Assessing HER2 gene amplification as a potential target for therapy in invasive urothelial bladder cancer with a standardized methodology: results in 1005 patients

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Background: This study assessed the human epidermal growth factor receptor-2 (HER2) protein expression and its relationship with gene amplification in invasive bladder carcinoma, using the same criteria than for breast cancer.

Patients and methods: In 1005 patients, paraffin-embedded tissues of transurethral resection or cystectomy were evaluated by immunohistochemistry (IHC), using antibodies against HER2. All samples with a 2+ or 3+ HER2 overexpression were evaluated by FISH.

Results: HER2 overexpression was observed in 93 (9.2%) tumors (2+: 42 tumors and 3+: 51 tumors). Using FISH, all HER2 3+ tumors had a gene amplification, whereas no amplification was found in 2+ tumors. Intratumoral heterogeneity was observed in 95% of cases. These tumors showed the same heterogeneous pattern, with adjacent 3+ positive and negative areas by both IHC and FISH.

Conclusions: This study showed that 5.1% of invasive bladder carcinomas had a HER2 gene amplification. These findings may have clinical implications for the management of patients with HER2-positive locally advanced or metastatic bladder cancer, as they could be potential candidates for targeted therapy.

Key words: FISH, HER2, immunohistochemistry, targeted therapy, urothelial bladder carcinoma

Introduction

Urothelial bladder carcinoma is the fourth most common malignancy in men and the ninth most common in women. In the United States, 61,420 estimated new cases were expected in 2006 and >13,000 patients were likely to die. At the time of diagnosis, ~80% of urothelial bladder carcinomas are superficial and 20% of them will become invasive. Tumors that invade the deep muscle layer of the bladder are assigned stage T2, while T3 and T4 lesions invade the perivesical tissue and local structures, respectively. Standard treatment of muscle-invasive cancers has historically comprised surgical resection achieving 5-year recurrence-free survival rates from 44% to 81%. The treatment of locally advanced or metastatic disease is currently based on the M-VAC regimen (methotrexate, vinblastine, doxorubicin, cisplatin), in spite of a high rate of toxicities. Among new combinations, gemcitabine and cisplatin (GC) confers a similar survival advantage to M-VAC, improving the safety profile [1]. Although not designed as an equivalency trial, GC has become the first-line treatment of patients with locally advanced or metastatic urothelial bladder carcinoma in many institutions. The next step in the management of advanced urothelial bladder carcinomas is to know whether targeted therapies could positively impact the outcome of the disease.

The human epidermal growth factor receptor-2 (HER2) is known to contribute to physiologic mechanisms of cell proliferation by an intrinsic tyrosine kinase activity. The assessment of HER2 status is crucial for the management of breast cancer, for both prognosis and prediction of the response to targeted therapies [2]. The introduction of trastuzumab, a recombinant humanized mAb to the extracellular domain of HER2, has dramatically changed the treatment of HER2-amplified breast tumor in the adjuvant and metastatic setting [3–5]. In breast cancer, HER2 overexpression is the result of an amplification of the HER2 gene, leading to an increase in HER2 messenger RNA levels and a concomitant overexpression of the HER2 receptor on the tumor cell surface [6]. The HER2 gene is amplified in ~18% of all breast cancers [7]. In invasive urothelial bladder carcinomas, amplification and/or overexpression were also found. However, the true incidence of HER2 overexpression and/or amplification remains uncertain ranging from 23% to 80% for overexpression [8–15] and from 0% to 32% for amplification [10–13, 15–18]. This variability could be explained by the small number of patients evaluated in

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each series and by the heterogeneity in laboratory tests. As in breast cancer, the assessment of HER2 status has to be standardized in terms of antibodies and interpretation of the results. In this setting, the American Society of Clinical Oncology (ASCO) guidelines have clearly defined a positive HER2 status as an immunohistochemistry (IHC) score of 3+, a negative HER2 status as an IHC score of 0 or 1+, and an IHC score of 2+ had to be tested by FISH confirming the HER2 status [19].

