Prolonged Response to Trastuzumab in a Patient With HER2-Nonamplified Breast Cancer With Elevated HER2 Dimerization Harboring an ERBB2 S310F Mutation

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Abstract

In the current genomic era, increasing evidence demonstrates that approximately 2% of HER2-negative breast cancers, by current standard testings, harbor activating mutations of ERBB2. However, whether patients with HER2-negative breast cancer with activating mutations of ERBB2 also experience response to anti-HER2 therapies remains unclear. This case report describes a patient with HER2-nonamplified heavily pretreated breast cancer who experienced prolonged response to trastuzumab in combination with pertuzumab and fulvestrant. Further molecular analysis demonstrated that her tumors had an elevated HER2 dimerization that corresponded to ERBB2 S310F mutation. Located in the extracellular domain of the HER2 protein, this mutation was reported to promote noncovalent dimerization that results in the activation of the downstream signaling pathways. This case highlights the fact that HER2-targeted therapy may be valuable in patients harboring an ERBB2 S310F mutation. (J Natl Compr Canc Netw 2015;13:1066–1070)

Case Report

A 51-year-old Caucasian female initially presented to an outside hospital with stage IIIA (pT3pN2aM0) invasive ductal carcinoma of the breast approximately 7 years before her presentation to our institute. The primary tumor was initially positive for estrogen (ER) and progesterone receptor (PR), but HER2-negative (1+) by immunohistochemistry (IHC). At that time, she underwent left lumpectomy with sentinel lymph node biopsy, which revealed a 5-cm moderately differentiated grade 2 infiltrating ductal carcinoma, and both of the sentinel lymph nodes (2 of 2) were positive for metastatic ductal carcinoma. She subsequently underwent axillary lymph dissection, which showed 2 additional involved axillary lymph nodes out of 22 lymph nodes. Thus, a total of 4 of 24 axillary lymph nodes were involved by metastatic ductal carcinoma.

She received adjuvant chemotherapy with dose-dense AC (doxorubicin and cyclophosphamide) followed by 1 cycle of paclitaxel, but developed an allergic reaction. The patient subsequently received 3 additional cycles of docetaxel. After adjuvant chemotherapy, she also completed adjuvant radiation and was on adjuvant tamoxifen for 3 years until she was found to have extensive liver metastases. At that time, she underwent an ultrasound-guided liver biopsy, which showed metastatic carcinoma consistent with breast primary. However, immunohistochemical staining of the liver biopsy showed no expression of ER (0%) or PR (0%) and no overexpression of HER2.
The patient subsequently received multiple lines of chemotherapy, including capecitabine, nab-paclitaxel, bevacizumab, gemcitabine, carboplatin, vinorelbine, pegylated liposomal doxorubicin, eribulin, CMF (cyclophosphamide, methotrexate, and 5-fluorouracil), and ixabepilone. Besides systemic chemotherapy, she also received liver-directed therapy with chemoembolization twice in the last 2 years. Despite these standard therapies, she developed progressive disease and was referred for possible clinical trials. Unfortunately, she was not eligible for the available phase I and II trials at that time because of abnormal liver and kidney function tests. The patient was recommended for hospice.

The patient presented with refractory metastatic breast cancer to our institute for a second opinion. At that time, a second liver biopsy was performed to reevaluate ER, PR, and HER2 status, and additional molecular studies were conducted. IHC staining of this liver biopsy was ER-positive (91% of tumor nuclei staining) and equivocal HER2 (2+ based on incomplete and/or weak to moderate circumferential membrane staining in >10% of invasive tumor cells). Further analysis using fluorescence in situ hybridization (FISH) showed no evidence of HER2 gene amplification based on a HER2/CEP17 ratio of 1.2. The patient was started on fulvestrant and everolimus. Given that she had liver dysfunction with a calculated Child-Pugh score of 10 (class C), she was started on 2.5 mg of oral everolimus daily. Nevertheless, her total bilirubin continued to increase from 5.8 to 10.4 mg/dL within 1 week, and everolimus was discontinued.

