Canadian Bladder Cancer Forum 2023 Meeting Abstracts – Poster Presentations

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Oncolytic virus VSV-d51-GM-CSF as an alternative for BCG treatment in non-muscle-invasive bladder cancer

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Introduction: Bladder cancer is the 4th and 15th most common cancer in men and women, respectively. Surgical resection followed by bacillus Calmette-Guérin (BCG) therapy can reduce this risk, and cystectomy (bladder removal) prior to muscle invasion provides the best option for survival. While these therapeutic approaches are effective in many patients, recurrences remain common and there is a lack of effective second-line bladder-sparing therapies. This is especially true for high-risk non-muscle-invasive bladder cancer (NMIBC) patients who do not respond to BCG. Two recent therapeutic advancements, immune checkpoint inhibitors and localized gene therapy, have attempted to treat high-risk NMIBC patients. Despite their success in other tumor types, most treated patients have failed to respond. This is due to overall immunosuppression and the small number of pre-existing immune-reactive cells within the bladder tumor microenvironment (TME), which limits their beneficial effects. We have previously shown an enhanced antitumor benefit of both the mouse and human versions of the novel oncolytic virus VSVd51-GMCSF vs. VSVd51 in preclinical models of bladder cancer. Moving forward from our proof-of-concept studies, we will study the translational potential of VSVd51-GMCSF in NMIBC. We hypothesize that treatment with VSVd51-GMCSF will initiate immunogenic cell death (ICD) of bladder cancer cells and potently activate bladder tumor-directed immune responses.

Methods/Results: In vitro, we observed that viability of human (T24, TCCSUP, UMUC3, and 5637) and mouse (MB49) bladder cancer cell line is not impacted by BCG treatment, while VSV-d51-VSV-GM-CSF triggers cell death. We analyzed ICD markers (HMGB1 and Hsp90 release, ATP essay, and calreticulin membrane exposure) and confirmed that VSVd51-GMCSF treatment induces ICD while BCG does not. In a mouse model, we compared the ability of VSVd51-GMCSF and BCG to attract effector immune cells in the bladder TME of MB49-implanted mice. We dissociated tumors and analyzed immune cells by flow cytometry. We observed a significant increase of CD8 T cells frequency in the bladder TME (mean ± SEM: 17.88 %±4.69 of CD3+ cells in VSVd51-GMCSF-treated mice vs. 20.93%±1.33 in BCG-treated mice, $p<0.01$, two-tailed Mann-Whitney, n=5), as well as NK cells (51.20%±0.86 of CD45+CD3- cells in VSVd51-GMCSF-treated mice vs. 11.25%±2.03 in BCG-treated mice, $p<0.05$, two-tailed Mann-Whitney). While results are significantly different between PBS and VSVd51-GMCSF-treated mice, they are not between PBS and BCG-treated mice. We also used a cohort of male mice previously treated with N-butyl-N-(4-hydroxybutyl)-Nitrosamyl (BBN) to induce bladder cancer and mimic smoking-induced bladder cancer. Animals were then treated with VSVd51-GMCSF or BCG when tumor size was 5–10 mm^3 (assessed by ultrasound). In VSVd51-GMCSF-treated animals, the tumor volume can be controlled; three of five animals have a tumor size still <10 mm^3 even if treatment failed for two animals, with tumor size reaching 150 mm^3 before euthanasia. In BCG-treated animals, two of four animals' tumors are still growing (reaching 50–70 mm^3) but for the other two animals, tumors grew very fast, reaching 130–150 mm^3 before euthanasia.

Conclusions: BCG is not able to induce cancer death, while VSVd51-GMCSF is. In an MB49 mouse model, BCG failed to attract effector immune cells, while VSVd51-GMCSF treatment induced an increased proportion of CD8 T cells and NK cells in the bladder TME. In a spontaneous bladder cancer model (BBN-induced), we are able to control disease progression in three (of five) animals treated by VSVd51-GMCSF, while BCG treatment failed or only slowed down the tumor growth.

