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TITLE: ^{64}Cu -DOTA-Trastuzumab PET Imaging in Women with HER2-Overexpressing Breast Cancer

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14. ABSTRACT Objectives. Patients with metastatic breast cancer identified as human epidermal growth factor receptor 2 (HER2)-positive (HER2+) routinely receive the anti-HER2 antibody trastuzumab plus chemotherapy. ⁶⁴ Cu-DOTA-trastuzumab/PET-CT has high tumor detection sensitivity in HER2+ patients [Bading, et al., JNM 2012;53 (Suppl 1):229P]. We are also imaging HER2- patients and present an initial comparison of tumor uptake between the two groups. Methods. Patients with biopsy-proven HER2+ (n=9) or HER2- (n=4) metastatic breast cancer who had not received anti-HER2 therapy for ≥ 4 mo were given trastuzumab i.v. (45 mg, to reduce liver uptake) prior to i.v. injection of ⁶⁴ Cu-DOTA-trastuzumab (364-512 MBq). PET-CT scans were obtained at 21-25 h (Day 1; 3-4 bed positions, 20 or 15 min each) and 47-49 h (Day 2; 1-2 bed positions, 60 or 30 min each) over fields of view based on recent (5-17 d prior) 18F-FDG scans. Tumor uptake was measured as body weight-normalized SUV [maximum voxel (SUVmax) and whole-tumor (SUVwt)]. Results. For Day 1, mean SUVmax was 77% higher in lesions from HER2+ vs HER2- patients [(n, mean, median, sd, range) = (57, 7.8, 6.6, 4.3, 2.4-22.5) and (25, 4.4, 4.1, 1.1, 3.0-6.8), respectively; p<1x10-6 by 2-tailed t test on log-transformed data]. Day 2 and SUVwt results were qualitatively the same as Day 1 SUVmax. Using median Day 1 SUVmax for a given lesion site and patient (pt), analysis for the two most common sites showed: bone [HER2+ (6 pts) 91% > HER2- (3 pts), p<0.05] and lymph nodes [HER2+ (8 pts) 54% > HER2- (3 pts), p<0.05]. Conclusions. Tumor uptake of ⁶⁴ Cu-DOTA-trastuzumab was higher on average in HER2+ vs HER2- patients, with significant intra-group and intra-patient variability. Our observations suggest that, by 1 d post-injection, uptake reflects binding of the radiotracer to HER2. Intra-group and intra-patient heterogeneity may have been due to variations in tumor HER2 expression, and/or other factors (blood clearance, blood-tumor transport, etc.) that influence uptake of ⁶⁴ Cu-DOTA-trastuzumab.					
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Table of Contents

	<u>Page</u>
1. Introduction.....	3
2. Keywords	4
3. Overall Project Summary.....	4
4. Key Research Accomplishments.....	6
5. Conclusion.....	6
6. Publications, Abstracts, and Presentations.....	6
7. Inventions, Patents and Licenses.....	6
8. Reportable Outcomes.....	6
9. Other Achievements.....	7
10. References.....	7
11. Appendices.....	7

1. INTRODUCTION:

The term “breast cancer” encompasses a number of different diseases that are clinically defined by hormone receptor status and *HER2* expression. *HER2* has tyrosine kinase activity that results in intracellular signaling and activation of genes for cell growth and survival. Women whose cancers overexpress the *HER2* protein have a distinct natural history and are candidates for treatment with trastuzumab. Trastuzumab is a humanized IgG-1 antibody that binds to the ectodomain of *HER2*. When combined with chemotherapy, trastuzumab significantly improves the survival of women with both early stage and advanced disease. The pathologic assessment of *HER2* status is made on the primary tumor or metastatic foci. Generally this is small sampling of a portion of one tumor and many not reflect the entire tumor or other sites of disease. *HER2* overexpression and candidacy for trastuzumab is defined as 3+ staining by immunohistochemistry (IHC) or gene amplification by fluorescence in situ hybridization (FISH).¹ In the randomized trials of *HER2*-directed therapy, a consistent group of women enrolled were actually *HER2* negative on central pathology review.² In both the adjuvant and metastatic settings, those who receive *HER2* directed therapy did better. This suggests that *HER2*-directed therapy should be expanded to include some women with *HER2* negative breast cancer.

The impact of *HER2* directed therapies on disease outcome is considerable. However, the cost, potential risk of cardiac toxicity, and inconvenience of intravenous administration make it imperative that an accurate assessment of *HER2* be made in all women with breast cancer. We hypothesize that a functional assessment of *HER2* will be more accurate than pathologic assessment.

Positron emission tomography (PET) provides a non-invasive way of studying tumor location, metabolic functions, and response to therapy. In breast cancer, PET imaging is used to stage women with advanced disease and to assess response to chemotherapy and endocrine therapy before tumor regression can be documented on clinical or radiological exam³⁻⁵. The ability to identify patients who may benefit from systemic therapy is critically important to the quality of life of patient; toxicities from ineffective therapies are prevented and medical costs are contained.

Radiolabeled trastuzumab has effectively been used to study *HER2* positive breast cancers in animal models and in humans, in part because trastuzumab has a high rate of internalization, which results in trapping of radiometals within the cell. ⁶⁴Cu-DOTA-trastuzumab, first developed at City of Hope, accurately identified *HER2* positive breast cancer in nude mice bearing an MCF7 *HER2* overexpressing xenografts.⁶⁻⁹ Others have been unable to demonstrate the utility of trastuzumab-based PET imaging. Because normal tissues that express *HER2* will bind the radiolabeled (hot) antibody, administration of a “cold dose” of antibody might improve the tumor specificity of the radiographic image. None of the studies that have utilized radiolabeled trastuzumab as an imaging agent have consistently administered a large “cold dose” of trastuzumab prior to radiographic imaging. We hypothesize that the dose of “cold antibody” is critical for image quality. We believe that too high a dose of “cold antibody” will reduce tumor uptake and thereby compromise the quality of the radiographic image.

The primary objective of this pilot study is to determine the optimal dose of cold antibody required for ⁶⁴Cu-DOTA-trastuzumab PET to produce a high image quality in women with metastatic *HER2* positive breast cancer. In addition, we will assess the impact of the

cold antibody dose on cardiac uptake of ^{64}Cu -DOTA-trastuzumab. To establish the optimal dose of cold antibody, we will image women with documented metastatic breast cancer with 3 different protein (trastuzumab) doses - 5 mg, 50 mg, and 4 mg/kg.

Once we have established the optimal dose of “cold antibody” the secondary objective was to correlate tumor uptake of ^{64}Cu -DOTA-trastuzumab PET with the degree of immunopositivity of *HER2* in women with metastatic disease. Women with metastatic breast cancer that is 1+, 2+, or 3+ positive by IHC assessment of *HER2* will undergo a ^{64}Cu -DOTA-trastuzumab PET imaging. We will correlate the standardized uptake values (SUVs) of tumor identified on ^{64}Cu -DOTA-trastuzumab PET with the degree of positivity of *HER2* by FISH.

We believe that functional imaging with ^{64}Cu -DOTA-trastuzumab PET has the potential to improve the accuracy of *HER2* assessment *in vivo*. Future studies will address tumor biomarker assessment with serial ^{64}Cu -DOTA-trastuzumab PET and [^{18}F]FDG/PET before and after treatment with trastuzumab to determine which patients are likely to benefit from the drug.

2. KEYWORDS: Provide a brief list of keywords (limit to 20 words).

^{64}Cu -DOTA-trastuzumab PET
Functional imaging
HER2 positive

3. OVERALL PROJECT SUMMARY:

Aim 1: Determine the dose of pre-administered cold antibody that optimizes image quality of ^{64}Cu -DOTA-trastuzumab PET without increasing the radiation dose to the heart in women with metastatic *HER2* positive breast cancer.

Study 1: Eight women underwent ^{64}Cu -DOTA-trastuzumab PET and ^{18}F -FDG/PET before and after treatment with trastuzumab. Initially we planned to image 15 women who would receive 3 different pre-administered doses of trastuzumab. The two women who received the lowest dose (5 mg) had poor image quality compared to those receiving 50 mg. Communications with investigators who preadministered larger doses (4 mg/kg) also had poor image quality. We anticipated that the higher dose may have resulted in binding to the tumor receptors precluded uptake of the radiolabel. Therefore we discontinued this study with 8 patients accrued

Findings as a result of Study 1:

- 1.) ^{64}Cu -DOTA-trastuzumab PET is feasible
- 2.) We identified no significant cardiac uptake on ^{64}Cu -DOTA-trastuzumab PET
- 3.) We found a high degree of concordance between ^{18}F -FDG PET and ^{64}Cu -DOTA-trastuzumab PET.
- 4.) A preadministered dose of 45 mg cold trastuzumab resulted in a 75% decrease in uptake of ^{64}Cu and improved image quality. This became the standard for subsequent ^{64}Cu -DOTA-trastuzumab PET imaging.

Aim 2: Determine whether tumor uptake on ^{64}Cu -DOTA-trastuzumab PET correlates with tumor expression of *HER2* in women with metastatic disease.

Study 2: Ten women whose *HER2* status was 1+, 2+, or 3+ underwent ^{64}Cu -DOTA-trastuzumab PET and ^{18}F -FDG/PET before and after treatment with trastuzumab. We

Findings as a result of Study 2:

1.) ^{64}Cu -DOTA-trastuzumab PET uptake was observed in patients whose tumors were considered by pathologic assessment to be HER2 negative.

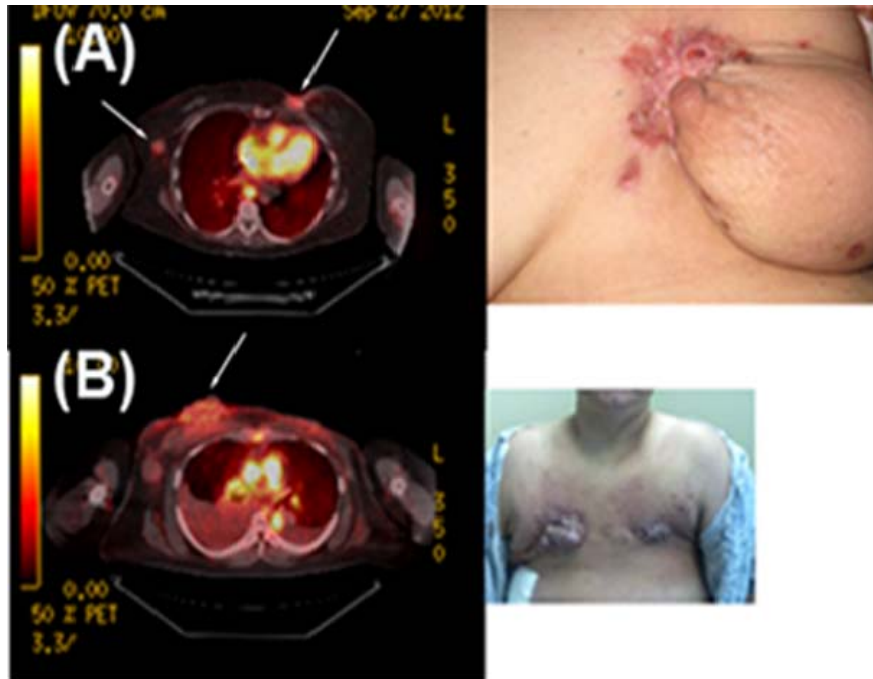
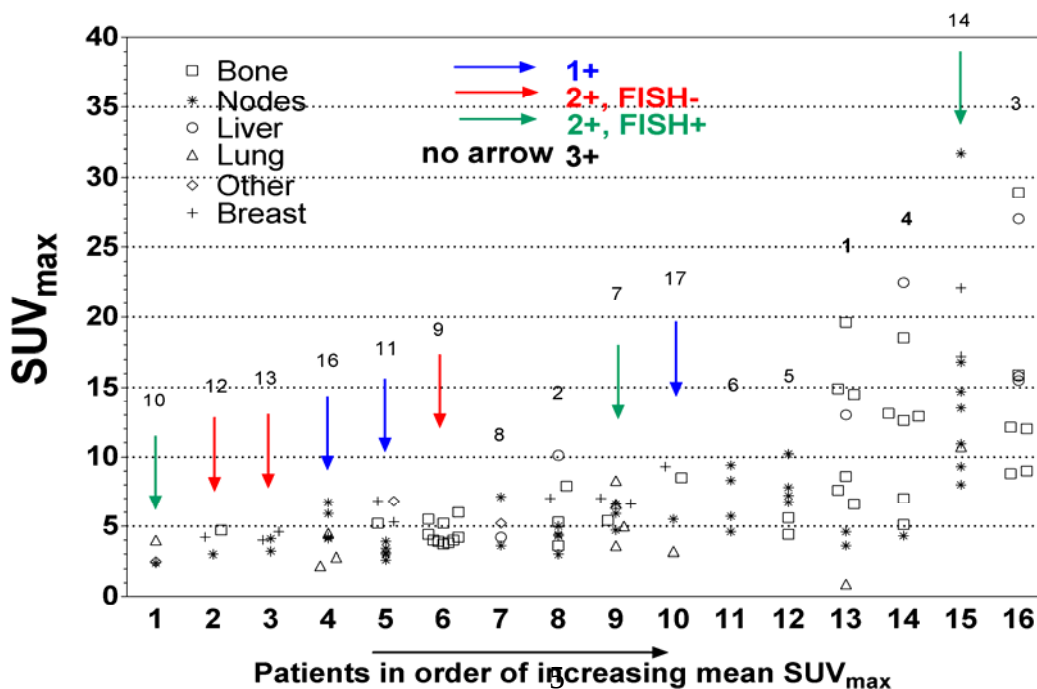


Figure 1: ^{64}Cu -DOTA-trastuzumab uptake in *HER2* negative patients. Arrows indicate ^{64}Cu -positive lesions in (A) the left breast and right axilla of a patient with *HER2* 2+/FISH- disease, and (B) the right mastectomy bed of a patient with *HER2* 1+ disease.

