

# Prognostic Factors of Non-Muscle Invasive Bladder Cancer: A Study Based on Next-generation Sequencing

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# Abstract

## Objective

To investigate the genetic prognostic factors for the recurrence of non-muscle invasive bladder cancer.

## Materials and Methods

The patients underwent transurethral resection of bladder tumor and received bacillus Calmette–Guérin (BCG) or epirubicin. Next-generation sequencing was performed and alterations of genes, pathways, and tumor mutation burden were recorded. Associations between these clinicopathological and genetic variants were estimated, and prognostic factor identified.

## Results

A total of 58 cases were included in our study, and 46 patients underwent treatment with BCG. *FGFR3* was the most frequently altered gene (48%), and more commonly detected in intermediate-risk patients. Univariate Cox analysis demonstrated that 10 genes were significantly correlated with BCG failure, while *NEB*, *FGFR1* and *SDHC* were independent recurrence predictors. Besides, epigenetic-related gene pathway mutations were negatively correlated with recurrence (hazard ratio: 0.198, P=0.023). DNA damage response and repair gene alterations were positively correlated with tumor burden, while altered *TP53* was most frequent among these genes and significant correlated with high tumor burden.

## Conclusion

BCG instillation significantly reduced the rate of recurrence compared with epirubicin in this population. Potential biomarkers and therapeutic targets were found with the help of next-generation sequencing; correlations between DDR genes alterations and high tumor mutation burden were also demonstrated.

# Introduction

Approximately 75% of patients with bladder cancer have non-muscle invasive bladder cancer (NMIBC; Ta or T1 disease) at the time of primary diagnosis<sup>1</sup>. For intermediate- and high-risk patients, transurethral resection of bladder tumor (TUR-BT) and subsequent intravesical drug instillation are the standard treatments. And it has been confirmed that treatment with Bacillus Calmette–Guérin (BCG) following TUR-BT is superior to TUR-BT alone or combined with chemotherapy in Caucasian patients<sup>2,3</sup>. However, despite the administration of maintenance therapy with BCG, 32.6% and 13.4% of patients continue to experience recurrence and tumor progression, respectively<sup>4</sup>.

With the help of next-generation sequencing (NGS), substantial information on genetic alterations can be acquired, which provide potential biomarkers for failure of intravesical therapy and therapeutic targets<sup>5</sup>. In this study, we analyzed the prognostic factors of recurrence based on a randomized controlled trial of

BCG and epirubicin (EPI), examined the predictive value of prognostic models, and investigated the genetic alterations of this cohort to define the potential clinical implications of these variants.

## **Patients And Methods**

### **Patients and treatment**

Patients were collected from a prospective randomized study of intravesical therapy after TUR-BT: those diagnosed as intermediate- or high-risk NMIBC were randomized to receive BCG or EPI for one-year regime by a ratio of 4:1 (phase IV clinical trial: CTR20150840 for Center for Drug Evaluation of National Medical Products Administration, China). The inclusion criteria were: age 20–75 years; TUR-BT with completely tumor resection. BT was completely resected with pathologically proven intermediate or high-risk non-muscle invasive bladder urothelium carcinoma according to the European Association of Urology (EAU) guidelines for risk stratification of NMIBC<sup>6</sup>. Exclusion criteria were: a. Eastern Cooperative Oncology Group performance status score >1; b. patients with active tuberculosis or receiving treatment for anti-tuberculous; c. immune deficiency or undergoing immunosuppressive therapy; d. severe complication (e.g., serious cardiovascular and cerebrovascular disease), or presence of other types of cancer; e. previous diagnosis with muscle-invasive bladder cancer; f. patients underwent treatment (e.g., chemotherapy, radiotherapy or immunotherapy) during the previous 4 weeks which may influence the research results; f. serious intraoperative and postoperative complications (e.g., bladder perforation, serious postoperative hematuria, bladder irritation, etc.); and g. patients not suitable to receive treatment or not able to participate in our trial due to pregnancy, severe disability, serious psychological problems, etc.

