

# Outcome of patients with micropapillary urothelial carcinoma following radical cystectomy: *ERBB2* (*HER2*) amplification identifies patients with poor outcome

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Micropapillary urothelial carcinoma exhibits amplification of the human epidermal growth factor receptor, *ERBB2*(*HER2*), and overexpression of the *ERBB2* protein product. The clinical significance of this has yet to be established. The objective of this study was to examine *ERBB2* amplification and protein expression in micropapillary urothelial carcinoma and stage-matched typical urothelial carcinoma treated by radical cystectomy to assess the frequency of amplification and protein expression, and to determine the association with cancer-specific survival. Pathologic material and data from patients undergoing cystectomy at Mayo Clinic between 1980 and 2008 were reviewed. *ERBB2* amplification by fluorescence *in situ* hybridization (FISH) and protein expression by immunohistochemistry were assessed. Univariate and multivariate Cox proportional hazards regression models were used to evaluate for associations of *ERBB2* amplification and protein expression with survival. *ERBB2* amplification was identified in 9 (15%) of 61 micropapillary carcinomas compared with 9 (9%) of 100 urothelial carcinomas. In patients with micropapillary carcinoma, *ERBB2* amplification was associated with a nearly threefold increased risk of cancer death. *ERBB2* amplification (hazard ratio 4.3;  $P=0.0008$ ) remained associated with an increased risk of death from bladder cancer among patients with micropapillary urothelial carcinoma on multivariate analysis. The association of cancer-specific survival and *ERBB2* amplification was not seen in patients with urothelial carcinoma. *ERBB2* immunohistochemistry correlated with *ERBB2* amplification but there was no association of *ERBB2* protein expression and survival. *ERBB2* amplification is more frequent in micropapillary urothelial carcinoma than typical urothelial carcinoma, and patients with micropapillary carcinoma who have *ERBB2* amplification have worse cancer-specific survival than those who do not. Identification of *ERBB2* amplification in micropapillary carcinoma could provide important prognostic information and possibly provide a role for *ERBB2* targeted therapy.

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Micropapillary urothelial carcinoma is a unique variant of urothelial carcinoma that comprises <6% of all bladder cancers.<sup>1–3</sup> Several studies demonstrated that micropapillary urothelial carcinoma is

diagnosed at a more advanced stage, and is associated with adverse outcome relative to typical urothelial carcinoma.<sup>4–8</sup> However, a recent study found that, although patients with micropapillary urothelial carcinoma presented at higher stage than patients with typical urothelial carcinoma, the outcomes following radical cystectomy were no different when matched for stage and other clinicopathologic variables.<sup>9</sup>

In addition to unique histologic features, studies have shown that micropapillary urothelial carcinoma

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exhibits amplification of the human epidermal growth factor receptor (*ERBB2* or *HER2*) in over 40% of cases.<sup>10</sup> *ERBB2* is a type-I transmembrane growth factor receptor that activates intracellular signaling pathways in response to extracellular signals. It was first implicated in the pathogenesis of human breast cancer 25 years ago.<sup>11</sup> In patients with breast cancer, *ERBB2* amplification is associated with poor prognosis.<sup>12</sup> However, *ERBB2*-targeted therapy with the anti-*ERBB2* antibody trastuzumab has resulted in significantly longer survival when combined with standard chemotherapy in patients whose breast tumors exhibit *ERBB2* amplification compared with patients whose tumor lack *ERBB2* amplification.<sup>12,13</sup> *ERBB2* amplification has been identified in other tumor types, although its role in treatment for these malignancies is yet to be defined.<sup>10,14–16</sup>

Although *ERBB2* amplification has been identified in micropapillary urothelial carcinoma, its frequency and association with outcome has not been established in patients undergoing curative surgery. The objective of this study was to examine *ERBB2* amplification and protein expression in a consecutive series of patients with micropapillary urothelial carcinoma undergoing radical cystectomy to determine the frequency of amplification and protein expression, and their association with outcome. In addition, *ERBB2* and protein status was compared between patients with micropapillary urothelial carcinoma and patients with typical urothelial carcinoma matched for the prognostic clinical and pathologic variables.

