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Platinum Priority – Bladder Cancer – Editor's Choice

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Molecular Subtyping of Clinically Localized Urothelial Carcinoma Reveals Lower Rates of Pathological Upstaging at Radical Cystectomy Among Luminal Tumors

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Abstract

Background: Upstaging of clinical T1-T2 urothelial carcinoma (UC) to non-organ-confined (NOC) pathological stage ≥T3 or N+ at radical cystectomy (RC) is common. Tools for stratifying patients who may have NOC disease are limited.
Objective: To determine an association of a genomic subtyping classifier (GSC) with pathological upstaging in multi-institutional cohort of patients with cT1-T2 UC treated with RC.
Design, setting, and participants: Precystectomy transurethral specimens from 206 patients with high-grade, cT1-T2, N0M0 UC, who underwent RC without neoadjuvant chemotherapy, underwent GSC testing.
Outcome measurements and statistical analysis: Uni- and multivariable logistic regression analyses evaluated GSC for upstaging, defined as pT3/T4 and/or pTanyN1–3 disease at RC.
Results and limitations: Pathological upstaging occurred in 23% of cT1 and 57% of cT2 cases. Lower rates of upstaging to NOC was seen for luminal versus nonluminal tumors (34% vs 51%, $p = 0.02$). The differences in upstaging were confined to T stage, with no difference in node positivity for luminal versus nonluminal patients (cT1: 13% for both [$p > 0.9$], cT2: 15% and 23% [$p = 0.6$], respectively). Fewer patients with luminal tumors were upstaged to ≥pT3Nany compared with nonluminal tumors (Mantel-Haenszel $p = 0.002$; cT1: 13% vs 30%, cT2: 34% vs 58%). On multivariable logistic regression analysis, nonluminal patients were more likely to be upstaged to ≥pT3 at RC ($p < 0.001$). Limitations include retrospective design and sample size.
Conclusions: Molecular subtyping of clinically localized UC demonstrated that luminal tumors have lower rates of upstaging to non-organ-confined disease compared with non-luminal tumors. If validated, these data can help inform which patients may need multimodal therapy.
Patient summary: Determining whether bladder cancer has spread beyond the bladder is challenging at diagnosis. In this paper, genomics helped identify patients who were more likely to have aggressive disease that has spread outside the bladder. These patients may benefit from chemotherapy prior to surgery.

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1. Introduction

Pathological upstaging of bladder cancer from precystectomy clinical stage ≤T2 to non-organ-confined pathological stage ≥T3 or N+ at radical cystectomy (RC) is common and significantly increases the risk of cancer-specific mortality (CSM) [1]. Neoadjuvant chemotherapy (NAC) is not recommended for patients who are clinical T1, while randomized controlled studies support the use of NAC in patients who are clinical T2 [2]. Despite level I evidence for improved survival using NAC prior to RC, rates of utilization are approximately 20–30% in the USA [3]. Underutilization of NAC is due to concerns for toxicity, perceived modest benefit, and the risk of overtreatment in patients with true organ-confined disease [3]. Approximately 80–90% of those with pathological stage T1 or T2 tumors who have negative lymph nodes will be cured with cystectomy alone [4,5]. On the contrary, patients with clinical T3/T4 disease showed a median survival increase from 24 mo without NAC to 65 mo with NAC [6].

Currently, approximately 40% of patients will be upstaged from clinical to pathological stage at the time of cystectomy in the absence of NAC [1]. There have been attempts to identify clinical factors that predict upstaging in order to improve stratification of those patients who may benefit most from NAC. The MD Anderson Cancer Center identified several risk factors for aggressive disease, including, but not limited to, clinical presence of hydroureteronephrosis, lymphovascular invasion (LVI), higher clinical stage (cT3b-T4a), and variant histological features (ie, micropapillary or neuroendocrine) [7]. Unfortunately, even in the absence of these features, 49% of low-risk patients in their cohort had pathological upstaging [7]. This strongly suggests that clinical features alone will be insufficient to risk stratify patients. Instead, biological features of the tumors may be critical for better risk stratification.

