” subsets. Expression analysis showed that SARCs are driven by downregulation of TP63 and dysregulation of cell cycle and EMT regulatory networks, and nearly half exhibited a heavily infiltrated immune phenotype with PD-L1 upregulation.

**Conclusions:** We conclude that SARCs are driven by profound dysregulation of the EMT network and that a large proportion of SARCs have an immune infiltration phenotype. Both these features present new avenues of therapeutic potential in patients with this highly lethal variant of bladder cancer.

## KEYNOTE LECTURE

### MIBC genomics in and beyond TCGA and the PanCancerAtlas

Gordon Robertson

Canada's Michael Smith Genome Sciences Centre  
British Columbia Cancer Agency  
Vancouver, Canada

I'll describe the TCGA muscle-invasive bladder cancer (MIBC) project and 2017 publication from the perspective of the BC Cancer Agency's Genome Sciences Centre, where we generated miRNA-seq data for over 11 thousand tumour and tissue normal samples across 33 cancer types. For the MIBC project, we also screened RNA and DNA sequence data for bacteria, fungi and viruses, and used *de novo* transcriptome assembly to assess viral genomic integration. Because lncRNAs can be specific to biological state, we used lncRNA expression profiles calculated from mRNA-seq data for subtyping analysis. We calculated regulon activity profiles for a subset of 23 bladder cancer-associated regulators, and used this RNA-based functional readout to characterize differences between subtypes. A multivariate survival analysis integrated diverse clinical and molecular covariates. Finally, from our ongoing work on bladder cancer genomics, I'll report on assessing results generated in TCGA PanCancerAtlas projects, and results from extended regulon analyses.

**SATURDAY OCTOBER 13, 2018**

# Inhibition of a G9a/EZH2 network triggers an immune-mediated bladder cancer regression

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**Objectives.** Advanced Bladder cancer (BC) has limited therapeutic options and only a limited fraction of patients benefit from immunotherapy. BC encompasses defined epigenetic drivers of the disease. We have studied the roles of G9a (EHMT2), a H3K9 methyltransferase, in human BC, and explored whether a novel, reversible, potent and dual inhibitor of G9a/DNMT methyltransferase activity (CM-272) could be of benefit for the management of advanced BC.

**Materials & Methods.** BC cell lines of known genomic features and derived xenografts, patient cohorts from our Hospital, TCGA database and metastatic BC transgenic mice were used

**Results.** The expression of G9a was associated with a poor clinical outcome in BC, and is associated with resistance to immune checkpoint inhibitors. CM-272 is effective in BC cells without *PIK3CA* mutations and inhibits an oncogenic loop mediated by G9a and EZH2. This promotes gene expression changes causing apoptosis and immunogenic cell death. Using a novel immunocompetent quadruple (*Pten*<sup>F/F</sup>; *Tp53*<sup>F/F</sup>; *Rb1*<sup>F/F</sup>; *Rbl1*<sup>-/-</sup>) knockout transgenic mouse model of highly aggressive metastatic muscle-invasive BC, we demonstrate that treatment with CM-272 and CDDP results in a significant tumor and metastasis regression accompanied with an *in vivo* activation of endogenous antitumor immune response and immunogenic cell death..

**Conclusions.** The expression of G9a may be a biomarker of response to immunotherapy and G9a inhibition may increase efficacy of checkpoint blockage, supporting new and promising opportunities for therapy in patients with BC.

## **RBM10: a new bladder tumor suppressor gene involved in alternative splicing**

Ana M Maldonado-Barragán, Ramón Tejedor, Göttfrid Sjö Dahl, Mattias Hoglund, Lars Dyrskjot, Tobias Hoffmann, Miriam Marqués, Juan Valcárcel, Francisco X Real

National Center of Cancer Research, Spain

**Introduction:** Urothelial bladder cancer is a heterogeneous disease both from the clinical and molecular standpoint. Whole-genome and -exome sequencing studies have recently uncovered a novel putative tumor suppressor gene, RBM10, which is mutated in 2-5% of bladder tumors as well as in lung adenocarcinoma, colon, and pancreatic cancer. RBM10 maps to the X chromosome; approximately 30% of the somatic mutations in tumors predict a premature stop codon and lead to loss of protein expression. RBM10 encodes an RNA-binding protein that modulates alternative splicing of genes involved in cell growth, proliferation, and apoptosis (i.e. NUMB, CREBBP, FAS, BCL-X).

**Objectives:** Identify the mechanisms by which RBM10 inactivation contributes to bladder cancer.

**Materials & Methods:** We have established new conditional Rbm10 knockout mouse models to investigate the role of Rbm10 in homeostasis and carcinogenesis. For in vitro studies, bladder organoids from Rbm10lox/lox; UbCreERT2; Rosa26mTmG mice we derived to elucidate Rbm10 role in the urothelium. Mouse data will be complemented by IHC analysis and RNA-Sequencing results from human bladder tumor biopsies and human-bladder tumor-derived organoids.

**Results:** Loss of RBM10 expression is observed across all bladder tumor stages and grades, suggesting that it is an early event in tumor progression. Ubiquitous Rbm10 inactivation in adult mice appears well tolerated, indicating that it is not absolutely required for tissue homeostasis. We are now characterizing the effects of Rbm10 inactivation in mouse bladder organoids by assessing proliferation, differentiation, as well as growth factor dependency. The organoids will also allow analyzing the effects of Rbm10 depletion on RNA splicing and will be combined with those from wild-type and RBM10-null human bladder tumors.

**Conclusions:** These studies will provide an understanding of the mechanisms through which RBM10 contributes to disease and pave the way to identify therapies for RBM10-mutant tumors.

## **Lentiviral Interferon: A Novel Method for Gene Therapy in Bladder Cancer**

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### **Introduction**

Gene therapy for bladder cancer (BLCA) is rapidly evolving. We reported that intravesical adenoviral interferon-alpha (Ad-IFN $\alpha$ ) produced a complete response in 35% of patients with BCG-unresponsive BLCA enrolled in a Phase II trial. Lentivirus (LV) is another potential vector for intravesical delivery of IFN $\alpha$ . Unlike the adenovirus, LV can infect non-dividing cells and integrate into the host's genome, making it one of the most efficient gene delivery vectors. The objective of this study was to investigate lentiviral interferon-alpha (LV-IFN $\alpha$ ) BLCA gene therapy in preclinical models.

### **Methods**

Murine BLCA cell lines were transduced in-vitro with LV-IFN $\alpha$  using a multiplicity of infection (MOI) of 2:1. IFN $\alpha$  levels were measured by ELISA. Cell viability was assessed using Trypan blue dye exclusion. qPCR was used to identify expression of IFN $\alpha$  target genes. A LV- $\beta$ Galactosidase reporter construct was delivered intravesically, and urinary IFN $\alpha$  levels were measured in mice treated with LV-IFN $\alpha$  or control virus to assess gene transfer. To assess survival benefit, p53+/- C57/B6 mice were exposed to N-butyl-N-(4-hydroxybutyl)-nitrosamine (BBN) to induce CIS and then treated with LV-IFN $\alpha$  or control virus, and sacrificed when moribund.

### **Results**

Efficient LV-IFN $\alpha$  transduction of BLCA cells was observed at an MOI of 2:1, resulting in increased expression of IFN $\alpha$  and its target genes PDL-1, TRAIL, and IRF7 ( $p < 0.001$ ), and reduced cell viability vs. controls ( $p < 0.001$ ).

$\beta$ Gal expression confirmed efficient transduction of murine urothelium. Urinary IFN $\alpha$  levels were elevated in mice receiving LV-IFN $\alpha$  compared with control virus. BBN mice treated with LV-IFN $\alpha$  had longer overall survival than mice treated with control virus ( $p = 0.04$ ). LV-IFN $\alpha$  induced intratumoral CD8+ T cell infiltration, high expression of PD-L1, and inhibited angiogenesis.

### **Conclusion**

LV-IFN $\alpha$  effectively upregulated IFN $\alpha$  target genes, was cytotoxic to murine BLCA cells, and improved the survival of BBN tumor-bearing mice. LV appears to be a promising vector for intravesical gene delivery.

## **Inhibitors of Metabolic Processes in Bladder Cancer Cells**

Garige M, Crooks R D, Oshima N, Simeonov A, Hall D M, Thomas C, Damien D, Lane N A, Fan WM F, Sourbier C, Krishna M, Neckers, L, Agarwal PK

National Cancer Institute, National Institutes of Health

**Introduction:** The Warburg Effect is seen in many tumors and provides opportunities for targeted inhibition of metabolism in tumors. We profiled 14 bladder cancer cell lines for their relative dependence on glycolysis or oxidative phosphorylation to establish their metabolic phenotype. We then targeted key metabolic enzymes, lactate dehydrogenase (LDH) and nicotinamide phosphoribosyl transferase (NAMPT), with specific novel inhibitors in pre-clinical bladder cancer models.

**Methods:** Using the extra cellular flux analyzer (Agilent Seahorse platform), we characterized cell lines based on their metabolic parameters, extracellular acidification rates (ECAR) and oxygen consumption rates (OCR), and determined whether they relied on glycolysis or oxidative phosphorylation relative to each other. Novel LDH inhibitors were tested in these cell lines for their effects on proliferation, invasion, and migration. These inhibitors were also tested on xenograft tumors either as a single agent or in combination with metformin. In a separate project, we also evaluated the effect of NAMPT inhibitors as single agents in cell lines and flank xenografts using similar methodology.

**Results:** Most bladder cancer cell lines depend on oxidative phosphorylation to some degree (based on high OCR) but a few are reliant on glycolysis (based on high ECAR). The more glycolytic cell lines were more sensitive to LDH inhibition; however, the other cell lines responded to LDH inhibition when made more glycolytic by either hypoxia or by cotreatment with metformin. The UMUC3 xenograft demonstrated decreased growth with LDH inhibition. Although NAMPT inhibition was only successful in a few bladder cancer cell lines, it was extremely potent in these cell lines. NAMPT (an enzyme involved in the repletion of NAD levels from nicotinic acid) deficient cell lines were sensitive to NAMPT inhibitor, whereas the cells with higher expression of NAMPT were resistant. In the UMUC3 flank xenograft, NAMPT inhibition was effective in inhibiting tumor growth.

**Conclusions:** These data describe the metabolic phenotype of bladder cancer cell lines and demonstrates that LDH inhibition in combination with metformin can be effective in inhibiting tumor growth in bladder cancer cell lines. NAMPT inhibition is more potent but only NAMPT-deficient cell lines are sensitive.

