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Original contribution

Human epidermal growth factor receptor 2 overexpression is frequently discordant between primary and metastatic urothelial carcinoma and is associated with intratumoral human epidermal growth factor receptor 2 heterogeneity☆,☆☆,☆☆☆

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Summary Human epidermal growth factor receptor 2 (HER2) overexpression occurs in 5–10% of primary urothelial carcinomas (UCs) but has not reliably predicted benefit from HER2-targeted agents in the metastatic setting. HER2 testing of primary tumors may not reflect the HER2 status of distant metastases. We assessed the concordance of HER2 expression in paired primary and distant metastatic UC lesions. Specimens from 149 patients with metastatic UC underwent immunohistochemical staining for HER2, including 79 paired primary and distant metastatic tumors. HER2 status was defined using 2018 ASCO/CAP guidelines. HER2 intratumoral heterogeneity (ITH) was defined as HER2 3+ expression in 5–50% of tumor cells. The HER2-positive, -equivocal, and -negative rates observed were 10.6%, 24.7%, and 64.7% for primary tumors and 9.8%, 12.6%, and 77.6% for metastatic tumors, respectively. HER2 ITH occurred in 44% of HER2-positive primary tumors. Low agreement of HER2-positive status between primary and metastatic tumors was observed ($\kappa = 0.193$, $P = 0.079$). Loss of HER2 overexpression in the metastatic lesion was observed in 55% (5 of 9 cases) of HER2-positive primary cases and was associated with the presence of HER2 ITH in the primary tumor (Fisher's exact $P = 0.048$). Change from negative primary to positive metastasis was seen in 2% (1 of 50) of cases. No differences

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in metastasis-free survival or overall survival were observed in accordance with HER2 status defined by either the primary or metastatic lesion. These findings are likely to impact patient selection for HER2 targeted therapies in UC. Confirmation and evaluation of the clinical significance of HER2 discordance is warranted, preferably in the context of a clinical trial.

1. Introduction

Human epidermal growth factor receptor 2 (HER2) is an oncogenic receptor tyrosine-kinase and known drug target that is overexpressed in 5−10% of urothelial carcinomas (UCs) [1,2]. Agents targeting HER2 are routinely used to treat breast cancer (BC) and gastroesophageal cancer (GEC), but trials of these drugs in UC have shown no meaningful benefit [3−7]. Still, some patients experience robust responses and, as newer HER2-targeted drugs show promise, there is renewed interest in targeting HER2 in advanced UC [8−10].

HER2 protein overexpression, determined by immunohistochemistry (IHC), and *ERBB2* gene amplification, quantified by *in situ* hybridization (ISH), are predictive biomarkers of benefit from HER2-targeted agents in BC and GEC [11,12]. HER2-positive disease has been classified in BC and GEC as those tumors showing 3+ protein overexpression in >10% of tumor cells or *ERBB2* gene amplification [11], although these definitions have been variably applied and poorly validated in UC. In large data sets, high concordance between 3+ protein expression by IHC and gene amplification in UC has been observed [1,2,11]. Heterogeneous intralesional HER2 expression has also been observed in UC; however, the clinical significance of this finding remains unknown [2,13−15].

In BC and GEC, intratumoral heterogeneity (ITH) with respect to HER2 expression/amplification has been variably defined yet is consistently associated with lack of benefit from HER2-targeted drugs, suggesting tumors with ITH more readily lose the HER2 target [16−20]. Given that ITH has been observed in UC, loss of HER2 overexpression between primary and metastatic sites could be responsible for failed trials of HER2-targeted agents in metastatic UC when HER2 testing is performed only on primary tumors [21]. This hypothesis is supported by prior biomarker observations from our group, in which discordant PD-L1 expression between primary and paired metastatic lesions was noted [22].

HER2 status is moderately to highly concordant between primary and metastatic lesions in BC and GEC [23−25]. In UC, previous publications also observed at least moderate concordance, but these studies applied nonuniform definitions of HER2 status and were limited to small sample sizes that only examined synchronous, regional lymph node metastases at cystectomy [13,14,26−28]. Based on the premise that interlesional HER2 expression can vary, testing in distant or metachronous UC lesions may more accurately describe metastatic biology as a result of temporal and spatial heterogeneity and provide more accurate predictive value for the use of HER2 targeted agents in UC.

Currently, no data exist describing HER2 expression patterns in primary and distant metastatic sites in advanced UC. Therefore, we sought to characterize HER2 expression in a large cohort of patients with metastatic UC using only metachronous or distant metastases and paired primary tumors. Given the failure of HER2-targeted therapeutic trials in UC to date and the association of ITH with lack of benefit from HER2-targeted therapies in other tumors, we hypothesized that discordant HER2 expression between primary and distant metastatic lesions might be common in UC.

