Impact of sex on response to neoadjuvant chemotherapy in patients with bladder cancer

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ABSTRACT

Objective: To assess the effect of patient's sex on response to neoadjuvant chemotherapy (NAC) in patients with clinically non-metastatic muscle-invasive bladder cancer (MIBC).

Methods: Complete pathologic response, defined as ypT0N0 at radical cystectomy, and downstaging were evaluated using sex-adjusted univariable and multivariable logistic regression modeling. We used interaction terms to account for age of menopause and smoking status. The association of sex with overall (OS) and cancer-specific survival (CSS) was evaluated using Cox regression analyses.

Results: A total of 1031 patients were included in the analysis, 227 (22%) of whom were female. Female patients had a higher rate of extravesical disease extension (p = 0.01). After the administration of NAC, ypT stage was equally distributed between sexes (p = 0.39). On multivariable logistic regression analyses, there was no difference between the sexes or [age of menopause] with regards to ypT0N0 rates or downstaging (all p > 0.5). On Cox regression analyses, sex was associated with neither OS (1.04, 95%CI 0.75 – 1.45, p = 0.81) nor CSS (1.06, 95%CI 0.71 – 1.58, p = 0.77).

Conclusion: Our study generates the hypothesis that NAC may equalize the preoperative disparity in clinical stage between men and women, suggesting a possible differential response between sexes. ~~suggesting that females might respond better to NAC.~~ This might be the explanation underlying the comparable pathologic stage and outcomes between sexes and needs to be tested prospectively.

Introduction

Patient sex (female vs male) has a differential effect on bladder cancer (BCa) presentation and survival[1, 2]. While BCa incidence is three to four times higher in males compared to females, the latter are more likely to be diagnosed with advanced disease and to suffer from worse survival outcomes despite standard treatment[3–5]. Neoadjuvant chemotherapy (NAC) is part of the standard of care therapeutic modalities delivered in patients with clinically non-metastatic muscle-invasive BCa (MIBC)[6, 7]. However, females are less likely to receive NAC which is partially explained by differences in health care factors like time to diagnosis and treatment modality[8–11].

To the best of our knowledge, there is no data on a potentially differential response to NAC according to sex in patients treated with radical cystectomy (RC). To fill this gap, we compared pathologic response rates and survival outcomes between sexes adjusting for the effects of smoking and age suggestive of menopause in a large multicenter dataset of patients treated with NAC followed by RC for BCa.

Material and methods

Study population

We performed a retrospective analysis of our multi institutional database comprising 1474 patients treated with NAC followed by RC for BCa from 2000 to 2013 [7].

Patients with clinically metastatic disease (N+ and/or M+) were excluded, leaving 1031 patients for final analysis. A total of 313 patients were lost to follow-up, leaving 718 patients for survival analyses. Clinical stage prior to the administration of chemotherapy was assigned by the treating physician based on transurethral resection of the bladder, bimanual exam and/or cross-sectional imaging.

Chemotherapy

NAC regimens consisted of cisplatin-based combination chemotherapy, or other. Chemotherapy regimen and number of cycles were administered at clinician discretion in accordance with institutional standards and guidelines at that time.

Radical cystectomy

Patients were treated with RC and lymphadenectomy. All procedures were performed by an open technique. The decision for the type of urinary diversions was based on patient and disease characteristics, patient's and surgeon's preferences as well as patient's performance status. All surgical specimens were processed according to standard pathologic procedures and staged according to the 1998 TNM classification. All tumors were high-grade.

Outcome measurement

Response to NAC was assessed by yTNM stage at RC. Complete pathologic response was defined as ypT0N0. Downstaging was defined as any stage migration from non-organ confined disease (NOC) to ypT2-N0, ypNMIBC-N0 or ypT0-N0 or from cT2 to ypNMIBC-N0 or ypT0-N0. Overall survival (OS) and cancer-specific survival (CSS) were calculated from the day of RC death of any cause for OS and death due to bladder cancer for CSS, respectively. Patients were censored at the time of last follow up.

