cell line: PC3 and human breast cancer cell line: MCF-7 were used in this study. Inhibition of basal autophagy was achieved using CQ, HCQ and Bafilomycin A1 (Baf A1) in multiple human bladder cancer cell lines. Cell viability was assessed by WST-1 assay. Western blot detection of LC3-II was performed to monitor autophagy, while detection of caspase 3/7 activities and DNA fragmentation were conducted to investigate apoptotic induction in treated cells. The disruption of mitochondria membrane potential (MMP), the generation of reactive oxygen species (ROS) and lysosome permeability were accessed by JC-1, H2DCFDA staining and IF detection of cathepsin D and E, respectively, in CQ- and HCQ-treated cells.

**Results:** Changes in LC3 flux, monitored by Western blot and IF, indicated inhibition of autophagy at the level of the autophagosome by CQ and HCQ. Both two autophagy inhibitors induced cytotoxicity in multiple human bladder cancer cell lines in time- and dose-dependent manner especially in advanced cancer cell lines. CQ and HCQ also significantly impacted the clonogenic formation of bladder cancer cells. However, the inhibition of cell viability was only observed in bladder cancer cell lines but not in SV-Huc-1, PC3 and MCF-7 cells that reported to be with low basal autophagy activity. Induction of apoptosis was found in cells treated with CQ and HCQ. We cannot detected the disruption of mitochondria membrane potential nor the generation of reactive oxygen species in CQ- or HCQ-treated cells. Translocation of cathepsin B in CQ-treated cells suggesting the change of lysosome permeability that leads to the blockage of autophagy and increasing apoptotic cell death by these agents.

**Conclusion:** Targeting autophagy with CQ or HCQ may be an effective cancer therapy in human bladder cancer.

**PD7-5:**
**INHIBITION OF AUTOPHAGY POTENTIATES APOPTOSIS IN BITC-TREATED HUMAN BLADDER CANCER CELLS**

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**Purpose:** Benzyl isothiocyanate (BITC) is contained in cruciferous plants which are part of the human diet, and has been shown to induce apoptosis in varies cancer cell lines including human bladder cancer cells. In our previous work, we showed that BITC induces protective autophagy via inhibition of mTOR signaling pathway in prostate cancer cells. And the induced apoptosis and autophagy in human prostate cancer cells were mediated by BITC-induced ROS generation. Here we investigated weather inhibition of autophagy enhances apoptosis in human bladder cancer cells as a novel therapeutic strategy.

**Materials and Methods:** Human bladder cancer cell lines including grade II, 5637, and grade III, T24, were used in this study. We also included mouse bladder cancer cell line, MBT2, for the development of orthotopic mice bladder cancer model for further study. Bafilomycin A1 (Baf A1) and chloroquine (CQ) were used as autophagy inhibitors. Cell viability in BITC-treated cells with or without the pretreatment of autophagy inhibitors were measured by WST-1 reagent. Detection of autophagy was conducted by measuring the LC3-II processing and the accumulation of p62 by Western blotting and immunofluorescent staining of these marker proteins. Detection of apoptosis was performed by the monitored the caspase 3/7 activity, Western blot of cleavage caspase 3 and cell flowcytometry of DNA fragmentation in treated cells. Detection of autophagy was conducted by measuring the LC3-II processing and the accumulation of p62.

**Results:** Exposure of 5637, T24 and MBT2 cells to pharmacologic concentrations of BITC resulted in the decrease of cell viability and autophagy induction. Although inhibition of basal autophagy by Baf A1 or CQ alone caused cell viability loss in these bladder cancer cell lines, pre-treatment of Baf A1 or CQ to inhibit BITC-induced autophagy further enhanced the inhibition of cell growth. Enhanced apoptosis judged by the increased caspase 3/7 activity, increased amount of cleaved caspase 3 and elevated level of DNA fragmentation in BITC-treated cell with Baf A1 or CQ pretreatment suggesting inhibition of autophagy significantly potentiates the anti-cancer effect of BITC in human bladder cancer cells.

**Conclusion:** This is the first study showed that inhibition of autophagy induced by BITC markedly enhance apoptosis in human and mouse bladder cancer cells. We are currently using orthotopic mice bladder cancer model to translate our results from in vitro to in vivo studies. Our data may be beneficial for further development of novel therapeutic strategies against bladder cancer.

**PD7-6:**
**THROMBOMODULIN EXPRESSION REGULATES TUMORIGENESIS IN BLADDER CANCER**

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**Purpose:** The identification of potential tumor markers will help improve therapeutic planning and patient management. Thrombomodulin (TM) is a sensitive urothelial marker. TM was reported to be one of the endogenous anti-metastatic factors and has diagnostic and prognostic values for the progression of carcinoma. In the present study, we examine the role of TM in bladder cancer.

