


HER-2 overexpression is a negative predictive factor for recurrence in patients with non-muscle-invasive bladder cancer on intravesical therapy

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Abstract

Objective: HER-2 is overexpressed in a variety of human malignant tumors and has been widely used in the prognosis and treatment of breast cancer. In urothelial cell carcinoma of the bladder, some reports have shown an association between HER-2 overexpression and worse outcomes. The aim of this study was to examine the association between HER-2 expression and other clinicopathologic parameters in 48 patients treated for primary non-muscle-invasive transitional cell carcinoma of the bladder.

Methods: The initial expression of HER-2 in tumor specimens and its expression upon disease recurrence following instillation therapy (BCG, Mitomycin, or Epirubicin) were studied.

Results: HER-2 expression was significantly increased between these two timepoints. In multivariate analysis, HER-2 expression at the time of diagnosis was found to be the only independent factor and was associated with reduced recurrence free survival.

Conclusions: HER-2 status could be an additional biomarker for predicting the outcome of non-muscle-invasive bladder cancer, which could help identify high-risk patients for recurrence and possible progression who require close observation and perhaps radical treatment, such as early cystectomy.

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Introduction

Urinary bladder cancer is the seventh most common malignancy in men and the seventeenth most common in women.¹ Bladder cancer originates from the epithelium in up to 98% of cases, and in 80% of cases the carcinoma is non-muscle-invasive at the time of diagnosis, which is associated with higher survival rates.² However, the clinical course of non-muscle-invasive carcinoma of the bladder (NMIBC) is unpredictable. The majority of patients with pTa and pT1 disease will develop a recurrence after the initial tumor resection. Furthermore, in 10% to 20% of patients the disease will progress to muscle-invasive disease, which has increased metastatic potential.¹ For this reason, novel prognostic markers that can help identify high-risk patients for recurrence and progression who will require closer observation and perhaps radical treatment, such as early cystectomy, would be valuable for the management of bladder carcinoma.

Human Epidermal Growth Factor Receptor 2 (c-erb-2 or HER-2) is overexpressed in a variety of human malignant tumors. HER-2 has been widely used in the prognosis and treatment of breast cancer. More specifically, the monoclonal antibody trastuzumab represents the most effective therapeutic option for patients with HER-2-overexpressing breast cancer, as this offers a targeted treatment for this disease subtype.^{3,4} There is also evidence that increased HER-2 expression is associated with a tendency towards metastatic activity.⁵ Moreover, HER-2 activation is

associated with more intensive mitotic activity, which leads to cellular transformation and the subsequent acquisition of advantageous properties for malignant cells.⁶ In urothelial cell carcinoma of the bladder, studies have reported overexpression of HER-2 in 17% to 76% of cases, and there is a possible association between HER-2 overexpression with early recurrence, high grade disease, and worse prognosis.⁷⁻¹⁰

The aim of this retrospective study was to evaluate HER-2 expression in a cohort of NMIBC patients, who underwent intravesical treatment and correlate the results with clinicopathologic characteristics.

Patients and methods

Patients

Forty-eight patients with recurrence of histologically proven non-muscle-invasive urothelial cell carcinoma of the bladder who were treated at Theagenio Cancer Hospital in Greece were included in this study. Additional inclusion criteria were intermediate or high-risk tumors with an expected EORTC recurrence score >5 that required further intravesical therapy, an ECOG performance status between 0 and 2, and complete follow-up data. The exclusion criteria were diagnosis of muscle-invasive carcinoma of the bladder \geq pT2, the presence of carcinoma in situ (CIS), remaining tumor after initial resection, metastatic disease, concomitant diagnosis of

another cancer, known immunodeficiency, medications affecting immune system function, and the development of life-threatening side effects from the administration of the intravesical treatment that required terminating therapy. Ethical approval for this study was obtained from Theagenio Cancer Hospital Research Ethics Committee. All participants gave written informed consent.