Unlike the situation in breast cancer, where the role of HER2-targeting agents has been well established in both metastatic and adjuvant settings, the use of HER2-targeting agents has only emerged recently in bladder carcinomas clinical research. A possible involvement of the HER2 receptor in the proliferation of invasive urothelial bladder carcinomas has led to initiate HER2-targeted therapy trials in locally advanced or metastatic disease [20]. However, this therapeutic approach requires a reliable evaluation of HER2 status on the basis of robust, reproducible IHC and FISH criteria.

The aim of this retrospective study was to evaluate the HER2 protein expression and HER2 gene amplification using the same criteria than those used in breast cancer in a large series of 1005 patients with invasive urothelial bladder carcinoma.

patients and methods
study population and tissue specimens
A series of 1005 tumors from 1005 patients diagnosed with primary invasive urothelial carcinoma of the urinary bladder was selected from the Cochin Pathology Department files from 2002 to 2008 (including cases collected from 10 centers in France for therapeutic purposes). Ten additional metastatic lymph nodes were also included. The material poorly fixed and/or with a low cellularity had been previously ruled out. Paraffin-embedded tissues were obtained after transurethral resection for bladder tumor or cystectomy. A histologic examination on hematoxylin–eosin and saffron (HES)-stained slides was carried out by a specialist in urologic pathology (AV) confirming the diagnosis of high-grade, muscle-invasive urothelial cell carcinoma (Figure 1A). Pathologic stage was evaluated according to the World Health Organization classification.

All patients provided written informed consent for central collection of their tissue material and clinical data for research purposes.

immunohistochemical analysis of HER2
Four-microns tissue sections prepared from a formalin-fixed and paraffin-embedded representative of the tumor sample were used. After deparaffinization, rehydration, and antigen retrieval in citrate buffer (10 mMol, pH 6.1), tissue sections were stained for HER2 (A0485 polyclonal antibody; Dako, Glostrup, Denmark; 1/1500). HER2 positivity was assessed using the ASCO scoring system, evaluating only membranous staining [19]. The interpretation of the results was on the basis of negativity of normal tissues. The level of HER2 protein expression was assessed semiquantitatively by the intensity and percentage of staining and scored on a scale of 0 to 3+. Scores of 0 and 1+ are categorized as negative, 2+ as equivocal, and 3+ as positive. The evaluation was carried out only on the invasive component of the tumor. A score of 1+ was defined as barely perceptible membrane staining in >10% of cells, a score of 2+ was defined as weak-to-moderate complete membrane staining present in >10% of tumor cells, and a score of 3+ was defined as strong complete membrane staining in >30% of tumor cells. A cytoplasmic staining was considered nonspecific. The in situ component was not evaluated. Tumors presenting 2+ or 3+ HER2 expression were centrally reasayed with IHC, using the CB-11 mAb (Novocastra, Newcastle upon Tyne, UK) as previously described [21], and FISH was carried out in a reference laboratory for breast cancer HER2 testing (>600 FISH and 2400 IHC per year). For this second analysis, IHC had been previously calibrated using FISH as the gold standard. Internal and external controls (0, 1+, 2+, and 3+ breast carcinoma samples with a known number of gene copies) were included in the experiments for each antibody. The primary antibody, CB-11, diluted to 1/800, was incubated for 1 h at 22°C. Heat-induced epitope retrieval buffer was sodium citrate (pH 6.1). Staining was revealed with the Vectastain Elite ABC peroxidase mouse IgG kit (Vector Laboratories, Inc., Burlingame, CA) using diaminobenzidine (DAB) with 0.05% hydrogen peroxide as a chromogen. Membranous HER2 staining was confirmed by FISH using the Spectrum™Orange dual-color break-apart rearrangement probe (Vysis, Abbott Laboratories, Abbott Park, IL) as previously described [21].

