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Platinum Priority – Bladder Cancer – Editor's Choice

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Genomic Differences Between "Primary" and "Secondary" Muscle-invasive Bladder Cancer as a Basis for Disparate Outcomes to Cisplatin-based Neoadjuvant Chemotherapy

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Abstract

Background: Cisplatin-based neoadjuvant chemotherapy (NAC) followed by radical cystectomy (RC) is the standard of care for patients with muscle-invasive bladder cancer (MIBC). It is unknown whether this treatment strategy is appropriate for patients who progress to MIBC after treatment for prior noninvasive disease (secondary MIBC).
Objective: To determine whether clinical and genomic differences exist between primary and secondary MIBC treated with NAC and RC.
Design, setting, and participants: Clinicopathologic outcomes were compared between 245 patients with clinical T2-4aN0M0-stage primary MIBC and 43 with secondary MIBC treated with NAC and RC at Memorial Sloan Kettering Cancer Center (MSKCC) from 2001 to 2015. Genomic differences were assessed in a retrospective cohort of 385 pre-chemotherapy specimens sequenced by whole-exome or targeted exon capture by the Cancer Genome Atlas or at MSKCC. Findings were confirmed in an independent validation cohort of 94 MIBC patients undergoing prospective targeted exon sequencing at MSKCC.
Outcome measurements and statistical analysis: Pathologic response rates, recurrence-free survival (RFS), bladder cancer-specific survival (CSS), and overall survival (OS) were measured. Differences in somatic genomic alteration rates were compared using Fisher's exact test and the Benjamini-Hochberg false discovery rate method.
Results and limitations: Patients with secondary MIBC had lower pathologic response rates following NAC than those with primary MIBC (univariable: 26% vs 45%, multivariable: odds ratio = 0.4 [95% confidence interval = 0.18 – 0.84] $p = 0.02$) and significantly

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worse RFS, CSS, and OS. Patients with secondary MIBC treated with NAC had worse CSS compared with cystectomy alone ($p = 0.002$). In a separate genomic analysis, we detected significantly more likely deleterious somatic *ERCC2* missense mutations in primary MIBC tumors in both the discovery (10.9% [36/330] vs 1.8% [1/55], $p = 0.04$) and the validation (15.7% [12/70] vs 0% [0/24], $p = 0.03$) cohort.

Conclusions: Patients with secondary MIBC treated with NAC had worse clinical outcomes than similarly treated patients with primary MIBC. *ERCC2* mutations predicted to result in increased cisplatin sensitivity were enriched in primary versus secondary MIBC. Prospective validation is still needed, but given the lack of clinical benefit with cisplatin-based NAC in patients with secondary MIBC, upfront RC or enrollment in clinical trials should be considered.

Patient summary: A retrospective cohort study of patients with "primary" and "secondary" muscle-invasive bladder cancer (MIBC) treated with chemotherapy before surgical removal of the bladder identified lower response rates and shorter survival in patients with secondary MIBC. Tumor genetic sequencing of separate discovery and validation cohorts revealed that chemotherapy-sensitizing DNA damage repair gene mutations occur predominantly in primary MIBC tumors and may underlie the greater sensitivity of primary MIBC to chemotherapy. Prospective validation is still needed, but patients with secondary MIBC may derive greater benefit from upfront surgery or enrollment in clinical trials rather than from standard chemotherapy.

1. Introduction

Neoadjuvant cisplatin-based chemotherapy (NAC) followed by surgical removal of the bladder (radical cystectomy [RC]) is the standard of care for muscle-invasive bladder cancer (MIBC) [1]. This practice is based on two large randomized clinical trials and a meta-analysis that demonstrated improved recurrence-free survival (RFS) and overall survival (OS) with the addition of NAC over cystectomy alone [1,2]. However, this survival advantage appears to be restricted to patients who achieve pathologic downstaging at RC, a surrogate of chemosensitivity [1–3]. Moreover, nonresponders to NAC may miss a potential window of opportunity for cure afforded by upfront cystectomy [4–6]. To optimize the selection of patients who are most likely to respond to NAC, several groups have used next-generation sequencing (NGS) to identify potential predictive biomarkers of NAC response [7–9]. These retrospective analyses of urothelial cancer patients treated in the neoadjuvant and metastatic settings identified alterations in DNA damage response (DDR) genes, including *ERCC2*, as associated with enhanced sensitivity to cisplatin-based chemotherapy.

