# Canadian Bladder Cancer Forum 2023 Meeting Abstracts – Podium Presentations

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## Phase 1 study of safety and immunogenicity of DPX-based products with or without intermittent low-dose cyclophosphamide in patients with non-muscle-invasive bladder cancer

*Alain Bergeron[1], Valérie Picard[1], Marjorie Besançon[1], Lisa D. MacDonald[2], Kabir Mody[2], Ashley Hilchie[2], Heather Hirsch[2], Lyne Lavoie[2], Stephan Fiset[2], Yves Fradet[1]*

[1]Centre de recherche du CHU de Québec-Université Laval, Quebec, QC, Canada; [2]IMV Inc., Dartmouth, NS, Canada

**Introduction:** Intravesical chemotherapy or immunotherapy to prevent recurrences fail in a significant proportion of patients with non-muscle-invasive bladder cancer (NMIBC) and, therefore, more effective therapies are needed. The DPX platform is a versatile, non-aqueous, lipid-based delivery platform that produces sustained T-cell responses against specific peptide antigens. In ovarian cancer and DLBCL, it has been shown to induce and maintain immune responses, leading to tumor regressions. DPX-based immunotherapies targeting survivin and MAGE-A9, two tumor-associated antigens expressed by NMIBC, could provide a novel way to treat these tumors.

**Methods:** This phase 1, multicenter study assesses the effect of three subcutaneous injections, prior to transurethral resection (TUR), of 0.25 ml of DPX-based products (Q 3 weeks) ± intermittent low-dose cyclophosphamide (CPA) as treatment for subjects with NMIBC who failed intravesical therapy. The primary objectives are to assess the safety and to evaluate induction of antigen-specific T-cell responses in ELISPOT assays. Additional objectives include measurement of T-cell infiltration changes using multiplex assays and number of patients achieving pT0 at TUR. Currently, arms using MVP-S (targeting survivin) and DPX-SurMAGE (targeting survivin and MAGE-A9) are enrolling subjects.

**Results:** As of January 2023, seven subjects have been enrolled: five have received MVP-S ± CPA, one has received DPX-SurMAGE without CPA, and four have completed TUR. Treatment has been well-tolerated, with observations of grade 1 fatigue and injection site reactions. H&E staining of baseline and post-treatment TUR tumor samples has shown a marked increase in immune cell infiltration in two of three post-MVP-S treatment specimens analyzed to date, suggesting local immune activity of MVP-S.

**Conclusions:** Ongoing recruitment, soon with more participating centers, will allow more subjects to confirm these early observations, with more analyses of the anti-tumor activity of DPX products to be presented at the meeting.

## Proteomic profiling of muscle-invasive bladder cancer treated with neoadjuvant platinum-based chemotherapy reveals unique biologic clusters with clinical relevance

*Alberto Contreras-Sanz[1], Gian L. Negri[2], Moritz J. Reike[1], Htoo Z. Oo[1], Sandra E. Spencer Miko[2], Karina Nielsen[2], Morgan E. Roberts[1], Joshua Scurll[1], Kenichiro Ikeda[1], Gang Wang[1], Roland Seiler[3], Gregg B. Morin[2], Peter C. Black[1]*

[1]Vancouver Prostate Centre, Department of Urologic Sciences, Vancouver, BC, Canada; [2]Canada's Michael Smith Genome Sciences Centre, BC Cancer, Vancouver, BC, Canada; [3]Department of Urology, Inselspital, University of Bern, Bern, Switzerland

**Introduction:** Neoadjuvant cisplatin-based chemotherapy (NAC) followed by radical cystectomy (RC) is recommended for muscle-invasive bladder cancer (MIBC); however, only ~40% of patients show an objective pathologic response, and the survival benefit is only 5–7% at five years. While DNA alterations and RNA classifiers may predict response to NAC in retrospective studies, the proteome has not been evaluated in this context. Here, we profiled the proteome of MIBC treated with NAC to identify markers of response and resistance to chemotherapy.