2.) ^{64}Cu -DOTA-trastuzumab PET uptake was directly related to the degree of HER2 positivity by IHC assessment. (Figure 2)



4. KEY RESEARCH ACCOMPLISHMENTS:

In this research grant we were able to demonstrate that:

- a. ^{64}Cu -DOTA trastuzumab PET/CT imaging is feasible.
- b. The pre-administration of trastuzumab 50 mg resulted in a 75% decrease in hepatic uptake of ^{64}Cu and resulted in improved image quality
- c. In women with metastatic HER2 positive breast cancer ^{64}Cu -DOTA trastuzumab PET/CT imaging was comparable to FDG-PET/CT in the identification of metastatic disease.
- d. In women with metastatic HER2 negative breast cancers, the uptake of ^{64}Cu -DOTA trastuzumab on PET/CT was less intense than for women with HER2 positive disease.

5. CONCLUSION: We were able to demonstrate the feasibility of using ^{64}Cu labeled trastuzumab for PET imaging. We were able to improve the quality of the PET images by pre-administering 45 mg of trastuzumab. Even HER2 negative breast cancers demonstrate uptake of ^{64}Cu -DOTA-trastuzumab PET.

6. PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS:

- a. Our first manuscript entitled "" is *In Press* in the Journal of Nuclear Medicine and will be published in the January 2014 issue. The second manuscript is in preparation.
- b. The data were presented at the following meetings as poster presentations:
 - a. ASCO May 2012 and 2013
 - b. San Antonio Breast Cancer Symposium 2012 and 2013
 - c. Society of Nuclear Medicine 2012 and 2013.
 - d. Radiologic Society of North America 2012
- c. Data were presented at two institutional Cancer Center Retreats for the Phase I Research meeting 2013.

7. INVENTIONS, PATENTS AND LICENSES: NA

8. REPORTABLE OUTCOMES:

Other investigators have tested the use of either Indium-111 or Copper-64 labeled trastuzumab. Prior clinical studies have generally included patients who were already on treatment with trastuzumab and the determination of HER2 status was based on the status of the primary cancer. Participants in our trial underwent biopsy of a metastatic focus prior to imaging and patients could not have received a HER2-directed therapy for more than 60 days. Therefore, we have knowledge of the HER2 status immediately prior to imaging and the co-administration of therapeutic trastuzumab did not confound our results. We were able to demonstrate that a small dose of pre-administered trastuzumab improved the image quality. We also demonstrated that the uptake of ^{64}Cu -DOTA-trastuzumab on PET imaging correlated with the intensity of HER2 staining by immunohistochemistry. ^{64}Cu -DOTA-trastuzumab PET uptake was observed in women whose cancers were considered HER2 negative by current pathologic criteria. Given this interesting observation, we hypothesize that ^{64}Cu -DOTA-trastuzumab PET uptake may help to identify women who might benefit from the therapeutic immunoconjugate, T-DM1, including women with HER2 negative breast cancers.

9. OTHER ACHIEVEMENTS: Nothing to report.

10. REFERENCES:

1. Wolff AC, Hammond MEH, Schwartz JN, et al. American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer. *J Clin Oncol*. January 1, 2007 2007;25(1):118-145.
2. Perez EA, Reinholz MM, Hillman DW, et al. HER2 and Chromosome 17 Effect on Patient Outcome in the N9831 Adjuvant Trastuzumab Trial. *Journal of Clinical Oncology*. October 1, 2010 2010;28(28):4307-4315.
3. Juweid ME, Cheson BD. Positron-Emission Tomography and Assessment of Cancer Therapy. *N Engl J Med*. February 2, 2006 2006;354(5):496-507.
4. Mortimer JE, Dehdashti F, Siegel BA, Trinkaus K, Katzenellenbogen JA, Welch MJ. Metabolic Flare: Indicator of Hormone Responsiveness in Advanced Breast Cancer. *J Clin Oncol*. June 1, 2001 2001;19(11):2797-2803.
5. Dedashti F MJ, Trinkaus K, Naughton MJ, Ellis M. Katzenellenbogen JA, Welch MJ, Siegel BA. . PET-based estradiol challenge as a predictive biomarker of response to endocrine therapy in women with estrogen receptor positive breast cancer. *Breast Cancer Res Treat*. 2008 Mar 9. [Epub ahead of print]. 2008.
6. 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):586-592.
7. Perik PJ, Lub-De Hooge MN, Gietema JA, et al. Indium-111-Labeled Trastuzumab Scintigraphy in Patients With Human Epidermal Growth Factor Receptor 2-Positive Metastatic Breast Cancer. *J Clin Oncol*. May 20, 2006 2006;24(15):2276-2282.
8. Williams L, Somlo G, Zhan J, et al. A pilot imaging trial of 111In-Herceptin in metastatic breast cancer patients receiving cold Herceptin therapy. *12th Conference on Cancer Therapy with Antibodies and Immunoconjugates*. 2008.
9. Wong JYC, Raubitschek A, Yamauchi D, et al. A Pretherapy Biodistribution and Dosimetry Study of Indium-111-Radiolabeled Trastuzumab in Patients with Human Epidermal Growth Factor Receptor 2-Overexpressing Breast Cancer *Cancer Biotherapy and Radiopharmaceuticals*. 2010;25(4):387-394.

11. APPENDICES

Functional Imaging of Human Epidermal Growth Factor Receptor 2–Positive Metastatic Breast Cancer Using ^{64}Cu -DOTA-Trastuzumab PET

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Women with human epidermal growth factor receptor 2 (HER2)–positive breast cancer are candidates for treatment with the anti-HER2 antibody trastuzumab. Assessment of HER2 status in recurrent disease is usually made by core needle biopsy of a single lesion, which may not represent the larger tumor mass or other sites of disease. Our long-range goal is to develop PET of radiolabeled trastuzumab for systemically assessing tumor HER2 expression and identifying appropriate use of anti-HER2 therapies. The purpose of this study was to evaluate PET/CT of ^{64}Cu -DOTA-trastuzumab for detecting and measuring tumor uptake of trastuzumab in patients with HER2-positive metastatic breast cancer. **Methods:** Eight women with biopsy-confirmed HER2-positive metastatic breast cancer and no anti-HER2 therapy for 4 mo or longer underwent complete staging, including ^{18}F -FDG/PET/CT. For 6 of the 8 patients, ^{64}Cu -DOTA-trastuzumab injection (364–512 MBq, 5 mg of trastuzumab) was preceded by trastuzumab infusion (45 mg). PET/CT (PET scan duration 1 h) was performed 21–25 (day 1) and 47–49 (day 2) h after ^{64}Cu -DOTA-trastuzumab injection. Scan fields of view were chosen on the basis of ^{18}F -FDG/PET/CT. Tumor detection sensitivity and uptake analysis were limited to lesions identifiable on CT; lesions visualized relative to adjacent tissue on PET were considered PET-positive. Radiolabel uptake in prominent lesions was measured as maximum single-voxel standardized uptake value (SUV_{max}). **Results:** Liver uptake of ^{64}Cu was reduced approximately 75% with the 45-mg trastuzumab predose, without significant effect on tumor uptake. The study included 89 CT-positive lesions; detection sensitivity was 77%, 89%, and 93% for day 1, day 2, and ^{18}F -FDG, respectively. On average, tumor uptake was similar for ^{64}Cu -DOTA-trastuzumab and ^{18}F -FDG (mean SUV_{max} and range were 8.1 and 3.0–22.5, respectively, for day 1 [$n = 48$]; 8.9 and 0.9–28.9, respectively, for day 2 [$n = 38$]; and 9.7 and 3.3–25.4, respectively, for ^{18}F -FDG [$n = 56$]), but same-lesion SUV_{max} was not correlated between the 2 radiotracers. No toxicities were observed, and estimated radiation dose from ^{64}Cu -DOTA-trastuzumab was similar to ^{18}F -FDG. **Conclusion:** ^{64}Cu -DOTA-trastuzumab visualizes HER2-positive metastatic breast cancer with high sensitivity and is effective in surveying disseminated disease. A 45-mg trastuzumab predose provides a ^{64}Cu -DOTA-trastuzumab biodistribution favorable for tumor imaging. ^{64}Cu -DOTA-trastuzumab

PET/CT warrants further evaluation for assessing tumor HER2 expression and individualizing treatments that include trastuzumab.

Key Words: ^{64}Cu -labeled trastuzumab; HER2; breast cancer

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Overexpression of human epidermal growth factor receptor 2 (HER2) is identified in 20% of breast cancers (1). Women with HER2-positive breast cancer are candidates for treatment with the humanized anti-HER2 antibody trastuzumab. When combined with chemotherapy, trastuzumab increases overall survival for all stages of HER2-positive breast cancer.

Trastuzumab is used in newly diagnosed HER2-positive breast cancer as adjuvant or neoadjuvant therapy and as treatment for metastatic disease at presentation or relapse if more than 6 mo since adjuvant trastuzumab. Response rates are at best about 25% and 50%, respectively, for first-line trastuzumab and trastuzumab plus chemotherapy (2). Furthermore, in the adjuvant setting, patients classified as HER2-negative sometimes benefit (3). There is a clear need to better identify those patients who may benefit from these costly and potentially toxic therapies.

The accurate and comprehensive assessment of tumor HER2 status is critical in determining treatment. However, the pathologic assessment of HER2 status suffers from interlaboratory discordance and lack of a clear definition of positivity (4). Furthermore, confirmation of recurrent disease is usually made by core needle biopsy of an accessible lesion and may not represent the larger tumor mass or other sites of disease. Differences in HER2 expression between primary and metastatic tumors have been observed in as many as 20% of patients, especially when metastasis occurs after adjuvant or neoadjuvant therapy (5–7).

We hypothesized that assessment of tumor HER2 status can be improved by supplementing pathologic evaluation with functional imaging of HER2. Radiolabeled trastuzumab has been used to image patients with HER2-positive breast cancer, initially with ^{111}In and single-photon imaging (8,9) and more recently with ^{89}Zr and PET (10). Although labeling with the positron-emitting isotope ^{124}I is also a possibility, radiometals are preferred given the known cellular internalization of trastuzumab and subsequent rapid efflux of radiolabel from cells when trastuzumab is labeled with

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isotopes of iodine (11). Tumor visualization has been variable, perhaps because the women were on active trastuzumab treatment, which may have inhibited radiolabeled trastuzumab binding to HER2.