2. Materials and methods

2.1. Patients and specimens

This research protocol was approved by the institutional review board (Advarra). Patients with metastatic UC treated between January 1, 2007 and February 1, 2018 were identified from an institutional tumor database as previously described [22]. We identified 149 subjects with archival metastatic tumor specimens; paired primary bladder tumor specimens were obtained when available. Six metastatic tissue samples were subsequently deemed inadequate quality for analysis, leaving a final cohort of 79 pairs of primary and metastatic specimens, 64 unpaired metastatic specimens, and 6 unpaired primary specimens.

Primary tumors included specimens obtained by cystectomy or transurethral resection. Metastatic lesions included excisional biopsies, core-needle biopsies, fine needle aspirate (FNA) cell blocks, and body cavity washing cell blocks. Synchronous, regional lymph nodes extracted at cystectomy were excluded from testing. Metastatic biopsies were defined as synchronous if obtained < 60 days after the primary tumor biopsy, and metachronous if ≥ 60 days apart. Hematoxylin and eosin stained slides were reviewed by two pathologists with genitourinary oncologic expertise (C.L., A.H.) to confirm diagnosis and assess adequacy for HER2 testing. Samples

containing ≥ 100 viable tumor cells were considered adequate for analysis.

2.2. Immunohistochemistry

Formalin-fixed paraffin-embedded cell blocks were sectioned at 4 microns on positively charged glass slides and immunostained for HER2 using the FDA-approved Ventana Pathway clone 4B5. Classification of HER2 status was defined in accordance with the current 2018 ASCO/CAP guidelines for BC [11]. HER2 ITH was defined in accordance with guidelines for genetic heterogeneity of HER2 in BC but substituting strong positive (3+) staining intensity in 5−50% of tumor cells for *ERBB2* amplification [11,29].

Among tumor pairs, concordant HER2 status was defined as having the same status in both specimens. Discordance between primary and metastasis was defined as having positive status in either specimen and negative status in the corresponding matched sample. Pairs in which either tumor had HER2 equivocal status were labeled as indeterminate.

2.3. Clinical data

Patient characteristics and cancer outcomes were obtained by chart review. The date of definitive local therapy was recorded as the first day of radiation treatment or the date of cystectomy. Metastatic diagnosis was defined as the first radiographic or pathologic confirmation of extravesical disease, excluding definitively treated regional nodal disease.

2.4. Statistics

Descriptive statistics were used to summarize patient characteristics. Fisher's exact test was performed to estimate correlation of HER2 status between paired primary and metastatic tumors and between ITH and loss of HER2 overexpression. To assess the degree of association of percentage HER2 cell expression between primary and metastatic lesions, Spearman's correlation coefficient (ρ) was estimated [22]. Cohen's kappa (κ) statistic was used to evaluate concordance of HER2 status between matched primary and metastatic tumors. A Kappa value less than 0 was considered to have no agreement; 0.01−0.20 considered low agreement; 0.21−0.40 considered fair agreement; 0.41−0.60 considered moderate agreement; 0.61−0.80 considered substantial agreement; >0.80 considered almost perfect agreement [22].

For the survival analysis, metastasis-free survival (MFS) was defined as the time from definitive local therapy to the date of metastasis or death. OS was measured separately for localized tumors (time elapsed from local therapy to death) and metastatic tumors (time from metastatic diagnosis to death). The probabilities of MFS and OS were estimated via the Kaplan-Meier method. Differences in MFS and OS between HER2 score groups were analyzed using a log-rank test.

3. Results

3.1. Patient and tumor characteristics

Demographic details for 149 patient samples are listed in Table 1. There were 79 paired tumors and 70 unpaired tumors: 64 metastatic and 6 primary tumors. The population was primarily Caucasian (82%), male (82%), and current or former smokers (75%). Primary tumor staging was pTis (n = 3, 3.5%), pTa (n = 4, 4.7%), pT1 (n = 17, 20%), pT2 (n = 34, 40%), pT3 (n = 20, 24%), and pT4 (n = 7, 8.5%). A mixed variant histology was reported in 17 of 85 (20%) primary tumors. The most common metastatic biopsy site was lymph node (30%), and the most common nodal sites were retroperitoneal (n = 17), inguinal (n = 7), and mediastinal (n = 7; see Supplementary Table 1). Among paired biopsies, 85% were

Table 1 Patient characteristics.

