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Assessment of the expression pattern of HER2 and its correlation with HER2-targeting antibody-drug conjugate therapy in urothelial cancer

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ABSTRACT

Background: Human epidermal growth factor receptor 2 (HER2) overexpression is related to anti-HER2 therapy in many tumors. RC48- antibody-drug conjugate (ADC) has shown promising efficacy in patients with HER2-positive locally advanced or metastatic urothelial carcinoma (UC). The characteristic expression and scoring systems of HER2 are nonexistent in UC. We aimed to explore HER2 status and its correlation with the efficacy of HER2-targeting ADC therapy in UC.

Methods: A total of 137 and 43 patients were enrolled in cohort 1 and cohort 2, respectively, from March 2009 to December 2018. The patients in cohort 2 were enrolled in a phase II study of RC48-ADC. UC samples were tested for HER2 status using immunohistochemistry (IHC) and/or fluorescence *in situ* hybridization (FISH). The 2018 ASCO/CAP HER2 scoring system was adopted and modified to score HER2 expression in UC.

Results: The HER2-positive (IHC 2+ or 3+) rate was 24.1% (33/137). In HER2 IHC 2+ or 3+ patients, the *HER2* gene amplification rate was 31% (13/42). The objective response rates (ORRs) in RC48-ADC-treated patients with IHC 3+, IHC 2+ and FISH+, IHC 2+ and FISH- were 58.8%, 66.7% and 40%, respectively. The ORR showed a trend toward a better benefit for RC48-ADC therapy in patients with *HER2* amplification than in those without

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amplification (61.5% vs. 44.8%, $P = 0.059$). The heterogeneity of HER2 expression in the primary tumor was 55.5% (15/27), and the ORR was not significantly different between patients with tumor heterogeneity and homogeneity.

Conclusions: IHC testing should be performed to assess the HER2 status before the initiation of HER2-ADC therapy. There was a trend toward a better benefit for patients with *HER2* amplification, and tumor heterogeneity did not influence the drug efficacy.

1. Introduction

Human epidermal growth factor receptor 2 (HER2) abnormalities, including protein overexpression, gene amplification and mutation, play an important role in the carcinogenesis and development of many cancers, including urothelial cancer. Gene amplification or protein overexpression of HER2 occurs in 15%–20% of breast cancer cases,[1] 10%–30% of gastric cancer cases, 20%–30% of ovarian cancer cases[2] and 8% of renal collecting duct carcinoma cases[3]; it is associated with tumor progression and poor prognosis. In bladder carcinoma, HER2 overexpression is correlated with more aggressive behavior and poor outcome, with an overexpression [immunohistochemistry (IHC) 3+] rate of 12.4% (59/475).[4] Anti-HER2 therapy represents an important opportunity for HER2-positive urothelial cancer.

Urothelial carcinoma is a common cancer worldwide, with approximately 80,000 estimated new cases and 17,100 estimated deaths per year.[5] Therefore, it is very important to test new drugs to improve the prognosis of locally advanced or metastatic urothelial carcinoma patients. The systemic treatment of urothelial carcinoma mainly includes neoadjuvant and adjuvant chemotherapy. Moreover, combination chemotherapy with immune checkpoint inhibitors has become a standard of care in the first-line setting for patients with metastatic urothelial carcinoma.[6] Compared with standard chemotherapy, some recent trials have demonstrated a survival benefit in PD-L1-positive locally advanced or metastatic urothelial carcinoma patients receiving immune checkpoint inhibitors.[7,8] HER-2, mTOR and DNA damage repair genes have been found to be frequently altered in urothelial carcinoma.[9] The anti-HER2 monoclonal antibody trastuzumab and the tyrosine kinase inhibitor lapatinib have been approved for the treatment of HER2-positive breast cancer, and the use of continued anti-HER2 therapy significantly improved the median overall survival in patients with advanced HER2-positive breast cancer.[10] Although there is no significant survival benefit from trastuzumab and lapatinib in patients with HER2-positive metastatic urothelial cancer,[11,12] the patients who received dual HER2 blockade had an objective response rate (ORR) of 18%.[13] T-DM1 is a HER2-targeting antibody-drug conjugate (ADC), a nonreducible thioether heterogeneous lysine conjugate of trastuzumab with an average drug approved not only for patients with HER2-positive metastatic breast cancer progressing after trastuzumab and taxane,[14] but also for patients with HER2-positive early breast cancer with residual invasive disease after the completion of neoadjuvant therapy.[15] T-DM1 allows the delivery of a potent cytotoxic agent specific to HER2-positive tumor cells, and a study in preclinical models of HER2-positive bladder cancer cell lines showed significant growth inhibition effects.[16] RC48-ADC is another novel humanized anti-HER2 antibody conjugate, and a recent study showed that RC48-ADC has promising efficacy with a manageable safety profile in locally advanced or metastatic urothelial carcinoma patients with HER2 overexpression.[17]

The efficacy of RC48-ADC has prompted studies on its clinical benefit in urothelial carcinoma, and effectively screening the potential benefits of HER2-ADCs has become a new challenge. Nevertheless, a HER2 scoring system for urothelial carcinoma has yet to be developed. The present study aimed to develop a validated HER2 scoring system for urothelial carcinoma to identify patients suitable for HER2-ADC treatment.