Cause of death was recorded through patients charts and/or death certificates [12].

Molecular correlates of response to chemotherapy

Since both RNA expression subtypes and mutations in specific DNA damage response (DDR) genes have been shown to correlate with response to NAC in patients with MIBC, we investigated the prevalence of subtypes and DDR gene alterations according to sex using data from 395 chemo-naïve patients with MIBC from The Cancer Genome Atlas Program (TCGA)[13]. The TCGA subtypes (luminal papillary, luminal infiltrated, luminal, basal squamous and neuronal) were used. We selected ERCC2[14] as well as RB1, ATM and FANCC[15] as key DDR genes based on prior reports, but also added ATR, BRCA1, BRCA2, ERCC5, RAD51C, and REQLC4 based on the list of DDR genes selected as functionally important in three ongoing trials investigating bladder preservation (NCT03609216, NCT03558087, NCT02710734)[16–18].

Statistical analysis

We performed a stepwise approach to the statistical analyses. First, we performed multiple imputation by using chained equations to handle missing data that were assumed to be missing at random. Fifteen imputed data sets were generated using predictive mean matching for numeric variables, logistic regression for binary variables and Bayesian polytomous regression for factor variables. Second, we compared the distribution of patients' clinicopathologic features according to sex. Third, we evaluated the association of sex with pathologic response using univariable and multivariable logistic regression modeling. Due to the even distribution of the data between groups, adjustments using propensity score were not performed. Fourth, as pre-planned analysis, we introduced interaction terms in the logistic models to evaluate the synergistic effect of sex and smoking status or menopausal status. As the age of menopause was not available, we arbitrarily assigned the age of 50 as cut-off for menopause. Fifth, we investigated the association of sex with OS and CSS using Cox regression analyses and plotted survival curves using the Kaplan-Meier method. Sixth, we tested the validity of the Cox model assumption using Shoenfeld residuals. Due to the exploratory character of the study, statistical significance was considered at $p<0.05$, but not in a confirmatory manner. Therefore, no adjustment for multiplicity was performed. All tests were performed with R (R Foundation for Statistical Computing, v3.5.1).

Results

Clinico-pathologic features of the population are shown in Table 1. Overall, 804 (78%) patients were of male sex and 227 (22%) were of female sex. Females had more advanced clinical stage at presentation than their male counterparts (NOC disease 36.6 vs 32.6%

We observed an equal distribution of ypT stage between sexes after NAC (Figure 1). On univariable logistic regression analyses, we could not identify an association of sex with downstaging or complete pathologic response to NAC (all $p > 0.5$). Multivariable analyses which adjusted for the effects of clinical stage, administered NAC regimen, number of cycles, and smoking status, failed to identify a significant difference between females and males in downstaging or complete pathologic response to NAC when comparing the means between the two populations in the overall model (all $p > 0.5$) (Table 2).

Overall, 207 (91%) female patients were 50 years or older. Of these, 91 (44%) were never smokers, 109 (53%) former smokers and 7 (3%) current smokers. On univariable and multivariable logistic regression analyses, we could not identify an association of menopausal status with complete response to NAC or downstaging (all $p > 0.5$) (Table 3).

Within a median follow-up of 17 months (IQR 7 – 37), 297 (41%) patients died and 206 (29%) died of their BCa. On Cox regression analyses, female sex was neither associated with OS (HR 0.98, 95%CI 0.69 – 1.38, $p = 0.89$) nor CSS (HR 1.03, 95%CI 0.69 – 1.55, $p = 0.88$) (Figure 2). The validity of the proportional hazard assumption was supported by a non-significant relationship between residuals and time ($p = 0.99$).