Investigating sex differences in response to bacillus Calmette-Guérin immunotherapy in the four-core genotype murine model of non-muscle-invasive bladder cancer

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Introduction: The incidence of bladder cancer is three times higher in males compared to females. Despite the lower incidence, women generally present with advanced-stage disease and experience shorter progression-free survival. Most patients are diagnosed with non-muscle-invasive bladder cancer (NMIBC), for which intravesical bacillus Calmette Guérin (BCG) immunotherapy remains the gold standard treatment. We previously demonstrated the association between increased intra-tumoral CD79a+ B cells pre-treatment tumors from patients with NMIBC who exhibit early recurrence and progression. B cells are critical to mucosal immunity and response to BCG due to their antibody-producing and antigen-presenting functions. It is also established that B cells exhibit a sex- and age-dependent expansion and response to treatment.

Methods: To understand the gonadal hormonal and sex chromosome-associated differences in cancer progression and response to BCG, we used the Four Core Genotype (FCG) mouse model. In this model, a male mouse lacking the testes determining Sry gene ($XY^-_{Chr3}{}^{Sry+}$/XYM) is crossed with an XX female (XXF) mouse leading to the generation of offspring with four genotypes: two gonadal males (XYM, XXM) and two gonadal females (XXF and XYF). Bladder carcinogenesis was induced by exposing 12-month-old aging FCG mice to 0.05% N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN) ad libitum in drinking water. After seven weeks of continuous exposure to BBN, mice were treated with three intravesical doses of BCG at weekly intervals. Untreated and saline treated controls were included. Systemic and local immune profiling was performed one week post-third BCG on spleen, bone marrow, and formalin-fixed whole bladders.

Results: Hematoxylin and eosin staining of whole bladder sections revealed an overall higher immune cell infiltration and increased presence of tertiary lymphoid structure formation in the bladders of XXF mice compared to all other genotypes following chronic exposure to BBN. Multispectral flow cytometry of splenocytes and bone marrow-derived cells revealed significant differences in the proportions of total and atypical B cells (ABCs) post-completion of BCG across the four genotypes of mice compared to the mice in BBN exposed untreated and saline treated groups.

Conclusions: Overall, these findings provide evidence for a sex-associated role of ABCs in mediating response to BCG. Further investigations are warranted to understand the mechanisms underlying ABC-associated poor outcomes in patients who exhibit high intra-tumoral B cells in their pre-treatment tumors.

Prebiotics improve anti-PD1 immunotherapy efficacy in bladder cancer by modulating the gut microbiota

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Introduction: The gut microbiota is a critical factor for the response to immune checkpoint blockade (ICB) immunotherapy in many cancers. Targeting gut microbiota with dietary elements is a new strategy to improve ICB immunotherapy outcomes in cancer patients. The current study's objective was to improve the efficacy of anti-PD1 in bladder cancer via the modulation of gut microbiota with prebiotics.

Methods: C3H syngeneic mice were subcutaneously injected with MBT-2 mouse bladder tumor cells. Prebiotics were administrated daily by oral gavage two weeks before tumor cell injection and until the end of the experiment. Following tumor implantation, mice were treated with anti-PD1 monoclonal antibody or isotype control intraperitoneally. Tumor growth was monitored twice a week. Mice fecal samples were collected at baseline and after two weeks of supplementation with prebiotics to perform 16S rRNA gene sequencing and profile the gut microbiota composition. At sacrifice, blood and tumors were harvested for flow cytometry analysis.

Results: In comparison to control group, two prebiotic molecules significantly reduced MBT-2 tumor growth and improved overall mouse survival. The gut microbiota profiling revealed an enrichment of the *Bacteroides* genus in mice gavaged with prebiotic A, while the prebiotic B induced enrichment of the *Faecalibaculum* genus and *Lachnospiraceae* family. Interestingly, prebiotic A supplementation combined with anti-PD-1 immunotherapy also enhanced the systemic antitumor effect of ICB. Treatment with prebiotic A also resulted in a systemic expansion of circulating $CD8^+$ T cells, supporting a boosted immune response.

Conclusions: Overall, our findings support that promising prebiotics can induce an antitumor effect alone, and in combination with anti-PD-1 treatment by modulating the gut microbiota, in a bladder cancer mouse model. These data will have a significant impact to understand and improve the clinical response to ICB treatment for bladder cancer patients.

