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Case report

Selection of HER2/NEU negative tumor cells as a mechanism of resistance to trastuzumab in uterine serous carcinoma

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ABSTRACT

Background: Uterine serous carcinoma (USC) is an aggressive variant of endometrial cancer overexpressing HER2/neu in about 30% of cases. Trastuzumab, a humanized monoclonal antibody targeting Her2/Neu, in combination with carboplatin/paclitaxel, is considered the preferred regimen for the treatment of advanced or recurrent HER2/Neu+ USC per NCCN guidelines.

Case: We describe two USC patients with overexpression of HER2/neu at 2+/3+ level by immunohistochemistry and c-erbB2 gene amplification by fluorescence in situ hybridization (FISH) assay that, after an initial clinical response to trastuzumab, developed resistance/progression. Post-treatment biopsy (collected at the time of clinical progression on trastuzumab) demonstrated loss of HER2/neu overexpression in the recurrent/progressing tumor cells in both patients.

Conclusion: Selection of HER2/NEU negative tumor cells may represent a major mechanism of resistance to trastuzumab in USC patients.

1. Introduction

Uterine serous carcinoma (USC) is an aggressive variant of endometrial cancer. Although it represents less than 10% of endometrial tumors, it accounts for up to 39% of all endometrial cancer-related deaths (Hamilton, 2006). The mainstay of treatment of USC patients consists of a combination of comprehensive surgical staging followed by adjuvant chemotherapy (carboplatin-paclitaxel) plus/minus radiation treatment in the form of vaginal brachytherapy or pelvic radiation (Boruta et al., 2009).

The significance of HER2/neu (i.e., the human epidermal growth factor receptor 2 encoded by the c-erbB2 gene) overexpression and/or gene amplification has been well established in the pathogenesis and targeted treatment of breast, gastric, and gastroesophageal junction carcinomas. Furthermore, HER2/neu has been studied in endometrial cancer for over 20 years, and several studies from our research group as well as others have demonstrated that USC has the highest rate of HER2/neu expression among gynecological malignancies, with up to 35% of tumors harboring either strong (i.e., 3+) HER2/neu expression by immunohistochemistry (IHC) or c-erbB2 gene amplification by fluorescence in situ hybridization (FISH) (Buza et al., 2013). Importantly, HER2/neu overexpression and/or gene amplification have been previously correlated to worse overall survival in many human tumors, including USC patients (Santin, 2005).

Trastuzumab (Herceptin, Genentech, San Francisco, CA, USA) is a humanized monoclonal antibody targeting the HER2/neu receptor with proven clinical efficacy in the treatment of breast and gastric cancer patients. Accordingly, it is currently approved as a single agent or in combination with chemotherapy for the treatment of HER2/neu overexpressing metastatic breast cancer and gastric carcinoma. In contrast, for reasons that are currently poorly understood, single-agent trastuzumab has demonstrated very limited clinical efficacy (i.e., no partial or complete responses) in recurrent endometrial cancer patients overexpressing HER2/neu (Fleming, 2010).

Our group has recently completed a multicenter randomized phase II clinical trial comparing carboplatin-paclitaxel VS carboplatin-paclitaxel plus trastuzumab in HER2/neu positive USC patients (Fader, 2018). Sixty-one patients with advanced/recurrent disease were enrolled and tested positive for HER2/neu expression by IHC and/or c-erbB2 amplification by FISH before enrollment in the trial. The results of the randomized study demonstrated for the first time the experimental arm containing trastuzumab was not only well-tolerated but

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also able to significantly increase progression-free survival (PFS) by 4.6 months when compared to the standard chemotherapy control arm (12.6 VS 8 months, P = 0.005; HR 0.44; 90% CI, 0.26–0.76) (Fader, 2018). On the basis of these promising clinical results, the National Comprehensive Cancer Network (NCCN) Uterine Corpus Guidelines have endorsed the addition of trastuzumab to standard cytotoxic chemotherapy as the preferred regimen for the treatment of HER2/neu-positive, advanced or recurrent uterine serous carcinoma (National Comprehensive Cancer Network, 2019).

