Human Epidermal Growth Factor Receptor 2-Targeted PET/Single-Photon Emission Computed Tomography Imaging of Breast Cancer:

Noninvasive Measurement of a Biomarker Integral to Tumor Treatment and Prognosis

Kelly E. Henry, PhD[a,*], **Gary A. Ulaner, MD, PhD**[a,b], and **Jason S. Lewis, PhD**[a,c]

[a]Department of Radiology, Memorial Sloan Kettering Cancer Center, New York, NY 10065, USA

[b]Department of Radiology, Weill Cornell Medical College, New York, NY 10065, USA

[c]Program in Molecular Pharmacology and Chemistry, Memorial Sloan Kettering Cancer Center, New York, NY 10065, USA

Keywords

PET; SPECT; CT; Imaging; Radiotracers; HER2; Metastasis; Breast cancer

INTRODUCTION

Breast cancer is a heterogeneous disease in which tumor differentiation within lesions and in primary versus metastatic disease can be the basis of a poor prognosis and clinical outcomes.[1] Initial staging of breast cancer integrates pathologic analysis of receptor status in biopsied tissues. The three main biomarkers of interest in breast cancer include estrogen receptor (ER), progesterone receptor, and human epidermal growth factor receptor 2 (HER2).[2] These receptors are assessed using immunohistochemistry, fluorescence in situ hybridization, or chromogen in situ hybridization to direct therapy.[3]

HER2 overexpression is a hallmark of aggressive breast cancer, and its expression in a primary tumor directly influences treatment.[4] Because of this, HER2 has become a critical target for molecular imaging and therapy.[4,5] Approximately 20% of invasive ductal breast malignancies are classified as HER2 positive as a result of gene amplification and/or the subsequent overexpression of the HER2 protein on the surface of tumor cells.[6] Patients with HER2-positive breast cancer receive specific, targeted HER2 therapies to reduce the risk of death.[7] However, the current methods in the clinic to assess receptor status have issues when it comes to staging breast cancer, and consequently assigning therapy. Inherent errors include bias in and misinterpretation of immunohistochemistry or in situ hybridization results, sampling issues, and discordance between primary metastatic sites of disease.[8–10] Although not typically within the standard course of treatment, there have been reports of

*Corresponding author. Department of Radiology, Memorial Sloan Kettering Cancer Center, 1275 York Avenue, New York, NY 10065. henryk1@mskcc.org.

clinical benefit of HER2-targeted therapy even in patients with a primary HER2-negative tumor.[11] The hypothesis behind this phenomenon is owing to the heterogeneity of not just the primary tumors, but of metastatic disease as well.[12]

One approach to address these issues with current staging methods in HER2-positive breast cancer is to use targeted molecular imaging. Imaging modalities that target HER2 have the potential to not only diagnose HER2-positive breast cancer, but also detect distant metastases via a single, noninvasive procedure.[5,13,14] Molecular imaging, particularly with tracers used in PET, can address issues of heterogeneity and discordance between primary and metastatic disease in breast cancer. PET is especially useful when paired with methods for anatomic imaging (eg, MR imaging or computed tomography [CT]). These modalities used in tandem can detect exact location of disease, and allow more accurate surgical resection. PET imaging can also be used to select patients for targeted therapies and monitor treatment effects.[15]

PET is a highly sensitive, quantitative molecular imaging technique with high spatial resolution. PET is the select modality for imaging in the clinic owing to its favorable properties. Several groups have developed antibody-based radioligands over the past 15 years for PET imaging preclinically and clinically.[16–19] From this body of work, many of these targeted probes have been used to image HER2-positive breast cancer.[5,13] The clinical translation of antibodies has its drawbacks owing to often low tumor penetration because of high molecular weight (approximately 150 kDa) and slow clearance.[16] Also, conventional positron emitters (eg, ^{11}C, ^{13}N, ^{15}O, and ^{18}F) have half-lives that may be too short to permit adequate biodistribution of labeled antibodies before imaging. Pairing these targeting moieties with longer-lived radioisotopes such at zirconium-89 (^{89}Zr) for PET and indium-111 (^{111}In) for single-photon emission CT (SPECT) (^{89}Zr, $t_{1/2}$ = 78 hours; ^{111}In, $t_{1/2}$ = 67 hours) can be used to match the biological half-life of antibodies in vivo ($t_{1/2}$ approximately 72 hours), and allow radioactivity accumulation in tumors for up to 6 days.[20] Antibody fragments and affibody molecules (small proteins engineered to bind to a large number of target proteins or peptides with high affinity) can be labeled with shorter-lived isotopes such as gallium-68 (^{68}Ga) or fluorine-18 (^{18}F) (^{68}Ga, $t_{1/2}$ = 68 minutes; ^{18}F, $t_{1/2}$ = 110 minutes) for a faster turnaround and more transient detection of disease.[21–24] Isotopes with "medium" half-lives such as copper-64 (^{64}Cu, $t_{1/2}$ = 12 hours) are also used for both full-length and antibody fragments for PET imaging.[25–27]

Many of the PET and SPECT imaging tracers that have made it to the clinic (either in current clinical use, or have made it to phase I/II trials) to detect HER2-positive breast cancer and metastases, are summarized in Table 1. These agents are used for a more accurate diagnosis and patient selection for therapy in breast cancer.

Inaccurate knowledge of receptor status in metastases owing to tumor heterogeneity and differentiation throughout cancer spreading may lead to suboptimal selection of patients for HER2-targeted therapy.[12,28,29] Data show that more than 10% of patients with HER2-negative primary breast cancer may still benefit from HER2-targeted treatment.[11] This observation indicates that HER2-negative patients may have distant disease that is in fact HER2 positive. Targeting these metastases can reduce the risk of death in this aggressive

subtype of breast cancer. Current treatments targeted to HER2-positive breast lesions, including hormone therapy, are listed in Table 2.

Trastuzumab is a monoclonal antibody that targets HER2 and is the first-line therapy for HER2-positive breast tumors.[29–32] The history of trastuzumab dates back to 1998 when it was approved by the Food and Drug Administration, and has been used regularly in the clinic ever since.[31] Imaging modalities have been constructed using a functionalized version of this antibody to detect HER2-positive disease and metastasis.

PET AND SINGLE PHOTON EMISSION COMPUTED TOMOGRAPHY IMAGING WITH MONOCLONAL ANTIBODIES IN HUMAN EPIDERMAL GROWTH FACTOR RECEPTOR 2-POSITIVE BREAST CANCER

Zirconium-89-DFO-Trastuzumab

[89]Zr radiopharmaceuticals are an attractive option for antibody-based imaging agents owing to their compatible half-life (approximately 78 hours) to monoclonal antibodies (approximately 72 hours).[20] Dijkers and colleagues[33] were the first to label clinical-grade trastuzumab with [89]Zr and use it for immunoPET imaging in animals. This radiotracer was later implemented to gauge HER2 status in not just primary tumor, but in patients with nonaccessible metastases.[33] With this product, Oude and colleagues[34] used [89]Zr-DFO-trastuzumab to annotate response of HER2 to HSP90 therapy preclinically. From here, there have been numerous reports of preclinical and clinical studies with this tracer that have evolved both imaging and therapy within HER2-positive breast cancer.

[89]Zr-DFO-trastuzumab has been favored owing to a higher spatial resolution and a better signal-to-noise ratio than ^{111}In-trastuzumab, and ease of data quantification with PET versus SPECT. Preliminary results (Dijkers and colleagues[35]) with [89]Zr-DFO-trastuzumab immunoPET in HER2-positive breast cancer patients indicated exceptional tumor uptake and spatial resolution. Fourteen patients with HER2-positive metastatic breast cancer received 37 MBq of [89]Zr-trastuzumab at 1 of 3 doses (10 or 50 mg for those who had not received trastuzumab and 10 mg for those who were undergoing trastuzumab treatment). The patients underwent serial PET scans between days 2 and 5. The results of the study showed that the best time for assessment of the uptake of [89]Zr-trastuzumab was 4 to 5 days after injection. Fig. 1 represents the biodistribution of [89]Zr-DFO-trastuzumab, including detection of metastatic brain lesions that were not observed by fludeoxyglucose F 18 (^{18}F-FDG). Fusion PET/MR imaging scans in Fig. 2 further highlight this observation, revealing the true usefulness of this radiotracer in patients. This pilot study showed promise in the ability of [89]Zr-DFO-trastuzumab to detect not only primary tumor, but also metastatic disease that conventional PET was unable to accomplish.[36]

Zirconium-89-DFO-Trastuzumab to Detect Unsuspected Human Epidermal Growth Factor Receptor 2-Positive Metastatic Disease

Ulaner and colleagues[12] explored the potential of [89]Zr-DFO-trastuzumab to detect HER2-positive metastases in HER2-negative primary tumors. Nine patients with HER2-negative primary breast cancer were enrolled in this study, and imaged with [89]Zr-DFO-trastuzumab

PET/CT to detect for [89]Zr-DFO-trastuzumab–avid metastases. All patients enrolled were confirmed pathologically as HER2 negative. Metastases that were observed via [89]Zr-trastuzumab PET/CT were biopsied and pathologically examined to define HER2 status. Based on these findings, patients with HER2-positive metastases received HER2-targeted therapy to evaluate treatment response. An example of one such patient is represented in Fig. 3.