The positron-emitting isotope ^{64}Cu is regularly available from Washington University, St. Louis, and we have extensive experience labeling antibodies with radiometals via the chelating agent DOTA (12). Although its half-life (12.8 h) is short relative to the blood clearance of trastuzumab, ^{64}Cu has potential advantages over ^{89}Zr in terms of radiation safety and patient radiation dose. The critical issue, addressed in this study, is whether tumor uptake of ^{64}Cu -DOTA-trastuzumab is sufficiently rapid to support tumor imaging and quantification within the 48-h time window afforded by ^{64}Cu . On the basis of our previous clinical study with ^{111}In -MxDTA-trastuzumab (9) and promising results in athymic mice bearing HER2-expressing human breast adenocarcinoma xenografts (13), we have obtained an investigational new drug (IND) application for ^{64}Cu -DOTA-trastuzumab.

The primary objective of this pilot study was to evaluate the feasibility and potential utility of CT-supplemented PET scanning of ^{64}Cu -DOTA-trastuzumab (^{64}Cu -DOTA-trastuzumab PET/CT) for lesion detection and uptake measurement in HER2-positive metastatic breast cancer. Similar to other antibodies (14), liver uptake of intravenously administered trastuzumab is strongly dependent on antibody protein load. Thus, we sought to identify a trastuzumab dose that minimizes liver uptake of ^{64}Cu -DOTA-trastuzumab. Additional goals were to compare ^{64}Cu -DOTA-trastuzumab with the standard PET radiotracer, ^{18}F -FDG, and to confirm the safety of the ^{64}Cu -DOTA-trastuzumab PET/CT procedure.

MATERIALS AND METHODS

Patient Selection

Women with metastatic HER2-positive breast cancer who had not received HER2-directed therapy for 4 mo or more were considered for study participation after undergoing a staging workup that included echocardiogram, bone scanning, and whole-body ^{18}F -FDG/PET/CT. All candidates underwent biopsy of a metastatic lesion within 28 d before the ^{64}Cu -DOTA-trastuzumab procedure to confirm recurrent, HER2-positive disease by immunohistochemical staining or fluorescence in situ hybridization. Assessable disease outside the primary breast site, ipsilateral axillary region, and biopsy site was also required. The study protocol was approved by the City of Hope Institutional Review Board and Radiation Safety Committee, and an IND was accepted by the Food and Drug Administration. All patients signed a written informed consent.

^{64}Cu -DOTA-Trastuzumab Preparation

Trastuzumab is a recombinant humanized antibody that binds with high affinity to the extracellular domain of the HER2 protein. Radiolabeled trastuzumab was prepared according to procedures defined in IND #109971. The antibody (Herceptin, purchased from Genentech) was conjugated with the active ester of DOTA (Macrocyclics) under current good manufacturing-compliant conditions. ^{64}Cu (half-life, 12.8 h; 0.18 positrons/decay) was provided by the Mallinckrodt Institute of Radiology, Washington University School of Medicine. DOTA-conjugated antibody was incubated with ^{64}Cu for 45 min at 43°C, chased with 1 mM diethylenetriamine pentaacetic acid (DTPA), and purified on a size-exclusion, preparative column (Superdex-200). Radiolabeling efficiency was more than 93%. Appropriate fractions were pooled, filtered, and formulated with 1% human serum albumin for patient administration. The ^{64}Cu -DOTA-trastuzumab preparations were sterile, with endotoxin levels less than 0.05 EU/mL and immunoreactivity greater than 86%. The DOTA-trastuzumab protein dose per ^{64}Cu -DOTA-trastuzumab injection was approximately 5 mg.

Administration of Trastuzumab and

^{64}Cu -DOTA-Trastuzumab

Patients were closely monitored for acute adverse reactions during trastuzumab administrations. ^{64}Cu -DOTA-trastuzumab (364–512 MBq; mean, 450 MBq) was infused intravenously in 25 mL of saline over 10 min. Dijkers et al. found that, compared with 10 mg, 50 mg of trastuzumab substantially reduced blood clearance and liver uptake of ^{89}Zr -trastuzumab in trastuzumab-naïve patients (10). To match the trastuzumab dose found effective in that study, patients receiving non-radiolabeled trastuzumab were infused intravenously with the antibody (45 mg in 50 mL of saline given over 15 min) immediately before radioactive injection.

The first 4 patients in our study were randomly assigned to receive trastuzumab doses of 5 or 50 mg. When ^{64}Cu -DOTA-trastuzumab PET/CT of those patients confirmed the findings of Dijkers et al., we adopted the 50-mg dose for the remainder of the study.

PET/CT Imaging

Imaging was performed with a Discovery STe 16 PET/CT scanner (GE Healthcare) operated in 3-dimensional mode (septa retracted). The PET axial field of view is 15.4 cm (image slice thickness, 3.3 mm). PET images were reconstructed using an iterative, ordered-subsets expectation maximization algorithm with gaussian postsMOOTHing and standard corrections for nonuniform detector sensitivity, scanner dead time, random, and scattered coincidence events. Correction for photon attenuation was based on coregistered CT scans acquired during the same examination. The measured spatial resolution of the PET images was approximately 9 mm in full width at half maximum.

Patients underwent a standard ^{18}F -FDG/PET/CT examination 13 d or fewer before the ^{64}Cu -DOTA-trastuzumab procedure. Patients fasted 6 h or more before injection of ^{18}F -FDG. Serum glucose concentration measured at time of examination was high (184 mg/dL) for one patient and normal (<120 mg/dL) for the others.

Injected ^{64}Cu activity was limited to 555 MBq (15 mCi), based on radiation dose estimates calculated from the pharmacokinetics of ^{111}In -MxDTA-trastuzumab (9). One hour was chosen as a reasonable limit for PET scan duration. Within those constraints, disease location as judged from the preceding ^{18}F -FDG PET/CT examination was used in choosing the axial coverage for the ^{64}Cu -DOTA-trastuzumab PET/CT scans. The first (day 1) ^{64}Cu scan was performed 21–25 h after injection to allow radiolabeled antibody accumulation in tumor. A second (day 2) scan was obtained 47–49 h after injection. Day 1 scans comprised of 3 or 4 (39 or 51 cm axial extent, 20 or 15 min per bed position) and day 2 scans comprised of 1 or 2 (15 or 27 cm axial extent, 60 or 30 min per bed position) contiguous bed positions, respectively, depending on patient body thickness. Signal-to-noise characteristics of the ^{64}Cu -DOTA-trastuzumab images approximated those of the ^{18}F -FDG scans (Fig. 1).

[Fig. 1]

Image Analysis

PET/CT examinations were interpreted by a nuclear medicine boarded radiologist. Tumor detection sensitivity and uptake analysis were limited to lesions identifiable on CT; lesions visualized relative to adjacent tissue on PET were considered PET-positive. PET-positive lesions were disregarded if CT was judged inconclusive. PET-positive findings with ^{64}Cu -DOTA-trastuzumab but not ^{18}F -FDG, and having no correlated CT lesion, were scored as false-positives. Because of possible ^{18}F -FDG or nonspecific antibody uptake secondary to biopsy, biopsied tumor sites were not included in the analysis. A detailed description of the lesion detection analysis is given in the supplemental material (available only online at <http://jnm.snmjournals.org>).

Radiolabel uptake in as many as 10 of the most prominent lesions per patient, as well as selected nontumor tissues and organs, was measured in terms of standardized uptake value ($\text{SUV} = \text{tissue activity}$

[AQ6]

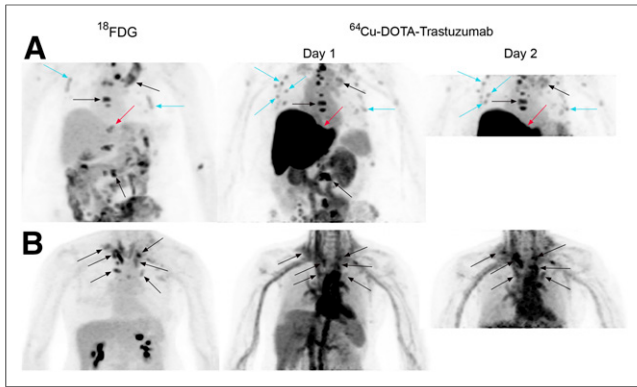


FIGURE 1. Visualization of HER2-positive metastatic breast cancer by PET of ^{64}Cu -DOTA-trastuzumab. Images are maximum-intensity projections with upper intensity thresholds corresponding to $\text{SUV} = 10 \text{ g/mL}$. (A) PET scans of ^{18}F -FDG, ^{64}Cu -DOTA-trastuzumab 23 h after injection (day 1) and ^{64}Cu -DOTA-trastuzumab 48 h after injection (day 2) in a patient (patient A) who received trastuzumab dose of 5 mg. Black arrows point out several of many corresponding CT-positive bone lesions seen both with ^{18}F -FDG and with ^{64}Cu -DOTA-trastuzumab, whereas turquoise arrows denote a few of many instances of ^{64}Cu -DOTA-trastuzumab, or ^{64}Cu -DOTA-trastuzumab and ^{18}F -FDG, focal uptake in rib regions too small to be evaluated on associated CT. Red arrows indicate intrahepatic lesion seen with ^{18}F -FDG but obscured by intense liver uptake in ^{64}Cu -DOTA-trastuzumab scans. (B) ^{18}F -FDG, ^{64}Cu -DOTA-trastuzumab 24 h after injection and ^{64}Cu -DOTA-trastuzumab 48 h after injection scans of patient (patient B) given trastuzumab dose of 50 mg. Arrows denote several lymph nodes visualized in both ^{18}F -FDG and ^{64}Cu -DOTA-trastuzumab scans. Liver uptake of ^{64}Cu was much lower for patient B than for patient A (compare day 1 images).

per $\text{cm}^3 \times \text{body weight [g]}/\text{injected activity decay-corrected to time of scan}$. Tumor uptake was parameterized as single-voxel maximum SUV (SUV_{max}) and background-adaptive whole-tumor average SUV (15). We found whole-tumor SUV to be closely and linearly correlated with SUV_{max} ($r^2 \geq 0.97$, $P < 0.001$). Therefore, tumor uptake results are presented only in terms of SUV_{max} .

Uptake analysis for blood, liver, spleen, kidney, and heart wall consisted of averaging the mean SUVs of circular or elliptical regions of interest of fixed size placed well within the tissue's or organ's PET image boundaries on 3 contiguous image slices. Blood measurements were obtained from PET images of the cardiac ventricles; heart wall was visualized as a region of relatively low uptake adjacent to the ventricles.

Pharmacokinetic Analysis and Radiation Dose Estimates

^{64}Cu activity concentration was measured in peripheral venous samples acquired 0–1, 23–24, and 47–48 h after injection from patients who received a 50-mg trastuzumab dose. Radiation dose estimates for these patients were obtained by combining blood activity and organ uptake measurements from the current study with blood and organ time–activity measurements (0–168 h) from our previous clinical study with ^{111}In -MxDTA-trastuzumab (9). Details of the radiation dose calculations are given in the supplemental material.

Human Anti-Trastuzumab Antibody Response

Serum samples obtained just before trastuzumab/ ^{64}Cu -DOTA-trastuzumab infusion and 1, 3, and 6 mo later, when possible, were evaluated for immune responses using a size-exclusion high-performance liquid chromatography shift assay. Samples (125 μL) were incubated with radiolabeled DOTA-trastuzumab (^{111}In , 9 $\mu\text{Ci}/\mu\text{g}$, 0.1 μCi) and then run on a Superose-6 size-exclusion column at 0.4 mL/min in phosphate-buffered saline/0.05% NaN_3 . A change in the elution pattern of the radiolabeled trastuzumab consistent with higher molecular weight was considered positive for an anti-antibody response.

Statistical Analysis

Statistical analysis was performed using R software (version 2.12.1, [AQ9] The R Foundation of Statistical Computing). Lesion detection sensitivities were compared by a 2-sided Fisher exact test. Comparison of tumor uptake between trastuzumab doses and among lesion sites used ANOVA to evaluate both dose/lesion site and patient effects, with Holm's method to adjust for multiple comparisons. Linear regression analysis was used to demonstrate correlation between whole-tumor SUV and SUV_{max} and lack of correlation between ^{18}F -FDG and ^{64}Cu -DOTA-trastuzumab. The effect of trastuzumab dose on organ uptake was evaluated by Wilcoxon rank-sum test. P values of less than 0.05 were considered statistically significant.

