

## Meeting Report

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# Proceedings of the 3rd Annual Albert Institute for Bladder Cancer Research Symposium

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**Abstract.** The Third Annual Albert Institute Bladder Symposium was held on September 8–10th, 2016, in Denver Colorado. Participants discussed several critical topics in the field of bladder cancer: 1) Best practices for tissue analysis and use to optimize correlative studies, 2) Modeling bladder cancer to facilitate understanding and innovation, 3) Targeted therapies for bladder cancer, 4) Tumor phylogeny in bladder cancer, 5) New Innovations in bladder cancer diagnostics. Our understanding of and approach to treating urothelial carcinoma is undergoing rapid advancement. Preclinical models of bladder cancer have been leveraged to increase our basic and mechanistic understanding of the disease. With the approval of immune checkpoint inhibitors for the treatment of advanced urothelial carcinoma, the treatment approach for these patients has quickly changed. In this light, molecularly-defined subtypes of bladder cancer and appropriate pre-clinical models are now essential to the further advancement and appropriate application of these therapeutic improvements. The optimal collection and processing of clinical urothelial carcinoma tissues samples will also be critical in the development of predictive biomarkers for therapeutic selection. Technological advances in other areas including optimal imaging technologies and micro/nanotechnologies are being applied to bladder cancer, especially in the localized setting, and hold the potential for translational impact in the treatment of bladder cancer patients. Taken together, advances in several basic science and clinical areas are now converging in bladder cancer. These developments hold the promise of shaping and improving the clinical care of those with the disease.

**Keywords:** Urinary Bladder Neoplasms, Molecular Targeted Therapy, Molecular Diagnostic Techniques, models, animal

## INTRODUCTION

The Leo and Anne Albert Institute for Bladder Cancer Care & Research is a non-profit organization

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with the mission to advance knowledge of bladder cancer and the care of those with the disease. The Third Annual Albert Institute Bladder Symposium was held on September 8–10th, 2016, in Denver Colorado. The 40 participants represented a multi-disciplinary group from academic centers in the United States and Europe. The symposium was organized into 5 major focus areas: Best Practices for Tissue Analysis and Use to Optimize Correlative

Studies, Modeling Bladder Cancer to Facilitate Understanding and Innovation, Targeted Therapies for Bladder Cancer, Tumor Phylogeny in Bladder Cancer and New Innovations in Bladder Cancer Diagnostics. Herein, we summarize the proceeding of the symposium (Table 1).

### Best practices for tissue analysis and use to optimize correlative studies

Chair: Donna Hansel, MD, PhD – University of California San Diego

One of the major challenges associated with clinical trial correlative studies is the compliant and judicious use of limited tissue samples obtained as part of the study. Successful completion of these correlative studies requires institutional review board (IRB) approval and proper informed consent, which is complicated in an era of genomic testing and emerging technology applications. Additional layers of oversight include proper custodianship of tissue by pathology departments and biorepositories. Once specimens have been approved for clinical trials use, numerous quality metrics must be applied to obtain high quality material and several approaches have been developed to maximize the use of bladder cancer tissue for these purposes. The session on *Best Practices for Bladder Cancer Use in Clinical Trials* included presentations from Dr. Scott Lucia and Dr. Dara Aisner, University of Colorado at Denver; Dr. Charles Guo, MD Anderson Cancer Center; Dr. Hikmat Al-Ahmadie, Memorial Sloan Kettering Cancer Center; and Dr. Donna Hansel, University of California at San Diego and addressed key issues relevant for bladder cancer tissue use in correlative studies.

Bladder cancer is a diverse disease at the morphological and genomic level, with numerous variants and subtypes. A subset of these variants appear to impact pathological and clinical stage and/or response to chemotherapy [1, 2]. In the majority of cases, however, variant morphology occurs in a background of conventional urothelial carcinoma (UC) and the response of variants to emerging therapies is largely unknown. In light of this, recent discussions have encouraged the enrollment of patients with variant histology into clinical trials, given that alternative therapies for these patients is limited and little evidence has been presented to rationally exclude these patients from clinical trial enrollment. Emerging molecular data have identified unique molecular alterations in a subset of variants, including HER2 amplification in micropapillary UC and E-cadherin deletions in plasmacytoid UC that may be useful in further defining these variants in future studies [3, 4].

The initial steps to obtaining tissue for correlative study use is successful IRB and informed consent approvals. Final tissue distribution for clinical trials use is regulated by pathology departments, however, who are required to properly maintain tissue obtained for diagnostic purposes and serve as a tissue custodian to avoid unnecessary depletion of specimens. Thus, it is recommended that pathologists with bladder-specific knowledge and with awareness of regulatory implications for tissue use be included early in clinical trials design to optimize tissue acquisition and use. As anatomic pathology oversees all tissue distribution from patients and allocates materials to the biorepository, close working relationships among anatomic pathology, the biorepository, the clinical trials office, and the IRB are necessary.

Given that tissue obtained from bladder cancer patients is often limited in the setting of biopsy or

Table 1  
2016 Albert Institute for Bladder Cancer Symposium

Working Group	Faculty Chair(s)
1. Best Practices for Tissue Analysis and Use to Optimize Correlative Studies	<ul style="list-style-type: none"> <li>● Donna Hansel, MD, PhD – University of California San Diego</li> </ul>
2. Modeling Bladder Cancer to Facilitate Understanding and Innovation	<ul style="list-style-type: none"> <li>● Molly Ingersoll, PhD – Institut Pasteur, Paris France</li> </ul>
3. Targeted Therapies for Bladder Cancer	<ul style="list-style-type: none"> <li>● H. Barton Grossman, MD – MD Anderson Cancer Center</li> <li>● Thomas Flaig, MD – University of Colorado</li> <li>● Cathy Mendelsohn, PhD – Columbia University</li> <li>● David DeGraff, PhD – Penn State</li> <li>● Joseph Liao, MD – Stanford University</li> <li>● Thomas Flaig, MD – University of Colorado</li> <li>● David DeGraff, PhD – Penn State</li> <li>● Ashish Kamat, MD – MD Anderson Cancer Center</li> <li>● John A. Taylor, III, MD, MS – University of Kansas</li> </ul>
4. Tumor Phylogeny in Bladder Cancer	
5. New Innovations in Bladder Cancer Diagnostics	
6. Scientific advisory board	

transurethral resection (TUR) specimens and there is an increasing frequency of pT0 disease in cystectomy specimens with the advent of neoadjuvant chemotherapy, approaches to allocate diagnostic and research tissue from each of these specimens is unique. For clinical purposes, diagnostic material is submitted for formalin-fixed paraffin embedded (FFPE) tissue analysis, with biopsy material fully submitted and TUR material initially submitted up to 10 blocks for detection of muscularis propria invasion, with additional blocks submitted as required. In this context, several unique approaches to obtain frozen or FFPE material from these limited specimens were discussed. One such method to obtain research FFPE material for molecular analysis includes saving “trimmings” from blocks as diagnostic slides are prepared. Another approach to obtain frozen material from TUR specimens would be to include frozen section analysis on bladder cancer chips and retain these slides in the permanent diagnostic record. Given the additional workload incurred by these potential protocols, cost-compensation for personnel must be accounted for in clinical trials when considering such approaches.