In the present study, we describe two patients diagnosed with advanced-stage USC and overexpressing HER2/neu at 3+ levels by IHC or at 2+ levels with a confirmatory positive FISH assay treated with trastuzumab. Both patients received the anti-HER2/neu targeted treatment either in combination with carboplatin-paclitaxel or as single agent and demonstrated clinical benefit on trastuzumab treatment. Of interest, both patients demonstrated on biopsy at the time of trastuzumab progression loss of HER2/neu overexpression in the recurrent tumor cells as confirmed by IHC and FISH assays. To our knowledge, these data represent the first evidence that the selection of HER2/neu negative tumor cells may represent a major mechanism of resistance to trastuzumab in USC patients.

2. Case series

Case # 1 was a 69 y.o. African American (AA) patient who underwent tumor staging through a robotic hysterectomy, bilateral salpingo-oophorectomy, pelvic node sampling, and omentectomy in 2016. Pathology revealed a USC metastatic to the omentum (FIGO stage IVB). HER2/neu IHC and FISH analysis were performed, demonstrating a HER2 expression score of 3+ by IHC as well as a FISH assay positive for c-erbB2 amplification (Fig. 1A and B). HER2/neu staining intensity was evaluated using the ASCO/CAP 2007 breast scoring criteria (as per the clinical trial protocol) (Wolff, 2013). Because of HER2/neu overexpression, she was enrolled in the NCT01367002 trial (carboplatin-paclitaxel vs. carboplatin-paclitaxel plus Trastuzumab) and randomized to trastuzumab arm. She completed 16 cycles of trastuzumab in October 2017 (6 cycles in combination with chemotherapy and 11 additional cycles every three weeks as a single agent). In November 2017, a CAT scan demonstrated ascites and new nonspecific abdominal nodularity. A diagnostic laparoscopy performed at that time with the collection of biopsies confirmed abdominal carcinomatosis secondary to a recurrent endometrial cancer histologically consistent with her original USC. HER2/neu expression on peritoneal biopsy demonstrated a score of 1+ by IHC and loss of c-erbB2 gene amplification by FISH in the trastuzumab-resistant tumor cells (Fig. 1C and D). Accordingly, she was removed from the study secondary to progression. At that time point, she received nine additional cycles of carboplatin and weekly paclitaxel (completed in May 2018). Secondary to progression, she was started on a dose-dense regimen based on Ixempra (Ixabepilone, 16 mg/m^2/weekly) plus Avastin (15 mg/kg/every three weeks) for three cycles. A CAT scan dated January 2019 demonstrated worsening carcinomatosis. She was then initiated on a dose-dense Abraxane (80 mg/m^2/weekly of a 28-day cycle) regimen in combination with Avastin (10 mg/kg every other week) and oral cyclophosphamide (50 mg q-day of a 28-day cycle). Unfortunately, she progressed on treatment. She was therefore started on Doxil (40 mg/m^2 every four weeks) and Avastin (10 mg/kg every other week) in May 2019, but after one cycle, the patient discontinued treatment secondary to increased nausea, fatigue, and weakness and was transitioned to hospice care. An additional HER2/neu IHC test performed on ascitic fluid demonstrated a HER2 score of 0. The patient eventually died of USC progression.

