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REVIEW

Advances in HER2-Targeted Treatment for Advanced/ Metastatic Urothelial Carcinoma

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Abbreviation used: UC, urothelial carcinoma; mUC, metastatic UC; HER, human epidermal growth factor receptor; T-DM1, trastuzumab emtansine; T-DXd, trastuzumab deruxtecan; OS, overall survival; ICIs, immune checkpoint inhibitors; NGS, Next-generation sequencing; ECD, extracellular domain; MAPK, mitogen-activated protein kinase; PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase; AKT, protein kinase B; PKC, protein kinase C; PLCγ, phospholipase C γ; ASCO/CAP, American Society of Clinical Oncology/ College of American Pathologists; IHC, immunohistochemistry; FISH, fluorescence in situ hybridization; ORR, objective response rate; PFS, progression-free survival; DCR, disease control rate; DRFS, distant relapse-free survival; CR, complete response; PR, partial response; SD, stable disease; MIBC, muscle-invasive bladder cancers; NMIBC, non-muscle invasive bladder cancers; AACR, American Association for Cancer Research; COSMIC, Catalogue of Somatic Mutations in Cancer; KD, kinase domain; TCGA, The Cancer Genome Atlas; SNV, single-nucleotide; UBC, urinary bladder cancer; TKI, Tyrosine kinase inhibitors; TCC, transitional cell carcinoma; BUC, bladder urothelial carcinoma; IgG1, immunoglobulin G1; ADCs, antibody-drug conjugates; DAR, drug-to-antibody ratio; DM1, emtansine; MMAE, monomethyl auristatin E; MTD, maximum therapeutic dosage; PK, pharmacokinetics; TLR8, toll-like receptor 8; APC, antigen-presenting cells; IGF1R, insulin-like growth factor-1 receptor

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ABSTRACT

Urothelial carcinoma (UC) represents a common malignancy of the urinary system that can involve the kidneys, ureter, bladder, and urethra. Advanced/metastatic UC (mUC) tends to have a poor prognosis. UC ranks third in terms of human epidermal growth factor receptor 2 (HER2) overexpression among all tumors. However, multiple studies found that, unlike breast cancer, variable degrees of HER2 positivity and poor consistency between HER2 protein overexpression and gene amplification have been found. Trials involving trastuzumab, pertuzumab, lapatinib, afatinib, and neratinib have failed to prove their beneficial effect in patients with HER2-positive mUC, and a clinical trial on T-DM1 (trastuzumab emtansine) was terminated prematurely because of the adverse reactions. However, a phase II trial showed that RC48-ADC was effective. In this review, we provided an in-depth overview of the advances in the research regarding HER2-targeted therapy and the role of HER2 in mUC. Furthermore, we also discussed the prospects of potential strategies aimed at overcoming anti-HER2 resistance, and summarize the novel anti-HER2 approaches for the management of mUC used in recent clinical trials.

Keywords: advanced/metastatic bladder cancer, advanced/ metastatic urothelial cancer, HER2, targeted therapy

INTRODUCTION

According to an estimate of GLOBOCAN 2020, over 573,278 new cases of bladder cancer emerged in 2020, causing approximately 212,536 deaths[1]. Advanced/metastatic urothelial carcinoma (mUC) has poor prognosis and is generally considered an incurable disease, with only about 5% of patients surviving longer than five years[2]. In the past decades, cisplatin-based chemotherapy, such as gemcitabine plus cisplatin (GC) and methotrexate, vinblastine, doxorubicin, and cisplatin (M-VAC) regimens, has been employed as the first-line treatment for advanced UC, leading to a median overall survival (OS) of 15 months[3]. In cisplatin-ineligible patients, carboplatin serves as a substitute option and achieves only an approximately 9-month median OS[2]. Recently, immune checkpoint inhibitors (ICIs), fibroblast growth factor receptor inhibitors (erdafitinib), and antibody-drug conjugates (enfortumab vedotin) have ushered in advances in the treatment of mUC[4]. Next-generation sequencing (NGS) and bioinformatics have provided numerous novel approaches to look into genomic landscape and actionable

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mutations of the condition. As a result, numerous putative therapeutic targets have been explored to offer potential opportunities for targeted therapy[5].

Human epidermal growth factor receptor 2 (HER2) is a member of the ERBB family of receptor tyrosine kinases. HER2 amplification/overexpression or activation of somatic mutations causes the abnormal activation of HER2 signaling and dysregulated cell proliferation in approximately 20% of patients with breast cancers[6]. Moreover, HER2 overexpression is correlated with increased lymph node-positive and disease-free survival in breast cancer patients[7]. The HER2-targeted therapy for patients with HER2 amplification/overexpression breast cancer has led to dramatic improvements in oncological outcomes[6]. Similar to breast cancer, UC also has HER2 amplification/overexpression, whereas HER2 expression is low or undetectable in normal urothelial tissues[8]. Thus, HER2 is a potential candidate for targeted therapy in UC. In this study, we conducted an in-depth overview about the role of HER2 in UC and advances in HER2-targeted therapy for mUC.