	Paired tumors, n (%)	Unpaired tumors, n (%)	Full cohort, n (%)
Total subjects	79	70	149
Gender			
Female	14 (17.7)	13 (18.6)	27 (18.1)
Male	65 (82.3)	57 (81.4)	122 (81.9)
Race			
Black	13 (16.4)	10 (14.3)	23 (15.4)
Caucasian	62 (78.5)	60 (85.7)	122 (81.9)
Other/ unknown	4 (5.0)	0 (0)	4 (2.7)
Current/former smoker			
Yes	58 (73.4)	54 (77.1)	112 (75.2)
No	19 (24.1)	16 (22.9)	35 (23.5)
Unknown	2 (2.5)	0 (0)	2 (1.3)
Metastatic biopsy site[a]			
Lymph node	22 (27.8)	21 (32.8)	43 (30.1)
Bone	18 (22.8)	10 (15.6)	28 (19.6)
Lung	14 (17.7)	10 (15.6)	24 (16.8)
Pelvic/GI	8 (10.1)	4 (6.3)	12 (8.4)
Liver	6 (7.6)	9 (14.1)	15 (10.5)
Brain	3 (3.8)	1 (1.5)	4 (2.8)
Other	8 (10.1)	9 (14.1)	17 (11.9)
Metastatic biopsy type[a]			
Excisional	28 (35.4)	13 (20.3)	41 (28.7)
Core needle	28 (35.4)	32 (50.0)	60 (42.0)
Fine needle aspirate	22 (27.8)	16 (25.0)	38 (26.6)
Other	1 (1.3)	3 (4.7)	4 (2.8)
Metachronous	67 (84.8)		
Synchronous	12 (15.2)		

[a] 6 of 70 unpaired tumors were primary lesions.

metachronous with a median time between biopsies of 1.15 years (range: 0.16–7.46).

3.2. HER2 expression patterns in primary and metastatic lesions

HER2 status for primary and metastatic tumor biopsies from the entire cohort is summarized in Table 2. Primary tumors exhibited HER2 status of negative, equivocal, and positive in 55 (64.7%), 21 (24.7%), and 9 cases (10.6%), respectively. Metastatic tumors exhibited HER2 status of negative, equivocal, and positive in 111 (77.6%), 18 (12.6%), and 14 (9.8%), respectively.

HER2 ITH was observed in 4 of 9 (44%) HER2-positive primary tumors and in 1 of 20 (5%) HER2-equivocal primary tumors. There was no observed ITH in 14 cases of HER2-positive metastatic UC. Representative images of HER2-positive and negative disease and HER2 ITH are illustrated in Fig. 1A–C.

3.3. HER2 status in paired primary and metastatic biopsies

Results from comparison of HER2 status in the 79 paired primary tumors and corresponding metastases are shown in Table 3. A moderate correlation was observed for percentage HER2 cell expression ($\rho = 0.47$, $P < 0.001$) and for HER2 status (Fisher's exact $P = 0.036$) between primary and metastatic tumors due to the high prevalence of HER2 negative biopsies. However, the level of agreement for HER2-positive tumors was low ($\kappa = 0.193$, $P = 0.079$). Among discordant tumor pairs, 5 of 9 (55%) primary HER2-positive tumors were HER2-negative in the metastatic setting, and 1 of 50 (2%) primary HER2-negative tumors was HER2-positive in the metastasis. Only 2 pairs were HER2-positive in both the primary and metastatic settings. All discordant metastatic biopsies were metachronous and were obtained >100 days after the primary tumor biopsy.

Table 3 HER2 status in paired tumors.

Primary tumors	Metastatic tumors (n)		
	Negative	Equivocal	Positive
Negative	43	6	1
Equivocal	16	1	3
Positive	5	2	2

HER2, human epidermal growth factor receptor 2.

To assess the impact of ITH on HER2 discordance between primary and metastatic lesions, all tumors showing any percentage of 3+ staining intensity and their corresponding paired biopsies were examined. This consisted of 14 tumor pairs (Supplementary Table 2): 6 discordant, 2 concordant, and 6 indeterminate (due to an equivocal lesion) pairs. All primary tumors with HER2 3+ staining between 10% and 60% of tumor cells exhibited loss of HER2 overexpression in the corresponding metastatic lesion. In contrast, all tumors with HER2 3+ expression in >70% of tumor cells maintained at least 2+ intensity in ≥70% of tumor cells (equivocal status) in the paired lesions. ITH in the primary tumor was significantly

Table 2 HER2 staining characteristics of all tumor specimens.