### **Follow-up and outcomes**

For intermediate-risk patients, cystoscopy was performed 3 and 6 months after surgery, then biannually until 5 years. For high-risk patients, cystoscopy was performed trimonthly until 2 years after surgery, then biannually until 5 years. The initiation time was the day TUR-BT was performed. The primary end point was recurrence during follow-up, while the secondary end point was progression to muscle-invasive bladder cancer or therapy-related discontinuation of treatment. Recurrence-free survival (RFS) and progression-free survival (PFS) times were recorded. BCG failure and intolerance were recorded according to the definitions of the EAU<sup>6</sup>

### **Data collection**

Recorded clinicopathological variants included gender, age, tumor size, site, and number, tumor histopathological type, T stage (8th American Joint Committee on Cancer TNM classification system)<sup>7</sup>, tumor grade (2004 World Health Organization grading system), and carcinoma in situ status. All pathological reports were provided by the Pathology Department of West China Hospital, Sichuan University.

Paraffin-embedded tissues obtained from the BCG treatment group were collected for NGS. Patients provided written informed consent. Sequencing was performed on a Nextseq500 sequencer (Illumina, Inc., San Diego, CA, USA) for 520 cancer-related genes, including the whole exons of 312 genes, as well as critical exons, introns, and promoter regions of the remaining 208 genes. Resultant sequences were analyzed for gene alternations. Nonsynonymous mutations, deletions, nonsense mutations, transcodng mutations, and splicing mutations were considered significant. In addition, the tumor mutation burden (TMB) was also recorded.

## Statistical methods

One-way analysis of variance and the chi-squared test were used for comparisons between groups. The chi-squared test was used to analyze categorical variants. The Pearson chi-squared test was used when  $n \geq 40$  and  $T \geq 5$ ; the continuously correcting chi-squared test was used when  $n \geq 40$  and  $1 \leq T < 5$ ; if  $n < 40$  or  $T < 0$ , Fisher's exact test was used. Kruskal–Wallis and Wilcoxon tests were used to analyze continuous variants. RFS and PFS were estimated using the Kaplan–Meier method. Differences in survival between two or more subgroups were evaluated using log-rank tests. Univariate and multivariate Cox regression analyses were performed to determine the clinicopathological parameters associated with the recurrence of patients with NMIBC. Patients were stratified using the EORTC risk tables<sup>8</sup> and the CUETO scoring model<sup>4</sup>.

Statistical analyses were performed using the SPSS Statistics version 25 (IBM, Armonk, NY, USA). A P-value  $< 0.05$  denoted statistical significance.

## Results

### Patient baseline data and prognosis

The clinicopathological data of 58 collected patients were shown in Table 1. The mean age was 61.91 years (standard deviation: 7.67 years), and the median follow-up time was 32.86 months (interquartile range: 21.00–44.49 months). There were no significant differences in baseline clinicopathological data between BCG and EPI subgroups except for T stage. For BCG subgroup, recurrence occurred in 9 patients, with 6 of them recurrent during course of intravesical BCG therapy. Two years RFS rate for BCG group was 82.2%; while for EPI subgroup, 7 patients recurrent and five occurred during EPI treatment course. Two years RFS rate for EPI was 58.3%. Univariate analysis revealed that significant better RFS was seen in

BCG treated subgroup ( $P=0.020$ ) and those with incipient tumor ( $P=0.001$  for all 58 patients and  $P=0.014$  for 46 BCG treated patients) (Additional file 1 table A1).

### Genomic landscape of patients with NMIBC and relationship with prognosis

NGS was performed on the total 58 patients, the most frequently altered genes were *FGFR3* (48%), *KDM6A* (47%), *KMT2D* (43%), *KMT2C* (34%), and *STAG2* (31%). Oncoprints for these genes are presented in Additional file 2 Figures A1–S7). Significant mutations of *FGFR3* were observed in 73.1% of intermediate-risk patients (19/26) and 15.6% of high-risk patients (5/32). For *STAG2*, these numbers were 46.2% (12/26) and 18.8% (6/32), respectively. Mutations of *FGFR3*, *STAG2*, and *PRKDC* were significantly associated with tumor risk stage ( $P<0.05$ , Additional file 1 Table A2). Moreover, we found that mutations of 17 genes (such as *KDM6A* and *ARID1A*) were significant correlated with a higher EORTC score, while mutations of other 17 genes (such as *TP53*) were significantly correlated with a higher CUETO score (Additional file 1 Table A3).