Several recommendations emerged following discussion with the participants, including the need for close working relationships among relevant working parties, early inclusion of pathology review to streamline and enhance tissue use, appropriate up front cost accounting for all aspects of tissue use in clinical trials, and use of innovative protocols to expand tissue use. One important topic that requires additional discussion is the description of future sample use in patient consent forms to allow for subsequent novel technology applications on patient materials. A second area of discussion focused on whether follow-up of germline genomic abnormalities identified during the course of a clinical trial should be required, as these would require patient sample testing as part of clinical care in a CLIA-certified lab.

### **Modeling bladder cancer to facilitate understanding and innovation**

Chairs: Molly Ingersoll, PhD – Institut Pasteur, Paris France

H. Barton Grossman, MD – MD Anderson Cancer Center

The aim of this session was to understand the roles of bladder cancer models in dissecting mechanisms of tumor development and host response to

established and experimental therapeutics. Modeling bladder cancer has permitted researchers to identify driver mutations, disease subsets, and mechanisms underlying therapeutic response. Although all models have their limitations, inherent in each model is the possibility to innovate through novel application or repurposing of established models.

### **CELL LINES TO MODEL BLADDER CANCER AND ASSESS THERAPEUTIC APPROACHES**

Dr. Grossman provided an introduction to immortalized cell lines. Numerous bladder cancer cell lines have been established that are well characterized and available through cell banks [5]. Normal urothelial cells can only be cultured for short term, however they are not identical to normal urothelium *in situ* and ultimately undergo senescence [6]. Some progress has been made through urothelial cell transformation by SV-40 [7] and h-TERT [8], providing *in vitro* comparators to bladder cancer cell lines. Advantages to using cell lines include ease of maintenance and relatively modest cost. Importantly, however, cell lines do not recapitulate tumor complexity and their behavior is shaped by culture conditions. Furthermore, currently available cell lines may not be representative of bladder cancers, as comparison of the genotype of a large panel of bladder cancer cell lines to bladder cancers revealed that cell lines are more likely to have *TP53* mutations and less likely to have *FGFR3* mutations [5].

Bladder cancer cell lines have provided valuable insight into tumor biology, despite their caveats. A panel of human bladder cancer cell lines with the p53-like molecular phenotype exhibited significantly lower rates of apoptosis when exposed to cisplatin than cell lines with basal or luminal phenotypes, suggesting that p53-like tumors were less likely to respond to neoadjuvant M-VAC due to innate cisplatin resistance [9]. Supporting this conclusion, bladder cancer patients with the p53-like molecular phenotype exhibit a decreased response to chemotherapy with M-VAC [9]. A study of a Smac mimetic in ten bladder cancer cell lines found that it increases apoptosis only in a subset of cell lines exposed to cisplatin and gemcitabine [10] highlighting the need for multiple cell lines, particularly when assessing response to potentially useful therapy.

Finally, Dr. Grossman further suggested taking a lesson from clinical investigators. Often researchers focusing on a specific cancer will only use cell lines

derived from that cancer. The so-called basket design enrolls patients not with a particular tumor but with a particular gene mutation [11]. In this way, we may advance our understanding of bladder cancer and therapeutics, but also make inroads into the treatment of related malignancies.

### **ORGANOID MODELS TO EVALUATE THERAPEUTIC APPROACHES**

Dr. Michael Shen, Columbia University, introduced the concept of patient-derived organoid tumor models. Patient-derived organoid models of bladder cancer have several major advantages for tumor biology and drug response studies, including ease of culture, handling, and cryopreservation; potential to model individual patient tumors; the ability to model rare subtypes and genomic alterations not represented in cell lines; and the potential for an organoid biobank representative of the full spectrum of human bladder cancer. Biobanks would provide the possibility for medium-scale drug screens and functional analyses or the generation of xenografts from established organoid lines.

Dr. Shen also highlighted the potential disadvantages of organoid models, most notably the lack of stromal microenvironment and immune system; inefficiency in establishing organoid lines; and that no definitive evidence exists demonstrating that drug response in organoid culture is translatable to patients. Despite these caveats, organoid culture shows considerable promise. In the field of prostate cancer, researchers have recapitulated the diversity of this disease and detailed methods to establish organoid cultures are available [12, 13]. Given the complex nature of bladder cancer, it is likely that organoid culture will provide an important platform to dissect diversity among patients and uncover mechanisms of therapeutic action on tumors.

### **ANIMAL MODELS TO UNDERSTAND BLADDER CANCER, HOST RESPONSE, AND THERAPEUTIC IMPACT**

Moving away from culture systems, Drs. Xue-Ru Wu, NYU School of Medicine, and Molly Ingersoll, Institut Pasteur, discussed the use of mouse bladder cancer models to investigate tumor development and mechanisms of immunity following immunotherapy, respectively [14].

Dr. Wu focused on the contribution of genetically engineered mouse models (GEMMs), which

have been instrumental in understanding the initiation and progression of bladder cancer over the past two decades [15, 16]. Remarkably, despite their prevalence, mutations in the RTK-RAS-PI3K pathway likely do not constitute driver mutations for disease [17–20]. Surprisingly, single mutations in p53/RB1 tumor suppressor pathways also appear to lack tumorigenicity [21, 22]. Urothelium-specific ablation of p53 and RB1 or PTEN does, however, lead to urothelial abnormality and tumor development [22, 23]. Interestingly, these studies and others suggest that, the RTK-RAS-PI3K pathway and the p53/RB1 pathways may intersect, leading to muscle invasive disease. This is relevant for human disease as whole-genome analyses showed that both pathways are altered in over 70% of human muscle-invasive bladder cancers [24].

Dr. Ingersoll described recent advances in understanding mechanisms of immunotherapy for bladder cancer. One of the most common approaches to study immunotherapy is orthotopic implantation of the bladder cancer cell line MB49 [25]. There are limitations to this approach, including inconsistent tumor implantation and a lack of tumor heterogeneity. Despite this, orthotopic models have advanced our understanding of mechanisms of BCG-induced tumor immunity. For example, BCG instillation induces cellular infiltration and cytokine expression [26], which can be accelerated by vaccinating mice with BCG prior to therapeutic intravesical instillation [27]. In addition, pre-existing immunity to BCG induces superior protection following tumor challenge and treatment than treatment alone [27]. Notably, this experimental observation was born out in patients, in which those who were PPD-positive at the start of therapy had improved recurrence-free survival compared to those who were PPD-negative at the onset of therapy [27]. Mouse models will likely play an even more important role in the years to come, by permitting testing of new therapeutics in heterogeneous tumors and intact immunity.

### **MINING GENOMICS TO CLASSIFY BLADDER CANCER AND IDENTIFY THERAPEUTIC TARGETS**

Dr. Lerner, Baylor College of Medicine, discussed the role of genomic analysis in classifying bladder cancer and identifying therapeutic targets. The Cancer Genome Atlas project (TCGA) has reported on the molecular characterization of muscle-invasive

urothelial bladder cancer [24], and several molecular bladder classification systems have been proposed that share the luminal/basal features discovered in breast cancer [9]. Notably, a more recent analysis has uncovered molecular subtypes in nonmuscle invasive bladder cancer, similar to those observed in other cohorts, but also containing distinct molecular signatures with respect to carcinoma *in situ* [28, 29]. SWOG has an ongoing clinical trial to validate predictive biomarkers in bladder cancer. Continued investigation into the genomics of bladder cancer offers the promise of defining novel prognostic and predictive biomarkers and therapeutic targets.

