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Original Article

Efficacy and safety of disitamab vedotin after trastuzumab for HER2 positive breast cancer: a real-world data of retrospective study

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Abstract: Disitamab vedotin (RC48) is a novel cleavable antibody-drug conjugate (ADC) that has shown promising preclinical activity in HER2-positive breast cancer. However, real-world data regarding its efficacy and safety is lacking, especially in patients previously treated with trastuzumab and heavily treated patients. This retrospective study aimed to evaluate the effectiveness and safety of RC48 in HER2-positive metastatic breast cancer (MBC) in non-clinical trial settings. Eighty-one patients with metastatic HER2-positive BC who received RC48 in Shandong Cancer Hospital and Institute between September 2021 to November 2022 were included in this study. The primary endpoints were real-world progression-free survival (RWPFS) and objective response rate (ORR), and the secondary endpoints included safety and exploratory subgroup analyses. Results showed that the median RWPFS was 5.9 months, and the ORR was 29.6%, including one patient who achieved complete remission. Two-thirds of the patients had received more than one line of prior anti-HER2 treatment, and 76.6% were exposed to anti-HER2 monoclonal antibodies and tyrosine kinase inhibitors. Patients who received RC48 in ≤3 lines of treatment had significantly longer RWPFS than those who received it in ≥4 lines of treatment. The median RWPFS of RC48 in patients with trastuzumab resistance and refractoriness was 6.5 months and 5.6 months, respectively. The sequence of pyrotinib and RC48 did not influence their total efficacy. To conclude, RC48 exhibited promising efficacy in HER2-positive MBC with manageable toxicity, particularly in patients previously treated with trastuzumab and those who had undergone extensive treatment. RC48 exhibited potent activity for patients regardless of trastuzumab resistance or refractory. The sequence of pyrotinib and RC48 did not influence their total efficacy, indicating no cross-resistance.

Keywords: Disitamab vedotin, HER2-positive, breast cancer, antibody-drug conjugate, trastuzumab

Introduction

Breast cancer is a commonly diagnosed cancer worldwide, with approximately 15%-20% of women having HER2-positive breast cancer, which is characterized by aggressive clinical behavior and a worse prognosis [1]. While the emergence of anti-HER2 targeted agents has improved the outcomes of patients with HER2-positive breast cancer, most patients with metastatic disease eventually develop relapse or disease progression [2].

In the first-line treatment of HER2-positive metastatic breast cancer (MBC), trastuzumab and pertuzumab with a taxane are recommended [3]. T-DM1 or T-DXd, ADCs, are preferred after initial treatment failure [3-6]. RC48, a new anti-HER2 ADC, is approved for patients who have undergone at least two rounds of chemotherapy for HER2-positive locally advanced or metastatic cancer [7]. In a xenograft tumor model of breast cancer resistant to trastuzumab and lapatinib, RC48 was more effective than other treatments [8]. The latest ASCO data showed that RC48 achieved good efficacy in both HER2+ and low-expression breast cancer patients, with no safety concerns [9].

However, to our knowledge, few studies have investigated the efficacy of RC48 in Chinese patients with HER2-positive MBC. Therefore, this study aims to provide a comprehensive assessment of the efficacy and safety of RC48

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in Chinese patients with HER2-positive MBC in a real-world clinical setting. By focusing particularly on patients who have previously undergone trastuzumab treatment and those who have been heavily treated with multiple lines of therapy, we seek to offer valuable insights into the potential benefits and risks of RC48 for this patient population. Through the analysis of treatment outcomes, survival rates, and adverse events (AEs), our study aims to contribute to a better understanding of the role of RC48 in the management of HER2-positive MBC and inform future therapeutic strategies in this area.

Methods

This study was a retrospective, real-world study conducted at the Shandong Cancer Hospital and Institute in Shandong Province, China.

Patients and treatment

The study enrolled female patients aged 18 years or older with histologically or cytologically confirmed MBC and documented HER2 overexpression (i.e., immunohistochemistry 3+ and/or fluorescent in situ hybridization-positive by local assessment), who received RC48 in 14-day cycles, in addition to other medications (by physicians' choice), from September 2021 to November 2022 in the hospital. Patients with incomplete medical data or treated with RC48 for less than two cycles were excluded. All patients provided written informed consent, and the study was reviewed and approved by the Research Ethics Committee of the Shandong Cancer Hospital and Institute, conducted by the Declaration of Helsinki.

All data were retrospectively collected from medical records and laboratory results of individual institutions and administered by Shandong Cancer Hospital and Institute. Patients were prescribed RC48 in routine clinical practice, and the starting dose, dose modification, and treatment discontinuation of RC48 were determined by physicians' choice based on previous clinical trial results, general health status, and patient willingness.