## Materials and methods

After Institutional Review Board approval was obtained, we reviewed the Mayo Clinic Cystectomy Registry of patients who underwent radical cystectomy at our institution between 1980 and 2008. Radical cystectomy was performed by various surgeons using standard techniques. Tissue slides and blocks are retained permanently on all patients, and all cystectomy specimens from 1980 to 2008 were re-reviewed by a urologic pathologist, and 61 cases of micropapillary urothelial carcinoma were identified. Inclusion of cases into the study required any micropapillary component in a urothelial carcinoma. These 61 cases were matched for stage, age of patient, ECOG status, receipt of perioperative chemotherapy and decade of surgery, with 100 cases of typical urothelial carcinoma. All cases were re-reviewed a second time by two pathologists (WRS and JCC) independently and blinded to the prior diagnoses to ensure the appropriate diagnosis of micropapillary carcinoma. Consensus agreement was reached on all cases in this study. Staging followed the 2010 American Joint Committee on Cancer/Union Internationale Contre le Cancer 7th edition TNM classification.<sup>17</sup> As all these specimens

were obtained before the 2010 staging system, retrospective staging was performed as described previously.<sup>18</sup> Briefly, pT1 and pT2 classification was obtained by microscopic slide review as staging parameters are based on microscopic features. In the case of pT3 tumors, the tissue slides (where gross features of the bladder and tumor are retained) were examined macroscopically, and the presence or absence of a gross lesion was determined, and findings confirmed microscopically.

The retrospective nature of this study precluded a standardized postoperative follow-up protocol in all patients. However, follow-up after radical cystectomy at our institution has generally been recommended quarterly for the first 2 years after surgery, semiannually for the next 2 years, and annually thereafter in patients without evidence of disease recurrence. Vital status was identified from death certificates or physician correspondence. For patients followed elsewhere, the Mayo Clinic Cystectomy Registry monitors outcomes annually by correspondence to the patient and treating physician.

Clinicopathologic demographics for patients with micropapillary cancer were reported and compared with a group of patients with typical urothelial carcinoma on the basis of age, gender, T-stage, N-stage, ECOG performance status, receipt of neoadjuvant chemotherapy, postoperative chemotherapy and radiation and surgical margin status.

## ERBB2 Testing Methods and Interpretive Criteria

*ERBB2* (*HER2*) by fluorescence *in situ* hybridization (FISH). *ERBB2* tests approved by the US Food and Drug Administration (FDA) were used for FISH. *ERBB2* amplification was assessed in formalin-fixed, paraffin-embedded 5- $\mu$ m sections using the PathVysion HER-2 DNA Probe Kit (*HER2* and centromere 17 probes; Abbott Molecular, Des Plaines, IL, USA), as described. Whole tumor sections from cystectomy specimens were used, and the complete section was scanned by certified cytogenetic technologists to detect any subpopulation of amplified cells. A total of 60 representative nuclei from the invasive tumor were scored, with an overall evaluation performed by a cytogeneticist.<sup>19,20</sup> A specimen with an *ERBB2*/centromere 17 ratio  $\geq 2.0$  in invasive cells was classified as *ERBB2* amplified in accordance with the criteria developed for classification of *ERBB2* and centromere 17 abnormalities as described previously.<sup>19,21</sup>

Chromosome 17 gains and losses were determined using centromere 17 signal patterns based on methodology and cutoffs that we previously validated using two large independent breast cancer sets. Accordingly, polysomy 17 (gain) was defined as three centromere 17 signals in more than 30% of nuclei; monosomy 17 (loss) was defined as one centromere 17 signal in more than 60% of nuclei; and all other cases were considered normal.<sup>20</sup> Both

**Table 1** Clinicopathologic features of patients with micropapillary urothelial carcinoma and typical urothelial carcinoma

	Micropapillary urothelial carcinoma		Typical urothelial carcinoma		P-value <sup>a</sup>
	Non-amplified (N = 52)	Amplified (N = 9)	Non-amplified (N = 91)	Amplified (N = 9)	
Median age (years) at cystectomy (range)	67 (43–88)	68 (57–79)	70 (43–92)	58 (47–79)	0.20
<i>Gender (%)</i>					0.31
Male	42 (81)	9 (100)	68 (75)	9 (100)	
Female	10 (19)	0	23 (25)	0	
<i>pT stage</i>					0.86
T1	6 (12)	0 (0)	7 (8)	1 (11)	
T2a, b	10 (19)	1 (11)	17 (19)	3 (33)	
T3a, b	30 (58)	5 (56)	56 (62)	5 (56)	
T4a, b	6 (12)	3 (33)	11 (12)	0 (0)	
<i>pN stage</i>					0.96
NX	6 (12)	1 (11)	10 (11)	2 (22)	
N0	21 (40)	1 (11)	33 (36)	1 (11)	
N1, 2 and 3	25 (48)	7 (78)	48 (53)	6 (67)	
<i>ECOG score</i>					0.62
0	40 (78)	6 (67)	70 (77)	7 (78)	
1	6 (12)	3 (33)	11 (12)	0 (0)	
≥2	4 (10)	0 (0)	10 (11)	2 (22)	
Receipt of perioperative chemotherapy	14 (27)	1 (11)	28 (31)	3 (33)	0.38
Positive surgical margin	6 (12)	1 (11)	4 (4)	0 (0)	0.07