2. Materials and methods

2.1. Case selection

A total of 137 patients and 43 patients in two studies were enrolled in cohort 1 and cohort 2, respectively. The patients in cohort 1 were enrolled at the Department of Pathology, Cancer Hospital, Chinese Academy of Medical Sciences (CAMS) from March 2009 to December 2016, including 25 biopsy specimens and 112 surgical resection specimens. Specimens in this cohort were used to analyze the expression status of HER2 in urothelial carcinoma. Cohort 2 consisted of patients with HER2+ (IHC 3+ or 2+) locally advanced (27/43, 62.8%) or metastatic urothelial carcinoma (16/43, 37.2%) who progressed after systemic chemotherapy. Patients in this cohort were enrolled in an open-label, multicenter, phase II study of RC48-ADC conducted by our group from December 2017 to November 2018 (ClinicalTrials.gov identifier: NCT03507166) (Fig. 1).[17] Cohort 2 is a clinical validation cohort of cohort 1. All archived pathological slides were reviewed by two senior pathologists (J.Y. and H.L.) according to the 2016 World Health Organization (4th edition) classification of urinary tumors.[18] Tumor grade was included as one of the observed variables and was defined as low-grade or high-grade. The primary endpoints were the ORR and progression-free survival (PFS). The clinicopathologic characteristics of these urothelial carcinoma patients were obtained from their medical records.

2.2. IHC

Tissue samples were routinely fixed in 10% formalin for approximately 24 h at 10 times the volume of the tissue liquid and then embedded in paraffin. Consecutive 4-μm-thick sections and wax rolls were prepared for IHC staining. IHC testing was performed using an anti-HER2 4B5 antibody (Roche) on a Ventana Benchmark Ultra (Roche) according to the manufacturer's instructions, and staining was performed with the Ultra View Universal DAB Detection Kit (Roche).

2.3. *Fluorescence* in situ *hybridization*

Fluorescence *in situ* hybridization (FISH) was performed only in cohort 2. Gene amplification of *HER2* was evaluated by FISH. According to the manufacturer's instructions, FISH staining was performed using the PathVysion HER2 DNA Probe Kit (PathVysion, Abbott Molecular, Des Plaines, Illinois, USA). The kit contains LSI HER2 probes and chromosome enumeration probe 17 (*CEP17*). The 20 nuclear signals of *HER2* and *CEP17* in at least two different tumor areas were counted using a Zeiss AxioImager M2 epifluorescence microscope (Carl Zeiss, Oberkochen, Germany) equipped with a 100 × oil immersion objective and 4′,6′-diamidino-2-phenylindole (DAPI)/Spectrum Green/Orange single and triple bandpass filters.

2.4. Scoring of IHC and FISH

HER2 IHC scores and FISH status were assessed according to the 2018 American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines[19,20] with minor modifications (0, no staining or membrane staining that is incomplete and faint/barely