## **Functional genomics with CRISPR/dCas9 and genetic screen identify multiple clinically actionable resistance mechanisms to CDK4/6 inhibition in bladder cancer**

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**Objectives:** Though CDK4/6 inhibition is a promising approach for treatment of BLCA, resistance mechanisms still are unclear. With functional genomics, we investigated relationship between transcriptional activation of genes and resistance to Palbociclib to understand the molecular mechanisms and further develop potential combination therapies for personalized therapy.

**Materials & Methods:** T24 cells, transduced with CRISPR/dCas9 SAM library containing 70291 sgRNAs were selected for Palbociclib resistance. Genomic DNA was extracted and sequenced by NGS. Enrichment of sgRNAs candidates was analyzed using MAGeCK-VISPR. The most significant 10 candidates were validated for mRNA expression, cell cycle distribution, cell proliferation and colony formation. Bioinformatic analysis of enriched sgRNA candidates was done using DAVID and signaling pathways. Suitable combination therapies were determined with cell viability, cell cycle progression, caspase3/7 activity in vitro and on the CAM xenograft model. Molecular mechanisms of resistance were preliminarily investigated with Western blot.

**Results:** We identified 3440 genes targeted by 3608 candidate sgRNAs that might confer resistance to Palbociclib. The 10 most significant candidates sgRNAs were all successfully validated. Oncogenic signaling pathways belong to 'pathways in cancer' containing clinically druggable targets were identified including Cell cycle, PI3K-AKT, MAPK, JAK-STAT, VEGF, Ras and PPAR $\gamma$ . Combination therapies with Palbociclib and inhibitors against these pathways revealed that targeting FGFRs, VEGFRs and PI3K/Akt pathway exhibited synergism with CDK4/6 inhibition. Further investigation on molecular mechanisms revealed that compensatory activation of PI3K, MAPK and CDK2 pathways induced resistance. Synergism of combination therapies was achieved via multiple mechanism including cell cycle arrest and apoptosis.

**Conclusions:** Application of a genome-scale transcriptional activation screen by an engineered CRISPR/dCas9 complex together with bioinformatic analysis are powerful approaches to identify mechanisms of resistance to Palbociclib and to develop novel directed combination therapies in bladder cancer.

## Multispectral imaging enables multiparametric (mp) cystoscopy and transurethral resection of bladder cancer

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**Introduction:** White light (WL) cystoscopy and transurethral resection (TUR) are the gold standard initial procedures for the detection and treatment of bladder cancer (BC). To overcome limited sensitivity of existing imaging techniques (e.g. PDD) we developed a real-time multispectral imaging (rMSI) device, enabling simultaneous imaging of reflectance and fluorescence modalities in up to 6 spectral bands.

**Methods:** Preclinical evaluation of rMSI included ex vivo and in vivo studies in porcine bladders. Different technical adaptations were required to meet standards for human cystoscopy. A pilot study in five patients who underwent TUR for BC was performed after instillation of HAL (Hexvix®) 1 hour before surgery. We used multiple LED light sources (Omicron-Laserage Laserprodukte GmbH) with different spectra to illuminate the bladder for fluorescence, NBI-like reflectance showing enhanced vascular contrast (EVC) and WL reflectance imaging. In a prospective cohort of 10 patients with bladder tumors we performed multiparametric (mp) cystoscopy with the rMSI device, simultaneously displaying six different modalities: WL, EVC, raw fluorescence, PDD-fluorescence, autofluorescence (AF) imaging, and merged mode.

**Results:** Multispectral images were recorded in porcine endoscopy and subsequently in all humans with simultaneous bladder imaging in different modalities (WL, NBI-like and raw fluorescence mode, which is unmixed into PDD and AF). Merged multiparametric endoscopic images were created from the EVC and the raw fluorescence signal. Overall, 24 suspicious lesions were identified, 18 of which were diagnosed as malignant. Detection rates of malignant lesions were 14/18 in WL, 16/18 in PDD, 15/18 in EVC and 15/18 in AF, respectively. When combining all modalities all malignant lesions (18/18) were detected.

**Discussion:** Multiparametric cystoscopy with rMSI is feasible and may substantially enhance the performance of WL cystoscopy for the detection of BC. Our results suggest that the distinct modalities may complement each other, enabling simultaneous real-time imaging with an increased sensitivity of cystoscopy.

## **Radical Cystectomy Provides Improved Survival Outcomes and Decreased Costs Compared With Trimodal Therapy for Patients Diagnosed With Localized Muscle-Invasive Bladder Cancer**

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**Objective:** While radical cystectomy is the guideline-recommended treatment for muscle-invasive bladder cancer, there has been a resurgence in trimodal therapy with limited data on comparative outcomes, and especially attributable costs. We assessed survival outcomes and costs of trimodal therapy versus radical cystectomy in older adults with muscle-invasive bladder cancer.

**Participants:** A total of 3,200 patients aged 66 years or older diagnosed with clinical stage T2-4a bladder cancer from January 1, 2002- December 31, 2011 from Surveillance, Epidemiology, and End Results (SEER)-Medicare data linked-data. Propensity score matching based on sociodemographic and clinical characteristics were used. The association of treatment with overall and cancer-specific survival was evaluated using the Cox proportional hazards regression and Fine and Gray's competing risk model.

**Results:** A total of 3,200 patients met inclusion criteria. After propensity score matching, 687 patients underwent trimodal therapy and 687 patients underwent radical cystectomy. Patients who underwent trimodal therapy had significantly decreased overall (Hazard Ratio (HR) 1.49, 95% Confidence Interval (CI), 1.31-1.69) and cancer-specific (HR 1.55, 95% CI 1.32-1.83) survival, respectively. While there was no difference in costs at 30 days, median total costs were significantly higher with trimodal therapy than radical cystectomy at 90-d (\$69,181 vs. \$80,174; Median Difference \$8,964, Hodges-Lehmann 95% CI, \$3,848-14,079) and 180-d (\$107,017 vs. \$179,891; Median Difference \$63,771, Hodges-Lehmann 95% CI, \$55,512-72,029), respectively. Extrapolating these figures to the total US population results in excess spending of \$335 million for trimodal therapy compared to less costly radical cystectomy for patients diagnosed in 2011.

**Conclusions:** Trimodal therapy was associated with significantly decreased overall and cancer-specific survival resulting in excess national spending of \$335 million in 2011 compared with radical cystectomy. These findings have important health policy implications regarding appropriate use of high-value based care among patients who are candidates for either treatment.

## **Agreement between FDA/EMA approved PD-L1 assays in muscle-invasive bladder cancer with emphasis on therapy stratification for first-line treatment with Atezolizumab and Pembrolizumab**

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**Background:** Four PD-L1 assays have been approved by the FDA/EMA to identify potential anti-PD-1/PD-L1 responders and eligible patients for first-line therapy with Atezolizumab and Pembrolizumab.

**Objective:** We analyzed the performance and agreement of four FDA/EMA-approved PD-L1 assays to detect PD-L1 expression in tumor and immune cells in muscle-invasive bladder cancer (MIBC).

**Patients and methods:** 173 formalin-fixed, paraffin-embedded MIBC were analyzed on tissue microarrays with four cores (1 mm diameter) of each tumor. Stains were performed in certified laboratories on Ventana Benchmark Ultra (Ventana-assays) and Dako Link 48 (Dako-assays) autostainers. Stains were read on an assay-by-assay basis by two trained pathologists. Overall percentage agreement (OPA) was calculated across preset cut-offs. Positive (PPA) and negative percentage agreements (NPA) were calculated across different scoring algorithms. Venn diagrams were constructed to illustrate discordance according to the recent FDA/EMA guidelines.

**Results and Limitations:** The Dako 28-8, 22c3 and the Ventana SP263 assays showed high inter-assay correlation (r-range 0.74-0.87). Inter-assay variability between the Ventana SP142 and the three other assays was moderate (r-range 0.47-0.67). OPA of 90.2% was achieved between the Dako and the Ventana SP263 assays at multiple cut-offs. OPA with the SP142 assay was 82.1%. Pooled PPA and NPA of different scoring algorithms was 81.3% and 97.1% for the Dako and the SP263 assays. With the SP142 assay pooled PPA reached just 43.4%. The SP142 assay identifies fewer patients as eligible for first line treatment with Atezolizumab or Pembrolizumab.

**Conclusion:** While the Dako and SP263 assays show comparable performance, the SP142 is an outlier which leads to a significantly reduced detection rate of eligible patients for first-line treatment with Atezolizumab and Pembrolizumab according to the new FDA/EMA restrictions.

# PD-L1 expression according to five monoclonal antibodies in urothelial cell cancer: concordance and clinical implications

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**Background.** High PD-L1 expression is frequently applied as an inclusion criterion or stratification factor in clinical trials on immune checkpoint inhibitors (ICIs). However, conflicting results have been published regarding the predictive and prognostic value of PD-L1 expression in urothelial cancer (UC), which may be confounded by the use of different PD-L1 companion diagnostics. The objective of this study was to compare PD-L1 expression of five commercially available PD-L1 antibodies in muscle-invasive UC.

**Methods.** Tissue Microarrays (TMA) containing samples of 139 muscle-invasive UC patients were stained with the anti-PD-L1 antibodies: 22C3, 28-8, SP142, SP263, and E1L3N on the Ventana Benchmark (SP142, SP263) and DAKO platforms (22C3, 28-8, E1L3N). PD-L1 expression was manually scored on tumor cells (TC) and infiltrating immune cells (IC). Next, the PD-L1 status was determined according to corresponding assay specifications used in clinical trials.

**Results.** PD-L1 expression was higher on TC than IC using antibodies 22C3, 28-8, SP263 and E1L3N, while SP142 demonstrated less PD-L1 expression on TC. PD-L1 status was positive in 20% to 27% of patients. The percentage agreement in PD-L1 status between individual antibody clones: i) varied from 60% to 90%, ii) was lowest for E1L3N and SP142, and iii) was better when based on a higher cutoff value for 22C3 ( $\geq 10\%$ ) and 28-8 ( $\geq 5\%$ ). Fleiss' Kappa as an index of inter-assay agreement was 0.506 for all antibodies (PD-L1 status identical in 64%), it improved to 0.617 considering 22C3, 28-8, SP-142 and SP263 (PD-L1 status identical in 78%), and was best considering only 22C3, 28-8 and SP263 (Fleiss' Kappa 0.674, PD-L1 status identical in 84%).