**Methods:** Pre-treatment tissue was included from 107 MIBC patients from two institutions who received NAC (including induction chemotherapy for cN1-3 MIBC) followed by RC. Residual tumor (≥ypT1N0-3M0-1) was present in the RC specimen in 66 (62%) patients after NAC, and was profiled for 55 (51%). Multiregional tumor sampling was conducted in 37/107 pre-NAC and 15/55 post-NAC samples. Benign ureter was used as control. Single-Pot, Solid-Phase-enhanced, Sample Preparation-Clinical Tissue Proteomics (SP3-CTP) was performed on formalin-fixed paraffin-embedded tissue (FFPE), followed by bioinformatic analysis. Immunohistochemistry (IHC) validation was conducted on matched tissues.

**Results:** We quantified 9769 proteins across samples. Unsupervised clustering of pre-NAC tissue established four clusters based on biology and survival outcomes, but with no difference in response by pathologic stage. Clusters were confirmed by IHC, and consisted of: Cluster 1 (CC1) with high metabolic activity and a luminal profile; Cluster 2 (CC2) with high nuclear activity; Cluster 3 (CC3) with high immune infiltration and basal profile; and Cluster (CC4) with high immune infiltration and stromal signature. CC3 showed worse survival outcomes ($p<0.01$). Multivariable analysis identified novel favorable (MAPK9 and MTIF3) and unfavorable (DVL2 and NES) markers of survival. Matched analysis of pre- and post-NAC tissue showed markers (AZGP1 and ORM1) and pathways indicative of chemotherapy resistance. In post-chemotherapy (i.e., resistant) tumors, we identified two clusters: post-NAC Cluster 1 was enriched for nuclear processes and had worse outcomes, whereas post-NAC Cluster 2 was enriched for immune pathways. Multiregional proteomic analysis of histologically similar pre-NAC tissue revealed that highly heterogeneous tumors are enriched for non-responders and have worse outcomes compared to homogeneous tumor specimens. Moreover, comparative analysis of pre- and post-NAC matched tumors highlighted the importance of heterogeneity in chemoresistance mechanisms.

**Conclusions:** We describe four pre-NAC and 2 post-NAC proteomic clusters with distinct biology and survival outcomes, alongside novel prognostic biomarkers. Future work includes IHC validation of clusters in larger, independent MIBC cohorts. A non-NAC cohort using pre-RC biopsy tissue will be used to confirm the prognostic vs. predictive relevance of these findings.

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## The predictive power of tertiary lymphoid structures in assessing response to trimodal therapy in muscle-invasive bladder cancer

*Nour Hassan[1], José Joao Mansure[1], Mina Farag[2], Ronald Kool[3], Eva Michaud[1], Mohammed Alessa[3], Luis Souhami[4], Fabio Cury[4], Fadi Brimo[2], Wassim Kassouf[1,3]*

[1]Cancer Research Program, Research Institute of the McGill University Health Centre, Montreal, QC, Canada; [2]Division of Pathology, McGill University Health Centre, McGill University, Montreal, QC, Canada; [3]Division of Urology, Department of Surgery, McGill University Health Centre, Montreal, QC, Canada; [4]Department of Radiation Oncology, McGill University Health Centre, Montreal, QC, Canada

**Introduction:** Radical cystectomy is the standard of care for muscle-invasive bladder cancer (MIBC). Radiotherapy (RT) is a bladder preservation option that offers patients comparable survival rates. Despite appropriate selection criteria, up to 30% of patients will require salvage cystectomy. Emerging evidence points to an important link between response to RT and the tumor microenvironment (TME). Notably, tertiary lymphoid structures (TLS) are