<sup>a</sup>P-values reflect comparison of patients with MP UC and UC.

cutoffs clearly distinguish chromosome 17 polysomic and monosomic cancers from cancers without chromosome 17 centromere anomalies.<sup>22</sup> All categorization thresholds were selected to reduce the rate of false-positive findings for gene amplification, gene deletion and chromosome loss or gain. Quality control of the *ERBB2* FISH test is routinely assessed according to standard CAP and American College of Medical Genetics guidelines.<sup>20,23–25</sup>

### ERBB2 Heterogeneity by FISH

Following CAP breast cancer guidelines,<sup>20</sup> a sample was considered to have heterogeneous *ERBB2* amplification if there were >5% but <50% infiltrating tumor cells with an *ERBB2*/centromere 17 ratio >2.2 (results were the same for a 2.0 cut-point). An *ERBB2*/centromere ratio was determined separately for the amplified and non-amplified subpopulation of each heterogeneous tumor. The primary *ERBB2*/centromere 17 ratio for each heterogeneous case was determined by calculating a mean ratio across the amplified and non-amplified subpopulations weighted by the percentage that each subpopulation comprised the entire tumor. *ERBB2* heterogeneous tumors were further characterized by the distribution of the amplified subpopulation—that is, clustered (amplified cells adjacent to each other, tending to be arranged in groups) versus diffuse (amplified cells scattered among the non-amplified cells and relatively evenly distributed throughout the tumor).

ERBB2 immunohistochemistry was performed using the HercepTest (Dako) according to the manufacturer's recommendations. Immunohistochemistry staining was scored according to recommendations of the American Society of Clinical Oncology/College of American Pathology guidelines.<sup>24</sup>

### Statistical Analysis

The Wilcoxon's rank-sum and  $\chi^2$  tests were used to compare variables between groups. For survival analysis, the outcome variable was cancer-specific survival, defined as the time from surgery to death related to index cancer and was censored at the date of death as a result of postoperative complications or other non-malignant causes. Cancer-specific survival was estimated using the Kaplan–Meier method and compared with the log-rank test. Associations of features with time to death from bladder cancer was evaluated using Cox proportional hazards regression models, and summarized with hazard ratios (HR) and 95% confidence intervals (CI). All P-values are two-sided, with a  $P < 0.05$  considered statistically significant. Analyses were conducted in SAS version 9.1 (SAS Institute, Cary, NC, USA).

### Results

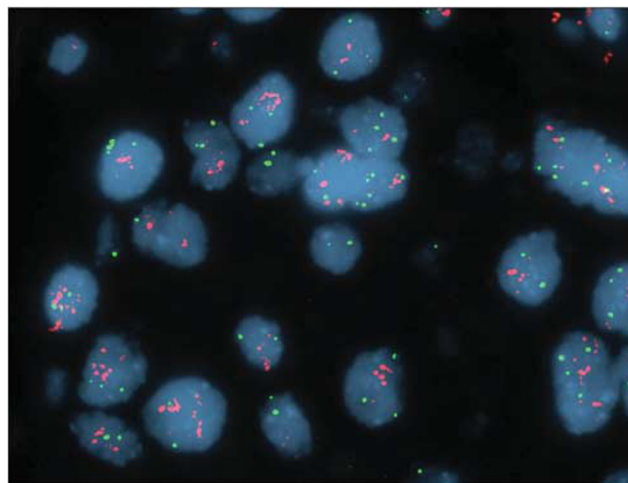
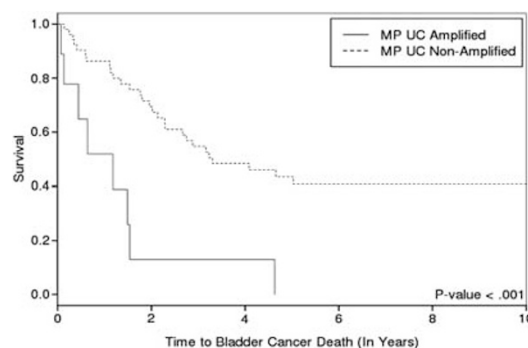
The clinicopathologic features of the patients with micropapillary carcinoma and typical urothelial carcinoma are listed in Table 1. There were 61