In this study, DV dose was calculated based on the BSA-based EC adopted in China. Abroad, the formula used for DV dose conversion is $\frac{(1.07 \times BSA\text{-}based\ EC\ dose)}{1.41}$. When DV dose is mentioned in the text, it is recommended to refer to the corresponding footnotes and conversion tables for interpretation. Abbreviations: BSA - bovine serum albumin, DV - disitamab vedotin, EC - extinction coefficient.

Outcome

The primary endpoint was real-world progression-free survival (RWPFS), defined as the time from initiation of RC48 treatment to disease progression or death of any cause, whichever occurred first. The secondary endpoints included objective response rate, overall survival (OS), and safety. Tumor evaluation was assessed by Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 criteria by radiologic scans, including computed tomography (CT) or magnetic resonance imaging (MRI). AE assessments were based on the National Cancer Institute Common Terminology Criteria for AEs (CTCAE, 4.03). AEs were collected based on a patient self-reporting system and by reviewing biochemical test results.

Trastuzumab resistance was defined as new recurrences diagnosed during or within 12 months after (neo)adjuvant trastuzumab or progression at first radiological reassessment or within 3 months after first-line trastuzumab in the metastatic setting. Trastuzumab refractoriness was defined as progression after two or more lines of trastuzumab-containing regimens that initially achieved disease response or stabilization at first radiological assessment.

Statistical analyses

Statistical analyses were performed using SPSS ver. 25.0 (SPSS Inc.). Categorical variables were assessed by Pearson's chi-squared test or Fisher's exact test in different groups of patients. Median RWPFS and OS were calculated using the Kaplan-Meier methodology, and univariate analyses were performed using the log-rank test. A Cox regression model was performed using a stepwise selection of all factors studied as candidate predictors of RWPFS. A p-value of <0.05 was considered statistically significant.

Results

Patient characteristics

A total of 89 patients were prescribed RC48 from September 2021 to November 2022 at Shandong Cancer Hospital and Institute. Eight

Table 1. Baseline characteristics of patients

Characteristics	Patients, No (%) N=81
Age, median (range years)	49 (34-83)
Menstrual status	
Pre-menopausal	52 (64.2)
Post-menopausal	29 (35.8)
ECOG performance status	
0-1	55 (67.9)
2	17 (21.0)
3	9 (11.1)
Pathological type	
IDC	78 (96.3)
Non-IDC	3 (3.7)
Grading	
1	1 (1.2)
2	29 (35.8)
3	51 (63.0)
HR status at metastatic setting	
Positive	41 (50.6)
Negative	40 (49.4)
HER2 status at metastatic setting	
2+	40 (49.4)
3+	41 (50.6)
Stage disease of first diagnosis	
I-II	36 (44.4)
III	32 (39.6)
De novo IV stage	13 (16.0)
(neo)Adjuvant chemotherapy	
Yes	54 (66.7)
No	27 (33.3)
Adjuvant radiotherapy	
Yes	33 (40.7)
No	48 (59.3)
Adjuvant endocrine therapy	
Yes	26 (32.1)
No	55 (67.9)
DFI type	
De novo IV stage	13 (16.0)
≤12 months	11 (13.6)
>12 months	57 (70.4)
Previous anti-HER2 drugs	
mAB	7 (8.6)
TKI	12 (14.8)
mAB+TKI	62 (76.6)
Metastatic Sites	
Local sites	18 (22.2)
Lymph node	36 (44.4)
Bone	43 (53.1)
Visceral	57 (70.4)
Brain	3 (3.7)

patients were excluded because they received treatment for less than two cycles or with incomplete medical data. Thus, 81 patients were included for efficacy and safety analysis. The baseline characteristics of patients are shown in **Table 1**. The median age of the patients was 49 years (range, 34 to 83 years), and 52 (64.2%) were premenopausal at first diagnosis. The ECOG status was 0-1 in 55 (67.9%) patients and 2 in 17 (21.0%) patients at the time of therapy. The positivity of HR status was 50.6%, and HER2 status with 2+ was 49.4%. Thirteen (16.0%) patients had de novo stage IV breast cancer at first diagnosis. Eleven (13.6%) patients had DFS time <1 year, and 57 (70.4%) patients had DFS time ≥1 year. The majority of metastatic sites were bone, lung, liver, and lymph nodes (43 (53.1%), 37 (45.7%), 36 (44.4%), and 36 (44.4%)) in order of decreasing frequency. Lung and liver metastases were integrated as visceral metastasis in **Table 1**. Only 3 patients had brain metastasis. Twenty-seven (33.3%) patients had received one or two lines of systematic therapy, including 10 patients who received RC48 as second-line and 17 patients as third-line treatment. Fifty-four (66.7%) patients had received more than three lines of prior treatment for MBC, representing the heavily pretreated MBC.