Molecular subtyping has shown that bladder cancer can be divided into several subtypes, each with distinct clinical and biological characteristics [8–12]. There is a general agreement between models regarding a high-level split into basal and luminal subtypes, with mounting evidence supporting a neuroendocrine-like subtype [8,10]. Basal tumors, while more aggressive, also showed the greatest improvements in survival outcome with platinum-based chemotherapy [9]. Conversely, luminal tumors tend to be less aggressive [8,9], but may also obtain less benefit from NAC [9]. These data suggest that molecular subtyping may provide valuable insights into stratifying treatment for patients with bladder cancer.

If accurate stage of patients could be prospectively identified based on analysis of tumor tissues obtained at transurethral resection of bladder tumor (TURBT) using biomarker signatures, high-risk patients may be counseled to receive chemotherapy, while low-risk patients might be spared the toxicity of chemotherapy. Significant cost savings to the health care system could be obtained through such individualized therapy [13]. In this study, we assess the association of a genomic subtyping classifier (GSC) with pathological upstaging to non-organ-confined disease in a multi-institutional cohort of patients with clinical T1-T2 bladder cancer treated with RC.

2. Patients and methods

2.1. Study patient population

Institutional Review Board approval was obtained from seven participating institutions before conducting this study. A total of 258 patients, diagnosed with cT1N0-cT2N0 bladder cancer and treated with RC (within 3 mo of diagnosis) and bilateral pelvic lymphadenectomy between 2009 and 2014 at selected US and Canadian tertiary care cancer centers, with formalin-fixed paraffin-embedded TURBT specimens available for genomic analysis, were compiled. All patients underwent cross-sectional imaging prior to cystectomy to determine whether they had extravesical disease and to assist with staging.

Inclusion criteria included the following:

1. cT1-cT2N0 disease
2. Undergone RC within 3 mo of diagnosis
3. Minimum standard lymph node dissection at RC

Exclusion criteria included the following:

1. Received any form of neoadjuvant systemic treatment
2. Prior pelvic radiation
3. Presence of hydronephrosis
4. Presence of cancer in a diverticulum (at a higher risk of becoming T3 since no muscle backing)
5. Presence of variant histology (ie, micropapillary) other than mixed urothelial with squamous or glandular differentiation

Thirteen patients were excluded due to the exclusion criteria, and quality control parameters were not met during genomic analysis in 39 patients (15%), rendering a final sample size of 206 patients.

2.2. Specimen collection and processing

Specimen collection and sample processing were conducted as described previously [9,14]. Decipher, a clinical-grade whole-transcriptome assay, was used to generate GSC scores for all specimens based on a validated signature (GenomeDx Laboratory, San Diego, CA, USA) [14]. The consensus subtyping and The Cancer Genome Atlas (TCGA) 2017 classifiers were downloaded from GitHub via links provided in bioRxiv 488460.

2.3. Molecular subtyping of TCGA patient cohort

The GSC was applied to the transcriptomes of 408 tumors downloaded from TCGA data portal, as described [9,12]. Cases with clinical T2 (and one patient with cT1) staging were retained for analysis.

2.4. Statistical analysis

The primary and secondary endpoints of the study were pathological upstaging at RC, defined as the presence of pT3–4 or pN1–3 disease, and CSM following surgery. Descriptive statistics with medians and interquartile range (IQR), or frequencies and proportions were presented as appropriate. Mantel-Haenszel (M-H) chi-square tests compared the association between subtypes and pathological upstaging, stratifying for

clinical stage. Cumulative incidence curves with Fine-Gray method were used to calculate survival functions and estimate the risks of CSM to account for death from other cause as a competing risk. Univariable and multivariable mixed-effect logistic regression analysis (UVA and MVA) models evaluated the association between genomic subtyping and pathological upstaging, with institutions adjusted as a random effect. Variables of continuous age, gender, smoking status, prior intravesical therapy, clinical T stage, and GSC (luminal vs nonluminal) were included in the UVA and MVA models. The area under the receiver operating characteristic curve (AUC) was bootstrap corrected and used to determine incremental improvement in model performance by adding GSC to the clinical risk model. The decision curve analysis (DCA) was used to evaluate the net benefit of clinical model, and the combined clinical and GSC models across clinically relevant threshold probabilities. All statistical tests were two sided; the reported significance level was 0.05. Analyses were performed in R v3.4.1 (R Foundation, Vienna, Austria).

