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MP-5.01

Outcome of 304 clinical T1G3 bladder cancer patients treated with radical cystectomy: the Canadian Bladder Cancer Network experience

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Introduction and Objective: Radical cystectomy may provide the optimal survival outcomes in the management of clinical T1G3 bladder cancer. We present our data from a large, multi-institutional, contemporary Canadian series of patients who underwent radical cystectomy for clinical T1G3 bladder cancer in a single-payer health care system.

Materials and Methods: We collected and pooled a database of 2287 patients who underwent radical cystectomy between 1993 and 2008 in 8 different centres across Canada. Three hundred and four of these patients had clinical T1G3 bladder cancer. Collected variables included age, race, gender, presence of hydronephrosis, clinical stage and nodal status, concomitant carcinoma in situ, histology, ECOG performance, smoking, pelvic lymph node dissection, pathological stage and nodal status, grade, surgical margins, postoperative chemotherapy/radiation, recurrence and salvage therapy. Survival data were analyzed using Kaplan–Meier method and Cox regression analysis.

Results: The median age of patients was 67 years with a mean follow-up time of 35 months. The 5-year overall, disease-specific and disease-free survival was 71%, 77% and 59%, respectively. The 10-year overall, disease-specific and disease-free survival was 60%, 67% and 50%, respectively. Pathological stage distribution was p0: 32 (11%), pT1: 78 (26%), pT2: 55 (19%), pT3: 60 (20%), pT4: 27 (9%), pTa: 16 (5%), pTis: 28 (10%), pN0: 215 (74%) and pN1–3: 78 (26%). Only 12% of patients were given adjuvant chemotherapy. On multivariate analysis, only margin status and pN stage were independently associated with overall, disease-specific and disease-free survival.

Conclusion: These results indicate that clinical T1G3 bladder cancer may be significantly understaged. Identifying factors associated with understaged and/or disease destined to progress, despite any prior intravesical or repeat transurethral therapies before radical cystectomy, will likely be critical to improve survival outcomes without overtreating clinical T1G3 disease that can be successfully managed with bladder preservation strategies.

MP-5.02

Stage review is indicated in pT1 bladder cancer

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Introduction and Objective: The clinical course of patients with pT1 bladder cancer is highly variable. We evaluated the impact of pathology review on the clinical outcome of a large series of primary pT1 bladder cancer patients treated with BCG.

Materials and Methods: The slides of 164 primary (first diagnosis) pT1 bladder tumours from 2 university hospitals (Rotterdam, the Netherlands, $n = 69$ and Toronto, Canada, $n = 95$) were reviewed by 1 pathologist for stage and grade (WHO 1973 and 2004). All 164 patients were initially managed conservatively (BCG). The clinical outcome (progression and disease-specific survival) was analyzed using Kaplan–Meier statistics. Multivariate analyses were done to compare the predictive value of variables (age, gender, hospital, CIS, tumour size, reviewed grade, reviewed stage).

Results: Mean follow-up was 6.4 (median 5.8; range 0.3–21.6) years, 29 patients were female. Mean age was 69 years at first diagnosis. CIS was found in 55 (34%) cases. Fifty-two patients remained recurrence-free (32%). Cystectomy was performed in 34 (21%) patients; muscle invasion was found in 27 of these patients. Progression to pT2 or metastasis was observed in 48 (29%) patients and 26 patients (16%) died of their disease. After review of the slides, 24 (15%) tumours were down-staged to pTa, 134 (82%) remained pT1 and 6 (4%) were up-staged to pT2. We found no difference in the 2 hospitals for stage review ($\chi^2 p = 0.33$) and outcome (log-rank $p = 0.69$ (progression) and 0.87 (disease-specific survival)). Grade review resulted in 74 G2 and 89 G3 lesions according to the 1973 system and 37 low-grade and 126 high-grade lesions according to the 2004 system. In multivariate analyses, reviewed stage ($p < 0.001$) and CIS ($p = 0.016$) proved of independent significance for progression and reviewed stage ($p < 0.001$) and CIS ($p = 0.023$) for disease-specific survival.