Investigating the role of tumor-associated B cells in bladder cancer progression

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Introduction: Bladder cancer is mostly diagnosed in older individuals (>65 years of age) and can be broadly categorized into non-muscle-invasive bladder cancer (NMIBC) and muscle-invasive bladder cancer (MIBC). Almost 75% of the incident bladder cancer cases are NMIBC and 25% present with de novo MIBC. Progression to secondary MIBC often occurs in patients with high-risk NMIBC. Our recent study on whole transcriptome analysis of tumors from 460 patients showed increased expression of B cell associated genes in high-grade tumors. Spatial immune profiling of 332 tumors demonstrated increased density of intratumoral B cells in patients who exhibited shorter recurrence-free survival. We hypothesized that specific B cell subsets expand due to carcinogen-induced chronic inflammation in the bladder mucosa, promoting tumor progression.

Methods: Female and male aging (12-month-old) mice were exposed to N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN) carcinogen with simultaneous B cell depletion using a panel of B cell depleting antibodies. Systemic immune profiling was conducted at multiple time points using flow cytometry. Whole bladder sections were subjected to hematoxylin and eosin (H&E) and multiplex immunofluorescence staining.

Results: Increased recruitment of atypical B cells (ABCs) was observed following continuous exposure to BBN. B cell depletion during BBN exposure resulted in reduced inflammation and delayed cancer progression. B cell depleted mice exhibited histologically benign or close to normal urothelium, whereas untreated and mice injected with isotype control antibodies showed reactive atypia or dysplasia. Both systemic and local immune profiling depicted sex differences, with female mice having a higher number of splenic total and atypical B cells and showing increased density of both populations in the bladder tumor immune microenvironment compared to their male counterparts.

Conclusions: These results suggest that long-term depletion of B cells in aging mice leads to reduced inflammation in the bladder mucosa and delays disease progression.

Circulating tumor DNA in urothelial cancer patients, and FGFR-targeted therapy eligibility and resistance

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Introduction: The pan-FGFR-inhibitor erdafitinib is the first targeted agent approved in patients with metastatic urothelial cancer (mUC), along with an archival-tissue companion diagnostic; however, longitudinal sequencing studies have shown changes in FGFR alterations over time. Furthermore, erdafitinib resistance mechanisms in mUC are relatively unexplored. This multicentre study evaluated the use of cell-free DNA (cfDNA) compared to archival tumor tissue in mUC to assess a patient's FGFR status, and through serial sampling, to evaluate genomic mechanisms of erdafitinib resistance.

Methods: Patients with progressing mUC undergoing standard tissue testing for FGFR1-3 mutations and/or fusions and who had blood samples drawn during the management of their disease were eligible. Clinical routine testing was applied for tissue testing. We assessed cfDNA using deep sequencing of UC-specific gene loci.

Results: As of January 2023, 109 patients from six sites were enrolled. Median age at diagnosis was 71, 36% had upper urinary tract primaries, and 78% were male. Most (73%) patients had detectable levels of circulating tumor DNA. Analysis of tumor tissue and cfDNA revealed a high concordance, with only four differing findings comprised of one negative cfDNA test in a patient whose tumor tissue showed a FGFR3-TACC3 fusion and three positive cfDNA test results in patients with FGFR alteration negative tissue tests. To date, four patients have had cfDNA samples taken upon progression after erdafitinib treatment. In one of these, we detected several tumor subpopulations developing variations of the same FGFR3 gatekeeper mutation V555L/M. Additionally, this patient gained another FGFR3 gatekeeper mutation, N540K, and a de novo FGFR3-TACC3 fusion under therapy.

Conclusions: In this preliminary analysis, cfDNA was a valuable addition to tissue-based assays for determining somatic FGFR alteration status — potentially giving additional patients a chance to benefit from FGFR-targeted therapy. Our explorative analyses help to decipher erdafitinib therapy resistance in mUC.

Classification of micropapillary and urothelial carcinoma using artificial intelligence-based histopathology image analysis

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Introduction: Micropapillary urothelial carcinoma (MPC) is a rare, aggressive histological subtype that frequently co-occurs with conventional urothelial carcinoma (UC) and comprises 2–5% of all bladder cancers. Proper identification and reporting of the proportion of MPC subtype are crucial for optimal risk stratification and treatment selection. Given the complex histological features of MPC and moderate (κ:0.54) interobserver agreement among genitourinary (GU) pathologists, an artificial intelligence (AI) tool that can not only reliably identify this subtype but also report the proportion of it within a sample would be a valuable resource for pathologists and clinicians.