RESULTS

Patient Characteristics

Eight of 10 women considered for study participation met the eligibility criteria. Biopsies of 2 patients previously treated for early stage HER2-positive breast cancer showed recurrent disease to be HER2-negative. Participating patients are characterized in Table 1. [Table 1]

Lesion Detection Sensitivity of ^{64}Cu -DOTA-Trastuzumab PET/CT

Figure 1 illustrates ^{64}Cu -DOTA-trastuzumab image quality and tumor visualization, compared with ^{18}F -FDG. Tumor-to-nontumor contrast for ^{64}Cu -DOTA-trastuzumab was generally high (Fig. 1,

TABLE 1
Patient Demographics and Clinical Characteristics

Characteristic	Trastuzumab protein dose (mg)		All patients
	5	50	
No. of patients	2	6	8
Age (y)			
Median	60	54	56
Range	44–75	39–69	39–75
Prior anti-HER2 therapy			
None		1	1
Adjuvant trastuzumab	1	2	3 (14, 18, 18)*
Trastuzumab for metastasis	1	3	4 (4, 6, 14, 18)*
Hormone receptor and HER2 status of recurrent disease			
ER or PR positive	1	3	4
ER and PR negative	1	3	4
HER2			
IHC3+	2	5	7
IHC2+/FISH positive		1	1
Sites of metastatic disease			
Bone	2	4	6
Lymph nodes	2	5	7
Liver	2	2	4
Lung	1	1	2
Pleural effusion		1	1
Breast		2	2

*Months since last anti-HER2 therapy administration

ER = estrogen receptor; PR = progesterone receptor; IHC = immunohistochemistry; FISH = fluorescence in situ hybridization.

patient A). Exceptions occurred for lymph nodes in the cervical, clavicular, and mediastinal regions due to high blood-pool activity (Fig. 1, patient B) and in the liver for the 5-mg trastuzumab dose (Fig. 1, patient A). Visualization of lymph nodes in regions of high blood activity improved between day 1 and day 2 but changed little for other lesion sites between the 2 scans (Fig. 2).

[Fig. 2] Lesion detection statistics are summarized in Table 2. Overall detection sensitivity with ^{18}F -FDG PET/CT (93%) was consistent with general experience in metastatic breast cancer (16). All 8 patients had CT-positive lesions that were detected with ^{64}Cu -DOTA-trastuzumab PET. There were no statistically significant differences in ^{64}Cu lesion detection sensitivity between 5- and 50-mg trastuzumab doses (data not shown). On day 1, ^{64}Cu detection sensitivity was lower for lymph nodes than for bone lesions. Overall, detection sensitivity for ^{64}Cu -DOTA-trastuzumab on day 1 was lower than for ^{18}F -FDG, with the difference being due primarily to the low sensitivity of lymph nodes in regions of high blood activity. There were 7 instances in which a CT-positive lesion was detected with ^{18}F -FDG but not ^{64}Cu -DOTA-trastuzumab on either day 1 or day 2. In 6 instances (3 bone, 2 liver, and 1 node), a CT-positive lesion was detected with ^{64}Cu -DOTA-trastuzumab but not with ^{18}F -FDG. ^{18}F FDG false-negative bone and liver lesions are illustrated in Figures 2 and 3B, respectively.

There was only 1 instance of a false-positive ^{64}Cu -DOTA-trastuzumab lesion, which occurred in the colon and may have been associated with diverticulitis. In 1 patient with numerous bone metastases, ^{64}Cu -DOTA-trastuzumab, or both ^{64}Cu -DOTA-trastuzumab and ^{18}F -FDG, produced hot spots in rib regions too small to be assessed on associated CT (Fig. 1, patient A).

Effects of Trastuzumab Protein Dose

Blood clearance was slowed, and liver uptake of ^{64}Cu -DOTA-trastuzumab was markedly decreased in patients preinfused with trastuzumab (45 mg) (supplemental material, Fig. 1). However, with only 2 patients at the lower protein dose, SUV differences between the 50- and 5-mg trastuzumab doses were not statistically significant ($P = 0.10$ and 0.05 on days 1 and 2 for blood; $P = 0.10$ and 0.07 on days 1 and 2 for liver). Trastuzumab predosing dramatically improved visualization of hepatic metastases (Fig. 3) and had little effect on ^{64}Cu -DOTA-trastuzumab uptake in heart wall (supplemental material, Fig. 1), kidney, or spleen (data not shown).

No statistically significant difference in tumor uptake of ^{64}Cu -DOTA-trastuzumab was observed between the 2 trastuzumab doses. Tumor SUV_{max} was generally higher for the 5- than for the 50-mg dose on day 1 (mean $\pm$ SD, 11.3 ± 5.9 , compared with 6.7 ± 2.4 , $P = 0.01$) but trended in the other direction on day 2 (mean $\pm$ SD, 5.9 ± 3.7 , compared with 9.6 ± 5.9 , $P = 0.11$). When ANOVA included both a patient effect (i.e., accounted for varying numbers of lesions among different patients) and a dose effect, there was no significant trastuzumab dose effect on either day.

Heterogeneity of ^{64}Cu -DOTA-Trastuzumab Uptake in Tumors

[Fig. 4] Uptake varied widely both among and within patients (Fig. 4). For the data included in Figure 4, mean SUV_{max} ranged from 5.5 to 15.0 g/mL among the 8 patients. Within patients, SUV_{max} varied between 2- and 5-fold in 7 patients and 22-fold in 1 patient.

[Fig. 5] The variability was, in part, associated with lesion site (Fig. 5).

Tumor Uptake Compared Between ^{64}Cu -DOTA-Trastuzumab and ^{18}F -FDG

Uptake of ^{64}Cu -DOTA-trastuzumab and ^{18}F -FDG was comparable when averaged over all lesions. For combined 5- and 50-mg

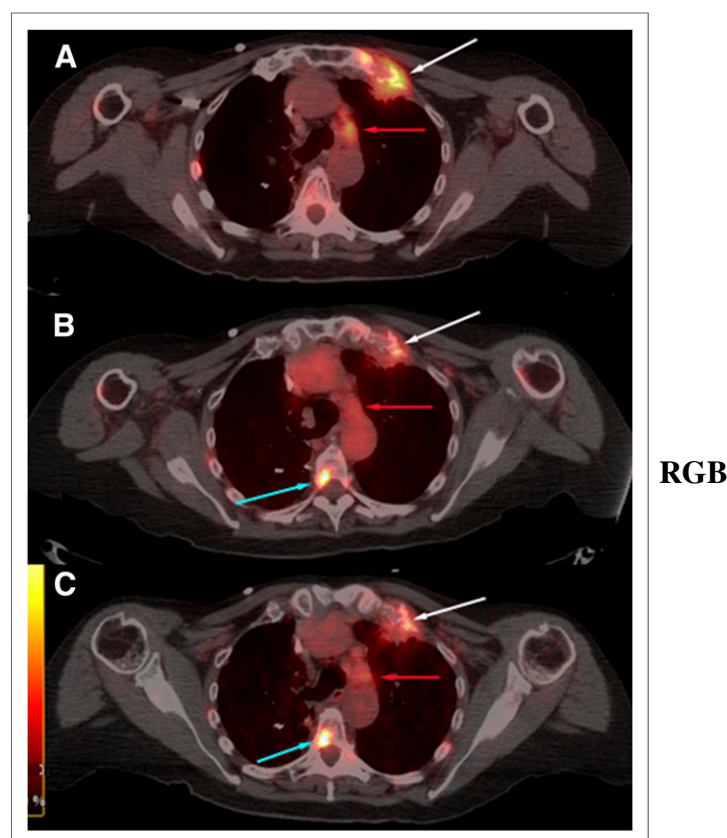


FIGURE 2. Visualization of bone and nodal metastases. Shown are transaxial PET/CT fusion images of ^{18}F -FDG (A), ^{64}Cu -DOTA-trastuzumab 23 h after injection (B), and ^{64}Cu -DOTA-trastuzumab 48 h after injection (C) from patient given 50 mg of trastuzumab. Upper intensity thresholds (white color) correspond to $\text{SUV} = 10$ g/mL. Lesion-to-nonlesion contrast improved modestly between day 1 and day 2 after ^{64}Cu -DOTA-trastuzumab injection. Lesion growing out of left first rib (white arrows) is well visualized on day 1 ^{64}Cu scan and little changed on day 2. Spinal metastasis (turquoise arrows) not seen with ^{18}F -FDG is well visualized on both day 1 and day 2 ^{64}Cu scans. On the other hand, nodal metastasis (red arrows) seen with ^{18}F -FDG is visualized only on day 2 with ^{64}Cu -DOTA-trastuzumab.

trastuzumab doses, SUV_{max} mean, median, and range results were 9.7, 9.3, and 3.3–25.4, respectively, for ^{18}F -FDG ($n = 56$); 8.1, 7.0, and 3.0–22.5, respectively, for ^{64}Cu -DOTA-trastuzumab day 1 ($n = 48$); and 9.0, 7.5, and 0.9–28.9, respectively, for ^{64}Cu -DOTA-trastuzumab day 2 ($n = 38$).

ANOVA including both lesion site and patient effects indicated significant lesion site effects for both ^{64}Cu -DOTA-trastuzumab and ^{18}F -FDG (Fig. 5). Pairwise comparisons between sites showed ^{18}F -FDG uptake in liver metastases to be less than in bone metastases ($P < 0.01$), whereas ^{64}Cu -DOTA-trastuzumab uptake on day 2 was higher in liver metastases than in bone metastases ($P < 0.02$).

Same-lesion SUV_{max} for ^{64}Cu -DOTA-trastuzumab and ^{18}F -FDG was uncorrelated ($P \geq 0.4$; correlation coefficients = -0.1). SUV_{max} ratios (^{64}Cu -dota-trastuzumab to ^{18}F -fdg) varied from 0.2 to 4.3 (supplemental material, Fig. 2).

Patient Safety

Trastuzumab infusion and ^{64}Cu -DOTA-trastuzumab PET/CT were well tolerated, with no unanticipated toxicity or adverse side effects observed. Antitrastuzumab antibody response assays were negative for 6 patients. Minor increases in higher-molecular-weight

TABLE 2
Detection of CT-Positive Lesions with PET

Lesion site	¹⁸ F-FDG*	⁶⁴ Cu-DOTA-trastuzumab†	
		Day 1	Day 2
All	83 of 89 (93%)	61 of 79 (77%)‡	54 of 61 (89%)
Bone	35 of 38 (92%)	33 of 36 (92%)	19 of 20 (95%)
Lymph nodes	30 of 31 (97%)	20 of 31 (65%)§,	19 of 23 (88%)
Liver	8 of 10 (80%)	1 of 3 (33%)	8 of 8 (100%)
Lung	5 of 5 (100%)	4 of 4 (100%)	4 of 5 (80%)
Pleural effusion	2 of 2 (100%)	0 of 2 (0%)	1 of 2 (50%)
Breast	3 of 3 (100%)	3 of 3 (100%)	3 of 3 (100%)

*Lesions evaluated for ¹⁸F-FDG were all included in and evaluated for either of or both ⁶⁴Cu-DOTA-trastuzumab day 1 and day 2 scans.

†Combined data for 5- and 50-mg trastuzumab doses.

‡*P* < 0.01 relative to ¹⁸F-FDG, all sites.

§*P* < 0.01 relative to ¹⁸F-FDG, lymph nodes.

||*P* < 0.05 relative to ⁶⁴Cu-DOTA-trastuzumab day 1, bone.

complexes were observed in the high-performance liquid chromatography shift assays of 2 patients at baseline and 6 mo or baseline, 3, and 6 mo after their ⁶⁴Cu-DOTA-trastuzumab PET/CT procedures. Estimated radiation doses (Table 3) were well within the range of those for established radionuclear imaging procedures.

DISCUSSION

Tumor HER2 status and trastuzumab exposure history were more clearly prescribed in the current investigation than in prior imaging studies with trastuzumab (8–10). All patients had biopsy confirmation of HER2 positivity at time of study, and none had received anti-HER2 therapy for at least 4 mo before imaging.