Conclusion: Stage-review is indicated in pT1 bladder cancer because it identifies patient-groups with a different prognosis. We confirmed that CIS is an unfavourable sign in pT1 bladder cancer.

MP-5.03

Does a stage-based surveillance strategy result in better survival outcomes after radical cystectomy?

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Introduction and Objective: Surveillance strategies following radical cystectomy for bladder cancer are implemented to identify long-term complications, metabolic abnormalities and renal deterioration and to achieve early detection and treatment of recurrent disease to optimize survival outcomes. Most surveillance strategies are stage-based, but it is not known if these strategies result in better survival outcomes over symptom-driven evaluation and therapy. Our objective was to determine if a stage-based surveillance strategy impacted on survival outcomes following radical cystectomy for bladder cancer.

Materials and Methods: A retrospective review of 187 patients that underwent radical cystectomy for bladder cancer from July 2001 to June 2008 was undertaken. All patients with disease recurrence were studied. The surveillance for all patients involved history, physical examination, CXR and blood work every 6 months. Urine cytology was performed annually. Computed tomography of the abdomen and pelvis was obtained on patients with pT3–4 disease annually. Additional investigations for symptomatic patients were directed toward their specific symptoms. Survival outcomes were analyzed using the Kaplan–Meier actuarial methodology and compared asymptomatic versus symptomatic patients.

Results: There were 47 patients who had disease recurrence occurring in the following sites (no. of patients): presacral (2), psoas muscle (1), pelvic sidewall (4), urethra (2), lung (4), bone (8), bowel (4), brain (1), multiorgan (2), retroperitoneal lymphadenopathy (7), pelvis (4), not documented (4), inguinal lymph nodes (2) and pelvis (2). Twenty-eight patients had symptoms at recurrence. Twenty-six of these patients had pain as a symptom. Nineteen patients had asymptomatic recurrences identified with routine surveillance. One patient with multiorgan recurrence also has

upper tract recurrent urothelial carcinoma. There was no difference in overall and disease-specific survival in asymptomatic versus symptomatic recurrences ($p = 0.36$ and $p = 0.29$, respectively). There was no difference in overall and disease-specific survival in patients with painful versus painless recurrences ($p = 0.60$ and 0.53 , respectively). The median follow-up for these 47 patients was 12.5 (range 0.3–50) months.

Conclusion: The stage-based surveillance strategy used in this patient population did not provide better survival outcomes over symptom-driven evaluation and therapy. Further prospective evaluations of stage-based surveillance strategies are necessary to determine if they result in better survival outcomes following radical cystectomy.

MP-5.04

Impact of histology on outcomes of radical cystectomy: a series from the Canadian Bladder Cancer Network

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Introduction and Objective: Urothelial carcinoma (UC) represents the most common bladder cancer pathology. Squamous and adenocarcinomas constitute the majority of variant pathology. These tumours are felt to have similar outcomes stage-for-stage compared with UC. We compare the outcome of patients with variant histologies using data from a large multi-institutional data set.

Materials and Methods: Between 1993 and 2008, 2287 patients underwent radical cystectomy at 8 different centres across Canada and had their data recorded in institutional databases. Of these, 2159 had the pathological type indicated. Patients were categorized into 3 groups, UC ($n = 1887$), UC + variant ($n = 62$) and variant ($n = 210$). Other variables recorded included age, gender, smoking history, clinical stage and nodal status, presence of hydronephrosis, pathological stage and nodal status, surgical margins, extent of pelvic lymph node dissection, grade, lymphovascular invasion and use of postoperative chemotherapy/radiation. Survival data were analyzed using Kaplan–Meier method and Cox regression analysis.

