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14. ABSTRACT

The purpose of this study is to determine if targeted imaging with a HER2-targeting PET tracer can detect HER2-positive metastases in patients with HER2-negative primary breast cancer. 43 of a potential 50 patients have been accrued to the trial. The first phase of the trial composed of 23 patients with HER2-negative primary breast cancer who were imaged with 89Zr-trastuzumab PET/CT. In 3 of these 23 patients, 89Zr-trastuzumab showed biopsy-proven HER2-positive metastases in patients with presumed HER2-negative primary disease, demonstrating the proof-of-concept that targeted imaging may help identify patients eligible for targeted therapies. However, in 6 additional patients, 89Zr-trastuzumab-avid foci were HER2-negative on pathology, and these false-positives limit the utility of 89Zr-trastuzumab imaging. A more specific radiotracer was needed. The second phase of the trial was first-in-human 89Zr-pertuzumab PET/CT, as a potentially more specific radiotracer. Six patients with HER2-positive metastatic breast cancer were imaged with 89Zr-pertuzumab PET/CT, demonstrating safety, dosimetry, and effectiveness of ^{89}Zr-pertuzumab to image HER2-positive disease. The third phase of the trial is 89Zr-pertuzumab PET/CT in patients with HER2-negative primary breast cancer, to identify unsuspected HER2-positive metastases. 14 patients have been accrued, with one patient demonstrating HER2-positive metastases. Three manuscripts have been published on this work (PMID 29146695, 28872549, 27151988), and a final manuscript will be prepared at its conclusion.

15. SUBJECT TERMS
Breast cancer, HER2, tumor heterogeneity, PET/CT, ^{89}Zr-trastuzumab

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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

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

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.

89Zr-Trastuzumab PET/CT

After retesting of the archived tissue, patients with confirmed HER2-negative tumors underwent 89Zr-trastuzumab PET/CT to assess for 89Zr-trastuzumab foci suggestive of HER2-positive disease. 89Zr-trastuzumab comprises the native HER2-targeted drug trastuzumab conjugated with desferoxamine and labeled with the positron-emitting metalloradionuclide 89Zr, 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 89Zr-trastuzumab imaging. 89Zr-trastuzumab was produced under good-manufacturing-practice conditions: trastuzumab was
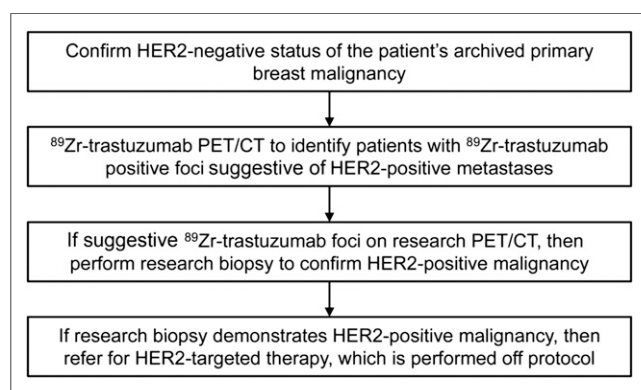

FIGURE 1. Protocol schema.

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.

chelated with desferoxamine and subsequently radiolabeled with 89Zr 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.

Patients received a nominal 185 MBq $\pm$ 10% of 89Zr-trastuzumab intravenously over 5–10 min. To optimize tumor targeting, radiolabeled 89Zr-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 89Zr-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 89Zr-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 [$\mu Ci/cm^3$]/(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.
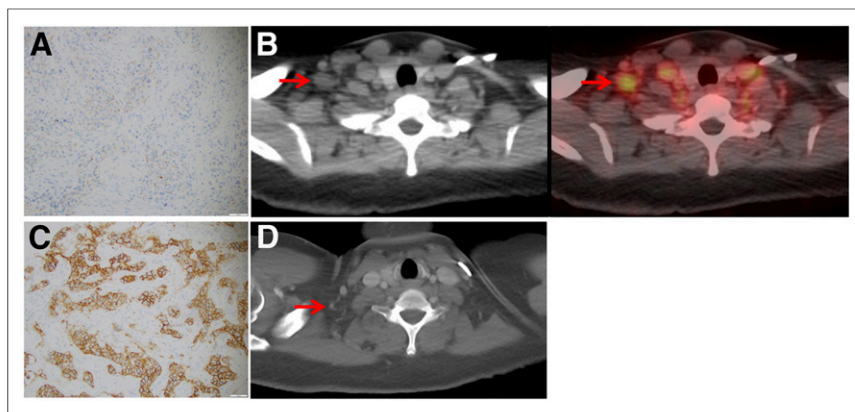All 9 women had primary invasive ductal breast cancer.

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
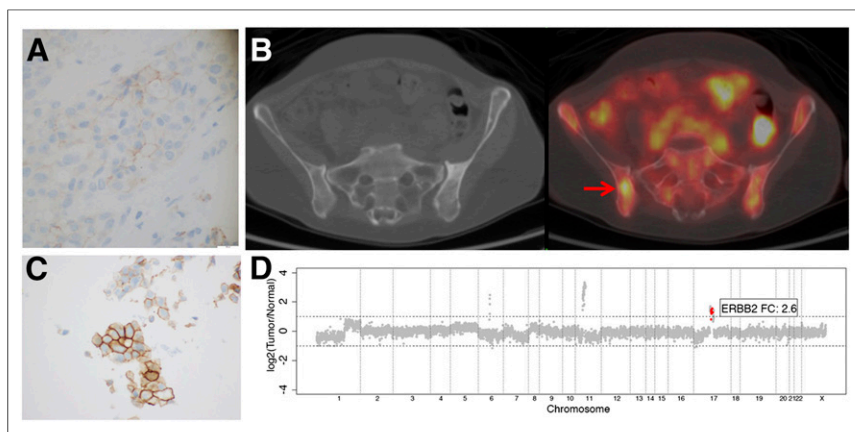

FIGURE 3. 38-year-old woman with primary ER-positive/HER2-negative invasive ductal breast carcinoma. (A) Immunohistochemistry score of primary breast malignancy was 1+ (at ×400 magnification), consistent with HER2-negative malignancy. (B) Axial CT and ^{89}Zr-trastuzumab PET/CT demonstrated ^{89}Zr-trastuzumab 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.

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.
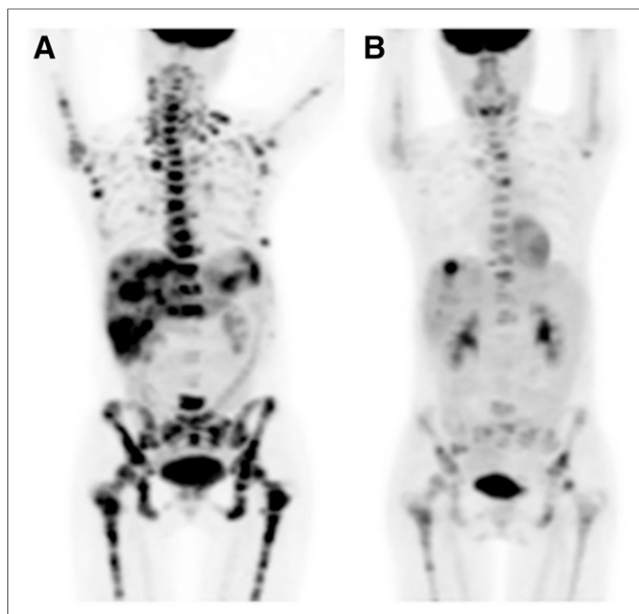
FIGURE 4. Patient from Figure 3 underwent HER2-targeted therapy after biopsy had demonstrated HER2 amplification in osseous metastasis. Maximum-intensity projections from ^{18}F-FDG PET/CT studies before (A) and after (B) 3 mo of systemic treatment including trastuzumab and pertuzumab demonstrate treatment 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
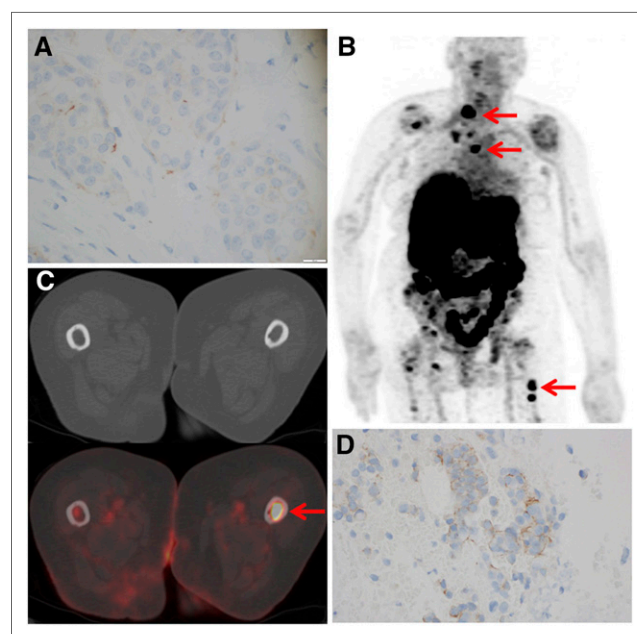
FIGURE 5. 83-y-old woman with primary ER-positive/HER2-negative invasive ductal breast carcinoma. (A) Immunohistochemistry score of primary breast malignancy was 1+ (at ×400 magnification), consistent with HER2-negative malignancy. (B) ^{89}Zr-trastuzumab maximum-intensity projection demonstrates several 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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The Journal of NUCLEAR MEDICINE

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

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1. **INTRODUCTION:**

Human epidermal growth factor receptor 2 (HER2) is a highly valuable biomarker in breast cancer, and its expression directly influences treatment. Growing evidence suggests that HER2 expression may change between the primary breast malignancy and metastases. This is an example of tumor heterogeneity. Inaccurate knowledge of receptor status in metastases due to tumor heterogeneity may lead to suboptimal treatment of metastatic breast cancer.

Our central hypothesis is that imaging with a targeted HER2 radiotracer will allow us to identify patients with HER2-negative primary breast cancers who develop HER2-positive metastases, and who may benefit from the addition of HER2 therapy.

2. **KEYWORDS:**

Breast cancer
Human epidermal growth factor receptor 2 (HER2)
Tumor heterogeneity
PET/CT
Targeted imaging
^{89}Zr-trastuzumab

3. **ACCOMPLISHMENTS:**

What were the major goals of the project?

Task 1. Submission and approval of regulatory documents
1a.   IND for ^{89}Zr-trastuzumab
1b.   IRB Protocol

Task 2. Determine the proportion of patients with HER2- primary breast cancer who develop imagable HER2+ metastases using a targeted HER2 radiotracer
2a.   Accrue 23 patients at a rate of 1-2 per month
2b.   Confirm HER2- status of archived pathology tissue samples
2c. ^{89}Zr-trastuzumab PET/CT to identify patients suspicious for HER2+ metastases
2d.   Biopsies to confirm HER2+ malignancy
2e.   Interim statistical analyses as predefined accrual numbers are met

Task 3. Among patients with HER2+ metastases discovered in Specific Aim #1, determine if HER2-targeted therapy results in a measurable treatment response
3a.   Pre-treatment baseline disease assessments with FDG PET and CT
3b.   HER2-targeted systemic therapy
3c.   Post-treatment disease assessments with FDG PET and CT
3d.   Final statistical analyses

Task 4. Submission and approval of regulatory documents for ^{89}Zr-pertuzumab. Six-to-ten patient first-in-human study of ^{89}Zr-pertuzumab in patients with HER2+ breast cancer.
4a.   IND for ^{89}Zr-trastuzumab
4b.   IRB Protocol
4c.   Six-to-ten patient first-in-human study of ^{89}Zr-pertuzumab

Task 5. Determine the proportion of patients with HER2- primary breast cancer who develop imagable HER2+ metastases using ^{89}Zr- pertuzumab
5a.   Accrue 21 patients at a rate of 1-2 patients per month
5b.   Confirm HER2- status of archived pathology tissue samples
5c. ^{89}Zr-pertuzumab PET/CT to identify patients suspicious for HER2+ metastases
5d.   Biopsies to confirm HER2+ malignancy

Task 6. Among patients with HER2+ metastases discovered in Task 5, determine if HER2 targeted therapy results in a measurable treatment response
6a.   Pre-treatment baseline disease assessments with FDG PET and CT
6b.   HER2 targeted systemic therapy
6c.   Post-treatment disease assessments with FDG PET and CT

What was accomplished under these goals?

Task 1. Submission and approval of regulatory documents
1a.   IND for ^{89}Zr-trastuzumab

         IND for ^{89}Zr was completed and maintained

1b.   IRB Protocol

         IRB for study protocol was completed and maintained.

Task 2. Determine the proportion of patients with HER2- primary breast cancer who develop imagable HER2+ metastases using a targeted HER2 radiotracer

2a.   Accrue 23 patients at a rate of 1-2 per month
          23 patients were accrued to this portion of the protocol as of 30-Sep-2016.

2b.   Confirm HER2- status of archived pathology tissue samples
         HER2- status of archived pathology samples was confirmed for all patients.

2c. ^{89}Zr-trastuzumab PET/CT to identify patients suspicious for HER2+ metastases
         ^{89}Zr-trastuzumab PET/CT was performed in all patients. Nine patients had ^{89}Zr-trastuzumab foci suspicious for HER2+ disease.

2d.   Biopsies to confirm HER2+ malignancy

Biopsies were performed in all nine patients with ^{89}Zr-trastuzumab foci suspicious for HER2+ disease.

- o Three of nine were positive for HER2+ disease on pathology (true positives)
- o Six of nine were not positive for HER2+ disease on pathology at any site (false positives)

2e.   Interim statistical analyses as predefined accrual numbers are met
Interim statistical analyses were performed.

Task 3. Among patients with HER2+ metastases discovered in Specific Aim #1, determine if HER2-targeted therapy results in a measurable treatment response

3a.   Pre-treatment baseline disease assessments with FDG PET and CT
Pre-treatment baseline disease assessments were performed in the two patients with biopsy-proven, HER2+ metastases.

3b.   HER2-targeted systemic therapy
HER2-targeted systemic therapy was performed in the two patients with biopsy-proven, HER2+ metastases.

3c.   Post-treatment disease assessments with FDG PET and CT
Post-treatment disease assessments were performed in the two patients with biopsy-proven, HER2+ metastases. Both patients demonstrated response to HER2-targeted therapy (one complete, one partial).

Tasks 1- 3 has resulted in 20 patients completing the protocol with ^{89}Zr-trastuzumab PET/CT.   All twenty had pathologic retesting that confirmed HER2-negative primary breast cancer. Nine demonstrated suspicious foci on ^{89}Zr-trastuzumab PET/CT. Of these nine with suspicious foci, three had biopsy proven HER2-positive metastases and went on to benefit from HER2 targeted therapy. Six of the nine patients with suspicious foci had biopsy without evidence of HER2-positive disease, and were considered false positive ^{89}Zr-trastuzumab PET foci. A protocol schema for these 20 patients is described in Figure 1. A tabulated summary of the results from these 20 patients is described in Table 1.

