Detection of HER2-Positive Metastases in Patients with HER2-Negative Primary Breast Cancer Using ^{89}Zr-Trastuzumab PET/CT

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Our objective was to determine whether imaging with a human epidermal growth factor receptor 2 (HER2)–targeted PET tracer can detect HER2-positive metastases in patients with HER2-negative primary breast cancer. **Methods:** Patients with HER2-negative primary breast cancer and evidence of distant metastases were enrolled in an Institutional Review Board–approved prospective clinical trial. Archived pathologic samples from the patient's primary breast cancer were retested to confirm HER2-negative disease. Patients with confirmed HER2-negative primary breast cancer underwent ^{89}Zr-trastuzumab PET/CT to screen for ^{89}Zr-trastuzumab metastases. Metastases avid for ^{89}Zr-trastuzumab by PET/CT were biopsied and pathologically examined to define HER2 status. Patients with pathologically proven HER2-positive metastases subsequently received off-protocol HER2-targeted therapy to evaluate treatment response. **Results:** Nine patients were enrolled, all of whom had pathologic retesting that confirmed HER2-negative primary breast cancer. Five demonstrated suggestive foci on ^{89}Zr-trastuzumab PET/CT. Of these 5 patients, 2 had biopsy-proven HER2-positive metastases and went on to benefit from HER2-targeted therapy. In the other 3 patients, biopsy showed no evidence of HER2-positive disease, and their foci on ^{89}Zr-trastuzumab PET were considered false-positive. **Conclusion:** In this proof-of-concept study, we demonstrated that ^{89}Zr-trastuzumab PET/CT detects unsuspected HER2-positive metastases in patients with HER2-negative primary breast cancer. Although these are only initial results in a small sample, they are a proof of the concept that HER2-targeted imaging can identify additional candidates for HER2-targeted therapy. More specific HER2-targeted agents will be needed for clinical use.

Key Words: breast; molecular imaging; oncology; PET/CT; HER2; trastuzumab

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Human epidermal growth factor receptor 2 (HER2) is a critical biomarker in breast cancer, and its expression directly influences treatment. Approximately 20% of invasive ductal breast malignancies are classified as HER2-positive as a result of *ERBB2* gene amplification or the subsequent overexpression of the HER2 protein on the surface of tumor cells (*1*). Patients with HER2-positive breast cancer receive specific targeted HER2 therapies that reduce the risk of death, whereas patients with HER2-negative breast cancer do not receive them (*2,3*).

Heterogeneity of tumors both within and across lesions in a single patient is increasingly being documented, with significant therapeutic implications (*4*). Evidence from tissue samples suggests that HER2 expression may change between the primary breast malignancy and metastases (*5–7*). Inaccurate knowledge of receptor status in metastases due to tumor heterogeneity may lead to suboptimal selection of patients for HER2-targeted therapy. Indeed, data suggest that 10%–15% of patients with HER2-negative primary breast cancer may still benefit from HER2-targeted treatment (*8*). It is currently unclear why some patients with HER2-negative breast cancer may benefit from HER2-targeted treatments or how to identify them.

We hypothesized that some patients with HER2-negative primary malignancies develop HER2-positive metastases that can be identified by imaging. Such identification would be difficult by conventional biopsies, as only small samples from a limited number of lesions could be evaluated. In contrast, specific radiotracers that identify HER2 could allow a whole-body evaluation of all identifiable lesions. Specifically, ^{89}Zr-trastuzumab is a PET radiotracer that allows visualization of HER2-positive lesions (*9*). ^{89}Zr-trastuzumab PET/CT has been used in patients with known HER2-positive breast cancer to help determine which patients will respond to HER2-targeted therapy (*10*). We performed a prospective clinical trial evaluating the ability of ^{89}Zr-trastuzumab PET/CT to detect HER2-positive metastases in patients with HER2-negative primary breast cancer, and in this article we report the initial results of that trial.