In a final step, we extracted TCGA data[13] and analyzed the prevalence of ten DDR genes (ATM, ATR, BRCA1, BRCA2, ERCC2, ERCC5, FANCC, RAD51C, RB1 and REQLC4) between males and females. We found that females have fewer DDR gene mutations overall compared to males (28.3% vs 44.6%, $p < 0.001$). However, there was no difference in the rate of single DDR gene mutations between sexes (supplementary Figure S1). With respect to RNA-based subtypes, basal-squamous was more frequent

121 in females (43.4% vs 37.7%) and luminal papillary in males (37.7% vs 28.3%). However, these
122 differences were not statistically significant (all $p \geq 0.05$, Supplementary figure S2).

Discussion

In a retrospective analysis of a large multicenter cohort of patients treated with NAC followed by RC for non-metastatic BCa, we could not observe any association of sex with pathologic complete response to NAC. While there was a small but statistically significant difference in clinical T stage at diagnosis, this difference between sexes could no longer be observed after NAC.

Although the incidence of BCa in females is lower than in males, female patients often present with more advanced disease and suffer from worse prognosis [1, 2, 19]. In this context, genetic, environmental, hormonal and health care differences are known to play a role in response to standard therapies and oncologic outcomes[8, 20]. However, a definitive and satisfactory explanation for these sex-based differences is still missing. We tried to shed light on this, by investigating the synergistic effect of smoking and cut-off age of 50, as surrogate for menopause[21], on response to NAC. We found no association of either age or smoking status with response to NAC. Notably, a meta-analysis showed that the magnitude of benefit to immune checkpoint inhibitors may be higher in men than women, but only one trial of patients with advanced urothelial carcinoma was included in that [22]. However, less than 10% of the women in this cohort were under age 50 and a difference may be difficult to identify.

Smoking is a well-known risk factor for BCa[23, 24]. Population-based studies have shown that among smokers, females have a higher risk of developing BCa compared to males (HR 2.75 for female vs 2.32 for male)[25]. However, the synergistic effect of smoking and sex is not consistent in the literature [26, 27]. In pre-clinical studies, smoking has been linked to chemo-resistance in human BCa cell line[28]. However, the clinical literature presents controversial results regarding smoking status as predictor of chemo-resistance, even when stratified by sex [29–32].

In our study, we expanded upon previous findings by investigating the synergistic effect of smoking and sex on the response to NAC in a large population with clinically non-metastatic MIBC. We could not identify a statistically significant association of smoking status with downstaging or complete response to NAC. This effect can partially be explained by the low patient number in relation to the difference between groups. Indeed, if we look at the reported effect in population-based studies[25,

33], a larger cohort would, probably, be needed to show a statistically significant difference between males and females.

Preclinical studies have shown that the modulation of circulating estrogen levels through the menopausal status leads to structural changes in the murine bladder [30, 31]. In clinical studies, sex-based differences in hormonal status have been linked to the development and progression of BCa [34, 35].

We investigated the association of age, using the cut-off of 50 years as surrogate for menopause, with pathological response to NAC. We, indeed, found no significant association with any of the outcomes. These findings are in line with the current literature. For example, in a case-control-study with a meta-analysis, Dietrich et al. found that postmenopausal females were at higher risk for developing a BCa, but this association was not statistically significant (OR 1.30, 95%CI 0.45 – 3.77). Those authors also reported that the OR increased with the age of menopause of <45 years (OR 1.33, 95%CI 0.72 – 2.47)[36]; but again, this association was not significant. Differences in tumor biology, change in sex steroid receptor after menopause and the potential association of BCa with sex steroid hormones may explain this phenomenon[37].

Somatic genetic alterations in DDR genes and molecular subtypes have been linked to clinical response to cisplatin-based NAC[14, 15, 38]. Choi et al. have also reported that tumors of the basal subtype, which appear to benefit most from NAC, are enriched in women[38]. In order to evaluate whether differences in these two molecular parameters could explain the differential response to NAC in female, we analyzed the TCGA data. We found that men had overall more DDR gene mutations than females; however, it can be hard to ascertain the functional impact of each mutation.. Moreover, we could not identify a ssignificant difference in the rate of single DDR gene mutations or prevalence of mRNA cluster between sexes. Altogether, these molecular findings do not clearly explain the modest differential response rate to NAC between males and females.