In the search for additional therapy options, the HERmark Breast Cancer Assay (Monogram Biosciences, South San Francisco, CA) was performed to quantitatively measure the total HER2 protein expression (H2T). Despite having equivocal HER2 status (2+) by IHC and lack of HER2 gene amplification by FISH, which was considered as HER2-negative by current standard HER2 testing, this patient’s HER2 total expression status by HERmark was positive (H2T=22.65 relative fluorescence [RF] units per mm²; Table 1, second liver biopsy). Using the VeraTag technology platform, this second liver biopsy also exhibited elevated levels of HER2-HER3 dimers, total HER3, phospho-HER3Y1289, and HER3-phosphoinositide 3-kinase (HER3-P13K) complex (Table 1).

Based on the HERmark HER2-positive result, the patient was started on trastuzumab and pertuzumab in combination with fulvestrant. Shortly after treatment initiation, the patient experienced a rapid reduction in total bilirubin from 14.0 to 6.8 mg/dL within 2 weeks, which declined to less than 2.0 mg/dL in less than 2 months (Figure 1A). Furthermore, levels of the tumor marker CA15-3 also significantly declined from 4669 to 1072 U/mL within 3 weeks after this treatment was started (Figure 1B). Four months later, her CA15-3 level completely normalized to below the abnormal cutoff of 31.3 U/mL. The patient experienced rapid symptomatic relief from malignant ascites and did extremely well with minimal side effects. Given that the patient’s disease recently progressed on the combination of fulvestrant, this remarkable response was likely due to trastuzumab and pertuzumab. She remained on trastuzumab and pertuzumab in combination with fulvestrant for almost 12 months.

Upon disease progression, a third liver biopsy was evaluated using the FoundationOne test in collaboration with Foundation Medicine (Cambridge, MA). Interestingly, the patient’s third liver biopsy was found to harbor the ERBB2 S310F mutation, which has been reported to be an activating mutation of the extracellular domain (ECD) of HER2. Retrospectively, the second liver biopsy specimen was tested and ERBB2 S310F was also identified in the pretreatment specimen. Following the third biopsy, her treatment was switched to lapatinib and trastuzumab. Shortly after, the patient unfortunately developed brain metastasis, progressive liver involvement, and liver failure. The patient passed away a few weeks later.

Retrospectively, we performed VeraTag assays on additional biopsied samples. We confirmed that her primary breast tumor was HER2-negative by HERmark (3.87 RF/mm²; Table 1). HERmark HER2 expression in a liver biopsy obtained after trastuzumab, pertuzumab, and fulvestrant treatment was 14.05 RF/mm², and upon repeat, was 17.68 RF/mm² (Table 1, third liver biopsy), both now HERmark HER2-equivocal. Similar increases in VeraTag RF/mm² for the HER3, phospho-HER3Y1289, and HER2-HER3 dimers from the primary breast biopsy to the second liver biopsy were followed by a subsequent reduction in levels of these analytes after trastuzumab, pertuzumab, and fulvestrant treatment at the time of the...
third liver biopsy. In contrast, less variation was seen in the HER3-P13K complex levels between the primary breast biopsy and the subsequent liver biopsies.