THE ROLE OF HER2

Four members of the HER family, HER1 (ERBB1), HER2 (ERBB2), HER3 (ERBB3), and HER4 (ERBB4), exist as monomers on the cell surface and their genes are localized on chromosome 17q. Receptor dimerization (pairing) is essential for HER function and the activation of a multitude of downstream signals[9]. All of the four HER receptors are made up of a cysteine-rich extracellular ligand binding site, a transmembrane lipophilic segment, and an intracellular domain consisting of a juxtamembrane domain, a typical tyrosine protein kinase segment, and a tyrosine-rich carboxyterminal tail[10]. Two different types of subdomains comprise the extracellular domain (ECD): domains I and III, which are the components involved in ligand binding, and cysteine-rich domains II and IV. Domain II participates in homo- and heterodimer formation with HER family members[10]. Several studies have described two different conformational states in the extracellular region of the HER family. In the absence of the ligand, the receptor is in a closed conformation, with domains I and III folded together. Upon ligand binding, HER1–4 takes on an open active conformation and interact with each other through domain II, which is known as "dimerization arm", to form various homo- and heterodimers[10-12].

Interestingly, HER2 can constitutively exist in an active conformation with no direct activating ligand[10]. Furthermore, HER2 has the strongest catalytic kinase activity and functions by creating spontaneous receptor homodimers or heterodimers with other HER family members, which initiates a wide array of signaling pathways, such as mitogen-activated protein kinase (MAPK), phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K), and protein kinase C (PKC), leading to cell-cycle progression and angiogenesis[9,10,13]. The formation of spontaneous receptor homodimers or heterodimers increases with HER2 protein overexpression. With breast cancer, several studies have demonstrated that the main mechanism responsible for HER2 overexpression is gene amplification[14,15]. Three principal mechanisms of HER2 signaling activation have been identified: HER2 amplification and overexpression; molecular alterations of the HER2 receptor, and inhibition of phosphatase activity[16].

HER2 ABERRATION IN UC

HER2 amplification and overexpression

Several investigators studied the HER2 positivity rate in UC and found that the rate varied, approximately ranging from 7% to 80%[17-26]. Studies examining the HER2 status and its prognostic value are summarized in **Table 1**.

Unlike for breast cancer, there are no standardized methodology and interpretation criteria for UC. Variability in the methods used to assess the HER2 status may be the most important factor impacting HER2 positivity rates. The most frequently cited scoring guidelines were ASCO/CAP guidelines for breast cancer (2007, 2013, and 2018) and gastric cancer. And some studies developed their own criterion or did not mention the criterion in their research methods. The HER2 status is significantly related to tumor stage and disease grade. In UC, HER2 expression is significantly increased in tumors of higher grades and stages[17,19]. A study discovered that tumors of a luminal molecular subtype had a significantly higher rate of HER2 alterations than those of a basal subtype, suggesting that HER2 activity is also associated with the subtype of tumors[18]. HER2 overexpression/amplification is often more common in a distant or regional metastatic lesion than in its primary counterpart[20,21]. Thus, further studies involving samples from distant metastases may be warranted to accurately determine the HER2 status, especially in patients with HER2-negative primary tumors[20].

With breast cancer, several studies have demonstrated that the main mechanism underlying HER2 overexpression is gene amplification[14,27]. However, most of the aforementioned studies failed to find an obvious agreement between HER2 overexpression and gene amplification in UC[21,22]. Numerous studies revealed amplification or borderline amplification in virtually all HER2 3+ tumors, whereas no amplification was found in HER2 2+ tumors[21,22]. In a study mainly involving invasive tumors, HER2 2+ tumors were found to be strongly associated with gene amplification[23]. Several studies have suggested that other mechanisms, such as polysomy 17, point mutations, and translocation or transcriptional upregulation, could contribute to HER2 protein overexpression[18,21,23,28,29]. Furthermore, the poor correlation may be also explained by some sort of heterogeneity[30]. Prior studies also found that the closest agreement between IHC and FISH existed when HER2 overexpression was defined as >50% tumor cells showing complete membranous

immunoreactivity. Therefore, determining an appropriate cut-off value for HER2 overexpression in UC is important[24,25]. These findings suggest that the biomarker assessment methods (IHC and FISH) used for breast cancer may not work as well for UC. Thus, standardized methodology and interpretation criteria are needed to evaluate the HER2 status in UC.

Table 1. Frequency of HER2 positivity and its predictive value in selected studies

Author, year	Sample size	Stage	Grade	Method	Definition of HER2 positivity	Positivity rate	Concordance rate between HER2 expression and amplification	Significant association
MATSUBARA *et al*. 2008	40	pT2: 22, pT3: 13, pT4: 5; pN0: 23, pN1: 4, pN2: 6, pNx: 7	G2: 3, G3: 37	IHC/FISH	IHC (2+/3+);	76.6%	29.4% (5/17)	-
Lae´ *et al*. 2010	1005	NA[&]	NA	IHC/FISH	2007 ASCO/CAP guidelines for breast cancer	5.1%	HER2 2+ tumours: 0% HER2 3+ tumours: 100%	-
Bellmunt *et al*. 2015	213[Ω]	NA	NA	IHC/FISH	2010 ASCO/CAP guidelines for breast cancer	Spanish cohort: 22%; Greek cohort: 4%	NA	Having no predictive value
Caner *et al*. 2008	36	NA	NA[Φ]	IHC/FISH/ Real-time quantitative PCR	IHC (2+/3+);	61.1%	IHC and FISH: 51.9% (14/27) IHC and PCR: 52.8% (19/36)	-
Olsson *et al*. 2012	201	T1: 159, Unknown: 42	G2: 31, G3: 170	IHC	IHC (1+, 2+)	12.4%	-	-
Kolla *et al*. 2008	90	Node negative: (T2: 30, T3a: 13, T3b: 13, T4: 8) Node positive: 26	G2: 22 G3: 68	IHC	IHC (2+/3)	IHC: 55.6%	-	Disease-free survival (DFS), Disease-related survival[#]
Tsai *et al*. 2007	114	pT3a: 36, pT3b/4: 78	G1/2: 33, G3: 81	IHC	IHC (1+, 2+) [ε]	41%	-	PFS, Disease-specific OS
Agrawal *et al*. 2020	93[θ]	NA	NA	IHC/FISH	2013 ASCO/CAP guidelines for breast cancer	33.3%	25.8% (8/18)[ω]	PFS
Chen *et al*. 2012	404	Ta: 271, T1: 133	PUNLMP: 36, LG-UC: 190, HG-UCL: 178	IHC/FISH	2007 ASCO/CAP guidelines for breast cancer	cut-off 30%: 7.4%; cut-off 40%: 5.9%; cut-off 50%: 42.5%	Cut-off 30%: 53.3% (16/30) Cut-off 40%: 66.7% (16/24) Cut-off 50%: 88.2% (15/17)	Recurrence and progression