**Conclusion.** We found a substantial concordance in PD-L1 status between the most frequently used PD-L1 companion diagnostics. In the majority of cases, the PD-L1 status was similar by each companion diagnostic antibody, indicating that the application of different companion PD-L1 antibodies may have limited implications on therapeutic decision making in ICI treatment for UC patients.

## HRAS mutations in early-onset bladder cancer

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**Background:** Bladder tumors of early-onset patients are rare and seem to exhibit unique features, both clinically and pathologically. The few available molecular studies observing a low frequency of the known alterations found in bladder cancer lead to the hypothesis of a different mutational profile between the various age groups. Recently, a study of few samples of young patients reported a higher percentage of HRAS mutations among the early-onset cohort compared to elderly samples. Due to the low sample size, the impact of these alterations of the age of onset and the frequency among young patients remain unclear and need further investigation.

**Objective:** The aim of our study was to test the distribution of HRAS mutations among the largest cohort of early-onset cases to date and to compare them with a consecutive group of bladder tumors.

**Materials and methods:** 141 early-onset patients aged 45 years old or younger and 144 consecutive samples were used for comparison. After microdissection, isolation of DNA and amplification the five established hotspot regions of the HRAS gene were examined by using SNaPshot approach. Chi-squared and Fisher's exact test were appropriately used for comparison between the two cohorts. Kruskal-Wallis non-parametric test was also calculated.

**Results:** A significantly higher number of HRAS mutations was found in tumors from the early-onset cohort compared to the consecutive samples (15.6% vs. 5.6%,  $p=0.01$ ). After stratifying the young samples according to the mutational HRAS status, we observed a significantly younger median age among the early-onset mutation carrier than for not mutated patients, with 32 and 40 years ( $p=0.01$ ) respectively. This difference was not found among the consecutive cohort.

**Conclusions:** We conclude that tumors of young patients showed a significantly higher frequency of HRAS mutations compared to the consecutive cohort. Thus, the tested mutations might play a more important role for the development of bladder cancer especially among the youngest cohort of early-onset patients. Our results strengthen the idea of a different molecular scenario between consecutive and early-onset bladder cancer tumors.

## Longitudinal Assessment of Multiplex Patient-Specific ctDNA Biomarkers in Bladder Cancer for Diagnosis, Surveillance, and Recurrence

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**Background:** The use of circulating tumor DNA (ctDNA) as a biomarker for disease staging at diagnosis, treatment response, and recurrence monitoring is an emerging field. In bladder cancer, the utility of ctDNA has shown promising results. Here we present a highly sensitive and specific NGS-based approach to ctDNA monitoring.

**Methods:** A prospective cohort of 68 MIBC patients treated with neoadjuvant chemotherapy were included. For each patient, a panel of 16 tumor-specific mutations was designed (Signatera™ RUO) based on whole-exome sequencing of tumor and germline DNA. In total, we analyzed ctDNA from longitudinally collected plasma samples from 637 time points procured at diagnosis, during treatment, at cystectomy, and during monitoring until disease recurrence or up to 2 years follow-up. Results of ctDNA analyses were compared to radiographic imaging and clinical outcomes. ctDNA from longitudinally-collected urine samples will also be analyzed for treatment response and disease recurrence.

**Results:** Results from the first 50 patients showed plasma ctDNA status was strongly prognostic of recurrence-free survival at diagnosis. Specifically, 62% (8/13) of the ctDNA+ patients at diagnosis recurred after neoadjuvant treatment and cystectomy; conversely, none (0/22) of the ctDNA- patients recurred ( $p < 0.0001$ ). In addition, a strong correlation was also observed between presence of ctDNA after cystectomy and disease relapse. Specifically, relapse after cystectomy was detected in 100% (10/10) of ctDNA+ patients ~120 days (0–245 days) prior to radiographic imaging, while 0% (0/38) of ctDNA- patients relapsed ( $p < 0.0001$ ). Results from all 68 patients will be presented.

**Conclusions:** We demonstrate a strong prognostic potential of ctDNA in bladder cancer at time of diagnosis, suggesting a potential role for ctDNA in the staging of bladder cancer. Incorporation of ctDNA analysis into routine follow-up may allow earlier initiation of alternate treatment modalities.

## **Association of circulating tumor (ct)-DNA genomic alterations (GA) with outcomes in metastatic urothelial carcinoma (mUC)**

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**Background:** Cell-free ctDNA profiling enables noninvasive identification of GA in mUC. We hypothesized that ctDNA GA correlate with outcomes and signify therapy targets.

**Methods:** Patients (pts) with UC who underwent ctDNA analysis for potentially actionable GA via Guardant360 were identified. A 73-gene ctDNA next generation sequencing (NGS) panel from CLIA-licensed, CAP-accredited laboratory (Guardant Health, Inc.) offers complete exon sequencing in 19 cancer genes, critical exons in 54 genes, amplifications (18 genes), fusions (6 genes) & indels (23 genes) from 10 mL of peripheral blood. KM method was used to estimate overall survival (OS) and failure-free-survival (FFS) since therapy initiation. Cox proportional hazards regression was used to assess the association of ctDNA GA present in > 10% of pts and clinical factors with OS and FFS in univariable analyses. All tests were 2-sided,  $p \leq 0.05$  was significant. We also evaluated GA in serial samples to assess genomic evolution.

**Results:** 557 patients with 673 samples were analyzed; 603 samples had ctDNA detected; most commonly altered genes were TP53, ARID1A, ERBB2, PIK3CA. 124 pts had available clinical data, of whom 65 had received prior platinum, 21 prior taxane and 10 prior PD1/PD-L1 inhibitor;  $\geq 1$  GA was detected in 112 pts. Median age at time of ctDNA collection was 72 and median (range) number of GA per sample was 4 (0-80); 110 pts had  $\geq 1$  SNV & 39 pts  $\geq 1$  CNV. Presence of RAF1 and BRCA1 GA correlated with shorter OS ( $p=0.007$ ;  $p=0.070$ , respectively). Serial samples showed new and resolution of some GA.

**Conclusions:** ctDNA GA were detected in most pts with mUC and appeared comparable to those from prior tumor tissue NGS studies. BRCA1 GA correlated with poor outcome suggesting that synthetic lethality with PARP inhibitors may be clinically useful (tested in clinical trials). Serial sample analysis revealed genomic evolution.

## **Molecular Markers (FGFR3 mutation; p53 & Ki-67 expression) and Clinical Outcome of Radical Cystectomy for Bladder Cancer: A Multi-center, Multi-lab Study**

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### **Objective:**

Radical cystectomy (RC) is standard treatment for BCG-refractory non-muscle invasive bladder cancer (BC) and muscle invasive BC. Fibroblast Growth Factor Receptor 3 (FGFR3) mutations were found to be associated with favorable prognosis. Various immunohistochemical (IHC) markers have gained attention as predictors of worse outcome. We have analyzed the prognostic value of the FGFR3 mutation and IHC markers (p53, Ki-67) in a multi-center, multi-lab setting.

### **Material and Methods:**

We included 904 cN0M0, chemotherapy-naïve patients who underwent RC with pelvic lymph-node dissection in 8 hospitals. The RC specimens were reviewed by 5 uro-pathologists. The FGFR3 mutation status was examined using PCR-SNaPshot in 5 labs. p53 and Ki-67 expression were determined by standard IHC (6 labs). FGFR3 mutation status, p53 (cut-off >10%) and Ki-67 (cut-off >20%) expression levels were correlated to clinic-pathological parameters and disease specific survival (DSS).

### **Results:**

pT-stage was <pT2 in 80, pT2 in 241, pT3 in 410 and pT4 in 173 patients, respectively. Cancer-positive nodes were found in 342 (38%) patients. The FGFR3 mutation was found in 102 RCs (11%), aberrant p53 in 639 (71%) RCs and aberrant Ki-67 in 502 (56%) RCs. The FGFR3 mutation was associated with lower pT-stage ( $P<0.001$ ), G2 ( $P<0.001$ ), pN0 ( $P=0.002$ ) and prolonged DSS ( $P=0.001$ ). Aberrant Ki-67 and p53 were associated with higher pT-stage and G3 tumors but not with pN+ or worse DSS. Significant predictors in multivariable analysis were pT-stage (HR 2.7, 95% CI: 1.6-4.5;  $P<0.001$ ), LVI (HR 1.5, 95% CI: 1.2-1.8;  $P=0.001$ ), pN-stage (HR 1.9, 95% CI: 1.5-2.3;  $P<0.001$ ) and FGFR3 mutation status (HR 1.6, 95% CI: 1.1-2.3;  $P=0.018$ ).

### **Conclusions:**

The FGFR3 mutation selectively identified patients with favorable BC at RC while p53 and Ki-67 expression were only associated with adverse tumor-characteristics. Tumor stage, LVI and nodal status remained strong predictors of DSS in this multi-center, multi-lab setting.

## **STAG2 is a Biomarker for Prediction of Recurrence and Progression in Papillary Non-Muscle Invasive Bladder Cancer**

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**Purpose:** Most bladder cancers are early stage tumors known as papillary non-muscle invasive bladder cancer (NMIBC). After resection, up to 70% of NMIBCs recur locally, and up to 20% of these recurrences progress to muscle invasion. There is an unmet need for additional biomarkers for stratifying tumors based on their risk of recurrence and progression. We previously identified STAG2 as among the most commonly mutated genes in NMIBC and provided initial evidence in a pilot cohort that STAG2 mutant tumors recurred less frequently than STAG2 wild-type tumors. Here we report a STAG2 biomarker validation study using two independent cohorts of clinically-annotated papillary NMIBC tumors from the US and Europe.

**Experimental Design:** The value of STAG2 immunostaining for prediction of recurrence was initially evaluated in a cohort of 82 patients with papillary NMIBC (“Georgetown cohort”). Next, the value of STAG2 immunostaining for prediction of progression to muscle invasion was evaluated in a progressor-enriched cohort of 253 patients with papillary NMIBC (“Aarhus cohort”).

**Results:** In the Georgetown cohort, 52% of NMIBC tumors with intact STAG2 expression recurred, whereas 25% of STAG2-deficient tumors recurred ( $p=0.02$ ). Multivariable analysis identified STAG2 expression as an independent predictor of recurrence ( $HR=2.4$ ;  $p=0.05$ ). In the progressor-enriched Aarhus cohort, 38% of tumors with intact STAG2 expression progressed within five years, versus 16% of STAG2-deficient tumors ( $p<0.01$ ). Multivariable analysis identified intact STAG2 expression as an independent predictor of progression ( $HR=1.86$ ;  $p=0.05$ ).