being investigated as a potential orchestrator of a local immune response. Mature TLS are lymph node-like structures that have an active germinal center (GC) and have been associated with improved outcomes in several cancers. Here, we explored the use of TLS and their associated TME as a predictive biomarker for response to RT in MIBC.
**Methods:** H&E-stained FFPE sections of pre-RT biopsies from 147 MIBC patients with known outcomes were examined to identify TLS presence, with confirmation from a pathologist. For further analysis, three representative tissue cores from each case were used to construct tissue microarrays (TMA). Gene expression profiles were obtained by NanoString's Digital Spatial technology, and immunohistochemical (IHC) staining of CD20, CD4, CD8, CD68, FoxP3, and Neutrophil Elastase was performed. Images were analyzed on the Halo platform.
**Results:** H&E revealed that 19.7% of patients (n=68) had TLS with a GC, 17.0% (n=25) had TLS without a GC, and 12.9% (n=19) had no TLS. In the remaining 50.3% of cases (n=74), confirmation of TLS presence required IHC staining, which is underway. Gene expression data showed that TLS marker CXCL13 is higher among complete responders to RT (p=0.0440). We also used a previously described 39-gene TLS signature to clustered patients into two groups, "TLS high" and "TLS low." Non-responders to therapy made up 40.4% of the "TLS low" group, compared to only 26.7% in the "TLS high" group. Further analysis will include the immune TME, as well as the assessment of TLS maturity and their effects on outcomes.
**Conclusions:** Our findings point to a potential role of TLS in predicting response to RT, but TLS presence alone is not sufficient to evaluate it. Further studies to assess the maturity of TLS and their interaction with the TME would be warranted to better delineate if the different state of TLS would be predictive of response.

## Development of NIMBLE – An artificial intelligence-based prediction tool for tumor progression of non-muscle-invasive bladder cancer using the WHO 2004/2016 grading system

*Jethro C.C. Kwong[1,2,3], Kellie Kim[4], Zizo Al-Daqqaq[4], Cynthia Kuk[5], Nathan Perlis[1,2], Jason Y. Lee[1,2], Robert J. Hamilton[1,2], Neil E. Fleshner[1,2], Antonio Finelli[1,2], Alexandre R. Zlotta[1,2,5], Alistair E.W. Johnson[3,6,7], Girish S. Kulkarni[1,2,3]*
[1]Division of Urology, Department of Surgery, University of Toronto, Toronto, ON, Canada; [2]Division of Urology, Department of Surgery, University Health Network, Toronto, ON, Canada; [3]Temerty Centre for AI Research and Education in Medicine, University of Toronto, Toronto, ON, Canada; [4]Temerty Faculty of Medicine, University of Toronto, Toronto, ON, Canada; [5]Division of Urology, Department of Surgery, Mount Sinai Hospital, Sinai Health System, Toronto, ON, Canada; [6]Division of Biostatistics, Dalla Lana School of Public Health, University of Toronto, Toronto, ON, Canada; [7]Vector Institute, Toronto, ON, Canada
**Introduction:** Several predictive models have been developed to estimate the risk of tumor progression in non-muscle-invasive bladder cancer (NMIBC); however, they do not reflect current practice, perform poorly, and are based on the World Health Organization (WHO) 1973 grading system. We aimed to develop NIMBLE, an artificial intelligence (AI)-based tool to better predict progression in NMIBC patients using the more widely used WHO 2004/2016 grading system in North America.
**Methods:** NIMBLE was trained on patients treated from January 2005 to October 2014 at the University Health Network in Toronto (n=564). Predictors included age, sex, history of urothelial cancer, stage, grade (WHO 2004/2016), concomitant carcinoma in situ (CIS), tumor burden and size, type of intravesical therapy, European Association of Urology (EAU) total progression score, and number of intermediate risk factors. Internal validation was performed on patients treated from October 2014 to December 2020 at the same institution (n=142). External validation was performed on a publicly available dataset of patients treated from October 2004 to December 2013 at Seoul National University in South Korea (n=198). Primary outcome was progression, defined as relapse of pT2 disease or higher. NIMBLE hyperparameters were tuned using a tree-structured Parzen estimator algorithm to optimize concordance index. NIMBLE was compared against the EAU risk groups and a previously published AI model trained on a multi-institutional European cohort.
**Results:** Mean age of the total cohort was 68 years and 23% were female; 52% of patients had pTa, 43% pT1, 5% primary CIS, 42% low-grade, and 58% high-grade disease. Median followup was 4.7 years (IQR 2.2–8.3). NIMBLE had the best performance in all cohorts (Table 1) and demonstrated excellent calibration (Figure 1).
**Conclusions:** Using the WHO 2004/2016 grading system, NIMBLE performed favorably compared to contemporary prediction tools. Ongoing work is being conducted to evaluate the safety and generalizability of NIMBLE in larger NMIBC cohorts.