Most patients diagnosed with bladder cancer initially present with non–muscle-invasive bladder cancer (NMIBC), which is usually managed by transurethral resection (TUR) with or without intravesical treatment, such as bacille Calmette-Guerin (BCG) immunotherapy [10]. These interventions are aimed at reducing the risk of recurrence and disease progression while maintaining a functional bladder [11]. However, up to 30% of patients with NMIBC experience progression to muscle-invasive disease; such patients are referred to as having secondary MIBC [12]. The effect of prior treatment of NMIBC on the clinical outcome and chemosensitivity of patients with secondary MIBC is unknown. Despite this lack of data, the standard recommendation of NAC followed by cystectomy is applied to all patients with MIBC who are candidates for cisplatin-based chemotherapy, regardless of prior history of treatment for NMIBC. We hypothesized that patients with secondary MIBC may have disparate clinical outcomes compared with those who present with de novo primary MIBC, possibly related to differences in tumor genomic profiles from treatment-induced tumor clonal selective pressure.

2. Patients and methods

2.1. Clinical cohort: patient characteristics and study design

Following Institutional Review Board approval, we performed a retrospective analysis of patients undergoing RC at Memorial Sloan Kettering Cancer Center (MSKCC) from May 15, 2001 (the date on which data from the seminal SWOG8710 NAC clinical trial was released) to July 1, 2015 (date of query) [2]. Supplementary Figure 1 lists the exclusion criteria for this analysis. Only patients with predominant urothelial carcinoma histology and clinical stage cT2-4aN0M0 were included. Patients who received fewer than three cycles of NAC or non–cisplatin-based therapy were excluded. Primary MIBC was defined as invasion into or beyond the muscularis propria (clinical stage ≥T2) on either initial or restaging TUR of first bladder tumor. Patients were considered to have secondary MIBC if they had an initial non–muscle-invasive tumor (Tis, Ta, or T1 with uninvolved muscularis propria in the specimen) that was confirmed by a restaging TUR or at least one follow-up cystoscopy prior to the eventual diagnosis of MIBC.

The primary outcome analyzed was pathologic response at RC, (≤pT1N0) [13]. Secondary outcomes included RFS, bladder cancer-specific survival (CSS), and OS. This cohort was used to examine clinical outcomes between primary and secondary MIBC independent of genomic analysis. Details of the statistical analysis for the clinical cohorts can be found in the Supplementary material (Methods section).

2.2. Genomic analyses

2.2.1. Retrospective discovery genomic cohort

To examine genomic differences between primary and secondary MIBC, we identified 385 muscle-invasive (≥T2) index pretreatment bladder specimens (330 primary and 55 secondary MIBC), derived from a combined cohort of 981tumors analyzed by NGS, including 412 tumors that underwent whole exome sequencing (WES) through the urothelial TCGA (the Cancer Genome Atlas) and 569 tumors sequenced

retrospectively at our institution for research purposes using WES or targeted exon capture sequencing. Sequenced specimens were fresh frozen or formalin-fixed paraffin embedded (FFPE), obtained by TUR or cystectomy; all were chemotherapy naïve. DNA from matched nonmalignant tissue or peripheral blood was also sequenced to filter out germline alterations. Details of the WES analysis have previously been described [8,14]. Targeted exon capture was performed using different versions of a customized multiplex gene panel of known cancer-associated genes (MSK-IMPACT) to sequence all protein-coding exons for 300, 341, or 410 genes (Supplementary Table 1 and Supplemental material, Methods) [10,15,16].

The frequency of somatic genomic alterations in genes previously implicated in platinum-based chemotherapy response in MIBC (*ERCC2*, *ATM*, *FANCC*, and *RB1*) was compared between primary and secondary MIBC specimens using Fisher's exact test [7–9]. Data on *FANCC* were missing for six patients (four primary and two secondary) sequenced using the 300-gene panel.

2.2.2. Prospective validation genomic cohort

Between January 2014 and November 2015, tumors from 214 patients with bladder cancer were prospectively sequenced using the MSK-IMPACT assay within the context of an institution-wide molecular profiling initiative (MSKCC IRB #12-245, NCT01775072). From this cohort, we identified 94 consecutive patients with MIBC (70 primary and 24 secondary) with chemotherapy-naïve, FFPE TUR, or cystectomy specimens to serve as an independent validation cohort. All tumor and matching germline specimens underwent targeted exon capture sequencing using the 341- or 410-gene MSK-IMPACT panel within a CLIA-certified laboratory (see the Supplemental material, Methods). Any significant differences in gene alteration frequencies between primary and secondary MIBC specimens in the retrospective cohort were verified in the validation cohort using Fisher's exact test.

