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The effectiveness and safety of RC48 alone or in combination with PD-1 inhibitors for locally advanced or metastatic urothelial carcinoma: a multicenter, real-world study

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Abstract

Background RC48 is an antibody-drug conjugate (ADC) specifically targeting HER2. Phase II and III clinical trials have proven its significant anti-tumor effect against locally advanced or metastatic urothelial carcinoma (la/mUC). This study aims to further assess the effectiveness and safety of RC48 for patients with la/mUC and provide insights for further clinical practice.

Methods Retrospective analysis for 42 patients with la/mUC who underwent RC48 alone or in combination with PD-1 inhibitors therapy between 18 October 2022 and 1 May 2024 were conducted to assess effectiveness and safety of RC48. Descriptive statistics were used to summarize baseline characteristics, treatment-related adverse events, etc. Cox proportional risk model and the Kaplan-Meier method were applied to analyze patients' survival.

Results We observed a median progression-free survival (mPFS) of 6.2 months, although median overall survival (mOS) has not been reached so far. An objective response rate (ORR) of 54.8% and a disease control rate (DCR) of 83.3% was also observed. Patients with first-line therapy, second- or later-line therapy and neoadjuvant therapy were observed disease remission with ORRs of 47.7%, 40.0% and 100.0%, respectively. The most common treatment-related adverse events (TRAEs) include hypoesthesia and elevated transaminases which affect over 90.0% of patients and mostly grade 1–2 in severity, and no treatment-related fatalities were found.

Conclusions This multicenter, real-world study confirms that RC48 alone or in combination with PD-1 inhibitors exerted a promising effectiveness and manageable safety for first-line, second- and post-line, and neoadjuvant therapy with la/mUC.

Keywords Antibody-drug conjugates, HER2, RC48, Real-world study, Urothelial carcinoma

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Introduction

Urothelial carcinoma (UC) is the ninth most common malignant tumor globally and there is a pressing need for the continual advancement and implementation of effective therapeutic strategies in clinical practice [1]. As a conventional therapy for locally advanced or metastatic UC (la/mUC), platinum-based chemotherapy is now largely limited by its severe hematological toxicity and systemic adverse effects, though the objective response rate (ORR) achieved 44.6% [2, 3]. Immune checkpoint inhibitors (ICIs) are recommended as a first- or second-line therapy for patients with UC who are intolerant to chemotherapy. However, their ORR remains relatively modest, at 20–30%. This is insufficient to provide survival benefits and lacks stable predictive biomarkers of effectiveness [4–7].

Antibody-drug conjugates (ADCs) represent a class of targeted anti-tumor drugs that possess both the high specificity of monoclonal antibodies and the potent cytotoxic activity of drugs. Enfortumab Vedotin (EV) and Sacituzumab Govitecan (SG), which respectively target Nectin-4 and Trop-2, are notable ADCs that have been approved for la/mUC. Nevertheless, at the time of writing, neither of them is available in China [8, 9]. Human epidermal growth factor receptor-2 (HER2) belongs to the family of EGFR tyrosine kinase receptors [10]. The overexpression of HER2 is frequently observed in UC and is strongly associated with tumor progression and adverse prognosis [11].

RC48 (Disitamab Vedotin), a novel humanized anti-HER2 ADC, has demonstrated promising effectiveness in clinical trials for HER2-positive la/mUC. Phase II clinical trials, RC48-C005 and RC48-C009, assessed the effectiveness and safety of RC48 monotherapy in patients who had previously received at least one line of systemic chemotherapy. Results revealed an ORR of 50.5%, a median progression-free survival (mPFS) of 5.9 months, and a median overall survival (mOS) of 14.2 months [12]. The recent clinical trial RC48-C014 investigated the effectiveness and safety of combining RC48 with ICIs in la/mUC patients. Following an eight-week course of treatment, a marked remission rate of 88.2% was observed, accompanied by an ORR of 71.8%, a disease control rate (DCR) of 92.3%, and a mPFS of 9.2 months [13, 14].

While clinical trials are conducted under strictly controlled conditions to establish drug effectiveness to a certain extent, their stringent inclusion and exclusion criteria can still introduce selection bias, thereby constraining the generalizability of their findings [15, 16]. Therefore, our study included patients with la/mUC who were receiving RC48 as either a first- or second-line therapy, as well as those with urothelial carcinoma of bladder (UCB) who were receiving RC48 as neoadjuvant therapy in real-world clinical settings. Our study is designed to further validate the effectiveness and safety of RC48 for urothelial carcinoma in real-world practice.

Materials and methods

Study design and patients

This retrospective, multicenter, real-world study included 42 patients with UC received RC48 therapy at the Affiliated Hospital of Qingdao University, Qingdao Central Hospital and Qingdao Hiser Hospital. The follow-up period spanned from October 18, 2022, when the initial patient commenced medication, until May 1, 2024. The median follow-up duration for patients was 6.2 months (range: 4.1 to 12.1 months). Inclusion criteria prior to first treatment were as follows: (1) Aged 18–85; (2) Primary UC confirmed by histopathology; (3) Have received at least one RC48 treatment; (4) Available response assessments; (5) Previous surgery, chemotherapy, or immunotherapy were allowed; (6) Patients with good cardiac, hepatic, and renal function, including transaminase, cardiac troponin, NT-proBNP and creatinine, and were assessed to be able to support treatment prior to the first dose of medication. RC48 therapy was administered at a dose of 2.0 mg/kg every 2 weeks (Q2W) in accordance with the protocol of the RC48-C014 study. ICIs therapy was administered at a dosage of 3.0 mg/kg Toripalimab and Tislelizumab. The research program was approved by the Ethics Committee of the Affiliated Hospital of Qingdao University, and all patients provided informed consent prior to undergoing treatment.