**Discussion**

The advent of targeted therapies against HER2 has drastically revolutionized the treatment of HER2-positive breast cancer in the past decade. However, it has been shown by previous clinical trials in the metastatic setting that the benefit of anti-HER2 therapies, such as trastuzumab and lapatinib, appears to be limited to patients with HER2-overexpressing tumors, measured either by IHC or FISH.1,2 HERmark is a novel assay that uses the proximity-based VeraTag platform to directly quantify proteins and protein complexes using a continuous scoring system. This technology uses the proximity-dependent release of a fluorescent reporter from a dual primary antibody immunoassay to precisely quantify proteins and protein complexes in formalin-fixed, paraffin-embedded tissue specimens.3 In contrast to current HER2 IHC testing, which is based on a subjective semiquantitative score, the HERmark assay produces a direct quantitative measurement of HER2 protein, measured as RF units per mm² of tumor (RF/mm²), over a 3-log dynamic range.4 Cutoffs have been established for HERmark HER2 status (positive, equivocal, negative) by comparison to independent central laboratory tests of IHC and/or FISH/chromogenic in situ hybridization from multiple cohorts involving more than 1000 samples from patients with breast cancer.4,5 In a study of patients with metastatic breast cancer treated with trastuzumab-based therapies, the HERmark assay was a better predictor of time to progression than standard HER2 FISH testing.6 This patient’s second liver biopsy was considered HER2-negative by IHC and FISH, but HER2-positive by HERmark, which provided her with the additional treatment option of HER2-targeted therapies.

The HER family of receptors dimerize and initiate a cascade of signaling events that result in the activation of the PI3K/AKT and MAPK pathways promoting survival and proliferation. The HER2-HER3 dimer is thought to be the most potent signal transducer of the HER dimers.7,8 Several preclinical studies underscore the importance of HER3 in the proliferation of HER2-positive breast cancer cell lines9,10; in a recent study, elevated HER3 expression, as measured by VeraTag (>3.5 RF/mm²), predicted resistance to trastuzumab therapy in HER2-positive metastatic breast cancer.12 In addition to HER3 protein expression, VeraTag assays can also measure activation of the HER3 signaling cascade, which includes HER2-HER3 dimers, the HER3-P13K complex, and phosho-HER3. These mea-

| Table 1 VeraTag Results Compared Between the Primary Breast Tumor and Subsequent Liver Metastases |
|-------------------------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Primary Breast Tumor in 2005 | First Liver Biopsy in 2008 | Second Liver Biopsy in 2012 | Third Liver Biopsy in 2013 |
| ER | ER+ (80%) | ER− (0%) | ER+ (91%) | ER+ (25%) |
| PR | PR+ (80%) | PR− (0%) | PR− (0%) | PR− (0%) |
| HER2 IHC | 1+ | 0 | 2+ | 3+ |
| HER2 FISH | 1.6 | Not tested | 1.2 | Not tested |
| VeraTag Assay | Normalized RPA (RF/mm²) | Normalized RPA (RF/mm²) | Normalized RPA (RF/mm²) | Normalized RPA (RF/mm²) |
| HERmark HER2 | 3.87 (negative) | NA+a | 22.65 (positive) | 14.05, 17.68 (equivocal) |
| P95HER2 | 0.75 | NA+a | 3.73 | 1.43 |
| HER3 | 1.09 | NA+a | 3.07 | 1.76 |
| Phospho-HER3Y1289 | 0.19 | NA+a | 1.34 | 0.79 |
| HER3-P13K dimer | 1.01 | NA+a | 0.71 | 0.86 |
| HER2-HER3 dimer | 0.77 | NA+a | 2.91 | 0.65 |

Abbreviations: ER, estrogen receptor; FISH, fluorescence in situ hybridization; IHC immunohistochemistry; NA, not available; PR, progesterone receptor; RF, relative fluorescence; RPA, relative peak area.

aFine-needle aspiration specimens are not acceptable for VeraTag assays.
Measurements may have clinical utility in the future, because patients with tumors activated by HER3 signaling may respond more favorably to addition of HER2 dimerization inhibitor, pertuzumab. However, further studies will be needed to further validate these results. VeraTag assays have demonstrated a strong correlation between H2T and downstream signaling complexes in HER2-positive breast tumors. The increase seen in the HER2-HER3 dimer and phospho-HER3Y1289 VeraTag measurements in this patient’s sample from the primary breast tumor to the second liver biopsy is consistent with the changes observed in HERmark HER2-negative versus HERmark HER2-positive breast cancer tumors. Finally, the observed decrease in HER2-HER3 dimer and phospho-HER3Y1289 VeraTag in the third liver biopsy is consistent with the mechanism of action of pertuzumab: that of inhibiting dimerization and resulting in downregulation of its downstream signaling.