Cox univariate analysis was performed to identify correlations of genomic mutations with recurrence. In all 58 cases, 18 genes were significantly correlated with recurrence (Table 2 and Additional file 1 Table A4); all these mutations were positively associated with poor prognosis. However, among these genes, only mutations of *NEB*, *MLH1*, *GATA3*, *FGFR1* and *RAF1* occurred in >5% of patients. Similar results were observed in the BCG subgroup (46 cases), with 10 genes significantly associated with recurrence ( $P<0.05$ , Table 2 and Additional file 1 Table A5). The multivariate Cox analysis included all genes significantly associated with recurrence and the results demonstrated that *NEB*, *MLH1*, *FGF12* and *FGFR1* were independently predicted poor prognosis for all 58 patients ( $P=0.001$ , 0.001, 0.001 and 0.007 respectively), while *NEB*, *FGFR1* and *SDHC* played a role as an independent prognostic predictor for recurrence in patients treated with BCG ( $P=0.001$ , 0.004 and 0.017 respectively) (Additional file 1 Table A6).

## Genomic pathways and recurrence

Canonical genomic pathways were analyzed to define whether mutations were associated with prognosis (Figure 1). Mutations of the receptor tyrosine kinases/RAS/phosphatidylinositol 3-kinase (RTK/RAS/PI3K) pathway were found in 87.9% of patients, and were associated with a higher risk stage ( $P=0.013$ , Additional file 1 Table A7). Epigenetic-related gene mutations were also frequently detected in patients with NMIBC (49/58, 84.4%), with markedly lower rates of mutations in the TP53/cell cycle pathway, Switch/sucrose nonfermentable pathway, DNA damage pathway, and alternative splicing pathway (50.0%, 31.0%, 32.8%, and 34.5%, respectively). Meanwhile, correlations between the number of pathway mutations and prediction models were also analyzed: larger numbers of mutated genes in the epigenetic pathway, histone modification pathway, and Switch/sucrose nonfermentable pathway were significant correlated with a poorer EORTC score, whereas there was no significant correlation found with the CUETO score (Additional file 1 Table A7).

Correlations of pathway mutations with recurrence were analyzed by Cox regression in qualitative and quantitative dimensions (i.e., whether mutations are present and the number of mutations in each pathway) (Table 3). In qualitative analysis, mutations in epigenetic-related genes, RTK-PI3K pathway, SWI/SNF pathway and alternative splicing pathway were protective factors (hazard ratio [HR]<1), while TP53/ cell cycle pathway mutations were risk factor, no matter in all 58 patients' cohort or in BCG subgroup. Besides, mutations in epigenetic-related genes were significantly associated with recurrence in

qualitative analysis ( $P=0.020$  and  $0.023$  for total patients and for BCG subgroup separately). Nevertheless, when quantitative analysis performed, we found that no pathways mutations were significantly correlated with recurrence ( $P>0.05$ , Table 3).

### DNA damage response and repair (DDR)genes and TMB

DDR genes have been associated with increased mutation load and the effect of immunotherapy against urothelial carcinoma<sup>9</sup>. We also analyzed the correlation between DDR, TMB, and prognosis (the list of DDR genes is provided in Additional file 1 Table A8 and the alteration status is shown in Figure S8). In our study, the percentages of DDR mutations in 58 cases with NMIBC and BCG subgroups were 86.2% and 84.8%, respectively (the number of mutations was 165 and 141, respectively). *TP53* was the most frequently mutated gene among the DDR genes (19/165 in the total cases and 17/141 in the BCG subgroups). Of note, missense variant was the most common alteration in *TP53* (16/19 and 14/17 in the total and BCG group, respectively) (Additional file 3 Figure A8). In our previous analysis, *TP53* mutation was correlated with a poorer CUETO score; however, there was no correlation found with tumor risk group stratification, EORTC score, or RFS (Additional file 1 Tables A2–5). When considering mutations in all DDR genes, there was no correlation demonstrated with the predicted models and RFS ( $P>0.05$ ) (Table 3 and Additional file 1 Table A7).

In the analysis of TMB, significant correlation was found with the EORTC model ( $P$ -values for correlation with risk group stratification, CUETO, and EORTC scores were 0.485, 0.706, and 0.037, respectively). There was no significant correlation between TMB and RFS (HR: 0.972,  $P=0.316$  for 58 NMIBC cases and HR: 0.976,  $P=0.497$  for 46 BCG subgroup cases). However, significant correlations were demonstrated between alterations in DDR and TMB, and between DDR mutated numbers and TMB (Figures 2A, B). In addition, *TP53* mutations were also significantly associated with TMB (Figure 2C).