All patients were previously exposed to anti-HER2 therapy in a metastatic setting, with 62 (76.6%) patients exposed to mAB and TKI, 12 (14.8%) exposed to TKI only, and 7 (8.6%) exposed to mAB only. Trastuzumab resistance and trastuzumab refractoriness occurred in 22 (27.2%) and 59 (72.8%) patients, respectively (**Table 1**).

Treatment administration

Treatment administration is described in **Table 2**, which shows that the majority of patients (69, 85.2%) were prescribed with RC48 alone, while others received combined therapy with pyrotinib (5, 6.2%), bevacizumab (2, 2.4%) and endocrine therapy (5, 6.2%), respectively. Most patients (71, 87.7%) started RC48 at the standard dose of 2.0 mg/kg, while 6 (7.4%) patients started at a dose of 1.5 mg/kg and 4 (4.9%) at a dose of 1.0 mg/kg. Three patients had their RC48 dose increased from 1.5 to 2.0 mg/kg or from 1.0 to 1.5 mg/kg. Six patients had a dose reduction in RC48 from 2.0 to 1.5 mg/kg. Four patients had RC48 treatment interrupted due to AEs, with three cases related to neutropenia and one case related to hypoesthesia (**Table 2**).

No. of Metastatic Sites	
≤2	45 (55.6)
≥3	36 (44.4)
Trastuzumab resistance status	
Resistance	22 (27.2)
Refractoriness	59 (72.8)
Lines of RC48 in metastatic setting	
≤3	27 (33.3)
≥4	54 (66.7)

Abbreviations: ECOG, Eastern cooperative oncology group; IDC, Invasive ductal carcinoma; HR, Hormone receptor; HER2, Human epidermal growth factor receptor 2; DFI, Disease-free interval; mAB, Monoclonal antibody; TKI, Tyrosine kinase inhibitor.

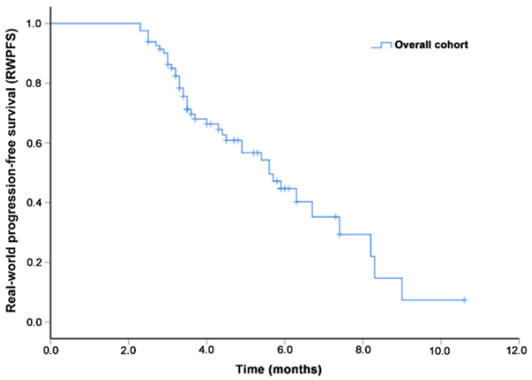

Figure 1. Median RWPFS of 81 patients over 5.9 months.

Table 2. Treatment administration

Treatment	Patients, No (%) N=81
Combined regimens with RC48	
Alone	69 (85.2)
Pyrotinib	5 (6.2)
Bevacizumab	2 (2.4)
Endocrine Therapy	5 (6.2)
RC48 dosage	
Starting dosage (mg/kg/day)	
2.0 mg/kg	71 (87.7)
1.5 mg/kg	6 (7.4)
1.0 mg/kg	4 (4.9)
Dose modification (mg/kg/day)	
2.0→1.5	6 (7.4)
1.5→2.0	2 (2.5)
1.0→1.5	1 (1.2)
Treatment discontinuation due to AEs	4 (5.0)

Abbreviations: RC48, Disitamab vedotin; AEs, Adverse events.

Efficacy in all patients

The median RWPFS for the total of 81 patients was 5.9 months (95% CI: 5.17-6.58 months) (**Figure 1**). Approximately half of the patients (49.4%) were still receiving treatment, while five patients had died of any cause. Median OS had not been achieved at the time of this study. The median RWPFS for the 61 patients who received RC48 alone was 5.7 months (**Figure 2A**).

All 81 patients were included in the ORR analysis, with an ORR of 29.6%. Only one patient achieved a complete response, who had isolated liver metastasis, and had received only pyrotinib and capecitabine as first-line therapy. 23 patients (28.4%) achieved a partial response, 44 (54.3%) had stable disease, and 13 (16.0%) experienced disease progression (**Table 3**). The one patient with CR response had only liver metastasis and received pyrotinib plus capetabine as first-line therapy, reaching a PFS of 5.3 months at the time of this study (**Figure 3**). A waterfall plot of tumor volume changes in patients with solid tumors treated with RC48 is provided as shown in **Figure 4**.