Figure 1. Pathologic immunohistochemistry labeling results of invasive urothelial bladder carcinoma. (A) Detrusor-invading urothelial bladder carcinoma (hematoxylin-eosin ×40 magnification); (B) HER2 2+ membranous expression (×40 magnification); (C) HER2 3+ membranous expression (×40 magnification); and (D) patterns of heterogeneous 3+ and 2+ HER2 membranous expression within the same tumor (×20 magnification).
amplification in tumors was classified as follows: low amplification (mean between low and high amplification was chosen because signals can almost American Pathologists [19]. The cut-off of 10 gene copies per nucleus color FISH, as it is the cut-off recommended by the ASCO/College of neither amplification nor polysomy 17, and to determine HER2 copy number. All samples presenting 2+ or 3+ HER2 protein expression were evaluated by FISH in the referral laboratory, carried out on a single block according to the instructions from the test kit manufacturer. Samples with 0/1+ staining were not tested. Formalin-fixed, paraffin-embedded 4-μm tumor tissue sections were deparaffinized and rehydrated. Sections were incubated in pretreatment buffer at 95°C for 10 min, then in proteolytic solution at 37°C for 8 min. Codenaturation of the probe and DNA of the tissue section was achieved by incubation at 82°C for 5 min using a Thermobrite automate (Abbott Molecular Diagnostics, Rungis, France). This was followed by 15-h hybridization at 37°C and by post-hybridization washes, according to the protocol. Slides were mounted in di amino phenyl indol (DAPI)/antifade. Internal and external controls were included in the experiments. Slides were viewed with a DAPI/rhodamine/fluorescein filter, and images were captured with a charge-coupled device camera, filtered and processed with Applied Spectral Imaging System using a Leica Microsystems microscope. The slides were first scanned at low magnification, using the DAPI filter, in order to locate invasive areas, referring to the HES staining when necessary. The number of green (corresponding to copies of chromosome 17) and red signals (corresponding to copies of HER2 gene) was counted in at least 100 nuclei of invasive tumor cells, in two distant areas of the section at high magnification (>1000). Three representative images per case were captured. Tumors were classified as amplified when they showed a mean of at least six HER2 signals, or a HER2/centromere 17 ratio >2.2, and as nonamplified when they showed a mean of less than four HER2 signals, or when the HER2/centromere 17 ratio was <1.8 [19]. The level of HER2 amplification in tumors was classified as follows: low amplification (mean 7–10 signals per nucleus) or high amplification (mean >10 signals/nucleus or uncountable due to clusters of signals). The cut-off value of seven copies between no amplification and amplification is appropriate for single-color FISH, as it is the cut-off recommended by the ASCO/College of American Pathologists [19]. The cut-off of 10 gene copies per nucleus between low and high amplification was chosen because signals can almost always not be precisely counted over this cut-off due to clusters.

results

immunohistochemistry

Moderate-to-strong protein expression (2+ or 3+) was observed in 115 of 1005 tumors (11.4%) using the first IHC protocol. An IHC 0/1+ score was detected in the remaining samples. In the reference laboratory, using IHC analysis previously calibrated by FISH as the gold standard, moderate-to-strong protein expression was observed in 93 of the 115 tumors tested, corresponding to 9.2% of the 1005 invasive urothelial bladder carcinomas. Results are summarized in Table 1. Staining was scored 2+ in 42 samples (2+ staining in 30%–100% of cells and the remaining cells were scored 0) (Figure 1B) and 3+ in 30 tumors (3+ staining in 30%–79% of cells in 32 tumors and 80%–100% of cells in 19 tumors) (Figure 1C). A heterogeneous 3+ staining was observed in 18 of 51 tumors (35.3%) (Figure 1D).

Table 1. Correlation between HER2 IHC staining and FISH in the 93 invasive bladder carcinomas showing HER2 overexpression (2+ and 3+). Significant overlap was seen in tumors classified as 3+ by IHC and 2+ by FISH.

| FISH | IHC positive | | |
|---|---|---|---|
| 2+ | 3+ | Total |
| HER2 amplification | 0 | 51* | 51 (5.1%) |
| No HER2 amplification | 42 | 0 | 42 (4.2%) |
| Total | 42 | 51 | 93 (9.2%) |

*Eighteen heterogeneous tumors among 51 HER2 3+ tumors. IHC, immunohistochemistry.

These tumors were characterized by areas scored 3+ (in 30%–60% of cells) strictly and sharply separated from areas scored 2+ (12 tumors) or areas scored 0’ (6 tumors) (Figure 1D).

HER2 expression was concordant in 100% of cases between metastatic lymph nodes (10 cases scored 3+ from 10 patients) and primary bladder tumor (10 cases scored 3+). FISH was not carried out on lymph node metastases.