Of the 9 patients, 5 exhibited metastatic uptake with [89]Zr-DFO-trastuzumab PET/CT. Of these 5 patients, 2 had biopsies taken of potential metastatic sites based from their PET scans. These 2 patients exhibited HER2-positive metastases (as confirmed via pathologic workup of the biopsied tissue) and subsequently benefitted from HER2-targeted therapy. The remaining 3 patients with suspicious uptake were also biopsied, and did not present evidence of HER2-positive disease at metastatic lesions, and were considered false positive for [89]Zr-DFO-trastuzumab PET. An example of a false-positive scan is presented in Fig. 4.[12]

The significant result of this study is that [89]Zr-DFO-trastuzumab PET/CT can detect unsuspected HER2-positive metastases in patients with a HER2-negative primary breast cancer. Although these preliminary results encompass a small sample size, it still gives strong evidence that HER2-targeted imaging can identify candidates for HER2-targeted therapy. These results provide a possible explanation for the hypothesis that a minority of patients with HER2-negative tumors benefit from HER2-targeted therapy because of the heterogeneity within distant disease. More specific HER2-targeting agents are necessary in the clinic to elucidate fully this relationship via imaging, and decrease the potential for false-positive uptake.

Annotating Treatment Response with Zirconium-89-DFO-Trastuzumab

Heath shock protein 90 (HSP90) is an ATP-dependent molecular chaperone protein that is involved in several oncogenic pathways, and has been a target for many cancer therapies.[37] HSP90 inhibitors have emerged as a promising anticancer strategy in many cancers, including HER2-positive breast cancer.[38] HSP90 inhibitors have been combined with trastuzumab (either as treatment or assessing treatment response) in HER2-positive metastatic breast cancer.[39]

The primary aim of a clinical trial led by Gaykema and colleagues[34] was to evaluate the usefulness of [89]Zr-DFO-trastuzumab PET (for HER2-positive breast cancer) or [89]Zr-DFO-bevaci-zumab PET (for ER-positive breast cancer) to determine the in vivo degradation of proteins owing to HSP90 inhibitor NVP-AUY922. Sixteen patients (10 HER2-positive and 6 ER-positive tumors) were included in this study. During this study, NVP-AUY922 (70 mg/m^2) was administered intravenously weekly to patients with advanced HER2- or ER-positive breast cancer. Serial PET imaging with ^{18}F-FDG, [89]Zr-DFO-trastuzumab, or [89]Zr-DFO-bevacizumab was performed to assess biomarkers of interest (HER2, ER, and ^{18}F-FDG used as a metabolic marker). Blood samples were collected to detect HSP90 levels and the extracellular form of HER2. One partial response was observed from NVP-AUY922 treatment, and 7 patients showed no response. Changes in the maximum standardized uptake value in patients with [89]Zr-trastuzumab PET before and after treatment was heterogeneous and correlated with size change on CT. Treatment with HSP90 inhibitor NVP-AUY922

showed a clinical response in HER2-positive metastatic breast cancer. Changes in ^{89}Zr-DFO-trastuzumab PET were concomitant with change in size of lesions, as measured by CT.

Another ongoing study to assess the usefulness of ^{89}Zr-trastuzumab to image therapy response is the ZEPHIR trial (Phase II Prospective Imaging Study Evaluating the Utility of Pre-treatment Zr^{89} Labelled Trastuzumab PET/CT and an Early FDG-PET/CT Response to Identify Patients With Advanced HER2+ BC Unlikely to Benefit From a Novel anti-HER2 Therapy: TDM1), led by the Jules Bordet Institute. This clinical trial is currently enrolling patients from Belgium and the Netherlands eligible to receive that antibody–drug conjugate T-DM1 for HER2-positive advanced disease. HER2-positive metastatic breast cancer patients with an immunohistochemistry 3+ or a fluorescence in situ hybridization score of greater than 2.2 eligible for T-DM1 treatment underwent a pretreatment PET/CT scan with ^{89}Zr-trastuzumab. ^{18}F-FDG PET/CT was performed at baseline and before T-DM1 cycle 2. Patients were grouped into 4 HER2–PET/CT patterns according to the proportion of ^{18}F-FDG–avid tumor concurrent with ^{89}Zr-trastuzumab uptake. In the 56 patients analyzed, 29% had negative ^{89}Zr-trastuzumab PET/CT and intrapatient heterogeneity was found in 46% of patients.[40] ^{89}Zr-trastuzumab imaging, combined with early metabolic response assessment with ^{18}F-FDG accurately predicted morphologic response after 3 treatment cycles with T-DM1. This methodology has the potential for improving the understanding of tumor heterogeneity in metastatic breast cancer and for selecting patients who may or may not benefit from T-DM1.

Currently, patients are still being recruited for many ^{89}Zr-DFO-trastuzumab PET imaging trials. The IMPACT trial (IMaging PAtients for Cancer Drug selecTion) for metastatic breast cancer, led by the University Medical Center Groningen, is using ^{89}Zr-DFO-trastuzumab for baseline scans to recruit patients for antibody therapy.[41] Another trial has been completed at the Jules Bordet Institute to assess ^{89}Zr-DFO-trastuzumab uptake in metastatic breast cancer,[42] and the ZEPHIR trial is still recruiting patients to increase the number of patients that may potentially benefit from T-DM1 therapy.[43] Patients are still being recruited at Memorial Sloan Kettering Cancer Center to extend the usefulness of ^{89}Zr-DFO-trastuzumab to detect metastatic disease in HER2-negative primary breast cancer.[44] The Washington University School of Medicine is recruiting patients headlining their own clinical trial to assess uptake and dosimetry of ^{89}Zr-DFO-trastuzumab in patients.[45] Overall, ^{89}Zr-DFO-trastuzumab is an extremely useful clinical tool for assessing HER2-positive breast cancer, and has proven useful as a method to detect heterogeneity within both primary and metastatic lesions. The future of this radiotracer lies in its capacity for patient selection, which is what the IMPACT trial and the Memorial Sloan Kettering Cancer Center study aim to accomplish.

Copper-64-DOTA-Trastuzumab

The transition to shorter-lived PET radioisotopes started with ^{64}Cu-labeling of trastuzumab. The first study was to determine the safety, distribution, internal dosimetry, and initial HER2-positive tumor images of ^{64}Cu-DOTA-trastuzumab in humans by Tamura and colleagues.[26] PET imaging was performed on 6 patients with primary or metastatic HER2-positive breast cancer at 1, 24, and 48 hours after injection of approximately 130 MBq of the

tracer [26]Cu-DOTA-trastuzumab. Samples were collected from the blood, urine, and nontarget tissue samples to assess the biodistribution and internal dosimetry of the probe.[26] The results from this study identified 48 hours as the best time point for ^{64}Cu-DOTA-trastuzumab after injection (Fig. 5).

An important outcome from this study was identifying that the radiation exposure during ^{64}Cu-DOTA-trastuzumab PET was comparable with that during ^{18}F-FDG PET, which is the conventional tracer for PET imaging in the clinic. Although the radioactivity in the blood was high, the uptake of ^{64}Cu-DOTA-trastuzumab in nontarget tissues was low, which is critical for improving detection. Brain metastases were observed in 2 patients that were administered ^{64}Cu-DOTA-trastuzumab PET, indicative of blood–brain barrier passage of the antibody tracer (Fig. 6). In 3 patients, ^{64}Cu-DOTA-trastuzumab PET imaging also revealed primary breast tumors in areas that were initially identified by CT. The findings of this study indicated that ^{64}Cu-DOTA-trastuzumab PET could detect HER2-positive lesions in patients with both primary and metastatic breast cancer.

Copper-64-DOTA-Trastuzumab in Metastatic Disease

Owing to successful identification of brain metastasis from HER2-positive primary breast tumors, a more focused study was done by Kurihara and colleagues[25] with ^{64}Cu-DOTA-trastuzumab PET in HER2-positive metastatic breast cancer. PET imaging was performed on 5 patients with confirmed brain metastases from HER2-positive breast cancer, at 24 or 48 hours after the injection of approximately 130 MBq of ^{64}Cu-DOTA-trastuzumab. ^{64}Cu-DOTA-trastuzumab PET visualized metasta-tic brain lesions in all 5 patients (Fig. 7). This study gave further evidence that trastuzumab may pass through the blood–brain barrier and is able to detect HER2-positive metastases. From these studies, ^{64}Cu-DOTA-trastuzumab PET could be a potential noninvasive procedure for serial identification of metastatic brain lesions in patients with HER2-positive breast cancer.[25]

In summary, ^{64}Cu-DOTA-trastuzumab has been successful in detecting both primary and metastatic disease in patients. Currently, patients are being recruited at the City of Hope Medical Center to use ^{64}Cu-DOTA-trastuzumab to predict treatment response with trastuzumab and pertuzumab before surgery in HER2-positive breast cancer.[46] The future of this tracer will be assessed by this trial and its involvement in predicting treatment response and facilitating patient selection for antibody therapy in HER2-positive breast cancer.

