

A Microdose Clinical Trial to Evaluate ^{64}Cu -NOTA-Trastuzumab as a Positron Emission Tomography Imaging Agent in Patients with Breast Cancer

Inki Lee

Korea Institute of Radiological and Medical Sciences

Ilhan Lim (✉ ilhan@kcch.re.kr)

Korea Institute of Radiological and Medical Sciences <https://orcid.org/0000-0002-5903-1659>

Byung Hyun Byun

Korea Institute of Radiological and Medical Sciences

Byung Il Kim

Korea Institute of Radiological and Medical Sciences

Chang Woon Choi

KIRAMS

Sang-Keun Woo

Korea Institute of Radiological and Medical Sciences

Kwang Il Kim

Korea Institute of Radiological and Medical Sciences

Kyo Chul Lee

Korea Institute of Radiological and Medical Sciences

Joo Hyun Kang

Korea Institute of Radiological and Medical Sciences

Min-Ki Seong

KIRAMS

Hyun-Ah Kim

Korea Institute of Radiological and Medical Sciences

Woo Chul Noh

Korea Institute of Radiological and Medical Sciences

Sang Moo Lim

KIRAMS

Original research

Keywords: HER-2, ^{64}Cu -NOTA-trastuzumab, breast cancer, positron emission tomography, computed tomography

DOI: <https://doi.org/10.21203/rs.3.rs-48411/v1>

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Abstract

Background: The purpose of this study was to evaluate the biodistribution and safety of ^{64}Cu -1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA)-Trastuzumab, a novel ^{64}Cu -labelled positron emission tomography (PET) tracer for human epidermal growth factor receptor 2 (HER2) in patients with breast cancer.

Methods: PET images at 1, 24, and 48 hours after injection of 296 MBq of ^{64}Cu -NOTA-Trastuzumab were obtained on seven patients with breast cancer. The maximum standardized uptake value (SUV_{max}) was evaluated in the tumors, including the primary tumor and metastatic lesions. The mean SUV_{max} (SUV_{mean}) was evaluated in the normal organs including the blood pool, liver, kidney, muscle, spleen, bladder, lung, and bone. In addition, the internal radiation dosimetry was calculated using the OLINDA/EXM software. Safety was assessed by gathering the feedback of adverse reactions and safety-related issues within 1 month after ^{64}Cu -NOTA-Trastuzumab administration.

Results: The overall values of SUV_{mean} in each normal organ decreased with time on ^{64}Cu -NOTA-Trastuzumab PET images. The average values of SUV_{mean} of the liver were measured at 5.3 ± 0.7 , 4.8 ± 0.6 , and 4.4 ± 0.5 on 1 hour, 24 hours, and 48 hours after injection. The average values of SUV_{mean} of the blood were evaluated as 13.1 ± 0.9 , 9.1 ± 1.2 , and 7.1 ± 1.9 on 1 hour, 24 hours, and 48 hours after injection. The SUV_{max} of HER2-positive tumors showed relatively higher than that of HER2-negative tumors (8.6 ± 5.1 and 5.2 ± 2.8 on 48 hours after injection, respectively). Tumor to background ratios were calculated higher in the HER2-positive tumors than those of HER2-negative tumors. No adverse events related to ^{64}Cu -NOTA-Trastuzumab were reported. The calculated effective dose with a 296 MBq injection of ^{64}Cu -NOTA-Trastuzumab was 2.96 mSv. The highest absorbed dose was observed in the liver (0.076 mGy/MBq), followed by the spleen (0.063 mGy/MBq), kidney (0.044 mGy/MBq), and heart wall (0.044 mGy/MBq).

Conclusions: ^{64}Cu -NOTA-Trastuzumab showed specific uptake at the HER2-expressing tumors. It suggests that ^{64}Cu -NOTA-Trastuzumab can be a feasible monitoring tool for HER2 tumor status in the patients with breast cancer with safe.

Trial registration: CRIS, KCT0002790. Registered 02 February 2018, <https://cris.nih.go.kr>

Background

The specific receptors that are expressed in cancer cells have been studied as targets for the treatment of tumors, resulting in the improved therapeutic performance of cancer patients [1]. Among them, human epidermal growth factor receptor (HER), which is involved in the growth of cancer cells, is a target of a representative molecular therapeutic agent [1, 2]. It is known that the overexpression of HER, an intrinsic protein tyrosine kinase, is closely related to rapid-progress tumors [3]. A member of the HER receptor family, HER2/neu (HER2) is overexpressed in breast cancer, ovarian cancer, bladder cancer, prostate cancer, and non-small cell lung cancer [3]. Recently, several therapeutic agents targeting HER2 have been developed to improve the patient's treatment outcomes, which include trastuzumab, lapatinib, and pertuzumab [4].

