In-111-Trastuzumab Scintigraphy in HER2-Positive Metastatic Breast Cancer Patients Remains Feasible during Trastuzumab Treatment

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RESEARCH ARTICLE

^{111}In-Trastuzumab Scintigraphy in HER2-Positive Metastatic Breast Cancer Patients Remains Feasible during Trastuzumab Treatment

Sietske B.M. Gaykema, Johan R. de Jong,* Patrick J. Perik, Adrienne H. Brouwers, Carolien P. Schröder, Thijs H. Oude Munnink, Alphons H.H. Bongaerts, Elisabeth G.E. de Vries, and Marjolijn N. Lub-de Hooge*

Abstract

Human epidermal growth factor receptor (HER)2 imaging with radiolabeled trastuzumab might support HER2-targeted therapy. It is, however, frequently questioned whether HER2 imaging is also possible during trastuzumab treatment as the receptor might be saturated. We studied the effect of trastuzumab treatment on ^{111}In-trastuzumab uptake. Patients received trastuzumab weekly and paclitaxel once every 3 weeks. ^{111}In-trastuzumab was injected on day 1 of cycle 1 and day 15 of cycle 4. Whole-body planar scintigraphy was acquired at different time points postinjection. Tumor uptake and organ distribution between the first and repeated scan series were calculated via residence times. Twenty-five tumor lesions in 12 patients were visualized on both scintigraphy series. Tumor uptake decreased (19.6%; $p = .03$). The residence times of normal organs remained similar except for the cardiac blood pool (+ 16.3%; $p = .014$). Trastuzumab treatment decreases tumor ^{111}In-trastuzumab uptake around 20%. HER2 imaging is feasible during trastuzumab treatment.

TUMOR OVEREXPRESSION or amplification of the human epidermal growth factor receptor (HER)2 occurs in 25 to 30% of patients with breast cancer. It is involved in tumor cell survival, proliferation, maturation, dissemination, and angiogenesis and has antiapoptotic effects.[1,2] Trastuzumab is a humanized monoclonal antibody approved for treatment in the (neo)adjuvant and metastatic setting of patients with HER2-positive breast cancer. The addition of trastuzumab to chemotherapy results in an increased time to disease progression, higher objective response rates, and longer overall survival.[3,4] Accurate characterization of HER2 expression is essential for optimal therapy. Therefore, HER2 status should be assessed in all patients with breast cancer to identify HER2-positive tumors. Ex vivo methods to determine the HER2 status of the primary tumor are immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH). These methods require biopsies, which are not always feasible. Furthermore, sampling error cannot be ruled out with repeated biopsies. This is particularly relevant in view of possible heterogeneous HER2 expression. Heterogeneity can exist between the primary tumor and the metastases,[5] between different metastases, and within a metastasis. Discordance of HER2 expression between primary tumors and metastases, as measured by IHC and/or FISH, varies between 10 and 24%.[6,7] Loss or gain of HER2 expression can have clear therapeutic consequences as patients with HER2-positive lesions benefit from anti-HER2 therapy.[3,8] This underlines the necessity to accurately assess HER2 status during the course of metastatic breast cancer. Noninvasive determination of HER2 expression can potentially be performed with molecular imaging. This might facilitate selection of patients for HER2-targeted therapy and assess the immediate response to therapeutic interventions. In the clinical setting, HER2 imaging may be performed with trastuzumab radiolabeled with the γ-emitter indium 111 (^{111}In) and with the positron emission tomography (PET) isotope zirconium 89 (^{89}Zr).[9,10] Experience with HER2 imaging increases; however, this comes with the frequently asked questions as to whether and how trastuzumab treatment affects the HER2 scan as the receptor may be already occupied. We previously reported about ^{111}In-trastuzumab scintigraphy in 17 patients

*Authors who contributed equally to this work.

From the Departments of Medical Oncology, Nuclear Medicine and Molecular Imaging, Radiology, and Hospital and Clinical Pharmacy, University of Groningen, University Medical Centre Groningen, Groningen, the Netherlands.

Address reprint requests to: Marjolijn N. Lub-de Hooge, PharmD, PhD, Department of Hospital and Clinical Pharmacy, University Medical Centre Groningen, PO Box 30001, 9700 RB Groningen, the Netherlands; e-mail: M.N.de.Hooge@umcg.nl.