**Table 2** Univariate associations with cancer-specific survival among patients with micropapillary urothelial carcinoma

Parameter	Hazard ratio	95% Confidence interval	P-value
Age at surgery	1.0	0.98–1.04	0.7
pT stage (reference $\leq$ T1)	1.6	0.8–3.3	0.16
pN stage (reference pN0)	2.2	1.1–4.6	0.03
ECOG	2.6	1.9–3.1	0.002
Perioperative chemotherapy	1.1	0.6–2.4	0.63
<i>ERBB2</i> amplification	4.2	1.9–9.7	<0.001

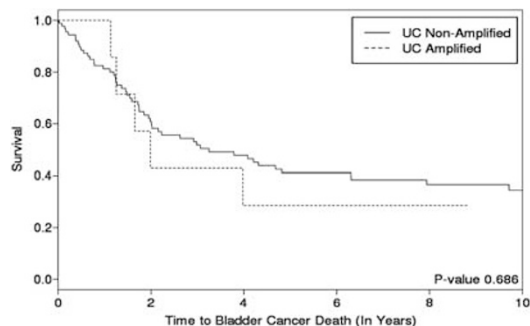
patients within the micropapillary (Table 2) cancer and 100 with urothelial carcinoma. Median follow-up of all patients was 12.8 years. The micropapillary urothelial carcinoma and urothelial carcinoma groups were matched such that there were no statistical differences between the cohorts with regard to age, gender, ECOG performance status, length of follow-up, stage or reception of perioperative (neoadjuvant) chemotherapy. With regard to postoperative treatment, 14 (23%) patients with micropapillary carcinoma received chemotherapy compared with 41 (25%) of patients with typical urothelial carcinoma, and postoperative radiation was given to 1 (1.6%) with micropapillary cancer and 2 (1.2%) patients with typical urothelial carcinoma. In the cases of micropapillary urothelial carcinoma, the majority of cases (55 of 73; 75%) were composed entirely of micropapillary carcinoma, and the range of the micropapillary carcinoma component in the remaining cases ranged from 5 to 90%. The other component in these cases was typical urothelial carcinoma.

*ERBB2* amplification was identified in 9 (15%) of 61 micropapillary urothelial carcinoma compared with 9 (9%) of 100 typical urothelial carcinomas (Figure 1). No heterogeneity of *ERBB2* amplification was identified. Patients with *ERBB2*-amplified micropapillary carcinomas were more likely to have positive lymph nodes at the time of cystectomy than patients with non-amplified micropapillary carcinomas, 78% versus 48%, respectively, but this did not reach statistical significance ( $P=0.078$ ). There were no differences in patient, age, gender, pT stage, ECOG status or margin status between patients with amplified and non-amplified tumors. In addition, there were no differences in the morphologic features (nuclear or architectural features) of tumors with and without amplification. The 5-year cancer-specific survival of patients with micropapillary urothelial carcinoma with *ERBB2* amplification was 0%, as opposed to patients with micropapillary urothelial carcinoma without amplification whose 5-year cancer-specific survival was 40% ( $P<0.001$ ) (Figure 2). There was no statistically significant difference in cancer-specific survival between

**Figure 1** A case of micropapillary urothelial carcinoma with *ERBB2* amplification. Red probe indicates copies of *ERBB2*; green probe indicates chromosome 17 centromere.**Figure 2** Cancer-specific survival of patients with *ERBB2*-amplified micropapillary urothelial carcinoma (MP UC) compared to patients with -non-amplified micropapillary carcinoma.

patients with typical urothelial carcinoma with amplification and without amplification (Figure 3). Associations of clinical and pathologic features and *ERBB2* amplification with cancer-specific survival are summarized in Tables 3 and 4. On univariate analysis, *ERBB2* amplification was associated with a nearly threefold increased risk of death from bladder cancer. On multivariate analysis, *ERBB2* amplification (HR 4.3;  $P=0.0008$ ) and ECOG score (HR 2.2;  $P=0.007$ ) remained significantly associated with death from bladder cancer.