## Discussion

Recurrence of NMIBC is affected by numerous factors, including tumor size, number of tumors, prior recurrence rate, T stage, grade, and presence of carcinoma in situ<sup>8</sup>. Since Morales first reported the irrigation treatment with BCG in bladder cancer<sup>10</sup>, the efficacy of this method has been widely demonstrated and became the standard therapy for intermediate- and high-risk NMIBC<sup>11</sup>. However, recurrence continues to occur in many patients treated with BCG; 32.6% as reported by Jesus et al.<sup>4</sup>. Even after a full course of maintenance therapy with BCG, 15.7% and 26.3% of patients continue to experience early and late recurrence, respectively<sup>12</sup>.

Pretreatment biomarkers can help to predict the therapeutic effect of BCG against NMIBC<sup>13</sup>. In fact, these biomarkers can be used as predictors and carry therapeutic value. For example, the protein encoded by the *MTOR* gene belongs to the family of phosphatidylinositol kinases, which mediate DNA damage and nutrient deficiency<sup>14</sup>. Hansel et al. reported that abnormal activation of phosphorylated *MTOR* and its downstream target was associated with increased pathological stage and poorer survival<sup>15</sup>. In our study,

we also found a correlation between *MTOR* mutations and tumor recurrence. Therefore, we suppose that *MTOR* can serve as a potential biomarker for recurrence of NMIBC. In addition, it was reported that inhibition of *MTOR* expression induced apoptosis of bladder cancer cells and inhibited cell cycle progression, cell growth, angiogenesis, and endothelial cell proliferation, indicating its therapeutic value<sup>16</sup>. In fact, *MTOR* protein is a key downstream protein kinase in the PI3K/AKT signaling pathway, with mutations occurring in approximately 40% of bladder cancer cases<sup>17</sup>. However, there was no correlation found between mutations of the RTK-PI3K pathway and recurrence in our study, and the underlying mechanism warrants further research.

Epigenetic regulations, which include DNA methylation, histone modification and chromatin remodeling, control gene expression and tumor biology<sup>18</sup>. Alterations of histone modification occurred in nearly 89% of invasive bladder cancer cases<sup>19</sup>, while DNA hypermethylation was correlated with grade and stage<sup>20,21</sup>. *In vitro*, tumor suppressor genes can be restored after treatment with DNA methyltransferases and histone deacetylase<sup>22,23</sup>, indicating the therapeutic significance of the epigenetic pathway in bladder cancer. Moreover, chromatin remodeling genes, such as *ARID1A*, *KDM6A*, etc., were frequently mutated in bladder cancer<sup>19,24</sup>. Pietzak et al., found that mutated *ARID1A* correlated with recurrence and could be used as a biomarker<sup>25</sup>. However, a negative correlation was found between mutations of epigenetic-related genes and recurrence in our study, and there was no prognostic significance demonstrated for *ARID1A*. In fact, epigenetic dysregulations occur in different stages of various tumors; this can be either a cause or an effect of tumor recurrence and progression. Further studies of the underlying mechanism are warranted to understand the significance of epigenetic regulation in NMIBC.

In our study, mutated *FGFR1* and *NEB* were shown to be independent predictors of recurrence, despite their low frequency of alterations observed in the present cohort (5.2%). In a study conducted by Rehab et al., overexpression of *FGFR1* was common in malignant bladder cancer, and the sensitivity and specificity of combined *FGFR1* and *CK20* for the differentiation between MIBC and NMIBC can be as high as 97.5% and 92.5%, respectively<sup>26</sup>. These results indicate that *FGFR1* can serve as a biomarker of tumor invasiveness. Although we suppose this is also the case for *NEB*, further investigations are warranted. However, except for the aforementioned independent predictors, we did not consider all the statistically significant gene mutations with low mutation rates as clinically significant.

Meanwhile, the clinical importance of some genes could not be ignored even in the absence of prognostic significance. The telomerase reverse transcriptase gene (*TERT*) is involved in DNA protection and cellular ageing processes<sup>27</sup>, and mutations of this gene occur in >70% of bladder cancer cases<sup>25,28</sup>. Although there was no correlation found between *TERT* mutation and tumor stage, grade, and prognosis in our and previous studies, this gene was also considered a potential biomarker for screening and surveillance owing to its high mutation rate in patients with bladder cancer<sup>28,29</sup>. *FGFR3* is also clinically significant, whose mutations have been identified as a key to tumor initiation<sup>30</sup>. *FGFR3* can serve as a potential biomarker, based on the negative correlation of mutated *FGFR3* and tumor malignant degree. *FGFR3* mutations were more commonly found in NMIBC than MIBC in a previous study<sup>5</sup>. In our study, these

mutations were also more common in the intermediate-risk than the high-risk NMIBC group. *FGFR3* is also a potential treatment target as it can activate many downstream kinase pathways, such as the RTK/RAS pathway<sup>30</sup>. The antitumor activity of BGJ398, a selective inhibitor of *FGFR*, has been demonstrated in patients with *FGFR3* mutation<sup>31</sup>. There is an ongoing clinical trial of BGJ398 in patients with NMIBC, and results are expected in the future.