Indium-111-Trastuzumab

Trastuzumab functionalized as an SPECT molecular imaging probe began with the pairing of the antibody to the SPECT isotope, indium-111 (^{111}In). ^{111}In-MxDTPA-trastuzumab imaging was done by Perik and colleagues[47] to assess effects and clinical benefit of trastuzumab therapy. The ultimate result from this study was to confirm that HER2 imaging is feasible during trastuzumab treatment, and can successfully annotate response. It was concluded that the same antibody could be used for both treatment and therapy benefit in the same patient. This was further confirmed in a follow-up study also done by Gay-kema and colleagues,[48] who showed that treatment with trastuzumab in patients resulted in decreased tumor ^{111}In-MxDTPA-trastuzumab uptake of about 20%.

Wong and colleagues[49] evaluated the organ bio-distribution, pharmacokinetics, immunogenicity, and tumor uptake of ^{111}In-MxDTPA-trastuzumab in patients with HER2-overexpressing breast cancers and to determine whether ^{90}Y-trastuzumab should be evaluated in subsequent clinical therapy trials. Patients with HER2-overexpressing breast cancers that were scheduled to receive trastuzumab therapy first underwent (unlabeled) trastuzumab therapy (4–8 mg/kg), followed by 185 MBq ^{111}In-MxDTPA-trastuzumab (10 mg, 4 hours after therapy). Blood and urine samples (24 hours) were obtained for further analysis. Nuclear scans were performed at defined time points for 7 days. Eight patients received ^{111}In-MxDTPA-trastuzumab, which was well-tolerated with no adverse side effects. Three of 7 patients with known lesions showed positive tracer uptake in PET imaging. No immunogenic responses were observed for 2 months after injection, indicating the safety of the tracer.

The outcome of this study indicates that ^{90}Y-trastuzumab may be an appropriate agent to evaluate further in the clinic. No immunogenic response to ^{111}In-MxDTPA-trastuzumab was observed, and the pharmacokinetics and organ biodistribution were comparable with other ^{90}Y-labeled monoclonal antibodies. Cardiac uptake was a slight concern/difference to other radioimmunotherapy antibodies in the clinic, but was found to be similar to hepatic uptake and not expected to cause dose-limiting cardiotoxicity. HER2-targeted therapy and subsequent interrogation of these treatment effects through imaging could be a useful tool for predicting patient response. This would allow physicians to identify patients that do and do not respond to targeted therapies, opening educated options of pursuing different avenues of treatment.

The National Cancer Institute also currently sponsors a clinical trial to assess the effects of ^{111}In-CHX-A DTPA-trastuzumab imaging in HER2-positive breast cancer. Fluorescence in situ hybridization at both primary and metastatic sites of breast cancer is used to assess HER2 status. The results of these studies have yet to be published.

Indium-111-DTPA-Pertuzumab

Pertuzumab is another monoclonal antibody marketed by Genentech for treatment for HER2-positive breast cancer.[50] This therapy is often used in tandem with trastuzumab and docetaxel.[51] Pertuzumab inhibits dimerization of HER2 with other HER2 receptors, which is thought to slow tumor growth.[50] A clinical trial was attempted to recruit patients with imaging with ^{111}In-DTPA-per-tuzumab to predict response to trastuzumab in HER2-positive metastatic breast cancer, but this trial was terminated owing to poor accrual of patients.[52] [89]Zr-DFO-pertuzumab has also been explored preclinically,[53] but has not yet reached clinical trials, likely owing to the high interest and success with [89]Zr-DFO-trastuzumab.

Iodine-131-SGMID Anti-Her2 VHH1

A monoclonal antibody directed against HER2 (VHH1) labeled with iodine-131 (^{131}I) with the radio-iodinating reagent *N*-succinimidyl 4-guanidinomethyl 3-iodobenzoate (SGMIB), is also being explored in the clinic for potential imaging of HER2-positive breast cancer.[54] SGMIB improves internalization and subsequent tumor retention of radioactivity, which has

potential to improve on the current state of antibody imaging. The primary goal of the aforementioned clinical trial is to evaluate the safety, biodistribution, and dosimetry of [^{131}I]-SGMIB anti-HER2 VHH1 in both healthy volunteers and patients with HER2-positive breast cancer. Tumor targeting potential of [^{131}I]-SGMIB anti-HER2 VHH1 in patients with HER2-positive breast cancer will also be explored in this clinical study.

Copper-64-MM-302

MM-302 is an antibody–drug conjugate of HER2-targeted liposomal doxorubicin, as a single therapy or in combination with trastuzumab or trastuzumab and cyclophosphamide, and is currently being explored in clinical trials.[55,56] Initial results showed that the drug was safe and efficacious in a group of pretreated women with HER2-positive metastatic breast cancer. MM-302 labeled with ^{64}Cu is currently being used as an imaging agent for patient selection for combination therapy with trastuzumab and MM-302 in HER2-positive metastatic cancer, including breast. Patients will receive standard imaging at baseline (^{18}F-FDG PET/CT), along with MR brain imaging. Patients will then receive ^{64}Cu-MM-302 after MM-302 administration, and continue to receive subsequent doses of unlabeled MM-302 plus trastuzumab every 3 weeks and to be assessed for disease progression.[57]

ANTIBODY FRAGMENTS IN CLINICAL PET IMAGING

^{68}Ga-DOTA-F(ab′)2-trastuzumab has been developed at Memorial Sloan Kettering Cancer Center as a PET imaging reagent for assessing HER2 expression status by in vivo imaging.[58] Preclinical studies demonstrated promising results in the monitoring of treatment response to HSP90-targeted drugs that inhibit HER2.[59] Initial clinical data were collected to assess the toxicity, pharmacokinetics, biodistribution, and dosimetry profile of ^{68}Ga-DOTA-F(ab′)2-trastuzumab with PET/CT. Beylergil and colleagues[58] led this study, where 16 women with breast cancer were enrolled in this clinical trial, with 1 patient who did not receive ^{68}Ga-DOTA-F(ab′)2-trastuzumab and was excluded from analysis. HER2-negative (n = 7) and HER2-positive (n = 8) cases were studied in this initial study. In the HER2-positive group, 7 had received trastuzumab treatment previously and 1 had not. It was determined that ^{68}Ga-DOTA-F(ab′)2-trastuzumab was well-tolerated, and tumor targeting was seen in 4 of the 8 patients with HER2-positive disease. Serial imaging of a single patient is represented in Fig. 8. ^{68}Ga-DOTA-F(ab′)2-trastuzumab was also successful in detecting metastatic disease (Figs. 9 and 10). It was hypothesized that high circulating levels of trastuzumab inhibiting with tumor targeting by ^{68}Ga-DOTA-F(ab′)2-trastuzumab, and could explain only 50% of targeting of known (^{18}F-FDG confirmed) lesions.

CLINICAL PET IMAGING WITH RADIOLABELED AFFIBODIES

Indium-111-ABY-025 and Gallium-68-ABY-025

Affibody molecules are small affinity ligands that are designed to mimic antibody-binding properties.[60] Affibodies are capable of binding to a wide range of protein targets and are used for diagnostic imaging and therapy applications. The first clinical studies using radiolabeled affibodies were performed by Baum and colleagues,[23] with ^{111}In- or ^{68}Ga-labeled ABY-002 for imaging HER2-expressing malignant breast tumors. Three patients

with metastatic breast cancer and known lesions (as identified by CT and/or ^{18}F-FDG PET/ CT) received approximately 80 to 90 mg of radiola-beled ABY-002. Two patients received both ^{111}In-ABY-002 and ^{68}Ga-ABY-002, and 1 patient received ^{68}Ga-ABY-002 only. Rapid blood clearance of radiolabeled ABY-002 was observed and allowed for quality images with both SPECT and PET radionuclides as quickly as 2 hours after injection. Radiolabeled ABY-002 was detected in metastatic lesions, along with background uptake in the kidneys and the liver. The majority of identified lesions (via ^{18}F-FDG PET) were successfully observed using ABY-002 imaging. However, 2 sites of metastatic lesions (near the kidney and liver) were positive on ^{18}F-FDG PET/CT scans, but not observed with ABY-002 imaging, owing to high background accumulation in these organs.[61] Therefore, these particular tracers were not pursued further, but the affibodies were modified to improve background radioactivity and clearance of the ligand. The benefit of affibodies in molecular imaging is the fast pharmacokinetics and overall turnaround, but further clinical studies are required to optimize the dose, time, sensitivity, and specificity of these ligands.

Another version of affibody molecule for HER2-targeted imaging (ABY-025) was developed by Sörensen and colleagues[22] ABY-025 is a reengineered affibody molecule that targets an exclusive epitope of the HER2 receptor that is not targeted by other HER2-targeted therapeutics. The advance of ABY-025 (vs ABY-002) lies in the improved tumor/ background ratios, allowing for successful detection metastatic disease (most importantly in the liver) to be observed. A preliminary study evaluated the biodistribution, safety, dosimetry, and tumor-targeting potential of ^{111}In-ABY-025 for assessing HER2 status in metastatic breast cancer. Seven patients with metastatic breast cancer and HER2-positive or negative primary tumors received approximately 100 mg (approximately 140 MBq) of ^{111}In-ABY-025. SPECT/CT imaging was performed after 4, 24, and 48 hours. Blood samples were taken to measure radioactivity, antibodies, serum HER2, and toxicity markers. HER2 status was verified by biopsies, and the metastases were detected by ^{18}F-FDG PET/CT 5 days before ^{111}In-ABY-025 imaging.[22]

^{111}In-ABY-025 produced high-contrast SPECT images within 4 to 24 hours. No anti-ABY-025 antibodies were observed ex vivo, indicating a non-immunogenic radiotracer, which is optimal for clinical translation. Background tissue uptake was highest in the kidneys, then the liver and spleen. ^{111}In-ABY-025 shows promise as a noninvasive tool for detecting HER2 in metastatic breast cancer, even during the occurrence of HER2-targeted antibody treatment.