Results: The mean age of patients was 66.7 years with a mean follow-up time of 2.92 years. The 5-year overall survival (OS) was 56%, 39% and 65% (log-rank $p < 0.0001$) for those with UC, UC + variant and variant histology. The 5-year disease-specific survival (DSS) was 66%, 52%, 74% (log-rank $p = 0.0011$) and the 5-year recurrence-free survival (RFS) was 49%, 33%, 50% (log-rank $p = 0.0003$) for those with UC, UC + variant and variant histology. In multivariate analysis controlling for all recorded variables, histology significantly predicted for OS and RFS and approached significance ($p = 0.06$) in the prediction of DSS. Patients with UC + variant had 1.6, 1.7 and 1.6 times the hazard of overall death, bladder cancer death and recurrence relative to those with UC alone. Patients with variant histology only did not significantly differ in any outcome compared with those with UC alone.

Conclusion: Patients with variant histology bladder cancer have similar outcomes to those with urothelial tumours when controlling for other clinical and pathological factors. However, the presence of both urothelial and other elements in bladder cancer is associated with significantly decreased survival. Whether such patients with mixed histology would benefit from neoadjuvant or adjuvant multimodal treatment are potential questions for clinical trials.

MP-5.05

Cost analysis of single instillation of mitomycin after transurethral resection of bladder tumour in the Quebec health care system

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Introduction and Objective: The currently recommended management strategy for recurrent superficial urothelial carcinoma (UC) of the bladder involves a single postoperative instillation of mitomycin-C; however, a minority of the patients receive it partly due to potential associated costs. We performed a 5-year cost-analysis of this strategy within the Quebec health care environment.

Materials and Methods: A decision-analytical model was used to evaluate the health-economic consequences of single-dose mitomycin after transurethral bladder tumour resection (TURBT). Input estimates for 5-year recurrence rates (50%) and mitomycin efficacy (absolute risk reduction of 15%) were identified via literature search, and data from meta-analyses was used. The 2008 treatment costs were determined from current Quebec health care policies, including physician fees, mitomycin drug costs, TURBT and cystoscopy costs, and institutional hospital admission. A sensitivity analysis was also performed. Costs associated with work absence and productivity losses were not included. The model was limited to a 5-year follow-up period, and inherent assumptions included low associated morbidity and mortality, and no crossover between groups. The indifferent rates of progression among treatment groups did not impact the cost analysis and were thus excluded from our model.

Results: Overall 5-year analysis reveals that the 5-year cumulative cost of the TURBT plus mitomycin strategy is more cost-effective, saving the Medicare system \$189.00/ patient over 5 years (\$8571.00 and \$8382.00 for TURBT v. TURBT + Mitomycin, respectively). Calculated differences take into account the avoidance of cystoscopic surveillance, urinary cytology and reoperative follow-up costs associated with multiple recurrences, as compared with less frequent occurrences in the mitomycin group. The sensitivity analysis revealed a dominance of mitomycin use over TURBT alone.

Conclusion: Routine use of mitomycin after TURBT is not associated with increased costs to the health care system. In fact, there is a significant cost savings to the health care system over a 5-year follow-up period. Additionally, nonquantified patient benefit and societal advantages of gained wages and productivity owing to decreases in recurrence and surgery could further alter the cost-analysis model, resulting in an increasingly favourable cost benefit. The clear sensitivity analyses further underscores the robustness of our findings.

MP-5.06

Evaluation of fluorodeoxyglucose positron emission tomography associated with computed tomography (¹⁸F-FDG-PET/CT) imaging for staging of bladder transitional cell carcinoma

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Introduction and Objective: Positron emission tomography with ¹⁸F-FDG has been considered of limited value because of the urinary excretion of the tracer. The purpose of this study was to investigate the role of PET associated with computed tomography (CT) in the staging of new diagnosed bladder cancer (BC) or in restaging of BC during follow-up after cystectomy or chemotherapy using furosemide and oral hydration to remove the excreted ¹⁸F-FDG from the bladder.