Methods: For the training dataset, we identified 29 MPC patients (with 128 whole slide images, [WSIs]) and 57 UC patients (with 134 WSIs). For the validation dataset, we obtained 88 MPC cases (with 88 WSIs) and 72 UC (with 72 WSIs) from across British Columbia. One GU pathologist annotated the MPC and UC-containing regions in all WSIs. Due to differences in scanners and staining conditions, the validation dataset occupies a slightly different color space than the original dataset. We fed the data into a ResNet18 model using three-fold cross-validation, which trained to classify these subtypes based on slide-level labels, with each WSI labelled as either MPC or UC. To improve model generalization to the validation dataset, we normalized the external color space based on reference images from the training dataset.

Results: Our classification of UC vs. MPC cases using AI attained 91.0% slide-level accuracy on the training dataset and 85.6% ($p<0.032$) slide-level accuracy on our validation dataset.

Conclusions: Using an AI-based approach for histopathology image classification, we have successfully classified MPC and UC without the use of manual pathologist annotations for identifying tumor regions. These findings demonstrate AI as a promising tool for the diagnosis of this rare and aggressive subtype of urothelial carcinoma. Future work will seek improvement of our AI algorithm to attain higher accuracy, and further validation in an external dataset. Furthermore, we will include additional histological subtypes.

Systemic BCG immunotherapy enhances antitumor immunity in a mouse model of bladder cancer: Novel role of trained immunity

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Introduction: Despite decades of intravesical bacillus Calmette-Guérin (BCG) administration for the management of higher-risk non-muscle-invasive bladder cancer (NMIBC), the mechanism of this immunotherapy is not fully understood. Mounting evidence indicates that systemic immune activation is required for maximal immunotherapeutic benefit, and intravesical BCG alone may not result in sufficient systemic immune activation and hence optimal antitumour responses. Here, we compared the effect of intravesical BCG vs. intravenous BCG (to maximize systemic immune activation) on antitumor immune responses.

Methods: Using a syngeneic orthotopic mouse model of NMIBC, we demonstrate a reduction in tumor volume and skew towards an antitumor microenvironment in mice that were treated intravenously with BCG compared with those that received intravesical BCG.

Results: Our results indicate that, in the absence of a tumor, intravesical BCG treatment leads to increased proportion of neutrophils in the bladder, which have been associated with a tumor-permissive niche. Systemic administration of BCG also led to antitumor tumor-draining lymph node and bone marrow microenvironments. The ability of BCG to induce trained immunity (TI), a heterologous form of memory acquired by innate immune cells, has recently been characterized. To investigate the potential contribution of TI to these effects, trained or untrained macrophages were co-instilled with MB49-OVA tumor cells into the bladders of mice. The presence of trained macrophages augmented adaptive immune responses, as a greater proportion of tumor-specific $CD8^+$ T cells were present in the bladders of these mice, compared with those instilled with untrained macrophages. Furthermore, BCG-trained dendritic cells exhibited enhanced antigen uptake, presentation, and were able to promote proliferation of $CD8^+$ T cells.

Conclusions: Understanding the link between systemic immunity, TI, and the TiME following BCG therapy may facilitate the development of new approaches to improve outcomes and reduce recurrence rates in patients with NMIBC.

The role of the urinary microbiome in determining response to intravesical bacillus Calmette-Guérin in high-risk non-muscle-invasive bladder cancer

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Introduction: Intravesical bacillus Calmette-Guérin (BCG) remains the first-line treatment for high- and intermediate-risk non-muscle-invasive bladder cancer (NMIBC); however, up to 40% of patients recur despite optimal BCG therapy. Given pre-existing evidence that commensal microbiomes can affect inflammatory and immune responses, we hypothesized that the urinary microbiome of the bladder may affect the BCG-induced antitumor immune response. The objective of this study was to determine whether the urinary microbiome composition impacts BCG responsiveness in patients and to understand its impact on the BCG-induced immune response using an in vivo mouse model.

Methods: Urine and stool samples were collected from patients with high-risk NMIBC before and after first BCG treatment to identify the composition of the urinary and gut microbiomes through metagenomic sequencing. To assess the role of the urinary microbiome on the BCG-induced inflammatory response, mice underwent ultrasound-guided instillation of BCG into the bladder lumen. The mice had either a healthy or a disrupted urinary microbiome. Disruption was achieved through gentamicin instillation prior to BCG treatment. Whole bladder, spleen, and bladder-draining lymph node tissue was collected to assess changes in immune cell populations through fluorescence-activated cell sorting (FACS) and histology.