We have clearly shown that, despite the relatively short half-life of the radiolabel, ⁶⁴Cu-DOTA-trastuzumab PET/CT can effectively detect and quantify tumor uptake in patients with known HER2-positive disease. Other than brain, all anatomic sites common to metastatic breast cancer were included in the patient cohort. Lesions were visualized in all 8 patients examined and were seen in bone, lymph nodes, liver, lung, pleural effusions, and breast. Detection sensitivity was 77% on day 1 and 89% on day 2 (Table 2). Tumor uptake was substantial by 24 h and, on average, increased modestly between 24 and 48 h. Detection of lymph nodes in the neck, upper thorax, and mediastinum is difficult at 24 h because of high blood background but improves by 48 h (Fig. 2). The chief limitation of ⁶⁴Cu-DOTA-trastuzumab PET/CT is that, because of the 13-h half-life of ⁶⁴Cu, it does not provide whole-body coverage with acceptable signal-to-noise ratio and scan duration. Nonetheless, as demonstrated here, ⁶⁴Cu-DOTA-trastuzumab PET can be used effectively in disseminated, HER2-positive breast cancer when disease location is defined in advance by ¹⁸F-FDG/PET or CT.

A second major objective was to establish a trastuzumab protein load that minimizes liver uptake without inhibiting tumor uptake of ⁶⁴Cu-DOTA-trastuzumab. We observed that adding 45 mg of trastuzumab to the 5 mg of DOTA-trastuzumab delivered with the radioactive injection approximately doubled blood SUV and reduced liver uptake by 75%–80% on days 1 and 2 after radiotracer injection (supplemental material, Fig. 1). These observations are quantitatively similar to those reported by Dijkers et al. for ⁸⁹Zr-trastuzumab given with trastuzumab loads of 10 and 50 mg (10).

Comparison with our ¹¹¹In-MxDTA-trastuzumab study (trastuzumab load 4–8 mg/kg) suggests that increasing beyond 50 mg of trastuzumab

dose would not yield further improvement in the pharmacokinetics or biodistribution of ⁶⁴Cu-DOTA-trastuzumab. We observed no statistically significant difference in tumor uptake between 5- and 50-mg doses in this small study. However, other investigations have demonstrated that a significant fraction of tumor binding sites can be occupied at antibody loading doses < 4–8 mg/kg (17). Furthermore, the dissociation constant for ¹¹¹In-DTPA-trastuzumab-HER2 binding is approximately 10 nM (18), a concentration that very likely would be exceeded in tumors at a trastuzumab load of 4–8 mg/kg. This suggests that HER2 saturation may have contributed to the relatively low tumor detection sensitivity in our ¹¹¹In-MxDTA-trastuzumab study (4 lesions visualized in 3 of 7 patients with known lesions).

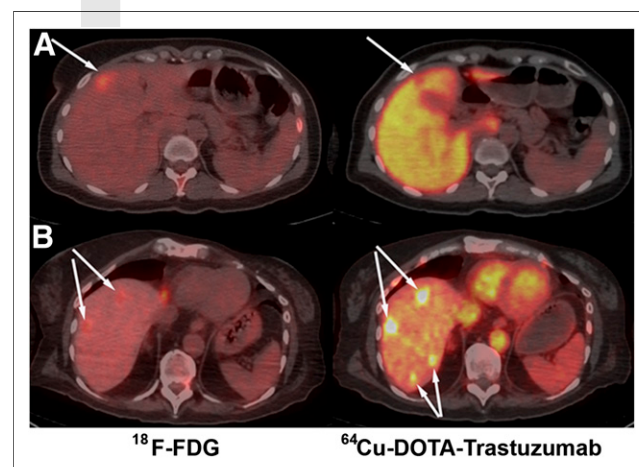


FIGURE 3. Visualization of hepatic metastases. (A) Fused transaxial PET/CT images of ¹⁸F-FDG and ⁶⁴Cu-DOTA-trastuzumab 21 h after injection for patient (patient A) given 5 mg of trastuzumab. Upper intensity thresholds (white color) correspond to SUV = 10 g/mL for ¹⁸F-FDG and 40 g/mL for ⁶⁴Cu-DOTA-trastuzumab. (B) Fused transaxial PET/CT images of ¹⁸F-FDG and ⁶⁴Cu-DOTA-trastuzumab 47 h after injection for patient (patient B) given 50 mg of trastuzumab. Upper intensity thresholds correspond to SUV = 10 g/mL. Arrows indicate detected lesions. For patient A, anterior hepatic lesion is visualized as cold spot on ⁶⁴Cu-DOTA-trastuzumab scan. Liver SUV was 27.6 g/mL in patient A, compared with 5.5 g/mL in patient B, for whom hepatic lesions are dramatically visualized as hot spots. For patient B, 2 lesions not seen with ¹⁸F-FDG are detected with ⁶⁴Cu-DOTA-trastuzumab.

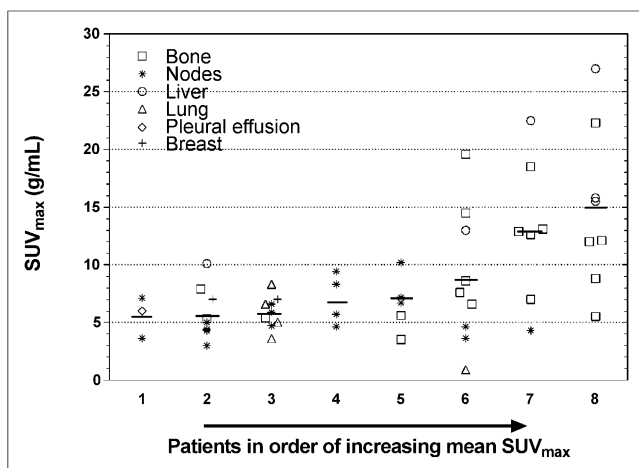


FIGURE 4. Inter- and inpatient heterogeneity of ^{64}Cu -DOTA-trastuzumab tumor uptake. Short horizontal lines indicate inpatient mean SUV_{max} . Data are from day 1 ($n = 49$) or day 2 ($n = 7$) for lesions not included in day 1 scan. Six of day 2 lesions are for patient 8, for whom data from 2 of 3 scanned bed positions on day 1 were lost due to scanner malfunction. Other day 2 lesion is for patient 6. Patients 6 and 7 received trastuzumab doses of 5 mg; others received 50 mg.

Heterogeneity of tumor HER2 expression within and among patients is poorly understood (5–7) and may be elucidated by imaging studies with radiolabeled trastuzumab. The high degree of tumor positivity observed in the current study suggests that most lesions in HER2-positive patients have HER2 expression adequate to render them detectable with ^{64}Cu -DOTA-trastuzumab PET/CT. On the other hand, tumor uptake was also highly variable among and within patients (Fig. 4). That heterogeneity suggests a potential role for ^{64}Cu -DOTA-trastuzumab PET/CT in the selection of patients for trastuzumab-based therapy.

Patient selection and scan design for the ^{64}Cu -DOTA-trastuzumab PET examinations relied on prior ^{18}F -FDG scans. Tumor uptake and detection sensitivity were only modestly lower for ^{64}Cu -DOTA-trastuzumab than for ^{18}F -FDG. Most lesions were positively visualized with both radiotracers, and 6 CT-positive tumors were

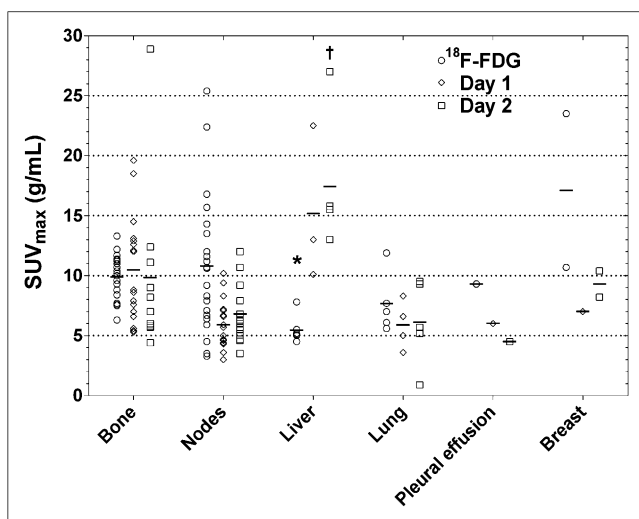


FIGURE 5. Tumor uptake of ^{18}F -FDG and ^{64}Cu -DOTA-trastuzumab (day 1 and day 2) vs. lesion site. Short horizontal lines indicate intrasite averages. * $<^{18}\text{F}$ -FDG bone, $P < 0.01$; +Day 2 bone, $P < 0.02$.

TABLE 3
Estimated Radiation Doses for ^{64}Cu -DOTA-Trastuzumab^{*,†}

Organ	Equivalent or effective dose per unit injected activity (mSv/MBq)	Equivalent or effective dose per PET examination (mSv) [‡]
Heart wall	0.16	71
Kidneys	0.09	42
Liver	0.12	53
Red marrow	0.04	17
Spleen	0.10	45
Whole body	0.02	10
Effective dose	0.03	12

*Trastuzumab protein dose = 50 mg.

†Calculations used averaged time-activity curves (8 patients) from our ^{111}In -MxDTA-trastuzumab study (9), which were normalized to averaged blood and organ uptake data from current ^{64}Cu -DOTA-trastuzumab study (6 patients).

‡Assumes ^{64}Cu injected activity = 450 MBq, average in current study.

detected with ^{64}Cu -DOTA-trastuzumab and not with ^{18}F -FDG. Same-lesion maximum SUVs for ^{64}Cu -DOTA-trastuzumab and ^{18}F -FDG were uncorrelated, and their ratios (^{64}Cu -DOTA-trastuzumab to ^{18}F -FDG) varied by a factor of 22 (supplemental material, Fig. 2). Tumor uptake of ^{18}F -FDG reflects density of glycolytic activity, which in turn depends on viable cell density and tissue oxygenation status (19–21). In breast cancer, high tumor uptake of ^{18}F -FDG is generally correlated with tumor aggressiveness but not with overexpression of the HER2 oncogene c-erbB-2 (22). For ^{64}Cu -DOTA-trastuzumab, the unproven assumption is that tumor uptake is closely related to HER2 density, which in turn is positively correlated with tumor growth rate and aggressiveness (23). However, the relationship between uptake and HER2 expression may be confounded by factors such as blood clearance and vascular permeability. Because glycolysis and HER2 expression are independently related to tumor aggressiveness, the observed lack of correlation between same-tumor uptake of ^{64}Cu -DOTA-trastuzumab and ^{18}F -FDG suggests that combining the 2 measurements may be useful in predicting patient outcomes.

The procedures used in this study were well tolerated. There were no unexpected toxicities associated with the trastuzumab or ^{64}Cu -DOTA-trastuzumab administrations. Two patients had assay results that might indicate low-level antibody responses after the ^{64}Cu -DOTA-trastuzumab procedure. However, both patients had positive pre- ^{64}Cu -DOTA-trastuzumab baseline assays and intermittently positive assays thereafter. This suggests positivity resulted from something other than the ^{64}Cu -DOTA-trastuzumab procedure, such as prior treatment with trastuzumab or the presence of circulating antigen (i.e., HER2 extracellular domain) in the serum, a possibility that we are currently evaluating.

Estimated radiation doses for ^{64}Cu -DOTA-trastuzumab (Table 3) are moderate, compared with ^{18}F -FDG and other imaging procedures with radiolabeled antibodies. For the mean administered activity in this study (450 MBq) and a 50-mg trastuzumab dose, estimated effective dose and maximum organ (heart wall) equivalent dose for ^{64}Cu -DOTA-trastuzumab are 12 and 71 mSv, respectively. ^{18}F -FDG has effective and critical organ (bladder wall) equivalent doses of 11 and 72 mSv, respectively, for the typical

injected activity of 555 MBq (15 mCi) (24). Monoclonal antibodies labeled with ^{111}In incur effective and critical organ (spleen and liver) equivalent doses of approximately 40 and 200 mSv, respectively, for the typical injected activity of 185 MBq (5 mCi) (24). Dijkers et al. estimated a radiation dose (presumably effective dose) of 18 mSv from a 37-MBq (1 mCi) injection of ^{89}Zr -trastuzumab (10).