Sandström and colleagues[21] produced a ^{68}Ga-labeled ABY-025 for diagnosis of HER2-positive breast cancer tumors with PET. The aim of the preliminary study with ^{68}Ga-ABY-025 was to measure the biodistribution and estimate the radiation dosimetry of ^{68}Ga-ABY-025 for 2 different peptide mass doses in the same group of patients (Fig. 11). Eight patients with metastatic breast cancer were included in this study. Each patient received a 45-minute dynamic (abdominal) and 3 whole-body PET/CT scans at 1, 2, and 4 hours after injection of a low peptide dose and a high peptide dose. The highest background uptake was observed in the kidneys and liver at all imaging time points.

In a phase I/II study, ^{68}Ga-ABY-025 to study effect of peptide mass and the effects on quantified uptake in tumors to pathologic analysis.[24] Sixteen women with metastatic breast cancer (confirmed by ^{18}F-FDG PET/CT) and receiving treatment were included. PET imaging was performed at 1, 2, and 4 hours after injection of 212 ± 46 MBq ^{68}Ga-ABY-025 (Fig. 12 for example).

In the cohort of patients (10 total, 6 with HER2-positive, and 4 with HER2-negative primary tumors), ^{68}Ga-ABY-025 PET/CT with 2 different doses of injected peptide was performed. The optimal time point for ^{68}Ga-ABY-025 PET was found to be 4 hours, and accurately quantifies whole-body HER2-receptor status in metastatic breast cancer, and showed a change in uptake among patients receiving targeted treatment in 3 of the 16 patients. Patients with widespread metastatic disease were used as an example of ^{18}F-FDG versus ^{68}Ga-ABY-025 PET (Fig. 13) to gauge the ability of the radiotracer to detect distant disease.

CLINICAL PET IMAGING WITH RADIOLABELED NANOBODIES

Gallium-68-Human Epidermal Growth Factor Receptor 2-Nanobody

Nanobodies directed against HER2 have also been developed as probes for molecular imaging. Nanobodies are produced from a heavy chain component of antibodies, are the smallest antigen-binding antibody fragments, and have ideal characteristics for PET imaging.[61]

Initial studies by Keyaerts and colleagues[62] assessed the safety, biodistribution, and dosimetry of a ^{68}Ga-HER2-Nanobody. In this study, 20 women with primary or metastatic breast carcinoma were injected with ^{68}Ga-HER2-Nanobody (average dose, 107 MBq) and dosimetry calculations were based from images obtained at 10, 60, and 90 minutes after tracer administration. Representative images of this study are shown in Fig. 14.

The tumor-targeting potential was assessed in primary and metastatic lesions. No adverse reactions to the tracer were recorded, and only 10% of injected dose remaining in the blood at 1 hour after injection. Background uptake was observed in the kidneys, liver, and intestines. Primary tumors were more variable in tracer accumulation (Fig. 15), whereas metastatic lesions showed specific, easily delineated tracer accumulation (Fig. 16).[62]

Overall, ^{68}Ga-HER2-Nanobody PET/CT is a well-tolerated procedure with a comparable radiation dose to other routinely used PET tracers. The biodistribution of this radiotracer is favorable, with the highest uptake in the kidneys, liver, and intestines but low background levels in all other organs, including where primary or metastatic disease is most often found. Tracer accumulation in HER2-positive metastases is high, can be easily distinguished from background tissues, and warrants further assessment in a phase II trial.

18F-Human Epidermal Growth Factor Receptor 2-Nanobody

Xavier and colleagues[63] have recently labeled the GMP-produced HER2-nanobody with ^{18}F with and shown success with tumor targeting preclinically. Specific uptake for HER2-positive xenografts ($5.94 \pm 1.17\%$ IA/g, 1 hour after injection) was observed with high tumor-to-tissue ratios. [^{18}F]-anti-HER2-nanobody showed rapid clearance through the

kidneys (4% IA/g at 3 hours post-injection). The probe was also able to image HER2-positive tumors when coadministered with trastuzumab, indicating the potential use of this tracer for patient selection and monitor patients undergoing therapy. With the success of this ^{18}F-labeled probe and the positive results from phase I/II ^{68}Ga-HER2-nanobody trials, this probe should enter the clinic with ease.

SUMMARY

HER2 receptor status is an important biomarker in patients with breast cancer, integral to treatment decisions and prognosis. HER2-targeted therapies have demonstrated clear survival advantages in patients with HER2-positive breast carcinoma. The possibility of heterogeneity of HER2 expression both within primary tumors and between primary and metastatic disease sites may limit the value of individual tumor biopsies and demonstrates a need for accurate, whole body assessment of HER2 status. Molecular imaging, most importantly PET, has been identified as a noninvasive tool to assess HER2-positive primary lesions and metastases. Radiolabeled antibodies, antibody fragments, and affibody molecules enable PET imaging to be a reliable and quantitative method for detecting HER2-positive cancer. HER2-targeted PET imaging has the potential to become a clinically valuable tool in the care of patients with breast cancer.

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KEY POINTS

- Knowledge of the receptor status of breast cancer is integral to patient treatment and prognosis.

- Expression of estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 (HER2) is assessed using immunohistochemistry, fluorescence in situ hybridization, or chromogen in situ hybridization.

- HER2 is expressed in low levels on the surface of normal cells but overexpressed in invasive breast cancers, a biomarker for aggressive breast cancer and response to HER2-targeted therapies.

- Monoclonal antibodies are used as HER2-targeted therapies, which can be labeled with positron-, gamma-emitting radionuclides for PET/computed tomography and single-photon emission computed tomography molecular imaging agents.

- PET imaging allows for noninvasive whole body analysis of tumors and may detect previously unsuspected HER2-positive metastases in patients with a HER2-negative primary breast tumor.
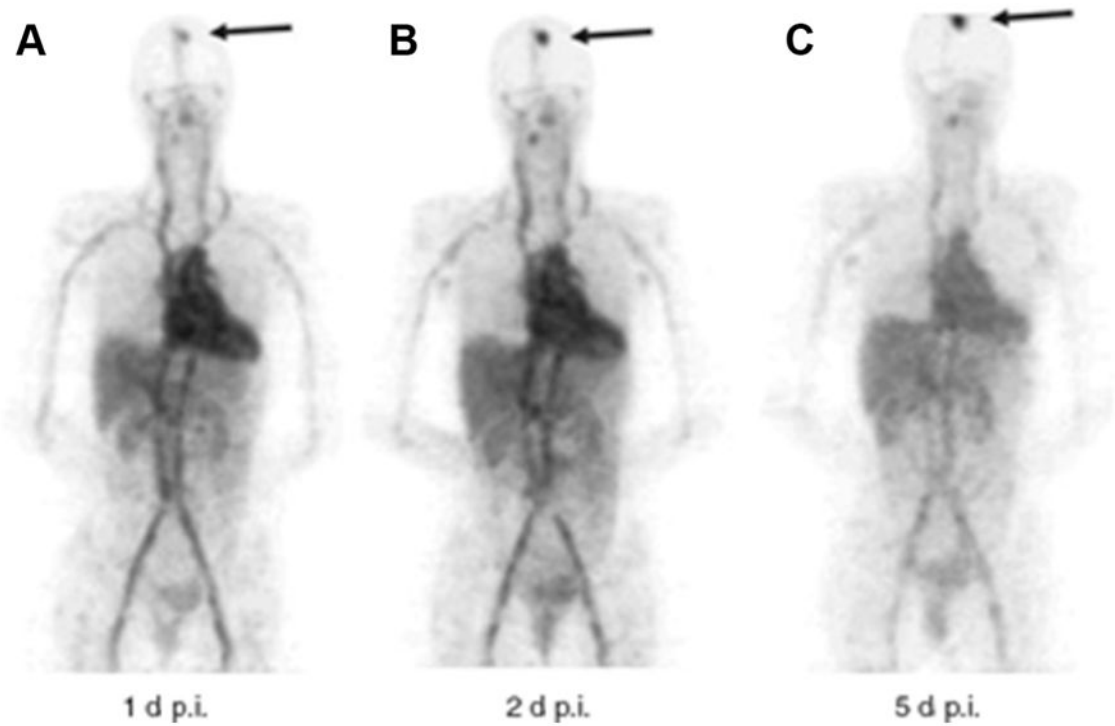
Fig. 1.
[89]Zr-trastuzumab biodistribution in time. (*A–C*) Three [89]Zr-trastuzumab scans of a patient already on tras-tuzumab treatment (cohort 3) show the increase over time in the tumor/nontumor ratio with regard to uptake of the tracer. Arrow indicates [89]Zr-trastuzumab uptake in the only lesion. p.i., postinjection; [89]Zr, zirconium-89. (*From* Dijkers EC, Oude Munnink TH, Kosterink JG, et al. Biodistribution of 89Zr-trastuzumab and PET imaging of HER2-positive lesions in patients with metastatic breast cancer. Clin Pharmacol Ther 2010;87(5): 588; with permission.)
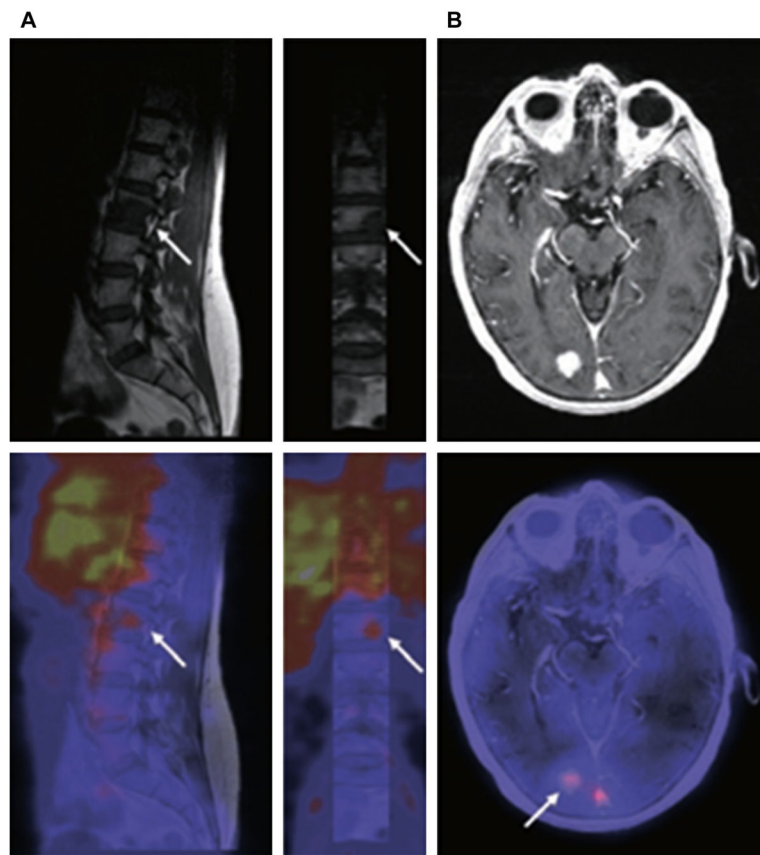
Fig. 2.