### Targeted therapies for bladder cancer

Chair: Thomas Flaig, MD – University of Colorado

At this year's symposium, a dedication session on *Targeted Therapies for Bladder Cancer* was introduced. Drs. Matthew Milowsky, UNC-Chapel Hill, and Ashish Kamat, MD Anderson, provided a summary of the current landscape for the treatment of advanced bladder cancer and non-muscle invasive bladder cancer, respectively. In this session, pharmaceutical representatives were invited to join the academic participants for this interactive exchange. It was emphasized that the treatment of advanced bladder cancer has largely relied on the use of platinum-based chemotherapy combinations and the treatment for non-muscle invasive bladder cancer centers around BCG immunotherapy. Dr. Noah Hahn, Johns Hopkins University School of Medicine, additionally detailed a multitude of distinct clinical states in UC (*e.g.*, BCG-relapsed, non-muscle invasive, neoadjuvant, post platinum, etc.) and highlighted the many opportunities for additional drug development, including in new clinical settings (*e.g.*, in the post checkpoint inhibitor state). Dr. Jonathan Rosenberg, Memorial Sloan Kettering, outlined the bladder cancer findings within the TCGA and how this and other related efforts have now transitioned bladder cancer into the realm of molecularly-characterized oncologic diseases [24]. Several molecular classification schemes to categorize bladder cancer now provide further insight into the bladder cancer subtypes, which may aid in therapeutic selection. After a very long dependence on platinum-based chemotherapy for advanced bladder cancer, these molecular insights and classifications provide a new road map for the development of a targeted therapy approach in advanced disease.

### IMMUNE CHECKPOINT INHIBITION IN BLADDER CANCER

With the approval of atezolizumab in May of 2016, a new era of immunotherapy for bladder cancer has begun. Drs. Elizabeth Plimack, Fox Chase Cancer Center, and Matthew Galsky, Tisch Cancer Institute/Mount Sinai School of Medicine, detailed recent advances in immune checkpoint inhibition, targeting the PD-1 and PD-L1 interactions. Atezolizumab is a programmed death-ligand 1 (PD-L1) blocking antibody approved for the treatment of locally advanced or metastatic UC in patients with disease progression during or following platinum-containing chemotherapy. In a phase 2 trial of patients with progressive disease after previous platinum-based chemotherapy, 310 received atezolizumab treatment. The objective response rate was 15% overall, with improved response rates observed in those with increased PD-L1 expression status on infiltrating immune cells. Notably, the majority of responding patients (84% of 45) continued to respond with a median follow-up of 11.7 months, suggesting a significant durability, not seen with traditional chemotherapy [30]. Data on the use of the PD1 inhibitors nivolumab, pembrolizumab, durvalumab and the CTLA-4 inhibitor tremelimumab in UC is also developing. Trials are currently underway exploring the use of PD-1/PD-L1 and CTLA-4 inhibitors in bladder cancer, encouraged by the success of these combinations in metastatic melanoma patients. The merits of upfront immune checkpoint treatment, as opposed to its use as second-line therapy as is indicated under the current label for Atezolizumab, is also being studied. The long-standing role of BCG immunotherapy in non-muscle invasive bladder cancer was acknowledged [31]. As such, investigations into the integration of checkpoint inhibitors in concert with BCG in earlier stages of bladder cancer are underway and open up new treatment possibilities to those with early-stage bladder cancer as well.

### FIBROBLAST GROWTH FACTOR RECEPTOR IN BLADDER CANCER

The alteration of the fibroblast growth factor receptor (FRFG) and the related pathway has been long known in the pathophysiology of bladder cancer. FGFR3 is known to be dysregulated in some forms of bladder cancer with the identification of the FGFR3-TACC3 fusion in both *in vitro* mod-

els and clinical samples [32]. Several agents are now emerging which target this pathway, including B-701, JNJ-42756493, and PRN1371. Dr. Arlene Siefker-Radtke, MD Anderson, detailed the background of FGFR dysregulation in bladder cancer and broad therapeutic potential in this area. Clinical investigations of the FGFR 1-4 inhibitor Erdafitinib (JNJ-42756493) which largely functions as a pan-FGFR inhibitor has shown initial activity in a small number of UC patients. A Phase 2 study: Two-arm Multicenter, Open-Label Study to Determine the Efficacy and the Safety of Two Different Dose Regimens of a pan-FGFR Tyrosine Kinase Inhibitor JNJ-42756493 in Subjects with Metastatic or Surgically Unresectable Urothelial Cancer with FGFR Genomic Alterations (NCT02365597), is now underway to better characterize the activity of this agent. B-701 is an anti-FGFR3 antibody, which is also being tested in UC. A clinical study is currently underway to investigate this agent: A phase 1b/2, Randomized, Double-Blind, Placebo-Controlled, Multicenter, Parallel-Group Study of B-701 Plus Docetaxel Versus Placebo Plus Docetaxel in the Treatment of Locally Advanced or Metastatic Urothelial Cell Carcinoma in Subjects Who Have Relapsed After, or Are Refractory to Standard Therapy (NCT02401542). PRN1371 is a pan-FGFR inhibitor with *in vivo* efficacy in the UC RT4/FGFR3:TACC3 model. A phase I trial is underway to investigate this agent, which includes patients with advanced urothelial carcinoma: A Phase I Open-Label, Multicenter, Dose-Escalation Study of PRN1371, a FGFR 1-4 Kinase Inhibitor, in Adult Patients with Advanced Solid Tumors, Followed by an Expansion Cohort in Patients with FGFR 1, 2, 3, or 4 Genetic Alterations (NCT02608125). There is an active effort to characterize the efficacy of FGFR inhibition in bladder cancer and ideally, to define the patient population most like to respond.

## OTHER THERAPEUTIC DEVELOPMENTS IN BLADDER CANCER

Dr. Primo Lara, UC Davis Comprehensive Cancer Center, described recent investigations with eribulin for UC. Eribulin was discovered in 1986 from the sponge *Halichondria okadai*, and the Halichondrin analog E7389 (eribulin) was subsequently developed. This compound is well tolerated in those with mild to moderate renal insufficiency [33]. Positive responses

in urothelial carcinoma patients were observed in the first-in-human trial, and a California Cancer Consortium trial, Phase II trial of eribulin in urothelial cancer patients with renal impairment (PHII-75), was subsequently performed, with an overall response rate of 37%. Additional clinical investigations of this agent in advanced UC are planned.

An emerging area of interest in UC therapeutics is the use of Antibody-Drug Conjugates (ADC). This approach combines the targeting specificity of the antibody coupled to a small payload of a potent cytotoxic agent in order to have a targeted delivery of the chemotherapy based on tumor surface markers. Two ADCs have been licensed and are in current clinical use in oncology: T-DM1 in breast cancer and brentuximab vedotin in classical Hodgkin lymphoma and systemic anaplastic large cell lymphoma. Enfortumab vedotin in an ADC targeting Nectin-4, which is highly expressed in bladder cancer, using the Microtubule-disrupting agent monomethylauristatin-E (MMAE) [34]. Activity is seen UC patients, including those with liver metastatic disease and post-checkpoint inhibition, with additional studies planned.