CONCLUSION

We have shown that, in patients with HER2-positive metastatic breast cancer, tumors rapidly accumulate ^{64}Cu -DOTA-trastuzumab to high concentrations, thus supporting both detection and measurement of tumor uptake by 1 d after injection. The rapid uptake, supplemented by prior knowledge of tumor location afforded by ^{18}F -FDG PET/CT, makes ^{64}Cu -DOTA-trastuzumab effective for surveying disseminated disease despite the limited half-life of ^{64}Cu . We have confirmed that 50 mg of trastuzumab protein dose provides a ^{64}Cu -DOTA-trastuzumab biodistribution favorable for tumor imaging. This study demonstrates that ^{64}Cu -DOTA-trastuzumab PET/CT is a practical and acceptably safe procedure in patients with metastatic breast cancer.

We will next broaden the study to include patients with metastatic breast cancer classified as HER2-negative on prescan biopsy and thus correlate tumor uptake of ^{64}Cu -DOTA-trastuzumab with HER2 expression. Beyond that, we envision using ^{64}Cu -DOTA-trastuzumab PET/CT to individualize treatment regimens that include trastuzumab and other HER2 directed therapies.

DISCLOSURE

The costs of publication of this article were defrayed in part by the payment of page charges. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 USC section 1734. This work was supported by the Department of Defense (grant 1024511) and by the National Cancer Institute of the National Institutes of Health under grant number P30CA033572. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. No other potential conflict of interest relevant to this article was reported.

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REFERENCES

- Wolff AC, Hammond MEH, Schwartz JN, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. *J Clin Oncol*. 2007;25:118–145.
- Hurvitz SA, Hu Y, O'Brien N, Finn RS. Current approaches and future directions in the treatment of HER2-positive breast cancer. *Cancer Treat Rev*. 2013;39:219–229.
- Paik S, Kim C, Wolmark N. HER2 status and benefit from adjuvant trastuzumab in breast cancer. *N Engl J Med*. 2008;358:1409–1411.
- Perez EA, Suman VJ, Davidson NE, et al. HER2 testing by local, central, and reference laboratories in specimens from the North Central Cancer Treatment Group N9831 intergroup adjuvant trial. *J Clin Oncol*. 2006;24:3032–3038.
- Simon R, Nocito A, Hübscher T, et al. Patterns of HER2/neu amplification and over-expression in primary and metastatic breast cancer. *J Natl Cancer Inst*. 2001;93:1141–1146.
- Zidan J, Dashkovsky I, Stayerman C, Basher W, Cozacov C, Hadary A. Comparison of HER-2 overexpression in primary breast cancer and metastatic sites and its effect on biological targeting therapy of metastatic disease. *Br J Cancer*. 2005;93:552–556.
- Lindström LS, Karlsson E, Wilking UM, et al. Clinically used breast cancer markers such as estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 are unstable throughout tumor progression. *J Clin Oncol*. 2012;30:2601–2608.
- Perik PJ, Lub-De Hooge MN, Gietema JA, et al. Indium-111-labeled trastuzumab scintigraphy in patients with human epidermal growth factor receptor 2-positive metastatic breast cancer. *J Clin Oncol*. 2006;24:2276–2282.
- Wong JYC, Raubitschek A, Yamauchi D, et al. A pretherapy biodistribution and dosimetry study of indium-111-radiolabeled trastuzumab in patients with human epidermal growth factor receptor 2-overexpressing breast cancer. *Cancer Biother Radiopharm*. 2010;25:387–394.
- Dijkers EC, Oude Munnink TH, Kosterink JG, et al. Biodistribution of ^{89}Zr -trastuzumab and PET imaging of HER2-positive lesions in patients with metastatic breast cancer. *Clin Pharmacol Ther*. 2010;87:586–592.
- Lub-de Hooge MN, Kosterink JGW, Perik PJ, et al. Preclinical characterization of ^{111}In -DTPA-trastuzumab. *Br J Pharmacol*. 2004;143:99–106.
- Li L, Bading J, Yazaki PJ, et al. A versatile bifunctional chelate for radiolabeling humanized anti-CEA antibody with In-111 and Cu-64 at either thiol or amino groups: PET imaging of CEA-positive tumors with whole antibodies. *Bioconjug Chem*. 2008;19:89–96.
- Bading JR, Ng TSC, Jacobs RE, Raubitschek A, Colcher D. ^{64}Cu -DOTA-Herceptin: radio-synthesis and initial evaluation in vivo [abstract]. *J Nucl Med*. 2009;50(suppl 2):404P.
- Patt YZ, Lamki LM, Haynie TP, et al. Improved tumor localization with increasing dose of indium-111-labeled anti-carcinoembryonic antigen monoclonal antibody ZCE-025 in metastatic colorectal cancer. *J Clin Oncol*. 1988;6:1220–1230.
- Cheebsumon P, Yaqub M, van Velden FH, Hoekstra OS, Lammertsma AA, Boellaard R. Impact of [^{18}F]FDG PET imaging parameters on automatic tumour delineation: need for improved tumour delineation methodology. *Eur J Nucl Med Mol Imaging*. 2011;38:2136–2144.
- Hong S, Li J, Wang S. ^{18}F FDG PET-CT for diagnosis of distant metastases in breast cancer patients: a meta-analysis. *Surg Oncol*. 2013;22:139–143.
- O'Donoghue JA, Smith-Jones PM, Humm JL, et al. ^{124}I -huA33 antibody uptake is driven by A33 antigen concentration in tissues from colorectal cancer patients imaged by immuno-PET. *J Nucl Med*. 2011;52:1878–1885.
- Costantini DL, Chan C, Cai Z, Vallis KA, Reilly RM. ^{111}In -labeled trastuzumab (Herceptin) modified with nuclear localization sequences (NLS): an Auger electron-emitting radiotherapeutic agent for HER2/neu-amplified breast cancer. *J Nucl Med*. 2007;48:1357–1368.
- Wahl RL, Jacene H, Kasamon Y, Lodge MA. From RECIST to PERCIST: evolving considerations for PET response criteria in solid tumors. *J Nucl Med*. 2009;50:122S–150S.
- Bos R, van der Hoeven JJM, van der Wall E, et al. Biologic correlates of ^{18}F fluorodeoxyglucose uptake in human breast cancer measured by positron emission tomography. *J Clin Oncol*. 2002;20:379–387.
- Minn H, Clavo AC, Wahl RL. Influence of hypoxia on tracer accumulation in squamous-cell carcinoma: in vitro evaluation for PET imaging. *Nucl Med Biol*. 1996;23:941–946.
- Groheux D, Giachetti S, Moretti JL, et al. Correlation of high ^{18}F -FDG uptake to clinical, pathological and biological prognostic factors in breast cancer. *Eur J Nucl Med Mol Imaging*. 2011;38:426–435.
- Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science*. 1987;235:177–182.
- International Commission on Radiological Protection. Radiation dose to patients from radiopharmaceuticals: addendum 3 to ICRP publication 53. ICRP Publication 106. *Ann ICRP*. 2008;38:1–197.

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- [AQ1] Please confirm that the correct city has been given for USC/Molecular Imaging Center.
- [AQ2] Please confirm that “On average,..” retains your meaning with edits.
- [AQ3] Please verify the data in any tables and figures, and please confirm that any radiation or radiopharmaceutical doses mentioned in the article are correct.
- [AQ4] Can MxDTPA be spelled out?
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San Antonio Breast Cancer Symposium 2013

Relationship between ^{64}Cu -DOTA-trastuzumab positron emission tomography uptake and assessment of HER2 by immunohistochemistry in women with advanced breast cancer. Joanne E. Mortimer, David Colcher, Paul Frankel, Andrew Raubitschek, Mary Carroll, Peter Conti, Shan Tong, Kofi Poku, Joshua Miles, and James Bading.

Background: We have utilized ^{64}Cu -DOTA-trastuzumab with PET imaging to assess the in vivo expression of HER2 in women with advanced breast cancer. We have demonstrated that a preadministered dose of trastuzumab 45 mg prior to injection of ^{64}Cu -DOTA-trastuzumab resulted in a 75% decrease in the hepatic uptake of Cu-64, resulting in improved image quality.

Methods: Patients with biopsy confirmation of recurrent disease located outside the breast and axilla considered for study. Complete staging workup included CT of the chest, abdomen, and pelvis, bone scintigraphy and ^{18}F FDG PET. At least 1 non-hepatic site of metastasis that was > 2 cm separate from the biopsy site was also required. HER2 status was assessed by both IHC and FISH. Index lesions were identified on CT imaging. After the first two patients, all patients received a cold dose of 45mg of trastuzumab immediately prior to ^{64}Cu -DOTA-trastuzumab to decrease liver uptake. ^{64}Cu -DOTA-trastuzumab PET imaging was performed at 24 and 48 hours. Uptake on ^{64}Cu -DOTA-trastuzumab was correlated with HER2 status by IHC and FISH.

Results: Fifteen women have undergone ^{64}Cu -DOTA-trastuzumab PET imaging and quantitative image analysis. 10 patients were HER2+ (7 IHC 3+, 3 IHC 2+/FISH+), 3 patients were IHC 2+/FISH-, and 2 patients were IHC 1+. Tumor uptake by ^{64}Cu -DOTA-trastuzumab PET max SUV was higher in HER2+ positive than HER2- patients (1.9-fold higher on day1, $p < 0.02$, and 1.7-fold higher on day2, $p < 0.05$). However, the lowest max SUV was in a HER2+ patient (HER2 2+/FISH+), demonstrating considerable heterogeneity. Conclusion: ^{64}Cu -DOTA-trastuzumab PET correlates with HER2+ status. However, due to high within and between patient variability, ^{64}Cu -DOTA-trastuzumab PET imaging could potentially enrich for HER2+ patients that respond to HER2-targeted therapy, and could also suggest some HER2- patients that may benefit from HER2-targeted therapy. This hypothesis needs to be further explored in patients undergoing HER2-targeted therapy. This work was supported by the Department of Defense grant # BC095002.



⁶⁴Cu-DOTA-trastuzumab positron emission tomography imaging of *HER2* in women with advanced breast cancer

Joanne E Mortimer, Jinha Mark Park, Mary I. Carroll, Kofi Poku, Joshua Miles, David Colcher,
Andrew A. Raubitschek, Peter Conti, Tri Tran, and James R. Bading

City of Hope Cancer Center/Beckman Research Institute, Duarte, CA; USC, Los Angeles, CA 91010

Abstract

Background: We have developed ⁶⁴Cu-DOTA-trastuzumab for PET imaging of *HER2*-positive breast cancer. We have determined that administering trastuzumab (45mg) prior to ⁶⁴Cu-DOTA-trastuzumab sharply reduces the liver uptake of the radiotracer. We are now testing whether tumor uptake of ⁶⁴Cu-DOTA-trastuzumab correlates with variable IHC staining in women with advanced breast cancer.

Methods: Eligibility criteria included biopsy confirmation of metastatic disease that was *HER2* 1+, 2+, or 3+ by IHC, no anti-*HER2* therapy within the prior 4 mo, and at least 1 non-hepatic site of metastasis >20 mm outside the biopsy site. Staging workup included ¹⁸F-FDG PET-CT. Patients received 45 mg of cold trastuzumab prior to ⁶⁴Cu-DOTA-trastuzumab. PET-CT scans were obtained at 21-25 h (Day 1) and 47-48 h (Day 2) over axial fields of view chosen in reference to ¹⁸F-FDG. Uptake is prominent lesions was measured in terms of maximum single-voxel SUV(SUV_{max}). Lesions identified on CT and judged to have image intensity > adjacent tissue by an expert radiologist were considered positive on PET.

Results: Fourteen women (median age 56, range 35-75 years) have undergone imaging. *HER2* status by IHC was 3+ in 9 pts, 2+ in 5 and 1+ in 1. Two women with IHC 2+ disease were FISH+. In the patients considered clinically *HER2*+ positive (IHC 3+ or 2+, FISH+), ⁶⁴Cu-DOTA-trastuzumab frequency was 75 and 90% respectively, on days 1 and 2, compared with 94% for ¹⁸F-FDG. Tumor uptake of ⁶⁴Cu-DOTA-trastuzumab was also readily visualized in *HER2*-patients (measured detection sensitivity 90%). There were no false positive findings with ⁶⁴Cu-DOTA-trastuzumab. Lesions uptake of ⁶⁴Cu-DOTA-trastuzumab was higher in *HER2*+ than in *HER2*- patients (SUV_{max} mean ± sem: Day 1 8.9±0.6 vs 4.3 ± 0.2; Day 2 9.9±0.8 vs 5.4±0.3, p<0.001).