3. Results

The clinical and pathological features of the patient cohort are described in Table 1. Our study population comprised 42% cT1 and 58% cT2 cases. The median time interval between TURBT and RC was 1.4 mo (range: 0.3–7.1 mo). Pathological upstaging to non-organ-confined disease (≥pT3 and/or ≥pN1) was observed in 23% of patients with cT1 and 57% with cT2, respectively. Forty-nine (24%) patients had positive lymph nodes at surgery, including 11 (13%) and 38 (32%) patients who were cT1 and cT2, respectively.

3.1. Molecular subtyping

Subtype calls were generated for the cohort ($n = 206$) by applying our single-sample GSC to these data [9], which showed an enrichment for luminal tumors (49%; Table 1). These luminal tumors showed strong expression of markers typically associated with the luminal subtype (ie, *PPARG* and *KRT20*) and lower expression of basal-associated (ie, *KRT5* and *KRT14*), EMT-associated (ie, *ZEB1* and *VIM*), immune-associated (ie, *CD274* and *CD8A*), and stromal-associated (*MHY11* and *DES*) markers (Fig. 1).

3.2. Luminal tumors had lower rates of pathological upstaging to non-organ-confined disease

To determine an association between molecular subtyping and upstaging, we evaluated the composite endpoint of T-stage upstaging (≥pT3) and nodal involvement (≥N1). There was a significantly lower rate of upstaging to non-organ-confined disease (≥pT3 and/or pTanyN+) for luminal versus combined nonluminal (basal, claudin low, and infiltrated luminal; 34% vs 51%, $p = 0.02$; Table 2). This was driven primarily by the rate of upstaging in T stage (24% vs 47%, $p < 0.001$ for luminal vs nonluminal) but not nodal status ($p = 0.4$; Table 2).

We hypothesized that the biological pathways driving upstaging and nodal involvement may be independent, prompting us to consider these endpoints separately. When stratifying for clinical stage and comparing luminal only with "nonluminal" (basal, claudin-low, and infiltrated luminal patients), we found that fewer patients with luminal tumors were upstaged than those with nonluminal tumors (M-H $p = 0.002$; Table 3). On MVA, nonluminal patients were significantly more likely to be upstaged at RC than luminal patients ($p < 0.001$ for both; Table 4). Adding genomic information to the clinical risk factors, MVA increased the AUC from 0.67 (0.62–0.70) to 0.72 (0.67–0.76). The prognostic value of the GSC was additionally reflected on DCA (Supplementary Fig. 1).

Table 1 – Clinical characteristic table

Variables	cT1	cT2
Total	87 (42%)	119 (58%)
Age		
Median (Q1, Q3)	71 (63, 76)	72 (64, 78)
Gender, *n* (%)		
Male	68 (78)	90 (76)
Smoking status, *n* (%)		
Never	26 (30)	38 (32)
Ever	61 (70)	79 (66)
Unavailable		2 (1.7)
CIS (at TURBT), *n* (%)		
No	63 (72)	82 (69)
Yes	20 (23)	28 (24)
Unavailable	4 (4.6)	9 (7.6)
Intravesical therapy, *n* (%)		
No	44 (51)	91 (76)
Yes	41 (47)	27 (23)
Unavailable	2 (2.3)	1 (0.84)
Pathological T stage, *n* (%)		
pT0	16 (18)	11 (9.2)
pTa/pTis	10 (11)	10 (8.4)
pT1	28 (32)	8 (6.7)
pT2	13 (15)	34 (29)
pT3	14 (16)	37 (31)
pT4	4 (4.6)	19 (16)
Unavailable	2 (2.3)	
Surgical margin, *n* (%)		
Negative	67 (77)	74 (62)
Positive	9 (10)	11 (9.2)
Unavailable	11 (14)	34 (29)
LVI, *n* (%)		
No	69 (79)	84 (71)
Yes	18 (21)	31 (26)
Unknown		4 (3.4)
LNI, *n* (%)		
Yes	11 (13)	38 (32)
CIS (at RC), *n* (%)		
No	55 (63)	65 (55)
Yes	31 (36)	52 (44)
Unavailable	1 (1.1)	2 (1.7)
No. of nodes removed		
Median (Q1, Q3)	21 (13, 32)	19 (13, 29)
Received adjuvant chemo, *n* (%)		
No	70 (80)	82 (69)
Yes	10 (11)	27 (23)
Unavailable	7 (8.0)	10 (8.4)
GSC subtype, *n* (%)		
Luminal	47 (54)	53 (45)
Basal	24 (28)	32 (27)
Infiltrated luminal	12 (14)	18 (15)
Claudin low	4 (4.6)	16 (13)