Complete pathologic response after NAC has been correlated with improved OS and RFS [39, 40]. We investigated the association of sex with survival and found no statistical difference in OS and CSS. In contrast to our findings, in a retrospective analysis of 4,216 patients treated with RC without NAC,

Messer et al. found a significant association of female sex with recurrence (p = 0.039) and CSS (p = 0.001) [41]. The explanation for these disparities is likely multifactorial[1, 2, 42]. In our study, all patients were treated with NAC, which might have potentially abrogated clinical differences in survival. Indeed, we observed no difference in pathologic T or N stage between sexes after NAC. This is an important finding which generates the hypothesis that sex-based differences in clinical and pathologic features in BCa might be potentially equalized through the administration of NAC, leading to comparable oncologic outcomes.

We acknowledge the limitations of our study, which are mainly inherent to its retrospective design and the short follow-up. Staging and the administration of NAC were not standardized. Moreover, we could not account for the quality of surgical techniques. Indeed, the extent of the resection may have possibly influenced outcomes. Previous reports could not show a significant difference between sexes in patients treated with incomplete or complete TURB before NAC. For example, James et al. investigated the association of maximal TURB with complete pathologic response to NAC. Among 81 patients who received NAC, those treated with maximal TURB were more likely to achieve complete pathologic response (OR 3.17, 95%CI 1.02 – 9.83). Stratified by sex, females were more likely to achieve complete pathologic response. However, this association was statistically not significant [43]. In addition, the anatomic difference in bladder wall thickness between males and females could also have influenced outcomes by allowing a more radical resection in females.

In this context, it can be argued that nodal staging could be a more accurate end-point to assess response to NAC, as lymph nodes are not affected by any surgical intervention prior to NAC administration. In a previous retrospective analysis of 304 patients with clinically N+ treated with induction chemotherapy followed by RC, we found that a complete pathological response can be achieved in 14.5% of the patients. However, the authors could not detect any differences in response to chemotherapy between sexes[44]. Finally, this study did not evaluate the association of sex with NAC related toxicity, morbidity and mortality.

Despite these limitations, our study provides clinically relevant information and generates the hypothesis that NAC could reduce the survival gap between males and females by equalizing sex-

specific differences in clinical stage emphasizing the adoption of multimodal treatment modalities in the era of personalized medicine[45].

Conclusion

We found that, in patients planned for NAC and RC, females have worse clinico-pathologic features compared to males at the time of diagnosis. After the administration of NAC there was no difference in pathologic stage. Our analyses generate the hypothesis of a differential response to NAC between sexes which could potentially equalize the clinical outcomes of patients with different prognosis. Further research should focus on testing that hypothesis, as well as on sex-based differences in response to novel systemic agents, such as immune checkpoint and FGFR inhibitors , as well as trimodality therapy[46].