Data collection and effectiveness evaluation

Data statistics included Baseline patient characteristics, Personal history, Imaging features, Primary lesion, Tumor characteristics, Site where tumor metastasis was first found, TNM stage, Prior therapy, Drug Using, HER2 expression, PD-L1 expression, Stage of drug using, Disease progression, Treatment-related adverse events, etc. Patients with la/mUC were treated with RC48 alone or RC48 in combination with PD-1 inhibitors until disease progression or treatment-related adverse events (TRAEs) that were intolerable and severely affected survival. Patients on neoadjuvant therapy undergo radical resection after a 12-week (6-cycle) course of RC48 combined with PD-1. The expression of HER2 is mainly detected by immunohistochemistry (IHC). HER2 IHC 3 + was considered HER2 positive. HER2 IHC 2 + specimens were further subjected to FISH to detect the amplification status; FISH positive specimens were considered positive for HER2 expression, and FISH negative specimens were considered negative for HER2 expression. HER2 IHC 1 + and 0 were considered HER2 negative. The HER2 score was developed according to the 2018 American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAPs) Breast Cancer Guidelines [17].

PD-L1 expression in tumor samples was assessed by the Ventana PD-L1 Assay (SP263, Ventana Medical Systems, Inc.). Patients were classified as PD-L1-positive if they met at least one of the following three criteria: At least 25% of tumor cells are stained for PD-L1; or at least 25% of immune cells are stained for PD-L1 (if more than 1% of immune cells are present in the tumor area); or 100% of immune cells are stained for PD-L1 (if no more than 1% of immune cells are present in the tumor area) [18].

An objective response was defined as a response lasting at least two consecutive imaging assessments separated by at least 4 weeks. Imaging included CT or CT-enhanced scans of primary and metastatic sites, magnetic resonance imaging (MRI) and bone scans. Imaging was performed before starting treatment and every 6–8 weeks during treatment. Routine assessment of blood counts, liver and kidney function, electrolytes, blood glucose, thyroid and adrenal function, cardiac function, and coagulation were performed before each treatment. The ORR, Complete response (CR), Partial response (PR), Stable disease (SD) and Progressive disease (PD) were evaluated by regular imaging. Both lesion selection and effectiveness evaluation were performed according to RECIST v1.1 criteria [19]. The DCR was calculated as the proportion of patients achieving a CR, PR, or SD. The ORR was calculated as the proportion of patients achieving a CR or a PR. The adverse events (AEs) were graded according to the Common Terminology Criteria for Adverse Events version 5.0.

Statistical analysis

SPSS 25.0 (IBM, Armonk, NY, USA), Prism 10.1 (GraphPad Software Inc., San Diego, CA, USA), and R 4.3.2 (The R Project for Statistical Computing, www.r-project. org) were used for statistical analysis. The population consisted of all patients receiving RC48 treatment in this study. Descriptive statistical methods were used to summarize patient characteristics, treatment-related adverse events and drug use. Kaplan-Meier method and Cox proportional risk model were used for PFS and OS analysis. Bilateral $P < 0.05$ was considered statistically significant.

Results

This multicenter retrospective study included patients with UC from the Affiliated Hospital of Qingdao University ($n = 37$), Qingdao Central Hospital ($n = 2$), and Qingdao Hiser Hospital ($n = 3$) who had been or were undergoing RC48 treatment. The patient cohort is aged between 50 and 85, with 27 patients falling within the ≥65 age bracket. Of the 42 patients, 17 patients (40.5%) received RC48 alone and 25 patients (59.5%) received RC48 in combination with PD-1 inhibitors, including Toripalimab ($n = 17$) and Tislelizumab ($n = 7$). 19 patients with la/mUC received RC48 as first-line therapy. 15 patients with la/mUC received RC48 as second- or later-line therapy. 8 patients received RC48 as neoadjuvant therapy. All 42 patients underwent at least one treatment response assessment, the overall treatment of the patients treated with RC48 was as follows. 4 patients achieved CR, 19 achieved PR, and 13 achieved SD, with an overall ORR of 54.8% and a DCR of 83.3%. The ORR was 47.1% (8/17) in patients receiving RC48 alone and 60.0% (15/25) in patients receiving RC48 combined with PD-1 inhibitors. The ORR for HER2 3+, 2+, and 1 + patients were 75.0% (6/8), 52.4% (11/21), and 46.2% (6/13), respectively. We performed FISH testing on 20 patients with HER2 IHC 2+, among whom 4 cases (20.0%) were FISH positive, while 16 cases (80.0%) were FISH negative. The demographic and baseline characteristics of these patients are detailed in Table 1.

In the cohort of patients receiving first-line therapy, 9 patients achieved PR, and 8 patients achieved SD, resulting in an ORR of 47.4% and a DCR of 89.5%. Specifically, among those treated with RC48 alone, 45.4% (5/11) achieved PR, while patients receiving RC48 combined with PD-1 inhibitors achieved a slightly higher ORR of 50.0% (4/8). Stratifying by HER2 status, patients with HER2 3+, 2+, and 1 + had ORRs of 66.7% (2/3), 42.9% (3/7), and 44.4% (4/9), respectively. The mPFS in patients receiving first-line therapy was 6.1 months. RC48 monotherapy demonstrated a mPFS of 5.0 months, while combination therapy with RC48 and PD-1 inhibitors showed a longer mPFS of 6.2 months (HR = 0.75, 95% CI 4.40–7.35, $P = 0.01$). 7 patients treated with Toripalimab had an ORR of 42.0% and achieved an mPFS of 6.1 months; 1 patient achieved PR after treatment with Tislelizumab. Patients with HER2 2 + FISH + and HER2 3 + had mPFS of 5.5 mouths, whereas those with HER2 1 + and HER2 2 + FISH- had a similar mPFS of 5.0 months ($P = 0.43$). Apart from this, all patients tested for PD-L1 in this cohort had negative results, with a corresponding mPFS of 6.2 months. (Fig. 1)