In the era of next-generation sequencing (NGS), numerous genomic alterations have been reported across multiple tumor types. FoundationOne is a test that uses NGS technology to sequence the entire coding regions of 182 cancer-related genes, along with 36 introns of 14 genes frequently involved in gene fusions. Nevertheless, the clinical significance and functional outcomes of these mutations remain largely unknown. Multiple mutations in the ERBB2 gene have been previously reported by several groups. Most of these mutations are primarily located in 2 main encoding regions in relation to the HER2 protein structure. Most of the mutations cluster in the kinase domain, which can result in autophosphorylation and activation of downstream signaling cascade. Some of these kinase domain mutations have been previously reported to confer resistance to tyrosine kinase inhibitors, such as lapatinib. Another less common mutation hotspot is located in the ECD region of the HER2 protein. These mutations, including amino acids 309 and 310, cluster in the subdomain II or the dimerization domain. The functional analysis of these ERBB2 ECD mutations (including S310F, S310Y, and G309E) demonstrates that these 3 mutations are activating mutations. Overexpression of any of these 3 mutations results in malignant transformation and increased colony formation in NIH 3T3 mouse fibroblasts, AALE human lung epithelial cells, and the Ba/F3 mouse lymphoid cell line. However, S310F mutant protein appears to have substantially higher protein phosphorylation and greater oncogenic activity compared to G309E. In other series, S310 mutations, including S310F and S310Y, were reported in approximately 1% of breast cancer cases. S310F and S310Y mutations have been speculated to result in hydrophobic interactions, which promote noncovalent dimerization and subsequent activation of the downstream signaling pathways. This is reflected in the present case, in which the second liver biopsy harboring S310F mutation showed increases in the HER2-HER3 complex, HER3-P13K dimers, and phospho-HER3Y1289. Given that these ECD mutations are located mainly in the dimerization domain, which is not the site for trastuzumab-binding, cells overexpressing these ECD mutant proteins retain sensitivity to both trastuzumab and small molecule inhibitors of HER family kinases, such as lapatinib, neratinib, and afatinib. Furthermore, irreversible inhibitors, such as neratinib and afatinib, appear to be more effective than lapatinib, because rebound increases in phospho-HER2 and phospho-AKT were observed after the removal of lapatinib.
but not in neratinib-treated cells. In the present case, this patient also did not appear to benefit from lapa
tinib upon progression. Given that ado-trastuzumab emtansine (TDM1) has a similar binding site as the
original trastuzumab, it is likely that TDM1 may also be effective in these ECD mutants. Unfortunately,
the present patient developed brain metastasis and rapid progressive liver failure and passed away before
receiving this therapy. The effects of pertuzumab on these ECD mutants are uncertain because it has not
been formally tested. However, the binding of per
tuzumab may be affected by these mutations, because
amino acids 309 and 310 are known to be part of the
erasmus binding epitope.15,18,24

Conclusions
This case report highlights the fact that biopsy, if it
can be safely performed, should be considered to reass
sess ER, PR, and HER2 status of distant metastases,
because changes in these molecular markers may provide
patients with additional treatment options. Further
more, novel molecular assays, including HERmark/
VeraTag assays and mutation analysis with NGS, may
shed light further into tumor growth activation and
potential resistant mechanisms, and thus provide ad
ditional treatment options. S310 mutations, like that
seen in this patient with breast cancer, have also been
reported in lung,26 ovarian,27 and bladder cancers.26,28
HER2-targeted therapies may serve as new treatment
options for these other patients. However, additional
studies are needed to further explore the efficacy of
these agents in other ERBB2 mutants in breast cancer
and other cancer types.

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