**Conclusions:** STAG2 IHC is a simple, binary, new assay for risk stratification in papillary NMIBC.

# **A non-invasive diagnostic urine assay to safely reduce the need for diagnostic cystoscopy in patients presenting with hematuria**

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## **Background**

Microscopic hematuria is reason for referral to the urology clinic. Only 2-5% of these patients are diagnosed with a urothelial carcinoma. Still the vast majority of all hematuria patients undergo cystoscopic evaluation to rule out bladder tumor presence. Hence, a non-invasive diagnostic test to rule in (micro)hematuria patients for cystoscopy is an unmet clinical need. In addition, a urothelial carcinoma diagnosis in women may be delayed as they are often treated for cystitis first, before they are referred to the urology clinic. An accurate rule in assay could reduce this diagnostic delay.

## **Objective**

To assess the accuracy to detect bladder cancer of a previously developed molecular assay in a large prospective cohort of patients referred for hematuria.

## **Methods**

We prospectively included 1003 patients referred to the urology clinic for hematuria who received a cystoscopy. Mutation status of the *FGFR3*, *TERT* and *HRAS* genes and methylation of the *OTX1*, *ONECUT2* and *TWIST1* genes were determined. The predictive capacity of the urine assay, potential confounders and the association between potentially predictive variables and the detection of bladder cancer were determined via logistic regression analyses.

## **Results**

Of all patients, 59% were male and 41% female, 55% presented with macroscopic hematuria compared to 45% with microscopic hematuria. A total of 115 patients was diagnosed with urothelial cancer. The assay resulted in an AUC of 0.95 and a sensitivity of 93%, a specificity of 81% and a NPV of 99%. All 4 upper tract tumors were identified.

## **Conclusion**

In patients referred for hematuria the urine assay was able to predict the absence of bladder cancer, especially in the microhematuria patient population, with very high accuracy. This assay seems ready to be implemented clinically to select patients for diagnostic cystoscopy.

## **POSTERS**

## **Long non coding RNA UCA1 as diagnostic and prognostic marker for bladder cancer.**

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Bladder cancer (BC) is one of the most common cancers of the urinary tract. Despite improvements in clinical treatment more than 50% of patients are relapsed within the next five years. Therefore we are still looking for the most sensitive and specific marker that would enable quick and precise diagnosis and monitoring of the disease. Long noncoding RNA UCA1 (lncRNA) plays a special role in the regulation of tumor cell proliferation, differentiation and apoptosis. It is highly expressed in diverse cancer types, such as breast cancer, pancreatic cancer and colorectal cancer, suggesting that high expression might serve as a molecular marker for predicting metastasis and prognosis also for BC. The presence of the UCA1 was confirmed by the real-time qPCR (quantitative polymerase chain reaction) method in 172 samples (tissue and serum) from patients diagnosed with BC. Analysis of relative gene expression levels was performed using the formula  $2^{-dCt}$  with  $dCt = Ct(\text{target gene}) - Ct(\text{control})$ . The tata box binding protein (TBP) was used as a housekeeping gene. Statistical analyzes were performed using U Mann-Whitney test and ANOVA Kruskal-Wallis test to determine the correlation between clinical and histopathological parameters and primary or recurrent tumor. We found that the lncRNA UCA1 was significantly higher in both tissue and serum samples of BC patients than in control group and in primary BC than in recurrent tumor. The increased expression was also associated with high grade and progression. In conclusion, our results indicated that lncRNA UCA1 could be useful diagnostic and prognostic marker for BC, however further studies with larger cohorts are necessary to evaluate it. One of the limitations of our research is low number of tissue samples from control group.

## Effect of the NAT2 Genetic Polymorphism on the p53 Mutagenic Spectrum in a Lebanese Urinary Bladder Cancer Cohort

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Urinary bladder cancer (UBC) is the eleventh most frequent cancer worldwide with higher incidence in industrialized countries, and much lower in developing countries. However, this epidemiological pattern is not observed in Lebanon, a Mediterranean middle-income country, where UBC incidence marks the second highest incidence in the world and the second most incident cancer in males. Genetic polymorphisms affecting metabolism of carcinogens may influence mutagenic risk in regulatory genes, leading to cancer development. We had previously reported slow N-acetylation, smoking, and diesel fume, as risk factors in Lebanese men. In this study, we investigated an association between N-Acetyltransferase *NAT2* genetic polymorphism and mutations in key carcinogenesis biomarkers. A cohort of 250 Lebanese males with histologically confirmed urothelial bladder cancer was identified. Archival tumors were used to extract DNA. A TaqMan real-time PCR assay was used to genotype *NAT2* for the following allelic variants: *NAT2*\*4 (wild-type), *NAT2*\*5, *NAT2*\*6, and *NAT2*\*7. PCR-RFLP was used to characterize tumors for *p53* mutations at *Codons 72 and 248*, and *RB1* 162238C>T mutation at *Exon 23*. Patients' clinical data were obtained from medical records. Pearson chi-squared tests were used to check for associations. The analysis was adjusted for multiple testing using Bonferroni correction. Our results showed that homozygous mutants for *p53* at *Codon 72* are prevalent in 20% of the samples, while *p53 Codon 248* and *RB1* 162238C>T are totally absent. *NAT2*\*4 (wild-type), \*5D, \*5E, \*5S, \*6B, \*6J, and \*7A, were found at frequencies of 17%, 27%, 13%, 7%, 25%, 5%, and 6%, in the total pool, respectively. Overall, there were no statistically significant differences in mutation frequencies of tested cancer biomarkers among carriers of the different *NAT2* alleles. In addition, *NAT2* polymorphism was not found to be associated with UBC muscle-invasiveness. Further studies are needed to examine the association of other drug-metabolizing enzymes with UBC.

## **Association of genetic variants with the occurrence of bladder cancer and adaptive selection in an arsenic-exposed population**

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**Objectives:** To identify genetic risk variants predisposing to bladder cancer (BC) due to chronic arsenic (AS) exposure and to detect Native-American genetic signatures of adaptation to AS according to our hypotheses of individual genetic susceptibility to BC due to AS exposure and genetic adaptation to selective pressure of AS exposure.

**Materials and Methods:** Genetic variants associated with BC occurrence were assessed by a case-control GWAS study among admixed subjects exposed to high AS levels in drinking-water in Northern Chile between 1958 and 1971. To address the second aim, we implemented two genome-wide tests of positive Darwinian selection: namely, PBSn1 test and an ancestry enrichment test, to detect adaptation signatures in local people that occurred pre- and post-admixture with Europeans, respectively.

**Results:** GWAS analysis detected several variants associated with BC development. The strongest association was for a SNP located close to CHL1, a gene involved in several cancers, including BC, possibly acting as a tumor suppressor. CHL1 has also been related cellular resistance to AS-trioxide in cell lines from different tumour types. Positive selection tests detected several adaptation signatures, some of them previously related to BC carcinogenesis. The highest-scoring SNP identified by PBSn1 is an intron variant of SMYD3, a gene with an active role in promoting BC progression. The ancestry enrichment test detected several significant SNPs in controls but not in cases considered separately, suggesting post-admixture adaptation to AS-induced BC. Some of these SNPs map genes (C7orf66 and TPMTP1) are associated with physiological response to cigarette smoke, which contains high levels of AS.

**Conclusions:** The results of this study contribute to a better understanding of the genetic factors affecting BC in subjects exposed to arsenic and shed new light into the recent evolutionary history of Native Americans. Candidate risk- and protective-SNPs identified need to be further validated in independent analyses.

## The rs351855T allele of *FGFR4* is associated with invasive growth of urothelial bladder cancer

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The Fibroblast growth factor receptor (*FGFR*) family plays a key role in the regulation of biological activities. Alterations of this family are very frequent in urothelial bladder cancer (UBC). Evidence is accumulating that the common variant rs351855 comprised in the *FGFR4* gene may act as a risk factor in many cancer types. Recently, it has been shown that the rs351855T allele leads to an enhanced activation of the signal transducer and activator of transcription 3 (STAT3). Due to the lack of studies, the association with the risk of regular- and early-onset UBC remains unclear. 252 patients consisting of an unselected cohort and a group of early-onset patients aged 45 years old or younger were used and compared with 147 controls. Restriction fragment length polymorphism was used as detection method. Results were exemplarily checked by Sanger sequencing. Fisher's exact and chi-squared tests were appropriately used. Regression models were calculated to test for differences between cases and controls. Overall, the rs351855T allele presented in a similar number in cases compared to healthy controls ( $p=0.73$ ). No difference for the risk of regular- and early-onset UBC was observed, with RRR=1.34 (95%CI=0.83-2.15) for regular and RRR=1.00 (95%CI=0.62-1.62) respectively. After grouping the samples based on the tumour stage in non- (PUNLMP, pTa, & Papilloma) and invasive tumours ( $\geq pT1$ ), the rs351855T allele (C/T + T/T) was significantly, more frequently detected among invasive bladder tumours compared to healthy controls, with RRR=1.95 (95%CI=1.14-3.36) respectively. Our findings suggest that the rs351855 polymorphism in *FGFR4* may not be associated with the overall risk of regular- and early-onset bladder cancer. The rs351855T allele was significantly associated with invasive tumour growth which might implicate the predisposition for the development of an aggressive disease. The reported association of the STAT3 pathway with invasive UBC and the rs351855 polymorphism might support our findings.

## **A comparison of pathologic and intermediate term oncologic outcomes following open and robotic radical cystectomy**

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**Objective:** Conflicting data regarding the oncologic efficacy of robotic surgery has lead to concerns for possible inferiority. Despite recent prospective results from the RAZOR trial demonstrating non-inferior progression free survival results from another prospective randomized trial from Memorial Sloan Kettering suggests a possible difference in the pattern of recurrences. We examined our extensive experience with both open and robotic radical cystectomy with the objective of establishing recurrence patterns and pathologic comparisons at a high volume tertiary referral center.

**Methods:** We performed a large retrospective cohort study at a high volume academic tertiary referral center for patients who underwent radical cystectomy for bladder cancer from 2005 to 2017. The surgical choice of robotic or open cystectomy is based on provider preference. A multivariable analysis was carried out to determine factors predictive of recurrence free survival and overall survival after radical cystectomy. All analysis was done with SAS 9.4.