**Materials and Methods:** We studied the role of TM in tumor behavior and related signaling pathways in vitro using the human bladder cancer cell lines HT1376, HT1197, 8J2 and T24, and in vivo using animal models. We also selected clinical specimens from 100 patients with bladder cancer for immunohistochemical staining to evaluate the predictive capacity of TM in tumor invasiveness.

**Results:** The data revealed that positive immunoreactivity for TM was inversely correlated with clinical stage and DNA methylation status 1 immuno-reactivity. Decreased TM expression could predict the aggressive tumor growth and advanced clinical stage in bladder cancer. When TM was inhibited, tumor growth rate and invasion ability were augmented in vitro and in vivo. The overall decreased expression of TM increased cell proliferation, enhanced epithelial-mesenchymal transition (EMT) and angiogenesis. Moreover, inhibition of NF-κB activation significantly increased TM expression and attenuated tumor aggressiveness in bladder cancer.

**Conclusion:** TM plays an important role in bladder cancer tumor aggressiveness in vitro and in vivo and is a clinically significant predictor that may represent a suitable therapeutic target for bladder cancer.

**Podium-8 Oncology**

**PD8-1:**
**URETERAL INVOLVEMENT AND DIABETES INCREASE THE RISK OF SUBSEQUENT BLADDER RECURRENCE AFTER NEPHROURETERECTOMY FOR UPPER URINARY TRACT UROTHELIAL CARCINOMA**

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**Purpose:** To investigate the prognostic factors for bladder recurrence after radical nephroureterectomy (RNU) in patients with upper urinary tract urothelial carcinoma (UUT-UC)

**Materials and Methods:** From 1994 to 2012, 695 patients with UUT-UC treated with RNU were enrolled in National Taiwan University Medical Center. Among them, 532 patients with no prior bladder UC histories were recruited for analysis. We assessed the impact of potentially prognostic factors on bladder recurrence after RNU.

**Results:** The median follow-up period was 47.8 months. In the Cox model, ureteral involvement and diabetes mellitus (DM) were significantly associated with a higher bladder recurrence rate in the multivariate analysis (hazard ratio [HR]: 1.838; p = 0.003 and HR: 1.821; p = 0.010, respectively). In the Kaplan-Meier analysis, DM patients with concomitant ureteral UC
experienced about a three-fold increased risk of bladder recurrence as compared to those without both factors (HR: 3.222; <p < 0.001). Patients with either one of the two risk factors experienced about a two-fold increased risk as compared to those without both factors (with DM, HR: 2.184, p = 0.024; with ureteral involvement, HR: 2.006, p = 0.003).

**Conclusion:** Ureteral involvement and DM are significantly related to bladder recurrence after RNU in patients with UUT-UC.

**PD8-2:**

**HYDRONEPHROSIS INDEPENDENTLY PREDICTS WORSE OUTCOME OF UPPER TRACT UROTHELIAL CARCINOMA IN PATIENTS PRESENTING WITH SIMULTANEOUS FLANK PAIN**

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**Purpose:** We aimed to investigate the impact of preoperative hydronephrosis (HN) and flank pain on prognosis of patients with upper tract urothelial carcinoma (UTUC).

**Materials and Methods:** In total, 472 patients with UTUC managed by radical nephroureterectomy were included from Kaohsiung Medical University Hospital Healthcare System. Clinicopathological data were collected retrospectively for analysis. The significance of HN, especially when combined with flank pain, and other relevant factors on cancer-specific survival (CSS) and overall survival (OS) were evaluated.

**Results:** Of the 472 patients, 292 (62%) had preoperative HN and 121 (26%) presented with flank pain. Preoperative HN was significantly associated with age, flank pain, tumor location, and pathological tumor stage. Preoperative HN and flank pain were significant predictors of non-organ-confined (NOC) UTUC (p = 0.020 and 0.013, respectively). Kaplan-Meier analysis showed significantly poorer OS and CSS in patients with preoperative HN (p = 0.005 and p = 0.026, respectively) and in patients with flank pain (p < 0.001 and p = 0.001, respectively) than those without. However, only simultaneous HN and flank pain independently predicted adverse outcome (HR = 2.47, p < 0.001 for OS and HR = 2.28, p = 0.002 for CSS, respectively) in multivariate Cox proportional hazards models. Notably, there was no difference in survival between patients with HN but devoid of flank pain and those without HN.

**Conclusion:** Both preoperative HN and flank pain predicted NOC UTUC. When accompanied with flank pain, HN represented an independent predictor for worse outcome in patients with UTUC.