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with HER2-overexpressing metastatic breast cancer after the first loading dose of trastuzumab.[9] Some of these patients underwent a second scintigraphy procedure during treatment. This gives us the unique opportunity to assess the feasibility of HER2 imaging in patients while on trastuzumab treatment. Therefore, the aim of this study was to quantitatively describe the biodistribution and uptake of ^{111}In-trastuzumab over time during 12 weeks of trastuzumab treatment.

Methods

Patients and Treatment

Eligibility criteria were described earlier.[9] In short, patients were women with histologically confirmed HER2-positive metastatic breast cancer eligible for treatment with paclitaxel and trastuzumab. After the loading dose of 4 mg/kg, trastuzumab was administered as a weekly intravenous infusion of 2 mg/kg. Paclitaxel (175 mg/m^2) was administered intravenously in 4 hours as an intravenous infusion, once every 3 weeks for six cycles. The study was approved by the local medical ethical committee. All patients provided written informed consent.

^{111}In-Trastuzumab Scintigraphy

Trastuzumab was radiolabeled with ^{111}In, as previously described.[11] A total of 100 to 150 MBq of 5 mg ^{111}In-trastuzumab was injected intravenously within 24 hours after the infusion of the trastuzumab loading dose at baseline. ^{111}In-trastuzumab injection was repeated after the trastuzumab dose on day 15 of the fourth cycle (14 weeks later). Whole-body planar scintigraphy was performed at four different time points at 15 minutes and 24, 72 to 96, and 168 hours postinjection (Figure 1). A dual-head Multispect-2 camera or dual-head E.Cam camera was used (Siemens, CTI, Knoxville, TN) as previously described.[9] Scintigraphy series for each patient series were performed on the same camera. Only patients with a first and a repeated scintigraphy series were analyzed. For quality control of dosimetry calculations, an aliquot of dose containing a known fraction of the injected radioactivity was positioned adjacent to the patient during scanning.

^{111}In-Trastuzumab Tumor Uptake, Organ Distribution, and Radiation Dosimetry

The uptake of ^{111}In-trastuzumab was determined by calculating residence times for tumors and organs. Residence time was defined as the area under the curve of radioactivity versus time (time-activity curve) and was calculated using the *SPRIND* (Radboud University Medical Center, Nijmegen, The Netherlands) software package.[12] The set of organs for which the residence times were calculated was limited to those organs that were clearly distinguishable on planar scintigraphy. Also, the residence time was used for internal radiation dose assessment according to the medical internal radiation dose (MIRD) scheme.[13] The radiation absorbed dose for all organs of interest and the effective dose were determined in accordance with International Commission on Radiological Protection Publication 60 (ICRP 60).[14] The estimated dose (ED) was calculated as a weighted mean of the absorbed radiation dose over organs defined within the ICRP framework. Organ-level internal radiation dose calculations using the MIRD and ICRP 60 are implemented in the *OLINDA/EXM* (Vanderbilt University, Nashville, TN) software package.[15] *OLINDA/EXM* incorporates a set of phantoms (e.g., adult male, adult female) that define the size of organs and the geometric relation between them. This determines the contribution from radioactivity in one organ to the absorbed dose in another organ. The quantitative results of the scintigraphy series were statistically tested for difference between the first and repeated scintigraphy series.

Statistical Analysis

Data are presented as mean $\pm$ standard deviation. Statistical analysis was performed using the Wilcoxon test for paired nonparametric data (*SPSS* version 19, IBM, Armonk, NY). A double sided p value $< .05$ was considered significant.

Results

Patient Characteristics

Seventeen patients were included between January 2002 and June 2004. In 12 patients, serial analyses of tumor uptake, organ absorbed dose, and radiation dosimetry, determined from ^{111}In-trastuzumab scintigraphy on first and second scintigraphy series, could be assessed. Of the remaining five patients, three were withdrawn from the study prematurely due to clinical deterioration and/or disease progression, and in two patients, the scintigraphy series were incomplete.

^{111}In-Trastuzumab Tumor Uptake

In these 12 patients, 25 tumor lesions in total were detected on ^{111}In-trastuzumab scintigraphy at the first and