## Tumor phylogeny in bladder cancer

Chairs: Cathy Mendelsohn, PhD – Columbia University

David DeGraff, PhD – Penn State

UC is the most common type of bladder cancer in the United States, affecting males 3-4 times more frequently than females. Major risk factors for UC include smoking and exposure to environmental toxins. UC arises from the urothelium, a slow cycling stratified epithelial barrier that lines the urinary tract stretching from the renal pelvis to the bladder neck [35, 36]. The urothelium consists of at least 4 known cell types that can be distinguished based on morphology and expression of combinatorial markers [37]. Superficial cells lining the luminal layer are a binucleated and polyploid cell types that produce the plaque that serves as the urothelial barrier. Intermediate cells, which can be mono or binucleated are a small population of self-renewing superficial cell that give rise to superficial “umbrella” cells during homeostasis and after acute damage reviewed in: [38, 39]. The basal cell population makes up most of the urothelium, and can be subdivided into K5-expressing basal cells that are negative for Krt14 and reside in the basal and suprabasal

layers, and Krt14-basal cells, a populations that resides exclusively in the basal layer expressing both Krt14 and Krt5 [40]. There is considerable controversy regarding the identity of progenitor populations in the urothelium. Fate mapping studies using Cre-Lox recombination to indelibly label urothelial sub-populations has produced conflicting results. Studies from the Mendelsohn lab used tamoxifen-inducible Upk3a and Krt5 lines to indelibly label intermediate and basal cells, respectively. These studies suggest that intermediate cells are a self-renewing population that produce umbrella cells during development, homeostasis and in response to acute injury from cyclophosphamide, while basal cells are self-renewing but generally unipotent [37]. On the other hand, work from the Klinakis groups using a tamoxifen-inducible Krt14 line to label Krt14-basal cells suggests that basal cells can produce umbrella cells during development, homeostasis and in response to severe injury [41]. These conflicting findings may reflect differences in lineage-tracing models, or more interestingly, may reflect changes in behavior of urothelial cells in response to acute or chronic injury.

UC is currently characterized based on histopathology, into classes that display distinct morphological features and clinical behaviors. Carcinoma *in situ* and muscle invasive tumors are often poorly differentiated, and thus classified as high grade, whereas non-muscle invasive UC often exhibits well differentiated, low grade papillary structures [42]. There are also a number of variant histologies such as micropapillary and sarcomatoid UC that are associated with adverse clinical outcomes. The differences in morphology and clinical behavior of UC has led to the suggestion that different types of tumors arise from different urothelial cell types of origin. Supporting this, lineage studies in mouse models of carcinogenesis suggest that intermediate cells give rise primarily to papillary carcinomas [43]. On the other hand, basal cells have the capacity to form several tumor types including carcinoma *in situ* (CIS), muscle invasive (MI) UC, and squamous lesions, suggesting that factors other than cell type of origin are likely to be critical determinants of tumor morphology and clinical behavior [41, 43–45]. However, the identity of these potential mechanisms, as well as the way they may operate in a cell subtype-specific manner in UC during cellular transformation and progression remains unresolved.

During the past few years, large-scale molecular characterization of tumors isolated from patients has

revolutionized the way we think about cancer. Exome sequencing and gene expression profiling reveals that lesions can be classified into tumor subtypes based on the mutational landscape and gene expression profiles [24, 46–54]. Analysis of muscle-invasive UC (MIUC) lesions reveals that they can be subdivided into 3 or more molecular subtypes depending on the study, including basal/SCC-like tumors and luminal-like tumors, which have distinct morphologies and patterns of gene expression, analogous to the subtypes observed in breast cancer [9]. These observations suggest that distinct features of UC lesions are likely to be governed by genomic changes. Hence, while cell type of origin may influence the types of tumors that arise in some instances, genomic alterations and gene expression changes are likely to be the “master regulators” of tumor subtypes in UC.

Despite the progress that has been made in the field, there are many questions that need to be addressed. There are accumulating data points related to the mutations that are acquired in UC, however there is incomplete understanding about the timing of particular genomic and gene expression alterations with respect to invasion, transformation, and metastasis. In addition, several studies suggest that tumors can undergo phenotypic shifts during metastasis. Whether this phenomenon is linked to epigenetic changes or acquisition of particular mutations is unknown. The increased tendency of males to develop UC compared to females is not well understood. The male and female genomes differ in a number of ways; males have a Y chromosome with a relatively small number of genes not present on the X chromosome. Notably, some of these genes regulate sexual development and hormonal signaling that may promote UC in males. On the other hand, genes on the X chromosome that escape inactivation may exert a suppressive effect with respect to tumor formation or progression in females. Tracing the phylogeny of UC, from the urothelium to metastases using genomics, gene expression analysis and mouse models will be important for understanding the sequence of events that lead to cancer, and will identify new potential targets for chemotherapy.

## INTRA-TUMOR HETEROGENEITY

Histopathological analysis of CIS, MIUC, and papillary lesions often reveals a fair degree of intra-tumor heterogeneity, including domains with variant histology, which recent studies suggest may have an

important impact on clinical behavior. For example, micropapillary morphology in non-muscle invasive UC predicts BCG failure, whereas squamous morphology may be predictive of differential response to neoadjuvant chemotherapy. In the context of molecular subtypes, basal-like lesions often contain pockets of squamous differentiation, which is considered a poor prognostic indicator. Dr. Warrick, Penn State, reviewed his efforts to study the relationship between expression subtype and variant histology using a retrospective cohort of over 300 consecutive cystectomy cases. These studies revealed that micropapillary and squamous variant patterns are often mutually exclusive supporting the idea that domains with variant histology have an important impact on tumor progression and behavior. Dr. Warrick's group investigated the distribution of the micropapillary and squamous histological variants in different tumor subtypes, using FOXA1 and KRT14, respectively, to distinguish lesions with luminal-like molecular subtype from lesions from basal-like subtype. These studies reveal that micropapillary domains tend to be associated with luminal like subtypes, while squamous histological variants tend to be associated with basal-like tumors. Interestingly, KRT14 was up regulated in domains with squamous histology. The findings support the idea that domains with variant histology may have an important impact on the behavior of basal and luminal lesions.

### MOLECULAR SUBTYPE STABILITY IN THE METASTATIC PROGRESSION

Dr. Gottfried Sjodahl, Lund University Sweden, discussed his recent investigation of molecular subtype stability in the metastatic progression in UC. In these studies, his group analyzed cells in tumors and local lymph node metastases, comparing immunohistochemistry, gene expression profiles, and mutations. Sixty-nine pairs were analyzed and for 58 of these pairs, there was no evidence of phenotypic shift between tumor cells between the two locations. Most of the tumors that did show phenotypic switching, went from a basal/SCC-like phenotype in the bladder tumor to luminal-subtype, uro-subtype or genomically unstable subtype in the matched lymph node metastases. Upon re-examination of full pathological sections, at least some degree of subtype heterogeneity was present in most trans urethral bladder tumor specimens, but heterogeneity could only explain a few of the phenotypic switches observed. In con-

clusion, Dr. Sjodahl found advanced UC samples and their corresponding matched lymph-node metastases to be more stable than expected. While genomic analysis still needs to be conducted, most cases did not exhibit a subtype shift, but pairs that switched lost the basal/SCC-like phenotype in the transition to node-metastasis, which may suggest that interaction of tumor cells with the malignant microenvironment within the bladder wall plays an important role in the maintenance of a basal/SCC-like state.