HER2 status	Primary tumors (n = 85)		Metastatic tumors (n = 143)	
	Total (%)	HER2 ITH (%)	Total (%)	HER2 ITH (%)
Negative	55 (64.7)	0 (0)	111 (77.6)	0 (0)
Equivocal	21 (24.7)	1 (4.7)	18 (12.6)	0 (0)
Positive	9 (10.6)	4 (44)	14 (9.8)	0 (0)

ITH, intratumoral heterogeneity; HER2, human epidermal growth factor receptor 2.

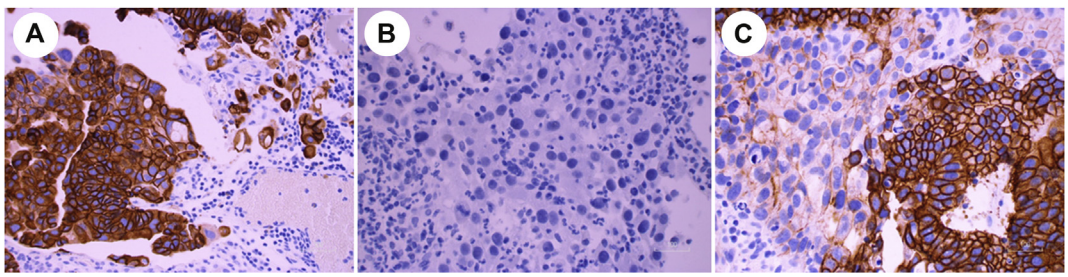

Fig. 1 Patterns of HER2 immunohistochemical staining. Representative photomicrographs of bladder tumor sections demonstrating (A, x400) HER2 positive and (B, x100) HER2 negative disease. Image C (x400) depicts spatial heterogeneity of HER2 expression. HER2, human epidermal growth factor receptor 2.

associated with loss of HER2 overexpression in the metastatic site (Fisher's exact $P = 0.048$).

Six of nine HER2 positive primary tumors harbored one or more variant histologies, including glandular (n = 3), signet ring (n = 1), plasmacytoid (n = 1), sarcomatoid (n = 1), squamous (n = 1), and small cell (n = 1) morphology. Five of the six corresponding paired metastatic lesions were HER2 negative. HER2 expression colocalized with histology in only tumor in which the glandular variant lacked HER2 expression.

3.4. Clinical outcomes by HER2 status

HER2 status has not been consistently associated with adverse survival in prior UC data sets based on a single biopsy for characterization [30], but given the poor agreement of HER2 status between primary and metastatic lesions observed in this study, we assessed the prognostic value of HER2 status from both primary and metastatic tumors separately. No differences in MFS were observed according to HER2 score from either primary (Fig. 2a, $P = 0.349$) or metastatic tumors (Fig. 2b, $P = 0.733$). OS from the date of local definitive therapy was similar when stratified by primary tumor HER2 score (Fig. 2c, $P = 0.707$). Similarly, no differences in OS from the date of metastatic diagnosis were observed when stratified by HER2 score in the metastasis (Fig. 2d, $P = 0.467$).

4. Discussion

Despite negative clinical trials to date, HER2 has remained an appealing therapeutic target in UC to many investigators based on anecdotal responses to HER2 targeted agents, and contemporary clinical trials have continued to use HER2 expression as a biomarker for patient selection. In our cohort, HER2-positive status occurred in 10.6% of 85 primary tumors and 9.8% of 143 metastatic tumors, consistent with other large data sets [2]. HER2 biomarker testing from pelvic nodal metastases has been previously characterized and considered a surrogate for metastatic lesions [13,14,26–28]. However, emerging