Aknowledgment

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Table 1. Clinicopathologic features of 1031 patients treated with neoadjuvant chemotherapy and radical cystectomy for clinically non-metastatic muscle-invasive bladder cancer, stratified by sex			
	Male	female	p
n (%)	804 (78)	227 (22)	
Age, median (IQR)	63 (57 – 71)	65 (58 – 72)	0.11
Smoking, n (%)			0.02
Never	292 (36.3)	100 (44.1)	
Former	451 (56.1)	119 (52.4)	
Current	61 (7.6)	8 (3.5)	
Histology, n (%)			0.98
Urothelial	705 (87.7)	201 (88.5)	
Mixed histological variant*	99 (12.3)	26 (11.5)	
Chemotherapy regimen, n (%)			0.73
Cisplatin-based	670 (83.3)	192 (84.6)	
Other	134 (16.7)	35 (15.4)	
Chemotherapy cycles, n (%)			0.13
1 to 2	76 (9.5)	32 (14.1)	
3 to 4	679 (84.5)	182 (80.2)	
5 to 8	49 (6.1)	13 (5.7)	
cT, n (%)			0.01
cT2	510 (63.4)	131 (57.7)	
cT3	195 (24.3)	77 (33.9)	
cT4	99 (12.3)	19 (8.4)	
ypT, n (%)			0.39
ypT0	190 (23.6)	48 (21.1)	
ypNMIBC	164 (20.4)	46 (20.3)	
ypT2	160 (19.9)	38 (16.7)	
ypT3/T4	290 (36.1)	95 (41.9)	
ypN, n (%)			0.76
ypN0	642 (79.9)	174 (76.7)	
ypN1	64 (8.0)	20 (8.8)	
ypN2	85 (10.6)	29 (12.8)	
ypN3	13 (1.6)	4 (1.8)	
Nodes removed, median (IQR)	18 (11 – 27)	16 (11 – 25)	0.11
Positive STSM, n (%)	65 (8.1)	16 (7.0)	0.71

IQR = interquartile range; GEM-CIS = gemcitabine cisplatin; DD-MVAC = dose-dense methotrexate, vinblastine, doxorubicin, cisplatin; GEM-CARBO = gemcitabine carboplatin; MVAC = methotrexate, vinblastine, doxorubicin, cisplatin; NMIBC = non-muscle invasive bladder cancer; STSM = soft tissue surgical margin

* Mixed histological variant includes adenocarcinoma, neuroendocrine carcinoma and squamous carcinoma

Table 2. Logistic regression analyses for the association of sex and smoking with downstaging and ypT0N0 status in 1031 patients treated with neoadjuvant chemotherapy and radical cystectomy for clinically non-metastatic muscle-invasive bladder cancer

Univariable analysis				
	Downstaging		ypT0N0	
	OR (95%CI)	P	OR (95%CI)	p
Female vs male sex	0.92 (0.68 – 1.24)	0.59	0.87 (0.60 – 1.23)	0.44
Smoker				
Never	Ref		ref	
Former	1.06 (0.82 – 1.38)	0.63	0.90 (0.66 – 1.22)	0.49
Current	0.74 (0.44 – 1.24)	0.25	0.73 (0.37 – 1.37)	0.35
Multivariable analysis				
	Downstaging		ypT0N0	
	OR (95%CI)	P	OR (95%CI)	p
Female vs male sex	0.82 (0.51 – 1.31)	0.40	1.18 (0.69 – 1.98)	0.54
Smoking status				
Never	Ref		ref	
Former	1.01 (0.75 – 1.36)	0.96	1.01 (0.72 – 1.45)	0.93
Current	0.77 (0.43 – 1.36)	0.37	0.75 (0.36 – 1.46)	0.41
Clinical T stage				
cT2	Ref		ref	
cT3	1.31 (0.98 – 1.75)	0.07	0.73 (0.51 – 1.03)	0.08
cT4	1.29 (0.86 – 1.93)	0.21	0.89 (0.54 – 1.41)	0.63
Cisplatin-based chemotherapy	2.09 (1.48 – 2.99)	<0.01	1.46 (0.96 – 2.28)	0.08
Chemotherapy cycles				
1 to 2	Ref		ref	
3 to 4	1.28 (0.85 – 1.94)	0.23	1.18 (0.73 – 1.98)	0.52
5 to 8	0.84 (0.43 – 1.60)	0.59	0.84 (0.36 – 1.87)	0.68
sexfemale:smokeformer	1.16 (0.62 – 2.15)	0.64	0.57 (0.27 – 1.21)	0.15
sexfemale:smokecurrent	1.41 (0.29 – 6.97)	0.66	0.50 (0.02 – 3.55)	0.55
	F-statistics 2.79, p = 0.002		F-statistics 1.12, p = 0.34	

