

Prognostic significance of HER2 status evaluation using immunohistochemistry in patients with urothelial carcinoma of the bladder: A retrospective single-center experience

XUESONG BAI, WEIYANG HE, HUBIN YIN, XINYUAN LI, XIANG ZHOU,
ZONGJIE WEI, HAITAO YU and XIN GOU

Department of Urology, The First Affiliated Hospital of Chongqing Medical University, Chongqing 400016, P.R. China

Received March 7, 2022; Accepted September 1, 2022

DOI: 10.3892/etm.2022.11640

Abstract. In recent years, antibody-drug conjugate (ADC) therapy targeting human epidermal growth factor receptor 2 (HER2) has been proven to be beneficial in patients with advanced urothelial carcinoma of the bladder (UCB); however, the role of HER2 in UCB remains obscure. Thus, the present retrospective single-center study was performed to evaluate the expression of HER2 in UCB and its prognostic significance. The HER2 status of 108 patients with UCB who underwent radical cystectomy was assessed using immunohistochemistry, and its association with the recurrence and survival rates of patients was analyzed. HER2 overexpression was observed in 57.4% of the patients; this was significantly associated with higher tumor grades ($P=0.006$) and stages ($P<0.001$). Kaplan-Meier analysis suggested that patients with HER2 overexpression had a shorter 5-year overall survival rate ($P=0.005$) and recurrence-free survival rate ($P=0.003$). Multivariate Cox regression analysis indicated that HER2 overexpression was a high-risk independent predictor of UCB recurrence (hazard ratio, 3.61; 95% confidence interval, 1.07-12.18; $P=0.039$). On the whole, these findings demonstrate that evaluating the HER2 status may improve the prediction of cancer recurrence and may thus guide the selection of patients that will benefit the most from HER2-ADC therapies.

Introduction

Bladder cancer (BCa) is the 10th most prevalent malignancy worldwide, with an estimated 573,278 new cases diagnosed and 212,536-related deaths worldwide in 2020 (1). The most

common type of BCa is urothelial carcinoma of the bladder (UCB). While radical cystectomy (RC) provides the local control of tumors for patients with muscle invasive BCa and high-risk non-muscle invasive BCa (NMIBC), 30% of patients experience relapse following RC (2). Moreover, the survival rate is only 12-15 months in patients with cancers of an advanced stage undergoing classical cisplatin-based systemic chemotherapy (3). However, the recent emergence of novel immunotherapies, targeted agents and antibody-drug conjugate (ADC) therapies have substantially broadened the treatment options for UCB, with the overall survival (OS) currently approaching 2 years (4). Currently, based on the excellent efficacy of study EV-201 (NCT03219333), TROPHY-U-01 (NCT03547973), and NCT03507166, three ADCs for UCB, enfortomab vedotin, sacituzumab govitecan and disitamab vedotin, have respectively been approved for application (5). The Cancer Genome Atlas database provides data enabling mapping the comprehensive molecular landscape of UCB, and accumulating evidence based on these data demonstrate the presence of distinct molecular subtypes of UCB, offering the potential to develop novel molecularly targeted therapies (6,7). In this context, identifying more specific tumor biomarkers would enable clinicians to assess cancer risk, predict disease development, and guide treatment more accurately in a personalized manner.

Human epidermal growth factor receptor 2 (HER2) is a transmembrane receptor of tyrosine kinase encoded by the *HER2/neu* oncogene, which participates in the processes of cell proliferation and tumorigenesis. The oncogenic role of HER2 has been most extensively studied in breast and gastric cancer, which has been confirmed as a poor prognostic factor; accordingly, trastuzumab, a humanized monoclonal antibody targeting HER2, is currently the cornerstone drug of targeted therapy for these types of cancer (8,9). In recent years, the overexpression of HER2 has also been detected in UCB, prompting the initiation of several clinical trials assessing the efficacy of HER2 inhibitors, such as trastuzumab and lapatinib, against this malignancy, with unsatisfactory results (10). However, the recent success of ADC therapies has brought HER2 back into focus in clinical and basic research regarding UBC.

Notably, there is no unified standard for the determination of the HER2 expression status in UCB, mainly due to issues

Correspondence to: Professor Xin Gou, Department of Urology, The First Affiliated Hospital of Chongqing Medical University, 1 Youyi Road, Yuzhong, Chongqing 400016, P.R. China
E-mail: gouxincq@163.com

Key words: human epidermal growth factor receptor 2, urothelial carcinoma of the bladder, prognosis, immunohistochemistry, antibody-drug conjugate

Table I. Descriptive characteristics for the cohort of 108 patients with urothelial carcinoma of the bladder treated with radical cystectomy.

