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Meeting Report

New Horizons in Bladder Cancer Research: Report of the 15th Meeting of the International Bladder Cancer Network (IBCN) in Lisbon, Portugal, October 21–23, 2017

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Fueled by recent progress of modern immunotherapy, bladder cancer has regained the interest of the scientific community and stimulated research exceeding the field of checkpoint inhibitors. The 15th meeting of the International Bladder Cancer Network (IBCN) took place at the Fundacao Champalimaud in Lisbon, Portugal, from October 21–23, 2017, 20 years after the first meeting in Barcelona. Once again, participants from different countries and with different scientific background discussed a broad spectrum of bladder cancer research spanning from basic research related topics (e.g. cancer models, biomarkers, molecular subtyping, molecular background of cancer progression and novel targets) to clinical aspects including tumor immunology [1]. A separate session tried to define "room to improvement" in clinical trial design.

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CANCER MODELS

Mullenders et al. *(Utrecht, The Netherlands)* successfully established organoid cultures in around 60% of bladder tissue samples. Organoids from both tumor and normal tissue could be expanded for prolonged periods of time (at least 9 months) and were found to contain both basal and superficial cells. This work was honoured with an award for outstanding presentation.

Fantini et al. *(Chicago, IL, USA)* presented a mutational analysis of the N-butyl-N-(4-hydroxybutyl)-nitrosamine (BBN) mouse model. They observed frequent mutations in Trp53 (80%), and the epigenetic master regulators MLL4 (Kmt2d) (70%), and MLL3 (Kmt2c) (90%), which also are commonly observed in human muscle invasive bladder cancer (MIBC). The group received a travel award for this excellent work.

The presence of different cell populations in normal mouse urothelial organoids was studied by

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Pereira Santos et al. *(Madrid, Spain)*. Looking at different markers they provided evidence that the Cd49f+ subpopulation contains urothelial stem cells, which may be an important step in studying bladder cancer development.

Gorill et al. *(Oxford, UK)* used the crypt assay to assess gut toxicity *in vivo* following whole abdominal radiation in a mouse model. They reported that a modified crypt assay is a promising method to determine the effects of chemoradiation combinations on the small intestine.

Southgate *(York, UK)* investigated normal urothelial cells in seeking drivers of bladder carcinogenesis. Based on this approach autocrine activation of EGFR was identified as a major driver of growth in NHU cell lines maintained in a non-differentiated or "basal" (CK5+, CK14+) state, whereas activation of the nuclear receptor PPARγ initiates a temporal programme of gene expression changes resulting in a differentiated (CK13+, CK20+, UPK+, CLDN3+) phenotype.

BIOMARKERS

Using data from genome wide association studies targeting patients with non-MIBC (NMIBC) from three large biobanks, Galesloot et al. *(Nijmegen, The Netherlands)* identified 2 single nucleotide variants (SNVs) predicting tumor recurrence and one more predicting tumor progression.

de Matos Simoes *(Boston, USA)* investigated the performance of an extensive range of urothelial cancer (UC) diagnostic classifiers across 156 patients presenting with haematuria in order to determine which patient characteristics were associated with a high probability of correct classification. The classifiers demonstrated favorable performance parameters in non-smokers, aged ≤ 65 years with microhaematuria.

Survivin and ERCC-1 expression were retrospectively investigated as potential prognostic markers in 290 patients with MIBC undergoing radical cystectomy. Kiani et al. *(Berlin, Germany)* demonstrated that low survivin and ERCC-1 expression are associated with longer overall survival after radical cystectomy irrespective of additional neoadjuvant or adjuvant chemotherapy. As an alternate option, Bazargani et al. *(Los Angeles, CA, USA)* prospectively measured serum levels of Carbohydrate Antigen CA 125, CA 19-9 and Carcinoembryonic Antigen (CEA) in 480 patients with MIBC receiving NAC. The group reported that patients with persistently elevated markers after NAC had a very poor prognosis following cystectomy.

Skowron et al. *(Düsseldorf, Germany)* studied long term bladder cancer cultures exposed to cisplatin. The cultured cells evaded cisplatin stress by phenotypic plasticity via EMT, induction of non-canonical WNT target genes and anti-apoptotic factors. Decreased DNA-damage could largely be attributed to an increased cisplatin inactivation by the NRF2 pathway.

MOLECULAR SUBTYPING

Zoidakis et al. *(Athens, Greece)* reported on a detailed proteomics analysis of bladder cancer specimens, aiming to investigate the question whether existing BC subtypes are reflected in the tissue proteome and to identify new therapeutic targets.