Figure 1: Protocol Schema

Confirm HER2-negative status of the patient's archived primary breast malignancy

↓

^{89}Zr-trastuzumab PET/CT to identify patients with ^{89}Zr-trastuzumab positive foci suspicious for HER2-positive metastases

↓

If suspicious ^{89}Zr-trastuzumab foci on research PET/CT, then perform research biopsy to confirm HER2-positive malignancy

↓

If research biopsy demonstrates HER2-positive malignancy, then refer for HER2-targeted therapy, which is performed off protocol

Table 1: Patient Demographics and Study Results

Patient	Age	Sites of distant metastases at enrollment	Confirmatory IHC of primary breast cancer	Suspicious foci on 89Zr-trastuzumab PET/CT?	Image guided biopsy results
1	46	Bone, Liver	HER2 0	None	
2	41	Nodes	HER2 1+	Nodes (SUVmax 4.6)	HER2 IHC 3+
3	58	Bone, Liver, Nodes	HER2 0	Bone (SUV max7.1)	HER2 IHC 1+
4	69	Bone, Liver, Nodes	HER2 0	None	
5	38	Bone, Liver, Nodes	HER2 1+	Bone (SUVmax 5.9)	HER2 IHC 2+ FISH Failed MSK-IMPACT

					amplified
6	42	Nodes, Adrenal	HER2 1+	Adrenal (SUVmax 9.2)	HER2 IHC 2+ FISH 1.4
7	83	Bone, Liver, Nodes, Lung	HER2 1+	Bone (SUVmax 9.7)	HER2 IHC 1+
8	54	Bone, Liver, Nodes, Lung	HER2 0	None	
9	48	Bone, Liver, Nodes, Pleura	HER2 1+	None	
10	54	Bone, Nodes	HER2 1+	None	
11	40	Bone, Liver, Nodes, Chest Wall	HER2 0	Bone (SUV 10.1) and Nodal (SUV 5.1)	HER2 IHC 2+ FISH 1.6
12	56	Liver	HER2 1+	None	
13	43	Nodes, Lung	HER2 1+	None	
14	47	Liver, Nodes, Pleura	HER2 2+ FISH 1.4	Liver (SUV 10.9) and Pleura (SUV 6.9)	HER2 IHC 2+ FISH 2.4
15	50	Bone, Liver	HER2 1+	None	
16	49	Bone, Liver	HER2 0	None	
17	51	Bone, Nodes, Pleura	HER2 0	Lung (SUV 11.3)	HER2 1+
18	48	Bone, Liver, Nodes	HER2 1+	None	
19	60	Liver	HER2 1+	None	
20	58	Bone, Liver, Nodes	HER2 1+	Liver (SUV 15.7)	HER2 1+

The major result from these 20 patients was the demonstration of unsuspected HER2-postive metastases in patients with HER2-negative primary breast malignancy. This resulted in addition of HER2-targeted therapy for these patients, with successful therapy response.

One example of successful identification of unsuspected HER2-positive metastases was patient 2 (Figure 2). Patient 2 was a 41-year-old woman who had undergone a right mastectomy in March 2014 with pathology demonstrating an 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 with mixed treatment responses and residual disease as determined by CT. In November 2014 CT imaging demonstrated enlarged thoracic nodes consistent with malignancy.   [89]Zr-trastuzumab PET/CT was performed in December 2014, and demonstrated [89]Zr-trastuzumab -avid thoracic nodes (Fig. 2B). The most avid node was a right supraclavicular node (SUVmax 4.6). In this patient biopsy of a right supraclavicular node demonstrated HER2 IHC of 3+ (Fig. 2C). This was considered a true positive [89]Zr-trastuzumab focus for a HER2-positive distant metastasis. She was then switched to treatment with trastuzumab, pertuzumab, and paclitaxel. Follow-up imaging for evaluation of treatment response was performed with CT of the chest, abdomen, and pelvis, which demonstrated resolution of previously enlarged lymph nodes as well as the absence of new lesions consistent with a complete response (Fig. 2D).
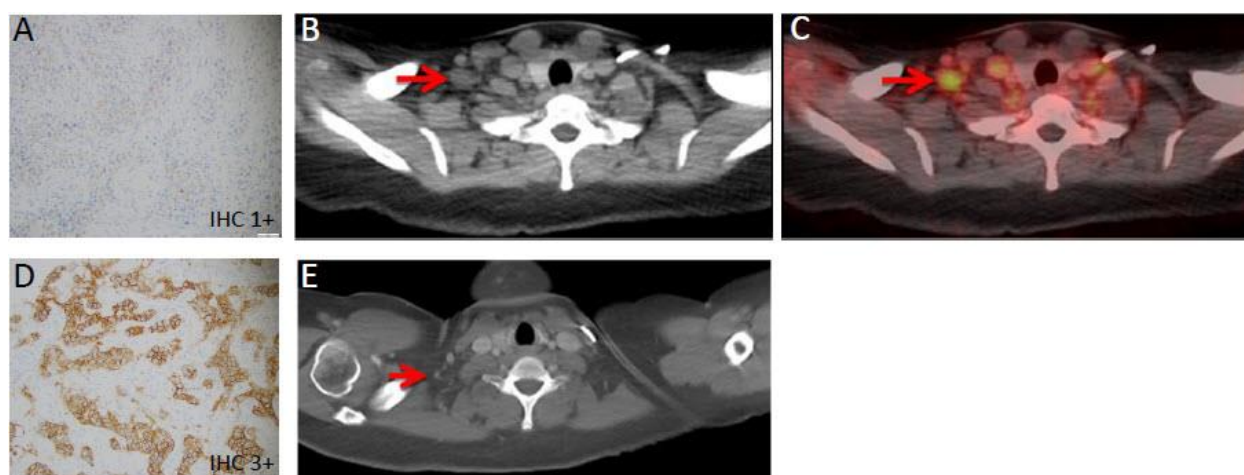
Figure 2: 41-year-old woman with primary ER-positive, HER2-negative invasive ductal breast carcinoma and recurrence in thoracic nodes. (A) HER2 IHC (at 20X magnification) of the primary breast malignancy was 1+, consistent with HER2-negative malignancy. (B) Axial CT and fused [89]Zr-trastuzumab PET/CT demonstrated [89]Zr-trastuzumab avidity in enlarged right supraclavicular nodes (arrows, SUVmax 4.6) and left internal mammary nodes (not shown). (C) Biopsy of a right supraclavicular node demonstrated metastatic breast carcinoma with HER2 3+ on IHC (at 20X magnification), consistent with HER2-positive disease as defined by ASCO guidelines. The patient began systemic treatment including trastuzumab and pertuzumab. (D) Follow-up axial CT after two months of treatment demonstrated resolution of the nodes on CT (short arrow).

A second example of successful identification of unsuspected HER2-positive metastases was patient 5 (Figure 3). Patient 5 was a 38-year-old woman who had undergone a left mastectomy in July 2011 with pathology demonstrating an 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, including fulvestrant, leuprolide, and a novel clinical trial therapeutic. In April 2015, [89]Zr-trastuzumab PET/CT was performed, which demonstrated multiple suspicious osseous foci (Figs. 3B). The most avid foci were in the right ilium and right proximal femur (SUVmax 5.9). In this patient, biopsy of the right ilium demonstrated equivocal HER2 findings based on ASCO guidelines. HER2 IHC was graded as equivocal (Fig. 3C), due to a small percentage of cells with incomplete membranous staining. HER2 FISH failed after repeated attempts. Given the equivocal results, further testing was performed with the MSK-IMPACT assay. *ERBB2* fold change on MSK-IMPACT was 2.6 ($\geq$2.0 is considered positive for amplification; Fig. 3D). Therefore, this was considered a true positive [89]Zr-trastuzumab focus for a HER2-positive distant metastasis (*14*). Patient 5 was then switched to treatment with trastuzumab, pertuzumab and docetaxel. Follow-up imaging for evaluation of treatment response was performed by FDG PET/CT. Following therapy, there was a decrease in size and FDG-avidity of liver and nodal metastases, as well as decreased FDG-avidity of osseous lesions, representing a partial response to treatment (Figs. 4A and 4B). In the liver, multiple FDG-avid lesions resolved after treatment, while a residual lesion in segment 4 decreased from SUVmax 8.2 to 5.6. In the osseous system, multiple FDG-avid lesions resolved after treatment, while other residual lesions decreased, such as the T11 vertebral body which decreased from SUVmax 9.1 to 4.7.

Figure 3: 38-year-old woman with primary ER-positive / HER2-negative invasive ductal breast carcinoma. (A) HER2 IHC (at 400X magnification) of the primary breast malignancy was 1+, consistent with HER2-negative malignancy. (B) Axial CT and fused [89]Zr-trastuzumab PET/CT demonstrated [89]Zr-trastuzumab avidity in the right ilium (arrow, SUVmax 5.9). Avidity in the bowel is considered

physiologic. (C) Biopsy of the right ilium demonstrated metastatic breast carcinoma with HER2 2+ on IHC (at 400X magnification), considered equivocal by ASCO guidelines (*11*). (D) MSK-IMPACT copy number plot demonstrating HER2 amplification. Each dot represents a probeset and the values on the y-axis show the log2 transformed ratio of tumor vs normal. *ERBB2* fold change was 2.6, consistent with HER2-positive disease.

The major limitation from Tasks 1-3 was that six patients demonstrated ^{89}Zr-trastuzumab foci on PET/CT which were expected to be HER2-postive disease, yet biopsy and pathologic analysis still yielded HER2-negative results. These results were either due to false positive ^{89}Zr-trastuzumab PET/CT scans, or false negative biopsy results. Given it was likely that false negative biopsies could result in this many discordant findings, we are presuming that their were false positive ^{89}Zr-trastuzumab PET/CT scans.

An example of a presumed false positive ^{89}Zr-trastuzumab PET/CT scans was patient 7 (Figure 4). Patient 7 was an 83 year-old woman who presented in June 2010 with metastatic ER-positive, HER2-negative invasive ductal breast cancer (Fig. 4A). Despite several courses of chemotherapy, she had persistent osseous, hepatic, nodal, and lung metastases on FDG PET/CT in 2015. ^{89}Zr-trastuzumab PET/CT was performed in July 2015, and demonstrated multiple suspicious osseous foci (Figs. 4B and 4C). The most avid osseous lesion was in the cervical spine (SUVmax 9.7), so a lesion more easily assessable in the proximal left femur (SUVmax 7.7) was chosen for biopsy. Pathology demonstrated metastatic breast cancer but HER2 IHC was only 1+, consistent with HER2-negative disease (Fig. 4D). This was considered a false positive ^{89}Zr-trastuzumab focus.
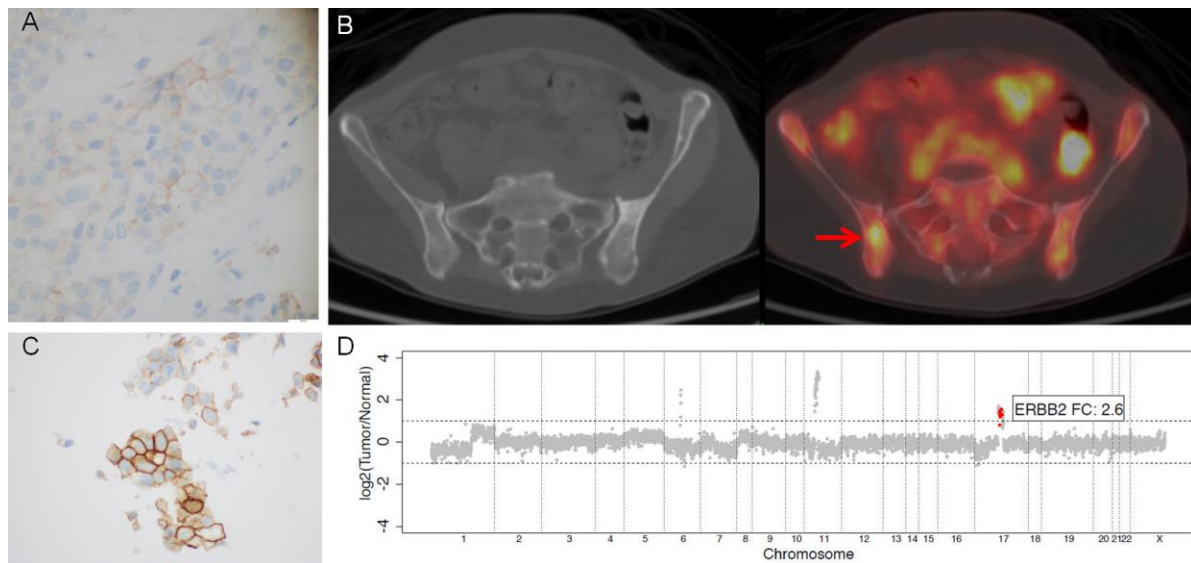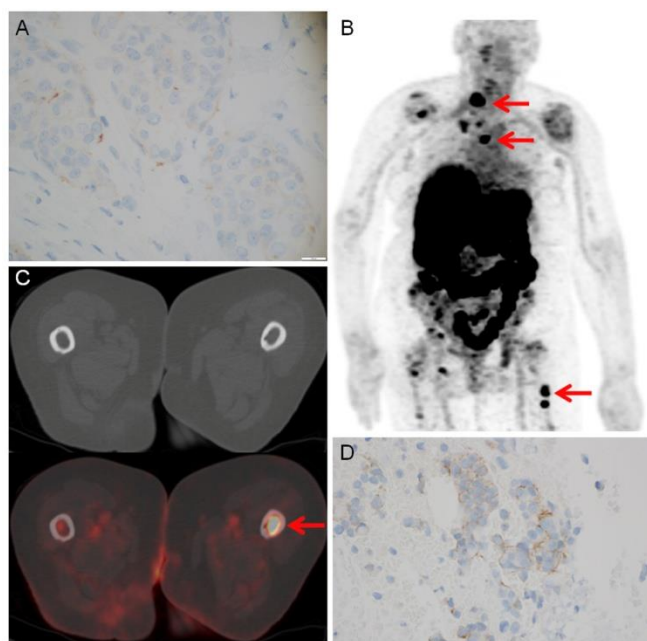

Figure 4. 83-year-old woman with primary ER-positive / HER2-negative invasive ductal breast carcinoma. (A) HER IHC (at 400X magnification) of the primary breast malignancy was 1+, consistent with HER2-negative malignancy. (B) ^{89}Zr-trastuzumab MIP demonstrates several foci of ^{89}Zr-trastuzumab avidity that localize to osseous structures. Avidity in the liver and bowel is considered physiologic. (C) Axial CT and fused ^{89}Zr-trastuzumab PET/CT demonstrated ^{89}Zr-trastuzumab avidity in the proximal left femur (arrow, SUVmax 7.7). (D) Biopsy of the proximal left femur demonstrated metastatic breast carcinoma with HER2 1+ on IHC (at 400X magnification), consistent with HER2-negative disease.

At the conclusion of Tasks 1-3, we had proven our initial hypotheses, that there is heterogeneity of HER2 expression in tumors, patients with HER2-negative primary malignancies may develop HER2-postive metastases, and non-invasive HER2-targeted imaging with ^{89}Zr-trastuzumab PET/CT could identify these patients. However, the false positive scans were a large limitation. By this time, there was evidence that pertuzumab, a newer monoclonal antibody to HER2, demonstrated greater specificity of HER2 binding than trastuzumab. We were able to produced a new HER2-targeted radiotracer, ^{89}Zr-pertuzumab, which was proven in animal models to accurately identify HER2-positive disease. At this time we petitioned the Department of Defense to allow us to stop further work with ^{89}Zr-trastuzumab, and perform a first-in-human study of ^{89}Zr-pertuzumab. If ^{89}Zr-pertuzumab could provide equal sensitivity for detection of HER2-positive disease, with better specificity, this would allow improvement in our protocol. **With the approval of the Department of Defense we initiated a first-in-human study of ^{89}Zr-pertuzumab PET/CT in patients with known HER2-positive disease, in order to demonstrate safety, dosimetry, and effectiveness of this radiotracer. This was then performed in Task 4.**

Task 4. Submission and approval of regulatory documents for ^{89}Zr-pertuzumab. Six-to-ten patient first-in-human study of ^{89}Zr-pertuzumab in patients with HER2+ breast cancer.

4a.   IND for ^{89}Zr-trastuzumab
         An IND for ^{89}Zr-trastuzumab was obtained

4b.   IRB Protocol
         An IRB Protocol for ^{89}Zr-trastuzumab was approved.

4c.   Six-to-ten patient first-in-human study of ^{89}Zr-pertuzumab
         Six patients were accrued to this portion of the protocol. This was adequate to demonstrate safety, dosimetry, and effectiveness of ^{89}Zr-pertuzumab to image HER2-positive disease. This portion of the protocol is complete as of 30-Sep-2017.