Variable	Patients, n (%)	HER2 expression, n (%)		P-value
		Low	High	
Total	108 (100)	46 (42.6)	62 (57.4)	
Age, years				0.740
≤65	52 (48.1)	23 (44.2)	29 (55.8)	
>65	56 (51.9)	23 (41.1)	33 (58.9)	
Sex				0.839
Female	11 (10.2)	5 (45.5)	6 (54.5)	
Male	97 (89.8)	41 (42.3)	56 (57.7)	
Tumor size				0.147
<3 cm	57 (52.8)	28 (49.1)	29 (50.9)	
≥3 cm	51 (47.2)	18 (35.3)	33 (64.7)	
No. of tumors				0.577
1	19 (17.6)	7 (36.8)	12 (63.2)	
>1	89 (82.4)	39 (43.8)	50 (56.2)	
T stage				0.001
Ta, T1	38 (35.2)	27 (71.1)	11 (28.9)	
T2	37 (34.3)	11 (29.7)	26 (70.3)	
T3	20 (18.5)	6 (30.0)	14 (70.0)	
T4	13 (12.0)	2 (15.4)	11 (84.6)	
G grade				0.006
G1/2	20 (18.5)	14 (70.0)	6 (30.0)	
G3	88 (81.5)	32 (36.4)	56 (63.6)	
Lymph node metastasis				0.156
Negative	100 (92.6)	45 (45.0)	55 (55.0)	
Positive	8 (7.4)	1 (12.5)	7 (87.5)	
Lymphovascular invasion				0.491
Negative	96 (88.9)	42 (43.8)	54 (56.3)	
Positive	12 (11.1)	4 (33.3)	8 (66.7)	
Adjuvant chemotherapy				0.643
Negative	89 (82.4)	37 (41.6)	52 (58.4)	
Positive	19 (17.6)	9 (47.4)	10 (52.6)	

with methodology (amplification detection vs. overexpression detection) and the diversity of available techniques [polymerase chain reaction, *in situ* hybridization and immunohistochemistry (IHC)]. A previous meta-analysis reported that HER2 levels in BCa varied from 9 to >80% as regards protein overexpression and from 0 to 32% in terms of gene amplification (11). Furthermore, previous studies investigating various BCa-associated genetic variants obtained conflicting results for the prognostic significance of HER2 status. Some studies revealed that upregulation of HER2 was associated with poor prognosis (12-17), whereas others indicated that HER2 status did not have prognostic significance (18-20). In addition, Gandour-Edwards *et al* (21) reported an increased cancer-specific survival of the HER2 (2+/3+) population in the context of paclitaxel-based chemotherapy. These conflicting findings mainly result from the different patient cohorts

between the studies, particularly regarding tumor stage and histological grade, and the methods used to evaluate HER2 status, meanwhile, it underlines the need to gain a better understanding of the expression and potential role of HER2 in UCB.

Based on this background, the aim of the present study was to evaluate the expression of HER2 in patients with UCB, as assessed using IHC, and to determine the association of HER2 status on recurrence-free survival (RFS) and OS.

Patients and methods

Patient selection. The present retrospective study was conducted following the approval from the Institutional Ethics Committee of The First Affiliated Hospital of Chongqing Medical University (approval no. 2022-K21; Chongqing,