DDR-related genes were frequently altered in high-grade NMIBC<sup>25</sup>, and these alterations were associated with increased mutation burden and better efficacy of chemotherapy and immunotherapy<sup>9,32</sup>. In a study conducted by Min et al., 60 patients with advanced urothelial cancer were treated with adjuvant PD-1/PD-L1 blockade therapy, and DDR alterations were investigated. The results showed that a higher response rate to immune therapy was observed in the DDR-altered group (67.9% vs. 18.8%), as well as longer PFS and overall survival<sup>9</sup>. Hence, we supposed that DDR alterations may influence the efficacy of BCG instillation, which is also based on the activation of the autoimmune system. However, we found that DDR alterations were only significantly correlated with TMB and there was no correlation with risk stage and prognosis.

*TP53* was the most frequently mutated DDR gene in our study. DNA damage can trigger the activation of p53, which maintains genetic stability and controls the transcription of DNA repair-related genes<sup>33</sup>. *TP53* mutation is one of the early events of bladder cancer pathogenesis and associated with tumor progression and poor prognosis<sup>34</sup>. However, there was no prognostic significance of *TP53* mutation or the mutated *TP53*/cell cycle pathway observed in our study. Interestingly, mutated *TP53* gene was associated with larger TMB, while these two variants were correlated with higher CUETO and EORTC score, respectively. This evidence indicates that prognostic models may serve as bridges for understanding the correlation between DDR alterations and NMIBC recurrence.

There were some limitations in our study. Although this research was based on a randomized controlled trial with limited heterogeneity among the subjects, the sample size was relatively insufficient for the analysis of the association between prognosis and different clinicopathological and genomic variants. Furthermore, the follow-up period was limited, with small number of patients reaching their follow-up endpoint; thus, PFS and overall survival of patients could not be analyzed.

## Conclusion

Maintenance treatment with BCG demonstrated better control of recurrence in Chinese patients with NMIBC after TUR-BT versus intravesical therapy with EPI. NGS of these patients found that *FGFR1* and *NEB* can be biomarkers of recurrence, while mutations of the *MTOR* gene and epigenetic pathway are potential treatment targets for patients who fail treatment with BCG. DDR alterations were positively correlated with higher TMB, and *TP53* may be the key to understanding the relationship between DDR and recurrence after treatment with BCG.

# **Declarations**

## **Ethics approval and consent to participate**

This study was approved by Center for Drug Evaluation of National Medical Products Administration (CDE-NMPA, China) and Ethics Committee of our institute, with the whole process supervised. The registration number in CDE-NMPA was CTR20150840 (website of CDE: <http://www.cde.org.cn/>). All the research registration and ethical approval documents were added below. Patients and their authorized family members had been fully informed before follow-up work was performed, with informed consent signed.

## **Consent for publication**

Consent for publication was obtained from all participants.

## **Availability of data and materials**

The dataset supporting the conclusions of this article is included within the article and its supplementary materials.

## **Competing interests**

None declared.

## **Fundings**

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## **authors contribution**

X.L. designed the study, guided experiments, carried out analysis and reviewed the manuscript. Y.S., X.H. and Z.Y contributed to the study design, collected the data, performed analysis, and wrote the manuscript. T.L., W.Y., K.W. and S.R. contributed to the data collection and analysis and reviewed the manuscript. S.X., W.D., S.F., Y.W., Y.L and K.W. helped with data collation and statistical analysis and reviewed the manuscript.