### SEX DISPARITIES IN UC INCIDENCE AND MORTALITY

Dr. Sean Li, Boston's Children Hospital and Harvard Medical School, discussed the latest studies aimed at understanding sex disparities in UC incidence and mortality. Men are much more likely than women to develop cancers arising in organs with non-reproductive functions. Specifically, UC is 3–5 times more common in men. The increased incidence in UC in men was originally attributed to increased frequency of tobacco use and environmental exposure to carcinogens. However, recent studies indicate that the sex disparity in UC and other cancers is linked to intrinsic differences between sexes [55–58]. Using the complete sex reversal mouse model to interrogate relative contributions of the sex chromosomes (XX in females vs XY in males) and gonadal hormones (testis vs ovary), Dr. Li has investigated whether genes encoded on the X-chromosome, of which there are two copies in females, and one copy in males, may have tumor suppressor activity rendering females more resistant to bladder cancer. Indeed, regardless of the gonadal type, mice with two copies of the X-chromosome are much less likely to develop bladder cancer. Lysine Demethylase 6A (*KDM6A*) is a X-chromosome-linked epigenetic regulator. It is expressed in significantly higher levels among females than males. By conditionally deleting *KDM6A* from the mouse bladder urothelium, Dr. Li observed that *Kdm6a* conditional knockout mice are significantly more susceptible to UC. Moreover, human *KDM6A* is frequently mutated in human UC; and mutations of *KDM6A* predicted poor outcome of disease-free survival in female bladder cancer patients. Collectively these findings demonstrate that the X-chromosome copy number difference is correlated with the sex disparity in UC, and further suggest that female-biased expression of the X-linked tissue-specific tumor suppressors, e.g., *KDM6A* in UC, underlies the sex difference observed.



## ENDOPHILIN A1 IN UROTHELIAL HOMEOSTASIS

Dr. Rosalyn Adam, Boston Children's Hospital and Harvard Medical School, outlined the role of endophilin A1 in urothelial homeostasis and in UC where its expression is lost during tumor progression. Endophilin A1 is a cytoplasmic protein and a component of the multi-protein complex that promotes endocytosis of receptor tyrosine kinases, including the EGFR and c-MET, to down regulate their activity [59, 60]. Investigations using RT4 cells and tumor xenografts to identify the consequences of endophilin A1 silencing on tumor progression have been performed. This work demonstrated that knock-down of endophilin A1 enhanced EGFR and c-MET signaling in RT4 cells, and was associated with an increase in survival, proliferation, and growth of tumor xenografts [3]. Additional studies suggested that loss of endophilin A1 expression arose, in part, from methylation-induced silencing. Dr. Adam hypothesized that loss of endophilin A1 may predict increased sensitivity to EGFR- and c-MET-targeted tyrosine kinase inhibitors.

## THREE-DIMENSIONAL ORGANOTYPIC CULTURE

A major barrier to understanding cancer growth, invasion, and metastasis is that these processes occur deep inside the body over years to decades. This inaccessibility limits our ability to screen for cancer in patients and limits our ability to understand the cellular and molecular basis of disease processes, thereby slowing the progress of new therapies for patients. The breast cancer research field has led the way in development of innovative "3D culture" experiments that enable researchers to study more realistic models of cancer progression *in vitro* [61]. Dr. Andrew Ewald, Johns Hopkins University School of Medicine, described how these 3D culture techniques could be used to test the importance of the tumor microenvironment [62], to isolate the effect of specific cancer genes [63], and to discover targetable molecular programs driving metastasis [64]. Interesting parallels emerged in the biology of breast and UC in terms of the heterogeneity in luminal and basal differentiation among different cancer cells populations both within the same tumors and between tumors [64]. Ongoing work seeks to determine whether the cancer cell (or cluster) that seeds new metastases is representative of the average cancer cell in the

primary tumor or whether it could be preferentially accomplished by cells in a specific (e.g., basal) differentiation state [65, 66].

## UROTHELIAL PROGENITOR CELLS AND TUMORIGENESIS

Dr. Mendelsohn provided an overview of basic aspects of urothelial cell biology, and the context-dependent role that various urothelial progenitor cells play in maintaining the barrier integrity of the urothelium and how urothelial progenitor populations become altered as a result of chronic inflammation in response to drugs and carcinogens. Previous findings from the Mendelsohn lab suggest that in the healthy bladder, intermediate cells are self-renewing superficial cell progenitors during homeostasis and regeneration after acute injury from urinary tract infection [37]. Interestingly, however, basal cells, that are largely unipotent in the healthy urothelium, acquire progenitor potential following chronic injury induced by treatment with cyclophosphamide (CPP), which causes hemorrhagic cystitis in humans and animal models and is sometimes used as a model of urothelial injury and regeneration in rodents [67, 68]. CPP-induced bladder damage is caused by acrolein, a carcinogenic metabolite of CPP that is a component of cigarette smoke [69]. Ongoing work from Dr. Mendelsohn is examining the effects of short-term exposure of mice to BBN, a chemical carcinogen that induces bladder cancer, which also contains acrolein. This work has shown a pattern of tissue injury following 1 month of BBN treatment that was very similar to that as observed after CPP treatment, including edema, granulation tissue and inflammation. These observations suggest that chronic inflammation may promote carcinogenesis in part, by altering the potential of basal cells. Basal cells contribute to several types of lesions, including CIS, MIUC, and SCC. The observation that severe or chronic urothelial damage can alter the progenitor properties of basal cells is an important finding that will help set the framework for future studies investigating the events that underlie tumorigenesis.

### New Innovations in bladder cancer diagnostics

Chair: Joseph Liao, MD – Stanford University

This session highlighted recent advances in bladder cancer diagnostics, particularly for high-risk

non-muscle invasive bladder cancer (NMIBC). Areas of focus included emerging optical imaging technologies, multiplex urinary biomarker panels, and *in vitro* diagnostics based on micro/nanotechnologies. As there are clear unmet needs in current diagnostics for NMIBC, new endoscopic technologies and urine-based diagnostics are complementary and synergistic. Emblematic of the interdisciplinary focus, session members included bladder cancer surgeons, clinician-scientists, and biomedical engineers experienced in translational research.

### **LIGHT-BASED TOOLS FOR BLADDER CANCER DETECTION AND THERAPY**

Dr. Liao highlighted the unmet needs in optical diagnosis of bladder cancer in which white light cystoscopy (WLC) and transurethral resection (TUR) are the standard. Well-recognized shortcomings include tumor enumeration, tissue characterization, flat lesion differentiation, and cancer staging, which in turn can lead to missed lesions, inadequate resection, and overall negative impact on cancer-specific outcomes. These clinical needs, in parallel with advances in imaging sciences, have motivated development of new imaging technologies that include wide field fluorescence and high resolution optical biopsy [70–73]. Dr. Audrey Bowden, Stanford University, further described her efforts to advance optical coherence tomography (OCT) for bladder cancer. OCT complements WLC through improved spatial resolution ( $<10\ \mu\text{m}$ ), sub-surface imaging to enable local cancer staging, and an established clinical utility in other disciplines (e.g., ophthalmology). A promising prototype has recently been introduced, which is capable of 3-D volumetric imaging and is the smallest (1.3 mm outer diameter) OCT endoscope with the fastest image processing capability reported to date [74]. In addition to instrumentation design, other novel approaches in this area are emerging including an imaging mosaicking and 3-D bladder mapping algorithm [75, 76], and a distensible bladder phantom [77, 78], with wide applicability for validation of other bladder imaging technologies.