Case #2 is a 76 y.o. Caucasian female who underwent a total abdominal hysterectomy and bilateral salpingo-oophorectomy, omentectomy, pelvic and periaortic lymphadenectomy in 2012. Final

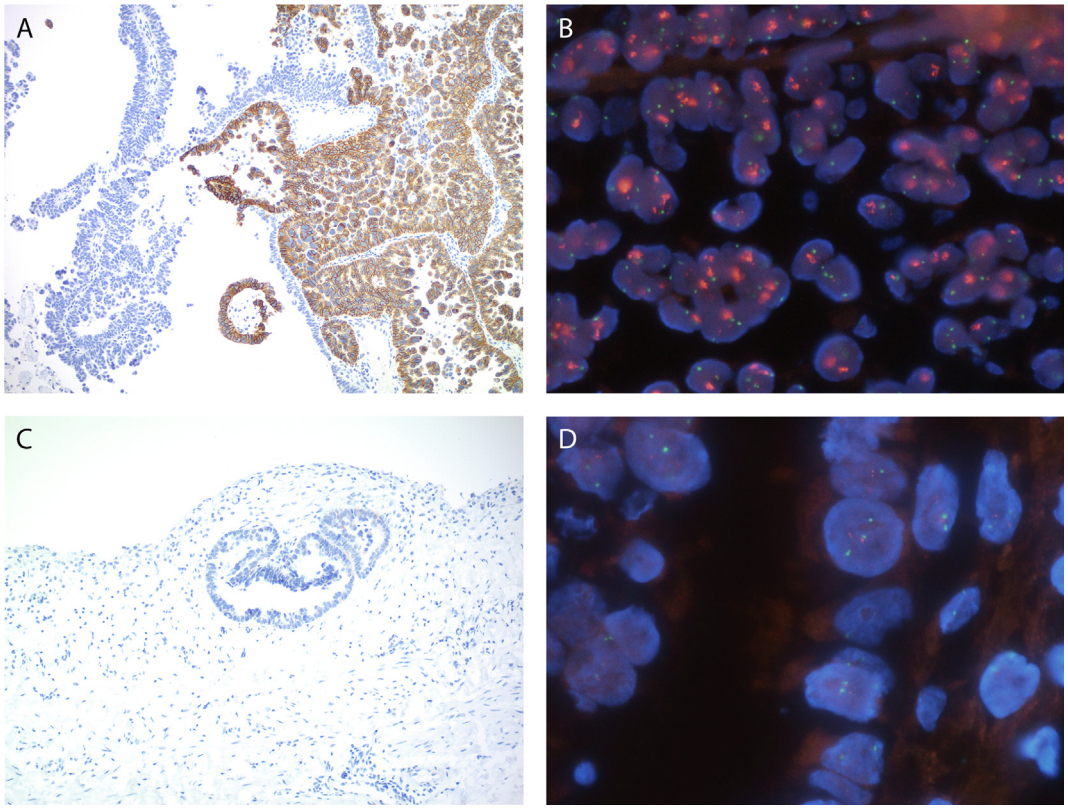

Fig. 1. Case 1. Representative immunostains demonstrating strong membranous staining (score 3+) with marked intratumoral heterogeneity (A) and Her2 gene amplification with a Her2/CEP17 ratio of 3.4 (B) before trastuzumab treatment. Her2 staining is only weak, focal (score 1+) in the recurrent tumor after trastuzumab treatment (C) and the Her2/CEP17 ratio is 0.6 (D). (Original magnification A, C: 100×; B, D: 600×).