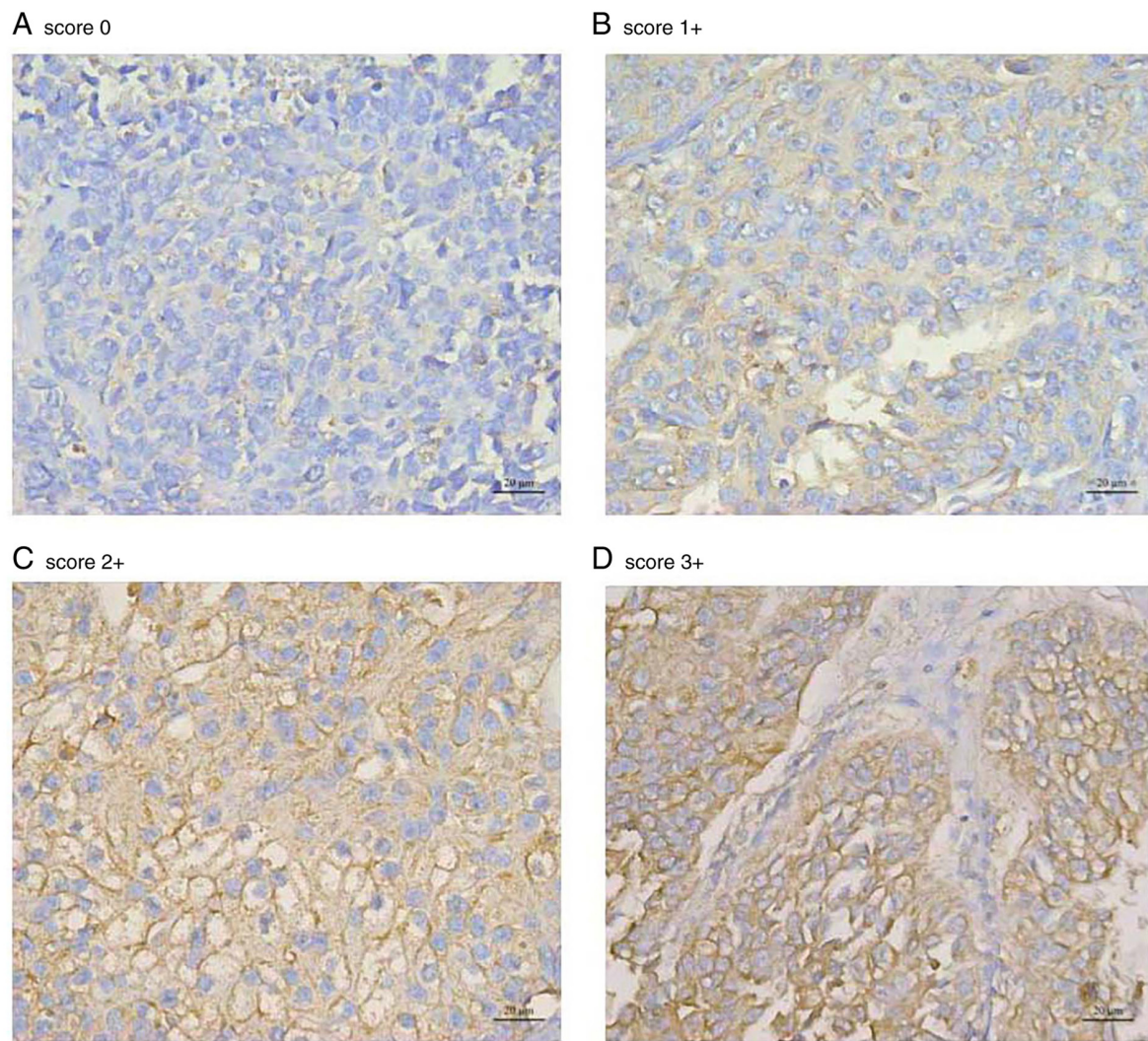


Figure 1. Immunohistochemical staining of urothelial carcinoma of the bladder illustrating different levels of human epidermal growth factor receptor 2 protein expression based on the allocated score. (A and B) low expression for HER2 (score 0 and 1+, respectively). (C and D) high expression for HER2 (score 2+ and 3+, respectively) (magnification, x400; scale bar, 20 μ m).

China), and written informed consent was signed by each participant prior to sample collection. A total of 108 patients who underwent RC and bilateral regional lymphadenectomy for UCB at the Department of Urology of The First Affiliated Hospital of Chongqing Medical University between 2015 and 2020 were included. None of the patients had received neoadjuvant chemotherapy prior to surgery, and all samples were subjected to pathological examinations that led to the identification of the tumors as urothelial carcinomas. The clinical data of the patients were collected from the medical record system of the hospital and included the following: sex, age, tumor size, number of tumors, pathological stage and grade, lymph node metastasis, lymphovascular invasion and adjuvant chemotherapy. The pathological specimens were re-examined by two experienced pathologists using the 2002 TNM system (22) for pathological staging and the 1973 World Health Organization system for pathological grading (23). The follow-up duration was defined as the period from the date the patient underwent RC until the date of recurrence of UCB, which was identified using computed tomography imaging or the date of death of the patient.

IHC. Tissues were fixed with 10% formalin at room temperature for 24 h and were embedded in paraffin. Subsequently, paraffin-embedded tumor sections (4 μ m) were successively subjected to dewaxing, antigen retrieval (achieved by boiling the sample in 0.01 mol/l sodium citrate buffer, pH 6.0, for 30 min), incubation with 3% hydrogen peroxide (IHC kit; cat. no. SP-9000; OriGene Technologies, Inc.) for 10 min at room temperature, and blocking with 10% goat serum (cat. no. SP-9000; OriGene Technologies, Inc.) for 15 min at room temperature. Subsequently, the slides were incubated with anti-HER2 polyclonal antibody (cat. no. 18299-1-AP; ProteinTech Group, Inc.; 1:50 dilution) at 4°C overnight, washed with phosphate-buffered saline three times, and incubated with the biotinylated goat anti-mouse/rabbit IgG secondary antibody (cat. no. SP-9000; OriGene Technologies, Inc.) for 20 min at room temperature. Then, sections were incubated with horseradish enzyme-labeled streptavidin working solution (cat. no. SP-9000; OriGene Technologies, Inc.) for 20 min at room temperature. Finally, the slides were stained using a 3,3'-diaminobenzidine kit (cat. no. ZLI-9018; OriGene Technologies, Inc.) for 1 min at room temperature, counterstained with hematoxylin

(cat. no. G1080; Beijing Solarbio Science & Technology Co., Ltd.) for 30 sec at room temperature, dehydrated and mounted in dibutylphthalate polystyrene xylene.