OR = odds ratio; CI = confidence interval

Table 3. Logistic regression analyses for the association of menopausal status and smoking with downstaging and ypT0N0 status in 227 female patients treated with neoadjuvant chemotherapy and radical cystectomy for clinically non-metastatic muscle-invasive bladder cancer

Univariable analysis				
	Downstaging		ypT0N0	
	OR (95%CI)	P	OR (95%CI)	p
Age ≥50 years*		0.88	1.05 (0.36 – 3.80)	0.93
Smoker				
Never	Ref		ref	
Former	1.19 (0.70 – 2.04)	0.51	0.57 (0.29 – 1.10)	0.09
Current	1.17 (0.26 – 5.21)	0.83	0.41 (0.02 – 2.44)	0.41
Multivariable analysis				
	Downstaging		ypT0N0	
	OR (95%CI)	P	OR (95%CI)	p
Age ≥50 years*	0.50 (0.11 – 2.11)	0.35	1.25 (0.27 – 9.02)	0.79
Smoking status				
Never	Ref		ref	
Former	0.37 (0.05 – 2.41)	0.30	0.88 (0.08 – 9.33)	0.91
Current	1.25e+06 (1.35e-72 – NA)	0.99	1.60e-06 (NA – 1.12e+72)	0.99
Clinical T stage				
cT2	Ref		ref	
cT3	1.20 (0.67 – 2.14)	0.54	0.85 (0.41 – 1.72)	0.67
cT4	0.55 (0.18 – 1.51)	0.26	0.34 (0.05 – 1.33)	0.17
Cisplatin-based chemotherapy	2.21 (1.02 – 5.08)	0.05	0.96 (0.40 – 2.51)	0.93
Chemotherapy cycles				
1 to 2	Ref		ref	
3 to 4	1.41 (0.65 – 3.19)	0.39	0.71 (0.29 – 1.83)	0.45
4 to 8	1.61 (0.41 – 6.35)	0.49	1.76 (0.38 – 7.73)	0.46
sexfemale:smokeformer	3.33 (0.47 – 25.6)	0.23	0.58 (0.05 – 6.79)	0.65
sexfemale:smokecurrent	7.07e-07 (NA – 4.28e+71)	0.99	3.37e+05 (7.43e-61 – NA)	0.99
	F-statistics 0.89, p = 0.54		F-statistics 0.71, p = 0.72	

OR = odds ratio; CI = confidence interval

Figure 1 – Alluvial diagram for the changes of cT stage to ypT stage in 1031 patients treated with neoadjuvant chemotherapy (NAC) and radical cystectomy for clinically non-metastatic muscle-invasive bladder cancer, stratified by sex