MATERIALS AND METHODS

Patients

The study was performed under a prospective single-center protocol approved by the Institutional Review Board of Memorial Sloan

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Kettering Cancer Center (MSKCC) (ClinicalTrials.gov identifier NCT02286843). All patients provided written informed consent. Patients receiving treatment for metastatic HER2-negative primary breast cancer at MSKCC were identified as potential candidates. The inclusion criteria were a biopsy-proven HER2-negative primary malignancy, biopsy-proven metastatic disease, foci of demonstrable metastases on imaging modalities within 6 wk of enrollment, age greater than 18 y for women, and Eastern Cooperative Oncology Group performance score of 0–2. The exclusion criteria were a creatinine level higher than 2 times the upper limit of normal, an aspartate aminotransferase/alanine aminotransferase level higher than 2 times the upper limit of normal, life expectancy less than 3 mo, pregnancy or lactation, and inability to undergo PET/CT scanning because of weight limits.

Retesting of Archived Tissue

After receiving written consent from the patients, we retested their archived samples of primary breast cancer tissue to ensure that they were HER2-negative (Fig. 1). HER2 protein overexpression was evaluated by immunohistochemical staining using a Food and Drug Administration (FDA)–approved monoclonal antibody (clone 4B5; Ventana) directed against the internal domain of the c-erbB-2 oncoprotein (HER2). The immunohistochemistry scores were categorized according to the guidelines of the American Society of Clinical Oncology (ASCO) as follows: 0 or 1+, negative; 2+, equivocal; 3+, positive (Table 1) (*11*). Tissues with a score of 2+ were assessed for HER2 amplification with fluorescence in situ hybridization (FISH) in accord with the ASCO guidelines (*11*), using an FDA-approved probe set (*HER2* IQFISH pharmDx; Dako), and a positive FISH result was defined as a HER2/CEP17 (chromosome enumeration probe 17) ratio of at least 2.0. Tissues with an immunohistochemistry score of 0 or 1+ or an immunohistochemistry score of 2+ with a concurrently negative FISH result were classified as HER2-negative.

^{89}Zr-Trastuzumab PET/CT

After retesting of the archived tissue, patients with confirmed HER2-negative tumors underwent ^{89}Zr-trastuzumab PET/CT to assess for ^{89}Zr-trastuzumab foci suggestive of HER2-positive disease. ^{89}Zr-trastuzumab comprises the native HER2-targeted drug trastuzumab conjugated with desferoxamine and labeled with the positron-emitting metalloradionuclide ^{89}Zr, which has a half-life of 78 h, long enough to allow favorable biodistribution of radiolabeled intact antibodies. Trastuzumab is an FDA-approved monoclonal antibody that disrupts HER2 receptor signaling. MSKCC has an acknowledged investigational new drug (119907) from the FDA for human ^{89}Zr-trastuzumab imaging. ^{89}Zr-trastuzumab was produced under good-manufacturing-practice conditions: trastuzumab was chelated with desferoxamine and subsequently radiolabeled with ^{89}Zr using a previously described methodology (*12*). The final drug product for human use was manufactured and quality control–tested by qualified personnel in conformance with the approved written standard operating procedures. The manufactured drug was tested before being released for patient administration to ensure that it conformed to the established acceptance specifications for appearance, pH, endotoxin content, residual solvent content, sterilizing filter integrity, radiochemical purity, and radiochemical identity. Sterility testing was initiated after the product had been released for patient administration.

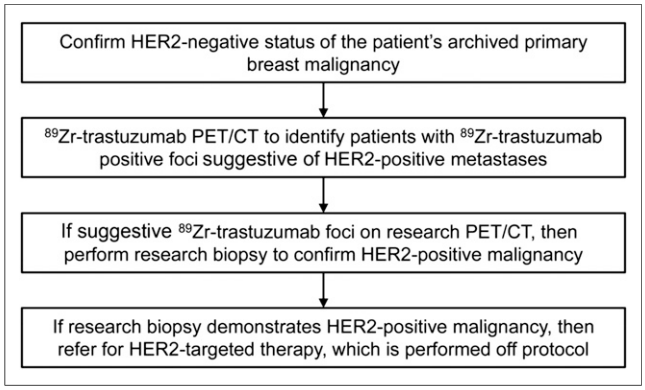

FIGURE 1. Protocol schema.

TABLE 1
Algorithm for Defining HER2 Expression in This Study

HER2 test result	Definition
Immunohistochemistry	
Score of 0 or 1+	Negative
Score of 2+	Equivocal
Score of 3+	Positive
FISH	
HER2/CEP17 ratio $\geq$ 2.0	Positive
HER2/CEP17 ratio $<$ 2.0	Negative

First, immunohistochemistry is performed. If result is 2+, FISH is performed.