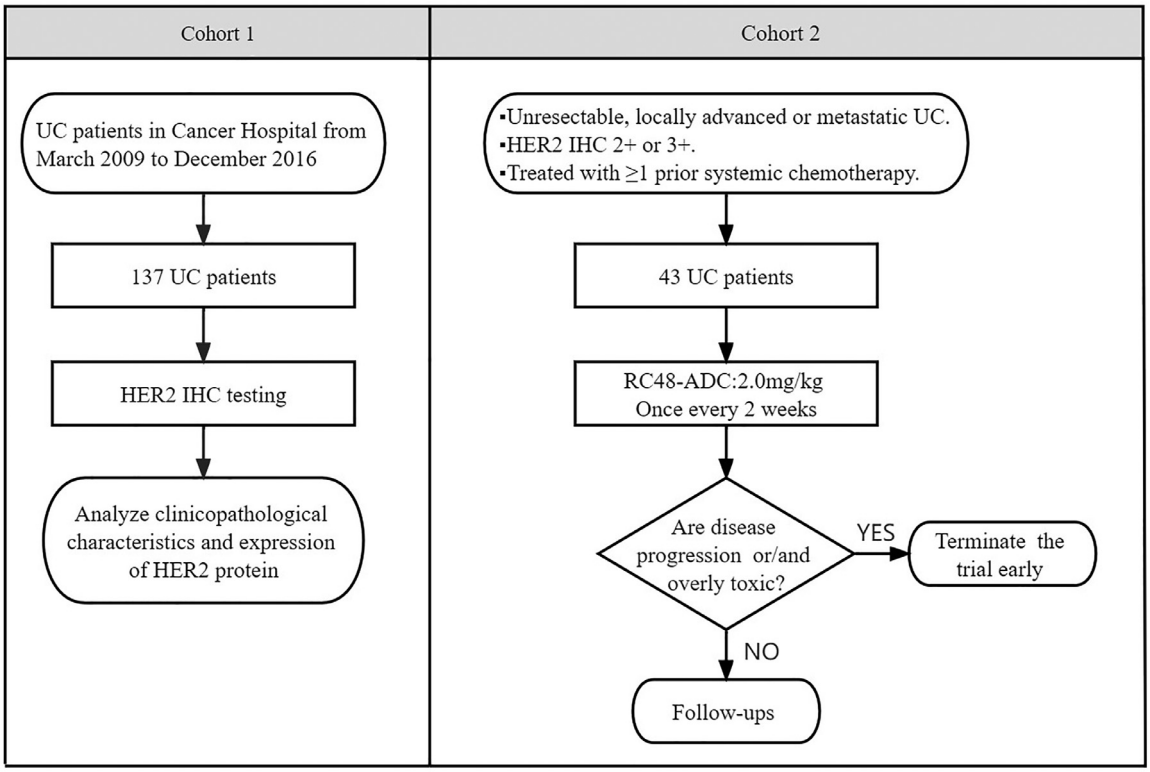

Fig. 1. Diagram of the cohort 2 study design. ADC, antibody-drug conjugates; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; UC, urothelial carcinoma.

perceptible and in < 10% of tumor cells; 1+, incomplete membrane staining that is faint/barely perceptible and in ≥ 10% of tumor cells; 2+, weak to moderate complete membrane staining observed in ≥ 10% of tumor cells or circumferential membrane staining that is complete, intense, and in < 10% of tumor cells; and 3+, circumferential membrane staining that is complete, intense, and in ≥ 10% of tumor cells). According to HER2 status, the samples were classified into two categories: HER2 heterogeneity and HER2 homogeneity. HER2 heterogeneity was defined as membranous staining 2+ and 3+ in 10%–90% of tumor cells in this study.[21]

2.5. Statistical analysis

The statistical analysis was carried out using SPSS version 24.0 software (IBM, New York, US). Frequencies of data were analyzed using Fisher's exact test. The Kaplan-Meier estimate was employed to calculate and plot survival curves. All tests were two-sided, and *P* values of < 0.05 were considered statistically significant.

3. Results

3.1. Patient characteristics

The clinicopathological characteristics of the patients in cohort 1 and cohort 2 are listed in Table 1 and Supplementary Table 1. The distribution of 137 HER2 IHC results was as follows: 75.9% (104/137) IHC 0 or 1+, 17.5% (24/137) IHC 2+, and 6.6% (9/137) IHC 3+. The expression of HER2 protein showed a tendency towards a male predominance ($P < 0.05$). According to the 2016 World Health Organization classification of the urinary system,[18] 15 (10.9%) and 122 (89.1%) patients had low-grade and high-grade invasive urothelial carcinoma, respectively. Clinical features were compared among the HER2 (3+), HER2 (2+) and HER2 (0 or 1+) groups. No significant differences were found in the majority of the parameters ($P > 0.05$) (Table 1).

3.2. HER2 heterogeneity

We observed HER2 protein expression heterogeneity in 27 primary tumors of cohort 2, of which 55.5% (15/27) had HER2 IHC score heterogeneity (Fig. 2). Among the 13 patients with HER2 FISH amplification, 5 patients (38.4%) had heterogeneous HER2 FISH status, and the remaining 8 patients (61.6%) had HER2 homogeneity.

3.3. Staining pattern of HER2 in urothelial carcinoma

We observed that most samples showed membrane staining (70%, 30/43), but some samples had both membranous and cytoplasmic expression of HER2 in urothelial carcinoma (30%, 13/43); the latter was confirmed by FISH to be positive (Fig. 3A and B). However, the case with only cytoplasmic staining was considered negative. We noticed that HER2 was negative (0) or low (1+) in the glandular differentiation region (Fig. 4A and B) and negative (0) in the squamous differentiation region (Fig. 4C and D). There was no significant difference between primary tumors and distant metastases tissues in HER2 protein expression.

3.4. Correlation between HER2 gene status and HER2 protein expression

In cohort 2, HER2 protein expression and *HER2* gene amplification status were determined by IHC and FISH in 27 primary tumors and 16 distant metastases (including lymph nodes, liver, bone, and lung). Except for one case in which FISH was unevaluable, all 42 appreciable cases were HER2 IHC 3+ or 2+. The *HER2* amplification rate was 31%. Among the cases with IHC 3+, 58.8% (10/17) had *HER2* amplification; among the cases with IHC 2+, 12% (3/25) had *HER2* amplification (Supplementary Table 2).

3.5. Correlation between HER2 and ORR

All cohort 2 patients ($n = 43$) participated in a phase II clinical trial of RC48-ADC. By the data cutoff date, one patient remained on treatment.

Table 1
Clinicopathological features of cohort 1 urothelial carcinomas.