2.2.3. Exploratory combined genomic analysis

The retrospective and validation cohorts were subsequently combined to increase the sensitivity to detect additional low-frequency differences in genomic alterations between primary and secondary MIBC specimens. This analysis included genes from the 341-gene targeted exon capture panel that were altered at ≥3% frequency in the combined cohort or urothelial TCGA or known *DDR* genes (Supplementary Table 1). Unadjusted *p* values were generated using Fisher's exact test. Adjusted *p* values were corrected for multiple comparisons using the Benjamini-Hochberg false discovery rate method.

2.3. Data availability

All genomic data used in the analyses presented here are publicly available at cbioportal.mskcc.org [17,18].

3. Results

3.1. Clinical analysis

A total of 2105 patients with bladder cancer underwent RC at MSKCC between May 2001 and July 2015. Of these patients,1082 had clinical stage T2-4aN0M0 MIBC with urothelial carcinoma histology. Of these patients, 288 (245 primary and 43 secondary MIBC) received three to four cycles of cisplatin-based NAC and met all inclusion criteria for our analysis (Supplementary Fig. 1). Demographics of this 288-patient cohort were similar to those of other NAC-treated MIBC cohorts (Supplementary Table 2) [2,3]. As expected, lower pretreatment clinical tumor stage was associated with improved NAC response [2,3,6] (Table 1). Median follow-up among survivors was 4 yr (interquartile range [IQR]: 1.4–6.2); during this time, 103 patients died from any cause, 83 of which were deaths attributed to progressive bladder cancer.

The pathologic response rate to NAC was lower among patients with secondary MIBC than among patients with primary MIBC on both univariable (26% vs 45%, $p = 0.02$) and multivariable analyses adjusted for age, sex, and T stage (OR = 0.4 [95% confidence interval = 0.18 – 0.84], $p = 0.02$; Tables 1 and 2, and Supplementary Table 2). Further, secondary MIBC was associated with significantly worse RFS ($p < 0.001$), CSS ($p = 0.004$), and OS ($p = 0.006$) on univariable analysis (Fig. 1 and Supplementary Fig. 2). On multivariable analysis adjusted for age, sex, T stage, and adjuvant chemotherapy, this association remained

Table 1 – Multivariable associations between patient/disease characteristics and pathologic response, recurrence-free survival, cancer-specific survival, and overall survival among 245 patients with primary and secondary MIBC who were treated with three to four cycles of cisplatin-based neoadjuvant chemotherapy prior to cystectomy

	Pathologic response (≤pT1N0)		Recurrence-free survival		Cancer-specific survival		Overall survival	
	OR (95% CI)	*p* value [a]	HR (95% CI)	*p* value [b]	HR (95% CI)	*p* value [c]	HR (95% CI)	*p* value [b]
Age	0.99 (0.96–1.02)	0.4	1.01 (0.99–1.04)	0.4	1.00 (0.97–1.03)	0.9	1.01 (0.99–1.04)	0.3
Male gender	1.14 (0.63–2.06)	0.7	0.93 (0.57–1.52)	0.8	0.85 (0.51–1.43)	0.6	0.80 (0.51–1.23)	0.3
Clinical stage								
T3	0.31 (0.16–0.58)	<0.001	–	–	–	–	–	–
T4a	0.14 (0.02–0.51)	0.01	–	–	–	–	–	–
Pathologic stage								
T2	–	–	2.68 (1.16–6.23	0.022	2.81 (1.21–6.52)	0.016	1.74 (0.86–3.51)	0.1
T3/T4a	–	–	10.2 (5.26–19.6	<0.001	7.96 (4.14–15.3)	<0.001	5.15 (3.12–8.52)	<0.001
Adjuvant chemotherapy	–	–	1.61 (0.62–4.15)	0.3	1.63 (0.9–2.94)	0.1	1.51 (0.59–3.88)	0.4
Secondary MIBC	0.4 (0.18–0.84)	**0.019**	2.10 (1.23–3.57)	**0.007**	1.78 (0.99–3.20)	**0.054**	1.66 (1.01–2.73)	**0.048**

CI = confidence interval; HR = hazard ratio; MIBC = muscle-invasive bladder cancer; OR = odds ratio.
[a] *p* value from logistic regression.
[b] *p* value from Cox regression.
[c] *p* value from competing risks regression.