**Results:** 1813 patients were identified, 10% underwent RARC and no difference in recurrence patterns were found compared to ORC. There was no difference in the severity of pathology distribution between the two cohorts. There was no difference in positive surgical margin status, 2.4% in ORC and 1.1% in RARC. Peritoneal carcinomatosis was seen in 1.1% of ORC and 0.5% in RARC. Shorter recurrence free survival was associated with younger age (HR 1.04, 95%CI 1.03-1.05,  $p<0.001$ ), neoadjuvant chemotherapy (HR1.55 95%CI 1.32-1.82,  $p<0.001$ ), higher pathologic stage (stage T4 HR 4.38, 95%CI 3.17-6.06,  $p<0.001$ ), positive lymph nodes at cystectomy (HR 1.82 95%CI 1.53-2.17,  $p<0.001$ ) and positive surgical margins (HR 1.50 95%CI 1.19-1.89,  $p<0.001$ ). At a median follow up of 60 months neither progression free or overall survival for ORC compared to RARC was significantly different.

**Conclusion:** The data from this study supports continued use of RARC as a safe oncologic procedure with similar outcomes to ORC.

## Radical Cystectomy in the Neoadjuvant Immunotherapy Era

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**Introduction and objectives:** Emerging evidence has demonstrated the efficacy of neoadjuvant immune checkpoint blockade (nICB) for muscle invasive bladder cancer, with complete response rates ranging from 29-39.5%. In this study, we describe the intraoperative findings after nICB and the perioperative outcomes.

**Methods:** We performed an IRB approved review of our bladder cancer database. From 07/2017 to 05/2018, 14 patients underwent RC following nICB: 11 cisplatin-ineligible patients treated with Durvalumab/Tremilimumab; 1 patient treated with either pembrolizumab or nivolumab/ipilimumab after disease progression on neoadjuvant chemotherapy; 1 patient treated with pembrolizumab for high grade disease in a bladder diverticulum. Baseline clinicopathologic features, intraoperative and perioperative measures and complications, as well as pathologic staging were recorded.

**Results:** Baseline clinicopathologic features are shown in Table 1. Five and nine patients underwent robotic assisted and open radical cystectomy with pelvic lymph node dissection, respectively. Median nodal yield was 23 (7-35). Overall, 10/14 (71%) were found to have pathologic downstaging <pT2, with 6 achieving pCR. Interestingly, dense adhesions were encountered extravesically or over the pelvic lymph nodes in 10 (71%) of the patients, including 5 with pCR. Intraoperatively, dense adhesions surrounding the bladder and lymphatic tissue led to resection of the internal iliac artery in one patient and partial transection of the obturator nerve in another. Six (43%) patients encountered postoperative complications, including one with interstitial nephritis leading to acute kidney injury thought to be related to nICB treatment (Table 2). No perioperative mortality occurred.

**Conclusions:** Although providing great oncologic benefit, nICB may render surgical dissection more difficult due to desmoplastic reactions presumably from heightened immunologic response. Postoperative course may also be marred by untoward complications directly related to nICB.

| Table 1 Clinicopathologic features |                |
|------------------------------------|----------------|
| Age                                | 75 (42-84)     |
| Gender                             |                |
| M                                  | 12 (86)        |
| F                                  | 2 (14)         |
| BMI                                | 28 (23.2-50.1) |
| History of pelvic radiation        |                |
| No                                 | 11 (79)        |
| Yes                                | 3 (21)         |
| Clinical T stage                   |                |
| <cT2                               | 1 (7)          |
| cT2                                | 6 (43)         |
| cT3                                | 6 (43)         |
| cT4                                | 1 (7)          |
| Clinical N Stage                   |                |
| cN0                                | 13 (93)        |
| cN+                                | 1 (7)          |
| Variant histology                  |                |
| None                               | 10 (71)        |
| Squamous                           | 2 (14)         |
| Plasmacytoid                       | 1 (7)          |
| Sarcomatoid                        | 1 (7)          |
| CIS                                |                |
| No                                 | 12 (86)        |
| Yes                                | 2 (14)         |
| LVI                                |                |
| No                                 | 12 (86)        |
| Yes                                | 2 (14)         |
| Hydronephrosis                     |                |
| No                                 | 6 (43)         |
| Yes                                | 8 (57)         |

| Table 2 Postoperative complications |   |
|-------------------------------------|---|
| Clavien-Dindo I                     |   |
| Dehydration                         | 1 |
| Clavien-Dindo II                    |   |
| UTI                                 | 2 |
| Wound infection                     | 1 |
| Interstitial nephritis              | 1 |
| Clavien-Dindo III                   |   |
| Urine leak                          | 1 |

## **Low awareness, adherence and practice but positive attitudes regarding lifestyle recommendations among bladder cancer patients**

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**Objectives:** A healthy lifestyle may reduce the risk of non-muscle-invasive bladder cancer (NMIBC) recurrence. We examined patients' awareness of (bladder) cancer risk factors, and their adherence to lifestyle recommendations for cancer prevention. Also, we evaluated whether they receive lifestyle advice, and their attitudes towards receiving lifestyle advice from their physician.

**Materials and Methods:** Patients with newly diagnosed NMIBC in 2014-2017 participating in the UroLife cohort study filled out questionnaires on lifestyle habits at 6 and 12 weeks after diagnosis. Also, a survey was completed on awareness of (bladder) cancer risk factors and reception of and attitudes towards physicians giving lifestyle advice.

**Results:** A total of 969 patients were included. Eighty-nine percent of patients were aware that smoking is a risk factor for cancer in general, but only 44% knew that smoking is a risk factor for bladder cancer. Knowledge of other risk factors for cancer in general varied between 29% (fruit and vegetable consumption) and 67% (overweight). Adherence to cancer prevention recommendations varied between 34% (body weight) and 84% (smoking), and differed by age, sex, and education. Of the smokers, 70% reported they were advised by their physician to quit. Only 19% of all patients indicated they received other lifestyle advice. More than 80% of patients had a positive attitude towards receiving lifestyle advice from their physician.

**Conclusion:** Most NMIBC patients were unaware of cancer risk factors, except for smoking. The degree of adherence to the cancer prevention recommendations varied widely. Patients were not routinely advised about a healthy lifestyle by their physician. Since the majority of NMIBC patients had a positive attitude towards receiving lifestyle advice from their physician, information provision by physicians should be improved.

## **Characteristic mutational pattern and chromosomal arrangements in long-term cisplatin-treated urothelial cancer cell lines**

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Cisplatin reacts with DNA purine bases, inducing monoadducts, intra- and interstrand crosslinks, and eventually apoptosis. The compound is commonly used in the treatment of various cancer types, including urothelial carcinoma, but its efficacy is limited by the development of resistance. While several resistance mechanisms have been identified, it is unclear to which extent they are caused by direct cisplatin effects on the cancer cell genome. We therefore performed whole-exome sequencing, array-CGH, and chromosomal karyotyping for four long-term cisplatin-treated urothelial carcinoma cell lines (LTTs) which had been developed by continuous cisplatin treatment with escalating doses over several months. These were each compared to their parental cell lines (RT-112, J82, 253J and T-24).

New point mutations were observed in 720 - 7479 genes in the four LTTs, which exhibited a distinctive mutational spectrum dominated by C>A transversions and C>T transitions, single base deletions, and double base exchanges. Even though no evidence for positive selection of mutations was obtained, several mutations were in genes involved in cisplatin resistance, like KEAP1 and ATP7B. Additionally, multiple chromosomal alterations as well as segmental gains and losses were observed in all LTTs. Their extent, like the number of mutations, increased with the maintenance concentrations of cisplatin among the LTTs. While chromosomal changes likewise appeared largely unselected, increases and losses in a few specific chromosomal regions were common to all LTTs. Most copy number changes were already established after the initial selection for resistance.

In summary, cisplatin induced characteristic point mutations with specific mutagenic signatures and distinctive chromosomal changes in urothelial carcinoma cell lines, most of which appeared to occur early during selection for resistant cell clones. Cisplatin treatment thus provoked broad genomic changes, of which a few could contribute to the development of resistance.

## **Viability of using adjusted tumour tissue instead of healthy tissue expression profiles for eQTL analysis**

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**Background:** Obtaining gene expression profiles from certain healthy tissues, such as bladder urothelium, for the identification of disease-specific expression quantitative trait loci (eQTL) is not always practical whereas tumour tissue may be readily available. In this study we examined the feasibility of using tumour-derived gene expression profiles adjusted for tumour methylation (Met) and copy number variation (CNV) as substitutes for expression profiles from healthy tissue in eQTL analyses.

**Methods:** We extracted data on blood-derived germline single nucleotide variants (SNVs), tumour Met, tumour CNV, and tumour and normal tissue gene expression profiles from 83 breast cancer (BRCA) and 67 clear cell renal cell carcinoma (ccRCC) patients from The Cancer Genome Atlas. We generated Met and CNV adjusted tumour gene expression profiles using multivariable regression analysis and compared genome-wide cis eQTL analysis results for adjusted tumour tissue expression levels against those for normal tissue.

**Results:** For BRCA, adjusted tumour and normal tissue eQTL analyses identified 601 and 1546 SNV-gene pairs, respectively, at an FDR adjusted p-value of  $<0.05$ ; 436 SNV-gene pairs were found in both analyses. Similar results were observed for ccRCC with 696 and 1879 SNV-gene pairs for adjusted tumour and normal tissue eQTL analyses, respectively, and an overlap of 449 SNV-gene pairs. Almost all of the overlapping SNV-gene pairs were identified in unadjusted tumour tissue eQTL analyses as well (95% for BRCA and 92% for ccRCC).

**Conclusion:** The use of tumour expression levels adjusted for Met and CNV as a substitute for normal tissue expression levels leads to underreporting of statistically significant eQTLs. Furthermore, similar results can be obtained with the use of unadjusted instead of adjusted tumour tissue expression profiles.

## Understanding muscle-invasive bladder cancer subtypes using *regulon* analysis

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Regulons consist of regulatory units comprised of a regulator and its targets. Many regulons together form transcriptional networks, which can be inferred from microarray or RNA-seq expression data. One of the novel analyses in the characterization of TCGA's muscle-invasive bladder cancer cohort involved regulon activity for 23 BLCA-associated regulators. In ongoing work, activities of these 23 regulons, calculated in 14 cohorts, are being used in characterizing proposed consensus MIBC subtypes. Following the TCGA project, we have extended the list of regulators, computing regulons for over 500 transcription factors, for each of which we calculate a regulon activity profile for individual samples in a cohort. We will describe how we use regulon activity profiles as a functional readout to characterize differences in regulatory states between subtypes. We will also demonstrate using regulon activity profiles as predictors of outcome in MIBC. Finally, we will show results for MIBC from our novel *dual* regulon inference method, which identifies targets jointly influenced by two regulators as a *dual regulon*, and assesses the effect of dual regulon interaction on survival analysis.