The expression of HER2 is evaluated with tumor tissue and to obtain tumor tissue is inevitably invasive [2, 5, 6]. It has been reported that the discordance rate of HER2 expression between primary tumors and distant metastatic lesions is 4.9–17.7% [7]. For this reason, it is necessary to re-evaluate HER2 expression in metastatic tumors. It is also reported that HER2 expression may change over time after cancer develops [8]. Therefore, continuous HER2 evaluation is necessary. However, repeated biopsies are difficult for the patient as it causes discomfort. In order to overcome this

limitation, the need for a non-invasive evaluation of HER2 expression is growing, and one of the proposed methods is the non-invasive evaluation of the expression of HER2 using radioisotopes [2, 5, 6, 9].

Various attempts have been made to non-invasively evaluate the expression of HER2 using radioisotopes. One of them is evaluating HER2 expression using the single-photon emission computerized tomography (SPECT) with ^{111}In -Trastuzumab [10, 11]. This study showed the possibility of evaluating HER2 expression, but had low sensitivity and limited spatial resolution [10]. To overcome these limitations, a diagnostic method using positron emission tomography (PET) has been studied. Clinical trials of isotopes, such as ^{124}I and ^{89}Zr that were labeled with antibodies such as trastuzumab, have been conducted [9, 11, 12]. The trials were able to demonstrate that they can quantify HER2 expression of lesions in patients with HER2 expressing tumors [9, 11, 12]. In addition, the use of HER2 targeted PET imaging using ^{64}Cu -tetra-azacyclododecanetetra-acetic acid (DOTA)-Trastuzumab has been attempted [5, 6, 11, 13, 14]. Clinical trials using this agent in the United States and Japan showed effective identification of HER2 expression in breast cancer patients [5, 6, 13, 14].

We have previously developed ^{64}Cu -1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA)-Trastuzumab targeting HER2-expressing tumor and investigated in *in vitro* and *in vivo* experiments, which showed that ^{64}Cu -NOTA-Trastuzumab can be used as a PET-diagnostic application for HER2-positive breast cancer [2]. In this study, we evaluated the safety and pharmacokinetics of ^{64}Cu -NOTA-Trastuzumab in the breast cancer patients.

Methods

Participants

We recruited a total of 7 patients with breast cancer between September 2017 to 2019. The selection criteria for the subjects were: 1) aged 40-80 years, 2) with at least one measurable lesion, 3) with histopathologically diagnosed breast cancer with HER2 expression, 4) an Eastern Cooperative Oncology Group score of 2 or lower.

This study was approved by the by the Korean Ministry of Food and Drug Safety (MFDS), and the Institutional Review Board of KIRAMS (IRB No.: KIRAMS 2017-09-006-020). All procedures were performed following the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study. This preliminary clinical trial is registered with the Clinical Research Information Service (<https://cris.nih.go.kr>), registration number KCT0002790.

Preparation of ^{64}Cu -NOTA-Trastuzumab

^{64}Cu -NOTA-Trastuzumab was produced from the immunoconjugate (NOTA-trastuzumab) radiolabeled with ^{64}Cu from 50-MeV cyclotron irradiation as described previously [2]. Briefly, trastuzumab (Herceptin[®]; F. Hoffmann-La Roche, Basel, Switzerland) was dissolved in 0.1 M 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (pH 8.5) at a concentration of 10 mg/ml and mixed with a 20-fold molar excess of *p*-SCN-Bn-NOTA in 100% ethanol. The immunoconjugate (NOTA-Trastuzumab) was purified after incubation at 4°C overnight and concentrated to 5 mg/mL with 0.1 M ammonium acetate buffer (pH 6). For radiolabeling, 370 MBq of $^{64}\text{CuCl}_2$ was added to 5mg of NOTA-Trastuzumab. The reaction mixtures were incubated at room temperature for 1 hour. Radiolabeling efficiency was more than 95%. The reaction mixtures formulated with saline were sterilized by filtration through a 0.22- μm Millex GV filter (Merck Millipore, Billerica, MA, USA).

PET protocol

The ^{64}Cu -NOTA-Trastuzumab PET images were acquired using a GE Discovery 710 PET/ computed tomography (CT) (GE Healthcare, Milwaukee, WI, USA). After intravenous injection of 45mg of Trastuzumab for at least 15 mins, subjects were intravenously injected with ^{64}Cu -NOTA-Trastuzumab (296 MBq). The mean administered activity was 278.4 ± 13.0 MBq (range, 259.0-297.0 MBq). There were no adverse or clinically detectable pharmacologic effects in any of the seven subjects. No significant changes in vital signs or the results of laboratory studies. PET images were obtained at 60 minutes after intravenous injection of ^{64}Cu -NOTA-Trastuzumab. Delayed PET images were obtained between 20 to 25 hours and 46 to 49 hours after injection of ^{64}Cu -NOTA-Trastuzumab. All subjects were scanned from the mid-thigh to the vertex of the skull.

^{18}F -fluorodeoxyglucose (FDG) PET/CT was performed one day before ^{64}Cu -NOTA-Trastuzumab PET. After 6 hours of fasting, 370 MBq of ^{18}F -FDG was intravenously injected. The blood glucose level before ^{18}F -FDG injection did not exceed 7.2 mmol/L. One hour after injection, PET images were acquired using a GE Discovery 710 PET/CT (GE Healthcare, Milwaukee, WI, USA).