Results: 16s rRNA sequencing from 32 patients demonstrated a loss in alpha diversity of both urinary and gut microbiome after treatment. Furthermore, BCG resulted in a significant change of urinary microbiome beta diversity. FACS analysis from in vivo studies observed a significant shift in immune cell populations. Gentamicin instillation induced a pro-inflammatory environment through elevated M1 and reduced M2 macrophages, compared to control; however, subsequent BCG treatment shifted the environment towards an anti-inflammatory response by increasing M2 and reducing M1 macrophages, which was also observed in mice with a healthy microbiome.

Conclusions: In patients, BCG induces changes to the composition of the urinary and gut microbiomes. Preliminary findings from in vivo studies demonstrate that disruption of the urinary microbiome alters the immune

cell environment, which can be shifted following BCG treatment. This suggests that the urinary microbiome may play a role in mediating the BCG-induced immune response.

Association between histone H3 lysine 4 trimethylation in circulating monocytes and recurrence-free survival of patients with bladder cancer following BCG induction therapy

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Introduction: The mode of bacillus Calmette-Guérin's (BCG) action in the treatment of patients with non-muscle-invasive bladder cancer (NMIBC) is not fully understood. We recently provided evidence supporting an association between the acquisition of BCG-induced trained immunity (TI) in circulating monocytes and disease-free survival. Trained immunity is a form of epigenetic memory acquired by innate immune cells following exposure to inflammatory molecules. Acquisition of TI often involves histone 3 lysine 4 trimethylation (H3K4me3) at promoter regions of inflammatory genes.
Methods: We conducted chromatin immunoprecipitation followed by DNA sequencing (ChIP-seq) on monocytes from seven patients treated with BCG (four with early recurrences and three recurrence-free after one year) to determine the genome-wide distribution and abundance of H3K4me3 prior to and after five weeks of induction therapy.
Results: Genome-wide H3K4me3 profiles obtained before or after BCG induction distinguished patients who suffered from early recurrence from those remaining recurrence-free after one year. Furthermore, levels of H3K4me3 at genes involved in specific pathways were increased in the recurrence-free group vs. the early recurrence group. When independently quantified at single-gene levels, elements of the Wnt and AMPK signaling pathways showed increased levels of H3K4me3 in the recurrence-free group before initiation of BCG treatment, while elements of the MAPK showed increased levels after five weeks of BCG induction in the same group. Validation of these genes on an independent cohort of seven more NMIBC patients (four recurrence-free and three with early recurrences) undergoing BCG immunotherapy showed that increased levels of H3K4me3 on genes from the MAPK pathway were maintained after five weeks of BCG treatment in the recurrence-free group.
Conclusions: These findings indicate that recurrence-free survival following BCG immunotherapy for NMIBC is associated with the accumulation of H3K4me3 at specific gene loci and could lead to the identification of prognostic biomarkers.

Molecular subtyping to stratify the treatment of muscle-invasive bladder cancer: A cost-effectiveness analysis

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Introduction: The gold standard treatment for muscle-invasive bladder cancer (MIBC) is neoadjuvant chemotherapy (NAC) followed by radical cystectomy; however, response to NAC is unpredictable. Molecular subtypes allow for an improved ability to select a tailored treatment course. Our study aimed to assess the cost-effectiveness of molecular subtyping in the management of MIBC.
Methods: A two-dimensional Markov microsimulation model was created to evaluate the management of MIBC using TreeAgePro2019. Three strategies were included in the primary analysis: universal use of NAC, NAC at current usage rates (36%), and molecular subtype-directed care. Seiler classification of subtyping in the molecular subtype directed care arm, as it had an operational commercially available test. Our base case was an adult patient with MIBC (cT2-4N0M0) appropriate for NAC and RC.
Results: A total of 150 000 simulations were completed. The predicted QALYs were 8.73, 8.34, and 9.14, with costs of $76 962, $62 478, and $62 579 for universal NAC usage, NAC at current usage rates, and subtype-directed care, respectively. When comparing subtype-directed care to current rates of NAC usage, the ICER was $127/QALY. Subtype-directed care dominated universal NAC usage. The probabilistic sensitivity analysis scatterplot is demonstrated in Figure 1, illustrating that subtype-directed care, on average, provides the greatest number of QALYs at consistently lower costs than universal NAC use and similar costs to the current NAC usage rate strategy.
Conclusions: We demonstrated that in patients with MIBC, a molecular subtype-directed approach to the administration of NAC can result in improved overall survival, greater QALYs, and be cost-effective within