Table 2 – Clinical and pathologic characteristics of patients with primary and secondary MIBC who were treated with three to four cycles of cisplatin-based neoadjuvant chemotherapy prior to cystectomy

Variable	Primary MIBC NAC + RC	Secondary MIBC NAC + RC	*p* value
n (%)	245 (85)	43 (15)	
Age at RC	65 (59, 70)	68 (63, 73)	0.004
Gender			0.6
Male	181 (74%)	34 (79%)	
Female	64 (26%)	9 (21%)	
Clinical stage			0.7
cT2	174 (71%)	32 (74%)	
cT3	58 (24%)	8 (19%)	
cT4a	13 (5.3%)	3 (7.0%)	
Variant histology on TUR	86 (35%)	14 (33%)	0.9
Neoadjuvant chemotherapy			0.4
Gemcitabine and cisplatin	229 (94%)	41 (95%)	
Gemcitabine, cisplatin, and sunitinib	10 (4.1%)	1 (2.3%)	
MVAC	6 (2.0%)	1 (2.3%)	
NAC cycles			0.4
3	30 (12%)	7 (16%)	
3.5	3 (1.2%)	1 (2.3%)	
4	212 (87%)	35 (82%)	
Pathologic response (≤pT1N0)	110 (45%)	11 (26%)	0.02
Pathologic stage			
pT0	36 (15%)	0 (0)	
pTis	49 (20%)	8 (19%)	
pTa	6 (2.4%)	0 (0)	
pT1	23 (9.4%)	4 (9.3%)	
pT2	37 (15%)	11 (26%)	
pT3	75 (31%)	10 (23%)	
pT4	19 (7.8%)	10 (23%)	
Positive lymph nodes	44 (18%)	11 (26%)	0.3
Variant histology on cystectomy specimen	57 (23%)	12 (28%)	0.6
Positive soft tissue margins	9 (3.7%)	4 (9.3%)	0.1
Adjuvant chemotherapy	7 (2.9%)	0	0.6

MIBC = muscle-invasive bladder cancer; MVAC = methotrexate, vinblastine, adriamycin, and cisplatin; NAC = neoadjuvant chemotherapy; RC = radical cystectomy; TUR = transurethral resection.
Numbers presented are *N* (%) when categorical and median (interquartile range) when continuous.

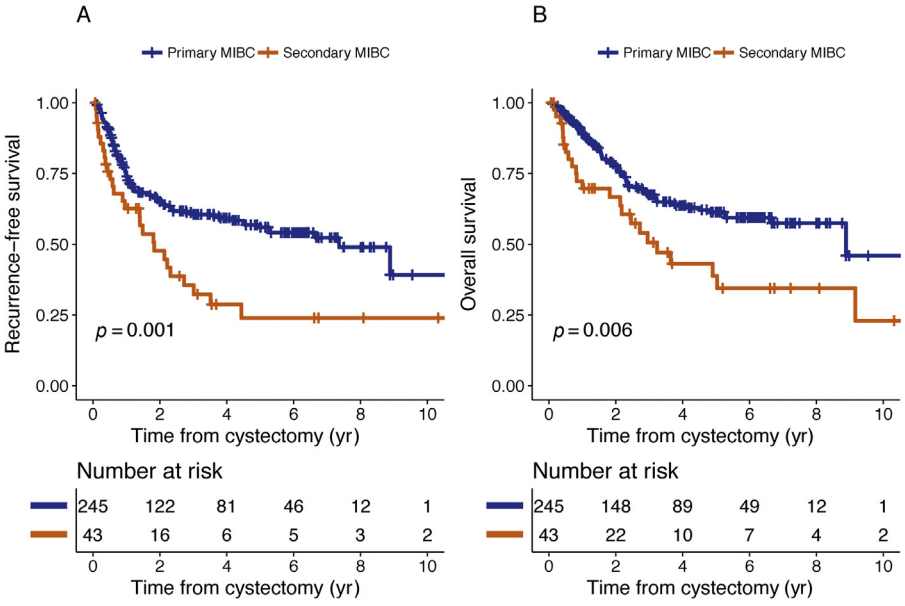

Fig. 1 – Recurrence-free survival (RFS) and overall survival (OS) in patients with primary and secondary MIBC. (A) Comparison of RFS in patients with primary versus secondary MIBC treated with neoadjuvant chemotherapy followed by radical cystectomy. (B) Comparison of OS in patients with primary versus secondary MIBC treated with neoadjuvant chemotherapy followed by radical cystectomy. MIBC = muscle-invasive bladder cancer.