Of the patients who received second- or later-line therapy, 6 patients achieved PR, and 4 patients achieved SD, with an ORR of 40.0% and a DCR of 66.7% in this cohort. Among these patients, the ORR for those receiving RC48 alone and combination therapy with ICIs was 33.3% (2/6) and 44.4% (4/9), respectively. The ORR was 66.7% (2/3) for HER2 3+, 28.6% (2/7) for HER2 2+, and 50.0% (2/4) for HER2 1+. The mPFS for patients undergoing second- or later-line therapy was 5.5 months. Specifically, patients treated with RC48 monotherapy demonstrated a mPFS of 6.6 months, which was higher compared to those receiving RC48 combined with PD-1 inhibitors (HR = 0.79, 95% CI 5.55–8.65, $P = 0.80$). The ORR for the 5 patients treated with Toripalimab was 40.0%, with an mPFS of 5.7 months; the ORR for the 4 patients treated with Tislelizumab was

Table 1 Characteristics of the patients

Characteristics	Values(%)
Male	36 (85.7)
Female	6 (14.3)
Age	
<65	15 (35.7)
≥65	27 (64.3)
Smoking	
Yes	14 (33.3)
No	28 (66.7)
ECOG	
0	30 (71.4)
1	12 (28.6)
Primary site	
Bladder	26 (61.9)
Renal Pelvis and Ureter	16(38.1)
Metastatic site	
Non-occurrence	3 (7.1)
Lung	8 (19.0)
Liver	4 (9.5)
Bone	5 (11.9)
Lymph node	14 (33.3)
Adjacent tissues and organs	13 (31.0)
Prior surgery	
Yes	35 (83.3)
Radical	26
Local	9
No	7 (16.7)
Prior chemotherapy or immunotherapy	
Yes	15 (35.7)
No	27 (64.3)
HER2	
IHC 0	1 (2.4)
IHC 1+	13 (31.0)
IHC 2 + FISH-	16 (38.1)
IHC 2 + FISH+	4 (9.5)
IHC 3+	8 (19.0)
PD-L1	
Positive	13 (40.6)
Negative	19 (59.4)
HER2&PD-L1	
HER2 IHC (2+/3+), PD-L1(+)	11 (26.2)
HER2 IHC (2+/3+), PD-L1(-)	11 (26.2)
HER2 IHC (1+), PD-L1(+)	2 (4.8)
HER2 IHC (1+), PD-L1(-)	8 (19.0)
RC48 treatment lines	
Neoadjuvant therapy	8 (19.0)
First-line therapy	19 (45.2)
Second- or later-line therapy	15 (35.7)

ECOG Eastern Cooperative Oncology Group, IHC immunohistochemistry, PD-L1 Programmed cell death 1 ligand 1

25.0%, with an mPFS of only 3.3 months. Patients with HER2 2 + FISH + and HER2 3 + had mPFS of 8.0 mouths, whereas those with HER2 1 + and HER2 2 + FISH- had a similar mPFS of 5.5 months ($P = 0.21$). Among patients who underwent PD-L1 testing, those with positive results had a mPFS of 6.8 months, whereas those with negative results had a mPFS of 5.3 months. (Fig. 1)

All the patients with neoadjuvant therapy received RC48 in combination with PD-1 inhibitors. 6 patients were treated with Toripalimab and 2 patients with Tislelizumab. Among them, 4 patients achieved CR, and 4 patients achieved PR, resulting in an ORR of 100%. All patients in this cohort exhibited HER2 expression levels of 2 + or 3+. Among the patients who achieved CR, 3 patients showed no residual tumor components in the radical cystectomy specimen, achieving pathological complete response (pCR), while 1 patient demonstrated complete tumor disappearance on imaging, achieving radiographic complete response (rCR). The swimming plot for all patients was shown in Fig. 2. And the ORR and mPFS of all cohorts were shown in Table 2 [20].

Treatment-related adverse events (TRAEs) occurred in more than 90% of patients, mostly grading 1 or 2. (Table 3) Common TRAEs included hypoesthesia (34.3%), anemia (8.6%), pruritus (14.3%), urinary tract infection (8.6%), elevated transaminases (22.9%), nausea (11.4%), leukopenia (5.7%), anorexia (17.1%), hyperlipidemia (8.6%), alopecia (8.6%), rash (14.3%), fatigue (11.4%) and thrombocytopenia (5.7%). During long-term follow-up, most of the patients suffered from hypoesthesia after medication, mainly manifested as decreased sensation in the limbs. 3 patients had difficulty walking and standing due to severe hypoesthesia (Grade 3–4), and eventually discontinued their intravenous therapy. Usually, we reduce the occurrence of adverse events by using glucocorticoid prophylactically. Most patients experienced effective relief after symptomatic treatments including anti-allergy, antiemetics, and antibiotics, or upon discontinuation of the drug. We observed no drug-related fatalities, indicating a favorable safety profile for RC48. At the end of follow-up, 13 patients were still receiving treatment while 29 patients had stopped. The main reasons for discontinuation of treatment were disease progression ($n = 21$), changes in treatment for personal reasons ($n = 4$), severe TRAEs ($n = 3$), and death due to influenza virus infection($n = 1$). The summary of TRAEs was in Table 3 (Fig. 3).

It was interesting to note that the heterogeneity of HER2 expression in pathological tissues of bladder cancer patients was also found in our study. In a histopathological examination of a patient with penile metastasis following radical bladder cancer surgery, HER2 expression was 1 + in the primary bladder cancer and 2 + in the penile metastasis. Upon initial evaluation after 6 cycles of RC48 combined with ICIs, the patient experienced a reduction of more than 40% in penile metastases and achieved PR. A patient with retroperitoneal lymph node metastasis after radical surgery for renal pelvic cancer