Patients received a nominal 185 MBq ± 10% of ^{89}Zr-trastuzumab intravenously over 5–10 min. To optimize tumor targeting, radiolabeled ^{89}Zr-trastuzumab was brought up to a final mass dose of 50 mg by the addition of nonradiolabeled trastuzumab at the end of the production (*9*). The final mass dose of 50 mg was provided by the MSKCC radiochemistry service to the clinic for patient administration. Five or six days after ^{89}Zr-trastuzumab administration, the patients underwent PET/CT from the mid-skull to the mid-thigh on a dedicated research Discovery PET/CT 710 scanner (GE Healthcare), with an 80-mA CT component for attenuation correction and lesion localization. The PET/CT images underwent iterative reconstruction, were displayed in multiplanar reconstructions, and were interpreted by 2 different nuclear medicine experts, both of whom were experienced in the use of novel research PET radiotracers. Physiologic ^{89}Zr-trastuzumab uptake was expected in the blood pool, liver, gallbladder, bowel, kidney, and (at a low grade) bone. Radiotracer uptake in areas that are not physiologic was graded both qualitatively and semiquantitatively. For qualitative grading, the foci were graded as suggestive or not suggestive. Only those foci qualitatively graded as suggestive by both interpreters were considered suspected lesions. For semiquantitative grading, 3-dimensional volumes of interest were placed around these suspected lesions, and the tracer uptake was graded using SUV_{max} (decay-corrected mean activity in volume of interest [μCi/cm^3]/(injected dose [μCi]/body weight [g])).

Pathologic Confirmation

Image-guided biopsy of sites suggestive on PET/CT was performed in concert with an experienced oncologic interventional radiologist to minimize risk to the patient while obtaining high-quality samples. Biopsy specimens were evaluated by board-certified breast pathology specialists. Immunohistochemical staining was performed, and the results were categorized according to the ASCO guidelines (*11*) in the same way as for retesting of the archived

tissues. Specimens with an immunohistochemistry score of 3+ or with an immunohistochemistry score of 2+ and concurrently positive FISH results were classified as HER2-positive metastases. If the immunohistochemistry results were equivocal and FISH testing failed to produce a result after repeated attempts, the HER2 amplification status was also assessed using a hybrid capture-based next-generation sequencing assay, MSK-IMPACT (Memorial Sloan Kettering–Integrated Mutation Profiling of Actionable Cancer Targets), in a Clinical Laboratory Improvement Amendment–certified lab (*13*). This assay is designed to detect somatic genetic alterations in cancer-related genes, in addition to enabling the accurate assessment of genomewide copy number. Amplification of *ERBB2* is reported if the change is at least 2.0-fold on MSK-IMPACT.

Although therapy was not a component of this clinical trial, when HER2-positive metastases were identified and confirmed pathologically, this information was provided to the treating oncologists. HER2-targeted therapy was then initiated at the discretion of the treating oncologists per standard prescribing guidelines. Likewise, imaging to determine tumor response to HER2-targeted therapy was not defined by the protocol.

RESULTS

Patient Characteristics

Between December 2014 and July 2015, 9 patients, all women with estrogen receptor (ER)–positive and HER2-negative primary invasive ductal breast cancer, completed the study protocol. The patient characteristics are summarized in Table 2.

Metastatic Sites at Enrollment

All patients had at least one site of metastatic disease proven by biopsy. Once one site of metastatic disease was proven by biopsy, additional sites were determined from abnormalities on contrast-enhanced CT or ^{18}F-FDG PET/CT. The most common sites of distant metastases at the time of enrollment were nodes ($n = 8$), followed by bone ($n = 7$), liver ($n = 7$), lung ($n = 2$), adrenal gland ($n = 1$), and pleura ($n = 1$). Eight of 9 patients had metastatic involvement in multiple organ systems.

Retesting of Archived Tissue

On immunohistochemical retesting of the patients' archived primary breast cancer specimens, all 9 patients had confirmed HER2-negative primary malignancies. Four patients had an immunohistochemistry score of 0, whereas the other 5 had an immunohistochemistry score of 1+.