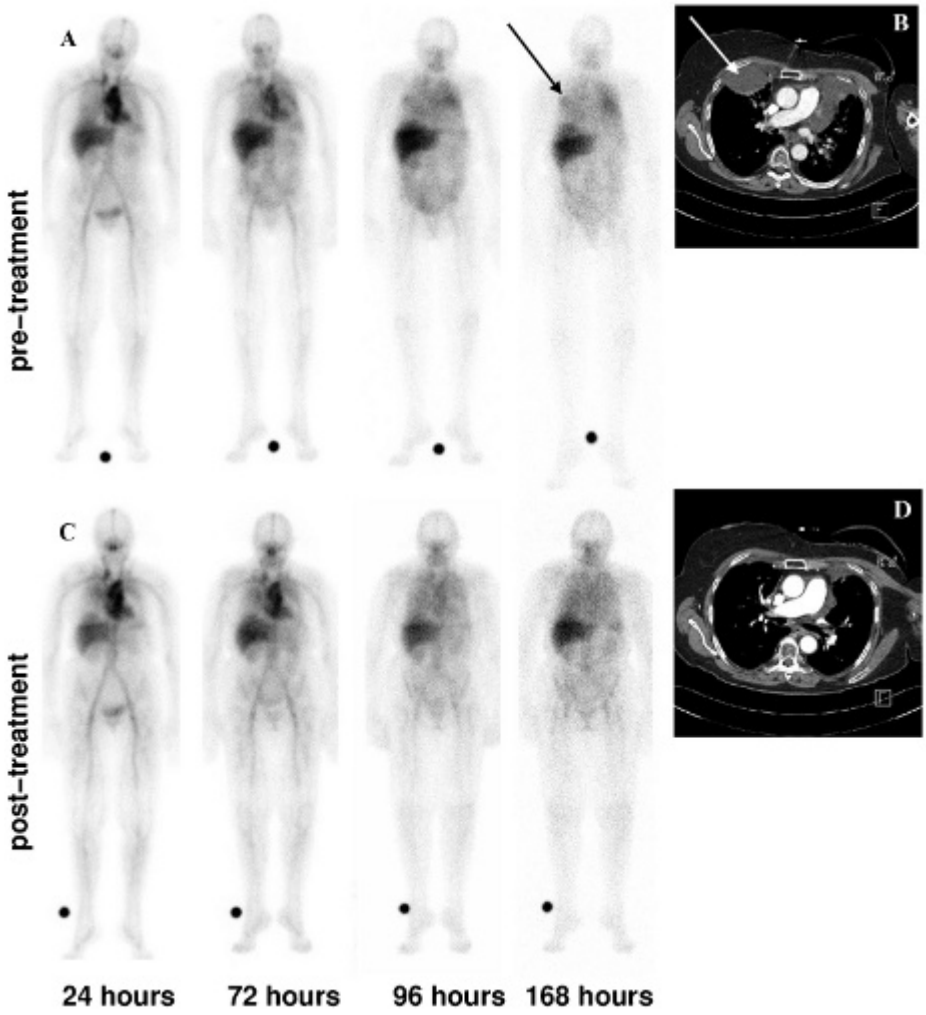

Figure 1. ^{111}In-trastuzumab scintigraphy at 24, 72, 96, and 168 hours postinjection in the first (A) and repeated (C) scan series. The pleural lesion on computed tomography (indicated with an *arrow*) pretreatment (B) is almost completely disappeared after treatment (D).

repeated scintigraphy series during treatment (see Figure 2 for an example). In 20 of the 25 tumor lesions, the ^{111}In-trastuzumab uptake was decreased at the repeated compared to the first scintigraphy series. The mean residence time in tumor lesions was 0.22 ± 0.30 hours (range 0.04–1.26 hours) at baseline and 0.14 ± 0.17 hours (range 0.02–0.79 hours) after 12 weekly therapeutic trastuzumab doses. Between the first and the repeated scintigraphy series, there was a mean reduction in ^{111}In-trastuzumab uptake per lesion of 19.6% ± 53.8 (range increase of 72.9% to a decrease of 188.5%; $p = .003$). The mean reduction per patient was 17.0% ± 35.0 ($p = .07$). In the imaging time series, the tumor to background ratio increased from the earliest time point up to the latest time point (168 hours). Also, the visibility of the lesions continued to increase at later time points. Within the time scale of imaging in the present study, the limiting factor is the radioactive decay, which causes an increase in noise in the images over time. Up to 72 hours, blood vessels are still clearly visible, indicating relatively high plasma levels of trastuzumab. For imaging or uptake assessment, a minimum time of 96 hours (4 days) between tracer injection and imaging is recommended.