Materials and Methods: Thirty-two patients (23 male, 9 female), with histologically proven muscle-invasive BC by transurethral resection of the bladder (TURB), were included in this prospective study. Mean age was 69 years and mean follow-up was of 4.7 (0.3–10.3) months. All underwent an ¹⁸F-FDG from the head to the upper thighs with additional pelvic images after 1 hour IV furosemide and oral hydration at least 3 months after TURB. In 25 cases a CT of the thorax and abdomen with contrast medium had also been performed within the 2 weeks before PET/CT. For CT scan, nodes greater than 1 cm or defined as suspicious by the radiologist were considered positive. The ¹⁸F-FDG-PET/CT lesions with

metabolic activity greater than 2.5 SUV on a confirmed anatomical structure were considered positive. Imaging findings were confirmed by histology or if not possible, by imaging follow-up.

Results: Of the 32 patients, 26 patients were studied with ^{18}F -FDG-PET/CT before radical cystectomy or, in 2 cases, only pelvic lymphadenectomy. In the same group, 19 CT were also performed. ^{18}F -FDG-PET/CT detected 21 of the 23 bladder lesions (91.3%) and 8/15 (53.3%) pelvic node metastasis, mostly N2. False negative were a pTis and a pT4. Computed tomography detected 12 of 17 bladder lesions (70.6%) and 4 out of 9 positive nodes (44.4%). ^{18}F -FDG-PET/CT showed prostate metabolic activity in 4 cases. In 2 cases BC invasion was histologically proven. Metabolic activity was seen in 2 para-aortic lymph nodes (LN), 3 mediastinal LN, 2 cervical LN and 4 pulmonary nodules. All these lesions showed progression on imaging follow-up. Computed tomography was negative in all prostate lesions, 1 para-aortic LN and 2 pulmonary nodules.

Conclusion: This prospective series shows that ^{18}F -FDG-PET/CT with furosemide wash out of the bladder was better than conventional CT for the detection of residual tumour in the bladder, pelvic LN metastasis and prostate infiltration. Moreover PET/CT detected earlier than conventional CT distant metastasis to retroperitoneal and cervical LN and pulmonary metastasis.

MP-5.08

Stage distribution at radical cystectomy during the last 2 decades

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Introduction and Objective: Improvements in diagnostic tools during the last 2 decades may have resulted in a more favourable stage distribution of bladder cancer at radical cystectomy (RC). We examined the pathological stage distribution at RC over the last 2 decades in a large population-based cohort and assessed the effect of the year of treatment on cancer-specific survival after RC for transitional cell carcinoma (TCC) of the urinary bladder.

Materials and Methods: We identified 5602 TCC cases treated with RC in 9 SEER registries. Survival plots and Cox regression models addressed the effect of year of treatment at RC on stage at RC. Covariates consisted of age, gender, race, pathological stage and grade.

Results: Distribution of non-organ confined disease at RC was 79.2% for patients treated between 1988 and 1992 ($n = 1355$, 24.2%), 82.8% in 1993–1997 ($n = 1535$, 27.4%), 85.8% in 1998–2001 ($n = 1545$, 27.6%) and 86.8% in 2002–2004 ($n = 1167$, 20.8) [χ^2 trend test $p < 0.001$]. The 2-year cancer-specific survival rates according to the year of treatment categories were as follows: 79.3% versus 76.1% versus 76.7% versus 71.5% ($p < 0.001$). In multivariable analyses, the risk of cancer-specific mortality was 1.02-fold higher in patients treated between 1993 and 1997 compared with those treated in 1988–1992, 1.006-fold higher in 1997–2001 and 1.3-fold higher in 2002–2004 ($p = 0.03$).

Conclusion: At radical cystectomy, a more advanced pathological stage was recorded in recent years compared with 2 decades ago. Our results show that efforts should be made to diagnose TCC at an earlier stage, with the hope of achieving better survival outcomes.