FISH

Ninety-three samples scored 2+ and 3+ were analyzed by FISH to evaluate HER2 gene copy number. Results are shown in Table 1. All FISH analyses were informative. All of the 51 cases with 3+ staining showed amplification by FISH (Figure 2). Twenty-one of the 51 tumors (41%) presented a low level of amplification (mean 7–10 signals per nucleus) and 30 tumors (59%) presented a high level of amplification (mean >10 signals/nucleus or uncountable due to clusters of signals indicating homogeneous staining regions). Noteworthy, the 18 tumors scored 3+ with heterogeneous staining showed exactly the same heterogeneous pattern with adjacent positive and negative areas, detected by both IHC and FISH methods. None of the 42 tumors with ’2+’ staining showed amplification by FISH (Figure 2). Chromosome 17 polysomy was detected in 13 of the 42 tumors (31%). The remaining 29 tumors showed two copies of HER2 and two copies of centromere 17.

discussion

In this study, 1005 primary invasive urothelial bladder carcinomas were screened for HER2 evaluation. To our knowledge, this is the largest multicenter series investigating invasive urothelial bladder carcinomas by calibrated IHC and FISH methods. A HER2 overexpression was found in 9.2% of tumor samples. This rate is lower than those previously described in the literature ranging from 23% to 80% [8–15]. Several hypotheses could explain these wide variations, as well as the relatively low rate of HER2 overexpression reported here. One of the major issues is the variability in IHC assays, related to the heterogeneity between kits, antibodies, protocols, interpretations or cut-off values. Discordant results reported in the literature highlight a need for standardized laboratory methods. The FISH method provides more objective results than IHC because of a numerical expression. In breast cancer, high levels of concordance (93%–100%) have been reported between FISH and IHC [21, 22]. The present study has the advantage of a large, retrospective, multicenter series of invasive
urothelial bladder carcinomas with extensive tumor sampling (one to two conventional slides of tumor when available). We used the CB-11 mAb, which has demonstrated its accuracy [21]. Our results highlight the complete concordance (100%) between IHC and FISH analyses in bladder carcinomas, when the same cut-off than for breast cancer is used. A quality assurance relying on internal and external controls for HER2 IHC determination contributed to the accuracy between IHC and FISH. IHC discordant results between two laboratories confirm that a calibration, according to FISH, appears mandatory to ensure the validation of a given HER2 IHC method.

Our series also indicate that a true HER2 overexpression in bladder carcinomas result from HER2 gene amplification, as in breast cancer. In the present study, 5.1% of invasive urothelial bladder carcinomas presented with a HER2 amplification as assessed by FISH, which was lower than rates observed in breast cancers. These results are in agreement with those described in the literature ranging from 0% to 32% [10–13, 15–18]. Interestingly, polysomy 17 is more common (31%) in high-grade, muscle-invasive urothelial bladder carcinomas than in invasive breast carcinomas (8%). Therefore, the inclusion of a chromosome 17 probe should be recommended.

An intratumoral heterogeneity, found in ~5% of invasive breast carcinomas scored 3+ [23, 24], was more frequent in our series of invasive urothelial bladder carcinomas scored 3+ (35%). This heterogeneity made immunohistochemical analyses more difficult to interpret than in breast cancers. Therefore, we recommend carrying out a FISH in a reference center in case of invasive urothelial bladder carcinoma showing heterogeneous HER2 IHC pattern. The results of the present study demonstrate that decision algorithms currently used in breast cancers is also appropriate in bladder cancers.

In ovarian and breast cancers, a good concordance was observed in HER2 status between primary tumor and distant metastases, indicating that a HER2 clonal selection occurred before tumor dissemination [25–27]. According to a recent study of 53 invasive urothelial bladder carcinomas and 42 paired lymph nodes metastases, the rate of concordance was 88% for HER2 overexpression and 100% for HER2 gene amplification between primary tumor of the bladder and metastatic sites, indicating that a dysregulation of this pathway was also maintained during bladder carcinoma metastatic spread [14]. Our findings were similar in the 10 couples of primary and metastatic bladder carcinomas. Thereby, the determination of HER2 status could be carried out either on the primary tumor or on metastatic sites. Nevertheless, the heterogeneity of HER2 overexpression in invasive urothelial bladder carcinomas may explain differences between primary tumor and metastases, as previously reported by Jimenez et al. [9].