CIS = carcinoma in situ; GSC = genomic subtyping classifier; LNI = lymph node involvement; LVI = lymphovascular invasion; RC = radical cystectomy; TURBT = transurethral resection of bladder tumor.

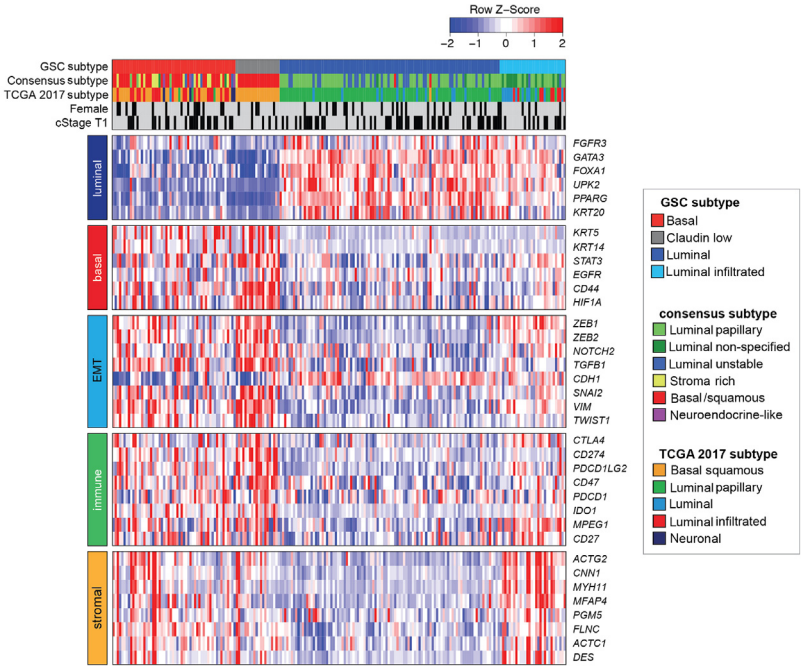

Fig. 1 – Forced-order heatmap for five biological categories (luminal, basal, EMT, immune, and stromal) of selected bladder cancer marker genes. Female gender, cStage T1, consensus (bioRxiv 488460), and TCGA [21] subtypes are indicated by black or colored bars in the respective covariate tracks. GSC = genomic subtyping classifier; TCGA = The Cancer Genome Atlas.

Table 2 – Rates of upstaging for luminal versus nonluminal (basal, claudin low, and infiltrated luminal) tumors in clinical T1/T2 patients from the upstaging cohort

Variables	Luminal	Nonluminal	*p* value
Total, *n* (%)	100 (48)	106 (52)	
Upstaging (≥pT3 and/or pTanyN+), *n* (%)			
No	66 (66)	52 (49)	0.02
Yes	34 (34)	54 (51)	
Pathological upstage (pT3–4 only), *n* (%)			
pT0–2	75 (75)	55 (52)	<0.001
pT3–4	24 (24)	50 (47)	
Unavailable	1 (1.0)	1 (0.90)	
Node positive (Tany, N+ only), *n* (%)			
No	79 (79)	78 (74)	0.4
Yes	21 (21)	28 (26)	

Table 3 – Rates of pathological T stage upstaging stratified by merged luminal versus nonluminal (basal, claudin low, and infiltrated luminal)