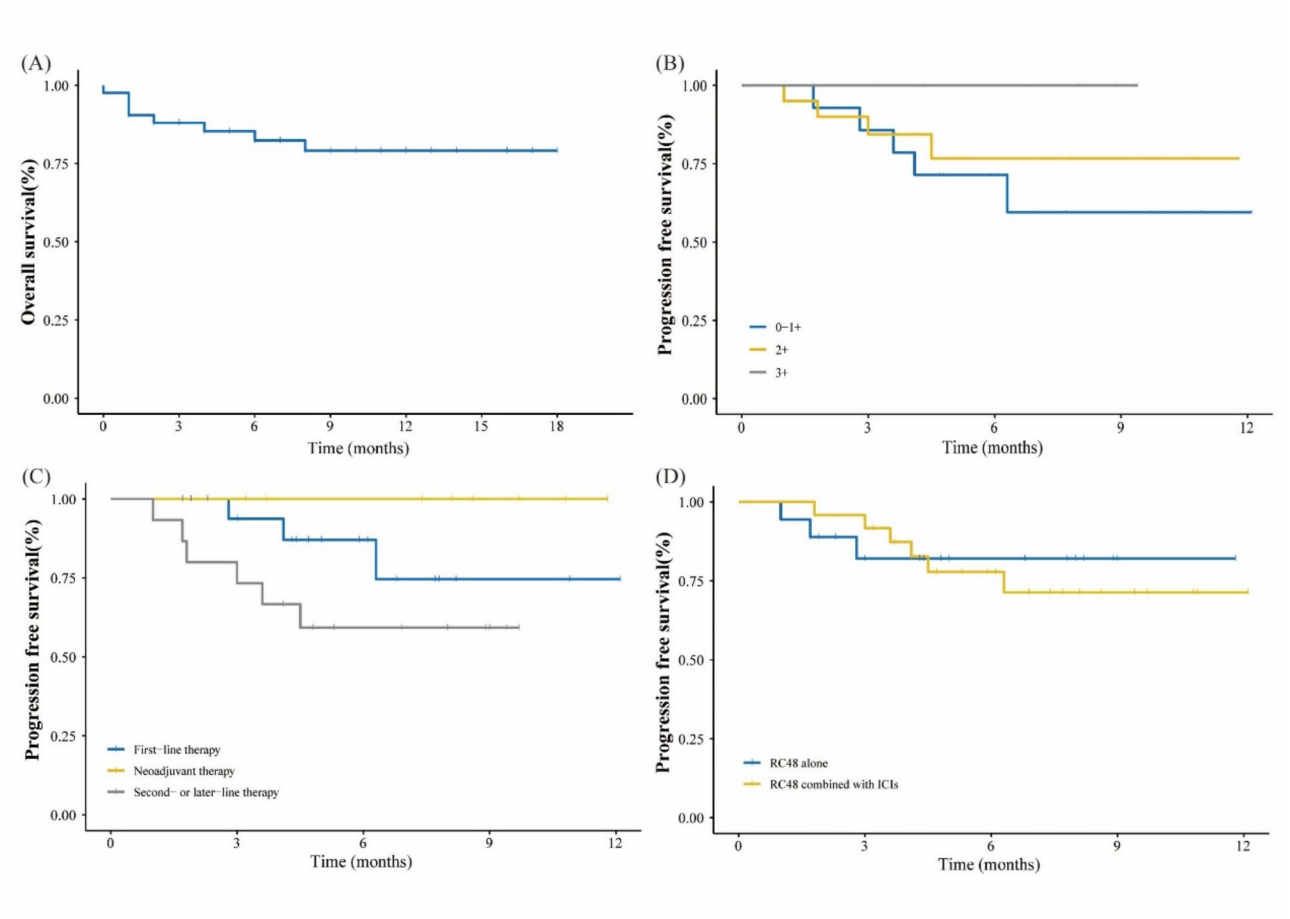


Fig. 1 Progression-free survival (PFS) of all patients (**A**). Progression-free survival (PFS) of the patients who have different drug stages (**B**). PFS of the patients receiving RC48 alone and combined with immunotherapy (**C**). PFS of the patients with HER2 0 and 1 + compared with HER2 2 + and 3+ (**D**)

had HER2 IHC 2 + at the primary site and punctured retroperitoneal lymph nodes with HER2 IHC 0. The patient did not achieve disease remission after 8 cycles of RC48 combined with ICIs. (Fig. 4) This observation implies heterogeneity in HER2 expressions between metastatic sites and the corresponding primary tumors. Furthermore, in another patient with early-stage tumor development undergoing Transurethral Resection of Bladder Tumor (TURBT), HER2 expression was detected as 1 + on pathological examination. Subsequent immunohistochemical staining of pathological specimens after radical cystectomy revealed HER2 IHC 2+. After 6 cycles of RC48 combined with ICIs, this patient also achieved PR. This result suggests that HER2 expression may also change during the course of different treatments received by the patients.

Discussion

This study examines the effectiveness and safety of RC48 and RC48 combined with ICIs in patients with la/mUC in the real world. The results demonstrated that RC48 monotherapy or in combined with immunotherapy showed promising anti-tumor activity and survival benefits in first-line, second- or later-line therapy, as well as neoadjuvant therapy in UC patients. Meanwhile, the incidence of TRAEs were also confirmed to be manageable.

The effectiveness of ADC in la/mUC has been preliminarily confirmed in clinical trials such as RC48-C005/RC48-C009 and RC48-C014 in the past. EV combined with Pembrolizumab has been reported to have positive clinical effect in either la/mUC suitable or unsuitable for cisplatin chemotherapy and is recommended as a first-line treatment option in guidelines. In this study, the ORR (47.4%) and mPFS (5.0 months) of patients receiving RC48 and RC48 combined with ICIs as first-line therapy were similar to the results of clinical trials and existing real-world studies. Patients who failed prior chemotherapy or immunotherapy and received RC48 as second- therapy had an ORR of 40.0%, which was higher than the study results of Chen et al., and mPFS was 4.8 months, which was lower than the study results of Chen et al. [21] These differences may relate to the discontinuity of medication due to adverse reactions or personal reasons. To achieve precise stratification of patients, we

