Genomic Characterisation and Response to Trastuzumab and Paclitaxel in Advanced or Recurrent HER2-positive Endometrial Carcinoma

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Abstract. *Background/Aim:* Human epidermal growth factor receptor 2 (HER2) positivity is associated with a worse prognosis in endometrial cancer (EC). Trastuzumab as a single agent did not demonstrate activity in such cases but there are no reports on its combined use with taxanes. We report the outcome in patients treated simultaneously with trastuzumab and paclitaxel for advanced or recurrent HER2-positive endometrial carcinoma and compared it to their microsatellite instability (MSI) status and PIK3CA mutational profiles. *Patients and Methods:* Patients with advanced or recurrent endometrial carcinoma showing HER2 overexpression (2+ or 3+ immunohistochemical staining) or HER2 amplification (fluorescence in situ hybridization (FISH) HER2/chromosome 17 centromere (CEP 17) ratio >2.0) were treated with trastuzumab (8 mg/kg) and paclitaxel (90 mg/m²) every three weeks. Evaluation of the response was assessed according to the response evaluation criteria in solid tumors (RECIST) guidelines. Endometrial tumors, sampled before the beginning of trastuzumab, were genotyped for PIK3CA hot spot mutations using Sequenom iPLEX Assay technology. *Results:* Two uterine serous adenocarcinomas and

one grade 3 endometrioid adenocarcinoma showing HER2 positivity were treated with trastuzumab and paclitaxel. Between three and seven months of treatment, the three cases showed progressive disease. The genomic analysis of the three cases showed different mutational profiles. One case was found to have MSI and had one PIK3CA mutation. The two others showed no hot spot mutation for PIK3CA. *Conclusion:* Even associated with paclitaxel, HER2-positive endometrial carcinomas poorly responded to trastuzumab. This report underlines the low accuracy of HER2 positivity to predict response of endometrial cancer to combined targeted therapy using trastuzumab and paclitaxel.

Type II endometrial cancer (EC), which includes uterine serous carcinoma (USC), clear cell cancer and grade-3 endometrioid carcinoma cases, typically occurs in older non-overweight patients, is not hormone-dependent and represents a particularly aggressive type (1). Development of more effective therapeutic strategies against this rare subset of endometrial cancer is mandatory.

Trastuzumab has been developed and proven to be efficient in breast cancer overexpressing Her2/neu (2). Various groups reported Her2/neu overexpression (by immunohistochemistry (IHC)) in patients harbouring USC with rates between 20-60% and overexpression of the Her2/neu has been found to be related to a worse prognosis (3, 4). These findings have provided a rationale to the use of Her2/neu-targeted therapies in patients harbouring aggressive EC subtypes.

A phase II study evaluated efficacy of trastuzumab alone against advanced or recurrent HER2-positive endometrial

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Table I. Management and outcomes of patients treated by trastuzumab and paclitaxel.

	Age	Stage and type of endometrial cancer	Initial surgery	Primary adjuvant therapy	Reason for trastuzumab	IHC/FISH on recurrence	Response to trastuzumab
Case 1	57	Stage IVA (bladder and rectal invasion) Grade 3 Endometrioid adenocarcinoma	Hysterectomy, rectosigmoid and small intestine partial resection, lymphadenectomy, partial cystectomy and left ureter resection	FEC 6 courses Disease-free for 14 months Carboplatin paclitaxel for 5 courses	Lung metastases diagnosed 7 months after the end of carboplatin and paclitaxel	IHC 3+ Ratio HER2/CEP17: 6.08	Progression after 4 courses
Case 2	69	Stage IVB (locoregional extension, lymph nodes and hepatic metastasis) USC	No	Weekly carboplatin paclitaxel 12 courses Adriamycin 4 courses	Progression disease under Adriamycin	HER2 (3+) and Ratio HER2/CEP17: 10	Progression after 3 courses
Case 3	70	Stage IVB (locoregional extension, bi-iliac, perirectal and left inguinal lymph nodes) USC	No	Carboplatin and epirubicin 6 courses	Recurrence one year after the end of chemotherapy	HER2 (3+)	Progression after 10 courses

USC, Uterine serous carcinoma; FEC, fluorouracil, epirubicin and cyclophosphamide; IHC, immunohistochemistry; FISH, fluorescence *in situ* hybridization; HER2, human epidermal growth factor receptor 2; CEP17, chromosome 17 centromere.

carcinoma: three of eight clear cell carcinomas (38%) and 7 of 25 USCs (28%) screened exhibited *HER2* amplification compared with 7% (2/29) of endometrioid adenocarcinomas (4). Thirty four women were finally enrolled and no major tumor responses were observed.