Conclusions: ⁶⁴Cu-DOTA-trastuzumab PET visualizes *HER2* 1+, 2+ and 3+ metastatic breast cancer with high sensitivity and specificity. Tumor uptake of ⁶⁴Cu-DOTA-trastuzumab-PET in IHC 1+ and 2+ patients implies possible benefit from anti-*HER2* therapies for individual whose cancers are currently considered *HER2* negative. This research was supported by Department of Defense grant BC095002.

Background

- 25-30% of all breast cancers overexpress the *HER2* protein as assessed by IHC or FISH.
- HER2* overexpression is used to guide the use of *HER*-directed therapy with trastuzumab.
- Following systemic therapy, the *HER2* status may change in 5-30% of patients and re-biopsy of recurrent tumor may be difficult.
- Functional imaging with PET provides information about tumor biology and may predict for drug resistance.
- Normal tissues express *HER2* and may uptake radiolabeled trastuzumab compromising PET image quality.

Objectives

- Determine the dose of pre-administered cold trastuzumab that optimizes tumor image quality of ⁶⁴Cu-DOTA-trastuzumab PET in women with advanced breast cancer. (Study 1)
- Determine whether ⁶⁴Cu-DOTA-trastuzumab PET uptake correlates with *HER2* status determined by IHC staining. (Study 2)

Material and Method

Eligibility

- Histological confirmation of metastatic invasive breast cancer outside the region of the primary tumor and axilla.
- At least 1 non-hepatic site of metastasis ≥ 2 cm in mean diameter in addition to the site that was biopsies
- Normal cardiac ejection fraction
- No trastuzumab therapy for ≥ 2 months
- Study 1 *HER2* positive by IHC and/or FISH
Study 2 *HER2* 1+, 2+, or 3+

Staging Workup

¹⁸F-FDG whole body CT/PET
Bone Scintigraphy

Imaging

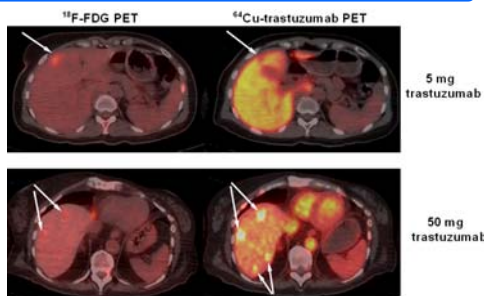
Day 0: Injection of cold dose trastuzumab (5 mg, 50 mg or 4 mg/kg)

Injection of 15 mCi ⁶⁴Cu-DOTA-trastuzumab

Day 1: ⁶⁴Cu-DOTA-trastuzumab PET imaging

Day 2: ⁶⁴Cu-DOTA-trastuzumab PET imaging

Study I Summary of Results



Visualization of hepatic metastases.

Conclusions

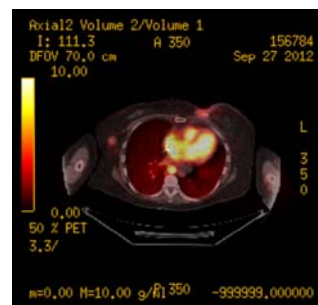
In eight women with documented *HER2* positive metastatic disease:

-⁶⁴Cu-DOTA-trastuzumab PET imaging is feasible

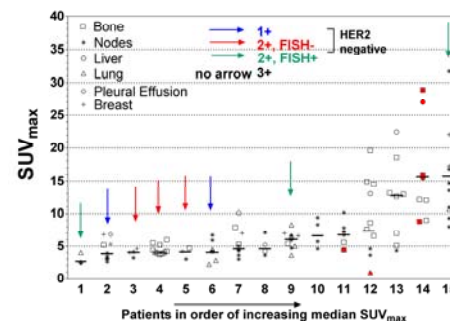
-The pre-administration of 50 mg trastuzumab resulted in a 75% decrease in hepatic uptake, improving image quality

Results - Study 2

⁶⁴Cu-DOTA-trastuzumab PET uptake and *HER2* status by IHC staining 1+, 2+, 3+



65 year old with *HER2* 2+ disease. Note ⁶⁴Cu-DOTA-trastuzumab uptake in skin and axilla



Inter- and intra-patient heterogeneity of ⁶⁴Cu-DOTA-trastuzumab tumor uptake. Data are from DAY 1 (n = 99), or DAY 2 (n = 9) for lesions not included in the DAY 1 scan. Seven of the DAY 2 lesions are for Patient 14, for whom data from 2 of the 3 scanned bed positions on DAY 1 were lost due to scanner malfunction.

Study 2 Summary

-In general uptake on ⁶⁴Cu-DOTA-trastuzumab PET correlated with *HER2* staining by IHC

-We continue to accrue patients to Study 2

All work was performed in accordance with IND# 10997.

Project funding was made possible by DOD grant BC095002.



Functional imaging of HER2-positive metastatic breast cancer using ^{64}Cu -DOTA-Trastuzumab Positron Emission Tomography (PET)

Joanne E. Mortimer, Peter Conti, Shan Tong, Jose Reyes, Mary I. Carroll, Kofi Poku, David Colcher, Andrew A. Raubitschek, James R. Bading, Joshua Miles,

¹Dept Medical Oncology and Experimental Therapeutics, ²Cancer Immunotherapeutics and Tumor Immunology

City of Hope Cancer Center/Beckman Research Institute, Duarte, CA; USC, Los Angeles, CA 91010

Background

- 25-30% of all breast cancers overexpress the *HER2* protein as assessed by IHC or FISH.
- *HER2* overexpression is used to guide the use of anti-*HER2* therapy with trastuzumab.
- Following systemic therapy, the *HER2* status may change in 5-30% of patients and re-biopsy of recurrent tumor may be difficult.
- Functional imaging with PET provides information about tumor biology and may predict for drug resistance.
- Normal tissues express *HER2* and may uptake radiolabeled trastuzumab compromising PET image quality.

Hypothesis

^{64}Cu -DOTA-Trastuzumab PET image quality can be improved by a pre-injection of cold trastuzumab.

Study Design

Eligibility

1. Histological confirmation of metastatic invasive breast cancer outside the region of the primary tumor and axilla.
2. At least 1 non-hepatic site of metastasis ≥ 2 cm in mean diameter in addition to the site that was biopsied.
3. The *HER2* positive by IHC and/or FISH.
4. No prior anti-HER therapy within 2 months
5. Normal cardiac ejection fraction.

Staging workup

- ^{18}F -FDG whole body CT/PET
- Bone Scan
- Cardiac Ejection Fraction

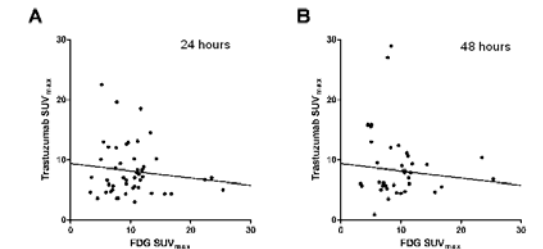
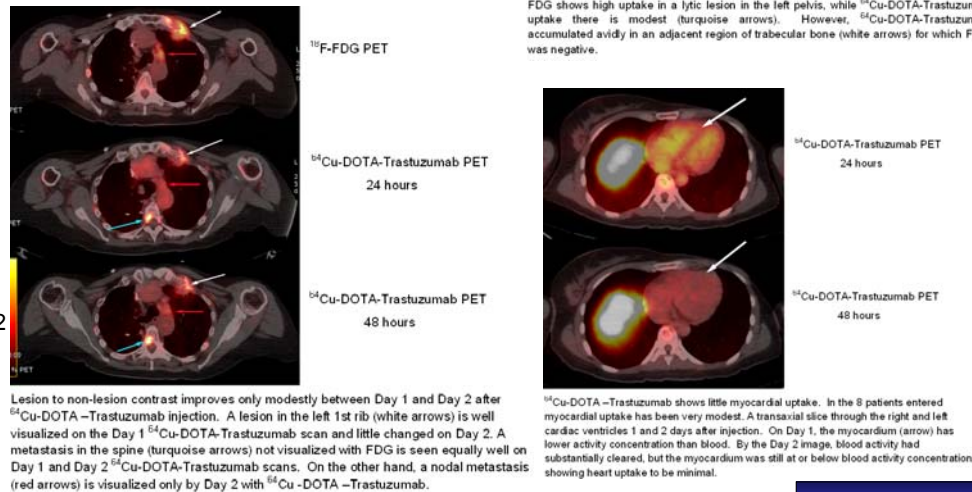
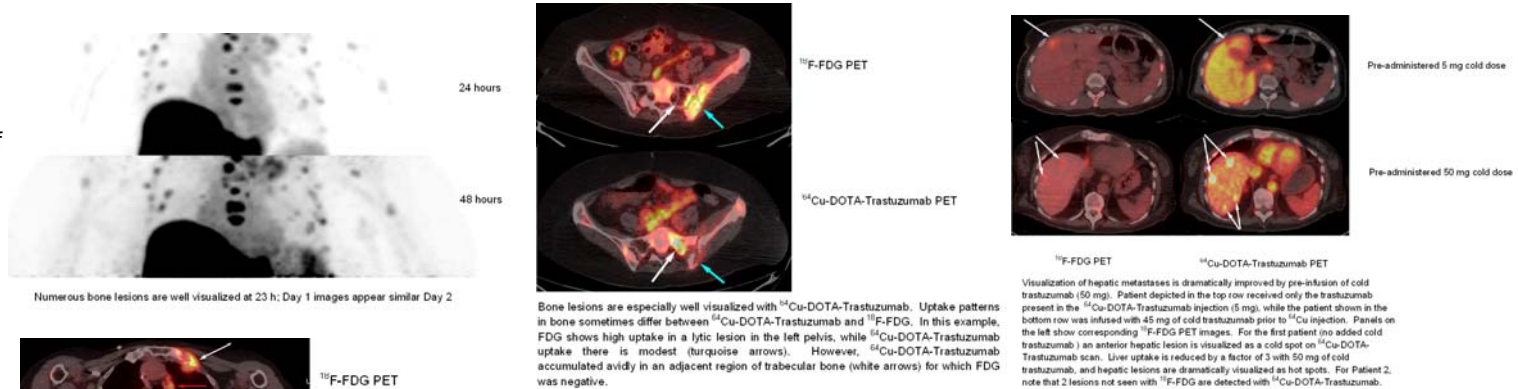
Imaging

Day 0: Injection of assigned cold dose (5 mg, 50 mg or 4mg/kg) trastuzumab
Injection of 15 mCi ^{64}Cu -DOTA-Trastuzumab

Day 1: ^{64}Cu -DOTA-Trastuzumab PET imaging

Day 2: ^{64}Cu -DOTA-Trastuzumab PET imaging

Results in eight patients



Non-correlation of tumor uptake between ^{18}F -FDG and ^{64}Cu -DOTA-trastuzumab. The plots compare maximum single-voxel standardized uptake values (SUV_{max}) within the same tumor between ^{18}F -FDG and ^{64}Cu -DOTA-trastuzumab Days 1 (A) and 2 (B). Slopes of the indicated linear regression lines are not significantly different from 0 ($P = 0.4$, $r^2 = 0.02$ for Day 1; $P = 0.5$, $r^2 = 0.01$ for Day 2).

Summary & Conclusions

- ^{64}Cu -DOTA-Trastuzumab PET imaging is feasible.
- The pre-administration of 50 mg Trastuzumab improves image quality.
- Myocardial uptake of ^{64}Cu -DOTA-Trastuzumab was not appreciated.

Phase II of the study will enroll women with advanced breast cancer that is 1+, and 2+ *HER2* positive.

This study was funded by a Department of Defense grant, BC095002.