For the 69 patients who received RC48 alone, the ORR was 31.9%, including 1 patient with CR and 21 patients with PR. Ten patients who received RC48 of 1.0 mg/kg or 1.5 mg/kg had not reached PFS events at the time of this study (**Figure 2B**) and presented stable disease (SD). For the 71 patients with 2.0 mg/kg dosage RC48, 1 patient achieved CR, 23 had PR, 34 had SD, and 13 had progression.

In the log-rank analysis, menstrual status, HER2 status (2+ vs. 3+), and the number of metastatic sites (≤2 vs. ≥3) did not show a significant correlation with RWPFS (P=0.621, P=0.911, P=0.508, respectively, **Figure 2C**). Hormone receptor status, the line number of RC48 (≤3 vs. ≥4), and prior exposure to anti-HER2 agents (mAB, TKI, or both) were significantly correlated with RWPFS in the log-rank analysis (P=0.028, P=0.011, and P=0.009, respectively, **Figure 2D-F**). However, the multivariable Cox regression analyses showed that none of these factors were independent predictors of RWPFS (**Table 4**).

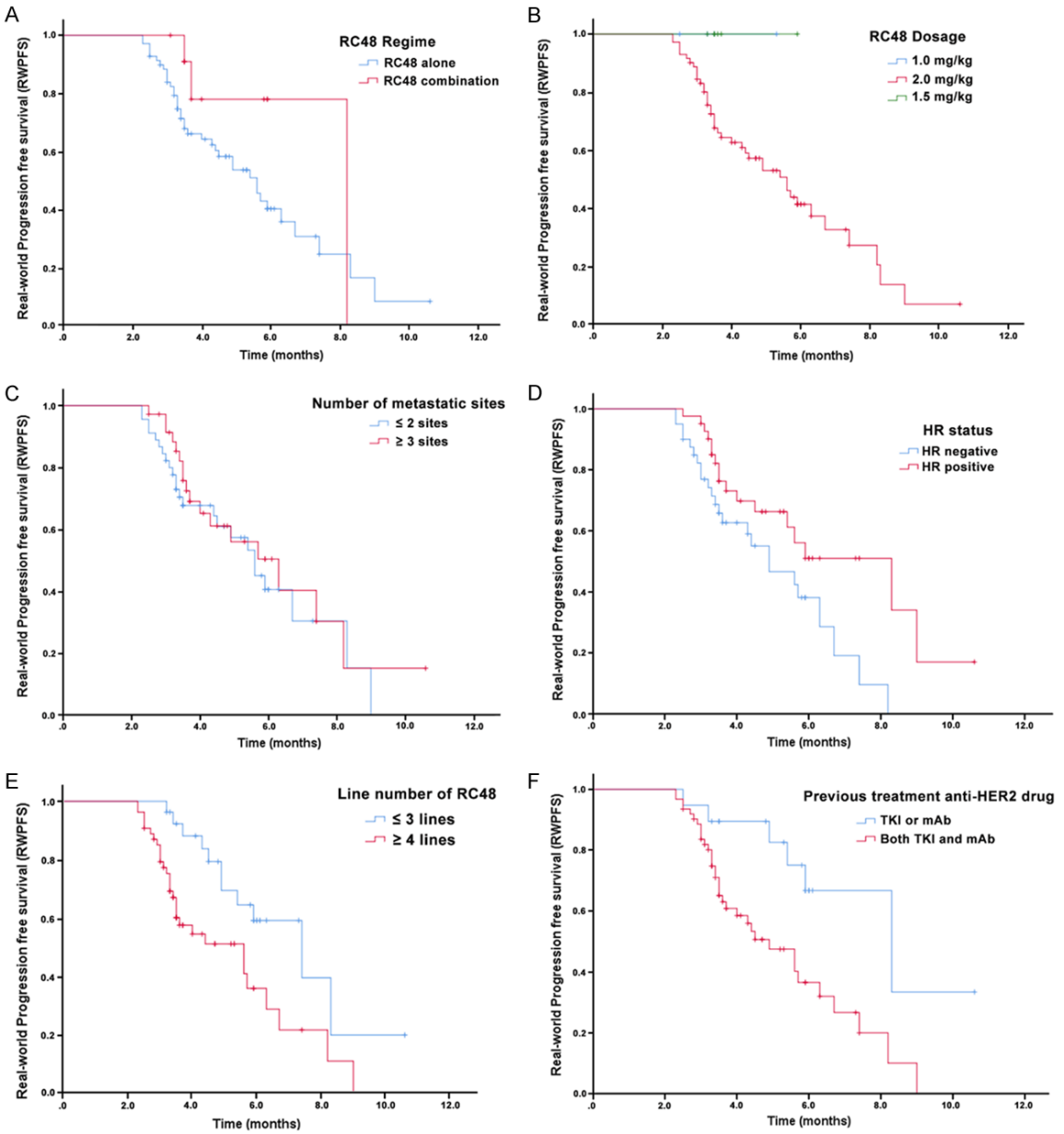

Figure 2. Correlation of clinical parameters with RWPFS in Log-rank analysis. A. Relationship between RC48 regime (alone vs. combination) and RWPFS, displaying no correlation. B. Influence of the dosage of RC48 (1.0, 1.5 vs. 2.0 mg/kg) on RWPFS, showing no correlation. C. Log-rank analysis of the number of metastatic sites (≤2 vs. ≥3) with RWPFS, displaying no correlation. D. Correlation between hormone receptor status and RWPFS, showing a significant association. E. Influence of the line number of RC48 (≤3 vs. ≥4) on RWPFS, indicating a significant correlation. F. Relationship between prior exposure to anti-HER2 agents (mAB, TKI, or both) and RWPFS, displaying a significant correlation.

Efficacy of RC48 therapy in trastuzumab-resistance patients

All 81 patients received trastuzumab in adjuvant or metastatic setting in previous therapy and later received RC48-based therapy. Median RWPFS in patients with trastuzumab resistance and trastuzumab refractoriness were 6.5 months and 5.6 months, respectively (P=0.469, **Figure 5A**).

Efficacy of TKI plus ADCs