Task 4 has resulted in 6 patients completing the protocol with ^{89}Zr-pertuzumab PET/CT.
No toxicities occurred. Dosimetry estimates from Organ Level Internal Dose Assessment (OLINDA) demonstrated safe levels of radiation exposure from ^{89}Zr-pertuzumab. Multi-time point PET/CT demonstrated optimal imaging was acheived 5-8 days post-administration. ^{89}Zr-pertuzumab was able to image multiple sites of malignancy, and suggest they are HER2-positive. In two patients with both known HER2-positive and HER2-negative primary breast cancers and brain metastases, ^{89}Zr-pertuzumab PET/CT suggested the brain metastases were HER2-positive. In one of two patients, subsequent resection of a brain metastasis proved HER2-positive disease, confirming ^{89}Zr-pertuzumab-avidity was true positive for HER2-positive malignancy. A tabulated summary of the results from these 20 patients is described in Table 2.

Table 2: Patient Demographics and Study Results

Patient	Age	Gender	Known Sites of Malignancy at Time of ^{89}Zr-pertuzumab Administration	Days Post-^{89}Zr-pertuzumab Administration of PET/CT Imaging	Sites of Demonstrably ^{89}Zr-pertuzumab-avid Disease
1	46	Female	Brain	1, 2, 6, 8	Brain
2	68	Female	Nodal	1, 2, 5, 7	Nodal
3	58	Female	Breast, nodal	1, 2, 6, 7	Breast, nodal
4	69	Female	Liver, bone, chest wall	1, 4, 5, 8	Chest wall
5	38	Female	Brain, lung, nodal, liver	1, 2, 5	Brain, lung, nodal
6	42	Female	Nodal	1, 4, 5, 7	None

An example of successful HER2-targeted imaging with ^{89}Zr-pertuzumab is demonstrated in patient 1 (Figure 5). Patient 1 had two known primary breast malignancies, a right breast estrogen receptor (ER), progesterone receptor (PR), and HER2-positive (HER2 IHC 3+) IDC diagnosed in 2014 and the other ER, PR, and HER2-negative (HER2 IHC 0) IDC diagnosed in 2015. She had received HER2 directed therapy, including TDM-1 in 2015 and was currently on trastuzumab therapy at the time of the ^{89}Zr-pertuzumab PET/CT. She had a recent diagnosis of brain metastases. ^{89}Zr-pertuzumab PET/CT demonstrated progressive increase in ^{89}Zr-pertuzumab avidity over PET/CT scans obtained on days 1, 2, 6, and 8 following tracer administration (SUVmax of the most avid lesion was 13.6, 16.6, 26.0, and 30.1 on these four days; see Fig. 5A-D). Blood pool activity, including activity in the superior sagittal sinus, demonstrated continued decrease over the scans. This allowed most optimal visualization of the known brain metastases (Fig. 5E) on the day 8 scan (Fig. 5F).
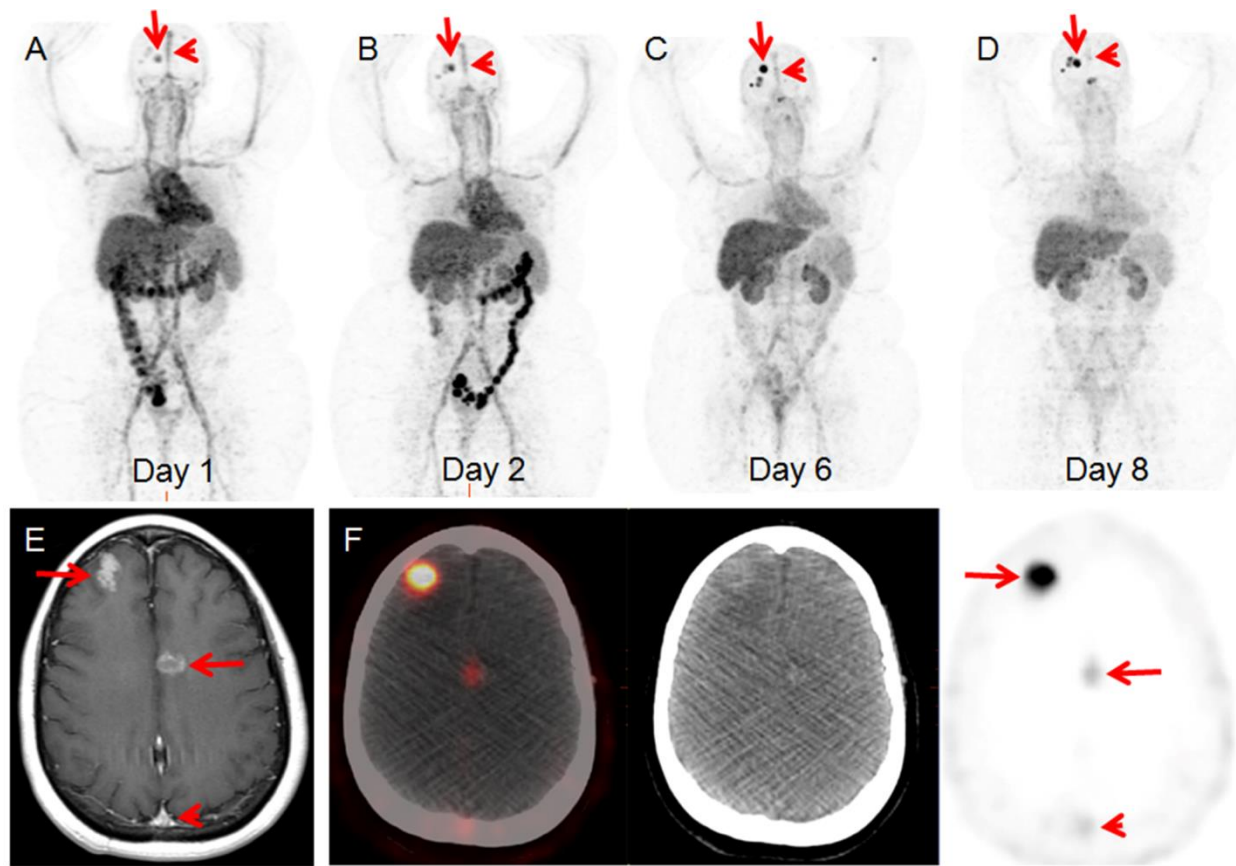
Figure 5: 46-year-old woman with both HER2-positive and HER2-negative primary breast malignancies and recently diagnosed brain metastases. Sequential maximum-intensity projection (MIP) images (A) 1 day, (B) 2 days, (C) 6 days, and (D) 8 days following administration of ^{89}Zr-pertuzumab. Blood pool and liver background clears on sequential images. Excreted bowel activity is seen on days 1 and 2. Bilateral kidney activity is visualized on all days. Increasing activity in foci overlying the skull is seen as time progresses (arrows). Decreasing activity is seen in the blood pool of the superior sagittal sinus (arrowheads). (E) Gadolinium-enhanced T1 weighed MR of the brain demonstrates enhancing brain metastases (arrows) and the superior sagittal sinus (arrowhead). (F) Axial fused PET/CT, CT, and PET images 8 days following ^{89}Zr-pertuzumab administration demonstrate avidity in the brain metastases (arrows) and minimal residual avidity in the superior sagittal sinus (arrowhead).

At the conclusion of Task 4, we had demonstrated first-in-human safety and effectiveness of ^{89}Zr-pertuzumab for imaging HER2-positive malignancy in patients with HER2-positive breast cancer. We now turned back to our initial goal of identifying HER2-positive malignancy is patients with presumed HER2-negative breast cancer. We will use ^{89}Zr-pertuzumab PET/CT is this patient population to determine if ^{89}Zr-pertuzumab PET/CT has equivalent ability to detect HER2-positive malignancy with fewer false positives than ^{89}Zr-trastuzumab PET/CT. This will be attempted in Tasks 5 and 6, which are currently being performed.

Task 5. Determine the proportion of patients with HER2- primary breast cancer who develop imagable HER2+ metastases using ^{89}Zr- pertuzumab

5a.   Accrue 24 patients at a rate of 1-2 patients per month
          24 patients were accrued to this portion of the protocol of 30-June-2019.

5b.   Confirm HER2- status of archived pathology tissue samples
          HER2-status of archived pathology samples was confirmed for all patients.

5c. ^{89}Zr-pertuzumab PET/CT to identify patients suspicious for HER2+ metastases
          ^{89}Zr-trastuzumab PET/CT was performed in all patients. Four patients had ^{89}Zr-trastuzumab foci suspicious for HER2+ disease.

5d.   Biopsies to confirm HER2+ malignancy
          Tissue sampling was performed in all three patients with ^{89}Zr-trastuzumab foci suspicious for HER2+ disease.

          o   Two patient had pathology demonstrates HER2+ disease
          o   Two patient had pathology which was HER2- disease
          o   One patient has a small lesion which underwent fine needle aspiration rather than biopsy, and there were too few cells in the sample to assess for HER2 status. This patient expired prior to repeat tissue sampling.

Task 6.   Among patients with HER2+ metastases discovered in Task 5, determine if HER2 targeted therapy results in a measurable treatment response.

6a.   Pre-treatment baseline disease assessments with FDG PET and CT
          Pre-treatment baseline disease assessments were performed in one patient with biopsy-proven, HER2+ metastases.

6b.   HER2-targeted systemic therapy
          HER2-targeted systemic therapy was performed in one patient with biopsy-proven, HER2+ metastases.

6c.   Post-treatment disease assessments with FDG PET and CT
          Post-treatment disease assessments were performed in one patient with biopsy-proven, HER2+ metastases. This patient demonstrated a substantial partial response to HER2-targeted therapy.

Tasks 5 and 6 has resulted in 24 patients completing the protocol with ^{89}Zr-trastuzumab PET/CT.   All twenty-four had pathologic retesting that confirmed HER2-negative primary breast cancer. Six demonstrated suspicious foci on ^{89}Zr-trastuzumab PET/CT. Of these six with suspicious foci, three had biopsy proven HER2-positive metastases and one went on to benefit from HER2 targeted therapy. Two of the five patients with suspicious foci had biopsy without evidence of HER2-positive disease and were considered false positive ^{89}Zr-trastuzumab PET

foci. One of the five patients with suspicious foci had an inclusive biopsy. A protocol schema for these 24 patients is described in Figure 6. A tabulated summary of the results from these 24 patients is described in Table 3.

Figure 6: Protocol Schema

Confirm HER2-negative status of the patient's archived primary breast malignancy

89Zr-pertuzumab PET/CT to identify patients with 89Zr-pertuzumab positive foci suspicious for HER2-positive metastases

If suspicious 89Zr-pertuzumab foci on research PET/CT, then perform research biopsy to confirm HER2-positive malignancy

If research biopsy demonstrates HER2-positive malignancy, then refer for HER2-targeted therapy, which is performed off protocol
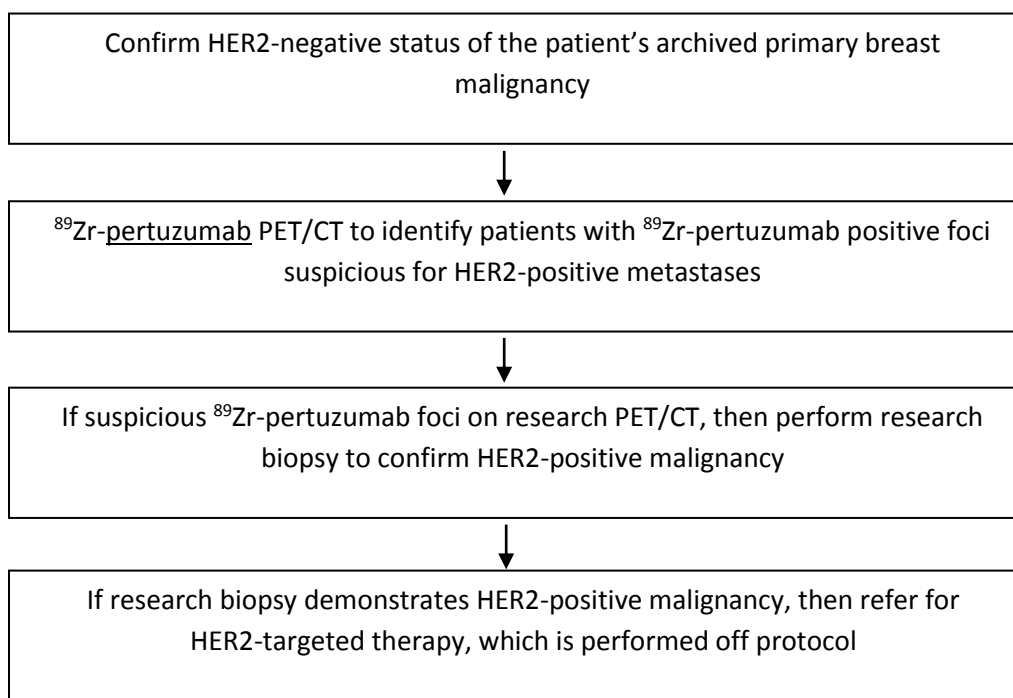
Table 3: Patient Demographics and Study Results

Patient	Age	Sites of distant metastases at enrollment	Confirmatory IHC of primary breast cancer	Suspicious foci on 89Zr-trastuzumab PET/CT?	Biopsy results
1	49	Liver	HER2 1+	None	
2	65	Nodes	HER2 0	None	
3	61	Bone, Liver, Nodes	HER2 0	None	

4	45	Bone, Liver, Breast	HER2 0	Bone	HER2 IHC 1+
5	64	Bone, Liver	HER2 0	None	
6	52	Pleura, Node, Bonr, Liver, Peritoneum	HER2 0	None	
7	54	Bone, Node	HER2 0	None	
8	44	Bone, Lung, Breast, Brain	HER2 0	Brain, Bone	HER2 IHC 2+ FISH 2.1
9	44	Bone, Lung, Nodes, Pleura	HER2 0	None	
10	68	Bone, Nodes. Liver, Lung	HER2 1+	None	
11	69	Liver, Nodes	HER2 1+	Node	Too few cells for HER2 analysis
12	47	Bone, Node, Liver, Breast	HER2 0	None	
13	69	Bone, Breast, Nodes	HER2 0	None	
14	66	Bone, Liver	HER2 1+	Bone, Liver	HER2 IHC 2+, FISH 3.0
15	76	Bone, Liver, Node, Pleura	HER2 0	None	
16	46	Bone, Node	HER2 IHC 2+ FISH 1.2	None	
17	42	Bone, Breast, Liver, Node, Peritoneum	HER2 0	None	
18	34	Bone	IHC 1+	Bone	HER2 IHC 2+ FISH 2.2

Could HER2 heterogeneity open new therapeutic options in patients with HER2- primary breast cancer?

19	69	Liver	HER2 1+	None	
20	51	Bone, Nodes	HER2 2+ FISH 1.4	None	
21	44	Node, Lung	HER2 0	None	
22	59	Breast, Liver	HER2 1+	None	
23	47	Bone	HER2 1+	Bone	HER2 IHC 2+ FISH 1.4
24	44	Bone, Liver, Node	HER2 1+	None	

<u>The major result from these 24 patients was the demonstration of unsuspected HER2-postive metastases with ^{89}Zr-pertuzumab in patients with HER2-negative primary breast malignancy, with far lower false positive PET scans compared with ^{89}Zr-trastuzumab.</u> This resulted in addition of HER2-targeted therapy with successful therapy response.

