

Assessment of Predictive Genomic Biomarkers for Response to Cisplatin-based Neoadjuvant Chemotherapy in Bladder Cancer

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DOI

[10.1016/j.eururo.2022.07.023](https://doi.org/10.1016/j.eururo.2022.07.023)

Publication date

2023

Document Version

Final published version

Published in

European Urology

Citation (APA)

Gil-Jimenez, A., van Dorp, J., Contreras-Sanz, A., van der Vos, K., Vis, D. J., Braaf, L., Broeks, A., Kerkhoven, R., van Kessel, K. E. M., Wessels, L. F. A., & More Authors (2023). Assessment of Predictive Genomic Biomarkers for Response to Cisplatin-based Neoadjuvant Chemotherapy in Bladder Cancer. *European Urology*, 83(4), 313-317. <https://doi.org/10.1016/j.eururo.2022.07.023>

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Platinum Priority – Brief Correspondence – Editor's Choice

Editorial by Srikala S. Sridhar, Scott A. North, Normand Blais on pp. 318–319 of this issue

Assessment of Predictive Genomic Biomarkers for Response to Cisplatin-based Neoadjuvant Chemotherapy in Bladder Cancer

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Article info

Article history:

Accepted July 26, 2022

Statistical Editor:

Rodney Dunn

Keywords:

Muscle-invasive bladder cancer
Neoadjuvant chemotherapy
DNA sequencing
Response prediction
Somatic mutations
Cisplatin-based chemotherapy



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Abstract

Cisplatin-based neoadjuvant chemotherapy (NAC) followed by radical cystectomy is recommended for patients with muscle-invasive bladder cancer (MIBC). It has been shown that somatic deleterious mutations in *ERCC2*, gain-of-function mutations in *ERBB2*, and alterations in *ATM*, *RB1*, and *FANCC* are correlated with pathological response to NAC in MIBC. The objective of this study was to validate these genomic biomarkers in pre-treatment transurethral resection material from an independent retrospective cohort of 165 patients with MIBC who subsequently underwent NAC and radical surgery. Patients with ypT0/Tis/Ta/T1N0 disease after surgery were defined as responders. Somatic deleterious mutations in *ERCC2* were found in nine of 68 (13%) evaluable responders and two of 95 (2%) evaluable nonresponders ($p = 0.009$; FDR = 0.03). No correlation was observed between response and alterations in *ERBB2* or in *ATM*, *RB1*, or *FANCC* alone or in combination. In an exploratory analysis, no additional genomic alterations discriminated between responders and nonresponders to NAC. No further associations were identified between the aforementioned biomarkers and pathological complete response (ypT0N0) after surgery. In conclusion, we observed a positive association between deleterious mutations in *ERCC2* and pathological response to NAC, but not overall survival or recurrence-free survival. Other previously reported genomic biomarkers were not validated.

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Patient summary: It is currently unknown which patients will respond to chemotherapy before definitive surgery for bladder cancer. Previous studies described several gene mutations in bladder cancer that correlated with chemotherapy response. This study confirmed that patients with bladder cancer with a mutation in the *ERCC2* gene often respond to chemotherapy.

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Neoadjuvant cisplatin-based chemotherapy (NAC) followed by radical cystectomy is recommended for patients with muscle-invasive bladder cancer (MIBC) [1]. Pathological response after treatment with NAC is strongly associated with recurrence-free survival (RFS) and overall survival (OS) [2]. Currently, clinicians are unable to identify which patients will benefit from NAC. Genomic biomarkers have been described to correlate with response to NAC, including somatic deleterious mutations in *ERCC2*, gain-of-function mutations in *ERBB2*, and alterations in *ATM*, *RB1*, and *FANCC* [3–7]. However, none of these biomarkers has been validated in larger independent cohorts and thus they are not used in clinical practice [1,8].