significant for RFS ($p = 0.007$) and OS ($p = 0.048$), and was marginally significant for CSS ($p = 0.054$; Table 1).

To explore whether these outcome differences were due to differences in NAC sensitivity, we compared the outcomes of primary and secondary MIBC patients treated with NAC with those treated with RC alone. The clinical and pathologic characteristics of these two groups are described in Supplementary Tables 3 and 4, respectively. In multivariable analysis, addition of NAC was associated with increased odds of pathologic downstaging among primary MIBC patients ($p < 0.001$) but not among secondary MIBC patients ($p = 0.3$; Supplementary Table 5). Furthermore, in multivariable analysis, addition of NAC was associated with improved RFS as compared with RC alone in primary MIBC patients ($p = 0.042$), whereas in patients with secondary MIBC, treatment with NAC was associated with worse RFS ($p = 0.007$), CSS ($p = 0.002$), and OS ($p = 0.013$) as compared with RC alone (Supplementary Table 5).

3.2. Genomic analysis

To determine whether tumor genomic differences might account for the contrasting clinical outcomes and chemosensitivity observed in patients with primary and secondary MIBC, we analyzed a separate retrospective cohort of bladder cancer patients (330 primary and 55 secondary MIBC) whose tumor had been analyzed using NGS methods to assess differences in the frequency of alterations in four genes previously implicated in NAC response (*ERCC2*, *ATM*, *FANCC*, and *RB1*; Supplementary Table 6). Among these genes, only *ERCC2* missense mutations were significantly enriched in the primary versus secondary MIBC tumors (11% vs 1.8%, $p = 0.044$; Fig. 2A and Supplementary Table 7).

Since *ERCC2* missense mutations have previously been associated with exquisite cisplatin chemosensitivity in patients with bladder cancer, we hypothesized that differences in the prevalence of *ERCC2* mutations may explain the observed differences in chemotherapy response between primary and secondary MIBC patients [8,19]. To validate this finding, we assembled an independent cohort of prospectively sequenced patients composed of 94 chemotherapy-naïve MIBC specimens (70 primary and 24 secondary MIBC tumors; Supplementary Table 6). We again found that *ERCC2* missense mutations were significantly enriched in primary MIBC specimens (17.1% vs 0% in secondary MIBC specimens, $p = 0.033$; Fig. 2C and Supplementary Table 7).

To increase the sensitivity to detect differences in the incidence of low-frequency genomic events between primary and secondary MIBC tumors, we analyzed the combined retrospective and validation cohorts. *ERCC2* missense mutations were significantly more common in primary versus secondary MIBC tumors (12% [48/400] vs 1.3% [1/79], unadjusted $p = 0.004$; Supplementary Table 8) and tended to cluster near or within conserved helicase domains (Fig. 2B). On univariable analysis, *KMT2D* alterations were also enriched in primary MIBC tumors (23% vs 10%, $p = 0.013$), whereas inactivating alterations in *STAG2* (7.8% vs 17%, $p = 0.014$) and *TSC1* (5.5% vs 14%, $p = 0.007$) were more frequent in secondary MIBC (Fig. 2D). However, no alteration remained significantly different between primary and secondary MIBC on the exploratory analysis after adjusting for multiple comparisons (Supplementary Table 8).