One example of successful identification of unsuspected HER2-positive metastases was patient 14 (Figure 7). Patient 14 is a 66-year-old woman diagnosed with ER-positive / HER2-negative primary invasive lobular breast cancer in November 2106 (Fig 7A), which was metastatic at presentation. She was treated with palbociclib and letrozole with pressive disease in July 2018. ^{89}Zr-pertuzumab PET/CT was performed in November 2018 and demonstrated ^{89}Zr-pertuzumab -avid osseous and hepatic lesions (Fig. 2B). In December 2018, A metastasis in the posterior left ilium was selected for biopsy due easy of accessing this lesion (Fig 2C). Pathology demonstrated HER2 IHC of 2+ (Fig 2D) and a FISH of 3.1 (Fig 2E), representing a true positive for HER2+ malignancy. The patient's therapy was changed to trastuzumab, pertuzumab, and paclitaxel. Follow up FDG PET/CT in March 2019 demonstrated substantial partial response to HER2-targeted therapy (Fig 2F and G), without continued reduction of disease on FDG PET/CT May 2019. As of June 2019 the patient is continuing on HER2-targeted therapy with trastuzumab and pertuzumab, and no other therapy.
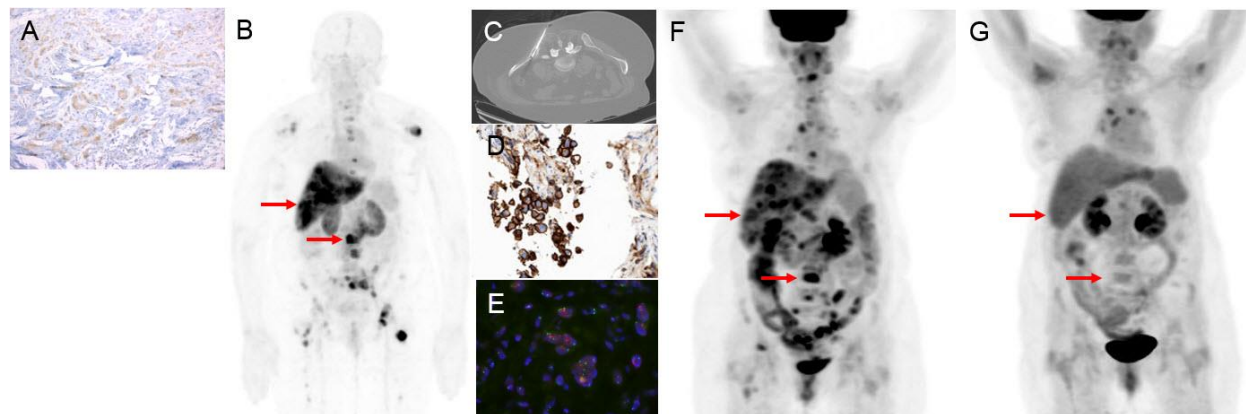
Figure 7: 66-year-old woman with primary ER-positive / HER2-negative invasive lobular breast cancer. (A) HER2 IHC (at 20X magnification) of the primary breast was 1+, consistent with HER2-negative malignancy. (B) Maximum intensity projection (MIP) image from the research ^{89}Zr-pertuzumab PET/CT demonstrated multiple ^{89}Zr-pertuzumab avid osseous and hepatic lesions (arrows). (C) Biopsy of a lesion in the posterior left ilium was performed, with pathology demonstrating metastatic breast carcinoma with HER2 IHC of 2+ (D, at 20X magnification), and FISH of 3.1 (E), consistent with HER2-positive disease as defined by ASCO guidelines. The patient began systemic treatment including trastuzumab and pertuzumab. Comparison of FDG MIP prior to initiation of HER2-targeted therapy (F) and FDG MIP 2 month following initiation of HER2- targeted therapy (G) demonstrated substantial partial response (arrows).

What opportunities for training and professional development has the project provided?

- Nothing to report

How were the results disseminated to communities of interest?

- Three manuscripts on the project have been published and are attached.

   1. Detection of HER2-positive metastases in patients with HER2-negative primary breast cancer using the ^{89}Zr-DFO-trastuzumab PET/CT. Ulaner et al, Journal of Nuclear Medicine 2016, Oct;57(10)1523-1528. PMID: 27151988. https://www.ncbi.nlm.nih.gov/pubmed/27151988

   2. 89Zr-Trastuzumab PET/CT for detection of human epidermal growth factor receptor 2-positive metastases in patients with human epidermal growth factor receptor 2-negative primary breast cancer. Ulaner et al,

Clinical Nuclear Medicine 2017, 42(12):912-917, PMID: 28872549.
https://www.ncbi.nlm.nih.gov/pubmed/28872549

3. First-in-human HER2-targeted imaging using [89]Zr-pertuzumab
PET/CT: Dosimetry and clinical application in patients with breast cancer.
Journal of Nuclear Medicine 2018, Epub ahead of print. PMID:
29146695. https://www.ncbi.nlm.nih.gov/pubmed/29146695

A final, fourth, manuscript is in preparation

- Six presentations have been made including the project. One was a
  keynote address at an international conference.

  1. Detection of HER2-positive metastases in patients with HER2-negative
  primary breast cancer using [89]Zr-DFO-trastuzumab. Presented by Gary
  Ulaner. Annual Meeting of the Society of Nuclear Medicine and
  Molecular Imaging, San Diego, California, June 2016.

  2. 89Zr-Trastuzumab PET/CT for detection of human epidermal growth
  factor receptor 2-positive metastases in patients with human epidermal
  growth factor receptor 2-negative primary breast cancer. Presented by
  Gary Ulaner. Radiologic Society of North America 2017, Chicago,
  Illinois, December 2016.

  3. Novel Radiotracers For Guiding Therapy for Patients with Breast
  Cancer, International **Keynote Speaker** for XII Professor Edwaldo
  Camargo Simpòsio on PET/CT and SPECT/CT in Oncology. São Paulo,
  Brazil. May 2017.

  4. Early phase positron emission tomography trials in patients with breast
  cancer. Presented by Gary Ulaner. Annual Meeting of the Society of
  Nuclear Meeting and Molecular Imaging, Denver, Colorado, June 2106.

  5. FDG and Novel Radiotracers for Patients with Breast Cancer,
  Society of Nuclear Medicine and Molecular Imaging Mid Winter Meeting,
  Orlando, Florida, January 2018.

  6. Incorporating Experimental Imaging Agents in Drug Clinical Trials.
  Medical Imaging in Clinical Research Conference, San Francisco,
  California, February 2018.

  A seventh presentation in planned at the San Antonio Breast cancer
  Symposium in Decemebr 2019.

- o **What do you plan to do during the next reporting period to accomplish the goals?**
  - All goals of been completed. The project is completed.

4. **IMPACT:**
   - o **What was the impact on the development of the principal discipline(s) of the project?**
     - The results proof that HER2-targeted imaging can be used to identify patients with unsuspected HER2+ metastatic disease. Six of 44 patients (14%) with HER2- primary breast cancer were found to express HER2+ metastatic disease by use of HER2-targeted PET/CT imaging. This has led to successful HER2-targeted therapy in multiple patients that otherwise would not have been offered these therapies.
     - This project successfully translated [89]Zr-pertuzumab from laboratory studies to clinical use in human patients. [89]Zr-pertuzumab was safe and effective in non-invasive imaging demonstration of HER2 expression in malignancy.

   - o **What was the impact on other disciplines?**
     - The results demonstrate that there is heterogeneity of HER2 expression between the primary malignancy and distant metastases. This adds to the growing knowledge of tumor heterogeneity.

   - o **What was the impact on technology transfer?**
     - Nothing to report.

   - o **What was the impact on society beyond science and technology?**
     - Nothing to report.

5. **CHANGES/PROBLEMS:**

   No current problems.

   - o **Changes in approach and reasons for change**
     - Nothing to report.
   - o **Actual or anticipated problems or delays and actions or plans to resolve them**
     - Nothing to report.
   - o **Changes that had a significant impact on expenditures**
     - Nothing to report.

- o **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
  - Nothing to report.
- o **Significant changes in use or care of human subjects**
  - Nothing to report.
- o **Significant changes in use or care of vertebrate animals.**
  - Nothing to report.
- o **Significant changes in use of biohazards and/or select agents**
  - Nothing to report.

6. **PRODUCTS:**
   - o **Publications, conference papers, and presentations**

     Journal publications.

     1. Detection of HER2-positive metastases in patients with HER2-negative primary breast cancer using the [89]Zr-DFO-trastuzumab PET/CT. Ulaner et al, Journal of Nuclear Medicine 2016, Oct;57(10)1523-1528. PMID: 27151988. https://www.ncbi.nlm.nih.gov/pubmed/27151988

     2. 89Zr-Trastuzumab PET/CT for detection of human epidermal growth factor receptor 2-positive metastases in patients with human epidermal growth factor receptor 2-negative primary breast cancer. Ulaner et al, Clinical Nuclear Medicine 2017, 42(12):912-917, PMID: 28872549. https://www.ncbi.nlm.nih.gov/pubmed/28872549

     3. First-in-human HER2-targeted imaging using [89]Zr-pertuzumab PET/CT: Dosimetry and clinical application in patients with breast cancer. Journal of Nuclear Medicine 2018, Epub ahead of print. PMID: 29146695. https://www.ncbi.nlm.nih.gov/pubmed/29146695

   - ▪ **Books or other non-periodical, one-time publications.**
     Nothing to report.

     Other publications, conference papers, and presentations.

     1. Detection of HER2-positive metastases in patients with HER2-negative primary breast cancer using [89]Zr-DFO-trastuzumab. Presented by Gary Ulaner. Annual Meeting of the Society of Nuclear Medicine and Molecular Imaging, San Diego, California, June 2016.

     2. 89Zr-Trastuzumab PET/CT for detection of human epidermal growth factor receptor 2-positive metastases in patients with human epidermal growth factor receptor 2-negative primary breast cancer. Presented by Gary Ulaner.

Radiologic Society of North America 2017, Chicago, Illinois, December 2016.

3. Novel Radiotracers For Guiding Therapy for Patients with Breast Cancer, International **Keynote Speaker** for XII Professor Edwaldo Camargo Simpòsio on PET/CT and SPECT/CT in Oncology. São Paulo, Brazil. May 2017.

4. Early phase positron emission tomography trials in patients with breast cancer. Presented by Gary Ulaner. Annual Meeting of the Society of Nuclear Meeting and Molecular Imaging, Denver, Colorado, June 2106.

5. FDG and Novel Radiotracers for Patients with Breast Cancer, Society of Nuclear Medicine and Molecular Imaging Mid Winter Meeting, Orlando, Florida, January 2018.

6. Incorporating Experimental Imaging Agents in Drug Clinical Trials. Medical Imaging in Clinical Research Conference, San Francisco, California, February 2018.

- **Website(s) or other Internet site(s)**
  Nothing to report.

- **Technologies or techniques**
  Nothing to report.

- **Inventions, patent applications, and/or licenses**
  Nothing to report.

- **Other Products**
  Nothing to report.

- **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**
- **What individuals have worked on the project?**
    - *Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change."*

Name:	Gary Ulaner (PI): No change

Name:	Hanh Pham (Research Assistant): No change

- o **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**
  - ▪ Nothing to report

- o **What other organizations were involved as partners?**
  - ▪ Nothing to report

7. **SPECIAL REPORTING REQUIREMENTS**
   - o **COLLABORATIVE AWARDS:** *Not applicable*
   - o **QUAD CHARTS:** *Not applicable.*

8. **APPENDICES:**

Three published manuscripts:

1. Detection of HER2-positive metastases in patients with HER2-negative primary breast cancer using the [89]Zr-DFO-trastuzumab PET/CT. Ulaner et al, Journal of Nuclear Medicine 2016, Oct;57(10)1523-1528. PMID: 27151988. https://www.ncbi.nlm.nih.gov/pubmed/27151988

2. 89Zr-Trastuzumab PET/CT for detection of human epidermal growth factor receptor 2-positive metastases in patients with human epidermal growth factor receptor 2-negative primary breast cancer. Ulaner et al, Clinical Nuclear Medicine 2017, 42(12):912-917, PMID: 28872549. https://www.ncbi.nlm.nih.gov/pubmed/28872549

3. First-in-human HER2-targeted imaging using [89]Zr-pertuzumab PET/CT: Dosimetry and clinical application in patients with breast cancer. Journal of Nuclear Medicine 2018, Epub ahead of print. PMID: 29146695. https://www.ncbi.nlm.nih.gov/pubmed/29146695

A final, fourth, manuscript is in preparation.

89Zr-Trastuzumab PET/CT for Detection of Human Epidermal Growth Factor Receptor 2–Positive Metastases in Patients With Human Epidermal Growth Factor Receptor 2–Negative Primary Breast Cancer

Gary A. Ulaner, MD, PhD,*† David M. Hyman, MD,‡§ Serge K. Lyashchenko, PhD,*†
Jason S. Lewis, PhD,*†‖ and Jorge A. Carrasquillo, MD*†

Purpose: The aim of this study was to determine if imaging with 89Zr-trastuzumab, a human epidermal growth factor receptor 2 (HER2)–targeting PET tracer, can detect HER2-positive metastases in patients with HER2-negative primary breast cancer.

Methods: As part of an institutional review board–approved, prospective clinical trial of 89Zr-trastuzumab PET/CT (ClinicalTrials.gov identifier NCT02286843), a second group of 11 patients with HER2-negative primary breast cancer and known metastatic disease were recruited. Patients with confirmed HER2-negative primary breast cancer underwent 89Zr-trastuzumab PET/CT to screen for 89Zr-trastuzumab–avid lesions suggestive of unsuspected HER2-positive metastases. 89Zr-trastuzumab–avid lesions on PET/CT were biopsied and pathologically examined to determine HER2 status.

Results: All 11 patients had confirmed HER2-negative primary cancer. Four patients demonstrated suspicious foci on 89Zr-trastuzumab PET/CT. Of these 4 patients, 1 patient had biopsy-proven HER2-positive metastases. The other 3 patients with suspicious 89Zr-trastuzumab–avid foci had biopsy demonstrating a metastasis that was HER2-negative and were considered false-positive 89Zr-trastuzumab PET foci. Combined with a published report of the first 9 patients, there have been a total of 20 HER2-negative primary breast cancer patients, with 3 patients (15%) having pathologically confirmed HER2-positive distant metastases and 6 (30%) with suspicious 89Zr-trastuzumab–avid foci that were HER2-negative on pathology, which were thus considered false-positive 89Zr-trastuzumab findings.

Conclusions: This second group of patients confirms the proof of concept that 89Zr-trastuzumab PET/CT detects unsuspected HER2-positive metastases in a subset of patients with HER2-negtive primary breast cancer. False-positive 89Zr-trastuzumab–avid foci present a challenge to using this tracer.

Key Words: breast cancer, HER2, PET/CT, trastuzumab

(Clin Nucl Med 2017;42: 912–917)

Approximately 20% of invasive ductal breast malignancies are classified as human epidermal growth factor receptor 2 (HER2)–positive because of overexpression of the HER2 protein on the surface of tumor cells or gene amplification.[1] Classification of a breast malignancy as HER2-positive or HER2-negative is an essential component of clinical care, as 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.[2,3] An important question is whether HER2 status could be heterogeneous between sites of malignancy in a single patient. If heterogeneity exists, then classification of HER2 status from 1 biopsy may inaccurately describe the extent of disease in a patient with the potential to respond to HER2-targeted therapy. Indeed, heterogeneity of tumors both within and across lesions in a single patient is increasingly being recognized.[4] Investigators have identified patients with HER2-positive primary malignancies in which metastases have lost HER2 positivity.[5] Potentially more clinically significant, investigators have identified patients with HER2-negative primary malignancies in which metastases have acquired HER2 positivity. These patients may benefit from HER2-targeted therapies that they are not receiving because they are not considered to have HER2-positive disease.

It is currently unclear how to best identify patients who have malignancy with heterogeneous HER2 expression. Identification by biopsy would be difficult because only small samples of tissue from a limited number of lesions are normally sampled. We hypothesized that HER2 heterogeneity may be identified by imaging with a HER2-targeting radiotracer and designed a prospective clinical trial to evaluate HER2 heterogeneity with 89Zr-trastuzumab PET/CT, which allows for visualization of HER2-positive malignancy.[6] We published the initial results from the first 9 patients and demonstrated the ability of 89Zr-trastuzumab PET/CT to identify HER2-positive metastases in patients with HER2-negative primary breast malignancy.[7] We present the results of the second group of patients, comprising 11 patients.