## **Inhibition of Urothelial Carcinoma through Targeted Type I Interferon-Mediated Immune Activation**

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Type I interferon (IFN) has potent antitumor effects in bladder cancer and provides an essential alternative treatment option for patients who do not respond to Bacillus Calmette-Guerin (BCG). However, the mechanism of the IFN-stimulated immune response in bladder cancer is not well understood. To examine effects of IFN- $\alpha$  in BCG-unresponsive patients, tumor samples from a Phase I Clinical Trials with Adenoviral Interferon- $\alpha$  (Instiladrin) were analyzed for RNA expression and immunohistochemical (IHC) staining both before and after treatment. Among these samples, 25% showed increased expression of T cell and checkpoint markers, similarly reflected in IHC analysis. We hypothesized that type I IFN enhances recruitment and activation of immune cells in murine bladder cancer tumors, and makes them more responsive to anti-PD-1 mAb therapy. To incite an IFN-driven inflammatory response, poly(I:C) was given to murine MB49 bladder cancer tumors peritumorally alone and in combination with anti-PD-1 mAb to determine effects on tumor growth and animal survival. IFN induction in MB49 tumors significantly inhibited tumor growth. This response was reliant on both innate and adaptive immune subsets, shown in the increased influx of intratumoral Ly6G<sup>+</sup> neutrophils and the increase in IFN $\gamma$ <sup>+</sup>CD8 T cells. In addition, loss of IL-6 signaling abrogated poly(I:C)'s antitumor benefits, however depleting specific immune cell subsets indicated that no one type of immune cell was critical for the IFN-mediated anti-tumor response. When used in combination with anti-PD-1 mAb, poly(I:C) prolonged mouse survival and enriched gene pathways involved in metabolism, extracellular matrix formation and organization, and PI3K and AKT signaling. Altogether, these findings suggest IFN-I's immune-driven antitumor response in UC is mediated by IL-6 and a collaboration of immune cells, and its use in combination with checkpoint blockade therapy can increase clinical benefit.

## **Concordance of PD-L1 expression in matched urothelial bladder cancer specimens**

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### Aims

Programmed Death-Ligand (PD-L1) expression has predictive value for response to immune-checkpoint inhibitor treatment in urothelial cancer patients. The consistency of PD-L1 expression amongst different specimen types is however unknown. The aim of the study is to compare PD-L1 expression in matched transurethral resections of the bladder (TURB), cystectomy specimens, and lymph node metastases of urothelial cancer patients.

### Methods and Results

We performed PD-L1 (SP142) immunohistochemistry on whole tissue slides of 115 urothelial carcinoma patients who had undergone TURB, followed by radical cystectomy and/or pelvic lymph node dissection. The PD-L1 assay was positive if PD-L1 expression in immune cells occupied  $\geq 5\%$  of the tumour area. PD-L1 was positive in 15/97 (15.5%) TURB, 17/98 (17.3%) cystectomies, and 9/49 (18.4%) lymph node metastases. Agreement of PD-L1 assay outcome between cystectomy and TURB (kappa 0.34;  $P=0.002$ ), and cystectomy and lymph node metastasis (kappa 0.35;  $P=0.034$ ) was fair; there was no agreement between TURB and lymph node metastasis (kappa 0.045;  $P=0.82$ ). Discordance of PD-L1 outcome in matched TURB and cystectomy specimens occurred more frequently after neo-adjuvant therapy (53.3% versus 25.4%;  $P=0.03$ ), and was not associated with other clinicopathologic parameters.

### Conclusion

Urothelial bladder cancer patients showed fair agreement of PD-L1 assay outcome in cystectomies and matched TURB or lymph node specimens. PD-L1 expression was discordant more often after neo-adjuvant therapy. Therefore, immune-checkpoint inhibitor studies should take into account specimen type and neo-adjuvant therapy in assessing the predictive value of PD-L1 expression.

## **Evolution of PD-1 and PD-L1 gene and protein expression in primary tumors and corresponding liver metastases of metastatic bladder cancer**

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**Background:** Demonstrated by previous clinical trials patients with liver metastases respond worse towards immune-oncological therapies.

**Patients and methods:** Immunohistochemistry and gene expression (RT-qPCR) of PD-1 and PD-L1 were analyzed in a total of 14 matched pairs of primary tumors (PT; radical cystectomy/transurethral resection specimens) and corresponding liver metastases (LM) in 14 patients with metastatic urothelial bladder cancer treated between 2001 and 2017. Stromal tumor infiltrating lymphocytes (%) were scored in corresponding H&E slides.

**Results:** Our main finding demonstrated that stromal tumor infiltrating lymphocytes (sTILs) were significantly decreased in LM (median 0%) compared with PT (median 7.5%). This indicates a significant change to an inactive immune state in LM. PD-L1 and PD-1 gene expression of tumor tissue, analyzed by RT-qPCR, was significantly higher in PTs than in LMs. Although PD-L1 protein expression on TC (IHC; Ventana SP263 assay) showed no significant difference between PTs and LMs, examining patient matched samples we observed individual changes. For example, a loss (21%) or a gain (21%) of PD-L1 expression was common in LM, whereas the remaining 57% of tumors showed no changes in metastasis. PD-L1 protein expression on immune cells in primary tumors (78%) was significantly expressed, but interestingly not PD-1 although sTILs were present. In a direct comparison to PTs, eight LMs (57%) showed a complete loss of sTILs thus consecutive absence of PD-1 and PD-L1 expression. This finding indicates a significant immunological change in the evolution to LM.

**Conclusion:** This result could be an explanation for reduced ORR in LMs and indicate that immune evasion mechanisms also include depletion of anti-tumoral immune infiltrates, thus absence of PD-L1 or PD-1 protein expression. The use of freshly obtained core biopsies from bladder cancer LM tissues might be more precise to predict responsiveness towards checkpoint-inhibitors since this would reflect the current immune state.

# Prognostic impact of tumor infiltrating lymphocytes and immune cell related gene expression after radical cystectomy in muscle-invasive bladder cancer

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**Background:** Development and progression of malignant tumors is characterized by interaction of tumor cells in the tumor microenvironment, for example with tumor infiltrating immune cells (TILs). Scoring of TILs is a well-established predictive and prognostic biomarker e.g. in breast cancer. The role in muscle-invasive urothelial bladder cancer (MIBC) is not yet clarified completely.

**Patients and Methods:** sTILs were scored continuously on HE slides in a cohort of 128 patients with MIBC treated by radical cystectomy (adjuvant chemotherapy n= 34) according to current recommendations (Salgado et al, 2015). In parallel, gene expression of PD-L1, PD-1, CD3Z, CD8 and CXCL9 was measured by RT-qPCR. Cut-offs were estimated by a decision-tree model. Kaplan-Meier-analysis as well as multivariate Cox regression were performed.

**Results:** Tumors with infiltration through sTILs of  $\geq 10\%$  (AUC=0.75) showed significantly better OS and DSS (Two-Step-System: 5-year OS 58% vs. 17.5%,  $p < 0.0001$ , HR=0.38 [CI: 0.21-0.66]; 5-year DSS 80% vs. 22%,  $p < 0.0001$ , HR=0.15 [CI: 0.06-0.33]). Furthermore, high amount of sTIL was significantly associated with higher infiltration through CD3, CD8 and PD-L1 positive immune cells and with high gene expression of PD-1 ( $p = 0.0001$ ), PD-L1 ( $p = 0.0012$ ), CD8A ( $p < 0.0001$ ), CD3Z ( $p = 0.0001$ ) and CXCL9 ( $p < 0.0001$ ).

**Conclusion:** High infiltration through sTILs is highly prognostic and strongly correlated with high amount of CD3, CD8 and PD-L1 positive tumor infiltrating immune cells as well as with genes to an INF-gamma driven immune response (CD3Z, CD8A, PD-1, PD-L1 and CXCL9). Scoring of sTIL scoring seems to be a suitable tool for identifying patients with highly activated anti-tumoral immunity and a better prognosis.

## **Combined Next Generation Sequencing and Flow Cytometry Analysis of an anti-PD-L1 Partial Responder over time: An exploration into mechanisms of PD-L1 activity and resistance**

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**Background:** Our objective was to explore mechanisms of anti-PD-L1 immunotherapy activation and resistance by tracking gene and protein expression of tumors over time. We report the first combined analysis utilizing next generation sequencing and flow cytometry of multiple tumor specimens over a five year period in a patient undergoing anti-PD-L1 therapy.

**Methods:** A single patient with urothelial carcinoma of the prostatic urethra with nodal metastases was enrolled in the IRB approved JHH biorepository. The patient was administered cisplatin based chemotherapy before being enrolled in an anti-PD-L1 clinical trial, and monitored with F-18 FDG PET and serum CEA (to which the tumor was sensitive) for treatment response. Tumor specimens were microdissected from FFPE slides for whole-exome and RNA sequencing, representing four time points: initial diagnosis, post chemotherapy resistance, early PD-L1 resistance, and late PD-L1 resistance. Whole exome sequencing analysis of tumor and normal samples was performed using the PGDX CancerXome platform to identify tumor-specific (somatic) sequence and copy number alterations. RNAseq was performed on all tumor samples to assess for gene expression patterns longitudinally across treatment time points. Fresh tissue was obtained from early and late PD-L1 resistance time points for flow cytometry to specifically assess protein expression of immune checkpoints.

**Results:** The patient had durable partial response to anti-PD-L1 immunotherapy over a 30 month period, and is currently 75 months out from initial diagnosis. Repeat PET-CT and CEA surveillance demonstrated the prostatic urethra consistently as the sole site of tumor recurrence, managed several times with transurethral resection and ultimately with radical cytoprostatectomy. Whole exome sequencing revealed 825 somatic mutations across tumor specimens, of which 69% were consistent across all time points. Notable somatic mutations across all time points included loss of p53 and FoxP3, a marker of regulatory T cells. RNAseq demonstrated that pre and post anti-PD-L1 treatment tumor samples were similar to one another. Gene expression timepoint analyses revealed notable declines in PD-1 Ligand 2 and IDO1 expression and an influx in FoxP3, CD8, CTLA4 and Tim-3 expression as the tumor became PD-L1 resistant. Flow cytometry demonstrated that as the tumor became more resistant to anti-PDL1 therapy, tumor infiltrating CD8 and regulatory T cells increased expression of alternate immune checkpoints LAG-3 and Tim-3.