IHC: immunohistochemistry; FISH: fluorescence *in situ* hybridization; PFS: progression-free survival; OS: overall survival; CEP: centromeric probe for chromosome; -: not mentioned; NA: not available; PUNLMP: papillary urothelial neoplasms of low malignant potential; LG-UC: low grade urothelial carcinomas; HG-UC: high-grade urothelial carcinomas; #: HER2 was as an independent predictor; &: all of the included patients with invasive urothelial bladder cancer; Ω: Spanish: 111, Greek: 102; Φ: high-grade invasive urothelial carcinoma; ε: - (staining reaction in 5% of tumour cells or the lack of any immunoreactivity), 1+ (staining reaction in 5–50% of tumour cells), 2+ (staining reaction in 50% of tumour cells); θ: 25 MIBCs and 68 NMIBCs; ω: none of the HER2 2+ tumours showed gene amplification.

Mixed results have been obtained on the prognostic value of HER2 in UC. In a study using IHC to evaluate the HER2 status in patients with advanced UC, a univariate analysis showed that the HER2 status was significantly associated with PFS and disease-specific OS in patients receiving methotrexate, vinblastine, epirubicin, and cisplatin (M-VEC) as adjuvant chemotherapy, but not in those receiving surgery alone[25]. Nedjadi *et al*.[19] found that, amplification of HER2/neu gene was associated with tumor grade, disease aggressiveness, and poor disease-specific survival. Moreover, Agrawal *et al*.[22] found that the worse PFS was significantly correlated with HER2 3+ protein overexpression in 93 bladder cancer patients, including 25 cases of muscle-invasive bladder cancers (MIBC) and 68 cases of non-muscle invasive bladder cancers (NMIBC)[22].

HER2 mutation

In addition to HER2 protein overexpression and gene amplification, mutations in HER2 have also been reported. HER2 mutants can also drive HER2 signaling by enhancing HER2 kinase activity or receptor dimerization[31,32]. According to the American Association for Cancer Research (AACR) GENIE dataset, the frequency of HER mutations in bladder cancer is 11.6%[33]. According to the Catalogue of Somatic Mutations in Cancer (COSMIC) database, 55 non-synonymous HER2 mutations have been identified[34]. HER2 mutations have been reported across the entire gene; however, they were primarily found in the ECD (primarily S310F/Y) and kinase domain (KD) (primarily exon 20 insertions/deletions, L755S, V777L, V842I, and D769N/Y/H). In bladder cancer, S310F/Y occurs most frequently[35,36]. The S310F mutation was first found in a 71-year-old female patient with IV high-grade UC using NGS, and it was identified as an activating mutation[37]. Madison *et al*.[38] observed a frequency of 23% and a frequency of 9.8% for S310F and S310Y mutations, respectively, in UC. In addition, HER2 exon 20 insertions have only been found in 3.6% of patients with UC, with the A775insYVMA mutation corresponding to 95% of the cases inclusively. Similar rates have been reported in the TCGA (The Cancer Genome Atlas) cohort, with 45 out of 407 (11%) MIBCs harboring missense single-nucleotide (SNV) variants in HER2, and almost half of the mutations falling into the Furin-like domain at the position 310 aa of the ECD[18].

In a study examining the impact of HER2 mutations on the *in vitro* sensitivity of bladder cancer to lapatinib, six ERBB2 mutations (L15F, R143Q, D277H, S310F, S653C, and R678Q) were found in 5 out of 33 advanced urothelial bladder cancer cell lines (15%). Overall, the urinary bladder cancer (UBC) cell lines harboring the HER2 mutations S653C, R678Q, and S310F were more sensitive to lapatinib than HER2 WT cell lines[39]. Nagano *et al*.[34] found that most of the ectodomain and C-terminal domain HER2 mutants conferred sensitivity to trastuzumab, whereas most of the tyrosine kinase domain mutants were resistant to the antibody. Furthermore, L755P/S mutants were found to be resistant to lapatinib, afatinib, and neratinib. Only osimertinib demonstrated good efficacy against L755P/S mutants and compound mutations involving L755S.

CLINICAL STUDIES

In the past decades, several studies evaluated HER2-targeted therapies for mUC, but only a few studies found they were clinically beneficial. The clinical trials with results on HER2-targeted therapy in HER2-positive advanced/metastatic UC are summarized in **Table 2**.