The samples were scored based on previous literature and according to the modified 2018 American Society of Clinical Oncology HER2 testing in breast cancer guideline (14,24). IHC staining was scored as follows: 0, no staining or <10% staining of tumor cells; 1, faint and partial membrane staining in >10% of cells; 2, weak to moderate, complete membrane staining in >10% of cells; or 3, strong, complete membrane staining in >10% of tumor cells. An IHC score of 2+ or 3+ was defined as a high expression or overexpression of HER2, while a score of 0 or 1+ was defined as a low expression. The scoring was performed by two pathologists who were blinded to the clinical data.

Statistical analysis. The Pearson's and continuous calibration Chi-squared tests were used to evaluate the association between HER2 expression status (overexpression vs. low expression) and clinicopathological parameters. A Kaplan-Meier curve was constructed to assess the prognostic significance of HER2 expression on RFS and OS, and differences between groups were statistically analyzed using the log-rank test. A Cox regression model was used for univariate and multivariate survival analyses. $P < 0.05$ was considered to indicate a statistically significant difference. All analyses were performed using SPSS version 22.0 software (IBM Corp.).

Results

Among the 108 patients included in this study, IHC scores of 0, 1+, 2+, and 3+ were observed in 6, 40, 47 and 15 patients, respectively. A total of 62 patients (57.4%) were found to have a high expression of HER2 (IHC 2+/3+). HER2 overexpression was significantly associated with a high tumor grade ($P = 0.006$) and an advanced stage ($P < 0.001$); however, it was not significantly associated with the patient's age ($P = 0.740$), sex ($P = 0.839$), tumor size ($P = 0.147$), the number of tumors ($P = 0.577$), lymph node metastasis ($P = 0.156$), or lymphovascular invasion ($P = 0.491$). The baseline characteristics of the patients are presented in Table I and representative images of the IHC staining intensities for the four different levels of scoring are presented in Fig. 1.

At the time of data analysis and with a median follow-up of 31.5 months [95% confidence interval (CI), 28-40], 24 patients (22.2%) experienced relapse and 22 succumbed to the disease (20.4%). Kaplan-Meier analysis revealed that patients with HER2 overexpression had a shorter OS ($P = 0.005$) and RFS ($P = 0.003$) than those with low levels of HER2 expression (Fig. 2). Univariate Cox regression analysis also revealed that HER2 overexpression was significantly associated with a poor RFS [hazard ratio (HR), 4.37; 95% CI, 1.49-12.79; $P = 0.007$] and OS (HR, 4.12; 95% CI, 1.39-12.21; $P = 0.011$) (Table II). In multivariate Cox regression analyses controlling for the effects of standard clinicopathologic variables, such as HER2 expression, pathological stage, pathological grade, lymph node metastasis and lymphovascular invasion, HER2 expression (HR, 3.61; 95% CI, 1.07-12.18; $P = 0.039$), pathological stage ($P = 0.003$) and adjuvant chemotherapy (HR, 0.09; 95% CI, 0.01-0.79; $P = 0.029$) remained