^{89}Zr-Trastuzumab PET/CT

All 9 patients underwent ^{89}Zr-trastuzumab PET/CT. They were monitored for side effects for 30 min after tracer injection, as well as being telephoned the following day, and no side effects were observed or reported. Vital signs were recorded before and after injection, and there were no changes that had a clinical impact.

In 5 of the 9 patients (56%), both interpreters observed foci of ^{89}Zr-trastuzumab avidity considered suggestive of HER2-positive disease. In no case were the suggestive foci seen by only one interpreter. Both interpreters were able to compare the ^{89}Zr-trastuzumab PET/CT results with prior imaging studies. In 3 patients, the suggestive organ system was bone, whereas one patient exhibited suggestive nodal foci, and another had a suggestive adrenal focus.

Pathologic Confirmation

Image-guided biopsy was performed on 5 patients with suggestive ^{89}Zr-trastuzumab foci.

Patient 2 was a 41-y-old woman who, in March 2014, had undergone a right mastectomy with pathologically demonstrated ER-positive/HER2-negative primary invasive ductal carcinoma (Fig. 2A). Thus, she was treated initially with ovarian suppression and tamoxifen and then with fulvestrant and aromatase inhibition, but the response to treatment was mixed. In November 2014, CT demonstrated enlarged thoracic nodes consistent with malignancy, and in December 2014, ^{89}Zr-trastuzumab PET/CT demonstrated ^{89}Zr-trastuzumab–avid thoracic nodes (Fig. 2B). The most avid was a right supraclavicular node (SUV_{max}, 4.6), which underwent biopsy and demonstrated an immunohistochemistry score of 3+ (Fig. 2C). This ^{89}Zr-trastuzumab focus was considered true-positive for HER2-positive distant metastasis. The patient was then switched to treatment with

TABLE 2
Patient Demographics and HER2 Expression Results

Patient no.	Age (y)	Metastatic sites at enrollment	Confirmatory HER2 IHC of primary breast cancer	Suggestive foci on ^{89}Zr-trastuzumab PET/CT?	Image-guided biopsy results
1	46	Bone, liver	0	None	
2	41	Nodes	1+	Nodes (SUV_{max}, 4.6)	IHC, 3+
3	58	Bone, liver, nodes	0	Bone (SUV_{max},7.1)	IHC, 1+
4	69	Bone, liver, nodes	0	None	
5	38	Bone, liver, nodes	1+	Bone (SUV_{max}, 5.9)	IHC, 2+; FISH, failure; MSK-IMPACT, amplified
6	42	Nodes, adrenal	1+	Adrenal (SUV_{max}, 9.2)	IHC, 2+; FISH, 1.4
7	83	Bone, liver, nodes, lung	1+	Bone (SUV_{max}, 9.7)	IHC, 1+
8	54	Bone, liver, nodes, lung	0	None	
9	48	Bone, liver, nodes, pleura	1+	None	

IHC = immunohistochemistry score.
All 9 women had primary invasive ductal breast cancer.