Tyrosine kinase inhibitors

Lapatinib, as a reversible TKI, can block both HER1 and HER2, thus inhibiting the activation of MAPK, PI3K-AKT (protein kinase B), and phospholipase C γ (PLCγ) downstream signaling pathways[34]. It can cross the blood-brain barrier and has been evaluated in various clinical settings for the management of mUC. However, it was not shown to have obvious efficacy[40-45]. A phase I study showed that lapatinib at 750–1250 mg combined with GC appeared to be safe and well-tolerated in the treatment of metastatic bladder cancer. Moreover, a promising ORR was demonstrated in 10 (58.8%) patients[41]. In contrast, docetaxel plus lapatinib failed to provide sufficient efficacy in a trial on patients with advanced UC (ORR: 8%, DCR: 39%)[44]. A single-arm, multicentre, open-label, prospective phase II study assessed lapatinib as the second-line treatment for patients with locally advanced or metastatic transitional cell carcinoma (TCC) and achieved a low response rate. The primary endpoint of an ORR >10% was observed in 1.7% of patients, and 18% patients attained stable disease (SD). The median OS was 17.9 weeks[40]. Another phase II study of lapatinib in patients with HER2-amplified solid tumors also yielded a low response rate and unsatisfactory clinical efficacy (ORR: 33.3%) in HER2-positive mUC[45]. In a study examining the efficacy of maintenance lapatinib in patients with metastatic bladder cancer after first-line chemotherapy, 232 patients with HER1/HER2 positive disease were randomly assigned (1:1) to either a maintenance lapatinib or a placebo group. As a result, the median PFS was 4.5 (95% CI: 2.8–5.4) and 5.1 months for the lapatinib and placebo arms, respectively. The OS for lapatinib and placebo lasted 12.6 and 12.0 months, respectively. However, subset analysis demonstrated no significant improvement in PFS or OS in patients with HER2-positive cancer[42]. Structural modelling was performed to elucidate the possible mechanisms underlying HER2 kinase domain mutations, such as T798M, L755S and L755P, that may be involved in lapatinib resistance. All of the mutants showed enhanced MAPK signaling compared to both the wild type and lapatinib-sensitive HER2 mutants[46]. Although lapatinib failed to show the survival benefits in the

aforementioned clinical trials, a recent study suggested that lapatinib in combination with piroxicam, as the first-line treatment, showed encouraging results in terms of the rate of durable response, survival, and tolerability in 44 dogs, as compared with piroxicam alone[47]. Thus, lapatinib should be further investigated as a therapeutic for human advanced urothelial carcinoma.

Table 2. Clinical trials on HER2-targeted therapy for patients with HER2-positive advanced/metastatic urothelial carcinoma

Treatment regimen		Trial	Phase	Patients	Lines of therapy	Definition of HER2 status	ORR, %	DCR, %	OS	PFS
TKIs	Lapatinib	Wülfing *et al*, 2009	2	59 (HER-2positive: 25)	Second–line	HER2positive: IHC (2+/3+)	2%	40%	NA	NA
	Lapatinib	Galsky *et al*, 2012	2	9	Refractory	HER2 amplification: FISH (HER2/CEP17 ≥2)	33.3%	NA	NA	NA
	Lapatinib + docetaxel	Tang *et al*, 2016	2	15	Second–line	Not selected for HER2 status	NA	NA	NA	NA
	Lapatinib + cisplatin + gemcitabine	Cerbone *et al*, 2016	1	17	First–line	Not selected for HER2 status	58.8%	82.4%	NA	NA
	Lapatinib	Powles et at, 2017	3 versus placebo	232 (HER-2positive: 130)	First–line	HER2 positive: IHC 2+/3+ or IHC + with FISH positive	NA	NA	HR: 0.96 (0.70-1.31)	HR: 1.07 (0.81–1.43)
	Afatinib	Kwak *et al*, 2013	2	3	Refractory	HER2 polysomy: FISH (≥4 genes copies in ≥40% of cells); HER2 amplification: FISH (HER2/CEP7 ratio >2.2)	0%	NA	NA	NA
	Afatinib	Choudhury *et al*, 2016	2	23 (HER-2positive: 4)	Refractory	2013 ASCO/CAP guidelines for breast cancer	8.7%	39.1%	NA	21.7% met PFS3 (75% for HER2–positive)
	Neratinib	Hyman *et al*, 2018	2	16	Refractory	HER2 mutation: NGS	0%	18.8%	NA	1.8 months
mAb	Trastuzumab + carboplatin + paclitaxel	Hussain *et al*, 2007	2	44	First-line	HER2 expressing: IHC (2+/3+) or serum HER2–ECD >16 ng/mL; HER2 amplification: FISH (HER2/CEP17 >2) HER2positive: IHC positive or serum HER2–ECD positive or FISH positive	70%	81.8%	14.1 months	9.3 months