PATIENTS AND METHODS

Patients

A single-center, prospective, institutional review board–approved protocol (ClinicalTrials.gov identifier NCT02286843) was performed. Patients receiving treatment for metastatic HER2-negative primary breast cancer were identified as potential candidates. Inclusion criteria were as follows: (1) biopsy-proven HER2-negative primary malignancy; (2) biopsy-proven metastatic disease; (3) foci of demonstrable metastases on imaging modalities within 6 weeks of enrollment; (4) women 18 or older; and (5) Eastern Cooperative Oncology Group performance score of 0 to 2. Exclusion criteria were as follows: (1) creatinine more than 2 times the upper limit of normal; (2) aspartate aminotransferase/alanine aminotransferase more than 2 times the upper limit of normal; (3) life expectancy of less than 3 months; (4) pregnancy or lactation; and (5) inability

TABLE 1. Algorithm for Defining HER2 Expression*

First, HER2 testing is performed with IHC. IHC results:

0 or 1+	HER2-negative
2+	Equivocal
3+	HER2-positive

If IHC is 2+ (equivocal), then dual-probe HER2 FISH is performed.
FISH results:

HER2/CEP17 ratio ≥2.0	HER2-positive
HER2/CEP17 ratio <2.0	HER2-negative

*As per ASCO guidelines.[8]
CEP17 indicates chromosome enumeration probe 17.

to undergo PET/CT scanning because of weight limits. All patients enrolled in the protocol provided written informed consent.

Confirmation of HER2 Status of the Primary Breast Malignancy

The primary breast cancer tissue sample for each patient was retested for HER2 status to ensure proper classification as HER2-negative. Human epidermal growth factor receptor 2 protein overexpression was evaluated by immunohistochemistry (IHC) using a US Food and Drug Administration (FDA)–approved monoclonal antibody (clone 4B5; Ventana, Tucson, AZ) directed against the internal domain of the c-erbB-2 oncoprotein (HER2). The IHC results were categorized as follows: 0, 1+ = negative result, 2+ = equivocal result, and 3+ = positive result, according to the published American Society of Clinical Oncology (ASCO) guidelines[8] (Table 1). Tissues with 2+ staining (equivocal) were assessed for HER2 amplification with fluorescence in situ hybridization (FISH) as per ASCO guidelines[8] using an FDA-approved probe set (*HER2* IQFISH pharmDx; Dako, Pittsburgh, PA) and defined as HER2/CEP17 ratio of 2.0 or greater. Carcinoma with 0 or 1+ IHC staining or 2+ IHC staining and concurrent negative HER2 FISH (HER2/CEP17 ratio <2.0) were classified as HER2-negative primary breast malignancies.

⁸⁹Zr-Trastuzumab PET/CT

Patients with confirmed HER2-negative primary breast malignancy underwent ⁸⁹Zr-trastuzumab PET/CT. ⁸⁹Zr-trastuzumab

is composed of the native trastuzumab, an FDA-approved HER2-targeting monoclonal antibody, conjugated with desferrioxamine and labeled with the positron-emitting metalloradionuclide ⁸⁹Zr. ⁸⁹Zr has a half-life of 78 hours, long enough to allow favorable biodistribution of radiolabeled intact antibodies. Our institution has an FDA Investigational New Drug for human ⁸⁹Zr-trastuzumab imaging (Memorial Sloan Kettering Cancer Center Investigational New Drug no. 119907). ⁸⁹Zr-trastuzumab was produced under Good Manufacturing Practice conditions using previously described methodology.[9] The final drug product for human use was manufactured, and quality control tested prior to being released for patient administration to ensure that it conformed to the established acceptance specifications for appearance, pH, endotoxin content, sterilizing filter integrity, radiochemical purity, and radionuclidic identity. Sterility and immunoreactivity determinations were performed after release.

Patients were intravenously administered 185 MBq ± 10% of ⁸⁹Zr-trastuzumab over 5 minutes. Radiolabeled ⁸⁹Zr-trastuzumab was brought up to a final mass dose of 50 mg by adding non-radiolabeled trastuzumab at the end of the production because this has been shown to help optimize tumor targeting.[6] The final mass dose of 50 mg was provided by the Radiochemistry and Molecular Imaging Probe Core at Memorial Sloan Kettering Cancer Center to the clinic for patient administration. Five or 6 days following ⁸⁹Zr-trastuzumab administration, patients underwent PET/CT from the midskull to midthigh on a dedicated research GE Discovery PET/CT 710 scanner (GE Healthcare, Chicago, IL), with an 80-mA CT component for attenuation correction and lesion localization. PET/CT images were reconstructed using iterative reconstruction and displayed in multiplanar reconstructions. ⁸⁹Zr-trastuzumab PET/CT scans were interpreted by 2 different nuclear medicine experts (G.A.U., J.A.C.), both experienced in the use of PET antibody radiotracers. Both readers were able to compare ⁸⁹Zr-trastuzumab PET/CT results with prior imaging studies. Physiologic ⁸⁹Zr-trastuzumab uptake was expected in the blood pool, liver, gallbladder, bowel, and kidney. Radiotracer uptake in areas that are not physiologic were graded both qualitatively and semiquantitatively. For qualitative scoring, lesions were scored as suspicious or not suspicious. Only those foci qualitatively scored as suspicious by both readers were considered suspicious foci. Semiquantitative analysis of tracer uptake was performed by recording the SUVmax of suspicious lesions. Three-dimensional volumes of interest were placed

TABLE 2. Patient Demographics and Study Results

Patient	Age, y	Sites of Distant Metastases at Enrollment	Confirmatory IHC of Primary Breast Cancer	Suspicious Foci on ⁸⁹Zr-Trastuzumab PET/CT	Image-Guided Biopsy Results
10	54	Bone, nodes	HER2 1+	None	
11	40	Bone, liver, nodes, chest wall	HER2 0	Bone (SUV 10.1) and nodal (SUV 5.1)	HER2 IHC 2+, FISH 1.6
12	56	Liver	HER2 1+	None	
13	43	Nodes, lung	HER2 1+	None	
14	47	Liver, nodes, pleura	HER2 2+ FISH 1.4	Liver (SUV 10.9) and pleura (SUV 6.9)	HER2 IHC 2+, FISH 2.4
15	50	Bone, liver	HER2 1+	None	
16	49	Bone, liver	HER2 0	None	
17	51	Bone, nodes, pleura	HER2 0	Lung (SUV 11.3)	HER2 1+
18	48	Bone, liver, nodes	HER2 1+	None	
19	60	Liver	HER2 1+	None	
20	58	Bone, liver, nodes	HER2 1+	Liver (SUV 15.7)	HER2 1+

Patients 11 to 20, all with primary invasive ductal breast cancer, are reported here. The first 9 patients were reported in the initial results of the trial.[7]

in these areas, and tracer uptake was quantified using SUV, calculated as decay-corrected mean region of interest activity (μCi/mL)/(injected dose (μCi)/body weight (g)).

Biopsy and Pathologic Evaluation of Suspicious [89]Zr-Trastuzumab Foci

Image-guided biopsy was selected in concert with an experienced oncologic interventional radiologist to minimize risks to the patient while obtaining high-quality samples. Biopsy specimens were evaluated by board-certified breast pathology specialists. The IHC results were categorized according to published ASCO guidelines,[8] as discussed previously and in Table 1. Carcinomas with 3+ IHC staining or 2+ IHC staining and concurrent positive HER2 FISH were classified as HER2-positive metastases.

RESULTS

Patient Characteristics

Between December 2015 and May 2016, a second group of 11 women with HER2-negative primary invasive ductal breast cancer completed the study protocol. These were designated patient numbers 10 to 20, following the first 9 patients described in the initial publication.[7] Patient characteristics and study results are summarized in Table 2.

Sites of Distant Metastases at Time of Protocol Enrollment

All 11 patients had at least 1 site of metastatic disease at the time of protocol enrollment. The most common sites of distant metastases were bone (n = 7), nodal (n = 7), and liver (n = 7), followed by lung (n = 2), pleural (n = 1), and chest wall (n = 1). Nine of the 11 patients had multiple organ system metastatic involvement.

Confirmation of HER2 Status of the Primary Breast Malignancy

On IHC retesting of the patients' archived primary breast cancer specimens, all 11 patients had confirmed HER2-negative primary malignancies according to published ASCO guidelines.[8] Three patients had HER2 IHC scores of 0, whereas 7 had HER2 IHC scores of 1+, and 1 had a HER2 IHC score of 2+ with a FISH of 1.4.

[89]Zr-Trastuzumab PET/CT

All 11 patients underwent [89]Zr-trastuzumab PET/CT. Patients were monitored for 30 minutes after tracer injection and called the following day; no adverse effects were observed or reported.

FIGURE 1. A 47-year-old woman with primary ER-positive, HER2-negative invasive ductal breast carcinoma and known metastases in the liver, nodes, and pleura. **A**, Axial CT and PET images from a contrast-enhanced FDG PET/CT through the chest demonstrate FDG-avid right pleural masses (SUVmax, 6.0; arrows). **B**, Axial CT and PET images from a non–contrast-enhanced [89]Zr-trastuzumab PET/CT at the same level demonstrate [89]Zr-trastuzumab avidity in the pleural lesions (SUVmax, 6.9; arrows).

Vital signs were recorded before and after injection, and no changes of clinical significance were observed.

In 4 (36%) of 11 patients, both readers observed foci of [89]Zr-trastuzumab avidity considered suggestive of HER2-positive disease. In 2 patients, the suspicious [89]Zr-trastuzumab foci were in pleura, whereas 1 patient exhibited suspicious [89]Zr-trastuzumab foci in the bone, and 1 patient exhibited suspicious [89]Zr-trastuzumab foci in the liver.

Biopsy and Pathologic Evaluation of Suspicious [89]Zr-Trastuzumab Foci

Image-guided biopsy was performed in all 4 patients with suspicious [89]Zr-trastuzumab–avid foci. Patient 11 was a 40-year-old woman who was diagnosed in October 2013 as having an estrogen receptor (ER)-positive/HER2-negative primary invasive ductal carcinoma. At the time of protocol enrollment, an FDG PET/CT demonstrated FDG-avid bone, liver, nodal, and chest wall malignancy. Bone and chest wall lesions had been pathologically confirmed as metastases. [89]Zr-trastuzumab PET/CT was performed in March 2016 and demonstrated [89]Zr-trastuzumab–avid osseous and nodal lesions. The most avid lesion was a right femoral focus (SUVmax, 10.1). Biopsy of the right femoral focus demonstrated HER2 IHC of 2+ and FISH of 1.6. As FISH was less than 2.0, this was considered a false-positive [89]Zr-trastuzumab focus.

Patient 14 was a 47-year-old woman diagnosed in June 2011 as having an ER-positive/HER2-negative primary invasive ductal carcinoma. At the time of protocol enrollment, an FDG PET/CT demonstrated FDG-avid liver, nodal, and pleural malignancy (Fig. 1). Hepatic and pleural lesions had been pathologically confirmed as metastases. [89]Zr-trastuzumab PET/CT was performed in May 2016 and demonstrated [89]Zr-trastuzumab–avid pleural (SUV, 6.9) and liver (SUV, 10.9) lesions. The pleural lesion was selected for biopsy as the lesion with the lowest risk for sampling. Biopsy of the pleural lesion demonstrated HER2 IHC of 2+ and FISH of 2.4. As FISH was greater than 2.0, this was considered a true-positive [89]Zr-trastuzumab focus for a HER2-positive distant metastasis.

Patient 17 was a 51-year-old woman diagnosed in November 2010 as having an ER-positive/HER2-negative primary invasive ductal carcinoma. At the time of protocol enrollment, an FDG PET/CT demonstrated FDG-avid bone, nodal, and lung malignancy (Fig. 2). A lung lesion had been pathologically confirmed as metastasis. [89]Zr-trastuzumab PET/CT was performed in May 2016 and demonstrated [89]Zr-trastuzumab–avid lung (SUV, 11.3) lesions. Biopsy of a lung lesion demonstrated HER2 IHC of 1+, which was considered a false-positive [89]Zr-trastuzumab focus.