Evidence from breast cancer indicates that only tumors with HER2 gene amplification respond to an anti-HER2-targeted therapy, such as trastuzumab. Using the same principle, ~5% of muscle-invasive urothelial bladder carcinomas should be suitable for such treatment. The potential involvement of HER2 in the proliferation of urothelial carcinoma led to the initiation of anti-HER2-targeted therapy protocols in advanced disease. Single-agent data with trastuzumab in urothelial cancer are not available or limited to case reports [28]. At first glance, it appears that the addition of trastuzumab to a three-drug regimen (paclitaxel, carboplatin, and gemcitabine) as first-line treatment did not significantly modify response rates and median survival [29]. Lapatinib, a dual inhibitor of HER1 and HER2 kinases, has been the subject of a single-arm, multicenter, open-label phase II study as second-line treatment of patients with locally advanced or metastatic urothelial bladder carcinomas. Negative results have been recently published with an objective response rate observed in only 1.7% of 59 patients [30]. These disappointing results may be due to unsuitable selection criteria, i.e. inclusion of HER2 2+ tumors not confirmed by FISH and inadequate FISH ratio cut-off. These preliminary results highlight the need for a better patient selection on the basis of robust and reproducible criteria. A good response to targeted therapies requires stringent assessment of the tumoral gene status. A French randomized, multicenter, phase II trial of gemcitabine plus platinum salt combined or not with trastuzumab in patients with HER2-overpressing advanced or metastatic bladder cancer is ongoing.

In conclusion, our study showed that 5.1% of invasive urothelial bladder carcinomas present with a HER2 amplification. This amplification can be assessed by a standardized IHC, using FISH in the ambiguous cases. These findings may have clinical implications for the management of...
patients with HER2-positive locally advanced or metastatic bladder cancer. In practice, all muscle-invasive urothelial bladder carcinomas should be tested for HER2 status according to the recommendations for breast carcinoma [19]. The proposed decision algorithm is to carry out calibrated HER2 IHC in all muscle-invasive urothelial bladder carcinomas as a screening test, confirmed by FISH in a reference center in case of equivocal or heterogeneous results.