MP-5.09

NMP22 is predictive of recurrence in high-risk superficial bladder cancer patients

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Introduction and Objective: High-risk urothelial carcinoma of the bladder (UC) that has not reached the muscularis propria include tumours that are high grade, T1, multiple, greater than 3 cm in size, recurrent at the 3-month cystoscopy and carcinoma in situ. The NMP22 assay has been shown to have greater sensitivity for the diagnosis and detection of recurrent bladder tumours over that of traditional urine cytology and NMP22 has shown increased predictive values if used in conjunction with behavioural and symptomatic data. We assessed the use of NMP22 to predict which

high-risk superficial bladder cancer patients will have recurrence, progression or disease-related death and compared this to standard urine cytology.

Materials and Methods: One hundred consecutive patients with high-risk UC were enrolled. During surveillance, urine was collected for cytology and NMP22 testing. Patients were subsequently followed up for at least 12 months. Retrospective chart review was undertaken to collect data on previous tumour history, tumour characteristics, disease recurrences, progression and death. Kaplan–Meier analyses were performed to determine the significance between NMP22 positive and negative patients in terms of recurrence-free, progression-free and overall survival. Similar analyses were performed for urine cytology.

Results: From 94 eligible patients, 15 were NMP22 positive and 79 were NMP22 negative. The baseline characteristics between the 2 groups were not significantly different in terms of patient characteristics or intravesical therapies received. Mean recurrence-free survival time was significantly lower in the NMP22 positive group ($p = 0.038$); however, mean progression-free and overall survival were not significantly different between the 2 groups ($p = 0.297$ and 0.519 , respectively). Urine cytology demonstrated no significant predictive power for disease recurrence, progression or survival.

Conclusion: NMP22 appears to have predictive value for future tumour recurrences, but not progression or survival in patients with high-risk superficial UC.

MP-5.10

What is the long-term prognostic value of proapoptotic, antiapoptotic, proliferation and invasiveness molecular markers in patients treated with BCG for high-risk non-muscle invasive bladder cancer?

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Introduction and Objective: To evaluate the long-term prognostic value of proapoptotic, antiapoptotic, proliferation and invasiveness molecular markers in predicting the outcome of high-risk non-muscle invasive bladder cancer treated with intravesical BCG therapy. Most previous series have analyzed the short-term prognostic value of these markers only.

Materials and Methods: A prospective study included 42 patients presenting with high-risk non-muscle invasive bladder cancer (high grade or T1 tumours or multiple rapidly recurrent tumours refractory to intravesical chemotherapy) treated with transurethral resection and intravesical BCG. Transurethral tumour resection samples were analyzed for the molecular markers p53, p21 waf1/cip, Bcl-2, Cyclin D1 and metallothionein 9 (MMP9) using immunohistochemical techniques. Frequency of positivity measured as a percentage was then assessed for interaction with clinical tumour characteristics (stage, grade, multifocality, size) and the tumour outcome variables of recurrence and progression using univariate and multivariate analyses as well as Kaplan–Meyer curves (SPSS statistical package).

Results: There were 38 men and 4 women (mean age 68.3 yr) and median follow-up was 88 (mean 99, range 14–212) months. In this high-risk population, the overall recurrence rate was 61.9% and progression rate was 21.4%. As expected, in multivariate analysis, grade was predictive of recurrence and stage predictive of progression ($p < 0.05$). In multivariate analysis adjusting for tumour stage, grade, multifocality and size, the only predictor of recurrence-free survival was p21 ($p = 0.041$), while progression-free survival was predicted by Cyclin D1 ($p = 0.024$) and MMP9 ($p = 0.054$). Interestingly, the statistically significant predictive value of these markers at 5 years was maintained at longer follow-up both for recurrence and progression, MMP 9 only becoming of borderline significance ($p = 0.054$).