Examples of fusion images from human epidermal growth factor receptor 2 (HER2) PET and MR imaging scans. (*A*) In a vertebral metastasis seen on MR imaging but unapproachable for biopsy, HER2 status was revealed by [89]Zr-trastuzumab uptake on PET imaging. (*B*) Example of HER2-positive brain lesion undetected by conventional scans, revealed by [89]Zr-trastuzumab PET imaging, and subsequently confirmed by MR imaging. Arrows indicate lesions. HER2, human epidermal growth factor receptor 2; [89]Zr, zirconium-89. (*From* Dijkers EC, Oude Munnink TH, Kosterink JG, et al. Biodistribution of 89Zr-trastuzumab and PET imaging of HER2-positive lesions in patients with metastatic breast cancer. Clin Pharmacol Ther 2010;87(5):589; with permission.)
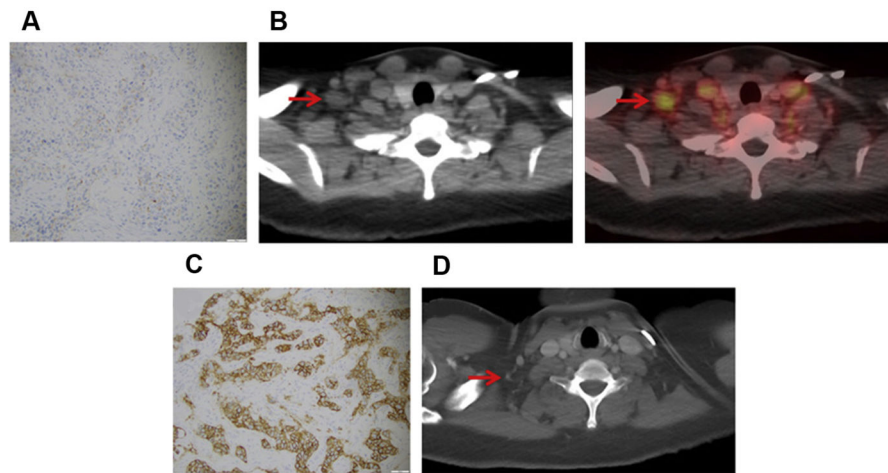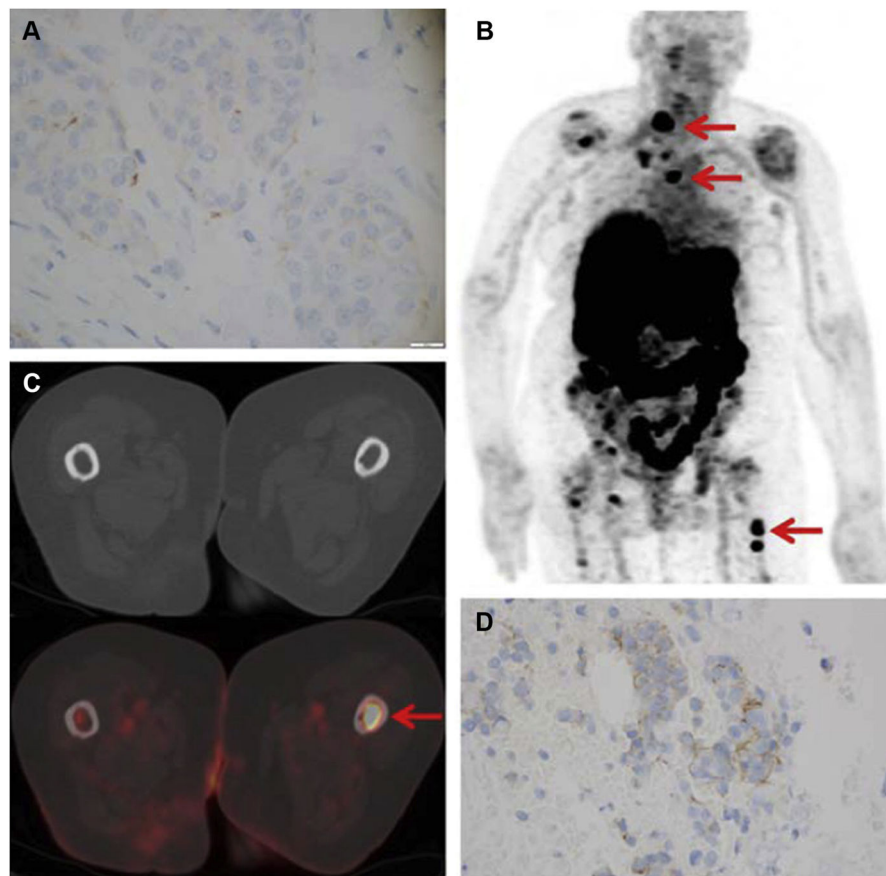
Fig. 3.

A 41-year-old woman with primary ER-positive, human epidermal growth factor receptor 2 (HER2)-negative invasive ductal breast carcinoma and recurrence in the thoracic nodes. (*A*) Immunohistochemistry score of primary breast malignancy was 11 (at $20 \times$ magnification), consistent with HER2-negative malignancy. (*B*) Axial computed tomography (CT) and ^{89}Zr-trastuzumab PET/CT demonstrated ^{89}Zr-trastuzumab avidity in enlarged right supraclavicular nodes (*arrows*, maximum standardized uptake value of 4.6) and left internal mammary nodes (not shown). (*C*) Biopsy of right supraclavicular node demonstrated metastatic breast carcinoma with immunohistochemistry score of 31 (at $20 \times$ magnification), consistent with HER2-positive disease. The patient began systemic treatment including trastuzumab and pertuzumab. (*D*) Follow-up axial CT after 2 months of treatment demonstrated resolution of nodes on CT examination (*arrow*). (*From* Ulaner GA, Hyman DM, Ross DS, et al. Detection of HER2-positive metastases in patients with HER2-negative primary breast cancer using 89Zr-trastuzumab PET/CT. J Nuc Med 2016;57(10):1526; with permission.)