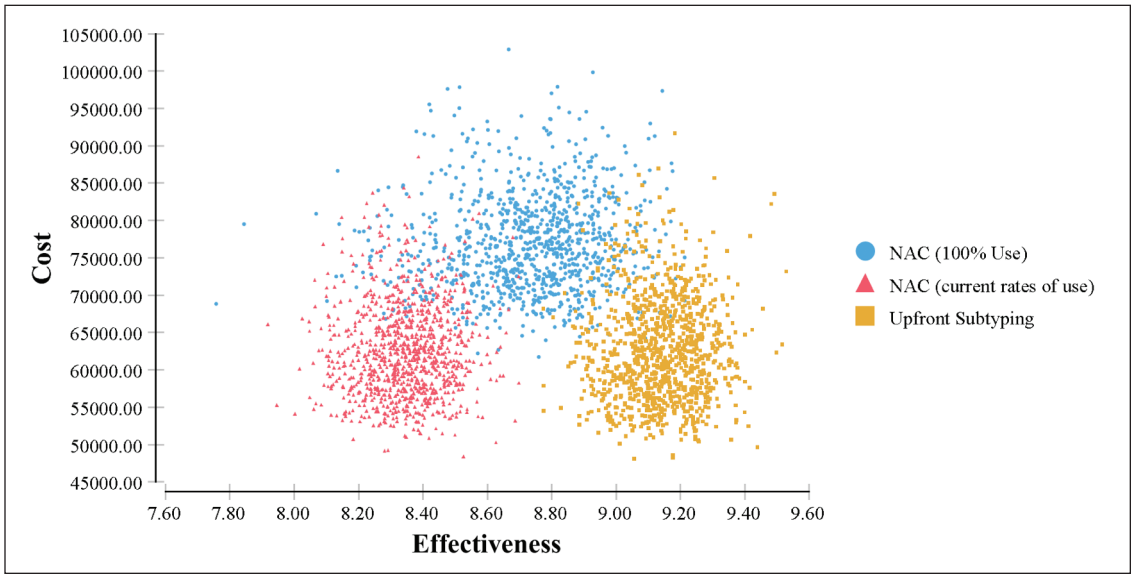

Figure 1 (Magee et al). Cost-effectiveness scatterplot.

a single-payer healthcare system. A push for the universal use of NAC will result in improved survival compared to what current rates of use achieve but is likely not the best approach considering the drawbacks of chemotherapy, including toxicity and unequal response. This model is built upon the available literature and requires further validation prior to clinical implementation but it demonstrates that personalized medicine is a feasible option.

Genomic dissection of *ERBB2* as a predictive biomarker in metastatic urothelial carcinoma

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Introduction: *ERBB2* (HER2) alterations are common in urothelial cancer (UC) and increase in frequency with disease stage; ~20% of metastatic tissue samples harbor *ERBB2* mutation or amplification. While prior clinical trials of HER2-targeted therapies in UC were largely unsuccessful, recent HER2 antibody-drug conjugates show greater promise. Accurate biomarker-driven patient selection will be critical to enable a new generation of clinical trials and optimize the clinical benefit of HER2-targeted therapy. Previously, we demonstrated that *ERBB2* alterations in circulating tumor DNA (ctDNA) can reflect clinically relevant tumor characteristics, including high HER2 protein expression. Here, we aimed to evaluate the heterogeneity of *ERBB2*-altered tumors via ctDNA, and to reveal critical genomic variables that could modulate response to HER2-directed strategies in UC.

Methods: In this retrospective analysis, we used a custom targeted sequencing panel covering >50 UC relevant genes to profile 412 plasma cell-free DNA samples from 236 metastatic UC patients. Patient-matched leukocyte DNA was profiled for all patients as a germline control.