Conclusion: Long-term response to BCG therapy of high-risk non-muscle invasive bladder cancer may be predicted by molecular markers. These represent different elements of the carcinogenic pathway and may offer improved prediction compared with grade and stage alone. Larger series are needed to confirm these findings including new pathways like the FGFR-3.

MP-5.11

The combination of antisense oligonucleotides targeting heat-shock protein-27 with HTI-286 as a novel intravesical treatment for high-grade bladder cancer

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Introduction and Objective: The results of current intravesical chemotherapeutics are unfortunately insufficient and, therefore, novel and safe intravesical options for early stage high-risk bladder cancer are absolutely required to prevent both recurrence and progression. In this study, we demonstrated the efficacy of the intravesical combination treatment of antisense oligonucleotides (ASO) targeting heat-shock protein (HSP)-27 (OGX427) with HTI-286, a synthetic analogue of the marine sponge product hemiamsterlin.

Materials and Methods: We screened several bladder cancer cell lines for the synergic effect of OGX427 with HTI-286 by MTS assay and flow cytometric analysis. Then, in bladder cancer cell lines which showed synergic effect, the molecular mechanisms underlying the change of HTI-286-induced cytotoxicity by OGX427 were analyzed by Western blot analysis. The chemosensitivity against HTI-286 was also compared between mock-transfected T24 (T24 mock) cells and Hsp27-overexpressing T24 (T24 Hsp27) cells. To evaluate the possibility of clinical use, monitoring of orthotopic murine xenograft model by in vivo imaging system (IVIS) were used in vivo.

Results: OGX427 significantly enhanced cytotoxicity of HTI-286 in bladder cancer cell lines ($p < 0.05$). In KU7-luc cells, combination treatment of OGX427 with HTI-286 induced Akt inactivation and Bcl-2 downregulation. This Akt inactivation was observed in KK47 cells receiving the combination treatment, and also in KU7-luc cells treated by the combination of OGX427 with other chemotherapeutic agents. T24 Hsp27 cells were more resistant to HTI286 than T24 mock cells, and showed stronger Akt activation after HTI-286 treatment compared with T24 mock cells. As the protective effect of Hsp27 against HTI-286 was suppressed by LY294002, a phosphatidylinositol 3-kinase (PI3K) inhibitor, in T24 Hsp27 cells, the intervention of Hsp27-Akt pathway was the key mechanism to enhance chemosensitivity by OGX427. The intravesical instillation of this combination also effectively inhibited orthotopic tumour growth without any side effect ($p < 0.05$).

Conclusion: These results suggest that OGX427 enhances cytotoxicity of HTI-286 through Akt inactivation, and provide strong preclinical proof for the intravesical administration of OGX427 in a combination with HTI-286 for high-grade bladder cancer.

MP-5.12

Blockade of sonic hedgehog pathway inhibits the proliferation and contributes to the enhancement of chemosensitivity of bladder cancer cells

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Introduction and Objective: Although the sonic hedgehog (Shh) pathway contributes to the initiation and progression of various tumours, its role in bladder cancer is still unclear. In this study, we investigate the effect of blockade of Shh pathway using cyclopamine, a Shh antagonist, or antisense oligonucleotide (ASO) targeting Gli1 or Gli2, in bladder cancer.

Materials and Methods: The expression of several proteins in Shh pathway was assessed in human bladder tissue and various benign and malignant human bladder cell lines. To examine the effect of blockade of Shh pathway, KU7 and T24 bladder cancer cell lines were treated with either cyclopamine or transfected with Gli1 or Gli2 ASO; the effect on cell viability of Shh pathway inhibition was assessed by MTS assay and rates of apoptosis was assessed by flow cytometry. Furthermore, the effect of combination treatment of cyclopamine or Gli2 ASO with several chemotherapeutic was also assessed. In vivo, the effect and safety of intravesical cyclopamine instillation and the chemosensitizing effect of Gli2 ASO with paclitaxel was assessed using KU7 orthotopic or subcutaneous xenograft model.