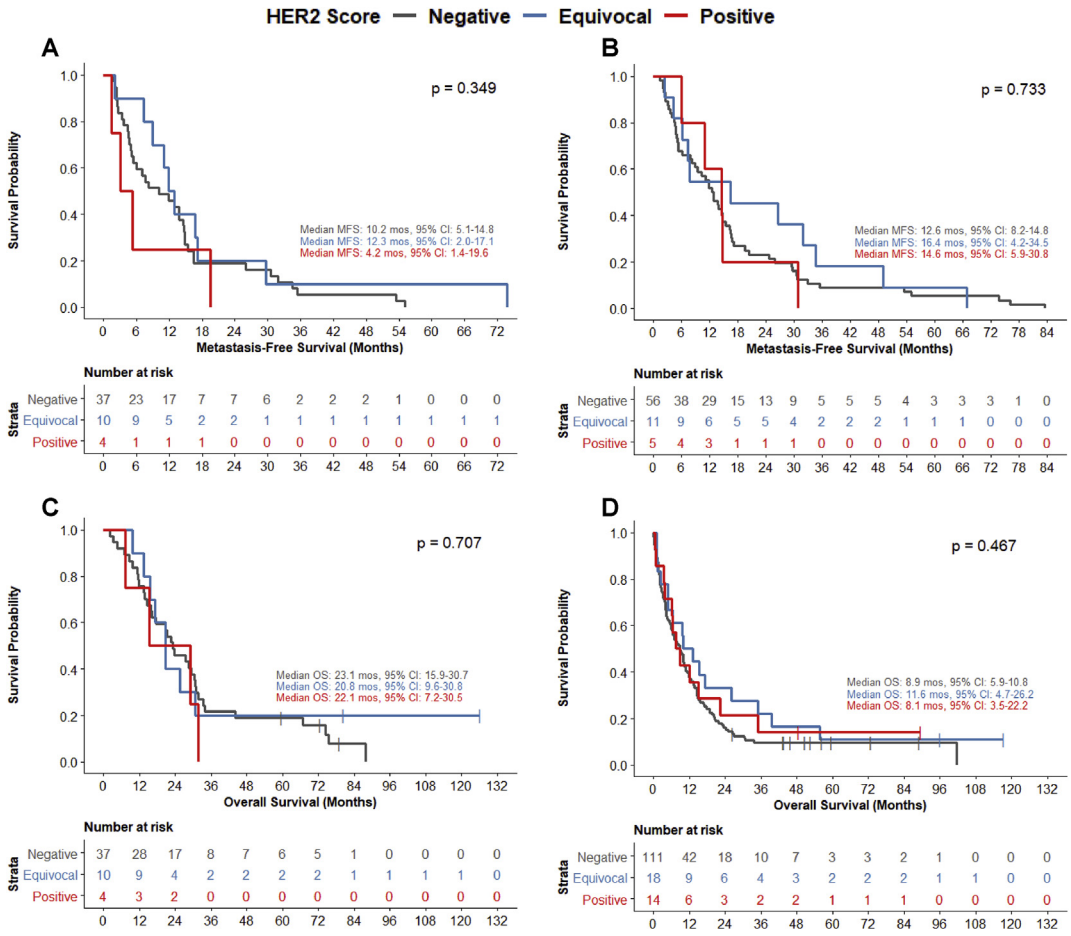

Fig. 2 Kaplan-Meier curves by HER2 status. Panel A, MFS by primary tumor HER2 score. Panel B, MFS by metastatic tumor HER2 score. Panel C, OS from local therapy by primary HER2 score. Panel D, OS from metastatic diagnosis by metastatic HER2 score. HER2, human epidermal growth factor receptor 2.

evidence suggests otherwise, and major genomic differences between nodal and distant metastatic sites can occur as a result of divergent clonal evolution originating within the primary tumor [21,31,32]. Consequently, sampling of distant or metachronous metastatic sites may be necessary for optimal testing of HER2 expression in advanced UC lesions, especially in the context of clinical trial screening with novel HER2-targeted agents.

In our cohort, we found that the rates of HER2 overexpression in primary and metastatic tumors were similar. However, agreement of HER2-positive status between matched primary and metastatic sites was poor, especially when ITH was present in the primary tumor. Loss of HER2 overexpression was frequent (55%), whereas gain of HER2 overexpression was rare (2%) in metastatic sites. These findings contrast with results of other cohorts which reported higher concordance and rare loss of HER2 overexpression in nodal metastases [13,14,26−28]. However, very limited data have been published describing HER2 expression patterns in paired primary and distant, metachronous metastatic lesions, and our cohort represents the largest reported series to date.

HER2 expression in distant or metachronous metastatic UC has been described in three small cohorts numbering 7, 12, and 43 patients [13,28,33]. These studies predate the standardization of HER2 scoring for BC by ASCO/CAP and included some cases in the HER2-positive groups that would now be characterized as HER2 equivocal (2+) [11]. Although the clinical relevance of this categorization has not been validated in UC, it is known that *ERBB2* gene amplification is very uncommon in UC with equivocal HER2 status [2]. The largest prior study, by Gardmark et al., did observe loss of HER2 overexpression in 34% of metastases, and HER2 concordance was inversely related to the distance of the metastasis from the primary tumor [33]. These findings support the observation from our larger, contemporary cohort that loss of HER2 overexpression is higher in distant metastases than in locoregional nodes.