Here, we set out to validate these genomic biomarkers in an independent multicenter retrospective cohort. Pretreatment tissue from five centers was sequenced at The Netherlands Cancer Institute (NKI cohort; $n = 117$) or Vancouver Prostate Centre (Vancouver cohort; $n = 48$; [Supplementary Fig. 1](#)). All patients were diagnosed with MIBC (cT2–4aN0M0 and/or cT1–4aN1–3M0) by transurethral resection (TUR) and were treated with at least two cycles of cisplatin-

based NAC, followed by radical cystectomy. The primary endpoint of this study was pathological response, defined as ypT0/Tis/Ta/T1N0 after surgery [2,9]. Seventy of 165 patients (42%) were categorized as responders. Pathological complete response after surgery, defined as ypT0N0, was used as a secondary endpoint and was observed in 51 of 165 patients (31%).

Baseline age, gender, chemotherapy regimen, and number of chemotherapy cycles did not differ between the response groups; however, cT stage at baseline was higher in the nonresponder group ([Table 1](#)). Furthermore, baseline cT stage and chemotherapy regimen differed between the cohorts ([Supplementary Table 1](#)). Tumor DNA extracted from TUR samples obtained before NAC was sequenced using a targeted capture-based panel for the NKI cohort and whole-exome sequencing for the Vancouver cohort. Somatic variants of *ERCC2*, *ERBB2*, *ATM*, *RB1*, and *FANCC* were inferred from population databases ([Supplementary material](#)). Mutations were predicted to be functional (deleterious or gain-of-function) using the OncoKB, ClinVar, SIFT, FATHMM, and PolyPhen-2 annotation databases ([Supple-](#)

Table 1 – Baseline characteristics and response of 165 patients with muscle-invasive bladder cancer treated with neoadjuvant chemotherapy and radical cystectomy

	NKI cohort ^a		Vancouver cohort ^a		p value ^b
	Responders	Nonresponders	Responders	Nonresponders	
Patients (n)	53	64	17	31	–
Median age, yr (IQR)	71.0 (61.0–75.0)	71.0 (61.0–77.3)	61.2 (56.0–66.0)	65.5 (58.3–73.0)	0.2
Male sex, n (%)	40 (76)	38 (59)	15 (88)	24 (77)	0.08
cT stage, n (%)					0.04
cT1	1 (2)	0 (0)	0 (0)	0 (0)	
cT2	27 (51)	19 (30)	2 (12)	4 (13)	
cT3	21 (40)	26 (40)	7 (41)	20 (65)	
cT4	4 (7)	19 (30)	8 (47)	7 (22)	
cN stage, n (%)					0.6
cN0	31 (59)	40 (63)	10 (59)	12 (39)	
cN+	22 (41)	24 (37)	7 (41)	19 (61)	
Ctx regimen, n (%)					0.16
Cis/Gem	40 (75)	41 (64)	14 (82)	28 (90)	
MVAC	11 (21)	23 (36)	3 (18)	3 (10)	
CMV	2 (4)	0 (0)	0 (0)	0 (0)	
Ctx cycles received, n (%)					0.8
2 cycles	2 (4)	2 (3)	0 (0)	2 (6)	
3 cycles	10 (19)	13 (20)	6 (35)	7 (23)	
4 cycles	39 (74)	46 (72)	10 (59)	17 (55)	
>4 cycles	2 (4)	3 (5)	1 (6)	5 (16)	
Pathological response, n (%)					
ypT0N0 (CR)	40 (75)	0 (0)	13 (76)	0 (0)	
ypTis/Ta/T1N0	13 (25)	0 (0)	4 (24)	0 (0)	
≥ypT2N0 (NR)	0 (0)	64 (100)	0 (0)	31 (100)	

Cis/Gem = cisplatin + gemcitabine; MVAC = methotrexate + vinblastine + doxorubicin + cisplatin; CMV = cisplatin + methotrexate + vinblastine; IQR = interquartile range; Ctx = chemotherapy; CR = complete response; NR = nonresponse.