Results: Gli2 expression was significantly higher in high-grade bladder

cancer tissues compared with normal urothelium in the TMA analysis ($p < 0.001$). In the bladder cancer cell lines tested, KU7 and T24 cells expressed high levels of Gli2. The blockade of SHH pathway suppressed antiapoptotic proteins, including Bcl-2, c-myc and cyclin D1, leading to the induction of apoptosis. Furthermore, blockade of Shh pathway enhanced chemosensitivity of those cells to cisplatin and paclitaxel. In vivo, the intravesical instillation of cyclopamine showed inhibitory effect against orthotopic KU7 tumour growth without any adverse effect ($p < 0.05$) and systematic administration of Gli2 ASO significantly enhanced chemotherapeutic effect of paclitaxel in a subcutaneous KU7 xenograft tumour model ($p < 0.016$).

Conclusion: The Shh pathway plays an important role in bladder cancer. The blockade of the Shh pathway inhibits cell proliferation, induces apoptosis and also enhances chemosensitivity in bladder cancer cells. The suppression of the Shh pathway, and especially Gli2, has the potential to be therapeutic target in bladder cancer and should be investigated further.

MP-5.13

Improved cancer specific-survival in patients with muscle-invasive bladder cancer expressing cyclo-oxygenase-2

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Introduction and Objective: In a large population-based case-control study, nonsteroidal anti-inflammatory drug (NSAID) users have been shown to have lower incidence of bladder cancer by 20%. Moreover, selective COX-2 inhibitors have been shown to have an antitumour effect in vitro and in vivo studies. The study objective was to evaluate the potential utility of COX-2 inhibition in secondary prevention of bladder cancer recurrence and progression.

Materials and Methods: The study population included 273 patients with muscle-invasive bladder cancers treated by cystectomy between 1983 and 2002. COX-2 expression was evaluated immunohistochemically with a monoclonal anti-COX-2 antibody (cat. 160112, Cayman Chemical Inc.). Results were correlated with risk factors influencing the disease-specific survival. Immunoreactivity was categorized as positive if COX-2 staining was greater than 5% tumour cells.

Results: The expression of COX-2 was not influenced by tumour stage, tumour grade or nodal status. The risk factors that influenced the disease-specific survival were patient age (HR 1.776 if age ≥ 70 yr; $p = 0.0011$), tumour stage (HR 2.245 for T4; $p < 0.0001$) and lymph node status (HR 1.668 for N1; $p = 0.045$ and HR 2.25 for N2; $p < 0.0001$) on multivariate analysis. Adjusting for these variables, COX-2 expression was associated with an increased disease-specific survival on univariate and multivariate analyses (HR 0.646 and $p = 0.0279$).

Conclusion: The improved survival observed in patients with COX-2 positive cancer and otherwise similar risk factors raises the hypothesis that these patients may have responded favourably to frequently used NSAIDs (noncontrolled in this study). These results if validated in other cohorts may justify a prospective randomized trial.

MP-5.14

Rationale combination intravesical therapy for bladder cancer: gemcitabine, BCG, and interleukin-2 in a rat model

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Introduction and Objective: BCG remains the intravesical treatment of choice for transitional cell carcinoma of the bladder (TCCB). Gemcitabine, interleukin-2 (IL-2), Interferon- α (IFN- α), are newer rationale agents for superficial TCCB. The benefits and risks of these agents as single or combination therapies were evaluated using an immunocompetent orthotopic TCCB rat model.

Materials and Methods: Rats with AY-27 TCCB (12 per group) received 6 instillations (twice weekly) escalating doses of gemcitabine or BCG, or combination of gemcitabine with BCG or BCG with IL-2 (dwelling time 1 hr). Gemcitabine was prepared in sterile water from stock solution. Aliquotted BCG was reconstituted in saline. Recombinant rat IL-2 was

dissolved in sterile water. All agents were freshly prepared before each experiment. Controls received saline instillations. Animals were monitored for tumour control, survival and side effects. At necropsy, tissue (bladder, kidneys) was examined grossly and histologically (H&E) for disease.