However, considering that poor response to trastuzumab alone observed in advanced or recurrent *HER2*-positive endometrial carcinoma has also been reported in breast cancer (5), we thought important to report a series of patients who were treated with trastuzumab associated with chemotherapy. Similar to breast cancer, we expected better response rates in patients treated with this association based on the principle that the concomitant delivery of drugs with different mechanisms of action may allow for synergistic effects. Moreover, to better understand the mechanisms underlying the response to trastuzumab associated with chemotherapy, endometrial tumors were genotyped for microsatellite instability (MSI) and *PIK3CA* hot spot mutations.

Patients and Methods

Patients with stage IVB or recurrent endometrial carcinoma showing *HER2* overexpression (2+ or 3+ immunohistochemical staining) or *HER2* amplification (fluorescence *in situ* hybridization (FISH) *HER2*/chromosome 17 centromere (CEP 17) ratio >2.0) were treated with trastuzumab and paclitaxel. Trastuzumab and paclitaxel were delivered together, 3 x weekly, 8 mg/kg loading, 6 mg/kg maintenance dose and at a dose of 90 mg/m², respectively. Evaluation of response was assessed according to response evaluation criteria in solid tumors (RECIST) guidelines.

Informed consent was obtained from the patients.

Genotyping. Trastuzumab-naïve endometrial tumors before the beginning of trastuzumab were genotyped for MSI and *PIK3CA* hot spot mutations. For each tumor block, an hematoxylin and eosin slide was prepared and the tumor area delineated. Tumor tissue was macro-dissected from the individual slides and DNA extraction was performed. The iPLEX technology on a Mass ARRAY Compact Analyser (Sequenom Inc., San Diego, CA, USA) was used for mutation analysis, as reported previously (6). MSI status was checked as previously described by Zhao *et al.* (7). Briefly, 59 mononucleotide homopolymers were assessed for single-nucleotide insertions and deletions using Sequenom Massarray. Tumors were determined as MSI if eight or more insertions or deletions were found.

Results

Three patients, aged 57 to 70 years old, diagnosed with stage IV EC, were included (Table I). One of them underwent primary surgery, while the two others received chemotherapy first. Because of recurrent disease and after verifying *HER2* overexpression on recurrent tumor, the three patients received paclitaxel/trastuzumab. However, progressive disease was observed for the three cases during treatment.

The three samples had different mutation profiles. One sample showed mutations in *PIK3CA* gene (c.241G>A) and MSI positivity. In the two other samples no *PIK3CA* hot spot mutations were detected.

Discussion

In the treatment of *HER2*-positive breast cancer, combination therapy with chemotherapy plus trastuzumab is generally

more effective than single-agent trastuzumab. Unfortunately, in this small series we observed no evidence of activity of trastuzumab associated with paclitaxel against HER2-positive EC. Various reasons underlying resistance to trastuzumab (single or associated with chemotherapy) have been proposed in breast and EC (8).

Potential mechanisms of resistance to trastuzumab include signaling from other members of the HER family, cross talk from the insulin-like growth factor-I receptor to HER2, Met activation and increased signaling through the PI3-kinase pathway by a variety of mechanisms, including *PTEN* or *PIK3CA* gene mutation (9-11). Recently, oncogenic *PIK3CA* mutations have been suggested to represent biomarkers to predict response to trastuzumab in breast cancer (12). For this reason, we conducted a genomic study on *PIK3CA* hot spot mutations to identify possible mechanisms underlying the poor response observed and, eventually, novel strategies for overcoming trastuzumab resistance.

USC have *ERBB2* amplification in 27% of tumors and *PIK3CA* mutations in 42%, which could provide other or combined translational opportunities for targeted therapeutics (13). In our series, we observed different genetic profiles for *PIK3CA* hotspot mutations. Since no response was observed for those cases, the present results suggest that identification of responders to Herceptin® and paclitaxel should not focus only on these mutations. p95HER2, a truncated variant of HER2 that lacks the extracellular domain (ECD) where trastuzumab binds, has been suggested to be involved in trastuzumab resistance in breast cancer (14). Prospective trials are underway to validate p95HER2 expression as a biomarker associated with response to specific HER2 therapies (15).

In conclusion, the present series suggests that, even associated with taxanes, trastuzumab has poor efficacy in patients with advanced HER2-positive endometrial cancer. Hopefully, development of new target therapy able to overcome inherent resistance to anti-HER2 therapy in endometrial cancer will improve prognosis for this aggressive disease.

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Conflicts of Interest

The Authors report no financial or commercial conflicts of interest. No financial support was received for this study. The Authors have nothing to disclose.

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