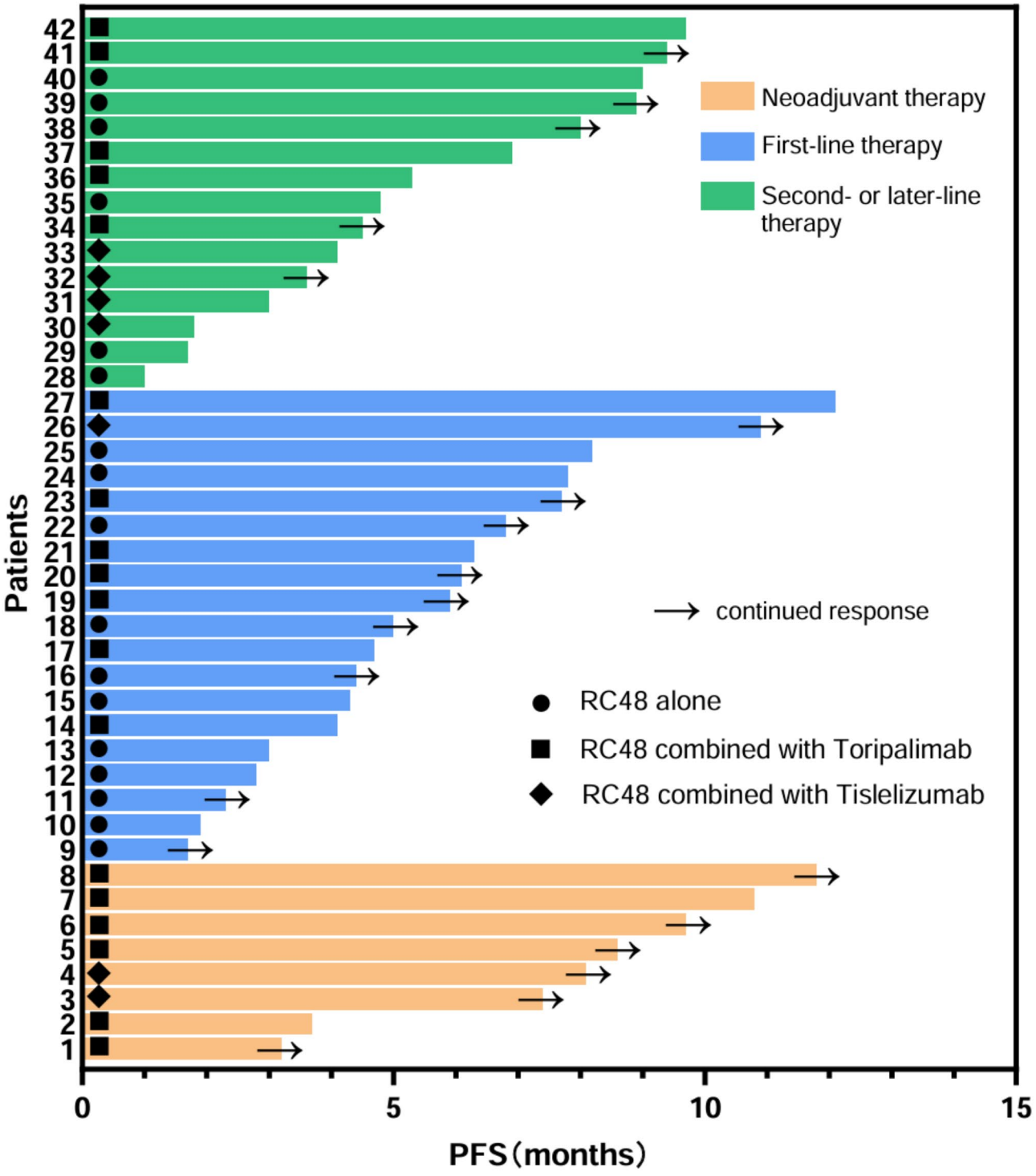


Fig. 2 Swimmer plot for patients who have different drug stages

conducted FISH testing on HER2 IHC 2 + patients. Based on experimental results and statistical analysis, no significant association was found between HER2 expression status and patients' disease response, which is consistent with data obtained from current clinical trials.

Neoadjuvant chemotherapy is the first-line standard treatment option for MIBC [22, 23]. In the latest reports, EV-103, RC48-C017 and HOPE-03 clinical trials have now achieved initial reliable effectiveness in the neoadjuvant therapy of UC [24]. An analysis of 8 patients who received neoadjuvant therapy with RC48 in combination

Table 2 ORR and mPFS of each treatment cohort

	mPFS	ORR
Neoadjuvant therapy	/	100.0%
RC48 alone	/	100.0%
RC48 combined with PD-1 inhibitors	/	100.0%
First-line therapy	6.1	47.4%
RC48 alone	5.0	45.4%
RC48 combined with PD-1 inhibitors	6.2	50.0%
Second- or later-line therapy	**5.5**	**40.0%**
RC48 alone	6.6	33.3%
RC48 combined with PD-1 inhibitors	4.5	44.4%

ORR: The proportion of patients who achieved pre-specified tumor volume reduction and maintained the minimum time limit was the sum of complete and partial responses; PFS: The time between the start of randomization and the progression of tumor development (in any respect) or death (for any cause)

