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ERBB2 Mutations Characterize a Subgroup of Muscle-invasive Bladder Cancers with Excellent Response to Neoadjuvant Chemotherapy

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Abstract

A pathologic complete response to neoadjuvant chemotherapy (NAC) containing platinum is a strong prognostic determinant for patients with muscle-invasive bladder cancer (MIBC). Despite comprehensive molecular characterization of bladder cancer, associations of molecular alterations with treatment response are still largely unknown. We selected pathologic complete responders (ypT0N0; $n = 38$) and nonresponders (higher than ypT2; $n = 33$) from a cohort of high-grade MIBC patients treated with NAC. DNA was isolated from prechemotherapy tumor tissue and used for next-generation sequencing of 178 cancer-associated genes (discovery cohort) or targeted sequencing (validation cohort). We found that 9 of 38 complete responders had erb-b2 receptor tyrosine kinase 2 (*ERBB2*) missense mutations, whereas none of 33 nonresponders had *ERBB2* mutations ($p = 0.003$). *ERBB2* missense mutations in complete responders were mostly confirmed activating mutations. *ERCC2* missense mutations, recently found associated with response to NAC, were more common in complete responders; however, this association did not reach statistical significance in our cohort. We conclude that *ERBB2* missense mutations characterize a subgroup of MIBC patients with an excellent response to NAC.

Patient summary: In this report we looked for genetic alterations that can predict the response to neoadjuvant chemotherapy (NAC) in bladder cancer. We found that mutations in the gene *ERBB2* are exclusively present in patients responding to NAC.

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A pathologic complete response to neoadjuvant chemotherapy (NAC) containing platinum is associated with superior clinical outcome in patients with muscle-invasive bladder cancer (MIBC) [1–3]. No molecular markers or baseline clinical characteristics that can predict the response to NAC are clinically validated. In the present study, we found an unexpected association between mutations in the erb-b2 receptor tyrosine kinase 2 (*ERBB2*) gene, also known as *HER2*, and a complete response to chemotherapy.

We collected pre- and postchemotherapy specimens from 110 prospectively registered MIBC patients treated with NAC. Good-quality DNA from the pretreatment transurethral resection specimens was available for 94 patients. In this cohort, we identified 38 pathologic complete responders (ypT0N0), 23 partial responders (ranging from a minor response to a near-complete response), and 33 nonresponders (higher than ypT2) to NAC. Full study methods are described in the supplement. No significant differences in baseline clinical characteristics were identified between the three groups (Supplementary Table 1). Complete responders had a superior recurrence-free and cancer-specific survival compared with nonresponders, whereas the partial responders had an in-between survival ($p < 0.001$) (Supplementary Fig. 1).

We sequenced 178 cancer-associated genes (Supplementary Table 2) on pretreatment tumor DNA from 16 complete responders and 16 nonresponders (discovery cohort). Genes with a differential mutation frequency in complete responders compared with nonresponders were identified by contrasting analysis (Fig. 1A). *ERBB2* had the highest enrichment for mutations in complete responders (Fig. 1A and 1B). We therefore tested the association between *ERBB2* mutations and chemotherapy sensitivity in a validation cohort consisting of the remaining 22 complete responders and 17 nonresponders to NAC in our patient series. We identified another five *ERBB2* missense mutations in four complete responders and none in the nonresponders. Taken together, we identified *ERBB2* missense mutations in 9 of the 38 complete responders (24%) and in none of the 33 nonresponders to NAC ($p = 0.003$) (Fig. 2).

Five of the 10 identified *ERBB2* missense mutations cluster at amino acid 310 in the extracellular domain (Fig. 1C; Supplementary Table 3). The S310 position is also a mutational hotspot in The Cancer Genome Atlas (TCGA) urothelial bladder cancer cohort because approximately 40% of all *ERBB2* missense mutations cluster at this position (TCGA Data Portal; http://cancergenome.nih.gov). Previous functional studies on *ERBB2* mutations have shown that the S310F, D769H, and V842I variants identified here are activating mutations that support cellular transformation [4,5]. To our knowledge, the R678L and V777M mutations have not been functionally characterized. However, a different amino acid substitution at the same V777 position was found to be activating [5]. It was recently reported that micropapillary urothelial carcinomas (UCs) carry a high frequency (40%) of activating extracellular domain *ERBB2* mutations [6]. We therefore reviewed all our *ERBB2*-mutant UCs. None of these had micropapillary variant histology.