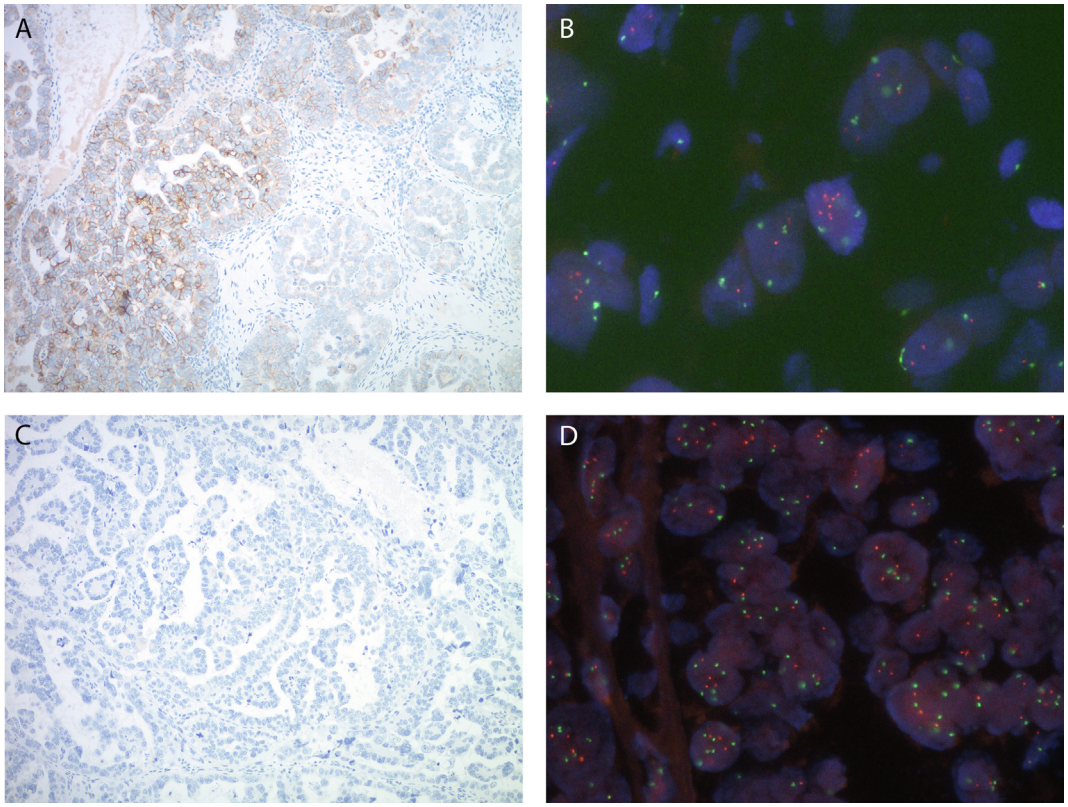

Fig. 2. Case 2. Representative immunostains demonstrating moderate membranous staining (score 2+) with intratumoral heterogeneity (A) and Her2 gene amplification with a Her2/CEP17 ratio of 1.8 (B) before trastuzumab treatment. Her2 staining is absent (score 0) in the recurrent tumor after trastuzumab treatment (C) and the Her2/CEP17 ratio is 0.8 (D). (Original magnification A, C: 100×; B, D: 600×).

pathology confirmed a USC with a minor (20%) clear cell component, FIGO Stage IVB due to omentum/peritoneum involvement. HER2/neu staining of the original tumor demonstrated a 2+ IHC score with a ratio of HER-2/neu to CEP17 of 3.69 to 1.8 (Fig. 2A and B) in different tumor blocks. She was enrolled in the NCT01367002 trial (carboplatin-paclitaxel versus carboplatin-paclitaxel plus Trastuzumab) and randomized to the control arm. She received six cycles of carboplatin-paclitaxel until December 2012 and then planned for surveillance. During follow up, in 2016, a CAT scan and a PET/CAT scan demonstrated the presence of new (hypermetabolic) nodule plaques in the left lower quadrant mesentery consistent with recurrent disease. She underwent an uneventful recto-sigmoid resection. Final pathology report confirmed the presence of recurrence/metastatic disease consistent with her original USC. On the patient's request, she was conservatively followed with frequent CAT scan imaging until March 2018 when a new 1 cm (hypermetabolic) nodule consistent with recurrent disease was detected adjacent to the ascending colon. Due to the tumor HER2/neu positivity, she was then started on trastuzumab single-agent. Response to treatment was followed with CAT scan imaging every three months. The disease remained stable until September 2019 (total 31 cycles of trastuzumab) when progression was diagnosed by the sudden increase in size of the ascending colonic nodule (ie, target lesion). A laparoscopic hemicolectomy was performed at that time. Pathology evaluation of the specimen demonstrated recurrent USC involving the ascending colon with loss of HER2/neu protein expression (IHC score = 0) and a negative FISH assay (Fig. 2C and D). The patient has been recently started on pembrolizumab in combination with lenvatinib. Her general conditions are stable at the time of writing this report.