PET images were reconstructed using a conventional iterative algorithm, ordered-subsets expectation-maximization, with parameters of four iterations and eight subsets. For attenuation correction, CT scans were obtained (130 kVp, 30 mA, 0.6 s/CT rotation, and 6 pitch), after voiding the bladder.

Radiation dosimetry

The internal dosimetry of ^{64}Cu -NOTA-Trastuzumab was evaluated using accumulated radioactivity in PET images. The organ time-activity curve of radioactivity in the target region (ID) divided by target mass (g) were acquired each organ for calculating residence time. The time activity curve was expressed by three time points at 1, 24, and 48 hours. The residence times were calculated by accumulated radioactivity divided by subject administered activity. S-value of source to target region energy deposited per unit mass was calculated using OLINDA/EXM version 1.1 software with an adult female as the model. The organ absorbed doses were calculated as the self-dose and cross-dose from each organ region.

Biodistribution

The biodistribution of ^{64}Cu -NOTA-Trastuzumab was evaluated using the maximum standardized uptake value (SUV_{max}) and the mean standardized uptake value (SUV_{mean}) from the three sequential PET images using GE AW software (GE Healthcare, Milwaukee, WI, USA). For normal-organ distribution, blood, liver, kidney, muscle, spleen, bladder, lung, and bone were analyzed. With tumors, the primary tumor, metastatic lymph nodes (LNs), and metastatic bone lesions were also evaluated. One 2–3 cm sized ellipsoidal volume of interest was drawn inside the organ on the PET images to calculate the SUV.

The lesion to background ratios were calculated to test the degree of ^{64}Cu -NOTA-Trastuzumab uptake at the lesion sites. The SUV_{mean} of the liver or blood was used as the background. The SUV_{max} of the tumors in the breast, metastatic LNs, and metastatic bone lesions were used to assess lesions.

Safety

Safety was assessed before and after the administration of ^{64}Cu -NOTA-Trastuzumab. Feedback such as adverse reactions and other safety-related issues was gathered within 1 month after ^{64}Cu -NOTA-Trastuzumab administration. Adverse events, vital signs, physical examination, and laboratory tests were all considered in the safety evaluation.

Results

Participant characteristics

Seven patients with breast cancer were recruited. One screened participant was excluded for failure of the radioisotope production. Six subjects were evaluated in total.

At the initial diagnosis, IHC results from core needle biopsy showed 3 patients with HER2-positive tumors and 3 patients with HER2-negative tumors. However, after neo-adjuvant chemotherapy, the final IHC results from tumor excision showed 2 patients with HER2-positive tumors and 4 patients with HER2-negative tumors. One subject with IHC score 3+ from core needle biopsy changed the result into IHC score 1+ from excision after neo-adjuvant chemotherapy and this patient was HER2-negative.

The cancer staging of patients were checked from IIA to IV. The period from evaluation of the histology to ^{64}Cu -NOTA-Trastuzumab imaging was 1 to 3 months. All subjects except for subject 1 underwent neo-adjuvant chemotherapy with adriamycin and cyclophosphamide before ^{64}Cu -NOTA-Trastuzumab PET/CT scan. The tumor size at the time of ^{64}Cu -NOTA-Trastuzumab PET/CT scan was measured from 1.7 cm to 14.0 cm.

Detailed subject characteristics are described in Table 1.

Safety

No adverse events were observed related to the use of ^{64}Cu -NOTA-Trastuzumab

Radiation dosimetry

The estimated radiation-absorbed dose for each organ is described in Table 2. The organ with the highest absorbed doses was liver at 0.076 ± 0.007 mGy/MBq. The effective dose was calculated as 0.010 ± 0.001 mSv/MBq. Thus, when injected with 296 MBq of ^{64}Cu -NOTA-Trastuzumab, the effective dose was calculated to be 2.96 mSv. Figure 1 shows the residence time for each organ.

Normal-organ biodistribution and tumor uptake

The uptakes of ^{64}Cu -NOTA-Trastuzumab in normal organs, including blood, liver, kidney, muscle, spleen, bladder, lung, and bone are presented in Fig. 2 as SUV_{mean} . Maximum intensity projection (MIP) images of subject 1 shows the whole-body distribution of ^{64}Cu -NOTA-Trastuzumab in Fig. 3.

The uptake of ^{64}Cu -NOTA-Trastuzumab in the blood showed a high value at 1 hour after injection and a decreasing pattern over time. The SUV_{mean} of the liver also showed a high value at 1 hour after injection and a gradual decrease over time. It was evaluated that the SUV_{mean} of the bladder was maintained at a low value from 1 to 48 hour after injection. Overall, it was confirmed that the uptakes of ^{64}Cu -NOTA-Trastuzumab in blood, liver, kidney, and spleen were relatively high.