The 2004 WHO grading system was used for histological classification of all tumours.¹¹ All patients had an immediate (within 24 hours) post-operation single intravesical treatment with mitomycin. A re-look transurethral resection of bladder tumor (TURBT) within 4 to 6 weeks after the initial resection was limited only to patients with doubt about completeness of the TURBT or if there was no muscle in the specimen from the initial resection. Follow-up cystoscopies were performed on all cases initially at 3-month intervals, which were subsequently increased to 6-month intervals according to individual tumor characteristics.^{12,13}

The patients were divided into three groups, according to the agent used for intravesical instillation (Bacillus Calmette–Guerin [BCG], Mitomycin, or Epirubicin). All patients had a satisfactory clinical follow up (age, sex, stage, grade, treatment, and survival). Patients with intermediate-risk tumors had an induction 6-week course plus 3-week instillations at 3, 6, and 12 months, while patients with high-risk tumors received additional 3-week instillations at 18, 24, 30, and 36 months.¹³ Recurrence was defined as biopsy-proven tumor relapse irrespective of tumor stage, while progression was defined as the presence of muscle invasive disease ($\geq T2$) or metastatic disease at the time of tumor recurrence.

HER2 expression

HER2 expression was assessed with immunohistochemistry in specimens from the

initial TURBT and in specimens of TURBT or positive bladder biopsies from the first recurrence. All specimens were examined in the pathology department of Theagenio Cancer Hospital. Confirmation of T stage, tumor grade, and HER-2 expression were assessed by a single pathologist (AN). The primary endpoint was defined as HER-2 expression at the time of diagnosis (the first TURBT that revealed primary NMIBCa). Correlations between HER-2 expression and demographic, clinical, pathologic, and follow-up parameters were analyzed.

Immunohistochemistry

Tissue samples from TURBTs were fixed in 10% neutral formalin solution and embedded in paraffin blocks. Immunohistochemical analysis was performed with the automated system BenchMark XT (Ventana BenchMark XT, Roche, Mannheim, Germany). Deparaffinization was performed with a combination of heat, a mild detergent solution (EZ prep), and spin-mixing. The Optiview DAB IHC Detection Kit, an indirect biotin-free system, was used to detect the primary antigens in paraffin sections. HER2 protein expression was studied with the prediluted VENTANA anti-HER-2 antibody (clone 4B5; Roche). This rabbit monoclonal antibody is directed against the internal domain of HER-2 protein. The staining was membranous, allowing a semi-quantitative detection of HER-2 antigen. Only membrane staining was scored according to the same standard criteria used in breast cancer.⁴ HER-2 positivity was assessed using the following scoring system:

0: no staining was observed or membrane staining that was incomplete and faint/barely perceptible was found in $\leq 10\%$ of tumor cells; 1+: incomplete membrane staining that was faint/barely perceptible in $>10\%$ of tumor cells (Figure 1);

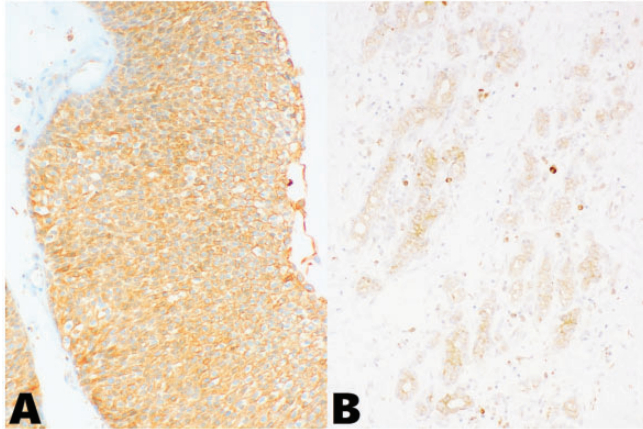


Figure 1. HER2 immunohistochemistry at 200 \times magnification; Score 1+. (a) Urothelial carcinoma. (b) Breast carcinoma-positive control.