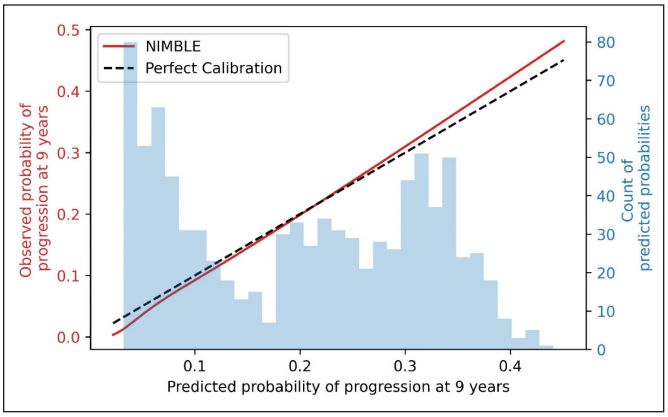

**Figure 1 (Kwong et al).** Calibration of NIMBLE at 9 years.

**Table 1 (Kwong et al). Performance of all models based on concordance index and integrated Brier score**

| **Concordance Index (higher is better)** | | | |
|---|---|---|---|
| **Cohort** | **NIMBLE** | **AI-EUR[a]** | **EAU risk groups[b]** |
| Training | 0.81 (0.77–0.85) | 0.67 (0.58–0.76) | 0.54 (0.50–0.57) |
| Internal validation | 0.79 (0.61–0.93) | 0.60 (0.51–0.80) | 0.77 (0.60–0.90) |
| External validation[c] | 0.78 (0.68–0.87) | 0.62 (0.51–0.74) | 0.63 (0.54–0.73) |
| **Integrated Brier Score (lower is better)** | | | |
| **Cohort** | **NIMBLE** | **AI-EUR[a]** | **EAU risk groups[b]** |
| Training | 0.08 (0.07–0.10) | 0.08 (0.05–0.10) | 0.09 (0.07–0.11) |
| Internal validation | 0.05 (0.02–0.08) | 0.07 (0.05–0.11) | 0.06 (0.03–0.09) |
| External validation[c] | 0.08 (0.05–0.11) | 0.10 (0.08–0.12) | 0.09 (0.05–0.12) |

NIMBLE was compared against a previously published AI model trained on a multi-institutional European cohort (AI-EUR) and the European Association of Urology (EAU) risk groups.
[a]https://doi.org/10.1016/j.euo.2021.05.006 (Extended model). [b]https://doi.org/10.1016/j.eururo.2020.12.033. [c]https://doi.org/10.1371/journal.pone.0189354.

## Quantitative nuclear prognostics to improve grading for patients with non-invasive bladder cancer

Katherine Lindale[1,2], Ava Slotman[1,2], Minqi Xu[1], Céline Hardy[1,2], Lina Chen[1], Chelsea L. Jackson[1,2], D. Robert Siemens[5,6], Robert J. Gooding[2,3], Amber L. Simpson[4,5,7], David M. Berman[1,2]

[1]Department of Pathology and Molecular Medicine, Queen's University, Kingston, ON, Canada; [2]Queen's Cancer Research Institute, Kingston, ON, Canada; [3]Department of Physics, Engineering Physics, and Astronomy, Queen's University, Kingston, ON, Canada; [4]School of Computing, Queen's University, Kingston, ON, Canada; [5]Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, Canada; [6]Department of Urology, Queen's University, Kingston, ON, Canada; [7]Centre for Health Innovation, Queen's University, Kingston, ON, Canada

**Introduction:** The qualitative nature of the non-muscle-invasive bladder cancer (NMIBC) grading system limits its reproducibility and ability to optimize prognostic value, compromising the potential for data-driven care decisions. Using machine learning (ML)-based image analysis, we constructed models that establish reproducible quantitative thresholds and feature importance for grade classification. We leveraged these tools to optimize grade for its ability to stratify risk of recurrence.