⁶⁴Cu-DOTA-TRASTUZUMAB (⁶⁴Cu-HERCEPTIN)/PET EFFECTIVELY VISUALIZES METASTATIC BREAST CANCER IN HER2-POSITIVE PATIENTS

J. Bading¹, D. Colcher¹, P. Frankel¹, S. Tong¹, J. Reyes¹, M. Carroll¹, E. Poku¹, J. Miles¹, J. Wong¹, J. Shively¹, P. Conti², A. Raubitschek¹, J. Mortimer¹. ¹City of Hope, Duarte, CA; ² University of Southern California, Los Angeles, CA

OBJECTIVES

1. Evaluate the feasibility and potential utility of ⁶⁴Cu-DOTA-trastuzumab/PET-CT for detecting and quantifying lesions in patients with HER2-positive metastatic breast cancer.
2. Investigate the effects of trastuzumab protein load on the pharmacokinetics, biodistribution and tumor uptake of ⁶⁴Cu-DOTA-trastuzumab.
3. Compare tumor uptake and visualization between ⁶⁴Cu-DOTA-trastuzumab and ¹⁸F-FDG.
4. Evaluate ⁶⁴Cu-DOTA-trastuzumab with regard to patient safety.

INTRODUCTION

HER2

- Human epidermal growth receptor 2 (HER2)
- Observed in 25-30% of breast cancers
- Associated with metastasis and decreased survival

TRASTUZUMAB (HERCEPTIN®)

- Murine-based, humanized monoclonal antibody
- Binds with high affinity to the extracellular domain of HER2
- Has significant anti-tumor effects
- Routinely used in combination with chemotherapy for Rx of HER2-positive breast cancer
- Internalizes after binding

PREVIOUS IMAGING STUDIES WITH RADIOLABELED TRASTUZUMAB

- Radiometals are trapped in cells following antibody internalization and therefore are preferred over radioiodine as labeling agents for trastuzumab.
- We previously evaluated ¹¹¹In-MxDTA-trastuzumab combined with conjugate-view planar imaging and SPECT in 8 patients with advanced HER2+ breast cancer (Wong, et al., Cancer Biother & Radiopharm 2010;25:387-94)
- Dijkers, et al., performed whole-body PET with ⁸⁹Zr-trastuzumab in 14 HER2+ breast cancer patients (Clin Pharmacol Ther 2010;87:586-92).

EFFECTS OF TRASTUZUMAB LOAD ON BLOOD CLEARANCE & BIODISTRIBUTION

- Dijkers, et al., showed large decreases in blood and hepatic clearance of ⁸⁹Zr-trastuzumab between trastuzumab protein doses of 10 and 50 mg.
- Wong, et al., used a 4 mg/kg trastuzumab dose and found liver uptake and blood clearance of ¹¹¹In-MxDTA-trastuzumab similar to that reported by Dijkers, et al., for 50 mg trastuzumab dose.

⁶⁴Cu: ADVANTAGES & DISADVANTAGES

- Availability
 - Routinely supplied by the Mallinckrodt Institute of Radiology, Washington University, St. Louis, MO
- Physical characteristics
 - Average β positrons/disintegration = 0.18
 - Half-life = 12.7 h
 - Half-life is short compared with trastuzumab blood clearance .

HYPOTHESIS

Used in conjunction with ¹⁸F-FDG/PET-CT and a 50 mg trastuzumab protein dose to suppress hepatic clearance, ⁶⁴Cu-DOTA-trastuzumab/PET-CT can be effective for characterizing extent of HER2-positive disease in patients with metastatic breast cancer.

METHODS

All procedures were performed in accordance with IND# 109971.

PATIENTS

- 8 women, age 39-75
- No exposure to trastuzumab for at least 6 mo
- Biopsy-proven (IHC & FISH) HER2-positive metastatic disease $\leq$ 28 d prior to study
- HER2 expression levels on IHC: 2+ (n = 1) or 3+ (n = 7)
- Normal cardiac ejection fraction
- ¹⁸F-FDG/PET-CT $\leq$ 2 wks prior to ⁶⁴Cu-DOTA-trastuzumab/PET-CT

ADMINISTRATION OF TRASTUZUMAB & ⁶⁴Cu-DOTA-TRASTUZUMAB

- Trastuzumab protein dose
 - 5 mg: n = 2 (concomitant with ⁶⁴Cu-DOTA-trastuzumab injection)
 - 50 mg: n = 6 (45 mg infused prior to ⁶⁴Cu-DOTA-trastuzumab injection)
- Biodistributions and blood clearance in the first 2 patients done at each dose level confirmed the findings of Dijkers, et al. with ⁸⁹Zr-trastuzumab. Thus we adopted the 50 mg dose going forward.
- ⁶⁴Cu-DOTA-trastuzumab injected activity: mean 450 MBq range 364-512 MBq

IMAGE ANALYSIS

- Lesion detection
 - Lesions judged to have intensity > adjacent tissue were considered positive on PET.
 - Biopsied tumor sites were excluded.
- Tumor uptake
 - Measured for up to 10 prominent lesions/patient
 - Metrics evaluated
 - SUV_{max}, SUV_{peak}, background-adaptive whole tumor average (SUV_{wt})
 - Normalization to body weight (BW) & lean body mass (LBM)
 - Metrics presented
 - Only SUV_{max}(BW) (Others were highly correlated with SUV_{max}(BW) $r^2 \geq 0.91$)

PHARMACOKINETIC ANALYSIS & RADIATION DOSE ESTIMATES

- Blood sampling (i. v., 50 mg trastuzumab only): 1, 24 & 48 h after with ⁶⁴Cu-DOTA-trastuzumab injection
- Radiation dose calculations:
 - Blood & organ time-activity curves (1-168 h) from our ¹¹¹In-MxDTA-trastuzumab study were scaled to ⁶⁴Cu-DOTA-trastuzumab blood & image data (24-48 h) from the current study.
 - The scaling factors were multiplied by ⁶⁴Cu blood & organ residence times derived from the ¹¹¹In-MxDTA-trastuzumab study.
 - These adjusted residence times were input to the dosimetry program OLINDA.

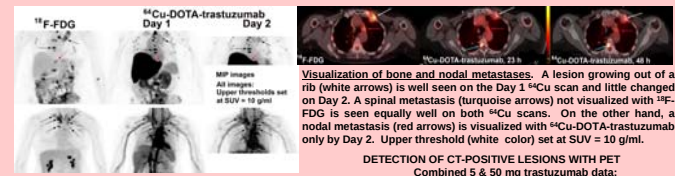
SUMMARY/CONCLUSIONS

1. Although the half-life of ⁶⁴Cu is less than ideal, ⁶⁴Cu-DOTA-trastuzumab/PET-CT effectively visualized and provided uptake measurements for presumably HER2-positive metastatic lesions in bone, liver, lung and, to a lesser degree, lymph nodes.
2. 50 mg trastuzumab protein dose reduced liver uptake of ⁶⁴Cu by approximately 75% relative to 5 mg dose, permitting positive visualization of intrahepatic lesions.
3. Uptake of ⁶⁴Cu-DOTA-trastuzumab and ¹⁸F-FDG was uncorrelated within a given lesion, but comparable in magnitude when averaged over all lesions. Liver metastases observed in this study had higher ⁶⁴Cu-DOTA-trastuzumab uptake relative to ¹⁸F-FDG than bone, nodal or lung metastases.
4. Administration of trastuzumab and ⁶⁴Cu-DOTA-trastuzumab was not associated with any adverse events and no more than low-level anti-trastuzumab antibody responses. Estimated radiation doses from ⁶⁴Cu-DOTA-trastuzumab were moderate compared with those incurred by radiopharmaceuticals routinely used in nuclear medicine.

NEXT STEP: Add patients with a wide range of HER2 levels on pre-scan biopsy to correlate HER2 expression with tumor uptake of ⁶⁴Cu-DOTA-trastuzumab.

RESULTS

⁶⁴Cu-DOTA-TRASTUZUMAB/PET-CT PROVIDES HIGH TUMOR DETECTION SENSITIVITY IN PATIENTS WITH HER2-POSITIVE DISEASE

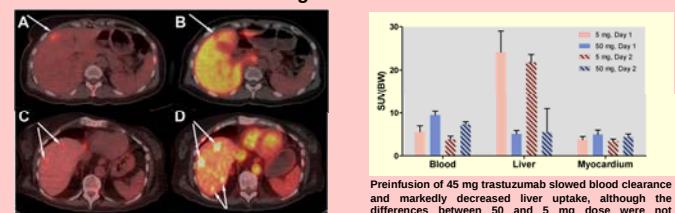


DETECTION OF CT-POSITIVE LESIONS WITH PET Combined 5 & 50 mg trastuzumab data:

Lesion site	¹⁸ F-FDG	⁶⁴ Cu-DOTA-trastuzumab Day 1	⁶⁴ Cu-DOTA-trastuzumab Day 2
all	83 of 89 (92%)	61 of 79 (77%)	54 of 61 (89%)
bone	35 of 38 (92%)	33 of 38 (87%)	19 of 20 (95%)
nodes	30 of 31 (97%)	29 of 31 (93%)	18 of 23 (78%)
liver	8 of 10 (80%)	1 of 3 (33%)	8 of 8 (100%)
lung	5 of 6 (83%)	4 of 4 (100%)	4 of 4 (100%)
pleural effusion	2 of 2 (100%)	0 of 2 (0%)	1 of 2 (50%)
breast	3 of 3 (100%)	3 of 3 (100%)	3 of 3 (100%)

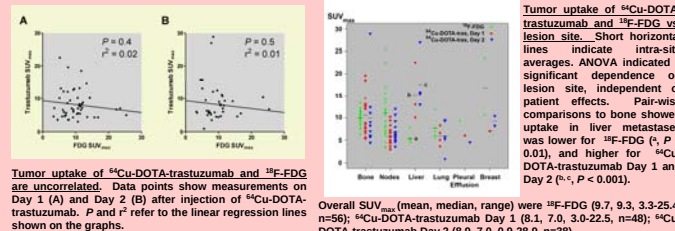
* P < 0.01 relative to ¹⁸F-FDG, all sites
* P < 0.01 relative to ¹⁸F-FDG, nodes
* P < 0.01 relative to ¹⁸F-FDG, liver

HEPATIC UPTAKE OF ⁶⁴Cu-DOTA-TRASTUZUMAB IS SHARPLY REDUCED AT 50 vs 5 mg TRASTUZUMAB PROTEIN DOSE



Preinfusion of 45 mg trastuzumab slowed blood clearance and markedly decreased liver uptake, although the differences between 50 and 5 mg dose were not statistically significant ($P = 0.16$ and 0.11 on Days 1 & 2 for blood; $P = 0.10$ and 0.07 on Days 1 & 2 for liver). Trastuzumab dose had little effect on myocardial uptake. SUVs (normalized to body weight) were determined for volumes of interest placed within organ boundaries on 3 consecutive PET slices. Blood SUVs were determined from the cardiac ventricle images on PET.

TUMOR UPTAKE: ⁶⁴Cu-DOTA-TRASTUZUMAB COMPARED WITH ¹⁸F-FDG



Tumor uptake of ⁶⁴Cu-DOTA-trastuzumab and ¹⁸F-FDG are uncorrelated. Data points show measurements on Day 1 (A) and Day 2 (B) after injection of ⁶⁴Cu-DOTA-trastuzumab. P and R refer to the linear regression lines shown on the graphs.

PATIENT SAFETY

ESTIMATED RADIATION DOSES FOR ⁶⁴Cu-DOTA-TRASTUZUMAB

Organ	Estimated dose per unit injected activity ^a (mSv/MBq)	Equivalent dose per PET examination ^b (mSv)
Myocardium	0.15	7.5
Kidneys	0.10	46
Liver	0.13	79
Red marrow	0.05	22
Spleen	0.13	60
Whole body	0.02	10
Effective dose	0.03	14

^a Trastuzumab protein dose = 50 mg
^b Values are averages based on data from 8 patients in the current study and 8 patients in the ¹¹¹In-MxDTA-trastuzumab study (Wong et al., Cancer Biother Radiopharm 2010;25:387-394).
^c Assumes ⁶⁴Cu injected activity = 450 MBq, the average in the current study.

- No adverse events
- Anti-trastuzumab antibody responses
- Possible low-level (< 1 µg/ml) response in 2 patients
- May be artifact due to circulating HER2
- Further analysis in progress

Work supported by DOD grant # 1024511