As a potential guide for future neoadjuvant/adjuvant treatment strategies, we also explored the prevalence and co-occurrence patterns of potentially actionable genomic alterations in the primary and secondary MIBC cohorts. Alterations in the receptor tyrosine kinase/mitogen-activated protein kinase pathway were identified in 42% of secondary MIBC specimens, with *FGFR3* (18%) and *ERBB2* (15%) being two of the most commonly altered genes (Supplementary Table 8). *TP53* pathway and cell cycle regulatory gene alterations were detected in 61% and 65% of the secondary MIBC specimens, respectively. Given reports that coalteration of *FGFR3* with the cell cycle regulatory gene *CDKN2A* may be associated with NMIBC progression to MIBC, we explored this relationship [20]. No statistically significant difference was seen in the rate of *FGFR3* and *CDKN2A* coalteration between primary and secondary MIBC (10% [8/79] vs 5.0% [20/400], unadjusted $p = 0.08$) or in coalteration of *FGFR3* with any examined cell cycle regulatory gene (*RB1*, *E2F2*, *CDKN1A*, *CDKN2A*, *CDKN2B*, *CCND1*, and *CCNE1*; 13% [10/79] vs 6.8% [27/400], unadjusted $p = 0.07$).

3.3. Assessment of mutation burden

The presence of a known or likely pathogenic alteration in DDR-associated genes has been shown to correlate with a high overall mutation burden [8,21,22]. We thus compared tumor mutation burden between primary and secondary MIBC, as well as between *ERCC2* mutant and wild-type tumors within the combined retrospective and validation cohorts. *ERCC2* mutant tumors had significantly higher median mutation counts as quantitated by both WES (14.7 mutations/Mb [IQR = 8.5, 23.2] vs 5.5 mutations/Mb [IQR = 3.2, 9.5], $p < 0.001$) and targeted exon capture sequencing (25.0 mutations/Mb [19.9, 29.4] vs 8.5 [6.1, 15.8], $p < 0.001$). Moreover, primary MIBC tumors had a higher median mutation burden than secondary MIBC by targeted exon capture sequencing (11.2 mutations/Mb [7.1, 19.4] vs 7.7 mutations/Mb [4.9, 13.3], $p = 0.012$), but this relationship was not confirmed by WES (6.1 mutations/Mb [IQR = 3.3, 10.6] vs 6.1 mutations/Mb [IQR = 3.9, 10.2], $p = 0.8$).

3.4. Assessment of molecular subtypes

Gene expression profiling has been used to define intrinsic molecular subtypes of bladder cancer (broadly categorized as basal and luminal) that may have prognostic and therapeutic implications [23–25]. To explore the possibility that differences in the prevalence of intrinsic molecular subtypes between primary and secondary MIBC contribute to differences in response to chemotherapy, we performed unsupervised clustering of the TCGA gene expression data from patients included in the discovery cohort. Using two previously published gene-classifying sets, we were unable to identify any enrichment of luminal or basal molecular subtypes within primary or secondary MIBC patients