^a Responders: ypT0/Tis/Ta/T1N0; nonresponders: ≥ypT2N0.

^b Differences in the overall cohort between responders and nonresponders. Fisher's exact test for binary predictors, t test for numerical predictors. All statistical tests were two-sided. No adjustments were made for multiple hypothesis testing. Significant associations are highlighted in bold.

mentary material). There was high concordance between the observed and The Cancer Genome Atlas (TCGA) mutation rates (Supplementary Table 2).

After filtering, deleterious mutations in *ERCC2* were found in nine of 68 (13%) evaluable responders and two of 95 (2%) evaluable nonresponders ($p = 0.009$; Fig. 1A). We found relevant gain-of-function mutations in *ERBB2* in nine of 69 (13%) evaluable responders and five of 95 (5%) evaluable nonresponders ($p = 0.09$; Fig. 1A). Of the 70 responders, 27 (39%) had at least one alteration in *ATM*, *RB1* or *FANCC* compared to 25/95 (26%) nonresponders ($p = 0.13$; Fig. 1A). Nine of the 11 patients (82%) with a deleterious mutation in *ERCC2* had a pathological response after NAC treatment, compared to 62/154 patients (40%) without any relevant *ERCC2* mutations (Supplementary Table 3). After correction for multiple hypothesis testing (three hypotheses), *ERCC2* mutations were significantly enriched in the responder group (false discovery rate [FDR] = 0.03; Fig. 1A). The association remained after adjustment for cT stage in a multivariable logistic regression model ($p_{ERCC2} = 0.008$, $p_{cT2} = p_{cT3} = p_{cT4} > 0.9$) and when patients who received fewer than three NAC cycles were excluded (Supplementary Fig. 2). Baseline clinical differences between the *ERCC2* mutant and wild-type groups were not identified (Supplementary Table 3).

By contrast, alterations in *ERCC2*, *ERBB2*, or any one of *ATM*, *RB1*, or *FANCC* were not associated with a pathological

complete response (ypT0N0) after correcting for multiple hypothesis testing ($FDR_{ERCC2} = 0.09$, $FDR_{ERBB2} = 0.07$, $FDR_{ATM/RB1/FANCC} = 0.07$; Supplementary Fig. 3).

The median follow-up for patients using reverse censoring was 7.2 yr. The 5-yr OS rate for patients with and without mutations in *ERCC2* was 75% (95% confidence interval [CI] 50–100%) and 52% (95% CI 45–62%), respectively ($p = 0.19$; Fig. 1B). The corresponding 5-yr RFS rates were 65% (95% CI 39–100%) and 49% (95% CI 42–59%; $p = 0.17$; Fig. 1C). Thus, while the Kaplan-Meier curves appear to separate according to *ERCC2* mutation status, we could not demonstrate a statistical difference for either OS or RFS, possibly because of the low frequency of *ERCC2* mutations.

Following earlier analyses by Plimack and colleagues [6], we assessed copy number alterations (CNAs) for *ATM*, *RB1*, and *FANCC* via shallow whole-genome sequencing for patients from the NKI cohort ($n = 117$; Supplementary material). CNAs for the Vancouver cohort could not be confidently assessed owing to a lack of germline data. We found seven CNAs in *ATM*, *RB1*, and/or *FANCC* in all evaluable patients. Together with the previously described mutations, 22/53 (42%) responders had at least one alteration in *ATM*, *RB1*, or *FANCC*, in comparison to 20/64 (31%) nonresponders ($p = 0.052$; Supplementary Fig. 4).