The average values of SUV_{max} for HER2 positive tumors were: 1.9 ± 0.8 at 1 hour; 6.3 ± 2.5 at 24 hour; 8.6 ± 5.1 at 48 hour after ^{64}Cu -NOTA-Trastuzumab injection. In case of HER2 negative tumors, the average values of SUV_{max} were: 2.1 ± 1.5 at 1 hour; 5.1 ± 3.4 at 24 hour; 5.2 ± 2.8 at 48 hour after ^{64}Cu -NOTA-Trastuzumab injection. The lesion to liver ratios at 48 hour after injection were 1.8 ± 1.0 and 1.3 ± 0.8 for HER2 positive and negative tumors, respectively. The lesion to blood ratios at 48 hours were 1.6 ± 0.9 and 0.7 ± 0.4 HER2 positive and negative tumors, respectively. Figure 4

shows changes in SUV_{max} of the tumors and tumor-to-background ratios depending on time. Overall, the values of SUV_{max} and tumor-to-background ratios were measured higher in HER2-positive tumors than that of HER2-negative tumors. The values of HER2-positive tumors increased over time up to 48 hours after injection. However, HER2-negative tumors did not show a remarkable increase of the values compared with those of HER2-positive tumors.

The representative images of ^{64}Cu -NOTA-Trastuzumab PET/CT are shown in Fig. 5, and are images of subject 1. Figure 5a (first column) is the ^{18}F -FDG PET/CT image and Figure 5b-5d (second to fourth columns) images are the ^{64}Cu -NOTA-Trastuzumab PET/CT images depending time (on 1, 24, and 48 hours, respectively). Subject 1 was a 45-year-old, left breast cancer patient with multiple metastatic lymph nodes and bones. The tumor showed HER2-positive expression (HER2 3+). The arrows in the upper row show the metastatic lymph node in the left neck. High ^{18}F -FDG uptake is visualized in the ^{18}F -FDG PET/CT (Fig. 5a upper row), and the uptakes of ^{64}Cu -NOTA-Trastuzumab increase over time in the same lesion with the FDG PET/CT images (Fig. 5b-5d upper row). The SUV_{max} of the metastatic lymph node are: 1.2 at 1 hour; 6.5 at 24 hour; 11.6 at 48 hour after injection. The lower row of Fig. 5 shows the primary tumor in the left breast. ^{18}F -FDG uptake can be seen in the left breast cancer (Fig. 5a, lower row, arrow head). The uptakes of ^{64}Cu -NOTA-Trastuzumab can also be observed in the same lesion with increases over time (Fig. 5b-5d, lower row, arrowhead). The SUV_{max} of the primary tumor is as follows: 2.2 at 1 hour; 5.8 at 24 hour; 9.7 at 48 hour after injection.

Discussion

The present study demonstrated that a novel HER2-targeted PET ligand, ^{64}Cu -NOTA-Trastuzumab, was safe, had no adverse effects, and provided a relatively low exposure to radiation (2.96 mSv from a 296-MBq injection). Moreover, the uptakes of ^{64}Cu -NOTA-Trastuzumab was observed in the HER2-expressing tumors including primary breast cancer, metastatic lymph nodes, and metastatic bones.

Due to the heterogeneity of the tumor, HER2 expression may be different between the primary lesion and the metastatic lesions, and may vary depending on the progression of the disease [7, 8]. For this reason, it is important to evaluate HER2 expression before HER2-targeted therapy in order to enhance the treatment efficacy of the patients.

^{64}Cu -DOTA-Trastuzumab PET, one of the methods for evaluating HER2 expression in a non-invasive method, was reported as a feasible modality with clinical trials [5, 6, 13, 14]. Compared to other PET agents, such as ^{89}Zr and ^{124}I , which have been used for evaluating HER2 expression, ^{64}Cu has the benefit of reducing radiation exposure with a relatively short half-life [2], and also has the advantage of being able to perform PET/CT in an outpatient setting. However, previous studies reported that ^{64}Cu -DOTA-Trastuzumab PET has difficulty in distinguishing metastatic lesions or tumors in liver and around blood vessels due to high physiologic uptakes of ^{64}Cu -DOTA-Trastuzumab [6]. It is also well known that the high uptakes of ^{64}Cu -containing agents in the liver and blood [15]. Therefore, we developed ^{64}Cu -NOTA-Trastuzumab using NOTA as a chelator to make a more stable ligand than ^{64}Cu -DOTA-Trastuzumab [2]. Our study showed that relatively higher uptake of ^{64}Cu -NOTA-Trastuzumab in HER2 positive tumors than in HER2 negative tumors. We determined the HER2 specific uptake of ^{64}Cu -NOTA-Trastuzumab from comparing the uptake at HER2 negative tumor. In contrast, previous studies of ^{64}Cu -DOTA-Trastuzumab assessed only HER2 positive tumors without HER2 negative tumors [6, 16]. With *in vitro* and *in vivo* studies, it was reported that ^{64}Cu -NOTA-Trastuzumab shows efficient targeting ability to HER2-expressing tumors [2].