Fig. 4.
An 83-year-old woman with primary ER-positive/human epidermal growth factor receptor 2 (HER2)-negative invasive ductal breast carcinoma. (*A*) Immunohistochemistry score of primary breast malignancy was 11 (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 (*arrows*). Avidity in liver and bowel is considered physiologic. (*C*) Axial computed tomography (CT) and ^{89}Zr-trastuzumab PET/CT demonstrate ^{89}Zr-trastuzumab avidity in proximal left femur (*arrow*; maximum standardized uptake value of 7.7). (*D*) Biopsy of proximal left femur demonstrated metastatic breast carcinoma with immunohistochemistry score of 11 (at 400 × magnification), consistent with HER2-negative disease. (*From* Ulaner GA, Hyman DM, Ross DS, et al. Detection of HER2-positive metastases in patients with HER2-negative primary breast cancer using 89Zr-trastuzumab PET/CT. J Nuc Med 2016;57(10):1527; with permission.)
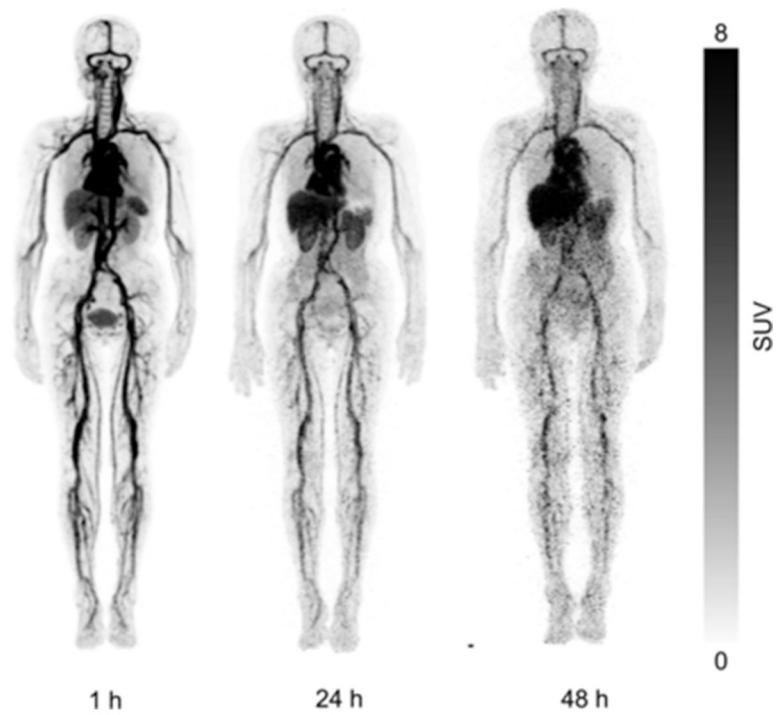
Fig. 5.
Whole-body copper-64-DOTA-trastuzumab PET images at 1, 24, and 48 hours after injection (patient 4). (*From* Tamura K, Kurihara H, Yonemori K, et al. 64Cu-DOTA-trastuzumab PET imaging in patients with HER2-positive breast cancer. J Nucl Med 2013;54(11):1871; with permission.)
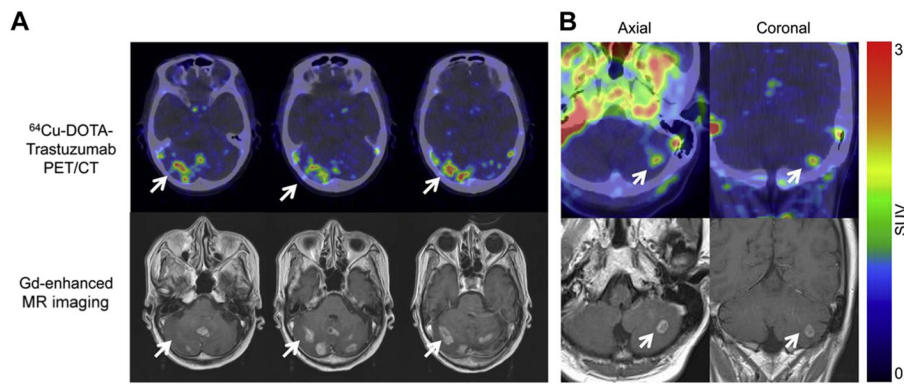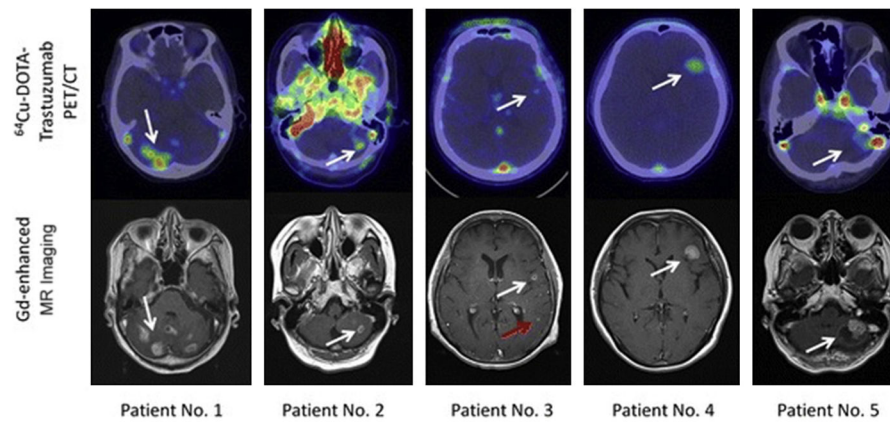
Fig. 6.
Copper-64 (^{64}Cu)-DOTA-trastuzumab PET images of human epidermal growth factor receptor 2 (HER2)-positive metastatic brain lesions (*arrows*). (*A*) Brain metastases were clearly visualized by ^{64}Cu-DOTA-trastuzumab PET in patient 1. Significant uptake values were found in areas corresponding to brain metastatic lesions that were detected by MR imaging. Some brain metastases could not be detected on conventional CT. (*B*) In patient 4, ^{64}Cu-DOTA-trastuzumab PET imaging could detect solitary brain metastasis that had also been identified in similar location by MR imaging. Gd, gadolinium. (*From* Tamura K, Kurihara H, Yonemori K, et al. 64Cu-DOTA-trastuzumab PET imaging in patients with HER2-positive breast cancer. J Nucl Med 2013;54(11):1874; with permission.)

Fig. 7.
Copper-64 (^{64}Cu)-DOTA-trastuzumab PET images of metastatic brain tumors in patients with human epidermal growth factor receptor 2 (HER2)-positive primary breast tumors. The white arrows show the metastatic brain tumors. Upper panels: ^{64}Cu-DOTA-trastuzumab PET images. Lower panels: gadolinium (Gd)-DTPA-enhanced T1-weighted MR imaging images. White arrows indicate metastatic brain lesions detectable by both MR imaging and ^{64}Cu-DOTA-trastuzumab PET, and the red arrow indicates a lesion detectable by MR imaging but not by ^{64}Cu-DOTA-trastuzumab PET. In the PET image from patient 2, nonspecific high uptake in the blood was noted. (*From* Kurihara H, Hamada A, Yoshida M, et al. 64Cu-DOTA-trastuzumab PET imaging and HER2 specificity of brain metastases in HER2-positive breast cancer patients. EJNMMI Res 2015;5(8):4; with permission.)
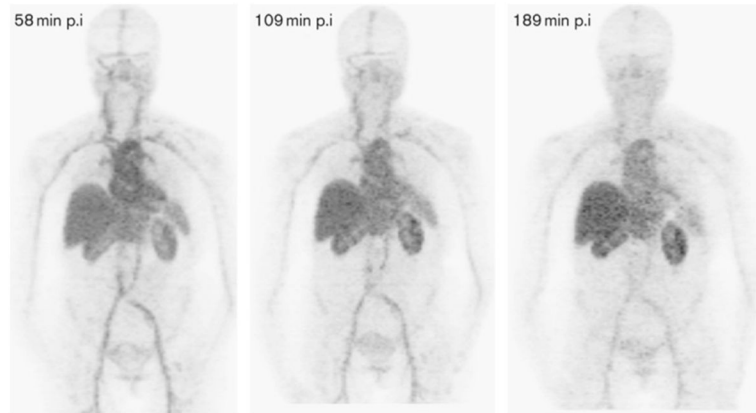
Fig. 8.
Serial MIP images (patient 6) displayed at the same relative intensity. Although a slight decrease in blood pool is present, blood pool activity dominates the distribution. At 58, 109, and 189 minutes, the blood pool has a maximum standardized uptake value of 16.9, 13.6, and 9.8, respectively. There is slight increased uptake over time in the liver and kidneys. The patient had human epidermal growth factor 2 (HER2)-positive disease and was receiving lapatinib. Tumors in the left adrenal, hilar nodes, and bones were not visualized on Ga-PET. HER2, human epidermal growth factor receptor 2; MIP, maximum intensity projection; p.i, postinjection. (*From* Beyergil V, Morris PG, Smith-Jones PM, et al. Pilot study of 68Ga-DOTA-F(abʹ)2-trastuzumab in patients with breast cancer. Nucl Med Commun 2013;34(12): 1162; with permission.)