Bridging optical diagnosis and therapy, Dr. Piyush Agarwal, National Cancer Institute, introduced photodynamic therapy (PDT) as a potential endoscopic therapeutic modality for NMIBC. PDT combines fluorescently labeled molecular targeting agents with laser excitation [79], thereby achieving greater precision in tumor ablation and reduced non-specific

injury compared to traditional photodynamic therapy (PDT). The strategy takes advantage of the expanding panel of therapeutic monoclonal antibodies that target molecules overexpressed by cancer cells. The therapeutic antibodies may also be tagged for optical molecular imaging, as exemplified by targeted imaging of CD47, a cell surface protein overexpressed by bladder cancer cells, in intact radical cystectomy specimens with promising diagnostic accuracy [80]. Another promising target is epidermal growth factor (EGFR), which is overexpressed in up to 74% of bladder cancer, particularly in basal phenotype [81]. Dr. Agarwal described his group's ongoing work on targeting EGFR in cultured bladder cancer cells and xenograft models using a combination of anti-EGFR conjugated with a near-infrared dye and a compatible laser source. A Phase I clinical trial is currently in the planning stage.

### **EMERGING URINARY BIOMARKERS AND *IN VITRO* DIAGNOSTIC PLATFORMS FOR BLADDER CANCER**

Dr. Jay Raman, Penn State, defined the rationale and need for better diagnostics for early-stage bladder cancer, given the suboptimal performance of current urine tests to replace surveillance cystoscopy even in patients with low-risk disease. In addition to the associated morbidity, the cost of cancer surveillance is a significant source of healthcare expenditure related to bladder cancer [82]. Shortcomings of standard urine cytology include poor sensitivity (12–48%), particularly for low-grade cancer, and the subjective and laborious nature of assay interpretation that requires trained cytopathologists [83]. The goals of new urine-based diagnostics are twofold: 1) cancer detection in a screening population (e.g., hematuria work-up), which requires a low false positive rate; and 2) cancer surveillance in patients with history of bladder cancer, which requires high sensitivity and negative predictive value. Many of the approved single biomarker immunoassays to date have not met these criteria, and hence the low clinical adoption rate. Improved understanding of cancer molecular pathways and wide availability of nucleic acid sequencing have rapidly expanded the pool of potential cancer biomarkers. Therefore, recent efforts in cancer diagnostics have focused on development of multi-gene panels to account for cancer heterogeneity, and quantitative reverse transcriptase PCR amplification as the detection strategy. Dr. Raman highlighted a

recently completed multicenter trial to validate a 5 mRNA panel which consisted of *MDK*, *HOXA13*, *CDC2*, *IGFBP5*, and *CXCR2* [84] for bladder cancer surveillance. The panel achieved a promising overall sensitivity of 92% and a 97% negative predictive value, which offers the possibility to reduce cystoscopy burden on low-risk patients.

In addition to biomarker panel development and ongoing efforts of biomarker discovery based on next generation sequencing technologies, Dr. Liao underscored the emergence of molecular diagnostic platforms based on microfluidics and nanotechnology that offer the potentials of ultra-sensitivity, integrated sample preparation, and near-patient testing. Significant efforts are underway to translate these ‘lab-on-a-chip’ technologies for cancer diagnostics, including bladder cancer. An integrated microfluidics cartridge capable of multiplex qPCR (Xpert®, Cepheid) of bladder cancer urinary markers has recently been approved in Europe. The platform is capable of ‘sample-in, answer-out’ within 90 minutes. Dr. Jeff T.H. Wang, Johns Hopkins University School of Medicine and Engineering, provided an exciting glimpse of various microfluidics and biosensor platforms at pre-clinical or early clinical validation stage for early cancer detection [85–87]. Examples presented include a fully integrated sample preparation and PCR on a biochip for ultrasensitive detection of methylated DNA from clinical samples [88]. It is anticipated that the platform can be repurposed for detection of bladder cancer urinary biomarkers.

In conclusion, the molecular characterization of bladder cancer has advanced substantially in recent years. To continue this progress, it is critically important that best practices for tissue collection and analysis be employed and standardized where possible. Robust and novel preclinical models of bladder cancer are needed and this is especially true of immune-competent models due to the importance and clinical relevance of immunotherapy in bladder cancer. The approval of an immune checkpoint inhibitor for advanced bladder cancer is a notable milestone on which further immune therapy integration may be built. In addition, there are many other drug development opportunities in bladder cancer with targeted agents including FGFR, additional immunotherapies, antibody drug conjugates and cytotoxic agents. The study of tumor phylogeny in bladder cancer continues to progress in the context of advances in the molecular characterization of bladder cancer. Technological advances are being studied and applied in

bladder cancer, in the areas of light-based tools and *in vitro* urine-based assays, with the goal of improved detection and treatment of early stage bladder cancer.

## REFERENCES

- [1] Dayyani F, Czerniak BA, Sircar K, et al. Plasmacytoid urothelial carcinoma, a chemosensitive cancer with poor prognosis, and peritoneal carcinomatosis. *J Urol* 2013;189:1656-61.
- [2] Kamat AM, Dinney CP, Gee JR, et al. Micropapillary bladder cancer: A review of the University of Texas M.D. Anderson Cancer Center experience with 100 consecutive patients. *Cancer* 2007;110:62-7.
- [3] Ching CB, Amin MB, Tubbs RR, et al. HER2 gene amplification occurs frequently in the micropapillary variant of urothelial carcinoma: Analysis by dual-color *in situ* hybridization. *Mod Pathol* 2011;24:1111-9.
- [4] Al-Ahmadie HA, Iyer G, Lee BH, et al. Frequent somatic CDH1 loss-of-function mutations in plasmacytoid variant bladder cancer. *Nat Genet* 2016;48:356-8.
- [5] Earl J, Rico D, Carrillo-de-Santa-Pau E, et al. The UBC-40 Urothelial Bladder Cancer cell line index: A genomic resource for functional studies. *BMC Genomics* 2015;16:403.
- [6] Liebert M, Wedemeyer G, Chang JH, et al. Comparison of antigen expression on normal urothelial cells in tissue section and tissue culture. *J Urol* 1990;144:1288-92.
- [7] Christian BJ, Loretz LJ, Oberley TD, Reznikoff CA. Characterization of human uroepithelial cells immortalized *in vitro* by simian virus 40. *Cancer Res* 1987;47:6066-73.
- [8] Chapman EJ, Hurst CD, Pitt E, Chambers P, Aveyard JS, Knowles MA. Expression of hTERT immortalises normal human urothelial cells without inactivation of the p16/Rb pathway. *Oncogene* 2006;25:5037-45.
- [9] Choi W, Porten S, Kim S, et al. Identification of distinct basal and luminal subtypes of muscle-invasive bladder cancer with different sensitivities to frontline chemotherapy. *Cancer Cell* 2014;25:152-65.
- [10] Lee EK, Jinesh GG, Laing NM, Choi W, McConkey DJ, Kamat AM. A Smac mimetic augments the response of urothelial cancer cells to gemcitabine and cisplatin. *Cancer Biol Ther* 2013;14:812-22.
- [11] Simon R, Geyer S, Subramanian J, Roychowdhury S. The Bayesian basket design for genomic variant-driven phase II trials. *Semin Oncol* 2016;43:13-8.
- [12] Gao D, Vela I, Sboner A, et al. Organoid cultures derived from patients with advanced prostate cancer. *Cell* 2014;159:176-87.
- [13] Drost J, Karthaus WR, Gao D, et al. Organoid culture systems for prostate epithelial and cancer tissue. *Nat Protoc* 2016;11:347-58.
- [14] Kobayashi T, Owczarek TB, McKiernan JM, Abate-Shen C. Modelling bladder cancer in mice: Opportunities and challenges. *Nat Rev Cancer* 2015;15:42-54.
- [15] Wu XR. Urothelial tumorigenesis: A tale of divergent pathways. *Nature reviews Cancer* 2005;5:713-25.
- [16] Wu XR. Biology of urothelial tumorigenesis: Insights from genetically engineered mice. *Cancer Metastasis Rev* 2009;28:281-90.