**Conclusions:** Our analysis demonstrates that as tumors become resistant to the PD1/PDL1 pathway, alternate immune checkpoints may be up regulated. This supports the concept of combined checkpoint blockade for urothelial carcinoma. The concept of the prostate and prostatic urethra as an immune sanctuary also warrants further study.

# Investigation of the role of inflammation in development of invasive bladder cancer

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## Objectives

Immune gene expressions revealed by the recent bladder cancer molecular classifications indicate that tumour immune environment significantly influence patient survival. However, the functional role of inflammation and immune cells in urothelial neopathogenesis is less well defined. Chemokine (C-X-C) receptor 2 (CXCR2) is known to contribute to tumorigenesis of various organs, including skin, colorectal and pancreatic cancers. Cxcr2 inhibition could suppress tumour progression by inhibiting Cxcr2-positive myeloid-derived suppressor cells that are pro-tumour and pro-metastatic. This study aimed to determine the role of Cxcr2 in development of bladder cancer in mice.

## Materials & Methods

The bladder phenotype of mice deleted with Cxcr2 in myeloid cells (LysMCre Cxcr2<sup>flox/flox</sup>, "Cxcr2 flox") were analysed compared to that of Wildtype mice. Eight-weeks old mice were administered with 0.05% 4-Hydroxybutyl(butyl)nitrosamine (OH-BBN) in drinking water for 10 weeks, then examined at time points of 2, 12 and 20 weeks from the first administration of OH-BBN.

## Results

Our results showed that Cxcr2 deletion in myeloid cells robustly increased tumorigenesis in mouse model of invasive bladder cancer, compared to Wildtype. This was unexpected as Cxcr2 inhibition had shown an overt anti-tumour effects in other cancer types. Our further histopathological studies revealed that tumours were highly infiltrated by neutrophils regardless of Cxcr2 deletion, likely to be owing to the compensatory mechanism developed through the tumour progression that are not present in other tissue types. Several growth factors and cytokines were up-regulated, indicative of such effects. Most interestingly, in the absence of Cxcr2, tumours became highly infiltrated with T-cells.

## Conclusions

We hypothesize that the long-term inhibition of Cxcr2 enhances tumour progression by increasing pro-tumour immune cells, and that such tumours may be targetable by signalling inhibitors and immune modulating agents, and by sensitizing them to anti-PD-1 immunotherapy.

## **BLASST-1 (Bladder Cancer Signal Seeking Trial), a phase II trial of neoadjuvant nivolumab with cisplatin and gemcitabine in muscle-invasive bladder cancer patients undergoing radical cystectomy**

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**Background:** Cisplatin-based neoadjuvant chemotherapy (NAC) in muscle-invasive bladder cancer (MIBC) improves survival in pts who achieve a pathologic response (PaR) and is the standard of care. Checkpoint inhibitors (CPI) are effective and approved in advanced bladder cancer. Addition of CPI to NAC can potentially improve PaR. BLASST-1 (Bladder Cancer Signal Seeking Trial-1; NCT03294304) is a single-arm, multi-site phase II study exploring the efficacy of nivolumab (N) and gemcitabine-cisplatin (GC) in MIBC.

**Methods:** 41 eligible pts with cT2-4, N0-1, M0 MIBC will be enrolled. Key eligibility criteria include ECOG PS of 0-1, Cr Cl  $\geq$  50 ml/min, no contraindications to receiving NAC or N. Four cycles of N+GC will be administered every 21 days (C 70 mg/m<sup>2</sup> IV D1, G 1000 mg/m<sup>2</sup> IV D1 and 8, N 360 mg IV D 8) followed by radical cystectomy (RC). The primary end point is pathologic response (PaR) of downstaging to  $\leq$  pT1N0M0. Secondary endpoints include safety of N+GC and progression-free survival (PFS). Pre and post-treatment tissues will be collected for biomarker correlates, including whole genome sequencing, molecular subtyping using GenomeDx and PD-L1 expression in pre-treatment tissues to study correlation with PaR. Nanostring pancan immune panel gene expression will be studied at baseline and at cystectomy.

**Results:** As of July, 2018, 12 patients have been enrolled with expected side effects and no additional toxicities were seen with the N+GC combination. No immune-related AEs were observed so far. PaR evaluation is ongoing and treatment will be worthy of further study if PaR is seen at RC in more than 19 pts. The novel exploratory analyses are aimed at understanding correlation between genomic and immunologic biomarkers in bladder cancer tissues with clinical outcomes, including PaR and PFS.

## **Influence of Chemotherapy on the relationship between urine and blood leukocytes in patients with muscle invasive bladder cancer.**

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### **Objectives**

To understand the immunological status of patients with muscle invasive bladder cancer (MIBC) and its relationship with the immunosuppressive properties of tumor cells, we analyzed the expression of immunocheckpoints on urine tumor cells and the leukocyte composition of peripheral blood and urine in two groups of patients segregated according to their radical treatment (Neoadjuvant chemotherapy + cystectomy vs. upfront cystectomy)

### **Material and Methods**

In this exploratory analysis we collected blood and urine from MIBC patients with and without neoadjuvant chemotherapy (n=9 in g1 and n=16 in g2 groups, respectively) before cystectomy. Blood and urine leukocyte subpopulations and the expression of PD-L1 on urine EpCAM+ tumor cells were analyzed by flow cytometry.

### **Results**

We have previously observed a different leukocyte composition in the urine but not in the blood of the two groups of patients. Here, we found that the percentages of neutrophils in urine and blood were significantly correlated in g1 ( $R=0.733$ ,  $p=0.02$ ) but not in g2. The percentages of lymphocyte subsets (CD4+, CD8+, B and NK cells) in urine and blood were comparable in the two groups but not correlated between urine and blood. We have previously found that tumor PD-L1 expression was higher in g2 than in g1 patients. We next analyzed the relationship between PD-L1 expression on urine tumor cells and the cellular composition. PD-L1 expression on urine tumor cells correlated inversely in g1 and directly in g2 with the percentage of urine monocytes (g1:  $R= -0.741$ ,  $p=0.03$ ; g2:  $R=0.682$ ,  $p=0.004$ ) and with the percentage of blood CD8+ T lymphocytes (g1:  $R= -0.588$ ,  $p=0.009$ , g2:  $R=0.707$ ,  $p=0.002$ ).

### **Conclusions**

The mechanisms responsible for the presence of different leukocyte subpopulations in urine are different in the two groups of patients. Our findings suggest that chemotherapy is not involved in the lymphocyte subset composition in urine and blood but rather in modulating the expression of PD-L1 on tumor cells.

## Integration of BK polyomavirus in (micropapillary) urothelial carcinoma – a role for pathogenesis?

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**Aims** - While integration of Merkel cell polyomavirus is considered oncogenic in Merkel Cell carcinoma, little is known about the association of highly prevalent human BK polyomavirus (BKV) and urothelial carcinoma (UC). An association with micropapillary urothelial carcinoma (MPUC) has been reported. BKV associated nephropathy and cystitis are major complications in immunosuppressed patients and few cases of BKV integration in UC have been documented.

**Methods** - Samples from MPUC and UC with micropapillary-like features (n=147) and post-transplant UC (n=14; 11 NTX, 2 HTX, 1 LTX; 4 MPUC and 10 conventional UC) were screened for SV40 polyomavirus large T antigen (SV40) expression by immunohistochemistry (IHC). BK viral load of positive tumors was determined by qPCR from FFPE-tissue extracted DNA. DNA from four FFPE- and one fresh frozen-tumor was converted into Illumina NGS libraries and sequenced on HiSeq 4000 or X-Ten (Macrogen Inc, ROK). Paired reads (between 313-880 million/sample) were aligned to the human genome, and BKV integration sites identified from broken paired reads mapped to human (hg19) and BKV genomes. RNA-Seq from the fresh frozen tumor sample was performed to test cellular and viral gene expression. All bioinformatic analyses were performed with CLC Genomics Workbench (Qiagen Aarhus, DK).

**Results** – Five tumors were positive by IHC (4 post-NTX, 1 post-HTX). Three were pure MPUC, two high-grade UC with unusual, mainly glandular and focal micropapillary features. Real time PCR detected BKV with a viral load of at least two BKV-DNA copies per cell in all tested tumors. Despite limited sample quality from FFPE-tissue, NGS analysis identified BKV integration in 4/5 tumor samples. Notably, the sample without detectable BKV integration had a high viral load of human herpesvirus 6. We detected one distinct BKV integration site each in three tumors, and at least two sites in one tumor. In 2/4 samples small deletions in the BKV noncoding control region (NCCR) were observed. Remarkably, one integration event might activate the Serine Protein Kinase A (PKA), which is tested in the tumor tissue.

**Conclusions** – Integrated BK polyomavirus may be involved in tumorigenesis of aggressive UC with frequent micropapillary morphology in immunocompromised patients.

## **Grainyhead-like 3 (GRHL3) affects migration and invasion of bladder cancer cells in vitro**

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Department of Urology, University of Ulm, Germany

### **Objectives**

Invasive urothelial carcinoma (UC) characteristically show a loss of differentiation-associated markers. The transcription factor GRHL3 plays a role in embryonal urothelial development and terminal tissue differentiation. We investigated the role of GRHL3 in proliferation, migration and invasion of UC cells in vitro.

### **Material and Methods**

Stable ectopic expression of GRHL3 was induced using lentiviral vectors in T24 and RT112 cells. Cell proliferation, migration (scratch wound assay) and invasion (Boyden chambers) were investigated. We seeded UC cell lines on deepithelialized porcine bladders in organ culture as a standardized organotypic invasion model. qPCR und western blot were applied for expression studies.

### **Results**

GRHL3 was significantly higher expressed in well-differentiated, non-invasive RT4 cells compared to moderately differentiated RT112 cells ( $p < 0.05$ ). Anaplastic T24 cells showed no expression of GRHL3. Following ectopic de novo expression of GRHL3, T24 cells showed significantly reduced migration and invasion in vitro, while cell proliferation was not affected. In organ culture, the diffuse invasion of T24 cells was completely reversible upon overexpression of GRHL3.