	Gemcitabine + platinum± trastuzumab	Oudard *et al*, 2015	2	61	First–line	2013 ASCO/CAP guidelines for breast cancer	53.2%	75.1%	NA	8.2 months
	Paclitaxel + irradiation±trastuzumab[δ]	Michaelson *et al*, 2017	1/2	66 (HER2+: 20)	Refractory	IHC (2+/3+)	NA[γ]	NA	NA	NA
	Trastuzumab + Pertuzumab	Hainsworth *et al*, 2018 (MyPathway)	2a	9	Refractory	HER2 expressing: IHC (3+) HER2 amplification: FISH/CISH (HER2/CEP17>2.0 or copy number>6); HER2 mutation: NGS[λ] HER2positive: IHC 3+ or FISH/CISH positive or NGS positive	33.3%	55.6%	NA	NA
	Trastuzumab+Pertuzumab	Meric-Bernstam *et al*, 2021	2a	22	Refractory	NA	18.2%	NA	NA	NA
ADCs	Ado–trastuzumab emtansine (T–DM1)	Li *et al*, 2018	2	NA	Refractory	HER2 amplification: NGS or FISH (HER2/CEP17 ≥2); HER2 expressing: IHC (3+)	0%	NA	NA	NA
	Trastuzumab deruxtecan (T–Dxd)	Galsky *et al*, 2022	1b	34 (HER2 high expressing: 30)	Refractory	HER2 high expressing: IHC (2+/3+); HER2 low expressing: IHC (1+)	36.7%[μ]	NA	6.9 months[μ]	11.0 months[μ]
	Trastuzumab deruxtecan (T–Dxd)	Meric-Bernstam *et al*, 2023	2	41	Refractory	2016 ASCO/CAP guidelines for gastric cancer	39.0%	NA	7.0 months	12.8 months
	Trastuzumab–duocarmazine	Banerji *et al*, 2019	1	16	Refractory	2013 ASCO/CAP guidelines for breast cancer	25%	93.8%	NA	3.5 months
	RC48–ADC	Xu *et al*, 2021	1	4	Refractory	2013 ASCO/CAP guidelines for breast cancer	0%	25%	NA	NA
	RC48–ADC	Sheng *et al*, 2023	2	107	Refractory	2013 ASCO/CAP guidelines for breast cancer	50.5%	82.2%	14.2 months	5.9 months
	RC48–ADC + toripalimab	Sheng *et al*, 2023	1b/2	41	Any line	2013 ASCO/CAP guidelines for breast cancer	73.2%	90.2%	NA	NA

mUC: metastatic urothelial carcinoma; IHC: immunohistochemistry; FISH: fluorescence *in situ* hybridization; NGS: next-generation sequencing; PFS: progression-free survival; OS: overall survival; CEP: centromeric probe for chromosome 17; ORR: objective response rate; DCR: disease control rate; +: with; ±: with or without; CEP: centromeric probe for chromosome; NA: not available; HR: hazard ratio; δ: patients with HER2-positive accepted trastuzumab; λ: well-recognized activating mutations or unusual mutations reported 2 times in COSMIC; μ: HER2 high expressing.

Afatinib, an irreversible pan-HER inhibitor, did not reach the primary endpoint in a phase II trial[48]. The study defined the primary endpoint as 3-month progression-free survival (PFS3), and 23 patients with HER1/HER2/HER3/HER4 alterations were enrolled. However, in the subgroup analysis of patients with HER2 alterations, three out of four patients with HER2 amplification achieved PFS3, and the median PFS was 6.6 months in patients with HER2/HER3 alterations, while that in HER2/HER3 negative patients was 1.4 months. No unexpected adverse events were observed. Notably, one patient with both HER1 and HER2 amplification as well as HER3 mutation had a strong clinical response[48]. In contrast, another study including only three UC cases with HER2-alteration failed to demonstrate promising clinical benefits (ORR: 0%)[49]. The small sample size may be a contributor to the failure of these studies.

Neratinib is a small-molecule, irreversible pan-inhibitor that binds to HER1, HER2, and HER4, inhibiting their tyrosine kinase activity[50]. The effect of neratinib on HER2-mutant tumors was tested in a basket clinical study involving 141 patients (125 with HER2 mutation and 16 with HER3 mutation)[36]. Neratinib demonstrated the greatest activity in breast cancer patients (ORR at eight weeks: 32%), while no responses were observed in 16 patients with bladder cancer, suggesting that histological difference may impact the efficacy of neratinib[36]. Dacomitinib is an irreversible tyrosine kinase inhibitor that blocks the catalytic domains of HER1, HER2, and HER4. Preclinical data showed that, compared to trastuzumab, cetuximab, and lapatinib, dacomitinib treatment enhanced growth inhibition in HER2-expressing bladder cancer cells and in a mouse xenograft model of UC[51].

Pyrotinib is a novel irreversible pan-receptor tyrosine kinase inhibitor targeting EGFR, HER2, and HER4, and has achieved significant improvement in progression-free survival when used with capecitabine in HER2-positive metastatic breast cancer[52]. Despite shortage of data about pyrotinib in the treatment of advanced UC, a case report described a patient with metastatic bladder urothelial carcinoma (BUC) carrying a HER2 V842I mutation who had been successfully treated with pyrotinib[53]. Based on this case, pyrotinib can be considered as an optional treatment for mUC and is worth further investigation.