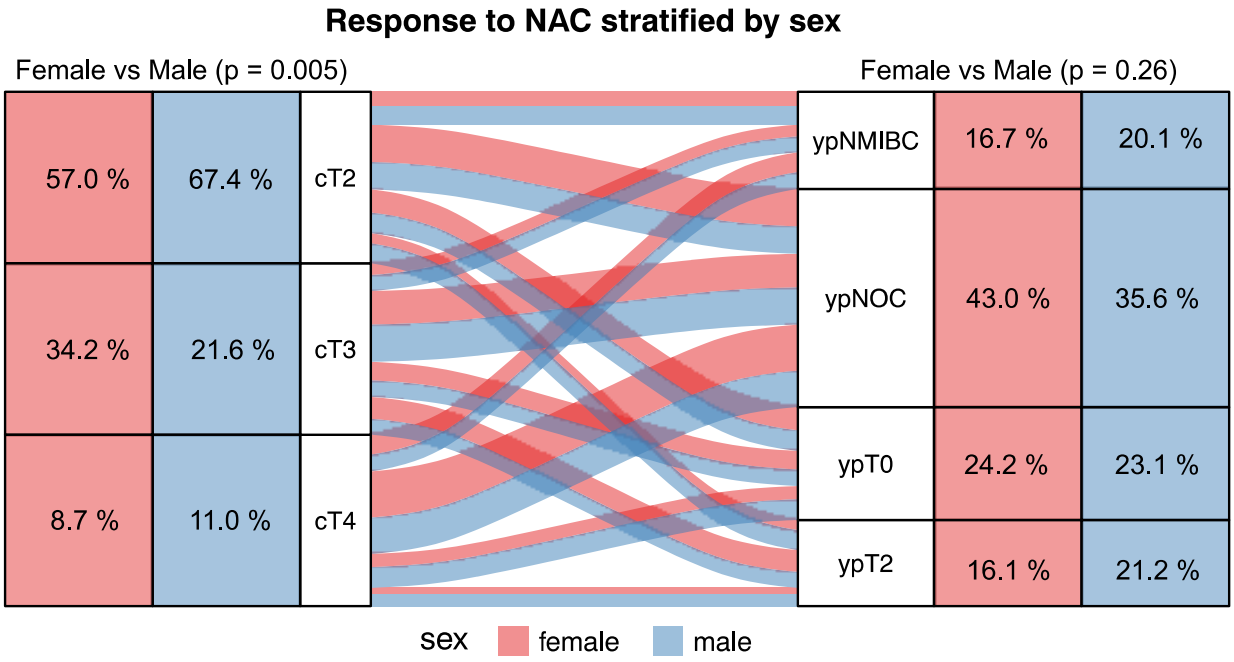
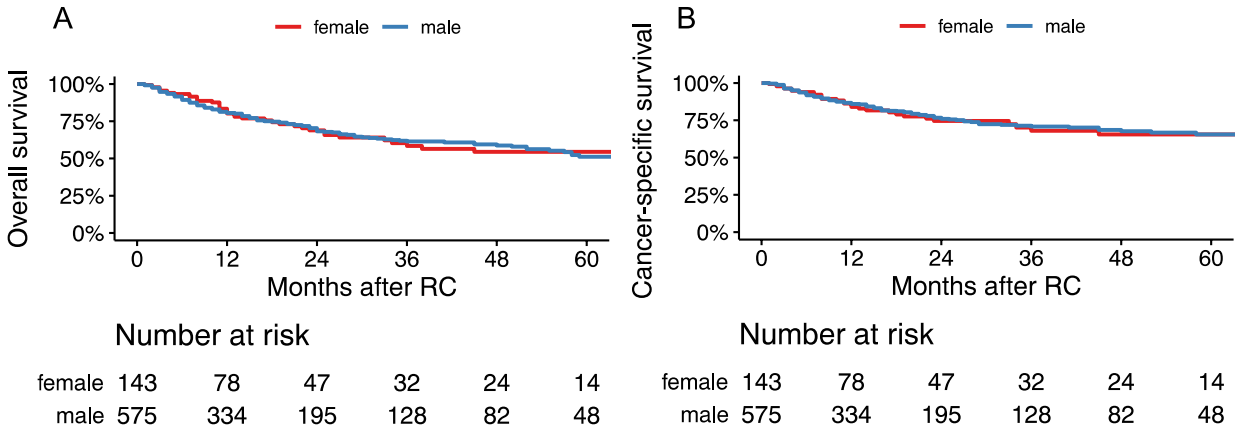
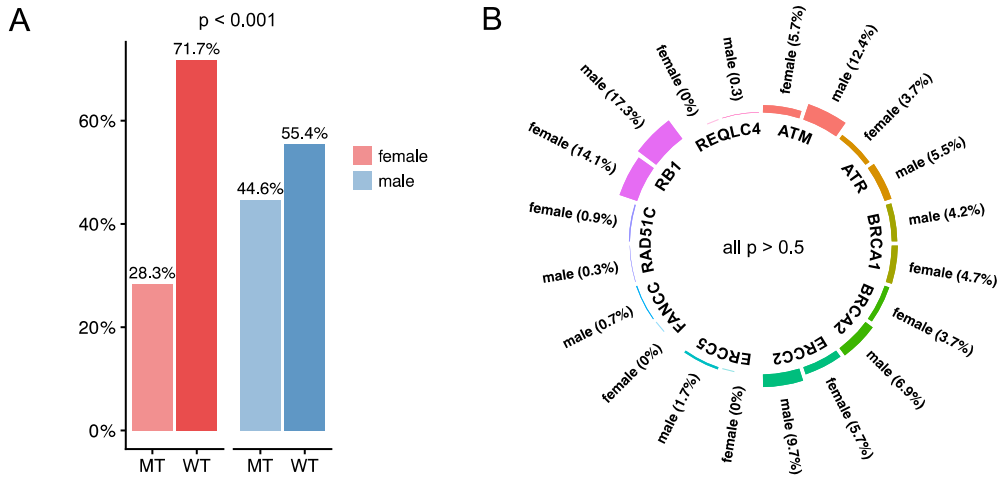