In a further exploratory analysis, mutations frequently occurring in MIBC were assessed for their correlation with response to NAC (Supplementary Fig. 5). This analysis

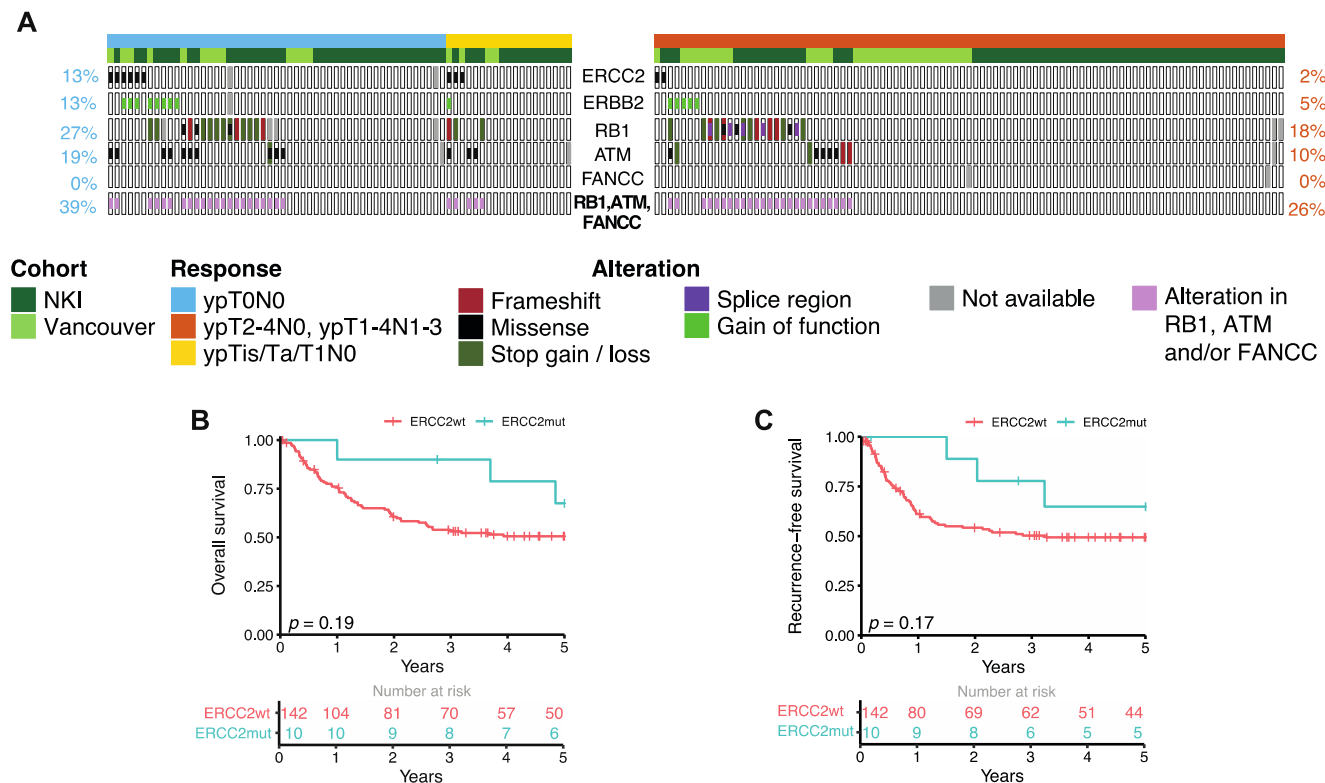


Fig. 1 – Somatic mutations in the *ERCC2*, *ERBB2*, *ATM*, *RB1*, and *FANCC* genes in patients with muscle-invasive bladder cancer treated with neoadjuvant chemotherapy. (A) Overview of relevant mutations for each patient. Details for variant calling and inference of somatic and functional variants are provided in the Supplementary material. The left panel shows results for patients with ypT0N0 (light blue; $n = 51$) or ypTis/Ta/T1N0 (yellow; $n = 19$) after neoadjuvant chemotherapy (responders), and the right panel shows results for nonresponders (orange; $n = 95$). Percentages represent the number of patients with a relevant mutation relative to the total number of eligible patients for that specific gene for responders (left) and nonresponders (right). Patients with an alteration in any one of *ATM*, *RB1*, or *FANCC* are indicated in the last row. The 5-yr (B) overall survival and (C) recurrence-free survival for patients with (blue) and without (red) a mutation in *ERCC2*. The p values indicate statistical significance for a log-rank test. NKI = patients from the NKI cohort; wt = wild type; mut = mutant; Not available = gene coverage <20 reads.