### **Conclusions**

Invasive UC cells show a loss of GRHL3 in vitro. De novo expression of GRHL3 in T24 cells significantly affected migration and invasion. The results indicate that GRHL3 may play a potential role in UC tumor progression and metastasis. In addition, we established an organotypic porcine organ culture model for invasion studies.

## **Targeting a sub-group of luminal muscle-invasive bladder cancer with a cytochrome P450 activated pro-drug.**

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<sup>3</sup>Division of Urology, Regensburg University Medical Centre, Regensburg, Germany.

### Introduction

Cytochrome P450 family 1 (CYP1) enzymes are transiently inducible by normal tissues to process specific toxins but are present constitutively in some tumours. Cytochrome P450 oxidoreductase (POR) is a critical electron donor for CYP1 enzymes and may act as master-regulator of total CYP1-activity.

We recently reported POR over-expressing tumours formed a subgroup of luminal muscle-invasive bladder cancer (MIBC) identified using GATA3 vs CK5/6 immunohistology. And furthermore, that in bladder cancer cell lines, CYP1-activity was undetectable in basal/POR<sup>lo</sup> T24 & SCaBER and high in luminal/POR<sup>hi</sup> RT4 & RT112 (Baker *et al.* PMID:29323757).

### Objectives

To establish whether CYP1-activity observed in a sub-group of luminal MIBC provides a novel prodruggable target.

### Materials & Methods

Principal component analysis of The Cancer Genome Atlas (TCGA) MIBC cohort was used to analyse transcriptomes. Quantitative immunohistology was used to evaluate reductase expression. CYP1-activity in bladder cancer cell lines was assessed with Ethoxyresorufin O-deethylation activity assays. The CYP1-activated pro-drug of duocarmycin “ICT2700” was used to evaluate chemosensitivity by alamar blue assay.

### Results

A sub-group of luminal tumours were enriched for *CYP1* gene expression in association with the relevant reductases (*POR*, *CYP5A* & *CYP5B*) in the TCGA cohort. The CYP1-prodrug ICT2700 did not induce its own activation by CYP1 supporting its selectivity for tumour cells with constitutive CYP1-activity. ICT2700 chemosensitivity was evaluated in 12 bladder cancer cell lines and showed luminal lines had a 25-fold lower mean LD<sub>50</sub> than basal lines (339nM vs 8354nM: *t* test; *p*<0.05). Furthermore, the ICT2700 LD<sub>50</sub> for all cell lines showed a significant negative correlation with quantified POR immunohistochemistry of cancer:stromal organoids (Pearson correlation; *R* = -0.62, *p*<0.05).

### Conclusions

This data suggests a sub-group of luminal MIBC would be susceptible to ICT2700, a CYP1-activated prodrug with potential as a treatment for bladder cancer and that POR immunohistochemistry provides a predictive biomarker capable of identifying responders for future clinical trials.

## **Targeting HER2 with T-DM1, an Antibody-cytotoxic Drug Conjugate, is effective in bladder cancer with HER2 IHC score 2+/3+**

Tetsutaro Hayashi, Roland Seiler, Htoo Zarni Oo, Wolfgang Jäger, Tilman Todenhöfer, Na Li, Ladan Fazli, Akio Matsubara and Peter C. Black

**Background and purpose:** Recent genomic studies suggest that urothelial carcinoma of the bladder (UCB) could potentially respond to HER2-targeted therapy if patients are selected optimally. T-DM1 is an antibody-drug conjugate consisting of trastuzumab linked to the cytotoxic agent DM1. We aimed to test T-DM1 in preclinical models of UBC.

**Results:** Higher HER2-expressing UBC cell line, RT4V6, showed higher growth inhibition with T-DM1 compared to trastuzumab. T-DM1 induced apoptosis after G2/M arrest. HER2 expression was higher in cell lines with acquired cisplatin-resistance compared to the corresponding parental cell lines, and the resistant cells showed higher sensitivity to T-DM1. In addition, cells cultured in anchorage independent conditions increased HER2 expression compared to cells cultured in adherent conditions, and T-DM1 significantly inhibited colony formation in soft agar. In an orthotopic bladder cancer xenograft model, tumor growth was significantly inhibited by T-DM1 via induction of apoptosis compared to treatment with either a control IgG or trastuzumab.

Next, we examined HER2 expression in UBC treated by radical cystectomy in immunohistochemistry (IHC). HER2 over-expression (IHC score 2+ or 3+) was detected in 59 of 159 (37%) patients. HER2 expression was higher in lymph node metastases than in primary tumors. To clarify the efficacy of T-DM1, we examined HER2 expression in several UBC cell lines, and found that BOY showed equivocal HER2 amplification by FISH (HER2/CEP17 ratio: 1.8, HER2 copy number: 4.7), which is known to correspond to HER2 IHC score 2+ in patient tissue. BOY responded most sensitively to T-DM1. This suggests T-DM1 could be effective in patients with HER2 over-expression (IHC score 2+/3+).

**Conclusions:** T-DM1 has promising anti-tumor effects in pre-clinical models of HER2-overexpressing bladder cancer. Furthermore, T-DM1 could have anti-metastatic potential and be a promising targeted therapy for patients with HER2 score 2+/3+ UBC. T-DM1 warrants clinical evaluation in these patients.

## **Molecular targeted therapy in bladder cancer: beyond tyrosine kinases**

Miriam Marqués<sup>1</sup>, Sonia Corral<sup>1</sup>, María Sánchez<sup>1</sup>, Antonio Cebriá<sup>2</sup>, Carmen Blanco<sup>2</sup>, Frank Hilberg<sup>3</sup> and Francisco X. Real<sup>1</sup>

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### **Objectives**

Clinical trials combining VEGFRs or FGFRs inhibitors and chemotherapy have yielded promising results in bladder cancer. The use of kinase inhibitors in cancer treatment has been challenging in part due to Tyr-kinase receptor crosstalk and the existing feedback mechanisms downstream the different signaling cascades. Recent data point to the combined targeting of epigenetic regulators and Tyr-kinases as an alternative for avoiding resistance. Our aim is to pre-clinically assess the use of Nintedanib, an angiokinase inhibitor which targets VEGFR, PDGFR and FGFR, for bladder cancer treatment.

### **Materials & Methods**

Nintedanib sensitivity was tested on a panel of genetically well-characterized human bladder cancer cell lines. Synthetic lethal screens were conducted combining Nintedanib with an anti-tumoral compound library. We also developed a negative screening based on the use of CRISPR-Cas9 knockout libraries directed to epigenomic regulators to identify targeted genes which confer sensitivity to Nintedanib in different human bladder cancer cells.

### **Results**

As single agent, Nintedanib has low cytotoxic activity on bladder cancer cells regardless of their FGFR3 gene status or mRNA levels. In vitro, Nintedanib treatment resulted in transient MAPK and PI3K downregulation. PI3K inhibitor BYL719, but not MEK inhibitor, synergized with Nintedanib, impairing bladder cancer cell growth in vitro and in vivo. Intriguingly, cell proliferation and apoptosis were similar in single- and Nintedanib/BYL719 treated tumours and the mechanism underlying this synergy remains to be identified. We also identified strong synergism between Nintedanib and HDAC inhibitors. The CRISPR-Cas9 screening is ongoing.

### **Conclusions**

Sensitivity to Nintedanib/BYL719 combination is not determined by FGFR3 alterations or expression. Combined targeting of Tyr-kinase/epigenomic regulators might be a feasible therapeutic strategy to prevent resistance. Understanding the mechanisms driven by these compound combinations might reveal response predictive biomarkers.

## **Mitochondrial dysfunctions in bladder cancer: exploring their role as disease markers and potential therapeutic targets.**

Antonella Cormio

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Bladder cancer (BC) is a major cause of mortality worldwide as it currently lacks fully reliable markers of disease outcome and effective molecular targets for therapy.

A large number of molecular markers have been explored in urine, blood and tissue in the search for some that could have reliable prognostic/predictive value or, most important, could represent a potential therapeutic target.

Among novel molecular markers, mitochondrial DNA mutations and some mitochondrial proteins may play an important role in BC prognosis and treatment. Specifically, screening the blood of men at risk for BC by testing those germline mtDNA mutations that seem to predispose to BC hold promise in identifying those harboring this cancer. Blood mtDNA content, urine tumor specific mtDNA mutations all hold promise as non-invasive tools for early detection of BC.

Finally, preliminary data suggest that neoplastic tissue expression of the mitochondrial proteins Lon protease, Mfn2 and TFAM might have prognostic/predictive value and, most important, represent a potential therapeutic target.

A deeper understanding of mitochondrial dysfunctions in BC could therefore provide novel opportunities for targeted therapeutic strategies.



11:34 AM

Amsterdam Airport Schiphol

Evert van de Beekstraat 202, 1118 CP Schiphol, Netherlands

11:34 AM

Schiphol Airport

Thalys Paris Nord

20 min (non-stop)  
Service run by Thalys

11:54 AM

Rotterdam Station

Walk

About 3 min

Use caution - may involve errors or sections not suited for walking

Head south on Stationsplein

29 m

Turn left

4 m

Walk for 87 m

87 m

Take the escalator up to the 1st floor

15 m

Walk for 31 m

31 m

12:05 PM

Rotterdam Centraal Station

D De Akkers

4 min (3 stops) · Platform 3 ·

Information

Za. 6 en zo. 7 okt rijden er 10-19 uur extra treinen op metro E i.v.m. spoorwerkzaamheden bij de NS. Vergeet

niet in- en uit te checken. In de metro geldt een ander tarief dan in de trein.  
"Za. 6 en zo. 7 okt rijden er 10-19 uur extra treinen op metro E i.v.m. spoorwerkzaamheden bij de NS. Vergeet niet in- en uit te checken. In de metro geldt een ander tarief dan in de trein."  
Effective from 10/2/18, 10:00 AM to 10/7/18, 7:00 PM  
Service run by RET

12:09 PM

Rotterdam, Leuvehaven

Walk

About 3 min , 220 m

Use caution - may involve errors or sections not suited for walking

Head south on Schiedamsedijk toward Vasteland/S100

94 m

Turn left onto Vasteland/S100

54 m

Turn left onto Leuvehaven

Take the stairs

Destination will be on the left

67 m

12:12 PM

Inntel Hotels Rotterdam Center

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**Tickets and information**

RET - +31 900 5006010  
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2/2