Fig. 9.
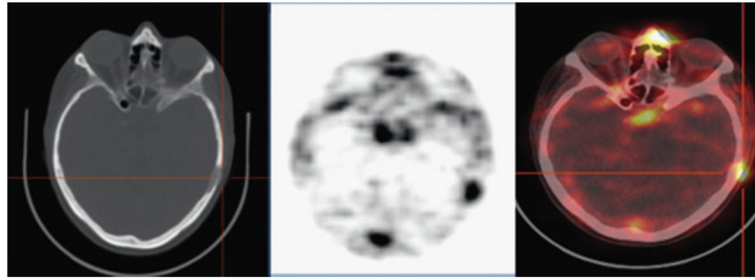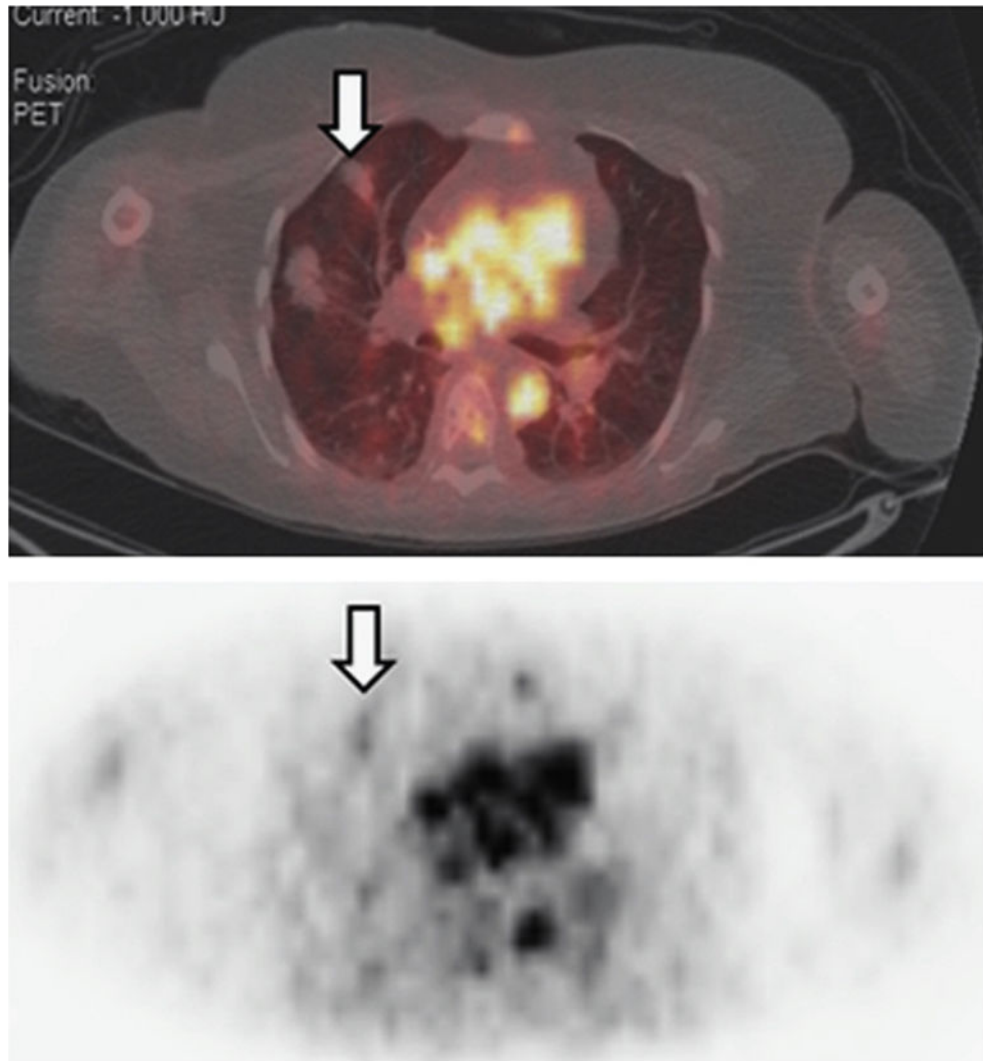Lytic lesion in the left calvarium with gallium-68 (^{68}Ga)-DOTA-F(ab′)2-trastuzumab uptake (patient 1). ^{68}Ga-DOTA-F(ab′)2-trastuzumab, ^{68}Ga-1,4,7,10-tetraazacyclododecane- N,N ′,N′,N‴-tetraacetic acid (DOTA)-F(ab′) 2-trastuzumab. (*From* Beyergil V, Morris PG, Smith-Jones PM, et al. Pilot study of 68Ga-DOTA-F(ab′)2-trastuzumab in patients with breast cancer. Nucl Med Commun 2013;34(12):1163; with permission.)

Fig. 10.
In patient 2, fused images obtained at B1 hours show mild ^{68}Ga-DOTAF(ab′)2-trastuzumab uptake in a lung metastasis (*arrows*). ^{68}Ga-DOTA-F(ab′)2- trastuzumab, ^{68}Ga-1,4,7,10-tetraazacyclododecane-N,N′,N′,N‴-tetraacetic acid (DOTA)-F(ab″)2-trastuzumab. (*From* Beyergil V, Morris PG, Smith-Jones PM, et al. Pilot study of 68Ga-DOTA-F(ab′)2-trastuzumab in patients with breast cancer. Nucl Med Commun 2013; 34(12):1163; with permission.)
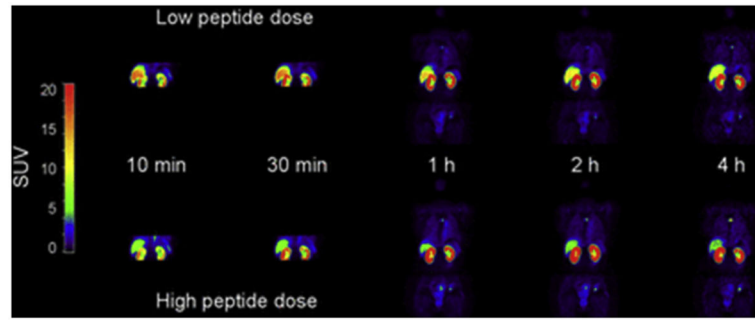
Fig. 11.
Representative whole-body images at 10, 30, 60, 120, and 240 minutes after injection of a low and high peptide dose in the same patient. (*From* Sandström M, Lindskog K, Velikyan I, et al. Bio-distribution and radiation dosimetry of the anti-HER2 affibody molecule 68Ga-ABY-025 in breast cancer patients. J Nuc Med 2016; 57(6):868; with permission.)
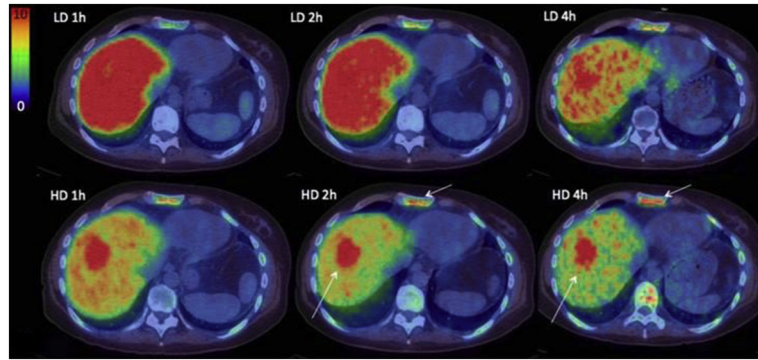
Fig. 12.
Uptake images of gallium-68 (^{68}Ga)-BY-025 with low (LD) and high administered peptide dose (HD) at 1, 2 and 4 hours after injection in patient 2. All images are normalized to the same scale of standardized uptake value 10. A rainbow color scale is used: red color, standardized uptake value of greater than 10. Normal soft tissue uptake was higher with LD. Note the gradually higher contrast of metastases in liver and bone (*arrows*), owing to both the disappearance of normal liver uptake and tumor uptake accumulation. (*From* Sörensen J, Velikyan I, Sandberg D, et al. Measuring HER2-receptor expression in metastatic breast cancer using [68Ga]ABY-025 affibody PET/CT. Theranostics 2016;6(2):266; with permission.)
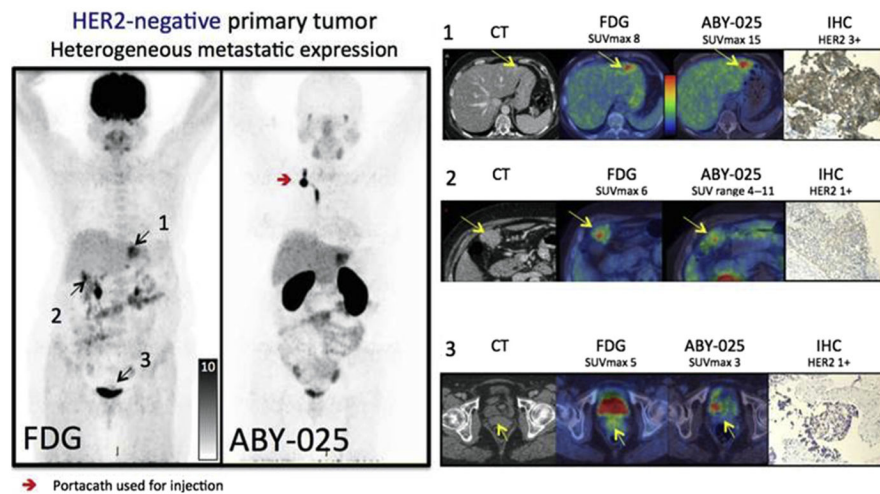
Fig. 13.