Clinicopathological features	Total, No.	HER2 IHC, No.			c^2 value	*P* value
		0 or 1+	2+	3+		
Cohort size	137	104	24	9		
Sex					6.241	0.035
Male	108	77	23	8		
Female	29	27	1	1		
Age, years					1.267	0.536
< 65	70	55	12	3		
≥ 65	67	49	12	6		
Smoking					3.114	0.209
No	79	63	10	6		
Yes	58	41	14	3		
Pathologic grade					0.394	0.889
Low grade	15	11	3	1		
High grade	122	93	21	8		
Pathologic T stage					2.736	0.247
T1/T2	79	56	16	7		
T3/T4	58	48	8	2		
Pathological subtype					14.82	0.082
Pure conventional UC	108	82	19	7		
UC with squamous differentiation	10	10	0	0		
UC with glandular differentiation	11	7	3	1		
UC with squamous and glandular differentiation	4	4	0	0		
Mixed micropapillary carcinoma	3	1	2	0		
Plasmacytoid carcinoma	1	0	0	1		
Vessel cancer embolus					4.013	0.174
Present	26	19	3	4		
Absent	111	85	21	5		
Nerve invasion					2.566	0.138
Present	20	18	1	1		
Absent	117	86	23	8		

Abbreviations: HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; UC, urothelial carcinoma.

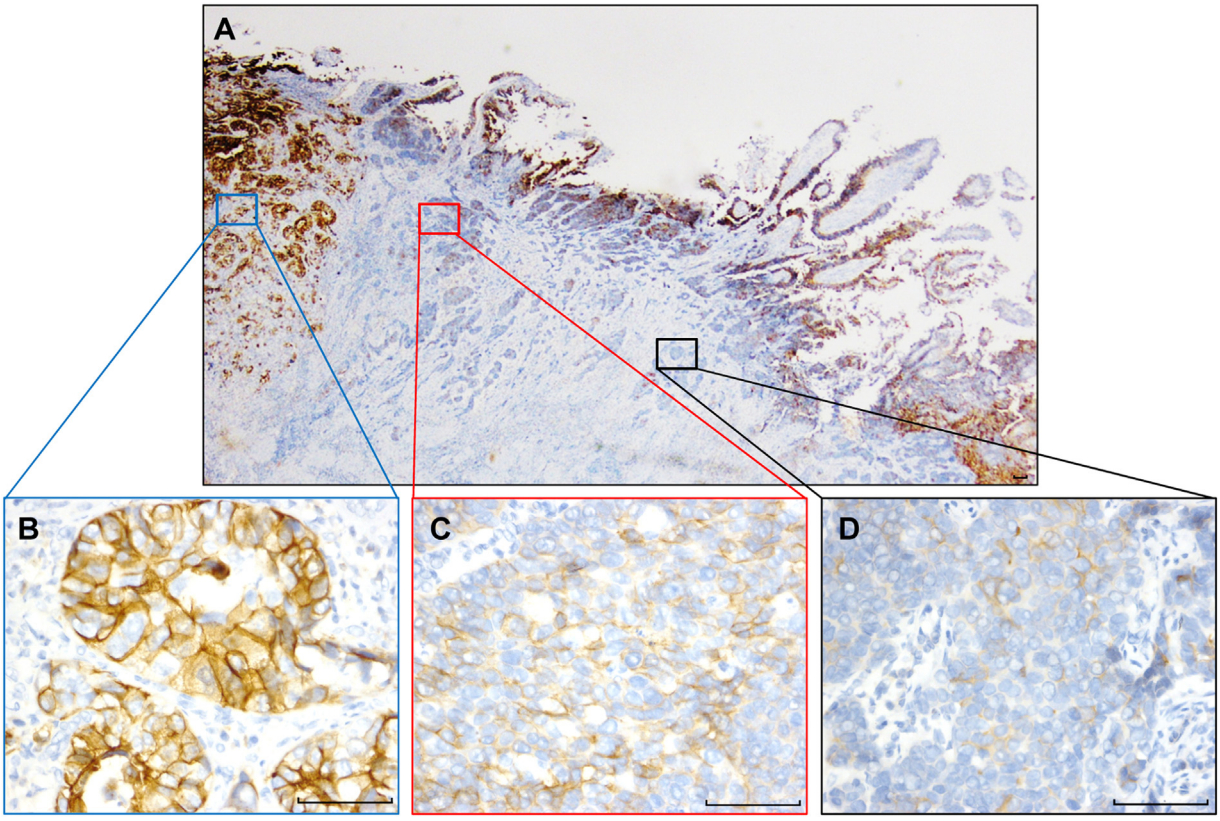

Fig. 2. HER2 protein heterogeneity. (A) One case of urothelial carcinoma (HER2 staining, original magnification 20 ×). (B) HER2 3+ (original magnification 400 ×). (C) HER2 2+ (original magnification 400 ×). (D) HER2 1+ (original magnification 400 ×). HER2, human epidermal growth factor receptor 2. Scale bar, 50 μm.