Thirty patients received pyrotinib and capecitabine first and then received RC48, while 6 patients received them in turn. The total

Table 3. ORR for overall cohort

Response	All No. (%) N=81	RC48 alone No. (%) N=69
Complete response	1 (1.2)	1 (1.4)
Partial response	23 (28.4)	21 (30.4)
Stable disease	44 (54.3)	34 (49.2)
Progressive disease	13 (16.0)	13 (18.8)
ORR	24 (29.6)	22 (31.9)

Abbreviation: ORR, objective response rate.

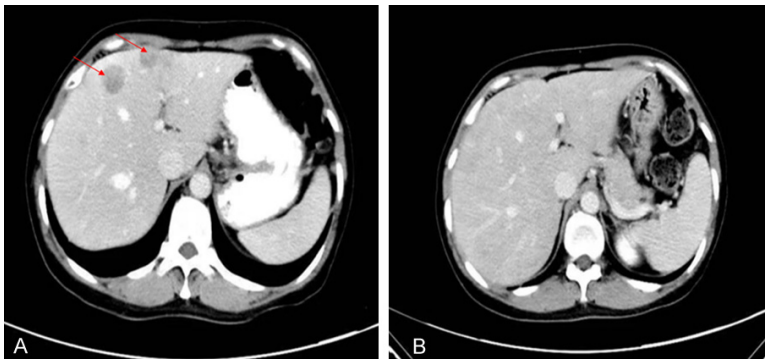

Figure 3. Case presentation of patients before and after RC48 treatment: evidence of efficacy in complete response (CR). Pre-treatment image: (A) shows the status of the tumor before starting RC48 treatment (The lesions were marked by red arrow). Post-treatment image: (B) shows the complete response of the tumor after treatment.

RWPFS of pyrotinib and RC48 have no significant difference in these two groups (**Figure 5B**).

Safety

Given the retrospective nature of our study, it was inevitable that some AE data might have been missed during data collection. We present the grades 3-4 AEs in **Table 5**. The most frequently reported AEs leading to dose reduction and treatment interruption were neutropenia, leukopenia, and hypoesthesia. No treatment-related deaths were reported, and the safety profile of RC48 was generally manageable and well-tolerated. For the patients with RC48 alone, the most common grades 3-4 AEs were neutropenia, leukopenia, and hypoesthesia. For the patients with RC48 and other regimes, no other grades 3-4 AEs were found. Most of the grades 3-4 AEs occurred in patients with 2.0 mg/kg dosage RC48, only 1 patient with 1.5 mg/kg had a grade 3 neutropenia.

Discussion

In this study, we evaluated the efficacy and safety of RC48 in a real-world cohort of heavily pretreated patients with HER2-positive MBC. Our findings demonstrate that (1) RC48 has promising efficacy in this population, with a median RWPFS of 5.9 months and an ORR of 29.6%, consistent with the results from previous clinical trials of RC48; (2) RC48 may have a greater impact when used earlier in the treatment course; (3) The order of administration of pyrotinib capecitabine, and RC48 does not significantly impact the overall RWPFS.