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## Tables

**Table 1. Clinicopathological data of patients with NMIBC**

Clinicopathological variants	BCG group (n=46)	EPI group (n=12)	Total (n=58)	P-value
<b>Gender</b>				0.432
Male	35	11	46	
Female	11	1	12	
<b>Preoperative age (years)</b>				0.336
≤60	18	7	25	
60–70	19	4	23	
>70	9	1	10	
<b>Smoking history</b>				0.331
No	25	4	29	
Yes	21	8	29	
<b>Prior recurrence rate</b>				0.233
Primary	27	5	32	
Recurrent, ≤1 rec/yr	11	2	13	
Recurrent, >1 rec/yr	8	5	13	
<b>T category</b>				0.012
Ta	29	12	41	
T1	17	0	17	
<b>Grade</b>				0.331
Low grade	21	8	29	
High grade	25	4	29	
<b>Tumor size</b>				0.759
<3 cm	26	8	34	
≥3 cm	20	4	24	
<b>No. tumors</b>				0.891
≤3	34	8	42	
>3	12	4	16	
<b>Risk stage</b>				0.291
Intermediate risk	19	5	26	

High risk	27	5	32
<b>EORTC risk tables</b>			0.956
1-4	17	3	20
5-9	24	9	33
10-17	5	0	5
<b>CUETO scoring model</b>			0. 866
0-4	13	3	16
5-6	16	5	21
7-9	15	4	19
10-16	2	0	2

NMIBC: non-muscle invasive bladder cancer; n: number of patients; yr: year; No: number; Recurrent,  $\leq 1$  rec/yr: prior recurrence rate of less than one per year; Recurrent,  $> 1$  rec/yr: prior recurrence rate of more than one per year; EORTC: European Organization for Research and Treatment of Cancer; CUETO: Spanish Urological Club for Oncological Treatment.

**Table 2. Univariate analysis of genetic mutations and recurrence survival rate**

Gene	Alterations (%)	Total patients (n=58)		BCG subgroup (n=46)	
		P-value	Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)
<b><i>NEB</i></b>	5.2	0.001	17.307 (3.305–90.639)	0.001	21.213 (3.466–129.823)
<b><i>MLH1</i></b>	5.2	0.011	18.580 (1.933–178.630)	NA	NA
<b><i>CDKN1B</i></b>	3.4	0.011	18.580 (1.933–178.630)	NA	NA
<b><i>FGF12</i></b>	1.7	0.011	18.580 (1.933–178.630)	0.012	21.995 (1.994–242.587)
<b><i>NKX2_1</i></b>	1.7	0.011	18.580 (1.933–178.630)	NA	NA
<b><i>RET</i></b>	1.7	0.011	18.580 (1.933–178.630)	NA	NA
<b><i>SDHB</i></b>	1.7	0.011	18.580 (1.933–178.630)	NA	NA
<b><i>TAF1</i></b>	1.7	0.011	18.580 (1.933–178.630)	0.012	14.493 (1.507–139.347)
<b><i>EPHB1</i></b>	3.4	0.019	13.794 (1.542–123.422)	NA	NA
<b><i>PIK3C3</i></b>	1.7	0.019	13.794 (1.542–123.422)	NA	NA
<b><i>ZNF703</i></b>	1.7	0.019	13.794 (1.542–123.422)	NA	NA
<b><i>PALB2</i></b>	3.4	0.028	5.421 (1.195–24.597)	0.056	8.100 (0.946–69.384)
<b><i>GATA3</i></b>	5.2	0.024	6.140 (1.263–29.846)	0.178	4.394 (0.510–37.818)
<b><i>FGFR1</i></b>	5.2	0.029	10.924 (1.276–93.521)	0.021	14.493 (1.507–139.347)
<b><i>MAX</i></b>	1.7	0.029	10.924 (1.276–93.521)	0.021	14.493 (1.507–139.347)
<b><i>SDHC</i></b>	12.1	0.074	3.917 (0.877–17.507)	0.003	11.892 (2.272–62.246)
<b><i>MTOR</i></b>	5.2	0.052	4.442 (0.989–19.956)	0.024	6.301 (1.278–31.072)
<b><i>RAF1</i></b>	5.2	0.042	8.986 (1.082–74.659)	0.034	10.686 (1.194–95.634)
<b><i>RECQL4</i></b>	1.7	0.042	8.986 (1.082–74.659)	0.034	10.686 (1.194–95.634)
<b><i>SDHAF2</i></b>	1.7	0.042	8.986 (1.082–74.659)	0.034	10.686 (1.194–95.634)

Mutated genes significantly correlated with prognosis are shown. BCG: *Bacillus Calmette–Guérin*; CI: confidence interval; n: number; NA: not available.