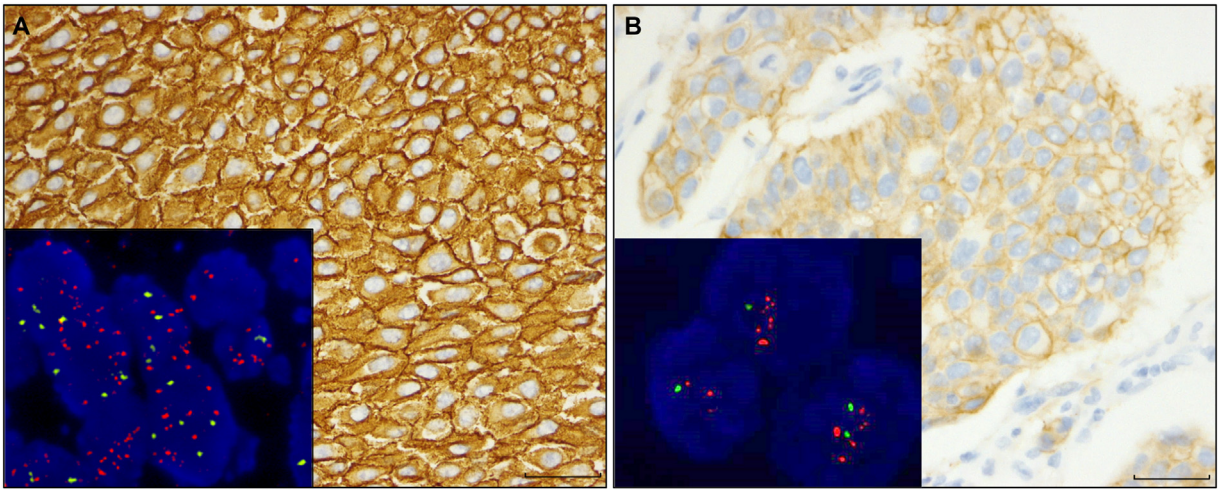

Fig. 3. Both membranous and cytoplasmic expression of HER2 in urothelial carcinoma. (A) HER2 IHC 3+ and FISH amplification (IHC original magnification 400 ×). (B) HER2 IHC 2+ and FISH amplification (IHC original magnification 400 ×). FISH, fluorescence *in situ* hybridization; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry. Scale bar, 50 μm.