The clinical relevance of discordant HER2 expression in advanced UC lesions remains undefined but has obvious implications for the use and development of HER2-directed therapies in this disease. In BC and GEC, HER2 testing is often repeated in the metastatic setting because HER2 expression status changes in approximately 10% of tumors [11,23−25]. Trials of HER2-targeted agents in metastatic UC have allowed for HER2 testing on archival primary tumor specimens. The observations from our cohort suggest that patients with HER2-negative metastatic disease, despite HER2-positive primary tumors, may have participated in these studies, which may have negatively confounded the trials' results and led to an inaccurate assessment of the drugs' activities in patients with true HER2-positive distant metastases. For example, two randomized trials in advanced HER2-positive UC failed to show benefit with the addition of HER2-directed therapy [5,6]. In both studies, archival tissue from the primary bladder tumor was principally used for characterization of HER2 status to determine molecular eligibility of patients to be enrolled in the study. Although the results of these two clinical trials imply that HER2-targeted therapies may be less effective in HER2-positive UC compared with BC or GEC, our study findings raise an alternate possibility that the metastatic disease burden of many patients enrolled in these studies may have lost HER2 expression and thus were unlikely to respond to HER2-targeted therapies.

We defined ITH in our study as HER2 3+ intensity expressed in 5−50% of tumor cells to match the definition for genetic heterogeneity in HER2-positive BC (defined as HER2 amplification by ISH in 5−50% of tumor cells) [29]. ITH was more common in primary positive tumors than in metastatic positive tumors in our data set and may be a reflection of the high degree of histologic and genomic heterogeneity previously reported in primary UC [21,31]. Given these findings, we hypothesized that primary tumors with HER2 ITH would be more likely to show discordant expression within metastatic lesions. As anticipated, all primary HER2-positive tumors with ITH, including those with up to 60% tumor cell expression, were HER2-negative in the metastatic setting, whereas no primary tumors with >70% expression showed loss of expression in metastatic site biopsies in this cohort. Loss of HER2 overexpression was also observed at a high rate among primary HER2 positive tumors with mixed variant histology, suggesting that other measures of ITH may also predict for HER2 discordance. Similar observations have been reported in BC and GEC, in which HER2 ITH has been associated with attenuated benefit from HER2-targeted therapies [16−20]. Thus, we propose that primary tumor HER2 ITH in UC may serve as a biomarker to identify patients at risk for discordant expression in metastatic sites and potentially lack of response to HER2 targeted therapies. Because HER2 ITH may be prevalent in primary UC, further investigation to validate these findings and the potential impact on the use of HER2-directed therapies should be pursued.

Neither HER2 overexpression nor *ERBB2* amplification in primary UC have been consistently associated with prognosis or response to therapy [30]. We sought to determine whether the lack of prognostic value of HER2 status in UC might be influenced by expression discordance. We examined MFS and OS outcomes by HER2 status from both the primary and metastatic lesions separately and found no differences in outcome (Fig. 2). Our findings reinforce prior observations that HER2 status is not prognostic in advanced UC, though we also note that due to the size of our cohort, we ultimately cannot make definitive conclusions about the prognostic value of HER2 based on the tumor site used for HER2 classification.

We acknowledge several limitations to our study. Despite reporting the largest cohort of paired distant metastases to date, the overall low incidence of HER2 overexpression in UC might affect the robustness of statistical tests and limit the generalizability of our findings. Second, without ISH testing for *ERBB2* amplification, we were unable to reclassify any HER2 equivocal tumors as positive which is routinely performed for BC and GEC. Most studies in UC, including an analysis of 1005 primary tumors, have reported that *ERBB2* amplification occurs almost exclusively in IHC positive tumors [2]. Furthermore, the biological relevance of *ERBB2* amplification without concurrent HER2 overexpression is unknown and has been questioned by a recent study suggesting that loss of HER2 expression in amplified tumors occurs through secondary genetic or epigenetic events [34]. Third, nonsurgical biopsies represented approximately two-thirds of metastatic biopsies in our cohort. However, the accuracy of HER2 testing in nonsurgical biopsies has been extensively evaluated in both BC and GEC, where >95% concordance is reported for core biopsies, FNA cell blocks, and surgical specimens when modern fixation techniques are used [11,12,35]. Thus, we believe HER2 testing using non-surgical biopsies to be appropriate. Finally, HER2 overexpression and *ERBB2* genomic aberrations have been reported at high frequencies in micropapillary UC, a rare histologic variant which carries a particularly poor prognosis [15,36,37]. Given that no cases of micropapillary UC were included in this study, our findings should not be generalized to this population and require confirmation in a separate cohort.

5. Conclusions

HER2-positive primary UC with ITH frequently shows loss of overexpression in metastatic sites. Loss of the HER2 target in metastatic disease may be clinically significant in predicting lack of response to HER2-targeted therapy. Future clinical trials assessing the efficacy of HER2-targeted therapy in metastatic UC should assess for HER2 ITH and make efforts to test HER2 status in both archival primary specimens and new metastatic biopsies to account for temporal and spatial heterogeneity of HER2 overexpression.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humpath.2020.10.006.