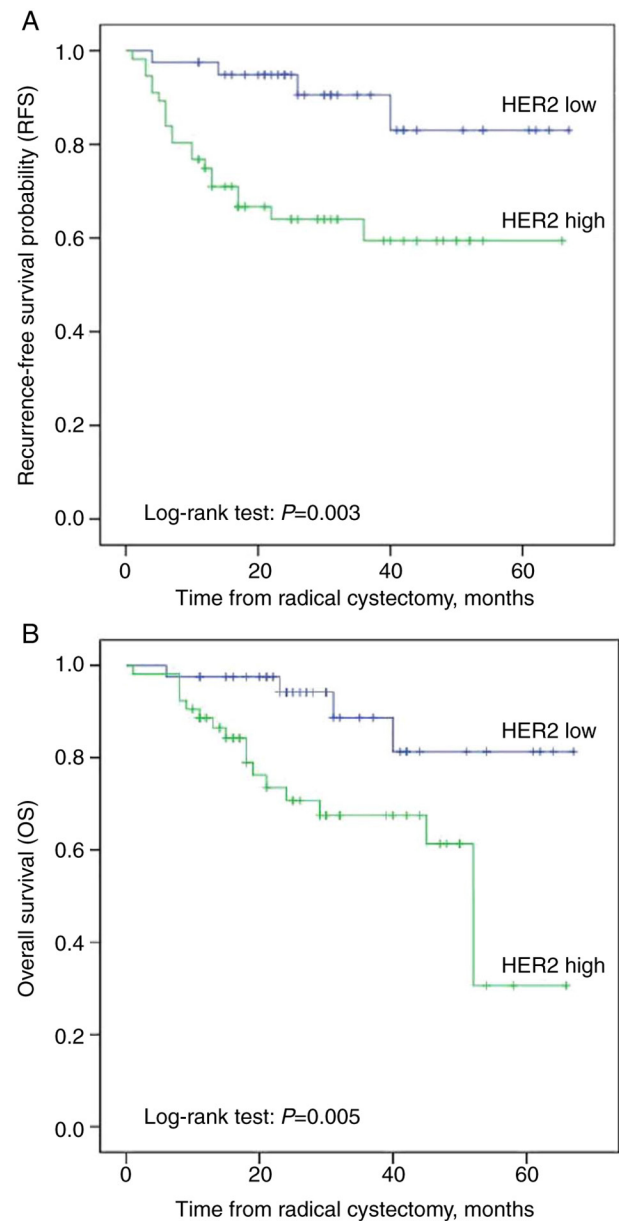


Figure 2. Kaplan-Meier plots of survival as regards HER2 expression. Patients with a high expression of HER2 (green curve) had a significantly higher risk of (A) mortality ($P = 0.003$) and (B) recurrence ($P = 0.005$) than those with a low expression of HER2 (blue curve). HER2, human epidermal growth factor receptor 2.

independent predictors of UCB recurrence. However, HER2 expression was not significantly associated with OS (HR, 3.03; 95% CI, 0.95-9.74; $P = 0.062$) (Table III).

Discussion

In the present study, it was found that HER2 was upregulated in 57.4% of patients with UCB, which is within the range of 27.8 to 85.2% reported previously (11). The wide range and discrepancies in the reported frequency of HER2 overexpression in UCB may be related to the combination of cases, varying definitions of 'positive', and the application of different evaluation techniques among studies (25). Although the present study did not perform fluorescence *in situ*

Table II. Univariate Cox regression model of pathological features for the prediction of RFS and OS in 108 patients with urothelial carcinoma of the bladder treated with radical cystectomy.

Variable	RFS		OS	
	HR (95% CI)	P-value	HR (95% CI)	P-value
Age	2.15 (0.92-5.04)	0.078	2.27 (0.93-5.50)	0.071
Sex	0.92 (0.27-3.09)	0.893	0.86 (0.25-2.93)	0.809
Tumor size	2.19 (0.96-5.01)	0.064	2.21 (0.94-5.19)	0.068
No. of tumors	0.77 (0.29-2.06)	0.602	0.58 (0.21-1.57)	0.281
G grade	1.89 (0.56-6.32)	0.304	6.86 (0.92-51.10)	0.060
T stage trend		<0.001 ^a		0.001 ^a
T2 vs. Ta, T1, Tis	0.94 (0.24-3.76)	0.929	1.27 (0.36-4.57)	0.711
T3 vs. Ta, T1, Tis	11.45 (3.59-36.51)	<0.001 ^a	7.88 (2.35-26.45)	0.001 ^a
T4 vs. Ta, T1, Tis	5.00 (1.33-18.76)	0.017 ^a	5.55 (1.36-22.68)	0.017 ^a
Lymph node metastasis	13.49 (5.04-36.12)	<0.001 ^a	13.76 (4.77-39.65)	<0.001 ^a
Lymphovascular invasion	2.70 (1.01-7.23)	0.049 ^a	3.30 (1.28-8.51)	0.013 ^a
Adjuvant chemotherapy	0.15 (0.02-1.13)	0.066	0.34 (0.09-1.21)	0.096
HER2 expression	4.37 (1.49-12.79)	0.007 ^a	4.12 (1.39-12.21)	0.011 ^a

^aDenotes statistically significant differences (P<0.05). RFS, recurrence-free survival; OS, overall survival; HR, hazard ratio; 95% CI, 95% confidence interval.

Table III. Multivariate Cox regression model of pathological features for prediction of RFS and OS in 108 patients with urothelial carcinoma of the bladder treated with radical cystectomy.

Variable	RFS		OS	
	HR (95% CI)	P-value	HR (95% CI)	P-value
HER2 expression	3.61 (1.07-12.18)	0.039 ^a	3.03 (0.95-9.74)	0.062
G grade	0.68 (0.18-2.60)	0.569	4.85 (0.56-41.66)	0.150
T stage trend		0.003 ^a		0.108
T2 vs. Ta, T1, Tis	0.74 (0.17-3.14)	0.683	1.54 (0.40-5.96)	0.533
T3 vs. Ta, T1, Tis	7.28 (1.83-29.00)	0.005	4.96 (1.08-22.82)	0.040
T4 vs. Ta, T1, Tis	4.20 (0.94-18.80)	0.062	5.24 (1.01-27.11)	0.048
Lymph node metastasis	2.91 (0.70-12.01)	0.140	2.13 (0.46-9.82)	0.332
Lymphovascular invasion	1.65 (0.40-6.84)	0.489	3.49 (0.97-12.51)	0.055
Adjuvant chemotherapy	0.09 (0.01-0.79)	0.029 ^a	0.13 (0.03-0.59)	0.008 ^a

^aDenotes statistically significant differences (P<0.05). RFS, recurrence-free survival; OS, overall survival; HR, hazard ratio; 95% CI, 95% confidence interval.

hybridization (FISH) to confirm the expression levels in the cases showing an IHC score of 2+, the main aim of the study was to assess the expression of HER2 in UBC as a preliminary analysis of an evaluation method for selecting potential ADC candidates and to compare the findings with similar reports on this topic, which have largely used IHC.