Subset	Variables	Luminal	Nonluminal
Clinical T1	Total	47 (54%)	40 (46%)
	pT0–2	40 (85%)	27 (68%)
	pT3–4	6 (13%)	12 (30%)
	Unavailable	1 (2.1%)	1 (2.5%)
Clinical T2	Total	53 (44%)	66 (56%)
	pT0–2	35 (66%)	28 (42%)
	pT3–4	18 (34%)	38 (58%)

Table 4 – Multivariable analyses for pathological T-stage upstaging (*n* = 199)

Upstaging	OR (95% CI)	*p* value
MVA		
Age at TURBT	1.03 (1.00–1.06)	0.085
Male vs female	0.70 (0.32–1.49)	0.4
Smoker vs nonsmoker	2.91 (1.36–6.22)	0.006
Prior intravesical therapy	0.86 (0.41–1.78)	0.7
cStage T2 vs T1	3.52 (1.73–7.19)	<0.001
Nonluminal vs luminal	3.10 (1.61–5.99)	<0.001

CI = confidence interval; MVA = multivariable logistic regression analysis; OR = odds ratio; TURBT = transurethral resection of bladder tumor.

To support these findings, we analyzed the TCGA muscle-invasive bladder cancer (MIBC) cohort to determine whether subtypes correlated with upstaging to non-organ-confined disease (pT stage ≥T3 or pN stage ≥N1), finding nonluminal tumors were significantly more likely to be upstaged compared with luminal tumors ($p = 0.006$; Table 5). When the upstaging endpoints were analyzed separately, we found that nonluminal tumors had significantly ($p = 0.01$) higher rates of primary tumor upstaging (pT3–4), but rates of nodal involvement (node positive) were similar ($p = 0.6$; Table 5).

3.3. *Luminal patients have lower rates of CSM*

Median follow-up time for patients without CSM was 43 mo (IQR: 25–72 mo), and the numbers of patients who died of bladder cancer were 17 and 37 for cT1 and cT2, respectively. Not surprisingly, patients who were upstaged had higher rates of CSM than those who were not upstaged (45% [34–56%] vs 9.6% [3.9–15%]; Supplementary Fig. 2). When stratified by subtype, patients with luminal tumors had lower CSM than patients with nonluminal tumors

Table 5 – Rates of upstaging for luminal versus nonluminal (basal, claudin low, and infiltrated luminal) tumors in clinical T1/T2 patients from the TCGA cohort

Variables	Luminal	Nonluminal	*p* value
Total, *n* (%)	22 (23)	74 (77)	
Upstaging (≥pT3 and/or pTanyN+), *n* (%)			
No	12 (55)	20 (27)	0.006
Yes	6 (27)	48 (65)	
Unavailable	4 (18)	6 (8.1)	
Pathological upstage (pT3–4 only), *n* (%)			
pT0–2	16 (73)	30 (40)	0.014
pT3–4	6 (27)	44 (60)	
Node positive (N+), *n* (%)			
No	14 (64)	42 (57)	0.6
Yes	4 (18)	21 (28)	
Unavailable	4 (18)	11 (15)	

TCGA = The Cancer Genome Atlas.

($p = 0.039$; Fig. 2A). Further stratification by clinical stage showed a non-statistically significant association with lower CSM for patients with luminal tumors (Fig. 2B and C).

4. Discussion

The role of NAC in bladder cancer is to improve survival in patients with true non-organ-confined disease, especially those with micrometastatic disease. There is a survival benefit in patients with cT2 disease due to a risk of understaging based both on extension beyond the bladder locally and on involvement of nodes and distant organs. In contrast, patients who truly have cancer in the bladder only should be curable with cystectomy alone. Identifying patients with non-organ-confined disease has been an ongoing challenge, with rates of upstaging at the time of cystectomy being routinely higher than 40%. Clinical factors such as hydronephrosis, palpable disease on examination under anesthesia, LVI, and variant histology have been proved insufficient to stratify patients, highlighting the unmet need for additional risk stratification markers.