The biodistribution of ^{64}Cu -NOTA-Trastuzumab in the normal organs showed high uptakes in the blood and liver, as was seen in the *in vivo* study. However, ^{64}Cu -NOTA-Trastuzumab shows an advantage in the relative low uptake in the liver compared to ^{64}Cu -DOTA-Trastuzumab. On the other hand, the uptake in the blood is relatively higher than that of to

^{64}Cu -DOTA-Trastuzumab. It is suggested that the relative low uptake in the liver is due to stable copper binding ability of NOTA than that of DOTA, which can reduce the accumulation of free copper. Furthermore, ^{64}Cu -NOTA-Trastuzumab shows a relatively low effective dose (0.014 mSv/MBq) compared to radiolabeled trastuzumab that has been studied so far (^{64}Cu -DOTA-Trastuzumab, 0.036 mSv/MBq; ^{89}Zr -Trastuzumab, 0.61 mSv/MBq), which can reduce radiation exposure in patients [6, 9].

The uptake of ^{64}Cu -DOTA-Trastuzumab in HER2-expressing tumors was not observed at 1 hour after injection. However, the specific uptake increased at 24 hours after injection, and further increase of specific uptake of ^{64}Cu -DOTA-Trastuzumab in HER2-expressing tumors could be observed after 48 hours after injection, showing a distinctive feature from the background. It is suggested that ^{64}Cu -DOTA-Trastuzumab PET at 48 hours after injection can evaluate the HER2 expression in the clinical setting. Mild diffuse uptakes were also observed in the tumors with a negative expression of HER2. This is because HER2 expression is not all-or-none as determined by the immunohistochemistry methods [5, 17]. In other words, even if HER2 expression of the tumor is negative, the SUV_{max} of the tumor increases in proportion to the IHC score of the tumor [5, 17]. Therefore, it is important to set the cut-off values of SUV_{max} or SUV ratio carefully between tumor and background for determining HER2 expression using ^{64}Cu -NOTA-trastuzumab PET image.

This study has some limitations. First, the number of enrolled subjects was relatively small. In addition, since all the patients except for subject 1 underwent neoadjuvant chemotherapy, the SUV of tumors after neo-adjuvant chemotherapy might reflect tumor cell suppression after chemotherapy. Therefore, the SUV might be less than expectation. Especially, in the case of subject 2 and 3 with HER2 positive tumors, it was confirmed that the size and metabolic activity of the tumors were reduced when comparing the ^{18}F -FDG PET images before the neoadjuvant chemotherapy to those performed with ^{64}Cu -NOTA-Trastuzumab PET (data not shown). For this reason, the SUV of HER2 positive tumors on ^{64}Cu -NOTA-Trastuzumab PET may be underestimated in these patients. Therefore, further study with a larger sample size and ^{64}Cu -NOTA-Trastuzumab PET before neoadjuvant chemotherapy is a need to evaluate the exact efficacy of ^{64}Cu -NOTA-trastuzumab PET image.

Conclusion

This preliminary clinical trial evaluated that ^{64}Cu -NOTA-Trastuzumab PET is safe and feasible. ^{64}Cu -NOTA-Trastuzumab showed specific uptake at the HER2-expressing tumors with relatively low uptake in the liver. ^{64}Cu -NOTA-Trastuzumab can be used for the evaluation of radiation dosimetry and prediction of treatment response in targeted therapy for HER2-positive breast cancer with HER2 targeted therapy.

List Of Abbreviations

^{64}Cu -NOTA-Trastuzumab: ^{64}Cu -1,4,7-triazacyclononane-1,4,7-triacetic acid-Trastuzumab, PET: positron emission tomography, HER2: human epidermal growth factor receptor 2, SUV_{max} : maximum standardized uptake value, SUV_{mean} : mean SUV_{max} , SPECT: single-photon emission computerized tomography, ^{64}Cu -DOTA-Trastuzumab: ^{64}Cu -tetraazacyclododecanetetra-acetic acid-Trastuzumab, CT: computed tomography, FDG: ^{18}F -fluorodeoxyglucose

Declarations

Ethics approval and consent to participate

This study was approved by the by the Korean Ministry of Food and Drug Safety (MFDS), and the Institutional Review Board of KIRAMS (IRB No.: KIRAMS 2017-09-006-020). All procedures were performed following the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This clinical trial has been registered with the Clinical Research Information Service (<https://cris.nih.go.kr>), registration number KCT0002790. Informed consent was obtained from all individual participants included in the study.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was supported by a grant from the Korea Institute of Radiological and Medical Sciences (KIRAMS), funded by the Ministry of Science and ICT, Republic of Korea (No. 50547-2020) and the National Research Foundation of Korea (NRF) grant funded by the Korea government (Ministry of Science and ICT) (No. 2019M2D2A1A02057204 and 2017M2A2A7A02070985)

Author contributions

All authors contributed to the study conception and design of this study. IL, BHB, BIK, CWC, MS, HK, WCN, SML, and ILim designed and performed clinical trial, analyzed data and wrote the manuscript; SW, KIK, KCL, and JHK made contributions to conception, analyzing and interpreting data; All authors gave final approval of the final content of the manuscript.