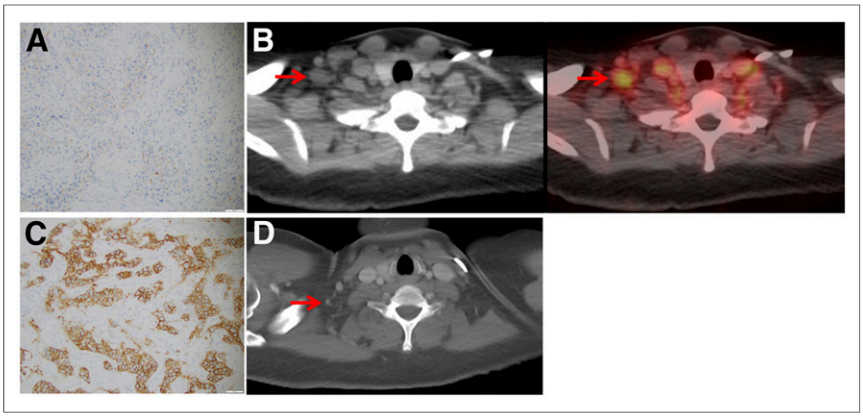

FIGURE 2. 41-y-old woman with primary ER-positive, HER2-negative invasive ductal breast carcinoma and recurrence in thoracic nodes. (A) Immunohistochemistry score of primary breast malignancy was 1+ (at ×20 magnification), consistent with HER2-negative malignancy. (B) Axial CT and ^{89}Zr-trastuzumab PET/CT demonstrated ^{89}Zr-trastuzumab avidity in enlarged right supraclavicular nodes (arrows, SUV_{max} of 4.6) and left internal mammary nodes (not shown). (C) Biopsy of right supraclavicular node demonstrated metastatic breast carcinoma with immunohistochemistry score of 3+ (at ×20 magnification), consistent with HER2-positive disease. Patient began systemic treatment including trastuzumab and pertuzumab. (D) Follow-up axial CT after 2 mo of treatment demonstrated resolution of nodes on CT (arrow).

trastuzumab, pertuzumab, and paclitaxel. Follow-up CT of the chest, abdomen, and pelvis demonstrated resolution of the previously enlarged lymph nodes and absence of new lesions, consistent with a complete response (Fig. 2D). As of November 2015, the patient remained on trastuzumab and pertuzumab alone and was without evidence of disease.

Patient 5 was a 38-y-old woman who, in July 2011, had undergone a left mastectomy with pathologically demonstrated ER-positive/HER2-negative primary invasive ductal carcinoma (Fig. 3A). Osseous, hepatic, and nodal metastases were identified beginning in October 2013 and were progressing despite systemic therapy with fulvestrant, leuprolide, and a novel clinical trial therapeutic. In April 2015, ^{89}Zr-trastuzumab PET/CT demonstrated multiple suggestive osseous foci (Fig. 3B). The most avid foci were in the right ilium and right proximal femur (SUV_{max}, 5.9). Biopsy of the right ilium demonstrated equivocal HER2 findings. The immunohistochemistry results were equivocal (Fig. 3C) because of incomplete membranous staining in a small percentage of the cells. FISH failed to produce a result after repeated attempts. Given the equivocal results, further testing was performed with the MSK-IMPACT assay (*13*). The change in *ERBB2* on MSK-IMPACT was 2.6-fold (Fig. 3D). Therefore, this ^{89}Zr-trastuzumab focus was considered true-positive for a HER2-positive distant metastasis (*14*). The patient was then switched to treatment with trastuzumab, pertuzumab, and docetaxel and was followed up with ^{18}F-FDG PET/CT, which showed a decrease in the size and ^{18}F-FDG avidity of the liver and nodal metastases, as well as a decrease in the ^{18}F-FDG avidity of the osseous lesions, representing a partial response to treatment (Figs. 4A and 4B). In the liver, multiple ^{18}F-FDG–avid lesions resolved after treatment, whereas a residual lesion in segment 4 showed an SUV_{max} decrease from 8.2 to 5.6. In the osseous system, multiple ^{18}F-FDG–avid lesions resolved after treatment and others showed an SUV_{max} decrease, such as a decrease from 9.1 to 4.7 in the body of T11.

Patients 3, 6, and 7 had suggestive foci on ^{89}Zr-trastuzumab PET/CT, but the pathologic findings from image-guided biopsy specimens were consistent with HER2-negative metastatic breast cancer (Table 2). For example, patient 7 was an 83-y-old woman who presented in June 2010 with metastatic ER-positive, HER2-negative invasive ductal breast cancer (Fig. 5A). Despite several courses of chemotherapy, in 2015 she had persistent osseous, hepatic, nodal, and pulmonary metastases on ^{18}F-FDG PET/CT. ^{89}Zr-trastuzumab PET/CT was performed in July 2015 and demonstrated multiple suggestive osseous foci (Figs. 4B and 4C). Because the most avid osseous lesion was in the cervical spine (SUV_{max}, 9.7), a more easily assessable lesion in the proximal left femur (SUV_{max}, 7.7) was chosen for biopsy. Pathologic examination demonstrated metastatic breast cancer, but the immunohistochemistry score was only 1+, consistent with HER2-negative disease (Fig. 4D). This ^{89}Zr-trastuzumab focus was considered a false-positive finding. Similarly, biopsy found that the foci in the left ilium of patient 3 were false-positive (SUV_{max}, 7.1), as were the foci in the left adrenal gland of patient 6 (SUV_{max}, 9.2).