We confirmed the association between *ERBB2* missense mutations and responsiveness to platinum-containing chemotherapy in the recently published MIBC data set of Van Allen et al [7]. In this external validation cohort, all three patients with an *ERBB2* missense mutation responded to NAC [7]. In addition, they reported a fourth complete responder who had an *ERBB2* mutation with an allelic fraction of 0.04.

ERBB2 missense mutations are significantly enriched in the chemotherapy responders from our cohort and the Van Allen et al cohort [7] compared with the unselected TCGA cohort (8% in TCGA; $p = 0.02$) (Fig. 1D). Conversely, we found that *ERBB2* missense mutations were significantly depleted in the nonresponder cohorts compared with the unselected TCGA cohort ($p = 0.02$) (Fig. 1D).

Having found an association between *ERBB2* missense mutations and platinum response, we next tested the association of *ERBB2* amplification with platinum response. *ERBB2* amplifications were identified in complete responders as well as in nonresponders and were not associated with response to NAC ($p = 0.52$) (Fig. 2). *ERBB2* amplification was always accompanied by protein overexpression. In four patients, amplification of *ERBB2* was found in combination with a missense mutation in *ERBB2*. Strikingly, in all of these cases, the *ERBB2*-mutant allele was found amplified, stressing once more the relevance of *ERBB2* mutations for MIBC oncogenesis.

Van Allen et al recently reported that missense mutations in *ERCC2*, a nucleotide excision repair gene, were selectively present in 9 of 25 MIBC patients with complete response to cisplatin-containing NAC, whereas *ERCC2* missense mutations were absent in 25 nonresponders [7]. In our discovery cohort, we found six *ERCC2* missense mutations present in four complete responders and in two nonresponders (Fig. 1B). Sanger sequencing of the postchemotherapy-resistant tumors of the two *ERCC2*-mutant nonresponders demonstrated in both cases that the *ERCC2* missense mutation was still present in the postchemotherapy-resistant tumor (Fig. 1E and 1F), indicating that the mutation was not counterselected during chemotherapy. In our validation cohort, we identified *ERCC2* missense mutations in two tumor samples from 22 complete responders and in none of the 17 nonresponders. In total, 6 somatic *ERCC2* missense mutations were identified in 38 complete responders (16%) and 2 in 33 nonresponders (6%; $p = 0.27$) (Fig. 2; Supplementary Table 4). Five of the six *ERCC2* mutations in complete responders were present in patients with wild-type nonamplified *ERBB2* (Fig. 2).

Finally, we also sequenced *ERBB2* and *ERCC2* in the pretreatment transurethral resection specimens from the remaining group of 23 patients who had responses to NAC ranging from a minor response to a near-complete response. We identified two *ERBB2* missense mutations in tumors from patients with a partial response. Both patients are still alive >10 yr after NAC without any signs of disease recurrence (Supplementary Table 5). This supports our finding that *ERBB2* missense mutations are associated with a favorable response to NAC. We identified four tumors (17%) with an *ERCC2* missense mutation in the remaining group of patients.