Patient 20 was a 58-year-old woman diagnosed in August 2003 as having an ER-positive/HER2-negative primary mixed invasive ductal and lobular carcinoma. At the time of protocol enrollment, an FDG PET/CT demonstrated FDG-avid bone, liver, and nodal malignancy (Fig. 3). Bone and liver lesions had been pathologically confirmed as metastases. [89]Zr-trastuzumab PET/CT was performed in June 2016 and demonstrated [89]Zr-trastuzumab–avid liver (SUV, 15.7) lesions. Biopsy of a liver lesion demonstrated
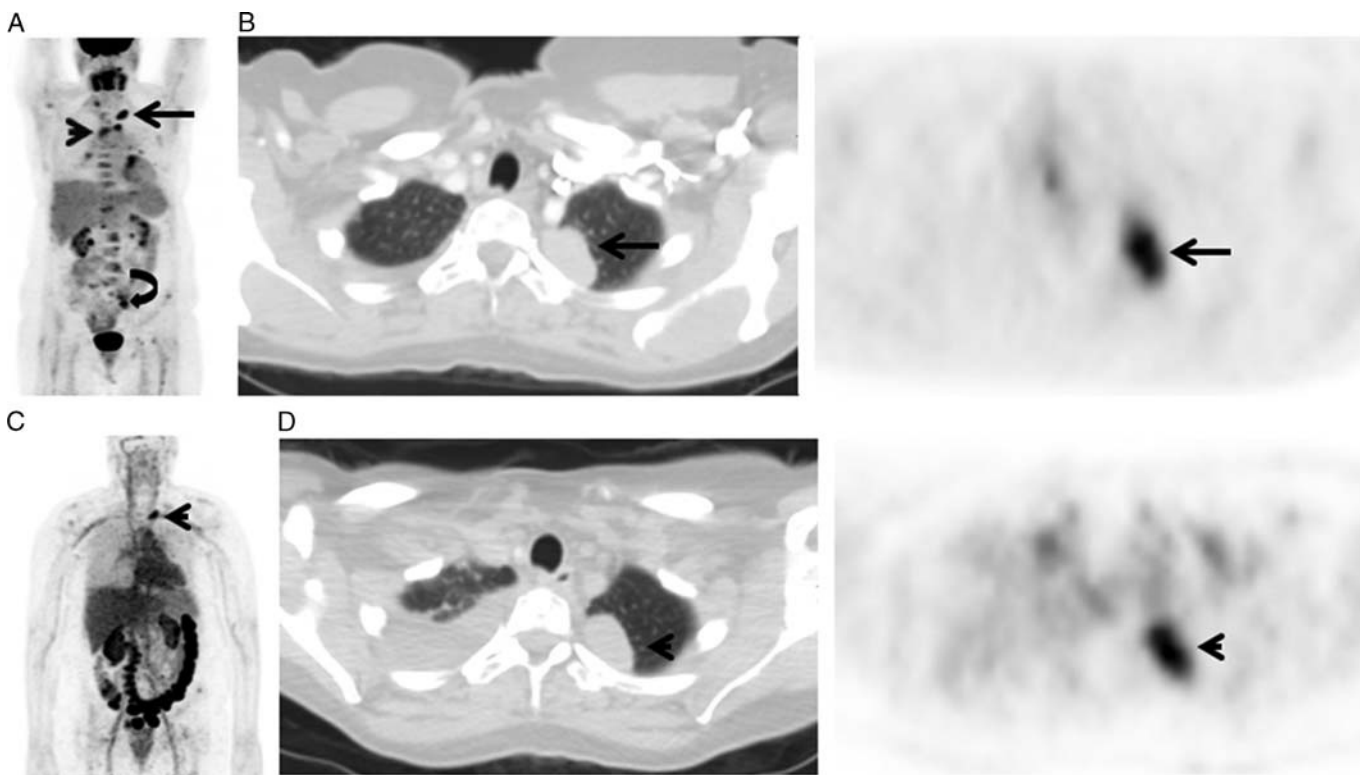
FIGURE 2. A 51-year-old woman with primary ER-positive, HER2-negative invasive ductal breast carcinoma. **A**, FDG PET MIP demonstrates multiple FDG-avid metastases, which correspond to lesions in the lung (arrow), nodes (arrowhead), and bone (curved arrow). **B**, Axial CT and PET images from a contrast-enhanced FDG PET/CT through the chest, displayed in lung CT windows, demonstrate an FDG-avid left lung mass (SUVmax, 7.3; arrow). **C**, [89]Zr-trastuzumab PET MIP demonstrates physiologic tracer in the blood pool, liver, and bowel, as well as a suspicious [89]Zr-trastuzumab–avid focus in the upper chest (arrow). **D**, Axial CT and PET images from a non–contrast-enhanced [89]Zr-trastuzumab PET/CT demonstrate that the suspicious [89]Zr-trastuzumab–avid focus corresponds to the left lung mass (SUVmax, 11.3; arrowhead).
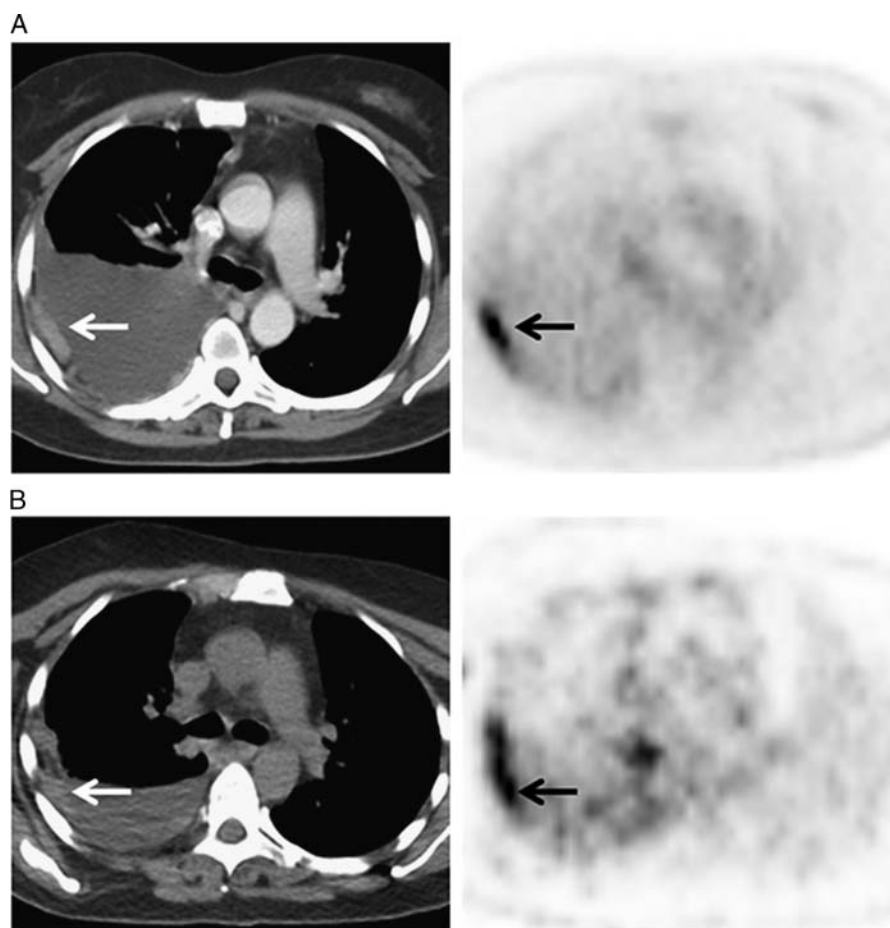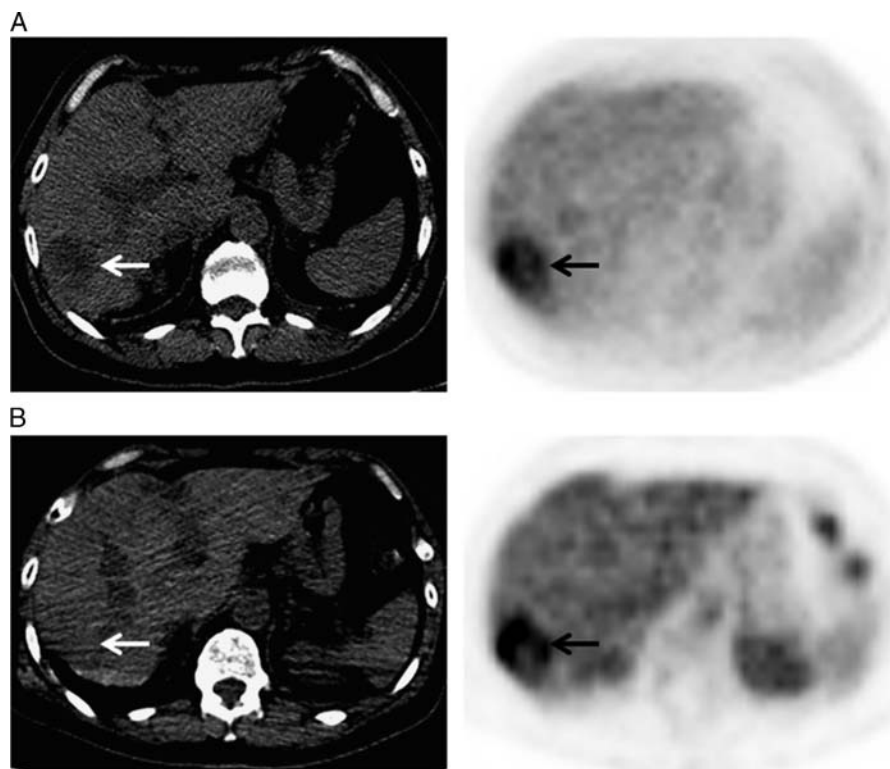
FIGURE 3. A 58-year-old woman with primary ER-positive, HER2-negative invasive ductal breast carcinoma and known metastases in the bone, liver, and nodes. **A**, Axial CT and PET images from a non–contrast-enhanced FDG PET/CT through the abdomen demonstrate an FDG-avid liver metastasis (SUVmax, 5.6; arrows). **B**, Axial CT and PET images from a non–contrast-enhanced [89]Zr-trastuzumab PET/CT at the same level demonstrate [89]Zr-trastuzumab avidity in the liver metastasis (SUVmax, 15.7; arrows).

HER2 IHC of 1+, which was considered a false-positive [89]Zr-trastuzumab focus. The remaining 7 patients did not have suspicious foci of [89]Zr-trastuzumab avidity.

DISCUSSION

This study describes a second group of 11 patients in a prospective clinical trial of HER2-targeted imaging with [89]Zr-trastuzumab PET/CT to evaluate for unsuspected HER2-positive metastases in patients with HER2-negative primary breast cancer. It demonstrates that in a small subset of patients with HER2-negative primary breast cancer HER2-targeted imaging can detect previously unsuspected HER2-positive disease.

Between the current group of 11 patients and a previously published initial group of 9 patients,[7] a completed cohort of 20 patients with HER2-negative primary breast cancer have now been evaluated. In total, 3 (15%) of 20 patients were demonstrated to have biopsy-proven unsuspected HER2-positive metastases. In another 6 (30%) of 20 patients, suspicious [89]Zr-trastuzumab–avid foci resulted in biopsies with HER2-negative pathology, which are being conservatively considered false-positive [89]Zr-trastuzumab–avid foci.

While medical imaging of oncology patients has traditionally been used for the detection of tumors, staging, and evaluation of treatment response, this study suggests that targeted medical imaging may be able to help select patients for targeted systemic therapies. More than 900,000 women are living with metastatic breast cancer, with more than 50,000 diagnosed each year.[10] Eighty percent of these women have HER2-negative primary malignancies.

If 15% of patients with metastatic HER2-negative primary breast cancer could be found to harbor HER2-positive metastases, this would represent a current population of 135,000 women. Thus, targeted HER2 imaging has the potential to play a clinically valuable role in identifying patients who may benefit from HER2-targeted therapies.

A limitation of this approach is the currently high level of presumably false-positive suspicious [89]Zr-trastuzumab–avid foci. This leads to unneeded workup and biopsies. The reason for these false-positive foci is unclear. One possibility is pathologic sampling or processing errors. For example, decalcification of osseous lesions may decrease the intensity of HER2 IHC staining, resulting in a false-negative pathologic result.[11] If a lesion exhibits heterogeneity of HER2 expression, it is possible that in any one biopsy the HER2-positive malignancy was undersampled. Although this could result in erroneous pathologic analysis, given the high number of false-positives, it is likely that at least some represent nonspecific [89]Zr-trastuzumab uptake in HER2-negative lesions. Another possible explanation is free [89]Zr being released from its chelator during the long 5-day uptake period. When loosely chelated, [89]Zr is known to be a bone seeker.[12] Indeed, evidence suggests that when [89]Zr is chelated to antibodies with desferrioxamine radioactivity accumulates in the bone.[13] Thus, nonspecific binding of potentially free [89]Zr at sites of osseous turnover associated with bone metastases could result in false-positive osseous foci. Another possible explanation for presumed false-positive [89]Zr-trastuzumab–avid foci could be related to in vivo HER2 internalization rates or affinity differences that would not necessarily be reflected by IHC or FISH.[14] More specific HER2-targeting radiotracers will need to

be explored. One potential alternative is [89]Zr-pertuzumab, which demonstrated HER2 specificity in animal models.[15]

Human epidermal growth factor receptor 2–negative patients have been reported to respond to trastuzumab therapy. It would be of interest to evaluate if patients with positive [89]Zr-trastuzumab scans but negative HER2 status by conventional criteria respond to trastuzumab. This would raise the potential of using [89]Zr-trastuzumab as a predictive biomarker[16] and selecting patients for HER2-target therapy based on imaging.

It is interesting to note that the intensity of [89]Zr-trastuzumab avidity did not discriminate between true-positive and false-positive lesions for HER2 expression. The most [89]Zr-trastuzumab–avid lesion in the study was a hepatic lesion (patient 20; SUV, 15.7), which had a HER2-negative pathology. Likewise, a substantially less [89]Zr-trastuzumab–avid lesion (patient 14; SUV, 6.9) had HER2-positive pathology and was a true-positive.

Another interesting issue was that even when a [89]Zr-trastuzumab–avid focus was considered a false-positive, there could be noticeable heterogeneity in HER2 expression between the primary malignancy and the avid metastasis. For example, patient 11 had a primary malignancy with an IHC of 0. The [89]Zr-trastuzumab–avid osseous metastasis that was biopsied had an IHC of 2+ and FISH of 1.6. While IHC 2+/FISH 1.6 is considered HER2-negative by ASCO guidelines, it still represents a change from the primary malignancy. The value of [89]Zr-trastuzumab PET/CT for evaluating this type of heterogeneity needs further evaluation.

The strength of this study is its design as a prospective clinical trial, whereas its weakness is still the small sample size. Ethical standards and logistical obstacles prevent biopsy of all [89]Zr-trastuzumab foci; thus, pathology is classified on the basis of a limited number of biopsies. Likewise, ethical standards prevent biopsy confirmation of HER2-negative status of metastases in patients without suspicious foci on [89]Zr-trastuzumab imaging.

In conclusion, [89]Zr-trastuzumab PET/CT can be used to detect unsuspected HER2-positive metastases in a subset of patients with HER2-negative primary breast cancer. This demonstrates that targeted imaging can identify patients amenable to targeted therapies. Presumed false-positive results currently limit the translation of these techniques, but there is great potential for more specific methodology to have substantial clinical value in the future.

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First-in-Human Human Epidermal Growth Factor Receptor 2–Targeted Imaging Using [89]Zr-Pertuzumab PET/CT: Dosimetry and Clinical Application in Patients with Breast Cancer

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In what we believe to be a first-in-human study, we evaluated the safety and dosimetry of [89]Zr-pertuzumab PET/CT for human epidermal growth factor receptor 2 (HER2)–targeted imaging in patients with HER2-positive breast cancer. **Methods:** Patients with HER2-positive breast cancer and evidence of distant metastases were enrolled in an institutional review board–approved prospective clinical trial. Pertuzumab was conjugated with deferoxamine and radiolabeled with [89]Zr. Patients underwent PET/CT with 74 MBq of [89]Zr-pertuzumab in a total antibody mass of 20–50 mg of pertuzumab. PET/CT, whole-body probe counts, and blood drawing were performed over 8 d to assess pharmacokinetics, biodistribution, and dosimetry. PET/CT images were evaluated for the ability to visualize HER2-positive metastases. **Results:** Six patients with HER2-positive metastatic breast cancer were enrolled and administered [89]Zr-pertuzumab. No toxicities occurred. Dosimetry estimates from OLINDA demonstrated that the organs receiving the highest doses (mean ± SD) were the liver (1.75 ± 0.21 mGy/MBq), the kidneys (1.27 ± 0.28 mGy/MBq), and the heart wall (1.22 ± 0.16 mGy/MBq), with an average effective dose of 0.54 ± 0.07 mSv/MBq. PET/CT demonstrated optimal imaging 5–8 d after administration. [89]Zr-pertuzumab was able to image multiple sites of malignancy and suggested that they were HER2-positive. In 2 patients with both known HER2-positive and HER2-negative primary breast cancers and brain metastases, [89]Zr-pertuzumab PET/CT suggested that the brain metastases were HER2-positive. In 1 of the 2 patients, subsequent resection of a brain metastasis proved HER2-positive disease, confirming that the [89]Zr-pertuzumab avidity was a true-positive result for HER2-positive malignancy. **Conclusion:** This first-in-human study demonstrated safety, dosimetry, biodistribution, and successful HER2-targeted imaging with [89]Zr-pertuzumab PET/CT. Potential clinical applications include assessment of the HER2 status of lesions that may not be accessible to biopsy and assessment of HER2 heterogeneity.

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 therapies that are targeted to HER2 and that reduce the risk of death, whereas patients with HER2-negative breast cancer do not receive them (*2,3*). This situation has resulted in considerable interest in HER2-targeted imaging (*4*). Recent work has demonstrated the ability to detect HER2-positive metastases in patients with HER2-negative primary breast tumors by HER2-targeted imaging confirmed with immunohistochemistry (IHC) (*5,6*) and molecular analyses (*7*). Thus, the ability to perform noninvasive, whole-body, HER2-targeted imaging may be valuable in the detection of otherwise unsuspected HER2-positive malignancies and may help direct appropriate HER2-targeted therapy.

Although there have been successes in HER2-targeted imaging with [89]Zr-trastuzumab, there have also been examples of nonspecific visualization of malignancies that are HER2-negative on pathology (*5,6*). More specific HER2-targeted agents may be needed for clinical translation of HER2-targeted imaging agents. Pertuzumab is a newer humanized monoclonal antibody that binds to the HER2 receptor at a site distinct from that bound to by trastuzumab and appears to be more efficient than trastuzumab (*8*). In vitro and in vivo models have demonstrated successful [89]Zr-pertuzumab targeting to HER2-positive malignancies and, notably, have demonstrated increased affinity for HER2 in the presence of trastuzumab (*9*)—as may be the case in patients who have HER2-positive malignancies and are receiving trastuzumab. Here we present the

results of what we believe to be a first-in-human study of HER2-targeted imaging with 89Zr-pertuzumab PET/CT, performed to document the safety, dosimetry, and potential clinical utility of this HER2-targeted imaging agent.