Table 3 Summary of treatment-related adverse events

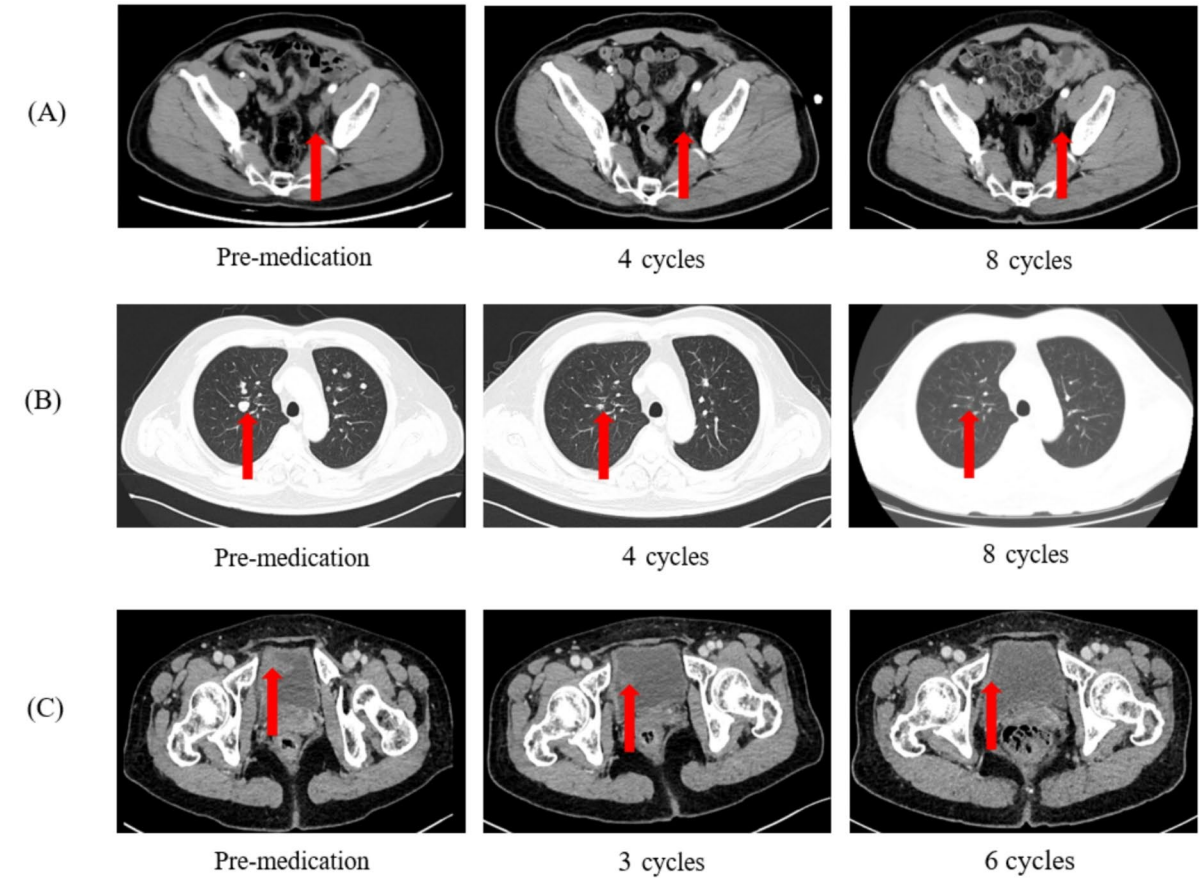
Adverse events	Patients ($n=35$)	
	Grade 1–2	Grade 3–4
Hypoesthesia	10 (23.8%)	3 (7.1%)
Anemia	3 (7.1%)	0
Pruritus	5 (11.9%)	0
Urinary tract infection	3 (7.1%)	0
Elevated transaminases	8 (19.0%)	0
Nausea	4 (9.5%)	0
Leukopenia	2 (5.7%)	0
Anorexia	6 (14.3%)	0
Hyperlipidemia	3 (7.1%)	0
Thrombocytopenia	2 (5.7%)	0
Alopecia	4 (9.5%)	0
Rash	5 (11.9%)	0
Fatigue	5 (11.9%)	0

Grade 1 Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated. Grade 2 Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental Activities of Daily Living (ADL). Grade 3 Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL. Grade 4 Life-threatening consequences; urgent intervention indicated. Grade 5 Death related to AE

with PD-1 inhibitors showed an ORR of 100% and a pCR of 50%, with sustained progression-free survival during follow-up. The favorable results achieved by RC48 neoadjuvant therapy go far beyond those of neoadjuvant chemotherapy, which may have a profound impact on the treatment strategy of UC and become a new trend in clinical practice. We also found that the incidence and severity of TRAEs was lower in patients receiving neoadjuvant therapy, which may result from better physical tolerance and shorter medication cycle. Our findings strongly suggest that RC48 combined with PD-1 inhibitors neoadjuvant therapy may be an effective alternative strategy for the treatment of UC.

ADCs combined with immunotherapy has gradually become the main direction of exploration UC treatment. By inducing immunogenic cell death (ICD), ADCs promote the release of damage-associated molecular patterns (DAMPs) in the body, activate innate immunity and trigger an anti-tumor T cell response, resulting in synergistic anti-tumor effects with PD-1 inhibitors and enhancing anti-tumor effects [25]. The ORR of EV combined with Pembrolizumab for la/mUC was 67.7% [26]. SG combined with Pembrolizumab achieved 41% ORR in second- or later-line therapy, with mPFS of 5.3 months and mOS of 12.7 months [27]. Our study found that ORR (60.0%) and mPFS (6.2 months) of RC48 combined with PD-1 inhibitors were improved compared with RC48 alone, but there was no statistical difference between the two options ($P=0.12$), which was consistent with existing real-world studies. This might be related to insufficient sample size and inconsistent research protocols in current study. In addition, our analysis of the impact of two different PD-1 inhibitors on effectiveness found no statistically significant difference between the effectiveness of RC48 in combination with Toripalimab and RC48 in combination with Tislelizumab in la/mUC, which was consistent with those of Pembrolizumab. This fully demonstrates that domestic ADCs combined with PD-1 inhibitors can also achieve satisfactory effectiveness, providing more treatment choices for UC patients in China.