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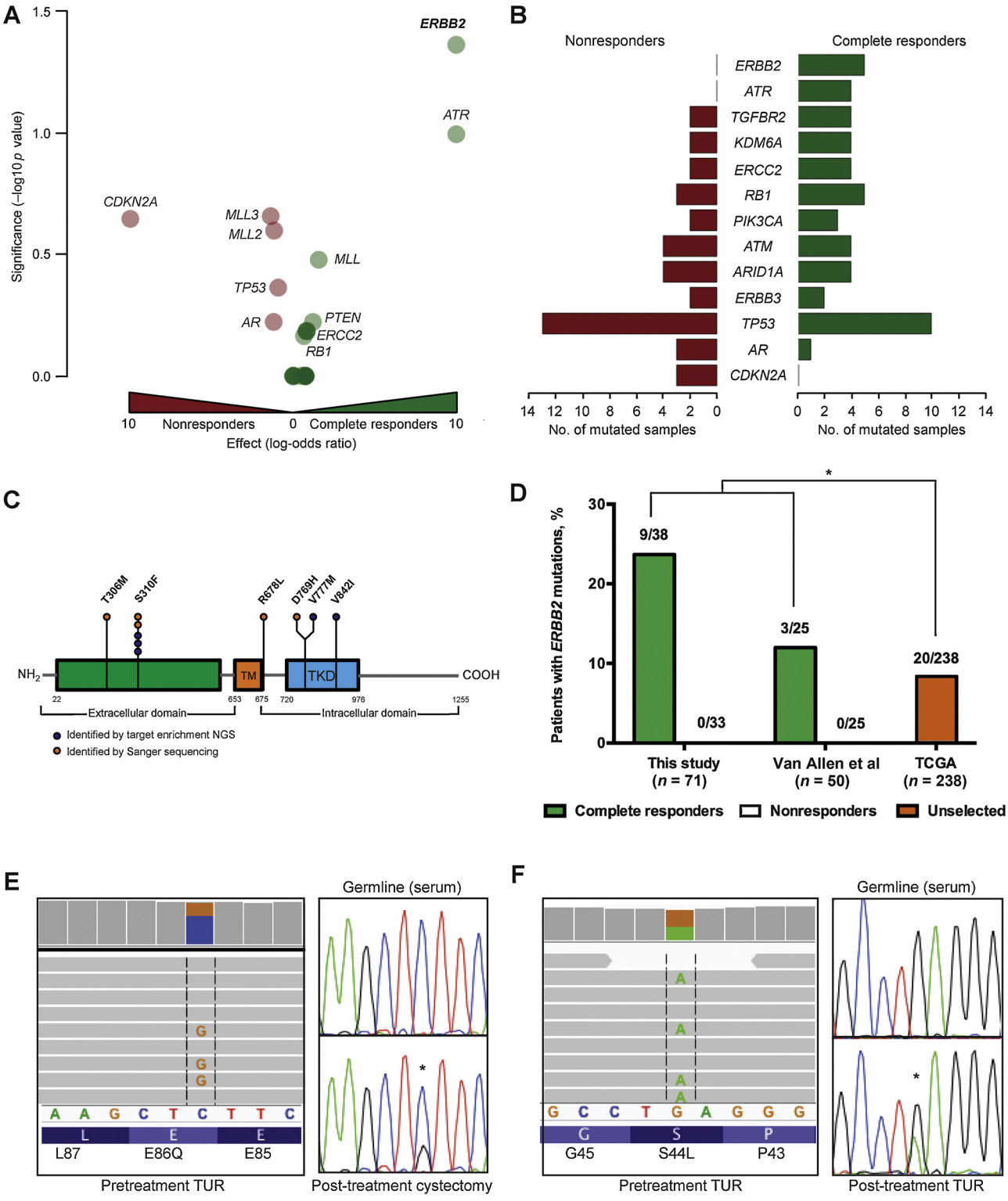

Fig. 1 – Gene enrichment analysis of mutated genes in complete responders and nonresponders. (A) Volcano plot of effect size (log-odds ratio) and significance (−log10 *p* value) of the 25 genes mutated in more than two samples. Mutated genes enriched in complete responders are labeled green; mutated genes enriched in nonresponders are labeled red. (B) Pyramid plot showing the number of mutated samples in 16 complete responders in green and the number of mutated samples in 16 nonresponders in red. (C) Plot showing the distribution of *ERBB2* missense mutations identified in this study by target enrichment next-generation sequencing (dark blue circles) or Sanger sequencing (orange circles). *ERBB2* missense mutations cluster at the S310 position in the extracellular domain and in the tyrosine kinase domain. (D) Graph showing that *ERBB2* missense mutations are significantly enriched in responders and significantly depleted in nonresponders to neoadjuvant chemotherapy compared with the unselected Cancer Genome Atlas urothelial bladder cancer cohort (*$p < 0.05$). (E, F) Sequencing results from two *ERCC2*-mutant nonresponders showing the *ERCC2* missense mutation (E86Q and S44L) in DNA isolated from the pretreatment transurethral resection and post-treatment tissue but not in the germline DNA. This demonstrates that these somatic *ERCC2* mutations were not counterselected during chemotherapy.
NGS = next-generation sequencing; TCGA = The Cancer Genome Atlas; TKD = tyrosine kinase domain; TM = transmembrane domain; TUR = transurethral resection.