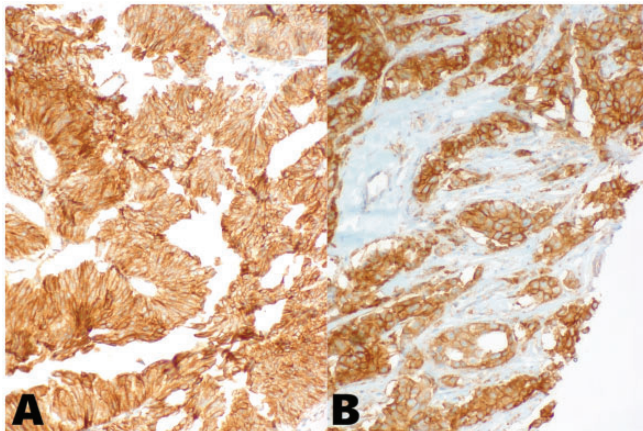


Figure 2. HER2 immunohistochemistry at 200 \times magnification; Score 2+. (a) Urothelial carcinoma. (b) Breast carcinoma-positive control.

2+: weak to moderate complete membrane staining was observed in $>10\%$ of tumor cells (Figure 2); 3+: circumferential membrane staining that was complete and intense was observed and in $>10\%$ of tumor cells (Figure 3).⁴

Statistical analysis

The association between HER-2 protein expression and other clinicopathologic

parameters was evaluated using the non-parametric Mann–Whitney U test (for numerical variables) or Pearson’s correlation test (for categorical variables). The comparison between HER-2 expression at initial diagnosis and on disease recurrence per patient was tested with the Wilcoxon Signed Ranks Test. The log-rank test was used to examine changes in outcomes between the three intravesical treatments, while multivariate Cox regression analysis

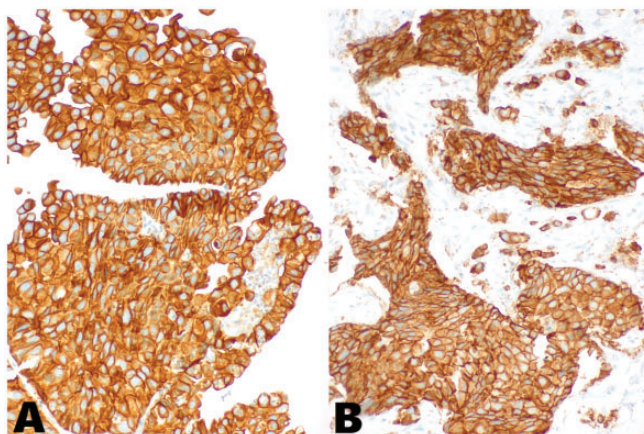


Figure 3. HER2 immunohistochemistry at 200 \times magnification; Score 3+. (a) Urothelial carcinoma. (b) Breast carcinoma-positive control.

was used to determine factors affecting the recurrence free survival of patients. Statistical significance was set to <0.05 . All analyses were performed using IBM SPSS Statistics for Windows, version 24.0 (IBM Corp., Armonk, NY, USA).

Results

The final cohort comprised 48 patients, among which 45 (93.7%) were men. The mean age at diagnosis was 68 years (range: 39–83). The patients were divided into three groups according to the agent used for intravesical instillation (BCG, Mitomycin, or Epirubicin); each group included 16 patients. At the time of initial diagnosis, 50% of patients²⁴ had a pT1 tumor and 33.3%¹⁶ had a high-grade urothelial cell carcinoma (UCC) (Table 1). There was a trend towards patients with higher grade and stage carcinomas receiving BCG rather than the other treatments (Mitomycin or Epirubicin), but this difference was not statistically significant.

The mean time for disease recurrence was 12 months and 13 days (range: 3–42 months). Four cases progressed to muscle invasive disease (T2), with two having a

pTa and two with pT1 stage at the time of diagnosis. Among all patients, 25% showed a decrease in stage, while 58.3% remained unchanged. Grade was improved in 29.1% of patients, while 16.6% showed a more aggressive grade upon disease recurrence. At the time of disease recurrence, there was no statistically significant difference in disease stage or in the grade of carcinoma before and after the implementation of intravesical treatment for all patients in the three treatment groups. None of the treatments were found to be more effective when compared by the log-rank test.