Patients 1, 4, 8, and 9 did not have suggestive ^{89}Zr-trastuzumab foci.

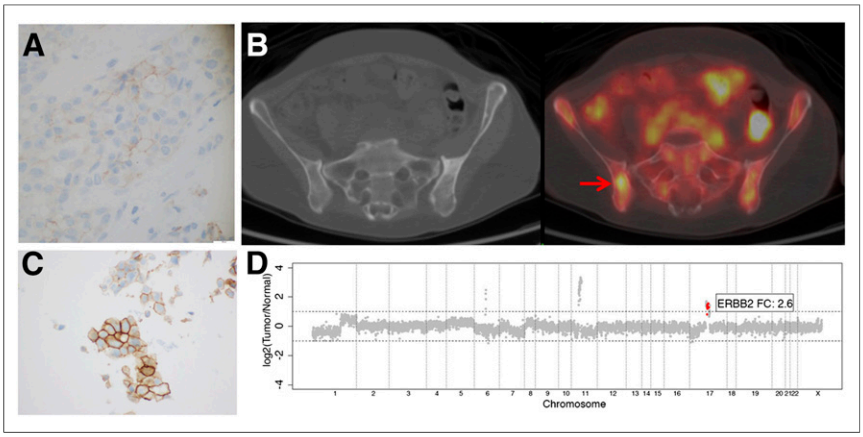

FIGURE 3. 38-year-old woman with primary ER-positive/HER2-negative invasive ductal breast carcinoma. (A) Immunohistochemistry score of primary breast malignancy was 1+ (at ×400 magnification), consistent with HER2-negative malignancy. (B) Axial CT and ^{89}Zr-trastuzumab PET/CT demonstrated ^{89}Zr-trastuzumab avidity in right ilium (arrow, SUV_{max} of 5.9). Avidity in bowel is considered physiologic. (C) Biopsy of right ilium demonstrated metastatic breast carcinoma with equivocal immunohistochemistry score of 2+ (at ×400 magnification). (D) MSK-IMPACT copy-number plot demonstrating HER2 amplification. Each dot represents probe set, and values on *y*-axis show log2-transformed ratio of tumor vs. normal. *ERBB2* change was 2.6-fold, consistent with HER2-positive disease.

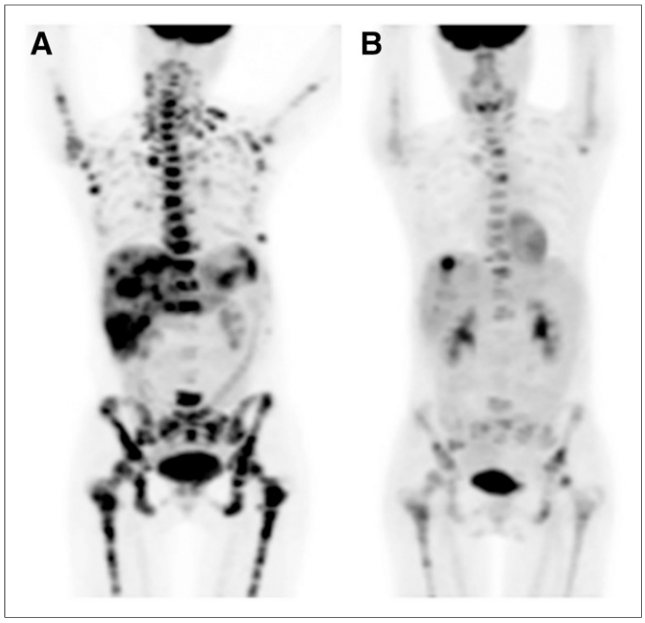

FIGURE 4. Patient from Figure 3 underwent HER2-targeted therapy after biopsy had demonstrated HER2 amplification in osseous metastasis. Maximum-intensity projections from ^{18}F-FDG PET/CT studies before (A) and after (B) 3 mo of systemic treatment including trastuzumab and pertuzumab demonstrate treatment response.