**Methods:** Small (1.0 mm diameter) histopathology images were obtained for 371 patients with stage Ta NMIBC, with clinical timelines for 163 patients. Nuclear measurements of 19 grade-based histological features were extracted using Visiopharm image analysis software (Hoersholm, Denmark). Quantitative grading models built using these features were analyzed to determine the variables that best classify histological grade. Cox proportional hazards (CPH) models for time to first bladder recurrence were cross-validated and tested for grade and quantified nuclear features (QNFs).

**Results:** The standard deviation of nuclear area and mitotic index were the QNFs that best predicted grade as univariate models (balanced accuracy up to 82%) and when used in combination with shape-related variables (mean ellipticalness, mean solidity, and standard deviation of form factor) in statistical and ML models (balanced accuracy up to 88%). Mitotic index, mean lesser diameter (size, shape), and mean variance HEM (texture) carried the most prognostic value. Upon validation, the CPH model constructed using these QNFs achieved a C-index of 0.73 (CI 0.56–0.88) compared to 0.55 (CI 0.40–0.69) using diagnostic grade alone.

**Conclusions:** Histological features in NMIBC can be quantified and used in grading classification algorithms, addressing the irreproducibility associated with grading. The prognostic value of QNFs supports grade's utility; however, findings indicate the opportunity to improve grading by reprioritizing these features. Establishing prognostically driven feature measurement thresholds and importance weights can optimize grade's contribution to risk scoring and clinical decisions.

## Exploring the impact of microbiome in the response of combined radiation with immune checkpoint blockade in muscle-invasive bladder cancer

Eva Michaud[1], Sabina Fehric[1], Cynthia Faubert[2], Irah King[2,3], José Joao Mansure[1], Wassim Kassouf[1,4]

[1]Urologic Oncology Research Division, McGill University Health Centre, Montreal, QC,Canada; [2]McGill Interdisciplinary Initiative in Infection and Immunity, Montreal, QC, Canada; [3]Meakins-Christie Laboratories, Department of Medicine, McGill University Health Centre, Montreal, QC, Canada; [4]Division of Urology, Department of Surgery, McGill University Health Centre, Montreal, QC, Canada

**Introduction:** Radiation therapy (RT) is a promising bladder-sparing option for MIBC treatment, yet 30% of patients do not respond and half will later die of metastasis. Improved antitumor responses when RT is combined with PD-1/ PD-L1 blockade (CT) have been described in mice, yet determinants of CT success remain flagrantly misunderstood. As such, gut microbiome composition influences PD-1 blockade efficacy and its modification potentiates combined RT and PD-L1 blockade activity. To add, responding patients with a favorable gut microbiome (i.e., enrichment in *A. muciniphila*, *Bifidobacterium*, and *Faecalibacterium*) have enhanced systemic and antitumor immunity. We thus aimed to document the role of patients' microbiome in polarizing antitumor immune responses to CT and use its composition as a predicting factor of CT success in MIBC.

**Methods:** Fecal material from a responder (R) and non-responder (NR) MIBC patient was gavaged into 20 germ-free mice. Three weeks after the last gavage, MB49 cells were delivered subcutaneously. Once tumors reached 0.1–0.15 $cm^3$, mice were randomized into four groups: control; anti-PD-L1; RT; RT+anti-PD-L1. Seven days later, tumors were dissociated for single-cell immune sequencing and stools collected for 16S sequencing. Correlation networks were built (TransNet, Microbiome R packages) and visualized in cytoscape.

**Results:** We show feasibility and robust engraftment of human FMT to germ-free mice in a MIBC tumor model. FMT from NR lessened the known beneficial effects of RT in the MB49 model compared to FMT from R. Transkingdom analysis of sc-RNA-seq and 16Sseq shows robust statistical interactions between immunosuppression and enrichment in microbes associated to poor outcome humans.

**Conclusions:** To our knowledge, this is the first study to use FMT as a modulator of response in the context of RT combinations in MIBC. These findings could be used to select patients who will benefit most from a personalized therapeutic approach.