The introduction of HER2-targeted therapies has been a paradigm shift in the management of HER2-positive breast cancer, substantially improving patient outcomes [10]. Among these therapies, RC48 represents a newer class of humanized anti-HER2 antibodies. It is distinguished by its conjugation to the cytotoxic agent MMAE, which is released upon cleavage at the tumor site [11]. Clinical trials (NCT03052634 and NCT03500380) have previously indicated the effectiveness of RC48 across varying levels of HER2 expression. In our study, RC48 demonstrated promising efficacy with a median RWPFS of 5.9 months and an ORR of 29.6% in metastatic HER2-positive breast cancer, consistent with previous clinical trial results [9, 12]. While our findings align with these trials in terms of efficacy, showing a median RWPFS of 5.9 months and an ORR of 29.6%, they also prompt consideration of how patient selection and treatment sequencing affect outcomes. Specifically, when juxtaposed with T-DXd outcomes reported in the DESTINYBreast01 trial [13], the observed differences in response rates and progression-free survival may reflect the impact of prior treatments and disease burden on treatment response. The lower drug-to-antibody ratio (DAR) of RC48 may also contribute to this variance, suggesting that payload delivery and the resulting bystander effect are critical determinants of efficacy. These findings underscore the necessity of optimizing treatment sequencing and personalizing therapy

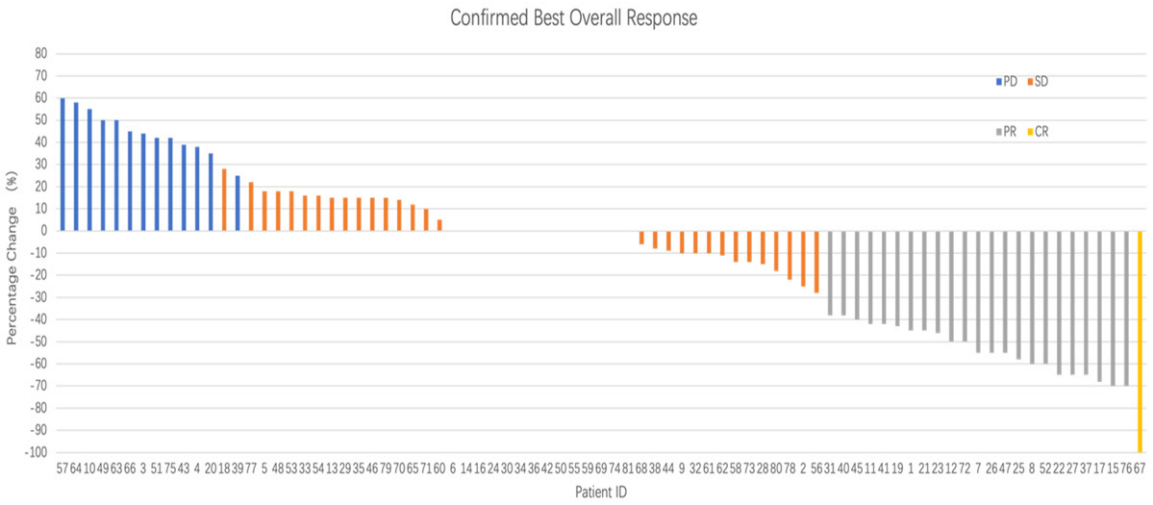

Figure 4. Waterfall plot of tumor volume changes in patients with solid tumors treated with RC48. Note: CR: Complete Response; PR: Partial Response; SD: Stable Disease; PD: Progression of Disease.

Table 4. Univariate and Cox multivariate analysis of factors associated with progression free survival

Characteristic	Univariate analysis		Cox multivariate analysis	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age group (<60 vs. ≥60)	1.236 (0.543-2.811)	0.613		
Menopausal status (Pre- vs. post-)	0.850 (0.445-1.626)	0.624		
HR status (HR+ vs. HR-)	0.493 (0.258-0.942)	0.032	0.608 (0.314-1.177)	0.14
HER2 status (2+ vs. 3+)	0.964 (0.499-1.862)	0.912		
Number of metastatic sites (≤2 vs. ≥3)	0.812 (0.435-1.515)	0.512		
Visceral metastasis (yes vs. no)	1.003 (0.482-2.090)	0.993		
Trastuzumab status (Resistance vs. Refractoriness)	1.292 (0.641-2.604)	0.473		
Lines of RC48 (≤3 vs. ≥4)	2.410 (1.196-4.855)	0.014	1.586 (0.710-3.541)	0.26
Previous exposure anti-HER2 drug (mAB/TKI vs. Both mAB and TKI)	3.037 (1.259-7.325)	0.013	2.007 (0.726-5.552)	0.179

Abbreviations: HR, Hormone receptor; HER2, Human epidermal growth factor receptor 2; RC48, Disitamab vedotin; mAB, Monoclonal antibody; TKI, Tyrosine kinase inhibitor.