Interestingly, we found that la/mUC patients with low expression of HER2 (IHC 1+) still achieved good outcomes after treatment with RC48 in our study. This may be related to the heterogeneity of HER2 in tumors and the bystander effect of RC48 [28, 29]. Several existing studies have confirmed significant heterogeneity in the expression of ADC targets such as HER2, Nectin-4, etc. between different histological subtypes of UCB. Tumors with neuroendocrine, sarcomatous and squamous histology had lower Nectin-4 expression, whereas histological subtypes that retained a luminal transcriptional profile such as micropapillary, plasmacytoid and nested histology had higher levels of Nectin-4 expression. HER2 had low expression levels in sarcomatous, neuroendocrine, adenocarcinoma, squamous, and nested tumors, heterogeneous expression among UC-NOS, glandular, and plasmacytoid tumors, and high expression among micropapillary tumors [30, 31]. Significant heterogeneities in molecular expression, as revealed by the lineage plasticity exhibited by histological morphological heterogeneity in bladder cancer, in turn plays an important role in tumor progression and mechanisms of tumor drug resistance [32]. The expression level of HER2 varies in different parts of tumor of the same patient, and also in the same part due to different time or location. This heterogeneity of HER2 expression in tumor tissues may alter our previous therapeutic strategies of predicting efficacy and screening the beneficiary population by the level of HER2 expression at the primary site. At the same time, we also found a higher positive rate of HER2 expression in UCB than in UTUC, which is consistent with previous genomic characteristics [33, 34]. In the



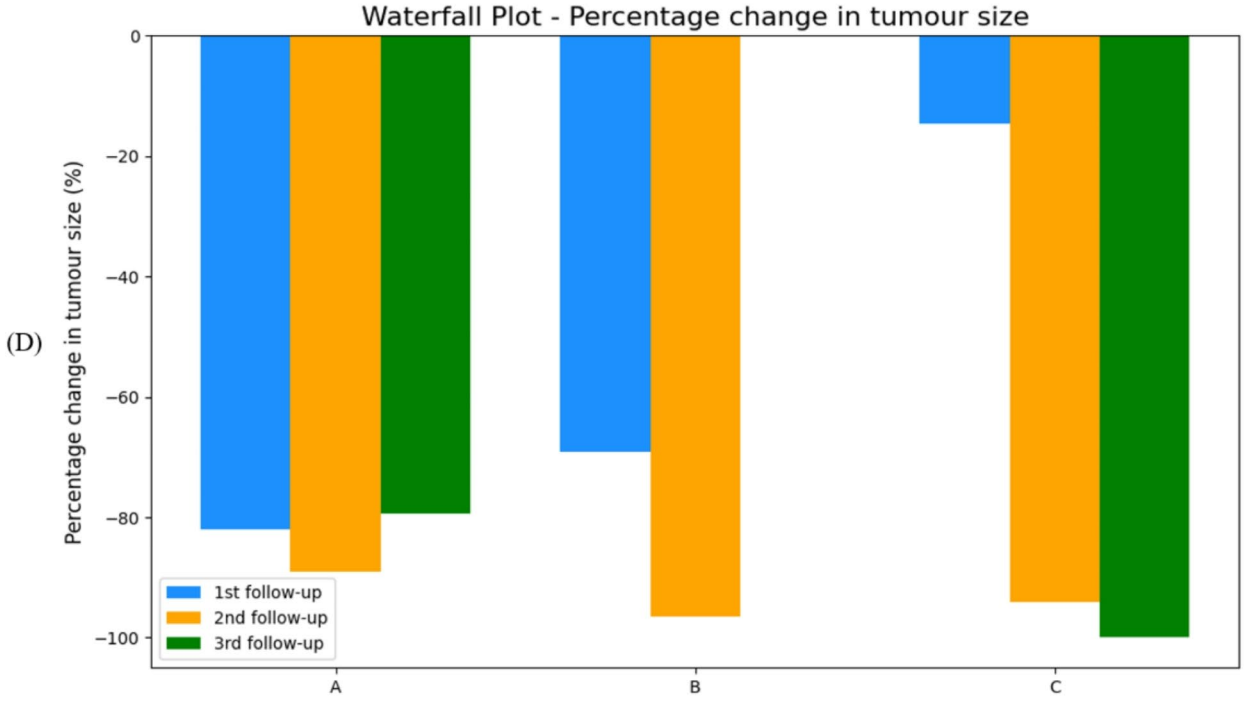


Fig. 3 **A** and **B** showed the remission of patients in first-line treatment and second- or later-line treatment groups at pre-medication, 4-cycles follow-up, and 8-cycles follow-up, respectively. **C** was the disease remission on imaging follow-up of a patient who achieved a pCR before receiving radical surgery at pre-medication, 3 cycles of medication and 6 cycles of medication. **D** was the percentage reduction in tumor size at follow-up for **A**, **B** and **C**