Monoclonal antibody

Trastuzumab is an immunoglobulin G1 (IgG1) monoclonal antibody against HER2. It interferes with HER2 signaling through various mechanisms: inhibiting ligand-independent intracellular signaling pathways by binding to domain IV of the ECD; binding to the Fcγ receptor of natural killer cells and triggering antibody-dependent cell-mediated cytotoxicity; inhibiting HER2 receptor dimerization, internalization and/or degradation[6,54]. In a phase II trial on mUC with HER2 overexpression, trastuzumab has been investigated in combination with gemcitabine, carboplatin/cisplatin in chemotherapy-naive patients. The study demonstrated a high initial response rate of 65.5% and 53.2% in the group given chemotherapy alone and the group treated with trastuzumab plus chemotherapy, respectively, but failed to show superiority of the combined treatment over chemotherapy. The main grade 3/4 toxicity was myelosuppression, and this was not enhanced by trastuzumab. However, an exploratory analysis showed that trastuzumab-treated patients receiving cisplatin had better outcomes than carboplatin (PFS: 10.6 versus 8.0 months; OS: 33.1 versus 9.5 months). The authors ascribed the negative result of this study to the low frequency of HER2 positivity in the screened population (75 positive patients among 563 screened patients, of which 61 met the eligibility criteria)[55]. However, in a phase II study, trastuzumab with other chemotherapeutics (carboplatin, paclitaxel, and gemcitabine) demonstrated a promising result in patients with HER2-overexpressing advanced UC (ORR: 70.5%, DCR: 81.8%, PFS: 9.3 months, OS: 14.1 months)[56]. Another study treated muscle-invasive UC patients who were not surgical candidates with radiation and paclitaxel for seven weeks, but trastuzumab was added in patients with HER2 overexpression. Finally, a comparable efficacy and toxicity were observed in patients with or without receiving trastuzumab (CR [complete response]: 61.5% versus 62.5%)[57].

Pertuzumab is a humanized monoclonal antibody against HER2 that efficiently blocks HER2 receptor dimerization by binding to domain II of the extracellular domain[54]. A basket trial (MyPathway) evaluated the efficacy and safety of several targeted therapies for various solid tumors. Pertuzumab plus trastuzumab were used to treat patients with HER2 alterations. Finally, three out of nine patients with advanced bladder cancer showing HER2 amplification/overexpression responded (one CR ongoing at 15 months; two PR [partial response] lasting 1 and 6 months), and two patients had SD over 120 days[58]. In another phase II basket trial evaluating the efficacy of pertuzumab with trastuzumab in HER2-positive advanced solid tumors, 22 patients with mUC were enrolled and four patients achieved objective response[59].

Antibody-drug conjugates (ADCs)

Antibody-drug conjugates (ADCs) comprise three major components, a monoclonal antibody recognizing a specific tumor-associated antigen, a cytotoxic payload, and a linker molecule. The technology aims to deliver cytotoxic therapeutic agents / therapeutics directly to tumor cells while avoiding offense to normal tissues. Upon binding to the target antigen by the antibody construct, the conjugated molecule is internalized by endocytosis and the linker is broken down, thereby releasing its cytotoxic payload (usually a DNA-damaging agent or microtubule inhibitor) inside the cell[8,14,15]. Several promising ADCs targeting HER2 for mUC are currently under evaluation.

Trastuzumab emtansine (T-DM1), with a drug-to-antibody ratio (DAR) of 3.5:1, is an ADC that consists of trastuzumab,

a non-cleavable linker, and microtubule inhibitor emtansine (DM1)[60]. In a preclinical model, exposure of HER2-expressing UC cell lines and xenograft models to T-DM1 resulted in a higher level growth inhibition than the control IgG or trastuzumab alone[23]. A phase II basket trial involving 58 patients examined the response of advanced solid tumors (non-breast non-gastric cancers) with HER2 amplification to T-DM1, with the ORR being 26%. However, no responses were seen in the small cohort of patients with bladder cancer[61].

RC48-ADC, consisting of hertuzumab conjugated to monomethyl auristatin E (MMAE) via a protease-cleavable linker, is a novel humanized anti-HER2 antibody that is thought to have stronger HER2 affinity than trastuzumab[29]. A phase I trial investigated the toxicity, maximum therapeutic dosage (MTD), pharmacokinetics (PK), and antitumor activity of RC48-ADC in HER2-positive metastatic solid carcinomas. The study demonstrated that RC48-ADC was well tolerated and had promising anti-tumor activity in late-stage solid tumors (including 4 UC)[62]. Thus, further studies have emerged to explore the benefits of RC48. A combined analysis of two phase II clinical trials, open-label, multicenter, single-arm (RC48-C005 and RC48-C009) was completed in 107 patients with HER2-positive (either 2+ or 3+ by IHC) mUC in whom at least one prior line of systemic chemotherapy failed[63]. The ORR was 50.5% and the PFS was 5.9 months and similar responses were observed in patients previously treated with anti-PD-1/L1 therapies or those with liver metastases. Toxicity was anticipated for a microtubule inhibitor, leading to sensory neuropathy (68.2%), leukopenia (50.5%), AST increased (42.1%), and neutropenia (42.1%). ADCs combined with immunotherapy have also attracted attention. The efficacy and safety of RC48-ADC combined with toripalimab were reported at the 2023 ASCO conference[64]. Forty-one patients were examined and HER2 expression was positive (IHC 3+ or 2+ ISH+) in 59% of the patients. The proportion of liver metastasis was 24%. The results were encouraging (ORR: 73.2%, DCR: 90.2%). The ORR was 83.3%, 64.3% and 33.3% for patients with HER2 2/3+, HER2 1+ and HER2 0, respectively. The most common adverse events were AST/ALT increase (68.3%), peripheral sensory neuropathy (61.0%), asthenia (61.0%), γ-glutamyl transferase increase (56.1%), hypertriglyceridemia (53.7%), and appetite decrease (51.2%). In this study, patients with low HER2 expression still benefited from RC48-ADC. Another phase II study began exploring the activity and safety of RC48-ADC in HER2-negative (IHC 0/1+) mUC in august, 2019. As of February 2022, 19 patients were enrolled, the ORR was 26.3% (17% in liver metastasis patients), the DCR was 94.7%, and the safety profile was similar to that reported previously[65].