References

[1] Yan M, Schwaederle M, Arguello D, Millis SZ, Gatalica Z, Kurzrock R. HER2 expression status in diverse cancers: review of results from 37,992 patients. Canc Metastasis Rev 2015;34(1):157–64.
[2] Lae M, Couturier J, Oudard S, Radvanyi F, Beuzeboc P, Vieillefond A. Assessing HER2 gene amplification as a potential target for therapy in invasive urothelial bladder cancer with a standardized methodology: results in 1005 patients. Ann Oncol 2010;21(4):815–9.
[3] Oh D-Y, Bang Y-J. HER2-targeted therapies — a role beyond breast cancer. Nat Rev Clin Oncol 2019;17:33–48.
[4] Hussain MHA, MacVicar GR, Petrylak DP, et al. Trastuzumab, paclitaxel, carboplatin, and gemcitabine in advanced human epidermal growth factor receptor-2/neu–positive urothelial carcinoma: results of a multicenter phase II national cancer Institute trial. J Clin Oncol 2007;25(16):2218–24.
[5] Powles T, Huddart RA, Elliott T, et al. Phase III, double-blind, randomized trial that compared maintenance lapatinib versus placebo after first-line chemotherapy in patients with human epidermal growth factor receptor 1/2-positive metastatic bladder cancer. J Clin Oncol 2017;35(1):48–55.
[6] Oudard S, Culine S, Vano Y, et al. Multicentre randomised phase II trial of gemcitabine+platinum, with or without trastuzumab, in advanced or metastatic urothelial carcinoma overexpressing Her2. Eur J Canc 2015;51(1):45–54.
[7] Wulfing C, Machiels JP, Richel DJ, et al. A single-arm, multicenter, open-label phase 2 study of lapatinib as the second-line treatment of patients with locally advanced or metastatic transitional cell carcinoma. Cancer 2009;115(13):2881–90.
[8] Bryce AH, Kurzrock R, Meric-Bernstam F, et al. Pertuzumab plus trastuzumab for HER2-positive metastatic urothelial cancer (mUC): preliminary data from MyPathway. J Clin Oncol 2017;35(6_suppl):348.
[9] Sheng X, Zhou A-P, Yao X, et al. A phase II study of RC48-ADC in HER2-positive patients with locally advanced or metastatic urothelial carcinoma. J Clin Oncol 2019;37(15_suppl):4509.
[10] Karass M, Bareja R, Shelkey E, et al. Oncogenic addiction to ERBB2 signaling predicts response to trastuzumab in urothelial cancer. J Natl Compr Canc Netw 2019;17(3):194–200.
[11] Wolff AC, Hammond MEH, Allison KH, et al. Human epidermal growth factor receptor 2 testing in breast cancer: American society of clinical oncology/college of American pathologists clinical practice guideline focused update. J Clin Oncol 2018;36(20):2105–22.
[12] Bartley AN, Washington MK, Colasacco C, et al. HER2 testing and clinical decision making in gastroesophageal adenocarcinoma: guideline from the college of American pathologists, American society for clinical pathology, and the American society of clinical oncology. J Clin Oncol 2017;35(4):446–64.
[13] Jimenez RE, Hussain M, Bianco Jr FJ, et al. Her-2/neu overexpression in muscle-invasive urothelial carcinoma of the bladder: prognostic significance and comparative analysis in primary and metastatic tumors. Clin Canc Res 2001;7(8):2440–7.
[14] Moktefi A, Pouessel D, Liu J, et al. Reappraisal of HER2 status in the spectrum of advanced urothelial carcinoma: a need of guidelines for treatment eligibility. Mod Pathol 2018;31(8):1270–81.
[15] Isharwal S, Huang H, Nanjangud G, et al. Intratumoral heterogeneity of ERBB2 amplification and HER2 expression in micropapillary urothelial carcinoma. Human pathol 2018;77:63–9.
[16] Hou Y, Nitta H, Wei L, et al. HER2 intratumoral heterogeneity is independently associated with incomplete response to anti-HER2 neoadjuvant chemotherapy in HER2-positive breast carcinoma. Breast Canc Res Treat 2017;166(2):447–57.
[17] Filho OM, Viale G, Trippa L, et al. HER2 heterogeneity as a predictor of response to neoadjuvant T-DM1 plus pertuzumab: results from a prospective clinical trial. J Clin Oncol 2019;37(15_suppl):502.
[18] Yagi S, Wakatsuki T, Yamamoto N, et al. Clinical significance of intratumoral HER2 heterogeneity on trastuzumab efficacy using