Immunohistochemistry results are provided in Table 4 and illustrated in Figure 4. In patients with micropapillary carcinoma and typical urothelial carcinoma, there was no significant association of immunohistochemistry scores with cancer-specific survivals when comparing intensity staining scores 0, 1 versus 2, 3 as typically done in breast cancer. There was a strong association of *ERBB2* amplification and *ERBB2* immunohistochemical scores 2 and 3. Fifteen (83%) of 18 bladder cancers that showed *ERBB2* amplification had *ERBB2* immunohistochemical scores of 2 and 3. Three cases of



**Figure 3** Cancer-specific survival of patients with *ERBB2*-amplified typical urothelial carcinoma (UC) compared to patients with non-amplified typical urothelial carcinoma.

**Table 3** Multivariate associations with cancer-specific survival in patients with micropapillary urothelial carcinoma

Parameter	Hazard ratio	95% Confidence interval	P-value
<i>ERBB2</i> amplification	4.3	1.8–10.2	0.0008
ECOG	2.2	1.4–3.9	0.0007

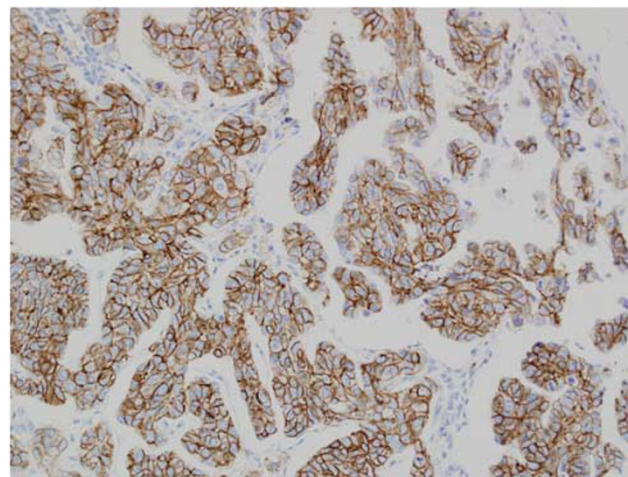
**Table 4** Immunohistochemical results in micropapillary urothelial carcinoma and typical urothelial carcinoma and comparison to *ERBB2* amplification

Immunohistochemical score	Micropapillary urothelial carcinoma no. (%), N = 61	Typical urothelial carcinoma no. (%), N = 100	No. of patients with <i>ERBB2</i> amplification (%), N = 18
0	9 (15)	54 (54)	2 (11)
1	21 (35)	22 (22)	1 (5)
2	15 (25)	14 (14)	3 (17)
3	15 (25)	10 (10)	12 (67)

*ERBB2*-amplified cancer had a score <2. However, immunohistochemical scores 2 and 3 were present in 39 (27%) of 143 patients with micropapillary carcinoma and urothelial carcinoma that did not have *ERBB2* amplification.

## Discussion

In this study involving radical cystectomy patients, *ERBB2* amplification was significantly associated with diminished cancer-specific survival among patients with micropapillary urothelial carcinoma. To our knowledge, this represents the first time that *ERBB2* amplification has been observed to predict for outcome among patients with the rare variant of urothelial carcinoma. Furthermore, these results suggest that therapeutic manipulation of *ERBB2* may hold promise for patients harboring the aggressive micropapillary urothelial carcinoma.



**Figure 4** Micropapillary urothelial carcinoma with *ERBB2* score of 3.

Micropapillary urothelial carcinoma is an unusual variant of bladder cancer that comprises 0.7 to 6% of all bladder cancers. It was initially described in 1994 and named for its resemblance to papillary serous carcinoma of the ovary.<sup>3</sup> Several studies have suggested that micropapillary urothelial carcinoma has a particularly aggressive course compared with typical urothelial carcinoma, but these studies have been limited by small patient numbers or non-standardized treatment and follow-up. Recently, we examined our experience of micropapillary urothelial carcinoma treated by radical cystectomy and showed that micropapillary urothelial carcinoma was associated with advanced locoregional spread at the time of surgery when compared to patients with typical urothelial carcinoma.<sup>9</sup> However, when matched for stage and other prognostic clinicopathologic features, patients with micropapillary carcinoma had the same outcome as patients with typical urothelial carcinoma. In this study, we further define the behavior of micropapillary urothelial carcinoma and show that *ERBB2* amplification identifies a group of patients with particularly adverse outcome.