^{111}In-Trastuzumab Organ Distribution and Radiation Dosimetry

The residence times of healthy organs remained the same between the first and the repeated scintigraphy series, except for an increase in the cardiac blood pool ($p = .014$) (Table 1). The absorbed dose for the organs and the effective dose thus also remained similar. The effective dose of ^{111}In-trastuzumab was 0.19 ± 0.02 mSv/MBq. The three organs with the highest absorbed dose were liver (0.60 ± 0.18 mGy/MBq), spleen (0.36 ± 0.08 mGy/MBq), and heart wall (0.34 ± 0.05 mGy/MBq). The three organs with

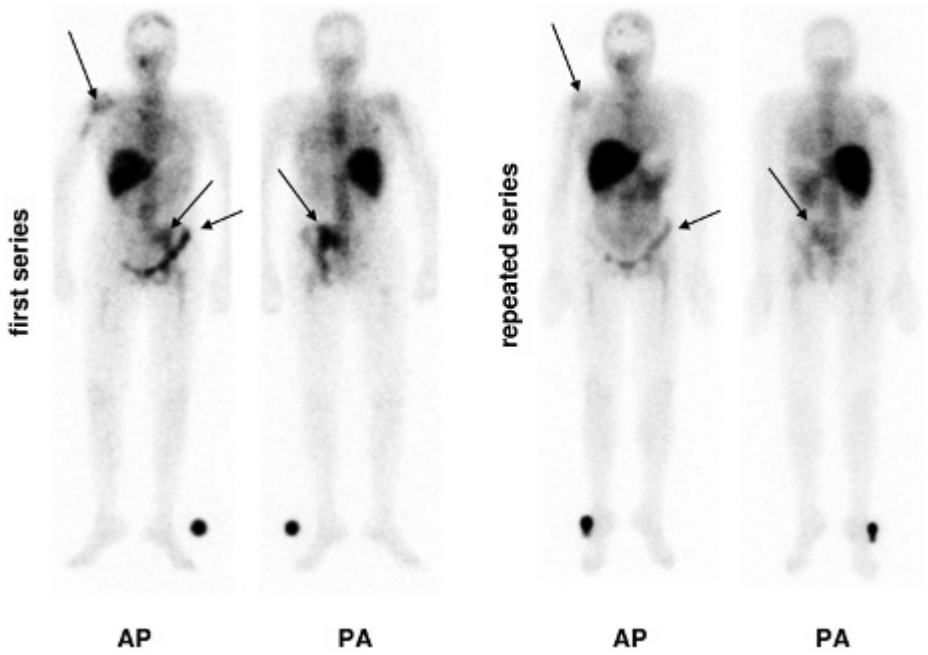

Figure 2. ^{111}In-trastuzumab scintigraphy in a patient with multiple bone lesions in the right humerus, and left os ilium (iliac crest and adjacent to sacroiliac joint) (*arrows*) at the first and repeated scan series.

the highest contribution to the effective dose were liver (0.030 ± 0.009 mSv/MBq), lungs (0.035 ± 0.007/mSv MBq), and ovaries (0.032 ± 0.004 mSv/MBq) (Table 2).

Discussion and Conclusions

This is the first study with serial ^{111}In-trastuzumab scintigraphy at the start and during trastuzumab treatment in patients with HER2-positive metastatic breast cancer. It showed persistent ^{111}In-trastuzumab uptake in all tumor lesions during trastuzumab treatment, with only a 20% lower absolute uptake after 12 weeks of trastuzumab treatment. This indicates that sufficient HER2 is constantly available at the tumor cell membrane to bind trastuzumab and that it is impossible to completely saturate the receptor. Furthermore, this is the first study to present radiation dosimetry data for ^{111}In-trastuzumab.

Table 1. Calculated Residence Times with Standard Deviations of Measured Organs in First and Repeated Scan Series

	Residence Time (h)	
Organ	*First*	*Repeated*
Blood pool	2.39 ± 0.71	2.78 ± 0.71
Kidney	0.86 ± 0.30	0.86 ± 0.31
Liver	12.96 ± 5.15	11.13 ± 4.29
Lung	3.26 ± 1.09	3.36 ± 1.25
Spleen	0.91 ± 0.33	0.92 ± 0.27
Red marrow	0.78 ± 0.19	0.90 ± 0.22

There are several potential explanations contributing to the 19.6% lower ^{111}In-trastuzumab uptake during trastuzumab treatment. It may be the consequence of the antitumor effect of paclitaxel and trastuzumab on the size of the tumor lesion or on HER2 expression itself. Unfortunately, rigorous evaluation of size effects of all tumor lesions was not possible. A feature of metastatic breast cancer is that, very frequently, metastases are located in the bones. This makes them most often not accessible for official analyses according to Response Evaluation Criteria in Solid Tumors (RECIST). Also, no serial biopsies were taken, so downregulation of HER2 expression could not be assessed. The final important explanation for the lower ^{111}In-trastuzumab uptake during treatment might be that therapeutic trastuzumab circulating in the blood competes with ^{111}In-trastuzumab for binding to HER2. Of the normal organs, only the cardiac blood pool showed a 16% higher uptake of ^{111}In-trastuzumab after the second injection during treatment. This can be explained by the fact that the trastuzumab elimination half-life is dose dependent; after multiple trastuzumab doses, half-life time increases.[16] However, we showed that tumor lesions could still be clearly visualized under trastuzumab treatment. Thus, we conclude that there is no complete downregulation or saturation of the HER2. Preferably, this would be confirmed with HER2 staining of tumor tissue in future clinical studies.