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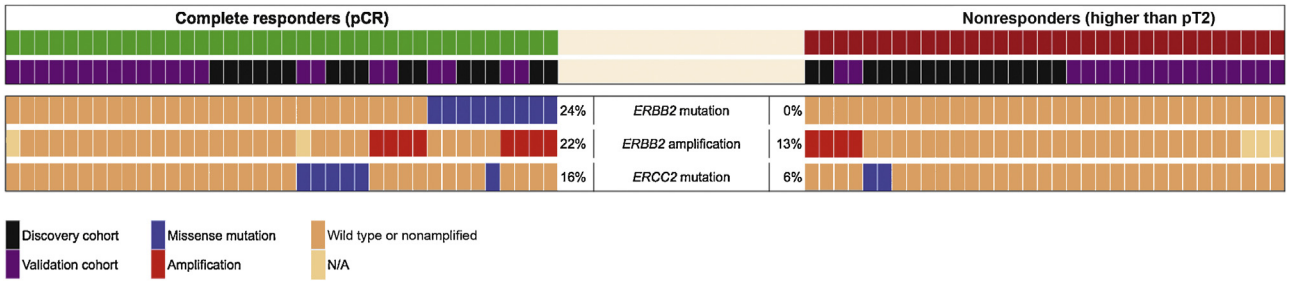

Fig. 2 – OncoPrint showing *ERBB2* missense mutations, *ERBB2* amplifications, and *ERCC2* missense mutations in the 38 complete responders and 33 nonresponders to neoadjuvant chemotherapy in this study. Individual patients are represented as columns. N/A = not available; pCR = pathologic complete response.

Two of these patients are alive without signs of disease recurrence, and two patients died due to distant recurrences (Supplementary Table 5).

Responses to NAC can also be plotted as complete response versus noncomplete response, as shown in Supplementary Figure 2. In this comparison, *ERBB2* mutations are strongly associated with response ($p = 0.006$), whereas *ERCC2* mutations are not.

Our findings indicate that *ERBB2* missense mutations could assist in selecting patients responding to NAC. Furthermore, these results suggest that *HER2*-directed therapies for *ERBB2* mutant bladder cancers are unlikely to replace chemotherapy in the neoadjuvant setting because these tumors have highly favorable responses to NAC containing platinum. However, despite a pathologic complete response to NAC, 3 of the 11 patients with *ERBB2*-mutant MIBC developed a distant recurrence. These patients may benefit from ERBB2 tyrosine kinase inhibitors, alone or in combination with chemotherapy. In contrast to the findings of Van Allen et al, we showed that the presence of an *ERCC2* mutation does not always confer sensitivity to platinum-based therapy.

A possible limitation of this study is the heterogeneity of our cohort. Patients were treated with different platinum-containing chemotherapy regimens: MVAC (methotrexate, vinblastine, doxorubicin, and cisplatin), gemcitabine and cisplatin, or gemcitabine and carboplatin. Although evidence for the benefit of gemcitabine and carboplatin in the neoadjuvant setting in terms of cancer-specific or overall survival is lacking, pathologic complete response rates appear to be similar [8]. Our cohort also contained more advanced cases than most neoadjuvant studies in bladder cancer. However, these patients reflect common clinical practice because many clinics would specifically treat this high-risk patient group with chemotherapy, followed by resection if possible.

In conclusion, we found that *ERBB2* missense mutations in MIBC are associated with an excellent response to NAC.

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: Groenendijk, de Jong, van Rhijn, Bernards, van der Heijden.
Acquisition of data: Groenendijk, van de Putte, Michaut, Schlicker.
Analysis and interpretation of data: Groenendijk, de Jong, van de Putte, Michaut, Velds, Bernards, van der Heijden.
Drafting of the manuscript: Groenendijk, Bernards, van der Heijden.
Critical revision of the manuscript for important intellectual content: de Jong, Michaut, Wessels, Broeks, van Rhijn.
Statistical analysis: Groenendijk, van de Putte, Michaut.
Obtaining funding: van der Heijden, Bernards.
Administrative, technical, or material support: Peters, Nieuwland, Kerkhoven, Broeks.
Supervision: Wessels, van Rhijn, Bernards, van der Heijden.
Other (specify): van de Heuvel (development of methodology).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.eururo.2015.01.014.

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