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references
1. von der Maase H, Senglev L, Roberts J et al. Long-term survival results of a randomized trial comparing gemcitabine plus cisplatin, with methotrexate, vinblastine, doxorubicin, plus cisplatin in patients with bladder cancer. J Clin Oncol 2005; 23: 4602–4608.
2. Goldhirsch A, Wood WC, Gelber RD et al. Progress and promise: highlights of the international expert consensus on the primary therapy of early breast cancer 2007. Ann Oncol 2007; 18: 1133–1144.
3. Slamon DJ, Clark GM, Wong SG et al. Human breast cancer: correlation of erbB-2 gene amplification with clinical behavior. Science 1987; 235: 177–182.
4. Smith I, Procter M, Gelber RD et al. 2-year follow-up of trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer: a randomised controlled trial. Lancet 2007; 369: 29–36.
5. Rimond EH, Perez EA, Bryant J et al. Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. N Engl J Med 2005; 353: 1673–1684.
6. Slamon DJ, Clark GM, Wong SG et al. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 1987; 235: 177–182.
7. Yaziji H, Goldstein LC, Barry TS et al. HER-2 testing in breast cancer using parallel tissue-based methods. JAMA 2004; 291: 1972–1977.
8. Ayoub D, Bartlett J, Reeves J et al. C-erbB-2 gene amplification: a molecular marker in recurrent bladder tumors? Cancer Res 1995; 55: 2422–2430.
9. Jimenez RE, Hussain M, Bianco FJ Jr 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 Cancer Res 2001; 7: 2440–2447.
10. Latiff Z, Watters AD, Dunn I et al. HER2/neu overexpression in the development of muscle-invasive transitional cell carcinoma of the bladder. Br J Cancer 2003; 89: 1305–1309.
11. Latiff Z, Watters AD, Dunn I et al. HER2/neu gene amplification and protein overexpression in G3 pT2 transitional cell carcinoma of the bladder: a role for anti-HER2 therapy? Eur J Cancer 2004; 40: 56–63.
12. Coogan CL, Estrada CR, Kapur S, Bloom KJ. HER-2/neu protein overexpression and gene amplification in human transitional cell carcinoma of the bladder. Urology 2004; 63: 786–790.
13. Caner V, Turk NS, Duzcan F et al. No strong association between HER-2/neu protein overexpression and gene amplification in high-grade invasive urothelial carcinomas. Pathol Oncol Res 2008; 14: 261–266.
14. Hansel DE, Swain D, Dreicer R, Tubbs RR. HER2 overexpression and amplification in urothelial carcinoma of the bladder is associated with MYC reexpression in a subset of cases. Am J Clin Pathol 2008; 130: 274–281.
15. Matsuura H, Yamada Y, Narse K et al. Potential for HER-2/neu molecular targeted therapy for invasive bladder carcinoma: comparative study of immunohistochemistry and fluorescent in situ hybridization. Oncol Rep 2008; 19: 57–63.
16. Miyamoto H, Kubota Y, Noguchi S et al. C-ERBB-2 gene amplification as a prognostic marker in human bladder cancer. Urology 2000; 55: 679–683.
17. Krüger S, Weltisch G, Böttner H et al. Overexpression of c-erbB-2 oncprotein in muscle-invasive bladder carcinoma: relationship with gene amplification, clinicopathological parameters and prognostic outcome. Int J Oncol 2002; 21: 981–987.
18. de Pinieux G, Colin D, Vincent-Salomon A et al. Confrontation of immunohistochemistry and fluorescent in situ hybridization for the assessment of HER-2/neu (c-erbB-2) status in urothelial carcinoma. Virchows Arch 2004; 444: 415–419.
19. Wolff AC, Hammond ME, Schwartz JN et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. J Clin Oncol 2007; 25: 118–145.
20. Mitra AP, Datar RH, Cote RJ. Molecular pathways in invasive bladder cancer: new insights into mechanisms, progression, and target identification. J Clin Oncol 2006; 24: 5552–5564.
21. Vincent-Salomon A, MacGrogan G, Couturier J et al. Calibration of immunohistochemistry for assessment of HER2 in breast cancer; results of the French multicentre GEFPICS study. Histopathology 2003; 42: 337–347.
22. Couturier J, Vincent-Salomon A, Nicolas A et al. Strong correlation between results of fluorescent in situ hybridization and immunohistochemistry for the assessment of the ERBB2 (HER-2/neu) gene status in breast carcinoma. Mod Pathol 2000; 13: 1238–1243.
23. Chivukula M, Bhargava R, Buskuyt A et al. Clinical importance of HER2 immunohistologic heterogeneous expression in core-needle biopsies vs resection specimens for equivocal (immunohistochemical score 2+) cases. Mod Pathol 2006; 21: 365–368.
24. Colto PH, Aseelah J, Lee M et al. Intratumoral heterogeneity of HER2/neu expression and its consequences for the management of advanced breast cancer. Ann Oncol 2008; 19: 595–597.
25. Simon R, Nocito A, Hüscher T et al. Patterns of her-2/neu amplification and overexpression in primary and metastatic breast cancer. J Natl Cancer Inst 2001; 93: 1141–1146.
26. Vincent-Salomon A, Jouve M, Genin P et al. HER2 status in patients with breast carcinoma is not modified selectively by preoperative chemotherapy and is stable during the metastatic process. Cancer 2002; 94: 2169–2173.
27. Tiuet JF, Couturier J, Pennault-Llorca F et al. HER2 status in ovarian carcinomas: a multicenter GINECO study of 520 patients. PLoS ONE 2007; 2: e1138.
28. Peyromaure M, Scottet F, Amsellem-Ouazana D et al. Trastuzumab (Herceptin) in metastatic transitional cell carcinoma of the urinary tract: report on six patients. Eur Urol 2005; 48: 771–775.
29. Hussain NH, MacWor 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: 2218–2224.
30. Wülfing 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: 2881–2890.