Results: In vitro gemcitabine exhibited a dose-dependent cytotoxicity of up to 10 μ M, beyond which there was no added effect. TCCB cells 253J, RT112, T24 and HT-1376 displayed differential sensitivity based on cellular Bcl-2 status, with no complete cell killing achieved. Animals could not tolerate more than 2 instillations of gemcitabine at doses greater than 10 mg/mL due to severe toxicities (GI bleeding, cystitis). Lower doses of gemcitabine showed significant benefit to saline ($p < 0.05$). The long-term survival/ tumour-free rates of animals receiving gemcitabine of 0.5, 1 or 2 mg/mL were 70%/60%, 100%/50% and 77%/67%, respectively, compared with 1% for saline group. Intermediate dose BCG (1×10^7 CFU) yielded 67%/58% survival/tumour-free rates. BCG combined with gemcitabine (0.5 mg/mL) caused more side effects (infection, urine retention) with no therapeutic gain. However, combining low dose BCG (5×10^5 CFU) with IL-2 achieved a better response than high dose BCG (5×10^7 CFU) alone.

Conclusion: Optimized gemcitabine dosing is needed for intravesical therapy of TCCB. Combination gemcitabine and BCG increased risk profile, while combination BCG and IL-2 increased therapeutic benefit. These data provide evidence for rationale treatment strategies for TCCB.

MP-5.15

Bovine α -lactalbumin complex (bLAC): a novel molecule for intravesical therapy of bladder cancer

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Introduction and Objective: Transitional cell carcinoma of the bladder

(TCCB) requires adjuvant therapies (Tx) to prevent recurrence. Current intravesical Tx carry the risk of infection or mutagenesis. Bovine α -lactalbumin (bLA) purified from milk and complexed with oleic acid (bLAC) selectively kills cancer cells. bLAC induces apoptosis independent of cell surface death receptors, p53, Bcl-2, or caspase status of the cells. We explored bLAC as a proapoptotic Tx for TCCB in vitro and in an orthotopic rat superficial TCCB model.

Materials and Methods: bLAC and its control protein without the oleic acid cofactor (bLA) were provided by NatImmune. BCG (OncoTICE) was obtained from Organon. A panel of TCCB cell lines was exposed to bLA or bLAC in serum-free culture media at escalating doses and time intervals (1–4 hr) while attached to multi-well plates. After overnight incubation in drug-free media, cytotoxicity was analyzed by MTT assay, and cell apoptosis determined by TUNEL assay, confocal microscopy and flow cytometry. Cocultured spheroids of TCCB and normal fibroblasts were used to assess bLAC selectivity. Anticancer effect of intravesical bLAC was studied using the syngeneic rat superficial bladder tumour model, which was compared with bLA, saline and BCG Tx.

Results: TUNEL and flow cytometry demonstrated a dose-dependent apoptosis induced by bLAC with IC_{50} of 0.21 mg/mL for a rat TCCB, and 0.22–0.61 mg/mL for human TCCB cells. No cell lines tested were resistant to bLAC. Fluorescent microscopy showed selective bLAC targeting of TCCB spheroids leaving normal fibroblasts unaffected. No cell killing was observed from bLA (control). In vivo results also showed a bLAC dose-dependent antitumour efficacy. With a high dose (10 mg/mL), intravesical bLAC was superior to BCG ($p = 0.15$) or bLA ($p = 0.065$) treatment. No toxicity was related to bLAC.

Conclusion: bLAC selectively induces apoptosis in a variety of TCCB cells. It also showed anticancer effect in animal bladder tumour model without toxicity. These data warrant further preclinical evaluation of bLAC for intravesical Tx of TCCB.