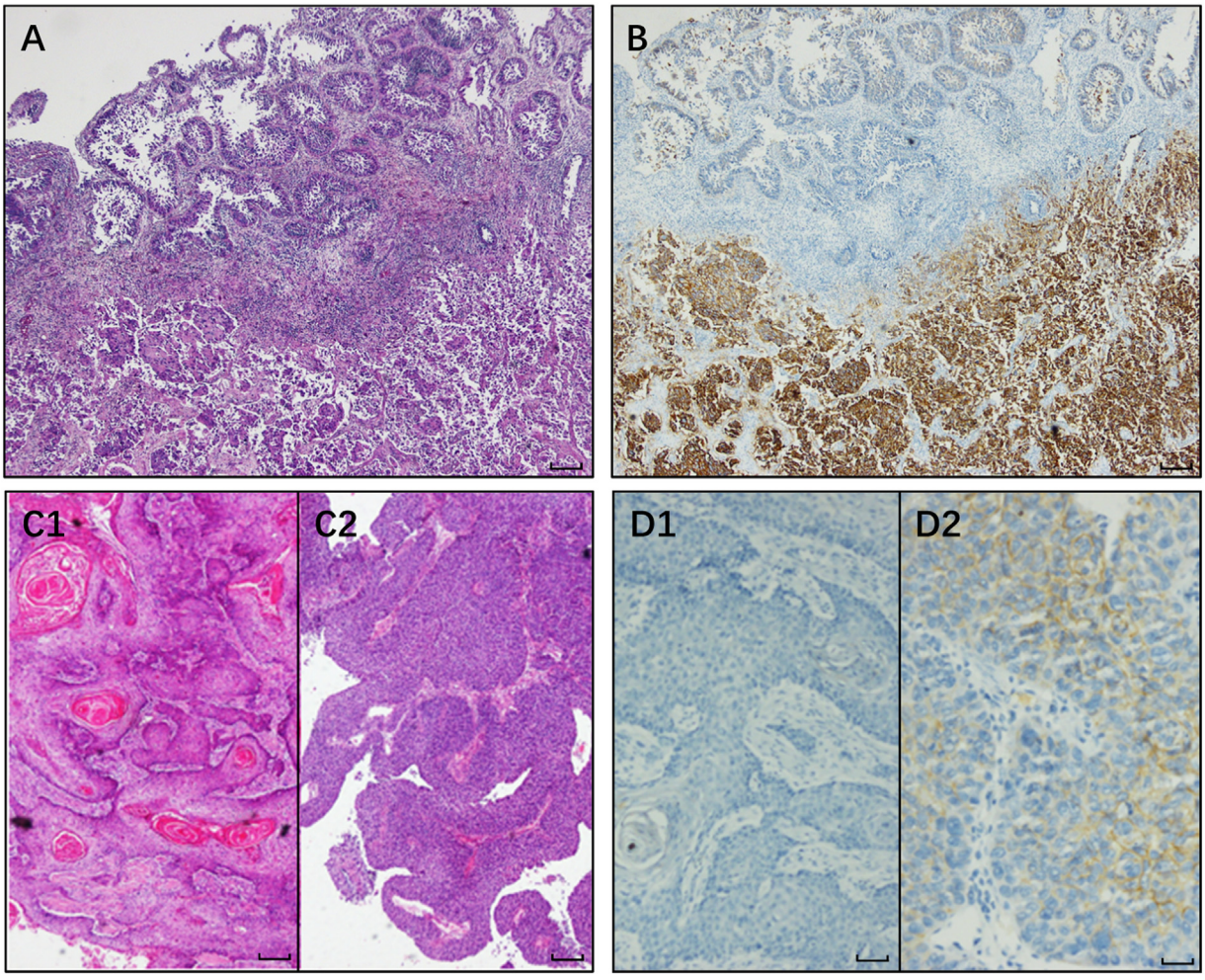

Fig. 4. HER2 expression in the glandular differentiation region and squamous differentiation region. (A) One case of UC with glandular differentiation (hematoxylin-eosin staining, original magnification 40 ×). (B) HER2 IHC 3+ in the conventional UC region and negative or low expression (1+) in the glandular differentiation region (original magnification 40 ×). (C) A tumor with a squamous differentiation region (C1) and a conventional UC region (C2) (hematoxylin-eosin staining, original magnification 40 ×). (D) The former case consisted of HER2 negativity (0) in the squamous differentiation region (D1) and HER2 IHC 2+ in the conventional UC region (D2) (original magnification 400 ×). HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; UC, urothelial carcinoma. Scale bar, 50 μm.

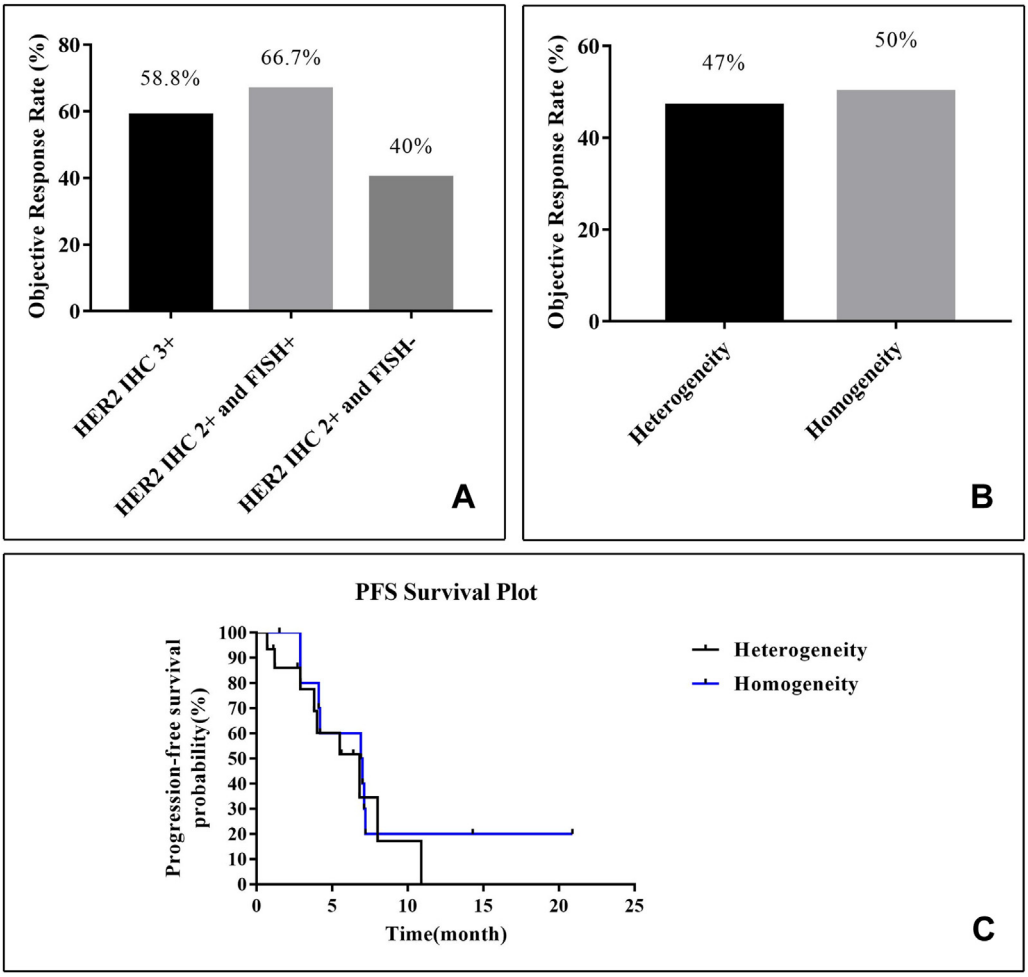

Fig. 5. (A) The correlation of the ORR and HER2 status in all patients (n = 42). (B) The correlation of the ORR and IHC heterogeneity in the primary tumor. (C) Kaplan-Meier estimates of PFS according to HER2 IHC heterogeneity and homogeneity. FISH, fluorescence *in situ* hybridization, HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ORR, objective response rate; PFS, progression-free survival.