3. Discussion

To our knowledge, this is the first report describing the loss of HER2/neu expression after trastuzumab treatment in USC patients. Trastuzumab, pertuzumab, and TDM-1 are some of the anti-HER2/neu targeted therapies currently used for the treatment of HER2-positive tumors such as breast cancer, gastric cancer, and uterine serous carcinomas. However, amongst these human malignancies, the subset of tumors overexpressing HER2/neu, their staining pattern and response to trastuzumab vary significantly according to the site of origin of the tumor. Consistent with this view, HER2/neu has been reported to be uniformly overexpressed throughout the tumor tissue in the majority of HER2/neu positive breast cancer patients, making tumor heterogeneity in breast cancer a rare event (Greer, 2013; Hanna et al., 2007).

In contrast, significant heterogeneity in HER2/neu expression is commonly observed in uterine serous and gastric carcinomas. Indeed, in gastric cancer, the frequency of intra-tumoral HER2/neu heterogeneity by IHC has been reported in up to 45–79% of the tumors (Kaito, 2019). Importantly, in this Japanese study evaluating the clinical significance of the HER2/neu heterogeneity in gastric cancer patients, a survival benefit of trastuzumab as adjuvant treatment was not observed. Accordingly, study authors suggested that intra-tumoral HER2/neu heterogeneity was the pivotal predictor of clinical response and a poor prognosticator for trastuzumab-based chemotherapy (Kaito, 2019).

Our group has previously reported significant heterogeneity of HER2/neu expression in USC patients (Buza et al., 2013). In a study comparing ASCO/CAP and FDA scoring criteria to assess HER2/neu in 108 endometrial carcinoma cases (85 pure serous carcinomas and 23 mixed carcinomas with a serous component), thirty-eight cases (35%) showed HER2/neu overexpression and/or gene amplification, 20 of which (53%) had significant heterogeneity of protein expression by IHC. Of interest, 74% of HER2/neu-positive USC lacked staining on their apical membrane surfaces, resulting in a lateral or basolateral ("U-shaped") pattern, an assessment observed more often in gastric/

gastroesophageal junction carcinomas than in breast cancer patients (Buza et al., 2013). Because of these results and in order to better select endometrial cancer patients benefitting the most from trastuzumab treatment (Fader, 2018), current recommendations for HER2/neu testing in USC and uterine carcinosarcoma (CS) patients suggest IHC to be performed on multiple tumor sections containing large areas of tumor (Buza et al., 2013; Rottmann, 2019).

In this study, we reported two advanced stage USC patients over-expressing HER2/neu and with proven c-erbB2 amplification treated with trastuzumab, which demonstrated loss of HER2/neu expression (i.e., scores 0 or 1 +) by IHC and negative FISH results in their recurrent tumors after trastuzumab treatment. Of interest, both tumor specimens demonstrated significant HER2/neu heterogeneity at the time of initial HER2/neu testing. We, therefore, speculated that HER2/neu heterogeneity under trastuzumab selective pressure may have led to the selection of HER2/neu-negative tumor clones able to survive over the HER2/neu-positive ones. HER2/neu loss of expression in this setting, similar to what has been recently described in breast cancer patients (Lee, 2015), may at least partially explain the rapid progression of metastatic disease in both uterine serous carcinoma patients. Taken together, our data suggest that intra-tumoral HER2-heterogeneity should be included in the assessment of HER2 positivity in future clinical trials in USC patients treated with anti- HER2/neu targeted agents.

Acknowledgments

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