- [17] Ahmad I, Singh LB, Foth M, et al. K-Ras and beta-catenin mutations cooperate with Fgfr3 mutations in mice to promote tumorigenesis in the skin and lung, but not in the bladder. *Dis Model Mech* 2011;4:548-55.
- [18] Chen X, Mitsutake N, LaPerle K, et al. Endogenous expression of Hras(G12V) induces developmental defects and neoplasms with copy number imbalances of the oncogene. *Proc Natl Acad Sci U S A* 2009;106:7979-84.
- [19] Zhou H, Wang X, Mo L, et al. Role of isoenzyme M2 of pyruvate kinase in urothelial tumorigenesis. *Oncotarget* 2016;7:23947-60.
- [20] Wu XR, Mendelsohn C, DeGraff DJ. Tumorigenicity of RTK/RAS in urothelium. *Oncoscience* 2015;2:739-40.
- [21] Gao J, Huang HY, Pak J, et al. p53 deficiency provokes urothelial proliferation and synergizes with activated Ha-ras in promoting urothelial tumorigenesis. *Oncogene* 2004;23:687-96.
- [22] He F, Mo L, Zheng XY, et al. Deficiency of pRb family proteins and p53 in invasive urothelial tumorigenesis. *Cancer Res* 2009;69:9413-21.
- [23] Puzio-Kuter AM, Castillo-Martin M, Kinkade CW, et al. Inactivation of p53 and Pten promotes invasive bladder cancer. *Genes & Development* 2009;23:675-80.
- [24] Cancer Genome Atlas Research N. Comprehensive molecular characterization of urothelial bladder carcinoma. *Nature* 2014;507:315-22.
- [25] Chan E, Patel A, Heston W, Larchian W. Mouse orthotopic models for bladder cancer research. *BJU Int* 2009;104:1286-91.
- [26] Zdimerova H, Albert ML, Ingersoll MA. Harnessing the Host Immune Response to Infection – BCG Immunotherapy for Bladder Cancer. In: Shurin MR, Thanavala Y, Ismail N, eds. *Infection and Cancer: Bi-Directional Interactions*: Springer International Publishing; 2015:387-403.
- [27] Biot C, Rentsch CA, Gsponer JR, et al. Preexisting BCG-Specific T Cells Improve Intravesical Immunotherapy for Bladder Cancer. *Science Translational Medicine* 2012;4:137ra72.
- [28] Lerner SP, Robertson AG. Molecular Subtypes of Non-muscle Invasive Bladder Cancer. *Cancer Cell* 2016;30:1-3.
- [29] Hedegaard J, Lamy P, Nordentoft I, et al. Comprehensive Transcriptional Analysis of Early-Stage Urothelial Carcinoma. *Cancer Cell* 2016;30:27-42.
- [30] Rosenberg JE, Hoffman-Censits J, Powles T, et al. Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: A single-arm, multicentre, phase 2 trial. *Lancet* 2016;387:1909-20.
- [31] Morales A, Eidinger D, Bruce AW. Intracavitary Bacillus Calmette-Guérin in the treatment of superficial bladder tumors. *J Urol* 1976;116:180-3.
- [32] Williams SV, Hurst CD, Knowles MA. Oncogenic FGFR3 gene fusions in bladder cancer. *Hum Mol Genet* 2013;22:795-803.
- [33] Morgan RJ, Synold TW, Longmate JA, et al. Pharmacodynamics (PD) and pharmacokinetics (PK) of E7389 (eribulin, halichondrin B analog) during a phase I trial in patients with advanced solid tumors: A California Cancer Consortium trial. *Cancer Chemother Pharmacol* 2015;76:897-907.
- [34] Challita-Eid PM, Satpayev D, Yang P, et al. Enfortumab Vedotin Antibody-Drug Conjugate Targeting Nectin-4 Is a Highly Potent Therapeutic Agent in Multiple Preclinical Cancer Models. *Cancer Res* 2016;76:3003-13.
- [35] Khandelwal P, Abraham SN, Apodaca G. Cell Biology and Physiology of the Uroepithelium. *Am J Physiol Renal Physiol* 2009;297:F1477-501.
- [36] Wu XR, Kong XP, Pellicer A, Kreibich G, Sun TT. Uroplakins in urothelial biology, function, and disease. *Kidney Int* 2009;75:1153-65.
- [37] Gandhi D, Molotkov A, Batourina E, et al. Retinoid signaling in progenitors controls specification and regeneration of the urothelium. *Dev Cell* 2013;26:469-82.
- [38] Airik R, Kispert A. Down the tube of obstructive nephropathies: The importance of tissue interactions during ureter development. *Kidney Int* 2007;72:1459-67.
- [39] Yamany T, Van Batavia J, Mendelsohn C. Formation and regeneration of the urothelium. *Curr Opin Organ Transplant* 2014;19:323-30.
- [40] Georgas KM, Armstrong J, Keast JR, et al. An illustrated anatomical ontology of the developing mouse lower urogenital tract. *Development* 2015;142:1893-908.
- [41] Papafotiou G, Paraskevopoulou V, Vasilaki E, Kanaki Z, Paschalidis N, Klinakis A. KRT14 marks a subpopulation of bladder basal cells with pivotal role in regeneration and tumorigenesis. *Nature Communications* 2016;7:11914.
- [42] Moch H, Cubilla AL, Humphrey PA, Reuter VE, Ulbright TM. The 2016 WHO Classification of Tumours of the Urinary System and Male Genital Organs-Part A: Renal, Penile, and Testicular Tumours. *Eur Urol* 2016;70:93-105.
- [43] Van Batavia J, Yamany T, Molotkov A, et al. Bladder cancers arise from distinct urothelial sub-populations. *Nat Cell Biol* 2014;16:982-91, 1-5.
- [44] Shin K, Lee J, Guo N, et al. Hedgehog/Wnt feedback supports regenerative proliferation of epithelial stem cells in bladder. *Nature* 2011;472:110-4.
- [45] Shin K, Lim A, Odegaard JI, et al. Cellular origin of bladder neoplasia and tissue dynamics of its progression to invasive carcinoma. *Nat Cell Biol* 2014;16:469-78.
- [46] Califano A, Alvarez MJ. The recurrent architecture of tumour initiation, progression and drug sensitivity. *Nat Rev Cancer* 2016;17:116-30.
- [47] DeGraff DJ, Cates JM, Mauney JR, Clark PE, Matusik RJ, Adam RM. When urothelial differentiation pathways go wrong: Implications for bladder cancer development and progression. *Urol Oncol* 2013;31:802-11.
- [48] Lawrence MS, Stojanov P, Mermel CH, et al. Discovery and saturation analysis of cancer genes across 21 tumour types. *Nature* 2014;505:495-501.
- [49] Lindgren D, Sjö Dahl G, Lauss M, et al. Integrated genomic and gene expression profiling identifies two major genomic circuits in urothelial carcinoma. *PLoS One* 2012;7:e38863.
- [50] Sjø Dahl G, Lauss M, Lovgren K, et al. A molecular taxonomy for urothelial carcinoma. *Clin Cancer Res* 2012;18:3377-86.
- [51] Choi W, Czerniak B, Ochoa A, et al. Intrinsic basal and luminal subtypes of muscle-invasive bladder cancer. *Nat Rev Urol* 2014;11:400-10.
- [52] Hurst CD, Knowles MA. Molecular subtyping of invasive bladder cancer: Time to divide and rule? *Cancer Cell* 2014;25:135-6.
- [53] Hoadley KA, Yau C, Wolf DM, et al. Multiplatform analysis of 12 cancer types reveals molecular classification within and across tissues of origin. *Cell* 2014;158:929-44.
- [54] Morrison CD, Liu P, Woloszynska-Read A, et al. Whole-genome sequencing identifies genomic heterogeneity at a nucleotide and chromosomal level in bladder cancer. *Proc Natl Acad Sci U S A* 2014;111:E672-81.