HER-2 immunohistochemistry at the time of initial diagnosis and upon disease recurrence is shown in Figure 4. No significant correlation was found between HER-2 expression and grade or stage of disease, both at the time of diagnosis or at the time of disease recurrence. There was a statistically significant increase in the intensity of staining before and after the implementation of treatment ($p=0.039$). Among the 48 cases, there was an increase in HER-2 expression in 23 cases (47.9%), 15 (31.2%) cases maintained the same intensity, while the remaining 10 (20.8%) cases showed

Table 1. Clinical and Pathologic features of the 48 patients.

No	Age	T stage at diagnosis	Grade at diagnosis	HER-2 at diagnosis	T stage following treatment	Grade following treatment	HER-2 following treatment	Type of intravesical therapy	RFS (months)
1	61	pTa	Low	+1	pT1	High	+2	BCG	24
2	70	pT1	High	+1	pT1	High	+3	BCG	6
3	75	pTa	High	+2	pTa	Low	+1	Mitomycin	6
4	56	pT1	Low	+2	pTa	Low	+1	Epirubicin	8
5	68	pTa	High	0	pTa	Low	+2	BCG	20
6	48	pT1	Low	+2	pT1	Low	+1	Mitomycin	5
7	52	pTa	Low	+2	pTa	Low	+1	Mitomycin	7
8	57	pTa	Low	+1	pT1	High	+1	Mitomycin	16
9	59	pTa	Low	+1	pTa	Low	+2	Epirubicin	24
10	82	pTa	Low	+2	pT1	High	+2	Epirubicin	10
11	66	pT1	High	+2	pT2	Low	0	BCG	7
12	24	pTa	Low	+1	pTa	Low	+2	BCG	3
13	52	pTa	Low	+3	pTa	Low	+2	Epirubicin	4
14	69	pTa	Low	+2	pT2	High	+3	Epirubicin	6
15	77	pTa	Low	+2	pTa	Low	+3	Mitomycin	7
16	61	pT1	High	+1	pTa	Low	+1	BCG	4
17	57	pTa	Low	+2	pTa	Low	+2	Epirubicin	5
18	57	pTa	High	+1	pTa	Low	+2	BCG	15
19	77	pT1	Low	+2	pT1	Low	+2	Mitomycin	27
20	78	pT1	High	+3	pTa	Low	+3	BCG	9
21	68	pT1	High	+3	pT1	Low	+3	Mitomycin	9
22	62	pTa	Low	+1	pTa	Low	+2	Epirubicin	10
23	46	pT1	Low	+2	pTa	Low	+2	Epirubicin	23
24	63	pT1	Low	+2	pTa	High	+3	Mitomycin	7
25	60	pT1	Low	+1	pT1	High	+2	BCG	7
26	46	pTa	Low	+2	pTa	Low	+2	Epirubicin	23
27	73	pTa	High	+2	pTa	Low	+2	Mitomycin	22
28	58	pT1	Low	+2	pTa	Low	+2	Epirubicin	30
29	69	pTa	High	0	pTa	Low	+2	BCG	8
30	47	pT1	Low	+2	pT1	Low	+1	Mitomycin	12
31	70	pTa	Low	+2	pT2	High	+3	Epirubicin	6
32	57	pTa	Low	+1	pT1	High	+2	Mitomycin	21
33	60	pT1	Low	+1	pTa	Low	+2	Epirubicin	21
34	81	pTa	Low	+2	pT1	High	+2	Epirubicin	3
35	56	pT1	High	+2	pT2	Low	0	BCG	6
36	34	pT1	Low	+1	pTa	Low	+2	BCG	4
37	51	pTa	Low	+3	pTa	Low	+2	Epirubicin	6
38	52	pTa	Low	+2	pTa	Low	+1	Mitomycin	10
39	77	pT1	Low	+2	pTa	Low	+3	Mitomycin	3
40	61	pT1	High	+1	pTa	Low	+1	BCG	11
41	56	pT1	Low	+2	pTa	Low	+2	Epirubicin	6
42	58	pTa	High	+1	pTa	Low	+2	BCG	27
43	76	pT1	Low	+2	pT1	Low	+2	Mitomycin	12
44	79	pT1	High	+3	pTa	Low	+3	BCG	3

(continued)

Table I. Continued.