endoscopic biopsy specimens in patients with advanced HER2 positive gastric cancer. Gastric Cancer 2019;22(3):518−25.
[19] Wakatsuki T, Yamamoto N, Sano T, et al. Clinical impact of intratumoral HER2 heterogeneity on trastuzumab efficacy in patients with HER2-positive gastric cancer. J Gastroenterol 2018;53(11):1186−95.
[20] Lee HJ, Seo AN, Kim EJ, et al. HER2 heterogeneity affects trastuzumab responses and survival in patients with HER2-positive metastatic breast cancer. Am J Clin Pathol 2014;142(6):755−66.
[21] Thomsen MB, Nordentoft I, Lamy P, et al. Spatial and temporal clonal evolution during development of metastatic urothelial carcinoma. Mol oncol 2016;10(9):1450−60.
[22] Burgess EF, Livasy C, Hartman A, et al. Discordance of high PD-L1 expression in primary and metastatic urothelial carcinoma lesions. Urol Oncol 2019;37(5):299. e19-.e25.
[23] Aurilio G, Disalvatore D, Pruneri G, et al. A meta-analysis of oestrogen receptor, progesterone receptor and human epidermal growth factor receptor 2 discordance between primary breast cancer and metastases. Eur J Canc 2014;50(2):277−89.
[24] Creemers A, Ter Veer E, de Waal L, et al. Discordance in HER2 status in gastro-esophageal adenocarcinomas: a systematic review and meta-analysis. Sci Rep 2017;7(1):3135.
[25] Niikura N, Liu J, Hayashi N, et al. Loss of human epidermal growth factor receptor 2 (HER2) expression in metastatic sites of HER2-overexpressing primary breast tumors. J Clin Oncol 2012;30(6):593−9.
[26] Hansel DE, Swain E, Dreicer R, Tubbs RR. HER2 overexpression and amplification in urothelial carcinoma of the bladder is associated with MYC coamplification in a subset of cases. Am J Clin Pathol 2008;130(2):274−81.
[27] Fleischmann A, Rotzer D, Seiler R, Studer UE, Thalmann GN. Her2 amplification is significantly more frequent in lymph node metastases from urothelial bladder cancer than in the primary tumours. Eur Urol 2011;60(2):350−7.
[28] Wester K, Sjostrom A, de la Torre M, Carlsson J, Malmstrom PU. HER-2–a possible target for therapy of metastatic urinary bladder carcinoma. Acta oncol 2002;41(3):282−8.
[29] Vance GH, Barry TS, Bloom KJ, et al. Genetic heterogeneity in HER2 testing in breast cancer: panel summary and guidelines. Arch Pathol Lab Med 2009;133(4):611−2.
[30] Koshkin VS, O'Donnell P, Yu EY, Grivas P. Systematic review: targeting HER2 in bladder cancer. Bladder Cancer 2019;5:1−12.
[31] Faltas BM, Prandi D, Tagawa ST, et al. Clonal evolution of chemotherapy-resistant urothelial carcinoma. Nat Genet 2016; 48(12):1490−9.
[32] Warrick JI, Sjödahl G, Kaag M, et al. Intratumoral heterogeneity of bladder cancer by molecular subtypes and histologic variants. Eur Urol 2019;75(1):18−22.
[33] Gårdmark T, Wester K, De La Torre M, Carlsson J, Malmström P-U. Analysis of HER2 expression in primary urinary bladder carcinoma and corresponding metastases. BJU Int 2005; 95(7):982−6.
[34] Kiss B, Wyatt AW, Douglas J, et al. Her2 alterations in muscle-invasive bladder cancer: patient selection beyond protein expression for targeted therapy. Sci Rep 2017;7:42713.
[35] Rakha EA, Pigera M, Shaaban A, et al. National guidelines and level of evidence: comments on some of the new recommendations in the American society of clinical oncology and the college of American pathologists human epidermal growth factor receptor 2 guidelines for breast cancer. J Clin Oncol 2015;33(11):1301−2.
[36] Zinnall U, Weyerer V, Comperat E, et al. Micropapillary urothelial carcinoma: evaluation of HER2 status and immunohistochemical characterization of the molecular subtype. Hum Pathol 2018;80: 55−64.
[37] Ross JS, Wang K, Gay LM, et al. A high frequency of activating extracellular domain ERBB2 (HER2) mutation in micropapillary urothelial carcinoma. Clin Canc Res 2014;20:68−75.