Results: 77% of patients had evidence of ctDNA in ≥1 blood collection. Protein-altering *ERBB2* mutations were identified in 14% of evaluable patients; 61% fell in known oncogenic hotspot loci. *ERBB2* copy amplification was detected in 8% of patients, or 9% when excluding patients with low tumor fractions, precluding accurate evaluation of copy number. Leveraging genome-wide and dense targeted probes, in a subset of patients we resolved ploidy and *ERBB2* locus amplification structure, revealing genomic breakpoints on chromosome 17 and cases of tandem duplication resulting in >50 copies of *ERBB2*. Clonality and allele-specific imbalance analyses are in progress.

Conclusions: Here, we demonstrated the feasibility of a ctDNA-based approach for determination of nuanced *ERBB2* biomarker status in metastatic UC. Ongoing work is comparing ctDNA results to mRNA and immunohistochemistry from archival tumor tissue samples. Moving forward, consideration of *ERBB2* as a non-binary biomarker will be important for a refined understanding of response to HER2-targeted therapy.

Clinical complete response as critical predictor for muscle-invasive bladder cancer outcomes and survival post-radiation-based bladder-preservation therapies

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Introduction: Bladder preservation using radiotherapy (RT), usually with radiosensitizing chemotherapy, for muscle-invasive bladder cancer (MIBC) is an alternative to radical cystectomy in select patients. This study investigated role of clinical complete response (CR) in predicting disease control and survival in a modern, node-negative MIBC cohort.

Methods: Between 2003 and 2017, 115 patients with cT2-4N0M0 MIBC were treated with curative intent RT (50–66 Gy in 20–33fractions) ± chemotherapy. Post-treatment CR was assessed with cystoscopy and imaging. Invasive locoregional recurrence-free survival (LRFS), distant metastasis-free survivals (DMFS), and overall survival (OS) at three years in patients with CR vs. non-CR were compared using Kaplan-Meier methods. Univariable (UV) and multivariable (MV) Cox proportional hazards (PH) were performed to assess association between outcomes with prognostic factors: CR, cT-stage (cT2 vs. cT3-4), carcinoma in situ (CIS) presence, tumor size <5 cm, hydronephrosis, concurrent chemotherapy, (neo)adjuvant chemotherapy, complete TURBT, and ECOG score.

Results: Median age was 76 (IQR 66–82) years. Median followup was 3.3 (IQR .2–6.7) years. Most patients had cT2 (77%), 17% had cT3, and 6% had cT4 disease. Concurrent chemotherapy was received by 72%, neoadjuvant in 28%, and adjuvant in 2% patients. CR was achieved in 64% of patients. At three years, patients with CR had superior LRFS (90.0% vs. 44.0%), DMFS (87.9% vs. 44.7%), and OS (92.0% vs. 63.2%) (all log-rank p<0.001). Non-CR was the strongest significant predictor of worse outcomes in UV (all p<0.001) and adjusted MV analyses: LRFS: hazard ratio (HR): 5.26 (95% confidence interval [CI] 2.76–18.61, p<0.001); DMFS: HR: 5.36 (95% CI 2.29–12.57, p<0.001); and OS: HR: 5.60 (95% CI 2.14–14.65, p<0.001). CIS presence also predicted LRFS: HR: 2.95 (95% CI 1.32–6.63, p=0.009); but CR was the sole significant DMFS and OS predictor in MV analyses. Concurrent chemotherapy use was the sole factor significantly associated with CR (odds ratio 3.43, p=0.011).

Conclusions: Post-treatment non-CR predicted for worse locoregional/distant recurrence and survival in this MIBC cohort. Concurrent chemotherapy with RT improves CR rates. Non-CR patients may benefit from further treatments, such as post-treatment immunotherapy and consideration for salvage cystectomy.

Multi-omic profiling of metastatic urothelial carcinoma in patients exhibiting exceptional response to systemic chemotherapy

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Introduction: Metastatic urothelial carcinoma (mUC) is an aggressive malignancy with limited therapeutic options and poor prognosis; however, a small subset of mUC patients have durable responses to systemic chemotherapy. We molecularly profiled tumors in a cohort of mUC patients with exceptional response (ER) to treatment (ER-mUC) to understand potential therapeutic vulnerabilities. We aimed to establish

the rationale for larger studies focusing on predictive and prognostic profiles of ER.