Cytochrome P450 family 1 (CYP1) enzyme activity in MIBC was studied by Baker et al. *(York, UK)*. This *in-vitro* study suggested that urothelial activation of pro-carcinogens by CYP1 likely contributes to bladder carcinogenesis. Furthermore, urothelial CYP1-activity might provide a new, unexploited route for bladder cancer therapy.

Dominguez et al. *(Madrid, Spain)* investigated the correlation between specific risk patterns for bladder cancer taxonomy-based subphenotypes. Analyzing over 800 patients they could correlate 3 bladder cancer clusters (basal/squamous, luminal-like and mixed) with different risk factors (e.g. tobacco smoking, risk-occupational exposures, NSAIDs consumption).

In an update from The Cancer Genome Atlas Research Network, Robertson et al. *(Vancouver, BC, Canada)* characterized microRNA (miR) and long non-coding (lnc) RNA expression patterns in a large cohort of patients with MIBC. Classifying the tumours based on miR or lncRNA generated clusters that were concordant with established mRNA-based subtypes, but also further discriminated within these subtypes. miR and lncRNA subtypes independently predicted survival.

The impact of genomic classifiers on prognosis in 257 patients with MIBC was investigated by Sundi et al. *(Houston, TX, USA)*. The group could validate earlier reports that a basal gene signature in bladder cancer patients undergoing NAC is associated with a clinical benefit, and that the p53-like gene signature appears to confer chemo-resistance as assessed by pathologic downstaging. The question

of plasticity of bladder cancer exposed to NAC was addressed by Seiler et al. *(Bern, Switzerland)* in a longitudinal study setting in 134 patients with MIBC. They observed a greater variability of post-NAC gene expression in previous basal-like tumors compared to luminal-like tumors. The group received a best presentation award for this outstanding contribution.

MOLECULAR BACKGROUND OF CANCER PROGRESSION

Majewski et al. *(Houston, TX, USA)* identified complex genomic alterations that involved areas of microscopically normal bladder mucosa adjacent to dysplasia and carcinoma *in situ* using a whole organ mapping strategy coupled with exome sequencing, genome-wide copy number analysis and whole genome methylation to define the genomic profile of bladder cancer progression from field effects to clinically evident disease.

Guo et al. *(Houston, TX, USA)* analyzed the expression profiles of RNA extracted from paraffin embedded tumor tissue of 28 patients with sarcomatoid bladder cancer and a reference set of 89 conventional urothelial carcinomas. The group observed that sarcomatoid bladder cancer is associated with widespread dysregulation of chromatin remodeling and cell cycle circuits.

Based upon the information that miRNAs packed in exosomes can affect cell-cell communication in the tumor microenvironment and play a crucial role in tumorigenesis, Baumgart et al. *(Homburg/Saar, Germany)* studied the functional role of exosomes in reprogramming normal bladder fibroblasts. Bladder cancer derived exosomes were found to stimulate the proliferation and migration of bladder fibroblasts.

Lapi et al. *(Madrid, Spain)* investigated the role of the STAG2 tumor suppressor gene in bladder cancer development *in vitro* and in organoids from transgenic animal models. The findings support that STAG2 inactivation contributes to bladder cancer development through the regulation of stemness, differentiation, and growth factor signalling.

NOVEL TARGETS

In particular in basal type tumors NOTCH2 is an oncogene driving bladder cancer (BC) progression. Contreras-Sanz et al. *(Vancouver, BC, Canada)* investigated whether NOTCH2 can promote BC development *in vivo* using a constitutively active NOTCH2 intracellular domain (N2ICD) mouse model. The results of this award winning submission (Travel Award) suggest that over-expression of wild-type N2ICD in the bladder wall could accelerate tumor development and possibly lead to more malignant phenotypes. Moreover, over-expression of N2ICD promotes metastasis *in vivo*.

Specific inhibitors of class-I histone deacetylases (HDACi) like romidepsin cause broad transcriptional changes, induce cell cycle arrest and cell death in urothelial carcinoma cell lines (UCCs). Combination of HDACi with JQ1, an inhibitor of bromodomain-containing acetylation reader proteins like BRD4 has previously shown efficacy in some cancers. Hölscher et al. *(Düsseldorf, Germany)* studied the synergy of these 2 agents in four UC cell lines. In the combination romidepsin could be applied in combination with JQ1 at relatively low, less toxic doses.

By establishing Bacillus Calmette-Guérin (BCG) resistant cell lines and conducting *in vitro* and *in vivo* studies, Jalalizadeh et al. *(Baltimore, MD, USA)* provided evidence that a dysregulation of the estrogen receptor ß pathway might be involved in BCG resistance in NMIBC.