No	Age	T stage at diagnosis	Grade at diagnosis	HER-2 at diagnosis	T stage following treatment	Grade following treatment	HER-2 following treatment	Type of intravesical therapy	RFS (months)
45	65	pT1	High	+3	pT1	Low	+3	Mitomycin	5
46	65	pTa	Low	+1	pTa	Low	+2	Epirubicin	16
47	71	pT1	High	+1	pT1	High	+3	BCG	24
48	63	pT1	Low	+2	pTa	High	+3	Mitomycin	38

HER-2: Human Epidermal growth factor Receptor 2, RFS: Recurrence Free Survival, BCG: Bacillus Calmette–Guerin.

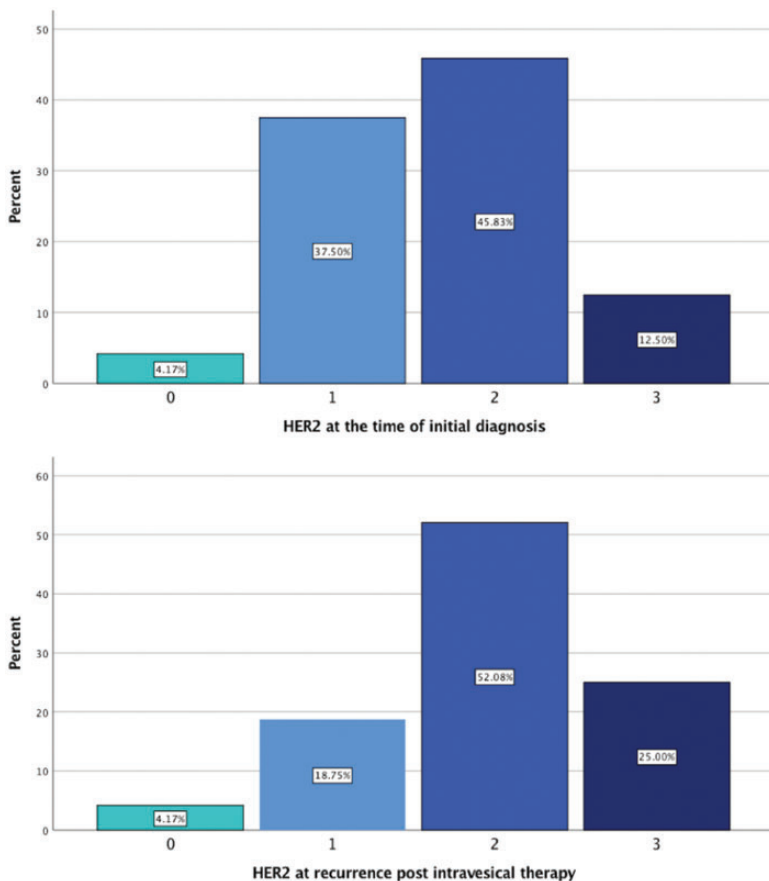


Figure 4. HER-2 expression at time of initial diagnosis and upon disease recurrence.

decreased HER-2 expression. Among the 23 patients with increased HER-2 expression, 12 were treated with BCG, five with Mitomycin, and six with Epirubicin.

Multivariate Cox regression analysis showed that the expression of HER-2 at the time of diagnosis was the only independent factor associated with lower recurrence

Table 2. Cox regression multivariate analysis for tumour recurrence.