Methods: Nine mUC patients with ER (defined as clinical complete or partial response to systemic chemotherapy lasting ≥18 months) were identified in British Columbia. We performed whole-transcriptome and targeted-DNA sequencing, and multicolor immunohistochemistry (mIHC) on all available specimens (N=18). For all patients, we included tumor tissues immediately preceding the metastatic event. For 6/9 patients we included historic (non-metastatic) serial surgical specimens. For comparison, we analyzed two published studies comprising a chemo-naive muscle-invasive bladder cancer (MIBC) cohort (MIBC-TCGA2017) and a non-ER mUC cohort (nonER-mUC).

Results: Targeted sequencing showed mutations or copy number alterations in 27/60 genes across the ER-mUC cohort. Similar to the MIBC-TCGA2017 and the nonER-mUC cohorts, the most commonly mutated genes were *TP53*, *KDM6A*, *KMT2D,* and *ARID1A*. Mutations or deletions in *TP53* were enriched in the ER-mUC compared to the MIBC-TCGA2017 and nonER-mUC cohorts (80%, 48%, and 60%, respectively). Mutations in DNA damage repair (DDR) genes were more frequent in ER-mUC patients compared to MIBC-TCGA2017 and nonER-mUC patient cohorts (50%, 28%, and 26%, respectively), with *ERCC2* being the most commonly mutated among those. Most (75%) ER-mUC tumors were classed as luminal by RNA analysis. In contrast, 47% and 44% of patients of the MIBC-TCGA2017 and nonER-mUC cohort, respectively, were luminal. mIHC revealed ER-mUC tumors had increased CD8+GzmB+/FoxP3+ cell populations compared to a cohort of MIBC patients treated with neoadjuvant chemotherapy (NAC).

Conclusions: Our results suggest that therapeutic success in the context of these rare ERs may be multifactorial and conferred by aggressive tumor profiles, with multiple molecular mechanisms, including DDR gene alterations, specific transcriptomic subtype, and immune microenvironment. Contrary to previous work in the context of NAC, luminal tumors are enriched among the ER-mUC cohort. This work highlights the need for multi-omic platform analyses of a larger number of ER-mUC cases.

Investigating sex differences in CD79a+ tumor-infiltrating B cells, tertiary lymphoid structures, and response to BCG in patients with non-muscle-invasive bladder cancer

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Introduction: Despite a higher incidence in males, female patients with NMIBC generally present with advanced disease and display earlier recurrence and progression post-treatment compared to males. Recurrence post-treatment with intravesical bacillus Calmette-Guérin (BCG) occurs in over 50% of patients. Our previous study showed that higher density of intra-tumoral CD163+ macrophages and B cells is present in patients with shorter recurrence-free survival (RFS). Given the established sex differences in B cell-associated mucosal immune physiology, in this study, we evaluated the patterns of B cell infiltration and tertiary lymphoid structure (TLS) formation in pre-treatment tumors from female and male patients with NMIBC.

Methods: Spatial immunophenotyping of pre-treatment tumors from 173 patients (n=34 females and 139 males) treated with BCG at the Kingston Health Sciences Centre, was performed using multiplex immunofluorescence assay. The density and spatial distribution of CD79a+ B cells, CD103+ tissue resident memory T cells, Ki67+CD8+ cytotoxic T cells, CD163+ M2 macrophages, including the checkpoints PD-1 and PD-L1, was determined via automated evaluation and manual validation. Hematoxylin and eosin-stained whole sections from a subset of immunophenotyped tumors were evaluated for the presence and organization of TLSs. Immune cell abundance was analyzed using R version 4.1.2. Survival and multivariate analyses were performed using survminer, survival, and finalfit packages. Differences in immune cell density between response groups were tested using the Mann-Whitney U test. Differences in survival were time-to-event analyses were performed using Kaplan-Meier and Cox proportional hazards analyses.

Results: In this cohort, female patients treated with BCG exhibited worse RFS and progression free survival (PFS) compared to their male counterparts. Tumors from BCG-refractory patients exhibited higher density of both stromal B cells and M2 like macrophages compared to BCG responders. Notably, high stromal CD79a+ B cell density was significantly associated with higher tumor stage and shorter PFS in a multivariable Cox proportional hazards model that considers gender, age, AUA risk score, and BCG treatment. High densities of CD8+ cytotoxic T cells, as well as CD103+ tissue resident memory T cells in either compartment associated with poor RFS.

Conclusions: Overall, this study suggests a potential role of B cells in mediating poor response to BCG immunotherapy with a more pronounced influence on female patients compared to males with NMIBC.