In this multicenter study of patients with cT1-T2 disease, we used molecular subtyping and found that luminal tumors had a significantly lower risk of upstaging than nonluminal tumors. This study is novel, as there is currently a lack of established biomarkers for predicting upstaging. Molecular testing could provide a valuable tool for patients and providers in deciding on the merits of proceeding with NAC if validated prospectively. This type of testing could be used in conjunction with information on the likelihood of response to NAC to enrich the population for both patients who likely have non-organ-confined disease and patients who are most likely to benefit from NAC. Molecular subtyping appears particularly promising in this regard because the basal subtype fulfills both these parameters [9,11].

One unique aspect of this study was the evaluation of patients with cT1 disease. These patients are not typically considered for NAC, but this study found that over 30% of patients with cT1 and nonluminal subtype had non-organ-confined disease (≥pT3). This may represent a missed opportunity to utilize NAC in these patients rather than

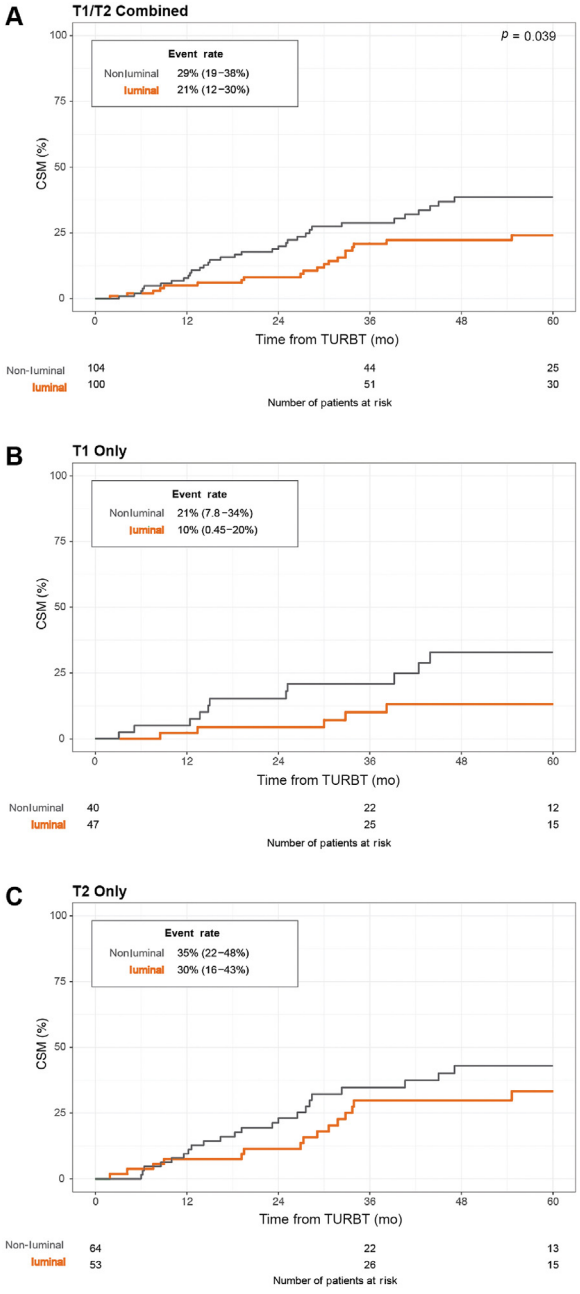

Fig. 2 – Cancer-specific mortality of luminal versus nonluminal patients: (A) T1/T2 tumors combined, (B) T1 tumors only, and (C) T2 tumors only. CSM = cancer-specific mortality; TURBT = transurethral resection of bladder tumor.

immediate cystectomy if clinical and/or molecular staging improved the accuracy of assessing clinical tumor and node status. On the contrary, even luminal patients with cT2 had a 34% risk of ≥pT3 disease, making it premature to recommend against NAC in these patients. However, luminal tumors tend to have better outcomes in both MIBC and non-muscle-invasive bladder cancer (NMIBC) [8,9,15]. A further consideration is that national rates of use of NAC are still relatively low, although this is increasing, and guideline recommendations are consistent in recommending NAC for all patients with MIBC [2]. This study found that 58% of

patients with nonluminal cT2 disease were upstaged to ≥pT3 disease, offering evidence that one could enrich for higher-risk patients using this classifier. It is likely that these findings after TURBT would encourage increased utilization of NAC in this high-risk group. To bolster this argument, studies suggest that patients with basal tumors tended to receive greater benefit from NAC [9,11].