Trastuzumab deruxtecan (T-DXd) is an ADC consisting of a trastuzumab, a topoisomerase I inhibitor- exatecan derivative, and a cleavable tetrapeptide-based linker. Compared with T-DM1, T-DXd has a higher drug-to-antibody ratio of 8:1, thus making it potent even in low HER2-expressing cancers due to the bystander killing effect[60]. Preclinical data have shown that T-DXd may induce immune responses other than the cytotoxic effect on tumor cells. In this study, T-DXd increased tumor-infiltrating CD8+ T cell levels and upregulated the expression of PD-L1 and MHC class, thus enhancing antitumor immunity. Therefore, a high payload specificity can improve ADC activation even in low HER2-expressing tumors. Furthermore, using antibodies with higher affinity for HER2 or ADCs with higher DAR may overcome resistance due to tumor heterogeneity, and may prove to be effective even in low HER2-expressing tumors[66]. T-DXd in combination with an anti-PD-1 antibody was shown to be more effective than either of the monotherapies[67]. DS8201-A-U105 was a phase Ib study exploring the application of T-DXd with nivolumab (nivo) in the treatment of UC with HER2 expression[68]. Until July 22, 2021, in the cohort of patients with high HER2 expression (IHC 2+/3+), the ORR was 36.7% (CR: 13.3%, PR: 23.3%), and mPFS and mOS were 6.9 and 11.0 months, respectively. Grade (G) ≥3 treatment-related adverse events took place in 73.5% of patients (44.1% related to T-DXd), and the most concerned adverse reaction was interstitial pneumonia, with an incidence rate of 23.5%. In addition, one patient developed grade 3 cardiac dysfunction. Therefore, this treatment should be carefully monitored for the occurrence of serious adverse events in clinical applications. DESTINY-PanTumor02 (DPT-02) is a global, multicenter, open-label phase II clinical study designed to evaluate the efficacy and safety of Enhertu (5.4mg / kg q3w) in the treatment of HER2-positive tumors[69]. Between October 7, 2020, and July 7, 2022, 267 patients were enrolled in multiple regions of Asia, Europe and North America totally. In the bladder cohort, 41 patients received treatment and demonstrated durable clinical benefit and meaningful survival outcomes (ORR: 39%, median OS: 12.8 months). Furthermore, the safety finds in this study were consistent with those previously reported by DS-8201, there is no new safety signal, and the overall safety is controllable and tolerable.

Other trastuzumab-based ADCs are also being explored for the treatment of mUC. Trastuzumab duocarmazine (SYD985) is comprised of trastuzumab, a cleavable linker, and a cytotoxic agent of duocarmycin (a DNA alkylator)[60]. Recently, a phase I dose-escalation and dose-expansion study assessed the safety and activity of SYD985 in advanced solid tumors. Partial responses were observed in four (25%, 95% CI, 7.3–52.4) out of 16 patients with local or advanced urothelial cancer[70].

In recent years, a new class of ADCs using immunotherapy has been developed. Margetuximab is an anti-HER2 antibody with an enhanced affinity to CD16A, an Fc receptor important for ADCC binding to tumor cells. It has promising activity levels even among patients with trastuzumab-resistant solid

tumors[71]. SBT6065 is an ADC that uses toll-like receptor 8 (TLR8) as a payload by linking to a HER2-directed monoclonal antibody. Preclinical studies in mice treated with SBT6065 demonstrated robust activation of tumor-associated myeloid cells, neutrophil infiltration, increased local cytokine and chemokine production in models resistant to checkpoint blockade, and no evidence of systemic toxicity, such as cytokine release syndrome[72]. DN24-02 is an autologous cellular immunotherapy targeting HER2, comprised of antigen-presenting cells (APC) cultured with BA7072 and a recombinant HER2-derived antigen (HER500) conjugated to granulocyte macrophage colony-stimulating factor. A study showed no statistically significant differences in terms of OS and distant relapse-free survival (DRFS) between the DN24-02 arm and the standard care arm in HER2-positive UC patients with high relapse risk after surgery[73]. Another promising ADC is HER2-targeted biparatopic antibody-drug conjugates, which combine payloads with bispecific antibodies that inhibit multiple pathways. ZW49 is a bispecific ADC that links ZW25, a HER2-targeting antibody binding trastuzumab and pertuzumab, to a novel auristatin payload[60,74]. A preclinical study demonstrated that this treatment elicited more potent anti-tumor activity in both low and high HER2-expressing cell lines than a monospecific trastuzumab-ADC[75]. Further clinical studies are needed to confirm its efficacy in mUC. In the past few years, immunotherapy and antibody-drug conjugates (ADCs) have improved the treatment result of advanced UC, and study RC48-C014 (NCT04264936) [64] have showed great promise in the field of ADC combined with immunotherapy.

The study on HER2-targeted therapies for mUC is a growing field. Apart from the above studies, various novel approaches are being currently studied. Ongoing trials targeting HER2 in metastatic/advanced urothelial carcinoma are summarized in **Table 3**.

CONCLUSION AND PERSPECTIVES

Over the past few years, substantial progress has been made in both basic and clinical research. UC ranks third in terms of HER2 overexpression, second only to breast cancer and gastric cancer. Studies on anti-HER2 treatment have not achieved any breakthrough but anti-HER2 therapy still shows great prospects, especially in the area of ADCs. RC48-ADC is effective and safe for the treatment of HER2-positive mUC after first-line chemotherapy, and it contains an antibody with a HER2 affinity stronger than trastuzumab, which can lead to increased HER2 selectivity for its antitumor effect[76].