- [55] Baris D, Karagas MR, Verrill C, et al. A case-control study of smoking and bladder cancer risk: Emergent patterns over time. *J Natl Cancer Inst* 2009;101:1553-61.
- [56] Dorak MT, Karpuzoglu E. Gender differences in cancer susceptibility: An inadequately addressed issue. *Front Genet* 2012;3:268.
- [57] Edgren G, Liang L, Adami HO, Chang ET. Enigmatic sex disparities in cancer incidence. *Eur J Epidemiol* 2012;27:187-96.
- [58] Freedman ND, Silverman DT, Hollenbeck AR, Schatzkin A, Abnet CC. Association between smoking and risk of bladder cancer among men and women. *JAMA* 2011;306:737-45.
- [59] Soubeyran P, Kowanetz K, Szymkiewicz I, Langdon WY, Dikic I. Cbl-CIN85-endophilin complex mediates ligand-induced downregulation of EGF receptors. *Nature* 2002;416:183-7.
- [60] Petrelli A, Gilestro GF, Lanzardo S, Comoglio PM, Migone N, Giordano S. The endophilin-CIN85-Cbl complex mediates ligand-dependent downregulation of c-Met. *Nature* 2002;416:187-90.
- [61] Shamir ER, Ewald AJ. Three-dimensional organotypic culture: Experimental models of mammalian biology and disease. *Nat Rev Mol Cell Biol* 2014;15:647-64.
- [62] Nguyen-Ngoc KV, Shamir ER, Huebner RJ, Beck JN, Cheung KJ, Ewald AJ. 3D culture assays of murine mammary branching morphogenesis and epithelial invasion. *Methods Mol Biol* 2015;1189:135-62.
- [63] Shamir ER, Pappalardo E, Jorgens DM, et al. Twist1-induced dissemination preserves epithelial identity and requires E-cadherin. *J Cell Biol* 2014;204:839-56.
- [64] Cheung KJ, Gabrielson E, Werb Z, Ewald AJ. Collective invasion in breast cancer requires a conserved basal epithelial program. *Cell* 2013;155:1639-51.
- [65] Cheung KJ, Ewald AJ. A collective route to metastasis: Seeding by tumor cell clusters. *Science* 2016;352:167-9.
- [66] Cheung KJ, Padmanaban V, Silvestri V, et al. Polyclonal breast cancer metastases arise from collective dissemination of keratin 14-expressing tumor cell clusters. *Proc Natl Acad Sci U S A* 2016;113:E854-63.
- [67] Bjorling DE, Elkahwaji JE, Bushman W, et al. Acute acrolein-induced cystitis in mice. *BJU Int* 2007;99:1523-9.
- [68] Romih R, Koprivec D, Martincic DS, Jezernik K. Restoration of the rat urothelium after cyclophosphamide treatment. *Cell Biol Int* 2001;25:531-7.
- [69] Cox PJ. Cyclophosphamide cystitis—identification of acrolein as the causative agent. *Biochem Pharmacol* 1979;28:2045-9.
- [70] Liu JJ, Droller MJ, Liao JC. New optical imaging technologies for bladder cancer: Considerations and perspectives. *The Journal of Urology* 2012;188:361-8.
- [71] Lopez A, Liao JC. Emerging endoscopic imaging technologies for bladder cancer detection. *Current Urology Reports* 2014;15:406.
- [72] Mertens LS, Neuzillet Y, Horenblas S, van Rhijn BW. Landmarks in non-muscle-invasive bladder cancer. *Nat Rev Urol* 2014;11:476-80.
- [73] Lerner SP, Goh A. Novel endoscopic diagnosis for bladder cancer. *Cancer* 2015;121:169-78.
- [74] Lurie KL, Gurjarpadhye AA, Seibel EJ, Ellerbee AK. Rapid scanning catheterscope for expanded forward-view volumetric imaging with optical coherence tomography. *Opt Lett* 2015;40:3165-8.
- [75] Lurie KL, Angst R, Zlatev DV, Liao JC, Ellerbee Bowden AK. 3D reconstruction of cystoscopy videos for comprehensive bladder records. *Biomed Opt Express* 2017;8:2106-23.
- [76] Lurie KL, Angst R, Seibel EJ, Liao JC, Ellerbee Bowden AK. Registration of free-hand OCT daughter endoscopy to 3D organ reconstruction. *Biomed Opt Express* 2016;7:4995-5009.
- [77] Lurie KL, Smith GT, Khan SA, Liao JC, Ellerbee AK. Three-dimensional, distensible bladder phantom for optical coherence tomography and white light cystoscopy. *Journal of Biomedical Optics* 2014;19:36009.
- [78] Smith GT, Lurie KL, Zlatev DV, Liao JC, Ellerbee Bowden AK. Multimodal 3D cancer-mimicking optical phantom. *Biomed Opt Express* 2016;7:648-62.
- [79] Mitsunaga M, Ogawa M, Kosaka N, Rosenblum LT, Choyke PL, Kobayashi H. Cancer cell-selective in vivo near infrared photoimmunotherapy targeting specific membrane molecules. *Nat Med* 2011;17:1685-91.
- [80] Pan Y, Volkmer JP, Mach KE, et al. Endoscopic molecular imaging of human bladder cancer using a CD47 antibody. *Science Translational Medicine* 2014;6:260ra148.
- [81] Rebouissou S, Bernard-Pierrot I, de Reynies A, et al. EGFR as a potential therapeutic target for a subset of muscle-invasive bladder cancers presenting a basal-like phenotype. *Science Translational Medicine* 2014;6:244ra91.
- [82] Yeung C, Dinh T, Lee J. The health economics of bladder cancer: An updated review of the published literature. *Pharmacoeconomics* 2014;32:1093-104.
- [83] Lotan Y, Shariat SF, Schmitz-Drager BJ, et al. Considerations on implementing diagnostic markers into clinical decision making in bladder cancer. *Urol Oncol-Semin Ori* 2010;28:441-8.
- [84] Lotan Y, Raman J, Shariat S, et al. The Development and Clinical Validation of a High Sensitivity Urine Biomarker Test for the Determination of Recurrence in Urothelial Carcinoma Patients. *J Urology* 2016;195:E1071-E.
- [85] Zhang CY, Yeh HC, Kuroki MT, Wang TH. Single-quantum-dot-based DNA nanosensor. *Nat Mater* 2005;4:826-31.
- [86] Zhang Y, Park S, Liu K, Tsuan J, Yang S, Wang TH. A surface topography assisted droplet manipulation platform for biomarker detection and pathogen identification. *Lab Chip* 2011;11:398-406.
- [87] Zhang Y, Wang TH. Full-range magnetic manipulation of droplets via surface energy traps enables complex bioassays. *Adv Mater* 2013;25:2903-8.
- [88] Stark A, Shin DJ, Pisanic T, 2nd, Hsieh K, Wang TH. A parallelized microfluidic DNA bisulfite conversion module for streamlined methylation analysis. *Biomed Microdevices* 2016;18:5.