included *FGFR3*, which was previously associated with negative outcome after chemotherapy (Supplementary Fig. 6) [10]. No association with response was identified after correction for multiple hypothesis testing (Supplementary Fig. 5).

There are several limitations to this study. The genomic data were obtained via different sequencing technologies at different centers, leading to potential biases in the mutation frequency. Furthermore, we lacked germline data and somatic variants were filtered with the help of population databases to remove benign germline variants. As germline DNA is often unavailable, this approach is common practice and was also used in the original studies for *ERBB2* and *ATM/RB1/FANCC* [4,6]. Multiple definitions of response have been used in previous studies, so there is heterogeneity among studies. Complete pathological response (ypT0N0) and pathological downstaging (ypT0/Tis/Ta/T1N0) are commonly used. The long-term clinical outcome is favorable in both groups, although patients with ypT0/TisN0 status may have a modest survival benefit over patients with ypT0/Tis/Ta/T1N0 disease [2,9].

In summary, we attempted to validate mutations in *ERCC2*, *ERBB2*, *ATM*, *RB1*, and *FANCC* as predictive markers of pathological response in a cohort of 165 patients treated with NAC. We confirmed a positive association of deleterious mutations in *ERCC2* with pathological response (ypT0/Tis/Ta/T1N0), but not with complete response (ypT0N0), OS, or RFS. Prospective evaluation of *ERCC2* mutations as a biomarker for response to NAC is needed to confirm our results.

Author contributions: Michiel S. van der Heijden 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: van Rhijn, Black, van der Heijden.

Acquisition of data: van Dorp, Contreras-Sanz, van der Vos, van Kessel, Ribal, Alcaraz, Seiler, Wright, Mengual, Boormans, van Rhijn, Black, van der Heijden.

Analysis and interpretation of data: Gil-Jimenez, Contreras-Sanz, Vis, Wessels.

Drafting of the manuscript: Gil-Jimenez, van Dorp.

Critical revision of the manuscript for important intellectual content: Gil-Jimenez, van Dorp, Contreras-Sanz, van der Vos, Vis, Broeks, van Kessel, Ribal, Alcaraz, Wessels, Seiler, Wright, Mengual, Boormans, van Rhijn, Black, van der Heijden.

Statistical analysis: Gil-Jimenez, Contreras-Sanz, Vis, Wessels.

Obtaining funding: van der Heijden.

Administrative, technical, or material support: Broeks, Braaf, Kerkhoven.

Supervision: Vis, Wessels, van der Heijden.

Other: None.

Financial disclosures: Michiel S. van der Heijden certifies that all conflicts of interest, including specific financial interests and relationships and affiliations relevant to the subject matter or materials discussed in the manuscript (e.g., employment/affiliation, grants or funding, consultancies, honoraria, stock ownership or options, expert testimony, royalties, or patents filed, received, or pending), are the following: Michiel S. van der Heijden has received institutional research funding from Bristol-