Since the use of GSC for the purpose of upstaging is novel, we wanted to validate our findings in an independent cohort. We explored the TCGA to determine whether subtyping was associated with upstaging and found similar lower incidence of primary tumor upstaging (≥pT3 disease) in patients with luminal tumors ($p = 0.01$). Interestingly, even the rates of upstaging for luminal (around 30%) and nonluminal (50–60%) tumors were similar between cohorts. While the TCGA data are supportive, there are several differences. First, the tissue was collected from both TURBT and cystectomy patients, so there could be discordance in subtypes. Second, there were very few patients with cT1 in the TCGA, and so these data are mostly applicable for cT2 patients.

In this study, almost half (48%) of the patients had luminal subtype. The luminal subtype as determined by the GSC model was observed in 20% of patients in the TCGA cohort, 21% in the Lund cohort, and 36% in the previously published NAC cohort. The use of cT1-T2 disease in this study makes it difficult to directly compare with the other cohorts that primarily had cT2-T4 disease. These results suggest that luminal tumors are more likely to be of lower stage, while nonluminal tumors are more likely to be clinically non-organ confined.

In this study, we did not determine an association between molecular subtyping and lymph node involvement. Although this may be related to the relatively low number of node-positive patients, it also suggests that (nodal) metastatic disease may progress through other pathway(s) than those defining the expression-based subtypes. In a prior report, molecular subtyping of both primary tumor and node tissues found higher levels of discordance in basal tumors compared with luminal tumors, further supporting the notion that subtyping may provide insufficient information regarding the likelihood of metastatic potential [16]. A number of signatures to predict lymph node involvement have been published [17–19]. At least one has failed validation on external testing [20], indicating that it is challenging to predict nodal status from TURBT samples. Collectively, these data suggest that further work is required to develop a subtype-independent predictive signature of lymph node involvement.

Limitations include retrospective design, limited sample size, and lack of a centralized pathological or radiographic review. There is a potential for confounding results when applying a subtyping model trained on MIBC to NMIBC, depending on the overlap in features between these disease states. Furthermore, the patients with clinical T1 disease may not be representative of all high-grade T1 patients since these patients were selected for cystectomy. Approximately 50% had prior intravesical therapy, and their clinical course may be different from patients who had immediate cystectomy. The sample size in this study is too small to assess this difference.

In this study, we have demonstrated that expression-based molecular subtyping of a bladder tumor not only may be predictive of outcome, but also holds utility in predicting upstaging from clinical T1–2 to pathological T3–4 at cystectomy. Prospective clinical trials to validate these findings will be necessary for this work to gain clinical utility.

5. Conclusions

Molecular subtyping revealed that luminal tumors have lower rates of upstaging than nonluminal tumors. Combined with previous reports that the benefit of NAC may be greatest in basal tumors, these data suggest that subtyping may have utility in identifying higher-risk patients who could be prioritized for NAC. Nevertheless, approximately one-third of luminal tumors with cT2 tumors are upstaged to non-organ-confined disease. These patients should continue to receive NAC until further studies evaluate the implications of immediate cystectomy.

Author contributions: Yair Lotan had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Lotan, Boorjian, Zhang, Bivalacqua, Porten, Lerner, Davicioni, Svatek, Black, Gibb.
Acquisition of data: Lotan, Boorjian, Bivalacqua, Porten, Wheeler, Lerner, Hutchinson, Francis, Svatek, Black.
Analysis and interpretation of data: Lotan, Boorjian, Zhang, Bivalacqua, Porten, Lerner, Davicioni, Svatek, Chen, Black, Gibb.
Drafting of the manuscript: Lotan, Boorjian, Zhang, Bivalacqua, Porten, Lerner, Davicioni, Svatek, Chen, Black, Gibb.
Critical revision of the manuscript for important intellectual content: Lotan, Boorjian, Zhang, Bivalacqua, Porten, Wheeler, Lerner, Hutchinson, Francis, Davicioni, Svatek, Chen, Black, Gib.
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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.eururo.2019.04.036.

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