Patients with HER2 IHC 3+ derived more benefits than those with HER2 IHC 2+. The ORRs of patients with IHC 3+, IHC 2+ and FISH+, and IHC 2+ and FISH- were 58.8%, 66.7% and 40%, respectively (Fig. 5A). For the ORR, there was a trend (P = 0.059) toward a better benefit from RC48-ADC therapy in patients with *HER2* amplification compared with those with nonamplification (61.5% vs. 44.8%). The location of HER2 protein staining does not affect the ORR of the patients. Moreover, there was no significant difference between primary tumors and distant metastases tissues in the ORR of the patients. The ORR and PFS were similar between patients with IHC heterogeneity and those with IHC homogeneity in the primary tumor (ORR, 47% vs. 50%; PFS, P = 0.48) (Fig. 5B and C). PFS was not significantly different between FISH-positive and FISH-negative patients and/or between IHC 2+ and IHC 3+ patients. We also noticed some special cases. First, patients with urothelial carcinoma with glandular differentiation (n = 1) and squamous differentiation (n = 1) had a shorter PFS (1.5 months and 1.2 months, respectively) than the median PFS (6.9 months). Second, patients with HER2 IHC3+ and FISH- (n = 3) had a shorter PFS than the median PFS. Third, patients with urothelial carcinoma mixed with micropapillary carcinoma (n = 1) had a shorter PFS (2.7 months) than the median PFS.

3.6. Specific histological types

Overexpression of HER2 (HER2 3+) was observed in a micropapillary urothelial carcinoma patient with partial response. In cohort 2, two patients with urothelial carcinoma with squamous differentiation were IHC 2+ and FISH-, in which all areas of squamous differentiation were HER2 negative. According to the follow-up, one patient experienced progression, and the other patient remained on treatment.

4. Discussion

HER2 overexpression is strongly associated with a poor prognosis in urothelial cancer.[22,23] As HER2-targeting ADCs demonstrate efficacy and safety with curative effects in HER2-positive urothelial carcinoma patients, the standard detection for HER2 status has become more important. The result might change when the 2013 and 2018 HER2 reporting guidelines are applied in the same patient.[24] However, there is no standard for HER2 evaluation in urothelial carcinoma.

This study showed that the updated 2018 HER2 testing scoring system in breast cancer with minor modifications could be applied to urothelial carcinoma to accurately determine the HER2 status.

First, we noticed that some samples had both membranous and cytoplasmic expression of HER2 in urothelial carcinoma, which was further confirmed by FISH to be HER2 positive. In addition, unlike gastric cancer and colorectal cancer, in which HER2 staining is incomplete, membranous and preferentially basolateral (U shaped),[25,26] complete membranous staining is required for positivity in urothelial carcinoma.

Second, the effectiveness of HER2-ADC was shown in both IHC 2+ and 3+ patients. In patients with HER2 IHC 2+, 88% (22/25) did not

show amplification. The ORRs of the IHC 2+ and FISH-negative groups were slightly lower than those of the IHC 3+ group and the IHC 2+ and FISH-positive group. The *HER2* amplification rate of HER2 IHC 2+ cases was similar to that in the study of Anissa Moktefi et al.,[27] but lower than that in breast cancer, gastric cancer and colorectal cancer.[25,26,28] *HER2* amplification analysis by FISH can accurately and promptly identify breast cancer patients who might benefit from anti-HER2 therapy. In urothelial cancer, the ORR had a trend toward a better benefit from RC48-ADC therapy in patients with *HER2* amplification compared with those with nonamplification (61.5% vs. 44.8%, $P = 0.059$). This result suggested that FISH detection could not completely guide the clinical application of RC48-ADC in urothelial carcinoma, but patients with *HER2* amplification seemed to derive more of a benefit. Considering that HER2 IHC 2+ patients have a low FISH amplification rate and that the ORR was not significantly different between the amplification and nonamplification groups, we recommend that patients with locally advanced or metastatic urothelial carcinoma should undergo IHC testing to assess HER2 status before the initiation of HER2-ADC therapy. The significance of detecting *HER2* gene amplification remains to be further discussed.

Latif et al.[29] showed that for patients with invasive urothelial bladder cancer, the *HER2* gene amplification rate was much lower than the protein overexpression rate, which suggested that *HER2* gene amplification is uncommon. Other molecular mechanisms may account for the high rate of protein overexpression. *HER2* gene mutations exist in many cancers and play an important role in carcinogenesis and development. The rate of *HER2* gene mutation is 12% in urothelial carcinoma,[30] and *HER2* gene mutation is more frequent in urothelial carcinoma than in any other common cancer.[31] In urothelial carcinoma, S310F and S310Y mutations were the most commonly observed mutations (183/3,753, 4.8%). *HER2* exon 20 insertion occurred in 0.53% of cases (20/3,753).[32] However, the correlation between *HER2* gene mutation and the drug effect of anti-HER2 therapy in urothelial carcinoma is still unclear.