Acknowledgements

Not applicable

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Tables

Table 1
Subject characteristics

Subject no.	Age (years)	Histology	Stage	IHC score* (CNB)	SISH	IHC score† (excision)	Neoadjuvant chemotherapy	Interval from CNB to ⁶⁴ Cu-NOTA-Trastuzumab (months)	Tumor size (cm)
1	45	IDC	IV	3+	N/A	N/A	-	1	7.3
2	42	IDC	IIIB	3+	N/A	3+	+	2	2.4
3	46	IDC	IIIA	3+	N/A	1+	+	3	1.7
4	45	IDC	IIA	-	N/A	-	+	3	2.2
5	56	IDC	IIIB	2+	-	1+	+	3	14.0
6	54	IDC	IIB	-	N/A	-	+	2	4.6
IHC, immunohistochemistry; SISH, silver-enhanced <i>in situ</i> hybridization; CNB, core needle biopsy; IDC, invasive ductal carcinoma; N/A, not applicable									
* IHC score with core needle biopsy at the initial diagnosis									
†IHC score with tumor excision after neoadjuvant chemotherapy									

Table 2
 Dosimetry of ⁶⁴Cu-NOTA-Trastuzumab (OLINDA)

Organ	Absorbed dose (mGv/MBq)		
	⁶⁴ Cu-NOTA-Trastuzumab	⁶⁴ Cu-DOTA-Trastuzumab [6]	[⁸⁹ Zr]Trastuzumab [9]
Adrenals	0.005 ± 0.001	0.031 ± 0.004	0.80
Brain	0.009 ± 0.002	0.015 ± 0.003	0.39
Breasts	0.002 ± 0.000	0.020 ± 0.001	0.42
Gallbladder Wall	0.006 ± 0.001	0.035 ± 0.008	0.86
LLI Wall	0.000 ± 0.000	0.018 ± 0.002	0.58
Small Intestine	0.001 ± 0.000	0.019 ± 0.001	0.57
Stomach Wall	0.008 ± 0.002	0.024 ± 0.002	0.63
ULI Wall	0.002 ± 0.000	0.022 ± 0.002	0.65
Heart Wall	0.042 ± 0.008	0.340 ± 0.046	1.11
Kidneys	0.044 ± 0.009	0.103 ± 0.034	1.23
Liver	0.076 ± 0.007	0.237 ± 0.117	1.63
Lungs	0.034 ± 0.004	0.057 ± 0.070	0.59
Muscle	0.001 ± 0.000	0.023 ± 0.006	0.49
Ovaries	0.001 ± 0.000	0.018 ± 0.002	0.59
Pancreas	0.005 ± 0.001	0.032 ± 0.003	0.78
Red Marrow	0.001 ± 0.000	0.017 ± 0.001	0.69
Osteogenic Cells	0.001 ± 0.000	0.035 ± 0.001	0.79
Skin	0.001 ± 0.000	0.015 ± 0.001	0.34
Spleen	0.063 ± 0.010	0.142 ± 0.040	0.86
Thymus	0.003 ± 0.000	0.030 ± 0.002	0.57
Thyroid	0.000 ± 0.000	0.016 ± 0.001	0.43
Urinary	0.003 ± 0.001	0.023 ± 0.006	0.42
Uterus	0.001 ± 0.000	0.018 ± 0.002	0.58
Total Body	0.004 ± 0.000	0.029 ± 0.004	0.55
Effective dose (mSv/MBq)	0.010 ± 0.001	0.036 ± 0.009	0.61

Figures

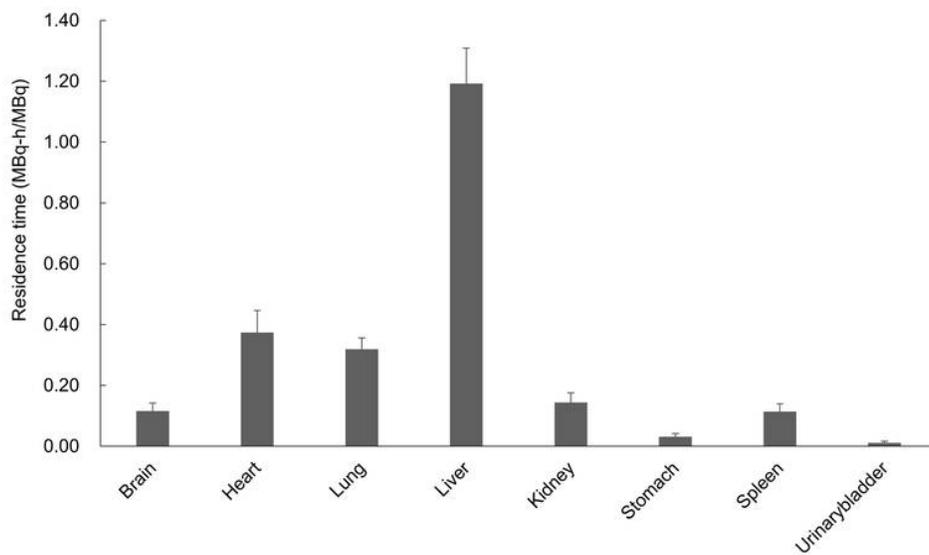


Figure 1

Residence time derived from serial positron emission tomography images (PET). Mean organ residence times (\pm standard deviation) for ^{64}Cu -NOTA-Trastuzumab.