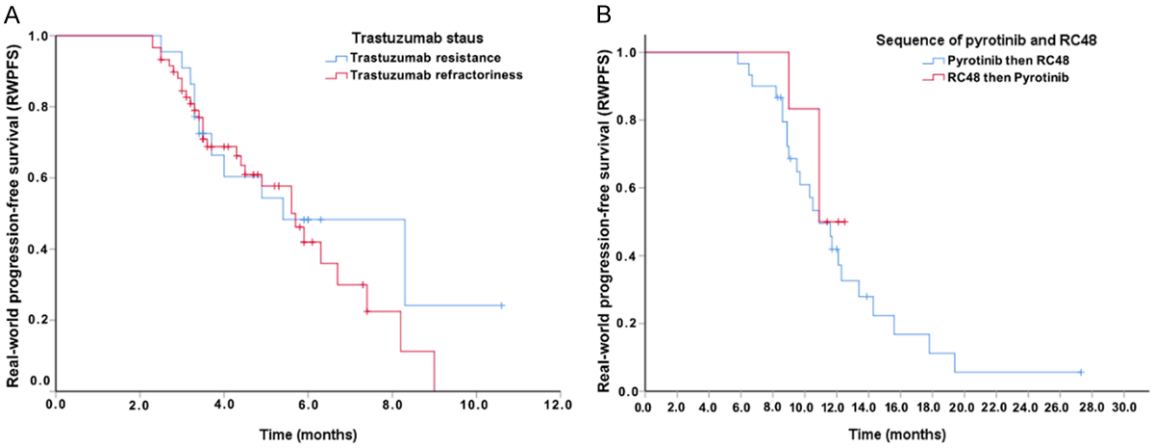

Figure 5. Analysis of RWPFS based on prior treatments and resistance patterns. A. Comparison of median RWPFS between patients with trastuzumab resistance and trastuzumab refractoriness after receiving RC48-based therapy. B. Analysis of total RWPFS in patients who first received pyrotinib and capecitabine followed by RC48, compared to those who received them in turn. No significant difference was observed between the two groups.

Table 5. Adverse events (grade 3/4)

AE (grade 3/4)	All No. (%) N=81	RC48 alone No. (%) N=69
Neutropenia	10 (12.3)	8 (11.6)
Leukopenia	7 (8.6)	5 (7.2)
Anemia	2 (2.5)	0 (0)
Thrombocytopenia	1 (1.2)	1 (1.4)
Hypoesthesia	9 (11.1)	7 (10.1)
Aminotransferase increased	4 (4.9)	2 (2.9)
γ-Glutamyl transferase increased	2 (2.5)	2 (2.9)
Asthenia	3 (3.7)	2 (2.9)
Nausea	2 (2.5)	2 (2.9)
Vomiting	2 (2.5)	1 (1.4)
All	42 (51.9)	39 (56.5)

Abbreviation: AE, Adverse event.

based on individual patient profiles and tumor biology.

The differential efficacy of RC48 observed in our study may shed light on the optimal timing of its administration in the treatment sequence for HER2-positive MBC. Patients treated with RC48 in earlier lines (≤3) exhibited more favorable outcomes than those receiving it later (≥4 lines), suggesting that earlier intervention with this therapy could be more beneficial. The comparison of median RWPFS achieved by RC48 in the ≤3 lines cohort with established therapies such as neratinib plus capecitabine and lapatinib plus capecitabine from the EMILIA study, and with T-DM1 from the TH3RESA study [14], suggests that RC48 has a competitive efficacy profile. These findings advocate for the inclusion of RC48 in treatment algorithms for earlier lines of therapy, although confirming this would require head-to-head comparisons in prospective studies. The reduced efficacy observed in patients previously treated with both monoclonal antibodies (mABs) and tyrosine kinase inhibitors (TKIs) raises important considerations about treatment-induced resistance. Such resistance could be multifactorial, involving the upregulation of alternative signaling pathways or other cellular adaptations, and underscores the complexity of managing advanced HER2-positive MBC. It also highlights the necessity of exploring the mechanisms underlying treatment resistance and the potential for RC48 to retain activity in this context. Ultimately, our findings contribute to a growing body of evidence that supports a more nuanced approach to the use of anti-HER2 therapies, considering the diversity of patient treatment histories and the need for personalized treatment strategies.