In addition, heterogeneity is associated with unfavorable clinicopathologic factors and a poor prognosis. In our study, the FISH amplification rate was 12% (3/25) in IHC 2+ patients, and of the three IHC 2+ and FISH+ patients, two had HER2 heterogeneity. The heterogeneity of HER2 protein expression in urothelial carcinoma was 55.5%, which was similar to that in gastric cancer (69.4%)[33] but much higher than that in breast cancer (18%).[34] The breast cancer scoring system refers to 10% as the cutoff in FISH, but our study showed no significant ORR difference when HER2 FISH heterogeneity cutoffs of 5% and 10% were used ($P = 0.77$). A similar result was observed in the IHC methodology; patients with small cohesive HER2+ clones can also benefit from RC48-ADC. This may be because RC48-ADC has a bystander effect and is beneficial in treating tumors with HER2 heterogeneity. This result suggests that HER2-directed ADCs can be used for patients with HER2 heterogeneity.

According to the literature on HER2 overexpression rates in urothelial carcinoma, the median HER2 positivity rate of 7 IHC studies was 34% (range 11%–56%, Supplementary Table 3). The median HER2 positivity rate of 6 FISH studies was 21.5% (range 10%–30%, Supplementary Table 4). The HER2-positive rate was similar to that in our study (24.1%). In patients with HER2 IHC 3+ or 2+, the *HER2* amplification rate was 31%, similar to that in the literature.[27,35] Several studies have shown that HER2 protein expression and gene alterations could be related to the histological type in urothelial carcinoma. A large cohort study that analyzed urothelial carcinoma mixed with micropapillary carcinoma reported that in addition to genetic alterations shared with conventional urothelial carcinoma, alterations in the *HER2* gene occurred at higher frequencies.[35] Bohyun Kim et al.[36] showed that plasmacytoid urothelial carcinoma has high HER2 overexpression (83%) and amplification (50%). In this study, we observed that two patients with urothelial carcinoma with squamous differentiation were IHC 2+ and FISH-, but all areas of squamous differentiation were HER2 negative. Michael Rose et al.[37] found that there are some genetic alterations in the ERBB signaling pathway in squamous bladder cancer, and we speculated that our finding may be related to this phenomenon. Gang Li et al.[38] demonstrated that patients with squamous differentiation of urothelial carcinoma had lower survival rates than those with pure conventional urothelial carcinoma. With increasingly intensive research on HER2, HER2-low expression and HER2 antibody-drug conjugates have become the focus of research. Therefore, higher requirements are needed for the accuracy of HER2 testing and evaluation. Cancer tissue obtained via surgical resection or biopsy is the main method for detecting HER2 expression. Some studies have attempted to detect HER2 expression in circulating tumor cells.[39] Based on this, we can reasonably speculate that precision detection and evaluation may become future research hotspots.

Of course, there are still several limitations in our study. First, we did not explore the efficacy of RC48-ADC in patients with IHC 0–1+ to reversely evaluate the feasibility of HER2 testing for screening potential beneficiaries of HER2-targeted therapy. Second, the relationship between *HER2* gene amplification or mutations and the potential benefit of HER2-ADCs in urothelial carcinoma still needs more thorough research. Third, due to our strict inclusion criteria for the phase II study, the number of enrolled patients was small, and future studies with a large sample size are needed to verify these results.

5. Conclusions

In conclusion, we suggest that the ASCO/CAP guidelines currently used in breast cancer could be widely applied to urothelial carcinoma with minor modifications. Patients with *HER2* amplification seemed to derive more of a benefit. The ORR was similar between patients with tumor heterogeneity and other patients. As the HER2-ADC showed efficacy in both IHC 2+ and 3+ patients, the significance of *HER2* gene amplification in guiding the use of HER2-ADCs still needs more investigation in evidence-based medicine.

Declaration of competing interest

Dr. Fang is a shareholder of RemeGen Co., Ltd. All of the authors have disclosed that they have not received any financial consideration from any person or organization to support the preparation, analysis, results, or discussion of this article.

Ethics statement

This study was conducted in compliance with the principles of the Declaration of Helsinki. Cohort 1 was approved by the Institutional Review Board of the Cancer Hospital, CAMS, and informed consent was not required (approval number: NCC201711018). In cohort 2, all patients provided written informed consent for treatment and the study has been approved by the institutional review board or ethics committee of each study center (ClinicalTrials.gov identifier: NCT03507166).

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Author contributions

H.L., Y.L., X.Y., L.W., Y.S., X.Y., H.L., B.S., J.L., Z.H., G.Y., Y.H., C.H., Z.C., C.C., L.S. and J.F. designed this study. H.L., Y.L., W.Y., L.W., Y.S., X.Y., H.L., B.S., J.L., Z.H., G.Y., W.H., C.H., Z.C., C.C., L.S. and J.F. conducted the methodology. A.Z., X.S., J.G. and J.Y. performed the investigation. H.L. and Y.L. conducted the visualization. A.Z., X.S., J.G. and J.Y. supervised this research. H.L., Y.L. and P.Y. wrote the original draft. H.L., Y.L., A.Z. and J.Y. revised and edited this manuscript.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jncc.2023.02.003.

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