**Table 3. Univariate analysis of mutated genetic pathways, DDR genes, and recurrence survival rate**

Genetic pathway		Total patients (n=58)		BCG subgroup (n=46)	
		P-value	Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)
<b>Qualitative</b>	<b>Epigenetic-related genes</b>	0.020	0.278 (0.095 - 0.814)	0.023	0.198 (0.049–0.797)
	<b>TP53/cell cycle pathway</b>	0.668	0.805 (0.299 - 2.170)	0.725	0.790 (0.212–2.942)
	<b>RTK-PI3K pathway</b>	0.830	0.849 (0.192 - 3.765)	0.351	0.473 (0.098–2.280)
	<b>SWI/SNF pathway</b>	0.182	0.424 (0.120 - 1.497)	0.397	0.507 (0.105–2.439)
	<b>DNA damage pathway</b>	0.554	0.710 (0.229 - 2.204)	0.965	0.969 (0.242–3.878)
	<b>Alternative splicing pathway</b>	0.059	0.240 (0.054 - 1.056)	0.289	0.427 (0.089–2.056)
<b>Quantitative</b>	<b>Epigenetic-related genes</b>	0.960	0.993 (0.756 - 1.303)	0.877	1.030 (0.710–1.494)
	<b>TP53/cell cycle pathway</b>	0.942	1.016 (0.665 - 1.553)	0.958	0.985 (0.566–1.716)
	<b>RTK-PI3K pathway</b>	0.096	0.686 (0.440 - 1.069)	0.308	0.756 (0.441–1.295)
	<b>SWI/SNF pathway</b>	0.170	0.567 (0.252 - 1.275)	0.379	0.671 (0.276–1.632)
	<b>DNA damage pathway</b>	0.319	0.700 (0.348 - 1.411)	0.582	0.810 (0.382–1.716)
	<b>Alternative splicing pathway</b>	0.101	0.400 (0.134 - 1.195)	0.410	0.629 (0.208–1.897)
	<b>DDR genes</b>	0.603	0.951 (0.788 - 1.148)	0.808	0.974 (0.789–1.203)

DDR: DNA damage response and repair; BCG: *Bacillus Calmette–Guérin*; CI: confidence interval; RTK: receptor tyrosine kinases; PI3K: phosphatidylinositol 3-kinase; SWI/SNF: Switch/sucrose nonfermentable; n: number.