CLINICAL TOPICS

Williams et al. *(Galveston, TX, USA)* investigated gender specific outcomes in non-metastatic MIBC in the SEER database. Analyzing nearly 10.000 patient records, they concluded that gender differences persist with women significantly more likely to undergo cystectomy independent of clinical stage. However, women have significantly worse survival than men.

Kukreja et al. *(Houston, TX, USA)* compared costs of open and robotic radical cystectomy using a propensity matched cohort. They concluded that the higher costs of robotic surgery are balanced by a lower transfusion rate and an increase in quality adjusted life years.

Results for local hyperthermic chemotherapy were reported by two groups. Mertens et al. *(Amsterdam, The Netherlands)* investigated a multimodal approach in patients with advanced urachal carcinoma. Combining cytoreductive surgery and hyperthermic intraperitoneal chemotherapy (HIPEC) in patients with peritoneal carcinomatosis, the group observed long-term survival in this fatal tumor entity. de Jong et al. *(Rotterdam, The Netherlands)* studied hyperthermic intravesical chemotherapy in 24

patients with BCG unresponsive or BCG ineligible NMIBC in a Phase II study setting.

Gupta et al. *(Minneapolis, MN, USA)* used the idea that the androgen receptor (AR) has been implicated in BC progression for a Phase I study combining enzalutamide with conventional chemotherapy (Gemcitabine/Cisplatin) in patients with metastastic BC.

TUMOR IMMUNOLOGY

Sweis et al. *(Chicago, IL, USA)* correlated different bladder cancer immunophenotypes with the genomic profile. Immunophenotypical analysis yielded tumors with presence or absence of T cells and BATF3-lineage dendritic cells. However, the group showed no difference in mutational density. β-catenin, PPARG, and FGFR3 pathways were found to correlate with T cell exclusion and are considered potential targetable mechanisms of immunotherapy resistance. Following a similar line of investigation, Sfakianos et al. *(New York, NY, USA)* conducted a molecular profiling of tumor infiltrating lymphocytes in NMIBC and MIBC specimens. They concluded from their analysis that TIM-3 and TIGIT might be possible novel targets for patients with BC. This submission also earned a Travel Award.

Cellular composition and expression of PD-L1/PD-1 in urine and blood cells from 18 patients with MIBC with or without NAC was examined by Rodriguez Faba et al. *(Barcelona, Spain)*. The group observed that in urine tumor cells PD-L1 expression was increased after NAC. In addition, urine concentrations of soluble PD-L1 were higher after NAC and correlated positively with CD3+CD8+ percentages in blood.

Dinney et al. *(Houston, TX, USA)* reported on a phase I Trial in patients with BCG refractory NMIBC using a newly developed adenoviral interferon-alpha (Ad-IFNα) for intravesical delivery along with with a novel adjuvant (Syn-3) to enhance viral transduction of the urothelium. This therapy induced PD-L1 expression in tumor cells, suggesting that combination with checkpoint blockade would be worthy of further study.

PD-L1 and PD-1 expression patterns in patients with NMIBC undergoing intravesical BCG were studied by Kates et al. *(Baltimore, MD, USA)*. Interestingly, no changes in PD-1+ or PD-L1+ tumor associated immune cells were found after BCG. Furthermore, there were no differences between BCG responders and non-responders, raising questions about the use of systemic immune checkpoint blockade in NMIBC.

First images of *in vivo* confocal laser endomicroscopy investigating histological grades of urinary upper tract tumors were presented by Palou et al. *(Barcelona, Spain)*.

A record attendance of over 100 participants suggests increasing acceptance of the IBCN meeting as a key conference in the field of bladder cancer. A key message of the meeting was the conclusion that the expanding spectrum and increasing complexity of diagnostic and therapeutic approaches in bladder cancer represent a new dimension of challenges that can only be met by large interdisciplinary and international consortia. The IBCN meetings provide a unique platform for exchange, discussion and intensifying multidimensional cooperation, thus satisfying this increasing need for the management of bladder cancer in all its facets.

The 16th meeting of the IBCN will take place in Rotterdam, The Netherlands, October 11–13, 2018.

HIGHLIGHTS

- The recent advent of checkpoint inhibitors and molecular classifications in bladder cancer has greatly stimulated also a variety of other areas of research in this tumor entity.
- Individualized therapy targeting suitable molecular pathways of a given tumor is moving into clinic.
- The expanding spectrum and increasing complexity of diagnostic and therapeutic approaches in bladder cancer represent a new dimension of challenges.
- These challenges can only be met by large interdisciplinary and international consortia.

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