Based on the results from gallium-68-ABY-025 PET/computed tomography (CT), mixed expression of human epidermal growth factor receptor 2 (HER2) in metastatic breast cancer was seen in several patients (*arrows*) and confirmed by biopsies in 2. Patient 9 had HER2-negative primary tumor and was enrolled as negative control. Fluorodeoxyglucose (FDG)-PET/CT showed metastases in left liver lobe, peritoneal lymph nodes and cervix of uterus (*arrows*). ABY-025 uptake was high in the liver metastasis, low in peritoneal metastases and absent in the cervical region (not shown). According to immunohistochemistry (IHC), the liver finding was true positive and both other sites were true negative. SUV_{max}, maximum standardized uptake volume. (*From* Sörensen J, Velikyan I, Sandberg D, et al. Measuring HER2-receptor expression in metastatic breast cancer using [68Ga] ABY-025 affibody PET/CT. Theranostics 2016;6(2):268; with permission.)
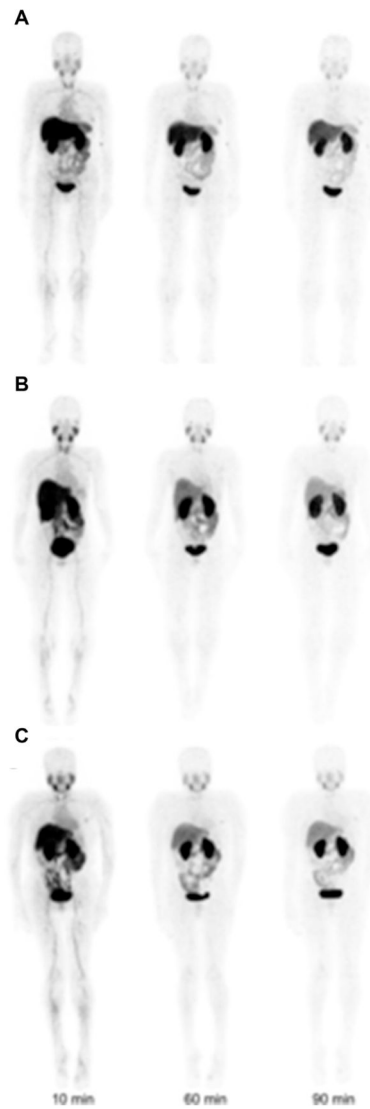
A

B

C

10 min   60 min   90 min

Fig. 14.
Representative maximum intensity projection images at 10, 60, and 90 minutes after injection of gallium-68 (^{68}Ga)-human epidermal growth factor receptor 2 (HER2)-nanobody for different mass subgroups. (*A*) Patient 4, injected with 0.01 mg of ^{68}Ga-HER2-nanobody. (*B*) Patient 12, injected with 0.1 mg. (*C*) Patient 17, injected with 1.0 mg. (*From* Keyaerts M, Xavier C, Heemskerk J, et al. Phase I study of 68Ga-HER2-nanobody for PET/CT assessment of HER2 expression in breast carcinoma. J Nuc Med 2016;57(1):30; with permission.)
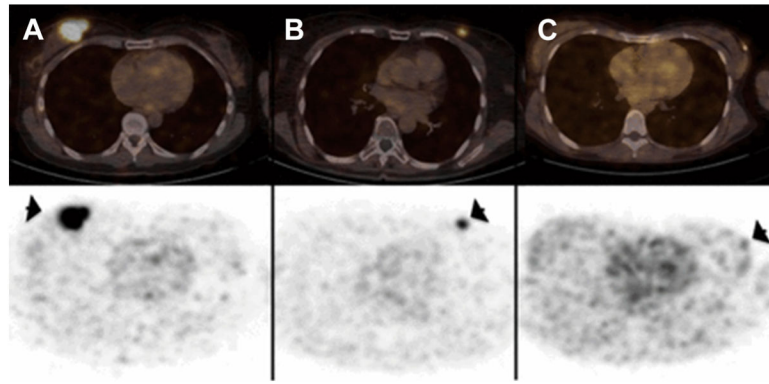
Fig. 15.
Uptake of gallium-68 (^{68}Ga)-human epidermal growth factor receptor 2-nanobody in primary breast carcinoma lesions (*arrows*) on PET/computed tomography (CT) images (*top*) and PET images (*bottom*). (*A*) Patient 14 showed highest tracer uptake (mean standardized uptake value, 11.8). (*B*) Patient 15 showed moderate tracer uptake, which was easily discernable from background (mean standardized uptake value, 4.9). (*C*) Patient 6 showed no uptake (mean standardized uptake value, 0.9), with CT showing marker clip at tumor region. (*From* Keyaerts M, Xavier C, Heemskerk J, et al. Phase I study of 68Ga-HER2-nanobody for PET/CT assessment of HER2 expression in breast carcinoma. J Nuc Med 2016;57(1):31; with permission.)
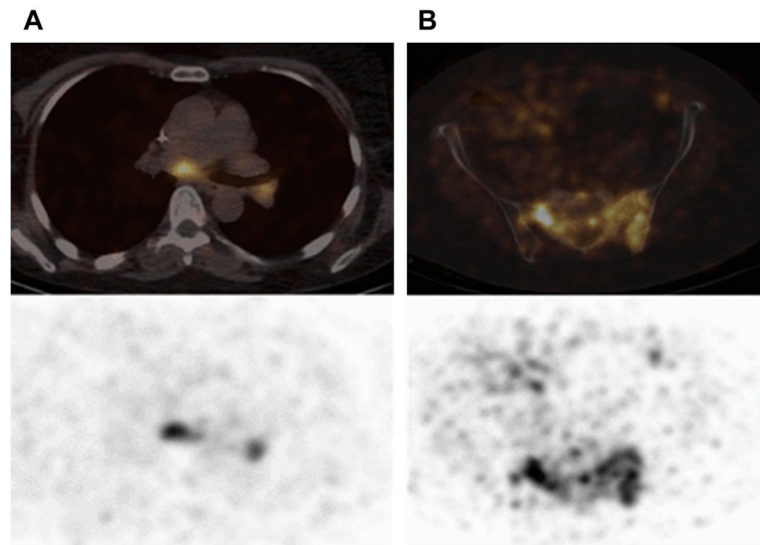
Fig. 16.
Uptake of gallium-68 (^{68}Ga)-human epidermal growth factor receptor 2-nanobody in metastatic lesions on PET/computed tomography images (*top*) and PET images (*bottom*). (*A*) Patient 18, with invaded lymph nodes in mediastinum and left hilar region. (*B*) Patient 20, with bone metastasis in pelvis. (*From* Keyaerts M, Xavier C, Heemskerk J, et al. Phase I study of 68Ga-HER2-nanobody for PET/CT assessment of HER2 expression in breast carcinoma. J Nuc Med 2016;57(1):32; with permission.)

Table 1

Summary of clinical trials with PET and SPECT radiotracers targeting HER2-positive breast cancer

Radiotracer	Description of Trial	Clinical Trial or Reference	Sponsor	Status
[89]Zr-DFO-trastuzumab	HER2+ BC	NCT01832051	University Medical Center Groningen	Completed
	Imaging the effect of HSP90 inhibition	NCT01081600	University Medical Center Groningen	Completed
	HER2+ MBC	NCT01420146	Jules Bordet Institute	Completed
	HER2+ BC	NCT02065609	Washington University School of Medicine	Recruiting
	HER2+ MBC	NCT02286843	Memorial Sloan Kettering Cancer Center	Recruiting
	IMPACT-MBC	NCT01957332	University Medical Center Groningen	Recruiting
[64]Cu-DOTA-trastuzumab	HER2+ MBC	NCT00605397	Memorial Sloan Kettering Cancer Center	Completed
	Predicting treatment response in HER2+ BC	NCT02827877	City of Hope Medical Center	Verified
^{111}In-CHX-A DTPA- trastuzumab	HER2+ BC	NCT01445054	National Cancer Institute	Completed
[111]In-MxDTPA- trastuzumab	HER2+ BC	Wong et al,[49] 2010	City of Hope Medical Center	Completed
	HER2+ MBC	Perik et al,[47] 2006	University Medical Center Groningen	Completed
	HER2+ MBC	Gaykema et al,[48] 2014	University Medical Center Groningen	Completed
[68]Ga-F(ab′)2- trastuzumab	HER2+ BC	NCT00613847	Memorial Sloan Kettering Cancer Center	Completed
^{68}Ga-ABY-025	HER2+ BC	NCT01858116	Biomedical Radiation Sciences	Completed
	HER2+ BC	NCT02095210	Dorte Nielsen	Recruiting
^{111}In-ABY-025	HER2+ MBC	NCT01216033	Biomedical Radiation Sciences	Completed
[64]Cu-MM-302	HER2+ MBC	NCT02735798	University of California, San Francisco	Verified
[^{131}I]-SGMIB anti- HER2 VHH1	Healthy Patients and HER2+ BC	NCT02683083	Camel-IDS NV	Recruiting
^{68}Ga-HER2-Nanobody	HER2+ BC	EudraCT 2012- 001135-31	Vrije Universiteit Brussels	Completed

Abbreviations: BC, breast cancer; HER2, human epidermal growth factor 2; IMPACT, IMaging PAtients for Cancer Drug selecTion; MBC, metastatic breast cancer; SGMIB, N-succinimidyl 4-guanidinomethyl 3-iodobenzoate; SPECT, single photon emission computed tomography.

Table 2

Drugs approved for anti-HER2 therapy in HER2-positive breast cancer

Drug (Chemical Name)	Classification	Mechanism of Action	Company	FDA Approved
Trastuzumab	Antibody	Binds to extracellular segment of the HER2/neu receptor	Genentech	1998
Pertuzumab	Antibody	Inhibits the dimerization of HER2 with other HER receptors	Genentech	2012
Ado-trastuzumab emtansine	Antibody–drug conjugate	Binds at plus ends of cellular microtubules and inhibits cell division in tumor cells	Genentech	2013
Lapatinib	Tyrosine kinase inhibitor	Interrupts HER2/neu and EGFR pathway	GlaxoSmithKline	2007

Abbreviations: EGFR, epidermal growth factor receptor; FDA, Food and Drug Administration; HER2, human epidermal growth factor 2.