MATERIALS AND METHODS

Patients

This study was performed under a single-center prospective Memorial Sloan Kettering Cancer Center Institutional Review Board–approved protocol (ClinicalTrials.gov identifier NCT03109977). All patients provided written informed consent. Patients with pathologically proven HER2-positive metastatic breast cancer were identified as potential candidates. HER2 positivity was defined according to American Society of Clinical Oncology guidelines (10), including 3+ HER2 IHC or 2+ HER2 IHC with HER2 amplification on fluorescence in situ hybridization (FISH) of 2.0 or more; 0 or 1+ IHC or 2+ IHC with HER2 amplification on FISH of less than 2.0 was considered HER2-negative. The 4 inclusion criteria were biopsy-proven, HER2-positive malignancy; foci of malignancy on imaging within 60 d of enrollment; women who were 21 years old or older; and Eastern Cooperative Oncology Group performance score of 0–2 (11). The 3 exclusion criteria were life expectancy of less than 3 mo; pregnancy or lactation; and inability to undergo PET/CT scanning because of weight limits. A biopsy demonstrating HER2-positive malignancy was required for inclusion. The HER2-positive biopsy was allowed at any time during the patient's disease course and could be from the primary breast malignancy or a site of metastatic disease. Patients were allowed to be on HER2-directed therapy. Sites of known malignancy were determined by medical imaging, including CT, MRI, and 18F-FDG PET/CT, within 60 d of protocol enrollment. As part of the inclusion criteria, the primary malignancy and at least 1 site of distant metastasis had to be pathologically proven.

89Zr-Pertuzumab Preparation

89Zr-deferoxamine-pertuzumab was manufactured at the Memorial Sloan Kettering Radiochemistry and Molecular Imaging Probes Core Facility in compliance with the requirements specified in the Chemistry, Manufacturing, and Controls section of a U.S. Food and Drug Administration–acknowledged Investigational New Drug application (134411). The preparation process involved conjugating clinical-grade pertuzumab (Perjeta; Genentech) with a bifunctional chelator, p-SCN-Bn-deferoxamine (Macrocyclics), and then radiolabeling with 89Zr, a radiometal positron emitter with a 78.4-h radioactive half-life. The conjugation and radiolabeling were performed using previously described methodology (12). Radiolabeling with 89Zr was chosen because of the favorable properties of this metalloradionuclide, such as radioactive half-life (which is long enough to allow for the imaging of radiolabeled antibodies after localization at the target site has occurred) as well as mild radiolabeling conditions (at an ambient temperature in 1 M ammonium acetate buffer; pH 7); these properties help to preserve pertuzumab protein integrity and immunoreactivity during the radiolabeling process (13,14).

The 89Zr-deferoxamine-pertuzumab final drug product batches underwent quality control testing before batch release for patient administration, to ensure conformance to the following acceptance specifications: radiochemical purity, as determined by radio–thin-layer chromatography and size-exclusion high-performance liquid chromatography; radioimmunoreactivity, as determined using a live antigen–expressing cell binding assay; endotoxin content, as measured using a portable test system supplied by Charles River Laboratories; sterilizing filter integrity, as measured by the bubble point method; pH, as measured using pH strips; appearance as a clear and particle-free solution, as determined by visual inspection check; and radionuclidic identity verification, as measured by radioactive γ-spectroscopy. Sterility testing using the direct medium inoculation method was performed after release.

89Zr-Pertuzumab Administration

An intravenous line was established and flushed with 5% human serum albumin solution. Nonradiolabeled pertuzumab (18 or 48 mg) was then intravenously administered over 5 min. Cold pertuzumab was administered to help reduce nonspecific uptake of the subsequently administered radiolabeled pertuzumab. Next, 74 MBq ± 10% of 89Zr-pertuzumab was intravenously administered in a mass of approximately 2 mg to bring the total pertuzumab antibody mass for each patient to 20 or 50 mg. The first 2 patients were administered 50 mg; the antibody mass was reduced to 20 mg for the next 2 patients. Visual analysis suggested that 50 mg of total antibody mass produced lower background uptake; thus, the total antibody mass was increased to 50 mg for the last 2 patients. Patients were monitored for side effects on the day of and on the day after 89Zr-pertuzumab administration.

89Zr-Pertuzumab PET/CT and Image Analysis

Up to 4 whole-body PET/CT scans were obtained for each patient on days 1, 2–4, 5 or 6, and 7 or 8 after the administration (day 0) of 89Zr-pertuzumab. The days of imaging were preselected before the protocol was opened to allow both comprehensive multiday imaging and flexibility of scheduling, particularly over the weekend.

Patients were imaged from the skull apex to the midthigh on a dedicated research PET/CT scanner (GE Discovery 710) in 3-dimensional mode with emission time per bed position extending from 4 min (day 1) to 8 min (day 7 or 8). Low-dose CT scans were acquired with an x-ray tube current of 80 mA. PET/CT images were reconstructed with attenuation, scatter, and other standard corrections applied and with iterative reconstruction. 89Zr-pertuzumab PET/CT scans were interpreted by a nuclear radiologist with experience in HER2-targeted imaging and knowledge of the patient's medical history and prior imaging. Nonphysiologic radiotracer uptake was considered suggestive of HER2-positive malignancy. Volumes of interest were drawn on PET/CT images over normal liver, kidney, spleen, and lung using a dedicated workstation (Hermes Medical Solutions). Normal tissue uptake was quantified as the mean SUV adjusted to lean body mass (SUV$_{LBM}$).

Whole-Body and Serum Clearance Measurements

Whole-body clearance was determined by serial measurements of counting rates using a 12.7-cm-thick NaI(Tl) scintillation detector at a fixed 3 m from the patient. Background-corrected geometric mean counts were obtained after infusion before and after voiding and, subsequently, at the times of the PET scans ($n = 6$). Counting rates were normalized to the immediate postinfusion value (taken as 100%) to yield relative retained activities (as percentages).

Multiple blood samples were obtained at approximately 15 min, 30 min, 1–2 h after injection and, subsequently, at the time of each PET scan ($n = 7$). Counts in aliquots of serum were obtained using a γ-well-type detector (Wallac Wizard 1480 γ-counter; Perkin Elmer), and measured activity concentrations were converted to percentage injected activity/L.

A monoexponential function was fitted to the whole-body probe data and a biexponential function was fitted to the serum activity concentration data using SAAM software (15). Areas under the curve (AUCs) and corresponding residence times were derived by analytic integration.

Normal Tissue Dosimetry

Normal tissue dose estimates were derived as described previously (16,17). In brief, image-derived SUV$_{LBM}$ was converted to activity concentration per unit of mass (kBq/g), and AUCs were estimated by trapezoidal integration. Whole-organ AUCs were estimated by multiplying the activity concentration AUCs by the projected organ

TABLE 1
Characteristics of 6 Women with IDC

Patient	Age (y)	Sites of known malignancy at time of ^{89}Zr-pertuzumab administration	Days after ^{89}Zr-pertuzumab administration on which PET/CT imaging was done	Sites of demonstrably ^{89}Zr-pertuzumab–avid disease
1	46	Brain	1, 2, 6, 8	Brain
2	68	Nodal	1, 2, 5, 7	Nodal
3	58	Breast, nodal	1, 2, 6, 7	Breast, nodal
4	69	Liver, bone, chest wall	1, 4, 5, 8	Chest wall
5	38	Brain, lung, nodal, liver	1, 2, 5	Brain, lung, nodal
6	42	Nodal	1, 4, 5, 7	None

mass. Residence times were derived by dividing whole-organ AUCs by the administered activity. Corresponding values for heart contents and red marrow were estimated from the serum AUCs (*18*). The residence time for the remainder of the body was derived by subtracting all individually estimated residence times from the whole-body residence time. Thereafter, absorbed radiation doses to individual organs were calculated using the OLINDA/EXM software application (*19*).

Comparison of ^{89}Zr-Pertuzumab Dosimetry with ^{89}Zr-Trastuzumab Dosimetry

Tissue dosimetry and total-body dosimetry were compared for the newly calculated values for ^{89}Zr-pertuzumab and for published values for ^{89}Zr-trastuzumab.

Statistics

Kinetic parameters and absorbed dose estimates were calculated for each patient on an individual basis. Subsequently, these data were summarized using descriptive statistics.

RESULTS

Patient Characteristics

Between April and June 2017, 6 patients, all women with biopsy-proven HER2-positive malignancies from invasive ductal breast cancer (IDC), completed the study protocol. All patients underwent imaging on days 1, 2–4, 5 or 6, or 7 or 8 as prescribed in the prospective protocol. Patient characteristics are summarized in Table 1.

Sites of Known Malignancy at Time of Protocol Enrollment

Sites of known malignancy were determined from medical imaging within 60 d of protocol enrollment. Known nodal disease was present in 4 patients, brain malignancies were present in 2, hepatic malignancies were present in 2, and malignancies involving the breast, bone, chest wall, and lung each were present in 1 patient. Sites of known malignancy are summarized in Table 1.

Adverse Events

All 6 patients underwent ^{89}Zr-pertuzumab administration. Patients were monitored for 2 h after tracer injection and evaluated on the following day when they returned for PET/CT imaging, and no side effects were observed or reported. Vital signs were recorded before and after tracer administration, and there were no changes with clinical significance. Safety data were reviewed and approved by the U.S. Food and Drug Administration as part of the Investigational New Drug application.

Pharmacokinetics

Whole-body clearance and serum clearance conformed to mono- and biexponential kinetics, respectively. Summed biologic clearance curves are shown in Figure 1. Summary statistics for the clearance parameters are provided in Table 2.

Biodistribution and Normal Tissue Dose Estimates

^{89}Zr-pertuzumab uptake was observed in the blood pool, liver, kidney, and spleen. There was little measureable change in whole-body activity after the first void (98% ± 1.8% [mean ± SD] of the prevoid measurement). The urinary bladder was not visualized on any PET/CT scan. Bowel excretion was visualized in 2 patients on day 1 and 2 scans. Uptake in the liver and kidneys, in terms of SUV_{LBM}, was relatively constant over the duration of imaging, whereas blood-pool and spleen uptake decreased over time. These sites of tracer visualization were considered physiologic. Absorbed dose estimates for normal tissues are provided in Table 3. The organs receiving the highest doses were the liver (1.75 ± 0.21 mGy/MBq), kidneys (1.27 ± 0.28 mGy/MBq), and heart wall (1.22 ± 0.16 mGy/MBq), with an average effective dose of 0.54 ± 0.07 mSv/MBq.

Comparison of ^{89}Zr-Pertuzumab Dosimetry with ^{89}Zr-Trastuzumab Dosimetry

The mean effective dose of ^{89}Zr-pertuzumab was 0.54 mSv/MBq. Figure 2 shows the comparative absorbed dose estimates for both antibodies.
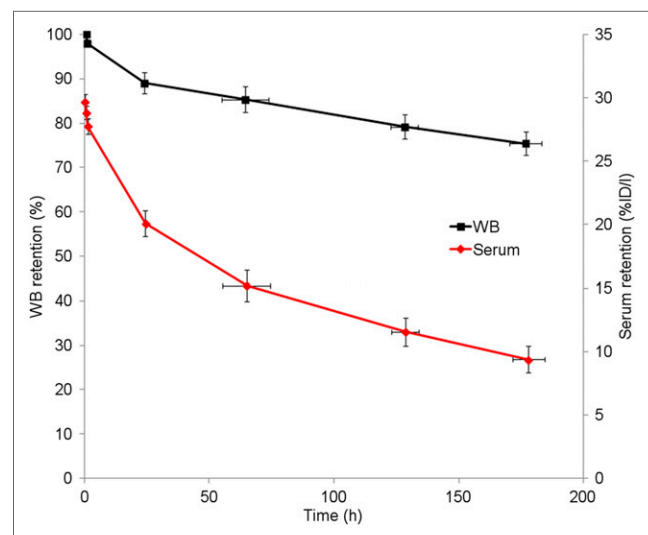

FIGURE 1. Summed whole-body (WB) and serum biologic clearance data for ^{89}Zr-pertuzumab in 6 patients. Error bars indicate SE of mean. %ID/l = percentage injected dose/L.

TABLE 2
Summary Statistics for Whole-Body and Serum Clearance

Measurement	Whole body		Serum						
	$t_{1/2}$ eff	$t_{1/2}$ bio	A1 (%/L)	$t_{1/2}$ α eff	$t_{1/2}$ α bio	A2 (%/L)	$t_{1/2}$ β eff	$t_{1/2}$ β bio
Mean	67.7	521	12.6	12.1	15.5	17.3	59.3	201
SD	2.3	145	5.3	7.8	11.5	5.0	10.3	72
Median	66.9	456	13.0	9.7	11.2	16.3	55.5	176
Minimum	65.2	387	5.6	3.1	3.3	11.7	50.4	141
Maximum	70.8	730	18.8	22.1	30.8	23.3	78.4	322

eff = effective half-life; bio = biologic half-life; A1 = partition coefficient for fast component of serum clearance; α = half-time for fast component of serum clearance; A2 = partition coefficient for slow component of serum clearance; β = half-time for slow component of serum clearance.

Whole-body clearance was monoexponential. Serum clearance was biexponential and conformed to the following equation: A1exp(−a1t) + A2exp(−a2t). A1 and A2 are partition coefficients, and respective $t_{1/2}$ values correspond to ln(2)/a. All half-lives are in hours.

Imaging of Lesions with ^{89}Zr-Pertuzumab PET/CT

Patient 1 had 2 known primary breast malignancies, a right breast estrogen receptor (ER)–positive, progesterone receptor (PR)–positive, and HER2-positive (HER2 IHC 3+) IDC diagnosed in 2014 and an ER-positive, PR-positive, and HER2-negative (HER2 IHC 0) IDC diagnosed in 2015. She had received HER2-directed

TABLE 3
Absorbed Dose Estimates for Normal Tissues

| Target organs or measurement	Absorbed dose estimates (mGy/MBq)				
	Mean	SD	Median	Minimum	Maximum
Adrenal glands	0.64	0.17	0.69	0.31	0.80
Brain	0.25	0.06	0.24	0.15	0.33
Breasts	0.33	0.08	0.35	0.18	0.41
Gallbladder wall	0.69	0.18	0.72	0.34	0.83
Lower large intestinal wall	0.42	0.06	0.40	0.36	0.52
Small intestine	0.39	0.09	0.40	0.22	0.51
Stomach wall	0.46	0.12	0.49	0.23	0.59
Upper large intestinal wall	0.46	0.09	0.47	0.30	0.57
Heart wall	1.22	0.16	1.24	0.98	1.37
Kidneys	1.27	0.28	1.18	0.98	1.64
Liver	1.75	0.21	1.73	1.54	2.13
Lungs	1.07	0.15	1.07	0.84	1.25
Muscle	0.34	0.08	0.35	0.19	0.44
Ovaries	0.38	0.10	0.38	0.20	0.50
Pancreas	0.62	0.16	0.66	0.30	0.77
Red marrow	0.48	0.09	0.50	0.34	0.60
Osteogenic cells	0.55	0.09	0.54	0.44	0.71
Skin	0.24	0.05	0.24	0.14	0.31
Spleen	1.04	0.16	1.01	0.87	1.26
Thymus	0.46	0.12	0.48	0.23	0.58
Thyroid	0.29	0.07	0.29	0.17	0.38
Urinary bladder wall	0.27	0.06	0.27	0.17	0.36
Uterus	0.37	0.10	0.37	0.19	0.49
Total body	0.40	0.08	0.41	0.25	0.49
Effective dose equivalent (mSv/MBq)	0.72	0.09	0.73	0.56	0.83
Effective dose (mSv/MBq)	0.54	0.07	0.56	0.41	0.62
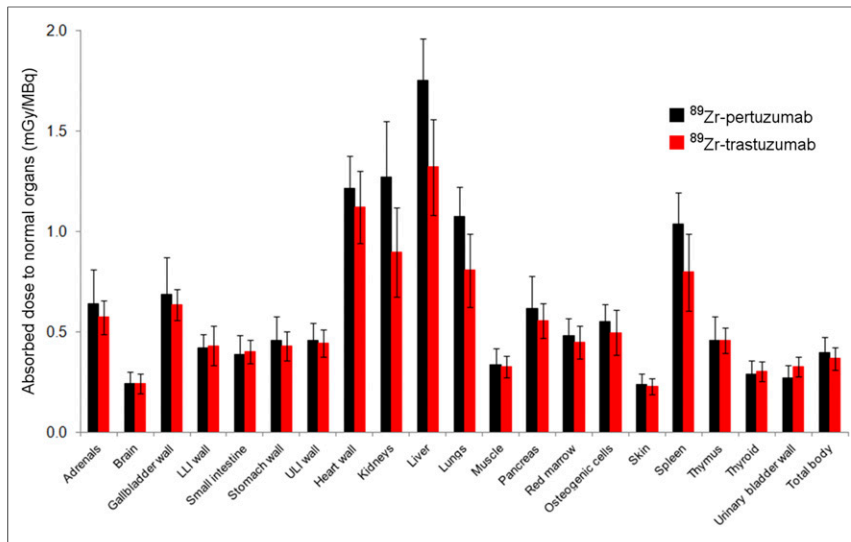
FIGURE 2. Comparative distributions of absorbed doses for ^{89}Zr-pertuzumab and ^{89}Zr-trastuzumab (16). Error bars denote SD. LLI = lower large intestinal; ULI = upper large intestinal.

therapy, including ado-trastuzumab emtansine in 2015, and was on trastuzumab therapy at the time of ^{89}Zr-pertuzumab PET/CT. She had a recent diagnosis of brain metastases. ^{89}Zr-pertuzumab PET/CT demonstrated a progressive increase in ^{89}Zr-pertuzumab avidity over PET/CT scans obtained on days 1, 2, 6, and 8 after tracer administration (the SUV_{max} of the most avid lesion was 13.6, 16.6, 26.0, and 30.1 on these 4 d) (Figs. 3A–3D). Blood-pool activity, including activity in the superior sagittal sinus, showed a continuing decrease over the scans. This feature allowed the most optimal visualization of the known brain metastases (Fig. 3E) on the day 8 scan (Fig. 3F).