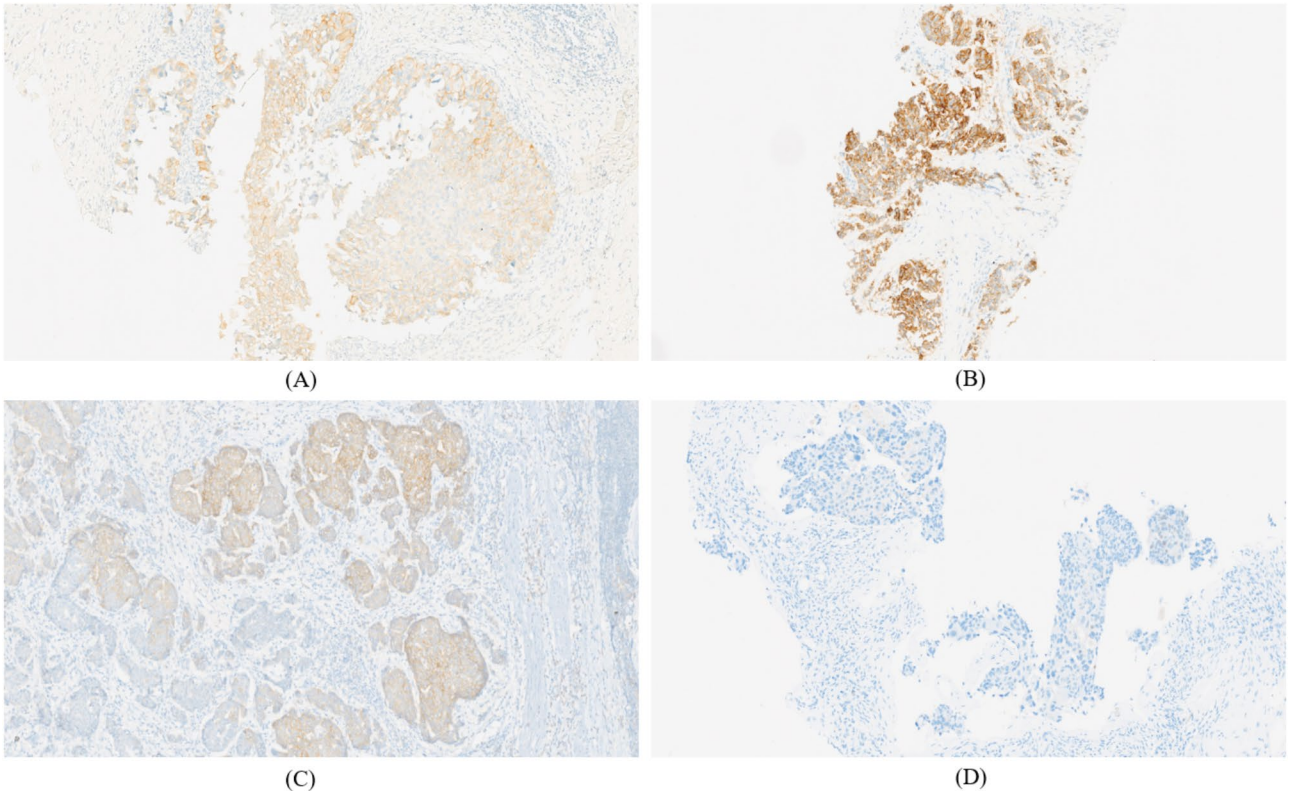


Fig. 4 **A** and **B** were HER2 immunohistochemical staining of bladder tumors and penile metastases in patients with penile metastases, respectively. **C** and **D** were HER2 immunohistochemical staining of renal pelvic tumors and lymph node metastases in patients with retroperitoneal lymph node metastases, respectively

future, further study is needed to assess HER2 expression comprehensively and accurately, so as to develop detailed and standardized HER2 expression scoring criteria to guide clinical screening, and to ultimately achieve precise treatment.

After receiving RC48 or RC48 combined with PD-1 inhibitor, the majority of patients developed TRAEs predominantly including hyperalgesia or sensory abnormalities and grading 1–2. From long-term follow-up, the incidence of peripheral neuropathy after RC48 treatment remains high. Almost all patients with long-term drug use suffer from hypoesthesia, which may be related to RC48-coupled micro-tubulin inhibitor MMAE, which is a common adverse reaction of micro-tubulin inhibitors [35, 36]. Due to the cytotoxic effect of MMAE, it not only effectively kills tumor cells, but also affects normal cells, leading to adverse reactions. We found that the incidence and severity of TRAEs were lower in patients with short treatment cycles than in patients with long-term therapy. In patients treated with RC48 in combination with PD-1 inhibitors, we did not observe the occurrence of serious immune-related adverse events (irAEs). The results suggest that PD-1 inhibitors did not significantly increase the adverse effects of ADC, and adverse effects from the combination of the two were generally manageable.

Compared with clinical trials, this real-world study was conducted in a real clinical practice setting, with no restrictions on the age of the patients or their previous treatment. A combination of statistical and epidemiological methods was used to integrate and analyze existing patients treated with RC48 in combination with PD-1 inhibitors to obtain more clinically relevant effectiveness and safety data. Meanwhile our finding of high affinity between RC48 and HER2 may further expand the use of RC48 in la/mUC, which is no longer limited to patients with positive HER2 expression. This study has its limitations. As RC48 has not yet been widely adopted in clinical practice, although we integrated multi-center resources and data in the study design, we were inevitably constrained by sample size and patient selection, which made it difficult to conduct larger-scale prospective randomized controlled trials at this stage. Given these limitations, we have developed a multicenter prospective study protocol that will rigorously and systematically enroll patients and include a standard treatment control group, with the aim of obtaining more reliable and generalizable results. We sincerely hope that future research will address the limitations of this study and provide stronger evidence for the clinical application of RC48.

Conclusions

This real-world study confirmed the favorable effectiveness and safety of RC48, either alone or in combination with ICIs, for treating UC. These findings underscore its potential as a promising therapeutic option, reinforce its clinical relevance and applicability in clinical practice. The extensive, profound, and enduring objective remission achieved by the implication of RC48 in combination with ICIs holds substantial clinical meanings, which is a promising new therapy for UC management.

Abbreviations

ADC	Antibody-Drug Conjugate
DAMP	damage-associated molecular pattern
EV	Enfortumab Vedotin
HER2	Human epidermal growth factor receptor-2
la/mUC	Locally advanced or metastatic urothelial carcinoma
ICD	Immunogenic cell death
ICI	Immune checkpoint inhibitor
IHC	Immunohistochemistry
irAE	Immune-related adverse event
MRI	Magnetic resonance imaging
PD-1	Programmed cell death protein-1
SG	Sacituzumab Govitecan
TRAE	Treatment-related adverse event
UC	Urothelial carcinoma
UCB	Urothelial carcinoma of bladder
UTUC	Upper tract urothelial carcinoma

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12967-025-06237-4.

Supplementary Material 1

Supplementary Material 2

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Declarations

Ethical approval

Research approval was obtained from the Ethics Committee of the Affiliated Hospital of Qingdao University.

Translational relevance

This multicenter, real-world study confirms that RC48 alone or in combination with PD-1 inhibitors exerted a promising effectiveness and manageable safety for first-line, second- and post-line, and neoadjuvant therapy with la/mUC. Interestingly, we found the heterogeneity of HER2 expression and followed up the patients rigorously. These findings may guide us to establish more standardized and rational HER2 scoring criteria to predict efficacy and screen potential beneficiary populations in clinical practice, contributing to the precision treatment of UC.

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