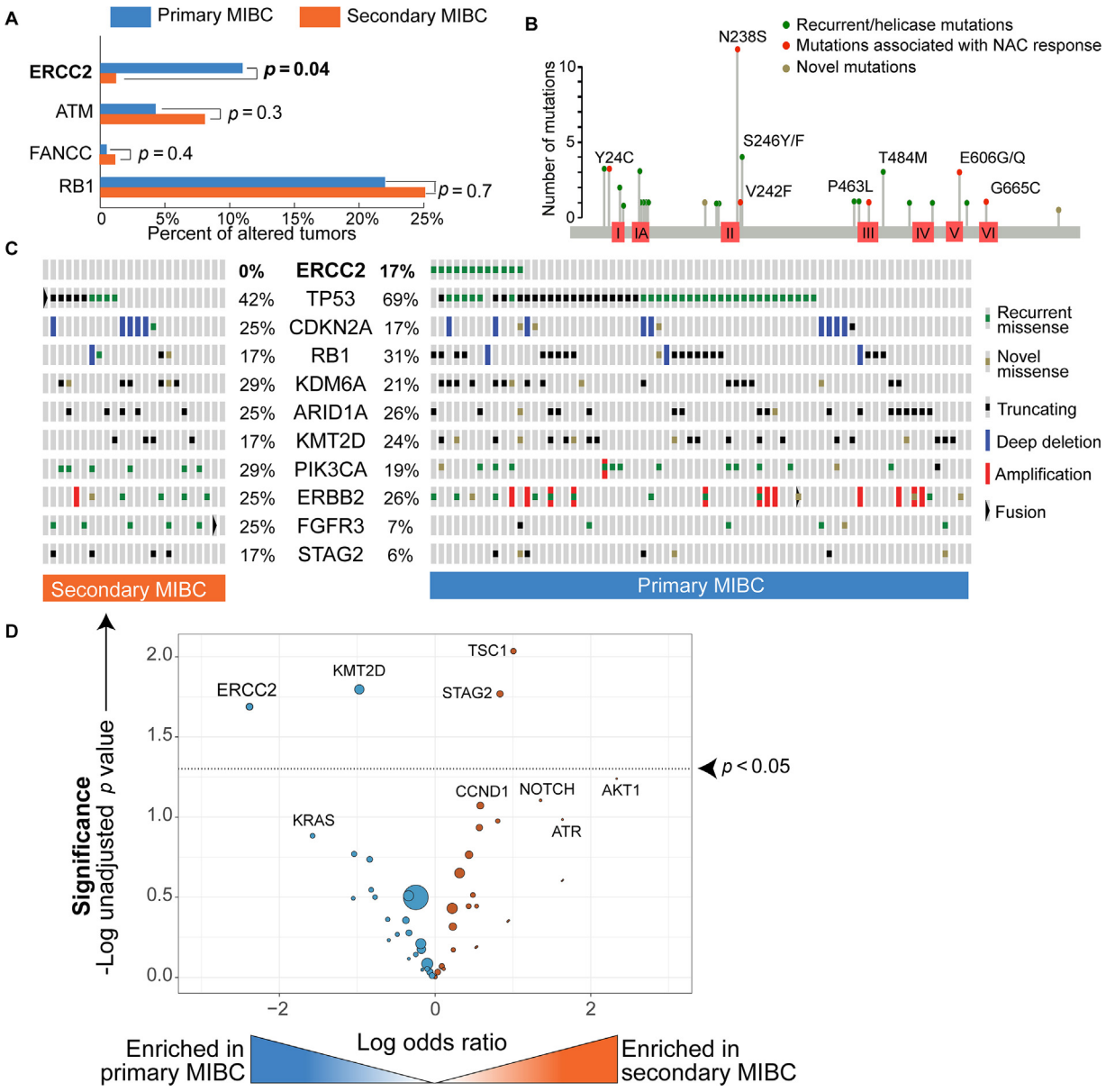

Fig. 2 – Genomic analyses of MIBC tumors. (A) Alteration frequency of genes previously associated with chemotherapy response in bladder cancer in primary versus secondary MIBC tumors in the initial genomic analysis. (B) Lollipop plot of ERCC2 missense mutations in primary MIBC in the combined genomic cohort. (C) Oncoprint comparing the frequency of mutations in ERCC2 and the 10 most commonly altered genes between primary and secondary MIBC patients in the prospective validation cohort. Genomic alteration frequencies include novel missense mutations of unknown significance. (D) Volcano plot of the association between mutations and secondary versus primary MIBC in the combined genomic cohort, shown as–log10 *p* value versus log odds ratio. Results are from exact logistic regression. Bubble size is proportional to the total number of alterations. The horizontal dotted line indicates an unadjusted *p* value of <0.05. MIBC = muscle-invasive bladder cancer; NAC = neoadjuvant chemotherapy.

[14,24] (Supplementary Fig. 3 and 4, and the Supplemental material, Methods). Thus, our clinical observation that secondary MIBC patients do worse with NAC appears to be independent of molecular subtyping.

4. Discussion

We find that MIBC can be dichotomized into primary and secondary disease states, with disparate clinical outcomes driven in part by differences in sensitivity to NAC. Although patients with MIBC have historically been offered NAC followed by RC regardless of whether they have a prior clinical history of treatment for NMIBC, our data indicate that patients with secondary MIBC have significantly lower response rates to chemotherapy and worse overall cancer-specific outcomes following cisplatin-based NAC. Indeed, secondary MIBC patients treated with NAC fared worse than those treated with cystectomy alone. The worse CSS observed following NAC in patients with secondary MIBC may in part stem from a significant delay in time to cystectomy (5.8 vs 1.7 mo, $p < 0.001$; Supplementary Table 4), which may increase the risk for micrometastatic

disease spread in this chemotherapy-refractory population. Until a molecular biomarker of NAC sensitivity in bladder cancer is validated and available for routine clinical care, secondary MIBC may serve as a surrogate to identify patients who may benefit from upfront cystectomy. Alternatively, patients with secondary MIBC may be ideal candidates for neoadjuvant clinical trials testing targeted agents in genomically selected patients, such as small molecule inhibitors of the FGFR signaling axis that have shown promising activity in chemotherapy-refractory metastatic bladder cancer, but have yet to be tested in the neoadjuvant setting [26].