Based on the overexpression of HER2, two HER2-ADC drugs, T-DM1, and DS-8201a, were successively approved by the United States Food and Drug Administration (FDA) mainly for the treatment of advanced HER2-positive (IHC 3+, and/or FISH positive) breast cancer, and the efficacy of these

treatments was demonstrated in the pivotal trials, EMILIA, TH3RESA and DESTINY-Breast01 (26-28). In contrast to research on breast and gastric cancer, the current clinical trials for UCB mainly highlight the assessment of HER2 expression using IHC, rather than FISH, which is attributed to the particular pharmacological characteristics of HER2-ADC. A phase 1b trial (NCT03523572) combining DS-8201a with nivolumab for the treatment of advanced breast or urothelial cancer is currently open for enrollment, and the main inclusion criterion is patients exhibiting any degree of expression of HER2 based on IHC (scores of 1+, 2+, 3+) or FISH

positivity (5). Another novel ADC drug, RC48-ADC, has exhibited outstanding efficacy and survival benefits when used in the treatment of patients with locally advanced and metastatic urothelial carcinoma with HER2 overexpression (IHC 2+/3+) who had failed systematic chemotherapy (29). This drug was then given accelerated approval by the National Medical Products Administration of China and FDA in June, 2020 and is currently used in routine clinical applications.

The association between clinical outcomes and HER2 expression in UCB has been reported in several single-center studies; however, the overall result remains controversial. Recently, Gan *et al* (30) comprehensively analyzed 14 studies that included a total of 1,398 patients with BCa with regard to the HER2 status determined using IHC. They found that the overexpression of HER2 was strongly related to an advanced pathological stage, high tumor grade and tumor recurrence, but not to OS, disease-specific survival, or progression-free survival. By contrast, a previous meta-analysis indicated that HER2 expression was associated with a poor disease-specific and disease-free survival (11). Consistent with the results of the study by Gan *et al* (30), the present study also found that the overexpression of HER2 was significantly associated with an advanced tumor stage and a high grade. In addition, the Kaplan-Meier curves and Cox regression analysis suggested a significant association between HER2 overexpression and disease recurrence. Although HER2 overexpression was found to be associated with a higher risk of mortality, this effect was not statistically significant, which may be attributable to the insufficient sample size and the inclusion of patients with NMIBC, who generally have improved survival rates.

HER2 can be activated through hetero- or homodimerization. The formation of heterodimers and subsequent HER2 activation are temporally and spatially controlled in normal cells and tissues, but the associated pathway is dysregulated in cancer cells, where upregulated expression of HER2 or HER1 offers a growth advantage (31). HER2 regulates the expression of multiple genes, such as those related to proliferation, differentiation and angiogenesis, mainly through the PI3K/AKT and MAPK/ERK signaling pathways (31). HER2 has also been identified as a metastasis-promoting factor; HER family members have been reported to play an essential role in promoting the metastatic potential of tumors, owing to their ability to enhance the release of matrix metalloproteases (32). These results may explain why UCB with HER2 overexpression tends to show malignant phenotypic characteristics and is associated with a poorer prognosis.

The present study has several limitations. The first is the technical restrictions of IHC, including the lack of standardization, semi-quantitative output and subjective scoring system. The present study opted to use IHC, as it is the first-level technique for HER2 detection in clinical practice. Nevertheless, since UCB presents unique characteristics of HER2 expression, the evaluation of HER2 alterations at the DNA, RNA and protein expression levels may provide a more exhaustive analysis and new insight into the relevance of HER2 as a tumor driver and potential therapeutic target in UCB. The second limitation refers to the assessment of lymph nodes. Previous studies have confirmed a strong association between HER2 overexpression and lymph node metastasis, which has also been found to be stronger than

that of the matched primary tumors (17,33). The present study was not able to verify this association owing to the small number of cases involving lymph node metastasis and the limitations of the available specimens. Despite these limitations, it is considered that the findings of the present study add to the mounting evidence demonstrating worse clinical outcomes for patients with UCB exhibiting HER2 overexpression. Additionally, these findings may prove to be useful in identifying patients who are at an increased risk of disease recurrence and would likely benefit from HER2-targeted therapies.