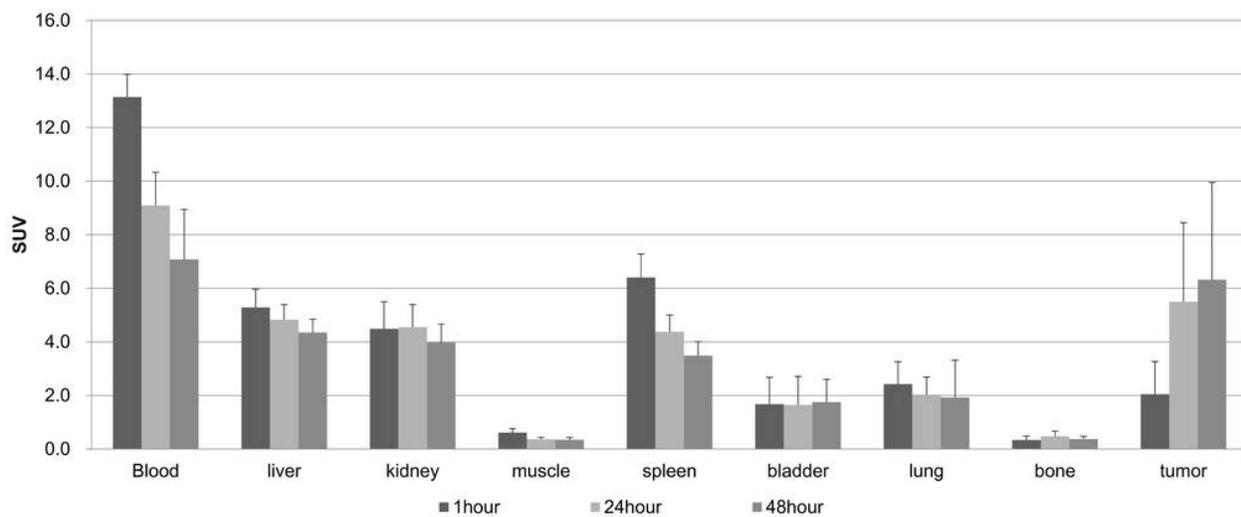


Figure 2

Mean standardized uptake value (SUV_{mean}) with standard error of ⁶⁴Cu-NOTA-Trastuzumab in normal organs and maximum standardized uptake value (SUV_{max}) with standard error of ⁶⁴Cu-NOTA-Trastuzumab in tumors

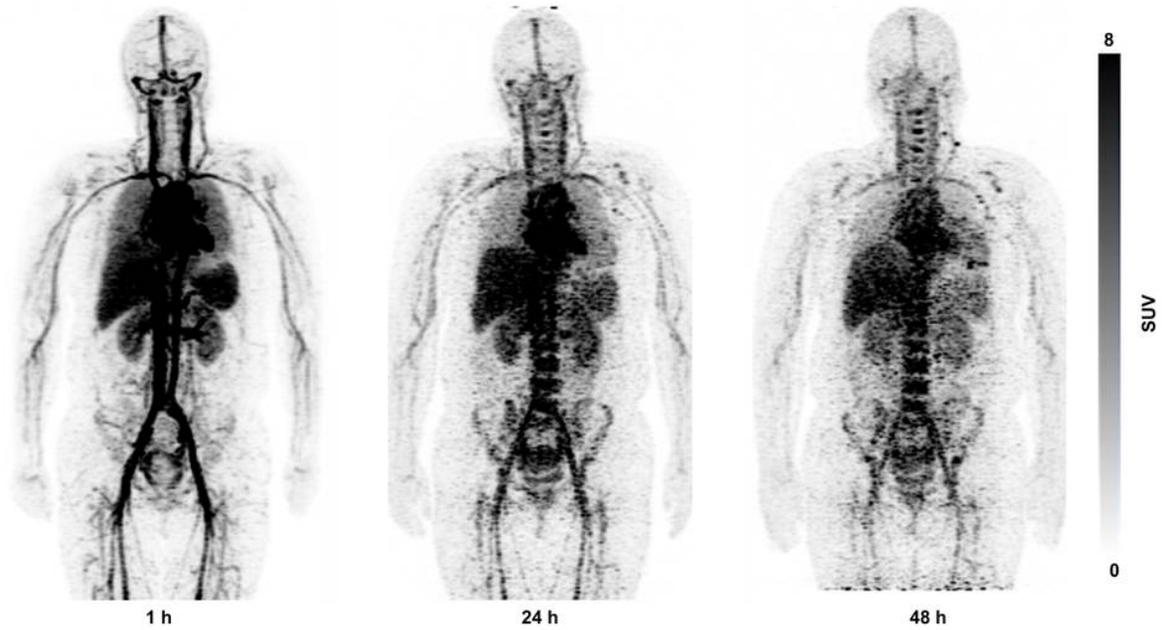


Figure 3

Maximum intensity projection images of ⁶⁴Cu-NOTA-Trastuzumab PET at 1, 24, and 48 hours after injection