Although this study requires external validation, our findings have several important clinical implications. For one, the assessment of *ERBB2* status could provide important prognostic information for patients under consideration for surgery. In a tumor that has been assumed to be particularly aggressive, the absence of *ERBB2* amplification suggests an outcome following cystectomy similar to patients with similar stage typical urothelial carcinoma. The presence of *ERBB2* amplification also suggests a higher likelihood of lymph node involvement that may alter the surgical approach and a greater probability of disease progression that would affect perioperative treatment and follow-up. There also implications with regard to targeted therapy. The role of trastuzumab in the treatment of breast cancer is well established. Recently, a survival benefit has

been reported in advanced *ERBB2*-amplified gastric cancer treated by anti-*ERBB2* monoclonal antibody.<sup>26</sup> There is potential that patients with *ERBB2*-amplified bladder cancers may benefit from trastuzumab therapy, and that the frequency of amplification can be expected to be higher in micropapillary carcinoma than typical urothelial carcinoma. It is important to note that, although relatively low, we detected a frequency of *ERBB2* amplification of approximately 9% in typical urothelial cancers similarly to other studies.<sup>16</sup> Although the number of patients with typical urothelial carcinoma and *ERBB2* amplification is small, we did not identify a worse outcome in those patients when compared with patients who had non-amplified tumors. Clinical trials are needed to determine the effectiveness of anti-*ERBB2* therapy in these patients, and trials would benefit from central pathology review to stratify patients by bladder cancer subtype and trials will require standardized assessment of *ERBB2* tumor status.

As expected, immunohistochemistry for *ERBB2* had a relatively high positive predictive value for the identification of bladder cancers that show *ERBB2* amplification with 15 of 18 (83%) *ERBB2*-amplified tumors showing high (score 2 and 3) *ERBB2* overexpression. However, a large proportion of tumors showing score 2 and 3 overexpression did not show *ERBB2* amplification (39 of 142 non-amplified cases). The significance of *ERBB2* overexpression in non-amplified tumors is not known. It will be important in future prospective clinical studies to explore whether there is any potential benefit from trastuzumab in patients not only with *ERBB2*-amplified tumors but also patients with *ERBB2*-non-amplified tumors that show *ERBB2* overexpression. It is of note that we did not identify an association of cancer-specific survival and *ERBB2* protein expression in patients with micropapillary or typical urothelial carcinoma.

We report a lower frequency of *ERBB2* amplification in micropapillary carcinoma than other groups. In particular, Ching *et al*<sup>10</sup> reported that 8 (42%) of 19 micropapillary carcinomas showed amplification and 13 (68%) exhibited score 2 and 3 by immunohistochemistry compared with 15% and 50%, respectively, in our series. In addition, Ching *et al* found no association of *ERBB2* amplification with outcome. There are several potential explanations for these differences. The study by Ching *et al* evaluated only 20 patients, four that received transurethral resection as the only surgical intervention, whereas 16 underwent radical cystectomy. Fourteen (88%) of the 16 patients had regional lymph node involvement at surgery and the remaining two were suspected of having involvement based on radiology, and 12 of their patients died over follow-up of 14 months. In our series, 32 (52%) patients with micropapillary carcinoma had regional lymph node involvement, and patients with lymph node involvement had a higher frequency of *ERBB2*

amplification. Of the 9 patients with amplified *ERBB2* tumors, 7 (78%) had regional lymph node involvement compared with 25 (48%) of 52 patients with non-amplified tumors. These findings suggest that the study by Ching *et al* consisted of patients with more advanced disease, and as a consequence their series showed a higher frequency of *ERBB2* amplification. There were also differences in methodology between the two studies. Ching *et al* used a modified chromagenic *in situ* hybridization technique applied to tissue microarrays in contrast to the FDA-approved FISH technique performed on whole tumor sections in our analysis. Finally, Ching *et al* performed immunohistochemistry with a primary antibody from Ventana while we utilized the FDA-approved Herceptest from Dako. It is difficult to assess the impact of these different methodologies on results but these differences emphasize the need for a standardized approach to determine *ERBB2* amplification across clinical trials.

There are several limitations to this study. Although the number of patients undergoing radical cystectomy for micropapillary urothelial carcinoma is relatively large compared with other studies, it remains quite limited. In addition, the study was retrospective and non-randomized. Our findings will, therefore, require validation either through a large multi-institutional retrospective study or preferably through a prospective clinical trial examining the prognostic significance of *ERBB2* amplification and the application of targeted therapy in the setting of cystectomy.

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## Disclosure/conflict of interest

The authors declare no conflict of interest.

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