In conclusion, the overexpression of HER2 is related to the pathological malignancy of UCB and may serve as an independent prognostic factor for recurrence in patients with UCB following RC. The present study provides a reference for the pre-treatment evaluation of HER2 as a therapeutic target for UCB; however, further prospective, large-scale, multi-detection studies are warranted to confirm these findings.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

XG, WH, HYi, ZW and XB designed the study. XB, HYi, XZ, HYu and XL performed the research and analyzed the data. XG, WH and XL wrote the manuscript. XZ, ZW and HYu revised the manuscript. XB and HYi confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

All procedures performed in the present study were in accordance with the ethical standards of the Ethics Committee of the Institutional Ethical Review Board of The First Affiliated Hospital of Chongqing Medical University (approval no. 2022-K21; Chongqing, China). Written informed consent forms were signed by each participant prior to sample collection.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

- Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A and Bray F: Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 71: 209-249, 2021.
- Stein JP, Lieskovsky G, Cote R, Groshen S, Feng AC, Boyd S, Skinner E, Bochner B, Thangathurai D, Mikhail M, *et al*: Radical cystectomy in the treatment of invasive bladder cancer: Long-term results in 1,054 patients. *J Clin Oncol* 19: 666-675, 2001.
- von der Maase H, Hansen SW, Roberts JT, Dogliotti L, Oliver T, Moore MJ, Bodrogi I, Albers P, Knuth A, Lippert CM, *et al*: Gemcitabine and cisplatin versus methotrexate, vinblastine, doxorubicin, and cisplatin in advanced or metastatic bladder cancer: Results of a large, randomized, multinational, multicenter, phase III study. *J Clin Oncol* 18: 3068-3077, 2000.
- Lenis AT, Lec PM, Chamie K and Mshs MD: Bladder cancer: A review. *JAMA* 324: 1980-1991, 2020.
- Sarfaty M and Rosenberg JE: Antibody-drug conjugates in urothelial carcinomas. *Curr Oncol Rep* 22: 13, 2020.
- Cancer Genome Atlas Research Network: Comprehensive molecular characterization of urothelial bladder carcinoma. *Nature* 507: 315-322, 2014.
- Kamoun A, de Reyniès A, Allory Y, Sjö Dahl G, Robertson AG, Seiler R, Hoadley KA, Groeneveld CS, Al-Ahmadie H, Choi W, *et al*: A consensus molecular classification of muscle-invasive bladder cancer. *Eur Urol* 77: 420-433, 2020.
- Yarden Y: Biology of HER2 and its importance in breast cancer. *Oncology* 61 (Suppl 2): 1-13, 2001.
- Bang YJ, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki A, Lordick F, Ohtsu A, Omuro Y, Satoh T, *et al*: Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): A phase 3, open-label, randomised controlled trial. *Lancet* 376: 687-697, 2010.
- Hindy JR, Souaid T, Kourie HR and Kattan J: Targeted therapies in urothelial bladder cancer: A disappointing past preceding a bright future? *Future Oncol* 15: 1505-1524, 2019.
- Zhao J, Xu W, Zhang Z, Song R, Zeng S, Sun Y and Xu C: Prognostic role of HER2 expression in bladder cancer: A systematic review and meta-analysis. *Int Urol Nephrol* 47: 87-94, 2015.
- Chen PC, Yu HJ, Chang YH and Pan CC: Her2 amplification distinguishes a subset of non-muscle-invasive bladder cancers with a high risk of progression. *J Clin Pathol* 66: 113-119, 2013.
- Kim D, Kim JM, Kim JS, Kim S and Kim KH: Differential expression and clinicopathological significance of HER2, indoleamine 2,3-dioxygenase and PD-L1 in urothelial carcinoma of the bladder. *J Clin Med* 9: 1265, 2020.
- Krüger S, Weitsch G, Büttner H, Matthiensen A, Böhmer T, Marquardt T, Sayk F, Feller AC and Böhle A: HER2 overexpression in muscle-invasive urothelial carcinoma of the bladder: Prognostic implications. *Int J Cancer* 102: 514-518, 2002.
- Abdelrahman AE, Rashed HE, Elkady E, Elsebai EA, El-Azomy A and Matar I: Fatty acid synthase, Her2/neu, and E2F1 as prognostic markers of progression in non-muscle invasive bladder cancer. *Ann Diagn Pathol* 39: 42-52, 2019.
- Kolla SB, Seth A, Singh MK, Gupta NP, Hemal AK, Dogra PN and Kumar R: Prognostic significance of Her2/neu overexpression in patients with muscle invasive urinary bladder cancer treated with radical cystectomy. *Int Urol Nephrol* 40: 321-327, 2008.