Resistance to HER2-targeting therapy can be seen in a great many tumors. Intrinsic and acquired resistance of metastatic cancer to HER2-targeted therapy was culpable for the failure of many studies. Major mechanisms of intrinsic resistance to anti-HER2 therapies include: (1) inactive target receptor, with HER2 carboxy terminal fragments (also known as p95HER2) being resistant to trastuzumab[77,78] in breast cancer (2) aberrant activation of target downstream components in the PI3K/Akt/mTOR and Ras-Raf-MAPK signaling pathways[51,79,80]; (3) overexpression of other HER ligands or receptors[6,9]; (4) alternative signaling from other receptors (such as the insulin-like growth factor-1 receptor (IGF1R)) [33,81]; (5) aberrant signaling caused by downregulation or loss of downstream controllers[13]; (6) influence of the tumor microenvironment[82]. Acquired resistance mostly occurs as a consequence of alterations taking place at the level of target signals or on an active target receptor[33]. HER2 loss owing to exposure to trastuzumab is an established phenomenon in both breast and gastric cancer patients[6]. In a previous study, HER2 loss was found in re-biopsied tumor tissues of 20 patients (60.6%) with refractory disease in 33 eligible HER2-positive patients, and the number of patients positive for IHC 3+ HER2 greatly dropped after therapy[35]. Therefore, re-evaluation of HER2 status may be necessary after HER2-targeted therapy. Combination therapy, such as combination with chemotherapy, cell signaling inhibitors (PI3K, Akt, and other proteins) or immunotherapeutic agents, is a promising strategy.

This review showed that the rate of protein overexpression and gene amplification varied substantially with UC patients. To some extent, this variability could be principally attributed to the difference in patients' features, such as race, molecular subtype, specimen selection spots, and intratumor heterogeneity. So, in specimen selection, paraffin-embedded tumor tissue obtained by surgical excision or biopsy (multipoint biopsy) should be used for HER2 detection. Furthermore, the lack of standardized methodology and interpretation criteria cannot be ignored since the detecting methods vary greatly. Thus, further studies are needed to explore the optimal cut-off value and mechanisms underlying HER2 protein overexpression (excluding gene amplification). Moreover, loss of HER2 positivity is frequently observed in solid cancers after HER2-targeted therapy. Therefore, reassessment of the HER2 status in primary tumors and metastases is very much warranted during disease progression.

The clinical benefit of HER2-targeted therapy remains to be clarified as various clinical trials failed to demonstrate significant clinical efficacy. Thus, further clinical studies are necessary to know whether HER2-targeted therapy is efficient in specific populations, which has significant implications for the precision and individualized treatments of UC.

Table3. Summary of ongoing clinical trials targeting HER2 in metastatic/advanced urothelial carcinoma

Drugs	Trial	Phase	Line	Definition of HER2 status	Status
Afatinib Dimaleate	NCT02122172	2	Refractory	NA	Recruiting
Afatinib Dimaleate Afatinib Pertuzumab Trastuzumab Trastuzumab Emtansine	NCT02465060	2	Refractory	NA	Active, not recruiting
Trastuzumab Pyrotinib	NCT05318339	2	Refractory	NA	Recruiting
BDTX-189	NCT04209465	1/2	Refractory	NA	Terminated
TAS0728	NCT03410927	1/2	Refractory	NA	Terminated
Trastuzumab Tucatunib	NCT04579380	1	Refractory	NA	Active, not recruiting
Trastuzumab emtansine	NCT02675829	2	Refractory	HER2 amplification: NGS or ISH (HER2/CEP17 ratio ≥2.0)	Recruiting
Trastuzumab deruxtecan Nivolumab	NCT03523572	1b	Refractory	HER2 expressing: IHC 2+ or 3+ HER2 amplification: ISH*	Unknown status
Trastuzumab deruxtecan	NCT04639219	2	Refractory	HER2 mutations only	Active, not recruiting
Trastuzumab deruxtecan	NCT04482309	2	Refractory	NA	Active, not recruiting
RC48-ADC	NCT04879329	2	Second line	HER2 expressing: IHC 1+, 2+ or 3+	Not yet recruiting
RC48-ADC	NCT04073602	2	Refractory	HER2 negative: IHC - or 1+	Unknow status
RC48-ADC Triplizumab Gemcitabine Cisplatin Carboplatin	NCT05302284	3	First line	HER2 expressing: IHC 1+, 2+ or 3+	Recruiting
BDC-1001 Nivolumab	NCT04278144	1/2	Refractory	NA	Recruiting
MRG002	NCT04839510	2	Refractory	HER2 expressing: IHC 2 + or 3+	Recruiting
PRS-343 atezolizumab	NCT03650348	1b	Refractory	NA	Unkown status
PRS-343	NCT03330561	1	Refractory	NA	Completed (Last Update posted: January 20, 2022)
CAdVEC/CART	NCT03740256	1	Refractory	HER2 expressing: IHC 2+ or 3+	Recruiting
CT-0508	NCT04660929	1	Refractory	NA	Recruiting
ACE1702	NCT04319757	1	Refractory	HER2 expressing: HER2 2+ or 3+	Recruiting
DF1001	NCT04143711	1/2	Refractory	NA	Recruiting

IHC: immunohistochemistry; ISH: in situ hybridization; NGS: next-generation sequencing; CEP: centromeric probe for chromosome; *: scored by American Society of Clinical Oncology/College of American Pathologists (ASCO-CAP) guidelines; NA: not available

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