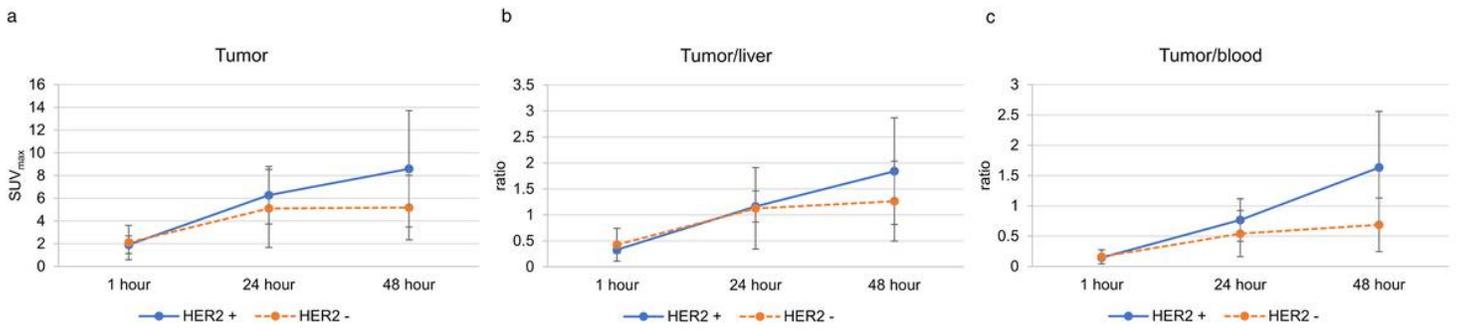


Figure 4

The changes of SUVmax (a), tumor-to-liver ratio (b), and tumor-to-blood pool ratio (c) of HER2-positive and HER2-negative tumors over time.

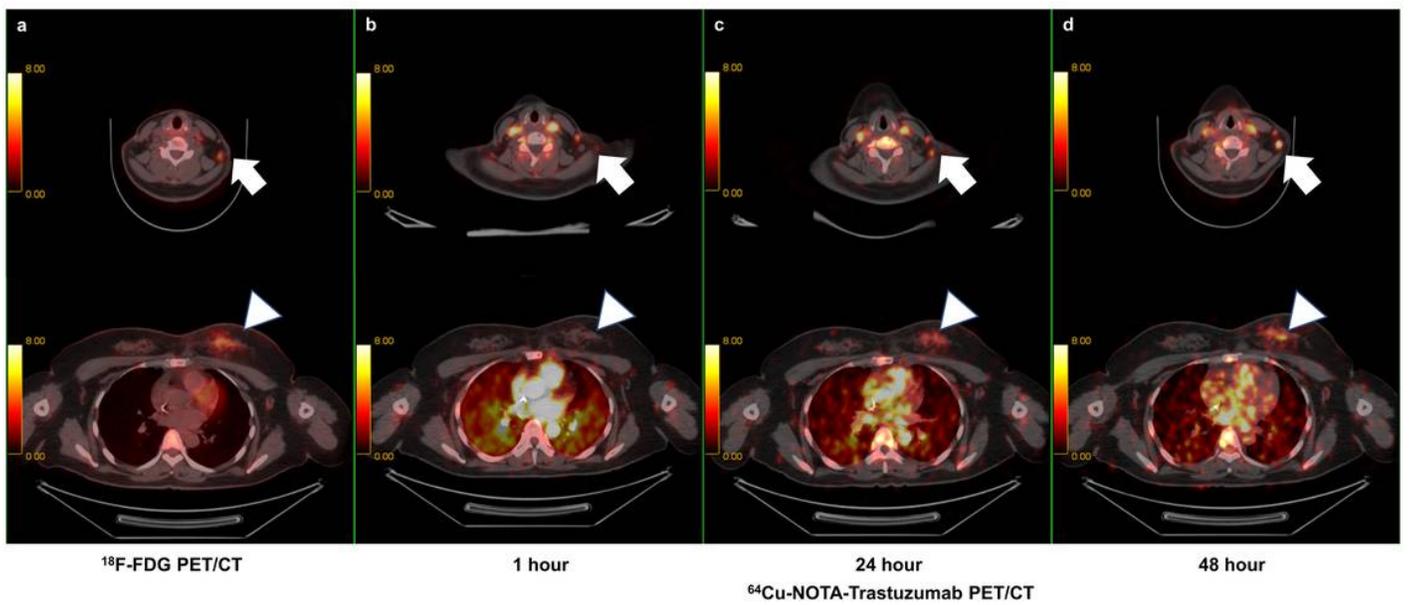


Figure 5

^{64}Cu -NOTA-Trastuzumab PET images of HER2-positive breast cancer (arrow heads, lower row) and metastatic lymph node (arrows, upper row). The primary tumor and metastatic lymph nodes were clearly observed by ^{18}F -FDG PET/CT (a) and ^{64}Cu -NOTA-Trastuzumab PET/CT (b-d). In HER2-expressing lesions, it was observed that the uptakes of NOTA increased over time to 48 hours after injection.