Prior exposure to both monoclonal antibodies and tyrosine kinase inhibitors was associated with reduced RC48 efficacy, potentially reflecting a treatment-induced resistance. This pattern, more pronounced in those treated with RC48 after ≥4 lines of therapy, indicates the need to understand the resistance mechanisms that could be influencing response rates in heavily pretreated HER2-positive breast cancer populations.

Our study found that RC48 treatment of trastuzumab resistance and refractory was similar in efficacy. RC48 contains the novel humanized anti-HER2 antibody-hertuzumab, which had a higher affinity to HER2 and had more potent antibody-dependent cell-mediated cytotoxicity (ADCC) activity than trastuzumab in vitro. Besides, the high potency of the payload cytotoxic agent (MMAE) can be cleaved from antibodies, and diffused into neighboring cells where they can induce a non-antigen dependent cytotoxicity, called the bystander effect. Based on the biomarker analyses from the EMILIA study, the activity of T-DM1 seems to be preserved in the presence of PI3K mutations, which is the main mechanism of trastuzumab resistance and refractory [15]. With the same payload and similar antibody, RC48 may had the same activity to overcome trastuzumab resistance. Further biomarker analyses of tissue samples might provide information about predictive factors and potential mechanisms of treatment insensitivity. Thus, RC48 is still an alternative for patients with trastuzumab resistance and refractory.

In our study, the sequence of pyrotinib and RC48 did not influence the total RWPFS, suggesting no cross-resistance between the two due to their different mechanisms of action. Docetaxel, pertuzumab, and trastuzumab are unanimously established as the optimal first-line therapy for advanced HER2-positive breast

cancer according to clinical guidelines. T-DM1 is the preferred therapy for patients who exhibit progression after prior trastuzumab-based treatment. The recent DESTINY-Breast03 study showed that T-DXd significantly improved the median PFS (28.8 vs. 6.8 months, P<0.001) and reduced the risk of progression or death when compared to trastuzumab emtansine [16]. Based on the PHOEBE and PHENIX studies, pyrotinib plus capecitabine has been approved as an alternative treatment option for patients with HER2-positive MBC previously treated with trastuzumab and/or chemotherapy in China [17, 18]. Multiple anti-HER2 agents have been approved for trastuzumab-treated advanced HER2-positive BC, each with different mechanisms of action and safety profiles, making the identification of the best sequence challenging [19]. This indicates that both ADC-to-TKI and TKI-to-ADC are alternatives for trastuzumab-treated HER2-positive MBC. Further prospective randomized controlled studies (ADC-to-TKI vs. TKI-to-ADC) are needed to confirm these results and identify the optimal patient population for individualized treatment.

Safety analyses indicated that RC48 had a manageable safety profile in previously treated advanced breast cancer, with fewer serious adverse events (AEs) and discontinuations due to AEs compared to historical data [20, 21]. Neutropenia, leukopenia, and hypoesthesia were the most common high-grade AEs, aligning with prior reports [22, 23]. These findings contribute to the growing evidence of RC48's tolerability [12, 24, 25]. Nonetheless, the retrospective design and reliance on self-reporting for AEs highlight the potential for underreporting and necessitate cautious interpretation of these results.

This study has several limitations, including its retrospective design, which might introduce selection bias. The limited sample size reduces the statistical power to detect significant differences and might not represent the broader patient population. Additionally, the lack of a control group makes it challenging to compare the efficacy and safety of RC48 with other treatment options directly. Moreover, the follow-up time was short, and OS data were not mature enough to draw firm conclusions. In addition, the patient self-reporting system used in reporting AEs and the retrospective nature of the study may result in underreporting or recall bias. Last but not least, in this study, we used RWPFS rather than PFS, which is routinely used in clinical trials, as the primary metric. RWPFS has the major advantage of being a broader reflection of real-world treatment effects. However, it also has some obvious limitations. Firstly, as the source of real-world data is not as controlled as in clinical trials, RWPFS may be affected by a variety of confounding factors, such as patients' baseline characteristics, comorbidities, and treatment sequence. In addition, data collection for RWPFS may not be as systematic and thorough as in clinical trials. Therefore, while RWPFS provides valuable real-world insights, direct comparisons to PFS in controlled clinical trials should be made with caution.

Despite these limitations, the present study provides valuable information for clinicians. RC48 demonstrated promising efficacy in HER2-positive MBC with tolerable toxicity, especially in trastuzumab-treated patients and even in heavily treated patients. RC48 exhibited potent activity for patients regardless of trastuzumab resistance or refractoriness. The sequence of pyrotinib and RC48 influenced their total efficacy, indicating no cross-resistance.

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Written informed consent was provided from the participants.

Disclosure of conflict of interest

None.

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