**PD8-3:**

**INFERIOR SURVIVAL, HIGHER TUMOR RECURRENTNESS AND PROGRESSION RATES IN PRIMARY NON-MUSCLE-INVASIVE BLADDERCANCER WITH IMPAIRED eGFR**

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**Purpose:** To evaluate the influence of preoperative patient-associated parameters and comorbidities, with special focus on preoperative renal function on non-muscle-invasive bladder cancer (NMIBC) recurrence, progression, upper urinary tract (UUT) recurrence, cancer specific survival and overall survival.

**Materials and Methods:** Medical records of 158 patients with first diagnosis of NMIBC from 2008 to 2010 treated at our department were reviewed for tumor stage and grade, comorbidities, and putative risk factors including squamous differentiation, tumor size and count, white blood cell (WBC), neutrophil to lymphocyte ratio (NL ratio), estimated glomerular filtration rate (eGFR) by chronic kidney disease-epidemiology collaboration formula, patient age and gender.

**Results:** Tumor recurrence was observed in 51 patients (32%) and UUT recurrence was 5 patients (3%); progression to higher pT was found in 9 patients (6%). Univariate analysis revealed count, grade, size, stage, eGFR and squamous differentiation as signification prediction determinants for bladder recurrence. Count, grade, stage, eGFR were signification prediction determinants for progression. Age, grade, stage and eGFR were signification prediction determinants for overall survival. Based on the results of multivariate analysis, a risk factor model was created to classify patients with high and low risk of recurrence, progression and survival.

**Conclusion:** eGFR, grade and stage are significant predictive variables of NMIBC recurrence, progression and survival. Integrating eGFR into NMIBC risk calculation helped to discriminate individuals with high and low risk of cancer recurrence, progression and survival. Confirmatory studies and external validation are needed to corroborate these findings.

**PD8-5:**

**THE PREDICTIVE FACTORS FOR CONCURRENT RENAL PELVIS TUMOR OF PATIENTS WITH URETER CARCINOMA: KAOHSIUNG CHANG GUNG MEMORIAL HOSPITAL EXPERIENCE**

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**Purpose:** The incidence of upper tract urothelial carcinoma (UTUC) and chronic kidney disease (CKD) are high in southern Taiwan. The renal sparing surgery (RSS) is an optional choice of treatment of UTUC. This study was designed to assess the predictive factors on multifocal UTUC.

**Materials and Methods:** This study was retrospectively designed to determine the predictive factors on concurrent renal pelvis urothelial carcinoma (RPUC). From 2004 to 2012, 263 patients with preoperative solitary ureter tumor underwent renal nephroureterectomy (RNU) at our tertiary medical center. Perioperative data were recorded by chart review. Multivariate binary logistic regression was used to analyze the impact of risk factors for concurrent RPUC by SPSS ver. 17.

**Results:** There are thirty-eight patients (14%) had postoperative findings of concurrent RPUC. There is no significant difference of grade of preoperative hydropneumosis between both groups (p = 0.533), and there are significant difference of proportion of preoperative CKD stage (p = 0.005) and past history of bladder cancer (p = 0.003) between both groups. Multivariate analysis disclosed both bladder cancer history (odds ratio: 2.948; p = 0.003) and preoperative CKD stage >3 (odds ratio: 2.207; p = 0.033) are independent risk factors for multifocal UTUC.

**Conclusion:** In addition to the EAU guideline of conservative treatment for UTUC, we supposed that past history of bladder cancer and preoperative CKD stage greater than stage 3 are both important and independent risk factors to synchronously multifocal UTUC. More consequent studies about the oncologic outcome of RSS are necessary.

**PD8-6:**

**IS THE CALCIFIED LESION OF BLADDER WALL AFTER INTRAVESICAL MITOMYCIN-C THERAPY FOR SUPERFICIAL BLADDER CANCER SHOULD BE RESECTED?**

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**Purpose:** Intravesical therapy with Mitomycin-C and Bacillus Calmette-Guérin is a standard treatment for superficial bladder cancer. Owing to the worldwide shortage of Bacillus Calmette-Guérin, the use of Mitomycin-C for intravesical therapy is increasing. Sometimes, calcified lesion of bladder wall at previous resection site were found during scheduled cystoscopy. Are these lesions should be resected?

**Materials and Methods:** We retrospectively included 43 non-muscle invasive bladder cancer patients who were treated with transurethral resection of bladder tumor followed by adjuvant intravesical mitomycin C intravesical therapy between 2011 and 2014 at Tri-service General Hospital. These patients were classified into calcification and non-calcification groups. This study analyzes the characteristic of these two groups in order to find the predictive factor of bladder wall calcification and the meaning of bladder wall calcification in clinical practice.