Using WES and targeted sequencing data from a retrospective discovery cohort and a separate prospective validation cohort, we found that likely deleterious *ERCC2* missense mutations were significantly enriched in patients with primary versus secondary MIBC. *ERCC2* plays a central role in the repair of DNA damage induced by cisplatin chemotherapy through the nucleotide excision repair (NER) pathway [8]. Missense mutations near or within conserved helicase domains of *ERCC2* have previously been shown to be predictive of response to cisplatin-based chemotherapy in urothelial carcinoma and may also underlie heightened sensitivity to radiation therapy [8,27]. *ERCC2* mutations were also associated with improved survival outcomes in two independent MIBC patient cohorts treated with cisplatin-based NAC [19].

ERCC2 missense mutations occur in approximately 12% of unselected MIBC tumors, which is more than any other tumor type studied by the TCGA [8]. Our group recently identified *ERCC2* missense mutations in approximately 17% of high-grade NMIBC, for which intravesical BCG is the standard treatment [10]. One possible explanation for the low incidence of *ERCC2* alterations in the secondary MIBC specimens analyzed here is that *ERCC2* mutant tumors may have heightened sensitivity to intravesical BCG, resulting in selective eradication of *ERCC2* mutant subclones [10]. Since *ERCC2* mutant tumors have a higher tumor mutational burden, the immune activating effects of BCG may have a similar relationship with tumor mutational/neoantigen burden to that seen with immune checkpoint blockade response in the metastatic setting [28]. Another possible explanation for the lack of *ERCC2* mutations in secondary MIBC is that *ERCC2*-mutated tumor cells may be more sensitive to BCG-induced cytotoxic reactive oxygen species, because the NER pathway is also critical for oxidative DNA damage repair [29].

Our finding of worse oncologic outcomes among patients with secondary MIBC confirms the results of a case-matched retrospective study comparing patients developing secondary MIBC or metastasis after BCG immunotherapy with those with primary MIBC or de novo metastatic disease (3-yr CSS: 37% vs 67% in primary MIBC, $p = 0.0015$) [30]. The authors also speculated that intravesical BCG immunotherapy might select for tumor clones that are resistant to subsequent systemic chemotherapy. At least 13 additional studies have compared cystectomy outcomes between patients with primary and secondary MIBC, but results are mixed (Supplementary Table 9). However, these studies excluded patients treated with NAC, so they did not address the hypothesis that intravesical BCG immunotherapy selects clones resistant to subsequent systemic chemotherapy.

This study has several limitations. The clinical cohort was retrospective with selection bias in terms of NAC treatment within primary and secondary MIBC patients. Further, most secondary MIBC patients had their initial NMIBC management at outside institutions before referral to our center; thus, we could not control for inadequate or delayed treatments. While we focused our efforts on somatic DDR gene alterations, the molecular basis of cisplatin sensitivity in DDR gene wild-type patients deserves further investigation, including germline, epigenetic, and transcriptomic factors that may modulate DDR function or are independent of DDR function. While our study did not show a clear correlation between primary and secondary MIBC and these RNA expression-based phenotypes, the size of our cohort limited our ability to detect such an association. Ultimately, prospective validation of our findings will be required, and might be possible in the context of the SWOG1314 COXEN study and the Alliance 017301 trial assessing bladder-sparing in patients with select DDR gene alterations who achieve clinical downstaging following cisplatin-based NAC.

5. Conclusions

Our results suggest that primary and secondary MIBC have disparate clinical outcomes and differential responses to cisplatin-based NAC. Genomic analysis of chemotherapy-naïve specimens reveals that primary MIBC, but not secondary MIBC, is more likely to harbor likely pathogenic *ERCC2* mutations predicted to be sensitizing to cisplatin-based chemotherapy. Although cystectomy should ideally be performed before NMIBC progresses to secondary MIBC, the findings of our study suggest that patients with secondary MIBC derive little benefit from current standard cisplatin-based NAC. Further investigation is warranted into whether upfront cystectomy or enrollment into clinical trials of novel agents may be a preferred clinical approach for secondary MIBC.

Author contributions: Eugene J. Pietzak 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.

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Appendix A. Supplementary data

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