## Figures

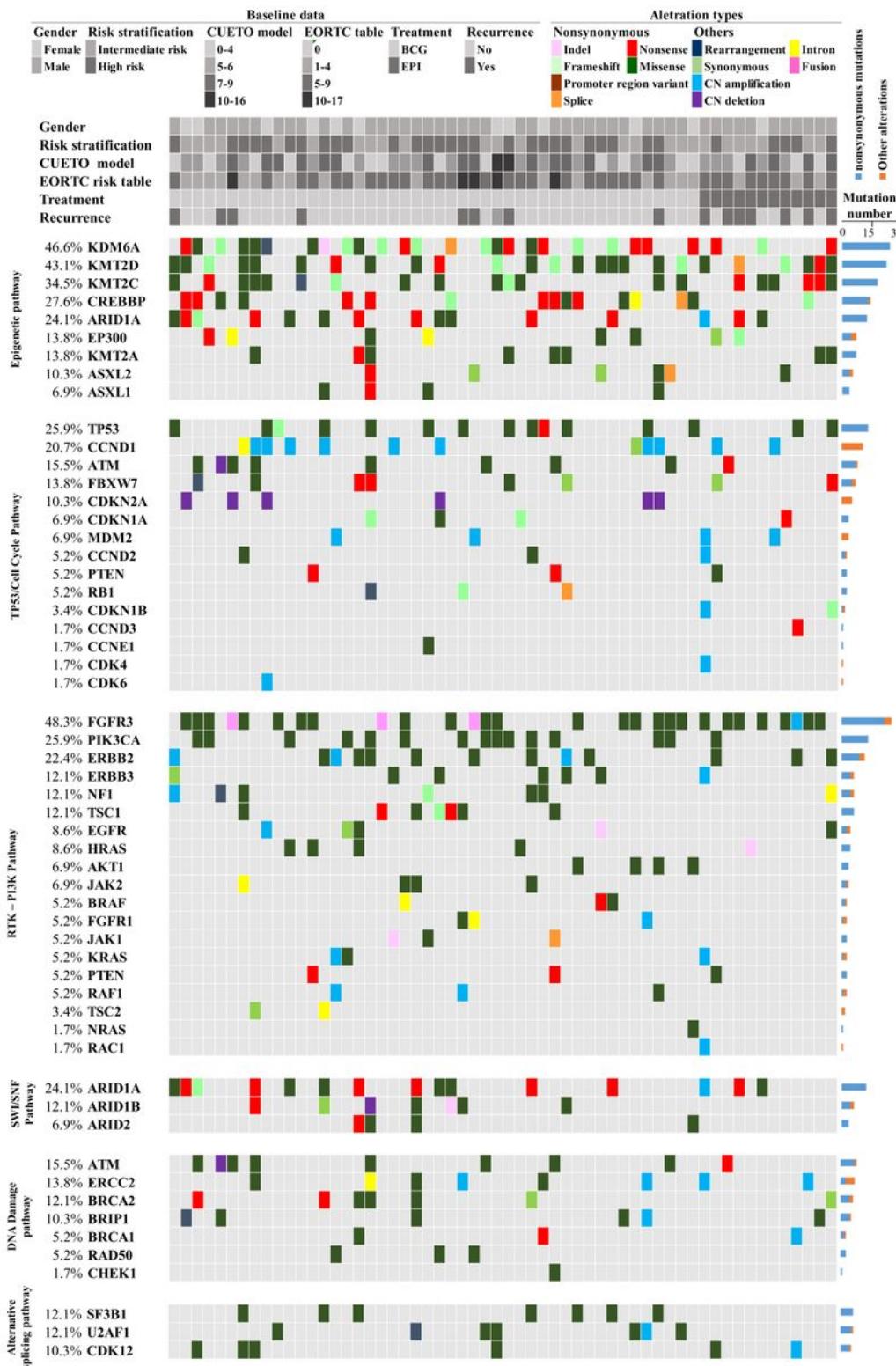
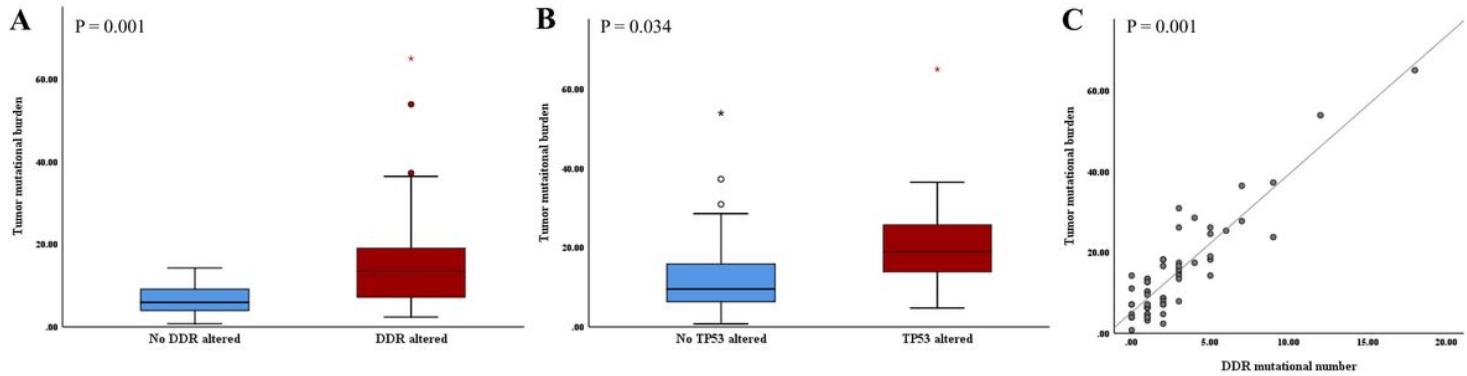


Figure 1

Oncoprint of genetic pathways. BCG: Bacillus Calmette–Guérin treated group; EPI: epirubicin treated group. CUETO model: Spanish Urological Club for Oncological Treatment scoring model; EORTC: European Organization for Research and Treatment of Cancer; CN: copy number; RTK: receptor tyrosine kinases; PI3K: phosphatidylinositol 3-kinase; SWI/SNF: Switch/sucrose nonfermentable.



**Figure 2**

Correlation analysis between DNA damage response and repair genes and TMB. (A) Correlation of the TMB and DDR alteration status. (B) Correlation of the TMB and DDR mutational numbers. DDR: DNA damage response and repair. (C) Correlation of the TMB and TP53 alteration status. TMB: tumor mutational burden.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Originaldataset.xlsx](#)
- [Additionalfile3FigA8.pdf](#)
- [Additionalfile2FigA17.pdf](#)
- [Additionalfile1.docx](#)