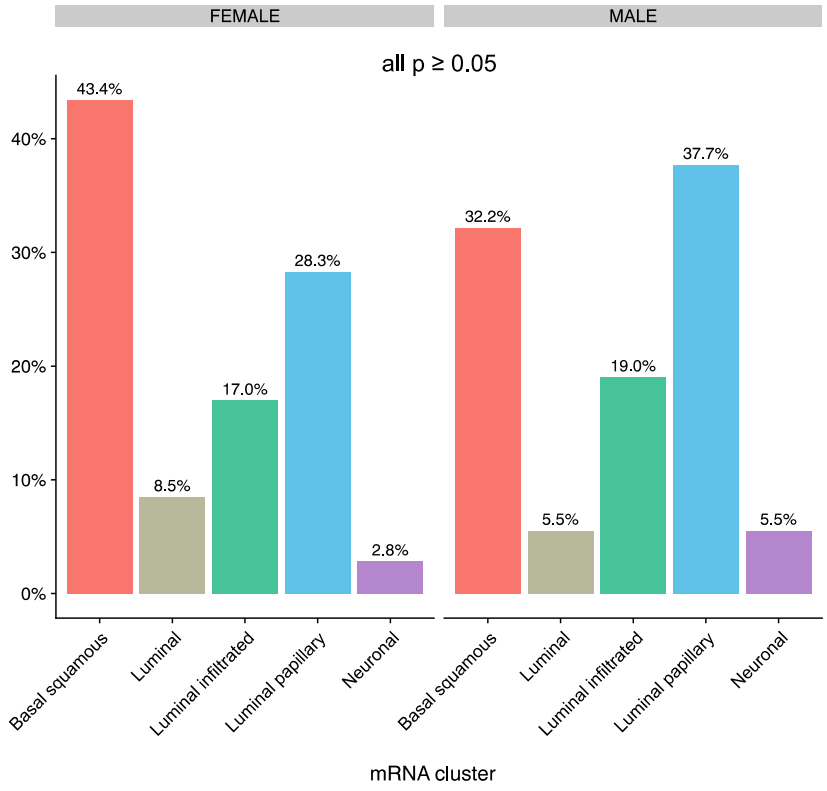
Figure 2 – Kaplan-Maier curves for the association of sex with overall (A) and cancer-specific survival (B) in 718 patients treated with neoadjuvant chemotherapy and radical cystectomy for clinically non-metastatic bladder cancer



Supplementary Figure S1 – Prevalence of mutated (MT) and wild-type (WT) DNA damage repair (DDR) genes in 106 female and 289 male patients with muscle-invasive bladder cancer. (A) Any DDR gene mutation stratified by sex. (B) Subanalysis of the single DDR genes stratified by sex. Data extracted from The Cancer Genome Atlas Program[13].



Supplementary Figure S2 – Prevalence of mRNA cluster in 106 female and 289 male patients with muscle-invasive bladder cancer. Data extracted from The Cancer Genome Atlas Program[13].



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