	95% CI		HR	P-value
	Lower	Upper		
Age	0.959	1.029	0.993	0.704
Treatment (BCG, Mitomycin, Epirubicin)	0.492	1.167	0.892	0.706
Grade (before treatment)	0.470	3.397	1.264	0.642
Grade (after treatment)	0.277	2.124	0.767	0.610
HER-2 (before treatment)	1.106	3.265	1.900	0.020
HER-2 (after treatment)	0.504	1.587	0.894	0.702
Stage (before treatment)	0.333	1.306	0.660	0.232
Stage (after treatment)	0.654	2.512	1.281	0.471

CI: confidence interval, HR: hazard ratio, BCG: bacillus Calmette–Guerin.

free survival time ($p=0.020$). All other factors examined (age, type of intravesical treatment, stage, grade, and HER-2 expression upon disease recurrence) did not significantly affect disease recurrence (Table 2). There was a statistically significant difference in recurrence free survival time between the different HER-2 expression groups at the time of diagnosis ($p=0.032$) (Figure 5).

Discussion

HER-2 protein is a member of the Epidermal Growth Factor Receptor (EGFR) family. The role of HER-2 is known in breast cancer because it is a prognostic factor and a classic therapeutic target when overexpressed.⁶ Enhancement of the *Her2* gene on chromosome 17q21 is the primary mechanism for its overexpression, and this subsequently activates pathways that promote the survival, motility, and proliferation of cancer cells.^{3,5} These oncogenic characteristics translate into reduced patient survival. The mechanisms of a successful anti-HER2 therapy are inhibition of HER-2 protein activity, as well as treatment with monoclonal chemotherapeutic agents.⁵ Recently, HER-2 alterations have been

found in bladder cancer, both in primary tumors and metastatic disease.^{7–10,14}

In 1990, Zhau et al.¹⁵ first reported increased amplification and overexpression of HER-2 in bladder carcinoma. Since then, several studies tried to confirm these findings and evaluate the role of HER-2 in patients prognosis. The incidence of HER-2 overexpression in muscle-invasive urothelial bladder carcinoma varies.^{8,16,17} It has been found to occur in 45% of cases, ranging from 23% to 80%.⁸ Additionally, some studies have found that HER-2 overexpression is predictive of bladder cancer-related death in patient with invasive tumours.^{7,9,18} Kolla et al.¹⁹ observed a significantly higher disease-free survival rate in HER-2 negative patients compared with HER-2 positive patients; this difference was more profound in patients with locally advanced disease (T2b–T4, N+). However, other studies did not agree with the these results and reported no significant difference in survival between HER-2 positive and negative patients.²⁰

Interestingly, Grivas et al. found that in 45% of tumors, HER-2 overexpression was negative at diagnosis and changed to positive when tumors becomes metastatic. This study also showed a median survival for HER-2 positive patients of 33 months

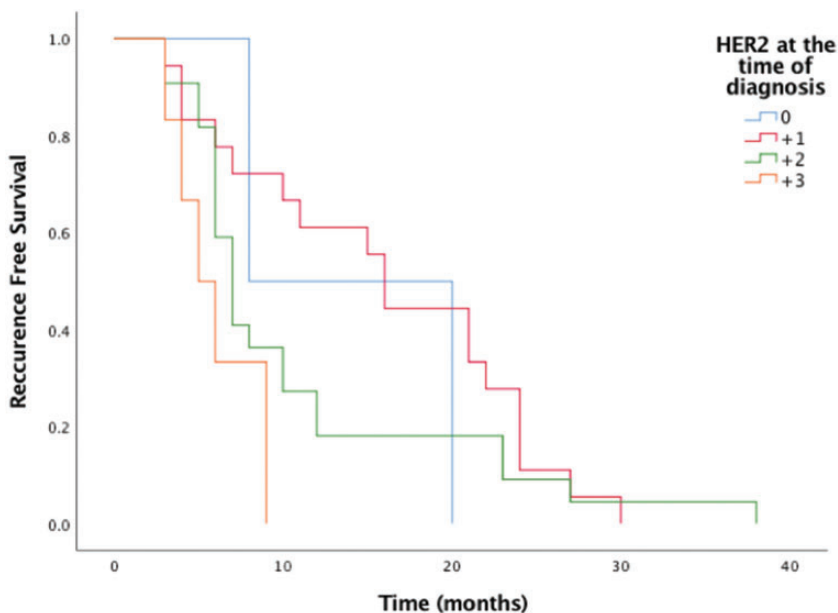


Figure 5. Recurrence-free survival by HER-2 expression at initial diagnosis.