DISCUSSION

Heterogeneity within a tumor, as well as across multiple tumors within a patient, has often been demonstrated. Thus, a limited number of small biopsies may not be able to accurately characterize multiple tumors in a single patient. This limitation has substantial implications for patients with breast cancer, which critically requires accurate documentation of receptor phenotype for selection of targeted systemic therapies. In this study, we demonstrated a proof of the concept that targeted HER2 imaging can detect unsuspected HER2-positive metastases in patients initially classified as having a HER2-negative primary breast malignancy. We also showed that these patients may go on to benefit from HER2-targeted therapy.

Although medical imaging of oncology patients has traditionally been used to detect tumors, determine stage, and evaluate treatment response, this study suggests that targeted medical imaging may help physicians select the particular targeted systemic therapy from which an individual patient can benefit. Over 900,000 women are currently living with metastatic breast cancer, with more than 50,000 new cases diagnosed each year (*15*). Eighty percent of these women have HER2-negative primary malignancies. If just 10% of the patients with metastatic HER2-negative primary breast cancer were found to harbor HER2-positive metastases, that would represent a current population of over 72,000 women. Thus, targeted HER2 imaging could substantially increase the number of patients who may be eligible for and benefit from HER2-targeted therapies.

Of the initial 9 patients with HER2-negative primary breast cancer in our study, 2 patients had suggestive ^{89}Zr-trastuzumab foci that were classified as HER2-positive metastases. One of these patients had a primary malignancy with a negative immunohistochemistry result and a metastasis with a positive immunohistochemistry result. The other patient had a primary malignancy with a negative immunohistochemistry result but a metastasis with an equivocal result. Use of the MSK-IMPACT assay in this patient after failure of FISH testing was positive for *ERBB2* amplification. Detection of *ERBB2* amplification by next-generation sequencing is not currently part of the ASCO guidelines (*13*); however, MSK-IMPACT detection of copy-number alterations correlates strongly with immunohistochemistry and FISH and thus, in this study, was considered evidence of HER2 positivity (*14*). A response to systemic HER2-targeted therapy was demonstrated in 2 patients, but because these patients also received concomitant taxane antimitotic chemotherapy, a definitive conclusion cannot be drawn about the HER2 therapy. Confirming the efficacy of HER2-targeted therapy in patients identified as eligible by HER2-targeted imaging will require further study.

Three patients had suggestive ^{89}Zr-trastuzumab foci that on biopsy were classified as HER2-negative metastases. Two of these patients underwent osseous biopsy demonstrating an immunohistochemistry score of only 1+. The third patient underwent an adrenal biopsy demonstrating an immunohistochemistry score of 2+ and a concurrent FISH ratio of 1.4, leading to classification as HER2-negative. Because only one site was biopsied in each patient, it was not proven that all ^{89}Zr-trastuzumab foci were negative on pathology. Of course, for ethical and logistic reasons it would not be possible to biopsy all ^{89}Zr-trastuzumab foci in a patient; thus, the available results from the biopsied sites were used to classify patients.

The explanation for ^{89}Zr-trastuzumab foci in lesions without high levels of HER2 expression by immunohistochemistry or FISH is still unknown. One possibility is that decalcification of osseous lesions may decrease the intensity of immunohistochemical staining, resulting in a false-negative pathologic result (*16*). This possibility may have accounted for the osseous biopsy results in this study but would