- Bolenz C, Shariat SF, Karakiewicz PI, Ashfaq R, Ho R, Sagalowsky AI and Lotan Y: Human epidermal growth factor receptor 2 expression status provides independent prognostic information in patients with urothelial carcinoma of the urinary bladder. *BJU Int* 106: 1216-1222, 2010.
- Jimenez RE, Hussain M, Bianco FJ Jr, Vaishampayan U, Tabazcka P, Sakr WA, Pontes JE, Wood DP Jr and Grignon DJ: Her-2/neu overexpression in muscle-invasive urothelial carcinoma of the bladder: Prognostic significance and comparative analysis in primary and metastatic tumors. *Clin Cancer Res* 7: 2440-2447, 2001.
- Kassouf W, Black PC, Tuziak T, Bondaruk J, Lee S, Brown GA, Adam L, Wei C, Baggerly K, Bar-Eli M, *et al*: Distinctive expression pattern of ErbB family receptors signifies an aggressive variant of bladder cancer. *J Urol* 179: 353-358, 2008.
- Soria F, Moschini M, Haitel A, Wirth GJ, Gust KM, Briganti A, Roupert M, Klatte T, Hassler MR, Karakiewicz PI and Shariat SF: The effect of HER2 status on oncological outcomes of patients with invasive bladder cancer. *Urol Oncol* 34: 533.e1-533.e10, 2016.
- Gandour-Edwards R, Lara PN Jr, Folkins AK, LaSalle JM, Beckett L, Li Y, Meyers FJ and De Vere-White R: Does HER2/neu expression provide prognostic information in patients with advanced urothelial carcinoma? *Cancer* 95: 1009-1015, 2002.
- Greene FL, Page DL, Fleming ID, Fritz AG, Balch CM, Haller DG and Morrow M: *AJCC cancer staging manual* (ed 6). New York, NY, Springer-Verlag, 2002.
- Mostofi FK, Sobin LH, Torloni H and World Health Organization (WHO): *Histological typing of urinary bladder tumours* / F. K. Mostofi, in collaboration with L. H. Sobin, H. Torloni and pathologists in fourteen countries. WHO, Geneva, 1973.
- Wolff AC, Hammond MEH, Allison KH, Harvey BE, Mangu PB, Bartlett JMS, Bilous M, Ellis IO, Fitzgibbons P, Hanna W, *et al*: Human epidermal growth factor receptor 2 testing in breast cancer: American society of clinical oncology/college of American pathologists clinical practice guideline focused update. *J Clin Oncol* 36: 2105-2122, 2018.
- Sanguedolce F, Russo D, Mancini V, Selvaggio O, Calò B, Carrieri G and Cormio L: Human epidermal growth factor receptor 2 in non-muscle invasive bladder cancer: Issues in assessment methods and its role as prognostic/predictive marker and putative therapeutic target: A comprehensive review. *Urol Int* 102: 249-261, 2019.
- Diéras V, Miles D, Verma S, Pegram M, Welslau M, Baselga J, Krop IE, Blackwell K, Hoersch S, Xu J, *et al*: Trastuzumab emtansine versus capecitabine plus lapatinib in patients with previously treated HER2-positive advanced breast cancer (EMILIA): A descriptive analysis of final overall survival results from a randomised, open-label, phase 3 trial. *Lancet Oncol* 18: 732-742, 2017.
- Krop IE, Kim SB, Martin AG, LoRusso PM, Ferrero JM, Badovinac-Crnjevic T, Hoersch S, Smitt M and Wildiers H: Trastuzumab emtansine versus treatment of physician's choice in patients with previously treated HER2-positive metastatic breast cancer (TH3RESA): Final overall survival results from a randomised open-label phase 3 trial. *Lancet Oncol* 18: 743-754, 2017.
- Modi S, Saura C, Yamashita T, Park YH, Kim SB, Tamura K, Andre F, Iwata H, Ito Y, Tsurutani J, *et al*: Trastuzumab deruxtecan in previously treated HER2-positive breast cancer. *N Engl J Med* 382: 610-621, 2020.
- Sheng X, Yan X, Wang L, Shi Y, Yao X, Luo H, Shi B, Liu J, He Z, Yu G, *et al*: Open-label, multicenter, phase II study of RC48-ADC, a HER2-targeting antibody-drug conjugate, in patients with locally advanced or metastatic urothelial carcinoma. *Clin Cancer Res* 27: 43-51, 2021.
- Gan K, Gao Y, Liu K, Xu B and Qin W: The clinical significance and prognostic value of HER2 expression in bladder cancer: A meta-analysis and a bioinformatic analysis. *Front Oncol* 11: 653491, 2021.
- Gutierrez C and Schiff R: HER2: biology, detection, and clinical implications. *Arch Pathol Lab Med* 135: 55-62, 2011.
- Ménard S, Pupa SM, Campiglio M and Tagliabue E: Biologic and therapeutic role of HER2 in cancer. *Oncogene* 22: 6570-6578, 2003.
- Fleischmann A, Rotzer D, Seiler R, Studer UE and Thalmann GN: Her2 amplification is significantly more frequent in lymph node metastases from urothelial bladder cancer than in the primary tumours. *Eur Urol* 60: 350-357, 2011.



This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.