compared with the significantly higher median survival of HER-2 negative patients of 50 months.¹⁷ Moreover, with regard to the grade and stage of UCC, Khaled et al. found a correlation between HER-2 overexpression and tumor stage ($p=0.011$). HER-2 overexpression was also more common in high-grade carcinomas but without statistical significance.¹⁶ Given these results, it seems that HER-2 overexpression is a reliable prognostic factor in muscle-invasive bladder cancer. However, its role in NMIBCa remains controversial. In 2013, Chen et al.²¹ showed that HER-2 amplification could distinguish a subset of NMIBCa patients with a high risk of disease progression. This was not in agreement with the findings from a larger study that included 285 patients with primary T1 NMIBCa, where HER-2 expression could not predict patient prognosis.²²

Our study population consisted of patients with NMIBCa, at intermediate- or high-risk for disease recurrence and

progression. Moreover, all selected patients received intravesical treatment with either BCG, Mitomycin, or Epirubicin, and a reassessment of HER-2 immunohistochemical expression was performed upon first recurrence. HER-2 expression at the time of diagnosis was found to be the only independent prognostic factor for disease recurrence, while carcinoma grade, disease stage, and type of intravesical treatment were not predictive. Stage and grade did not affect recurrence in our study. This could be explained by the small number of patients and the non-randomization of this study. Additionally, patients with positive expression of HER-2 did not appear to benefit from intravesical therapy, as HER-2 expression frequently increased after treatment.

Our results confirm recent well-designed studies, demonstrating that HER-2 overexpression is a significant predictor of disease recurrence and/or progression. Ding et al.²³ correlated HER-2 overexpression with

progression of tumors to muscle-invasive disease, especially in patients with intermediate- and high-risk EORTC scores, a similar population to our study. Cormio et al. tested the role of HER-2 expression in predicting recurrence and progression in 67 patients with T1G3 NMIBCa who underwent TURBT alone (33 cases) or TURBT + BCG instillations (34 cases). HER-2 overexpression was a significant predictor of disease free survival ($p=0.0013$) and progression free survival ($p=0.0322$) in the overall patient population, while BCG treatment was significant only for disease free survival ($p=0.0231$) but not progression free survival ($p=0.6901$).²⁴ The results from our study were did not show a statistically significant difference in disease progression, but this could be explained by the limited number of events and the fact that follow-up was ended upon first disease recurrence.

The drawbacks of this study include its retrospective nature and the small number of cases examined with immunohistochemistry, but this also applies to all relevant studies in the literature. Most importantly, we applied the algorithm of HER-2 expression for breast cancer to urothelial carcinoma. This may not necessarily be accurate, as urothelial cells are not breast ductal cells. Fluorescence in situ hybridization (FISH) could be a more appropriate methodology to detect *Her2* amplification in urothelial carcinoma. However, this test was not available in our hospital. Another limitation could be a bias in patient selection. However, the main criterion of this study was to involve equal numbers of patients who had different types of intravesical treatment to see if the type of treatment could play a role in changing the expression of HER-2. To our knowledge, this is the first study to investigate the role of HER-2 expression in patients receiving the three most commonly used intravesical treatments for NMIBCa.

In summary, this study indicated that HER-2 overexpression is a negative predictive factor for recurrence free survival in intermediate- and high-risk NMIBCa, independent of tumor stage, grade, and the type of intravesical therapy. This indicates that immunohistochemical evaluation of HER-2 may be valuable to identify the subset of patients at high risk of recurrence, for whom an early and more aggressive therapy should be considered.

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Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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