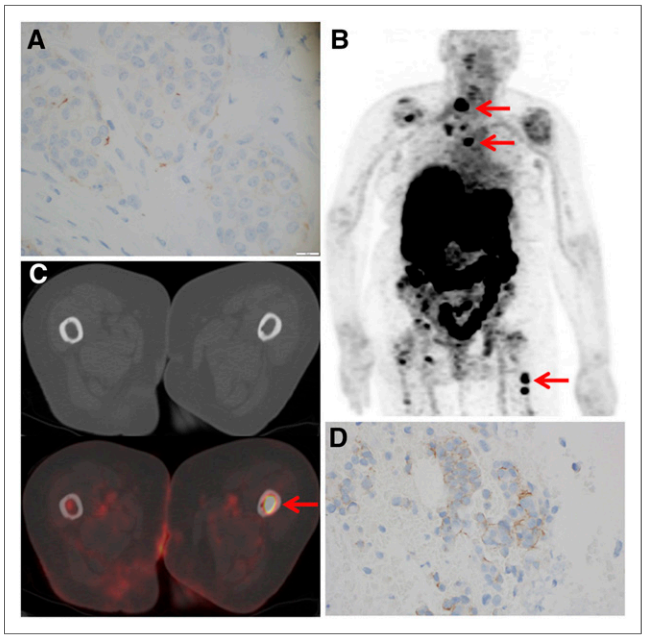

FIGURE 5. 83-y-old woman with primary ER-positive/HER2-negative invasive ductal breast carcinoma. (A) Immunohistochemistry score of primary breast malignancy was 1+ (at ×400 magnification), consistent with HER2-negative malignancy. (B) ^{89}Zr-trastuzumab maximum-intensity projection demonstrates several foci of ^{89}Zr-trastuzumab avidity that localize to osseous structures. Avidity in liver and bowel is considered physiologic. (C) Axial CT and ^{89}Zr-trastuzumab PET/CT demonstrate ^{89}Zr-trastuzumab avidity in proximal left femur (arrow, SUV_{max} of 7.7). (D) Biopsy of proximal left femur demonstrated metastatic breast carcinoma with immunohistochemistry score of 1+ (at ×400 magnification), consistent with HER2-negative disease.

not affect the adrenal biopsy result. Another possible explanation is release of free ^{89}Zr from its chelator during the long 5-d uptake period. When loosely chelated, ^{89}Zr is known to be a bone seeker (*17*). Indeed, evidence suggests that when ^{89}Zr is chelated to antibodies with desferoxamine, radioactivity accumulates in the bone (*18*). Thus, nonspecific binding of potentially free ^{89}Zr at sites of osseous turnover associated with bone metastases could be the reason for the false-positive osseous foci in this study. Nonetheless, patients 1, 4, 8, and 9, who had metastatic bone disease, were negative on ^{89}Zr-trastuzumab imaging. The high HER2 expression based on ^{89}Zr-trastuzumab imaging could be related to in vivo internalization rates or affinity differences that would not necessarily be reflected by immunohistochemistry or FISH (*19*). The development of more specific radiotracers to reduce false-positive foci on PET may be important. Potential alternatives include HER2-targeting Affibody molecules (*20*) and Nanobodies (*21,22*), which have the advantage of a rapid biodistribution that allows imaging within hours of tracer administration, rather than days as required after antibody tracers. The advantage of Affibody molecules and Nanobodies would be more pronounced if multiple scans were performed on a single patient, such as if there were a need to assess the response of HER2-positive disease at multiple time-points. Affibody molecules and Nanobodies labeled with shorter-half-life tracers may result in a lower radiation dose to patients and can often be imaged on the day they are injected, resulting in fewer patient visits.

Although this study had a limited sample, it is interesting to note that the intensity of ^{89}Zr-trastuzumab uptake did not correlate with the intensity of HER2 expression on immunohistochemistry. Indeed, the sites of highest ^{89}Zr-trastuzumab avidity were the false-positives, whereas the patient with the strongest immunohistochemistry score, 3+, demonstrated only moderate ^{89}Zr-trastuzumab uptake. The reason for this finding is currently unknown.

The strength of this study was its design as a prospective clinical trial, whereas its weakness was the relatively small sample size. Ethical and logistic reasons prevent biopsy of all ^{89}Zr-trastuzumab foci; thus, the patients had their pathology classified from a limited number of biopsies. Likewise, ethical reasons prevent biopsy confirmation of the HER2-negative status of metastases in patients without suggestive foci on ^{89}Zr-trastuzumab. This paper is presented as a proof of concept, not a demonstration of the accuracy of ^{89}Zr-trastuzumab for imaging HER2-positive and -negative metastases. These preliminary results are encouraging, but larger studies will be needed to further evaluate the value of ^{89}Zr-trastuzumab for the imaging of HER2-positive breast cancer.

CONCLUSION

^{89}Zr-trastuzumab PET/CT detects unsuspected HER2-positive metastases in patients with HER2-negative primary breast cancer and thus identifies patients who are eligible for highly effective HER2-targeted therapies but would otherwise be overlooked by conventional means. Although these are only initial results from a small sample, the study is a proof of the concept that targeted imaging may help identify patients with actionable targets. More specific HER2-targeted agents will be needed for clinical utility.

DISCLOSURE

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