Patient 2 had a left breast ER-positive, PR-positive, and HER2-positive (HER2 IHC 2+; HER2 amplification of 4.0 on FISH) IDC diagnosed in 2014. She had received HER2-directed therapy, including ado-trastuzumab emtansine in 2015–2016, and was on trastuzumab and pertuzumab therapy at the time of ^{89}Zr-pertuzumab PET/CT. She had known supraclavicular nodal metastases at the time of ^{89}Zr-pertuzumab PET/CT. There was relatively stable ^{89}Zr-pertuzumab avidity on days 1, 2, 5, and 7 after tracer administration (the SUV_{max} of the most avid lesion was 6.0, 4.7, 4.6, and 5.1 on these 4 d).

Patient 3 had a left breast ER-positive, PR-positive, and HER3-positive (HER2 IHC 3+) IDC that was diagnosed in 2014 and that was metastatic to the lung at the time of diagnosis. She had received HER2-directed therapy, including trastuzumab and pertuzumab, which she was receiving at the time of ^{89}Zr-pertuzumab PET/CT. She had demonstrable ^{18}F-FDG–avid disease in the left breast and left axillary nodes at the time of ^{89}Zr-pertuzumab PET/CT. There was mildly increasing ^{89}Zr-pertuzumab avidity on days 1, 2, 6, and 7 after tracer administration (the SUV_{max} of the breast lesion was 3.7, 4.7, 6.3, and 5.5). Decreasing background avidity made the avid lesions most visible on the day 6 and 7 scans (Fig. 4). ^{89}Zr-pertuzumab avidity was similar to ^{18}F-FDG avidity (SUV_{max}, 6.6), which was obtained 3 wk earlier.

Patient 4 was diagnosed with ER-positive, HER2-negative (HER2 IHC 1+) IDC in 2003. At the time of ^{89}Zr-pertuzumab PET/CT, she had demonstrable disease in the liver, bone, and chest wall on CT and MRI. She had previously received HER2-directed therapy, including trastuzumab and pertuzumab, which she was

receiving at the time of ^{89}Zr-pertuzumab PET/CT. A recent biopsy of a right chest wall mass was HER2-positive (HER2 IHC 2+; HER2 amplification of 2.4 on FISH). There was low-level avidity in the right chest wall lesion (the SUV_{max} was 2.8, 2.3, 2.4, and 2.4 on days 1, 4, 5, and 8 after ^{89}Zr-pertuzumab administration, respectively).

Patient 5 was diagnosed with 2 distinct right breast malignancies in 2014: an ER-negative, HER2-positive (HER2 IHC 3+) malignancy and an ER-positive, HER2-negative (HER2 IHC 0) malignancy. She had previously received HER2-directed therapy, including trastuzumab and pertuzumab, which she was receiving at the time of ^{89}Zr-pertuzumab PET/CT. She had demonstrable disease in the brain, lung, nodes, and liver at the time of ^{89}Zr-pertuzumab PET/CT. Mild ^{89}Zr-pertuzumab avidity in the brain, lung, and nodal lesions was greatest in the brain (the SUV_{max} of the most avid brain lesion was 2.9, 6.1, and 6.1 on days 1, 2, and 5 after tracer administration, respectively). A brain metastasis was resected and was HER2-positive (IHC 2+; HER2 amplification of 2.4 on FISH); these findings confirmed that this site of ^{89}Zr-pertuzumab-avidity was true-positive for a HER2-positive malignancy. Liver lesions did not show appreciable ^{89}Zr-pertuzumab avidity above the liver background.

Patient 6 had a right breast ER-negative, HER2-positive (HER2 IHC 3+) IDC diagnosed in 2008. She had previously received HER2-directed therapy, including trastuzumab and pertuzumab, and was on ado-trastuzumab emtansine therapy at the time of ^{89}Zr-pertuzumab PET/CT. She had only a small volume disease in thoracic and abdominal nodes at the time of ^{89}Zr-pertuzumab PET/CT. Avidity in small nodes (the greatest being 1.5×1.2 cm in the right common iliac chain) was difficult to appreciate on ^{89}Zr-pertuzumab PET/CT.

DISCUSSION

In this first-in-human trial, we demonstrated safety and dosimetry for intravenously administered ^{89}Zr-pertuzumab, which has been reviewed and accepted by the U.S. Food and Drug Administration. We also demonstrated successful HER2-targeted imaging in patients with HER2-positive metastatic breast cancer.

The mean effective dose of ^{89}Zr-pertuzumab was 0.54 mSv/MBq, which is comparable to those of other radiolabeled antibody PET tracers, such as ^{89}Zr-J591 (0.38 mSv/MBq) (16). In particular, the biodistribution and normal tissue dosimetry of ^{89}Zr-pertuzumab are comparable to those of ^{89}Zr-trastuzumab (0.48 mSv/MBq) (17). However, ^{89}Zr-pertuzumab does appear to have slightly higher uptake in the central parenchymal organs (liver, kidney, spleen, and lung) than ^{89}Zr-trastuzumab; this finding translates into a higher dose (mGy/MBq) by an average factor of approximately 1.3 for these organs. Because of their relatively slow kinetics, antibody-based PET tracers require radionuclides with relatively long physical half-lives. This requirement leads to radiation doses that are higher than those of small-molecule imaging agents that have fast kinetics and can use radionuclides with relatively short physical half-lives—the classic example being ^{18}F-FDG.
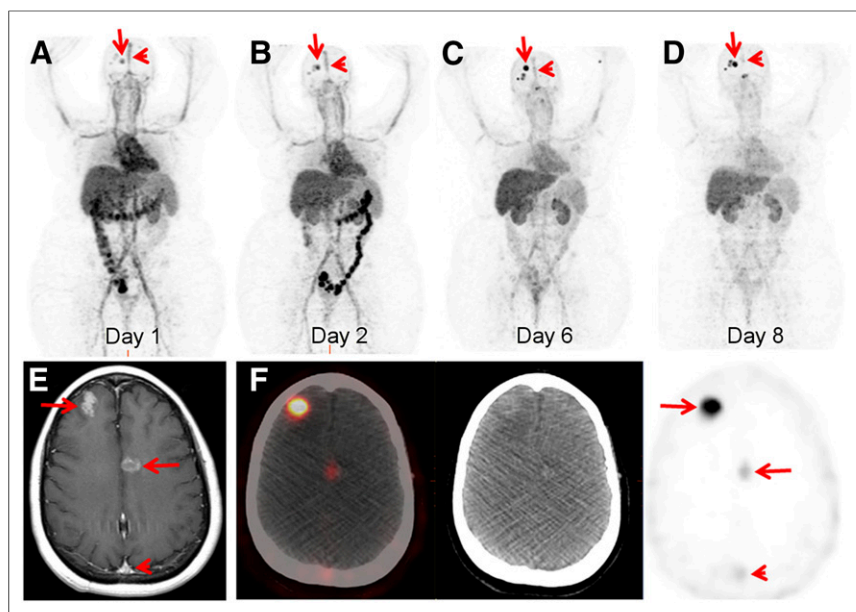
FIGURE 3. 46-y-old woman with both HER2-positive and HER2-negative primary breast malignancies and recently diagnosed brain metastases. Sequential maximum-intensity-projection images 1 d (A), 2 d (B), 6 d (C), and 8 d (D) after administration of 89Zr-pertuzumab. Blood-pool and liver background cleared on sequential images. Activity excreted by bowel was seen on days 1 and 2. Bilateral kidney activity was visualized on all days. Increasing activity in foci overlying skull was seen as time progressed (arrows). Decreasing activity was seen in blood pool of superior sagittal sinus (arrowheads). (E) Gadolinium-enhanced T1-weighed MR image of brain demonstrated enhancing brain metastases (arrows) and superior sagittal sinus (arrowhead). (F) Axial fused PET/CT, CT, and PET images 8 d after 89Zr-pertuzumab administration demonstrated avidity in brain metastases (arrows) and minimal residual avidity in superior sagittal sinus (arrowhead).

In the present study, we used a 89Zr activity (~74 MBq) that was lower than what we used in previous studies (~185 MBq). This activity was found to be adequate in terms of image quality for clinically feasible emission times, ranging from 4–8 min/bed position. As the radiation dose is directly proportional to the administered activity, this approach provided a significant reduction in the actual dose (mGy) compared with the doses used in our previous studies. Further, several investigators (20,21), including equipment manufacturers, are developing new, advanced methods of image

reconstruction that should result in further significant reductions in radiation doses. The radiation exposures generated by radiolabeled antibody PET tracers would be justified if they produced clinically valuable information.

Imaging of 89Zr-pertuzumab was best performed 5–8 d after tracer administration. Scans on earlier days had higher liver and blood-pool backgrounds and tended to have lower tumor uptake. The combination of higher tumor uptake and lower backgrounds for imaging performed 5–8 d after tracer administration has been observed with other antibody tracers (16,17). The multiple-day delay in blood-pool clearance of antibody tracers can be considered a limitation of this technology. Potential alternatives include Affibody (Affibody AB) molecules (22) and single-domain antibodies (23,24), which have a rapid biodistribution, allowing for imaging within hours of tracer administration. Affibody molecules and single-domain antibodies labeled with tracers with shorter half-lives may have the added advantage of lower radiation doses to patients.

Although limited activity was seen in the bowel, we anticipate that this was the primary route for any 89Zr-pertuzumab excretion that did occur. Little measurable change was observed in whole-body activity after the first void (98% ± 1.8% of the prevoid measurement), and there was no visualization of activity in the urinary bladder at any time.

One potential clinical application of 89Zr-pertuzumab PET/CT is assessment of the HER2 status of disease—information that may not be accessible by biopsy (e.g., brain metastases). This application becomes increasingly important given the recent finding that in patients who have HER2-negative primary breast cancer and develop brain metastases, 20% will acquire HER2-positive metastases (7). Thus, a method of noninvasive, whole-body screening for HER2-positive disease would be of clinical value as a predictive biomarker (25), aiding in the selection of patients for HER2-targeted therapy on the basis of imaging. In this small trial, patients 1 and 5 had both HER2-positive and HER2-negative primary breast cancers and brain metastases. 89Zr-pertuzumab PET/CT imaging suggested that the brain metastases were HER2-positive; histologic proof was available in 1 of these patients.

Another potential clinical application of 89Zr-pertuzumab PET/CT is the assessment of HER2 heterogeneity. Patient 4 had a biopsy-proven HER2-negative primary breast malignancy but a biopsy-proven HER2-positive chest wall metastasis. Two previous liver biopsies for this patient were HER2-negative. The only site of
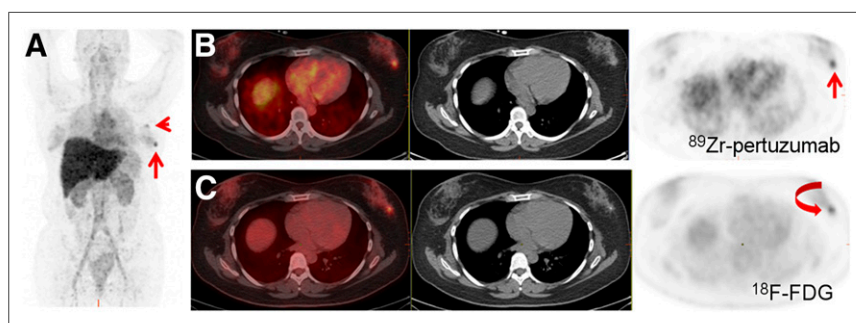

FIGURE 4. 58-y-old woman with HER2-positive breast cancer and current left breast and left axillary nodal disease. (A) Maximum-intensity-projection image 6 d after administration of 89Zr-pertuzumab demonstrated 89Zr-pertuzumab–avid left breast (arrow) and left axillary nodal (arrowhead) disease. (B) Axial fused PET/CT, CT, and PET images obtained 6 d after 89Zr-pertuzumab administration localized 89Zr-pertuzumab avidity in left breast (arrow). (C) Axial fused PET/CT, CT, and PET images from 18F-FDG PET/CT scan 3 wk earlier demonstrated corresponding 18F-FDG–avid breast lesion (arrow).

[89]Zr-pertuzumab avidity was the chest wall lesion, which was the only site of known HER2-positive disease, as defined by American Society of Clinical Oncology criteria. Of course, histologic analysis was not available for all sites of malignancy, but this early work suggests that [89]Zr-pertuzumab could be used to assess a heterogeneous HER2 tumor burden.

For this first-in-human pilot study of [89]Zr-pertuzumab PET/CT, the sample size of only 6 patients was primarily designed to provide normal tissue biodistribution and dosimetry data. Further information on the biodistribution of [89]Zr-pertuzumab in normal and malignant tissues will be generated in additional HER2-targeted imaging trials with this novel agent. Patients included in this trial had metastases that were treated with systemic therapy, often including HER2-targeted therapy, before trial enrollment. This scenario introduces difficulties in comparing the extents of active malignancies with radiotracer uptake. A treated HER2-positive metastasis may not have sufficient residual active tumor for visualization on PET. Despite this limitation, sites of disease that were avid for [89]Zr-pertuzumab were demonstrated in 5 of 6 patients. [89]Zr-pertuzumab–avid lesions were still demonstrated in patients who had previously received and were currently receiving HER2-targeted therapy. The effects of HER2-targeted therapy on [89]Zr-pertuzumab are not known; however, it was clear that HER2-targeted therapy did not prevent HER2-targeted imaging with [89]Zr-pertuzumab.

CONCLUSION

This first-in-human trial demonstrated that [89]Zr-pertuzumab PET/CT may be safely performed and that [89]Zr-pertuzumab has the potential to be a clinically valuable HER2-targeted imaging agent for patients with metastatic breast cancer. Potential clinical applications include assessment of the HER2 status of lesions—information that may not be accessible by biopsy—and the assessment of HER2 heterogeneity. [89]Zr-pertuzumab PET/CT will next be used in a prospective clinical trial of patients with HER2-negative primary breast cancer to analyze the ability of [89]Zr-pertuzumab to detect unsuspected HER2-positive metastatic disease and help direct HER2-targeted therapy to appropriate patients.

DISCLOSURE

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First-in-Human Human Epidermal Growth Factor Receptor 2–Targeted Imaging Using ^{89}**Zr-Pertuzumab PET/CT: Dosimetry and Clinical Application in Patients with Breast Cancer**

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