Myers Squibb, 4SC, Roche, Astellas Pharma Netherlands and AstraZeneca, and institutional consultancy fees from Bristol-Myers Squibb, Roche, Merck Sharp & Dohme, AstraZeneca, Pfizer, Janssen, and Seattle Genetics. Bas W.G. van Rhijn has participated in advisory boards for AstraZeneca, Ferring, and QED Therapeutics. Lodewyk F.A. Wessels has received institutional research funding from Genmab. Peter C. Black has been a consultant for AbbVie, Astellas Pharma, EMD-Serono, Pfizer, Janssen Oncology, Bayer, Merck, Sanofi Canada, Biosynt, Ferring, Roche Canada, MDxHealth, AstraZeneca, UroGen Pharma, Bristol-Myers Squibb, Fergene, Prokarium, Protara, QED, STIMIT, Theralase, and Verity; has received research funding from iProgen; and shares a patent with Decipher Biosciences. Joost Boormans has received institutional research funding from Decipher and Merck/Pfizer and institutional consultancy fees from Roche, Merck Sharp & Dohme, AstraZeneca, Pfizer, Janssen, AMBU, and Eight Medical. The remaining authors have nothing to disclose.

Funding/Support and role of the sponsor: This project was partially funded by the NWO (Dutch Research Council) and financial contributions from DUOS (Duth Uro-oncology group) and Astellas Pharma Netherlands. The sponsor played no direct role in the study.

Acknowledgments: We acknowledge the Genomics Core Facility (Roel Kluin), the Core Facility Molecular Pathology and Biobanking, and the Research High-Performance Computing facility, all at The Netherlands Cancer Institute; the sequencing facility at the Vancouver Prostate Center, and all the clinical teams involved in the five-center patient cohort.

Peer Review Summary

Peer Review Summary and Supplementary data to this article can be found online at <https://doi.org/10.1016/j.eururo.2022.07.023>.

References

- [1] Witjes JA, Bruins HM, Cathomas R, et al. European Association of Urology guidelines on muscle-invasive and metastatic bladder cancer: summary of the 2020 guidelines. *Eur Urol* 2021;79:82–104.
- [2] Rosenblatt R, Sherif A, Rintala E, et al. Pathologic downstaging is a surrogate marker for efficacy and increased survival following neoadjuvant chemotherapy and radical cystectomy for muscle-invasive urothelial bladder cancer. *Eur Urol* 2012;61:1229–38.
- [3] Van Allen EM, Mouw KW, Kim P, et al. Somatic ERCC2 mutations correlate with cisplatin sensitivity in muscle-invasive urothelial carcinoma. *Cancer Discov* 2014;4:1140–53.
- [4] Groenendijk FH, de Jong J, Fransen van de Putte EE, et al. ERBB2 mutations characterize a subgroup of muscle-invasive bladder cancers with excellent response to neoadjuvant chemotherapy. *Eur Urol* 2016;69:384–8.
- [5] Li Q, Damish AW, Frazier Z, et al. ERCC2 helicase domain mutations confer nucleotide excision repair deficiency and drive cisplatin sensitivity in muscle-invasive bladder cancer. *Clin Cancer Res* 2019;25:977–88.
- [6] Plimack ER, Dunbrack RL, Brennan TA, et al. Defects in DNA repair genes predict response to neoadjuvant cisplatin-based chemotherapy in muscle-invasive bladder cancer. *Eur Urol* 2015;68:959–67.
- [7] Miron B, Hoffman-Censits JH, Anari F, et al. Defects in DNA repair genes confer improved long-term survival after cisplatin-based neoadjuvant chemotherapy for muscle-invasive bladder cancer. *Eur Urol Oncol* 2020;3:544–7.
- [8] Becker REN, Meyer AR, Brant A, et al. Clinical restaging and tumor sequencing are inaccurate indicators of response to neoadjuvant chemotherapy for muscle-invasive bladder cancer. *Eur Urol* 2021;79:364–71.

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- [9] Ravi P, Pond GR, Diamantopoulos LN, et al. Optimal pathological response after neoadjuvant chemotherapy for muscle-invasive bladder cancer: results from a global, multicentre collaboration. *BJU Int* 2021;128:607–14.
- [10] Teo MY, Mota JM, Whiting KA, et al. Fibroblast growth factor receptor 3 alteration status is associated with differential sensitivity to platinum-based chemotherapy in locally advanced and metastatic urothelial carcinoma. *Eur Urol* 2020;78:907–15.