The disadvantage of biopsies is that there is only information on a small piece of a metastasis, although the tumor and its metastases can be heterogeneous.[5] However,

Table 2. Radiation Absorbed Dose Estimates for Organs and Effective Dose Contribution of ICRP Publication 60 Target Organs on the First Scan Series

Organ	*Radiation Absorbed Dose (μGy/MBq) ± SD*	*ICRP 60 Effective Dose Contribution (μSv/MBq) ± SD*
Adrenal gland	230 ± 17	1.1 ± 0.2
Brain	106 ± 16	0.5 ± 0.1
Breast	115 ± 12	5.7 ± 0.6
Gallbladder wall	277 ± 37	*
Lower colon wall	152 ± 21	18.3 ± 2.6
Small intestine	156 ± 16	0.7 ± 0.1
Stomach wall	181 ± 16	21.8 ± 1.9
Upper colon wall	180 ± 15	0.9 ± 0.2
Heart wall	339 ± 49	*
Kidney	268 ± 42	1.3 ± 0.3
Liver	598 ± 183	29.9 ± 9.1
Lung	291 ± 54	34.9 ± 6.5
Muscle	131 ± 14	0.6 ± 0.1
Ovary	158 ± 21	31.7 ± 4.1
Pancreas	238 ± 17	1.1 ± 0.2
Red marrow	132 ± 15	15.8 ± 1.8
Skin	84 ± 10	0.8 ± 0.1
Spleen	360 ± 78	2.8 ± 3.2
Thymus	167 ± 21	0.8 ± 0.2
Thyroid	117 ± 17	5.8 ± 0.8
Urinary bladder wall	140 ± 20	7.0 ± 1.0
Uterus	156 ± 21	0.7 ± 0.1
Total body	151 ± 13	*
Effective dose	*	185 ± 16

*Not defined.

preclinical data are available. Preclinical evaluation of change of HER2 status on trastuzumab therapy with molecular imaging was performed previously in two studies with a human breast cancer xenograft. Micro–single-photon emission computed tomography (SPECT) with ^{111}In-pertuzumab and ^{18}F-FBEM-$_{HER2:243}$ was performed pre- and posttrastuzumab treatment in mice bearing human tumor xenografts. Uptake of the tracers was decreased, but not completely blocked, after 3 weeks of treatment, which corresponded with changes in tumor size.[17,18] Distinct from our study, there was no competition between the tracer and the therapeutic agent in these studies because both tracers bind to another domain of HER2 than trastuzumab. Thus, extrapolating to our study results, we conclude that during trastuzumab treatment, visualization of tumor lesions with ^{111}In-trastuzumab is feasible and not completely blocked or saturated, despite this competition of the (subtherapeutic amount of) radiolabeled trastuzumab with therapeutic trastuzumab.

In a human HER2-overexpressing SKOV-3 ovarian tumor xenograft, there was higher uptake of ^{111}In-trastuzumab compared to a HER2-negative tumor xenograft,[11] indicating tumor-specific uptake.

As no significant differences in organ distribution were found, the radiation absorbed dose of organs between the two scintigraphy series is comparable. Radiation dose estimates with ^{111}In-trastuzumab were comparable to those calculated by others.[19] Trastuzumab can also be radiolabeled with the positron emission tomography (PET) isotope copper 64 (NCT01093612) or zirconium 89 (^{89}Zr) for clinical purposes. PET provides a higher spatial resolution, a better signal to noise ratio, and better quantification. No dosimetric results for ^{89}Zr-trastuzumab have been published so far. However, as the physical half-lives of ^{111}In and ^{89}Zr are comparable, the data for ^{111}In-trastuzumab can be used to estimate the ED for ^{89}Zr-trastuzumab, resulting in an ED of 0.5 mSv/MBq ^{89}Zr. For a typical administration of 37 MBq of ^{89}Zr-trastuzumab, the radiation dose amounts to 18 mSv, which is comparable to the radiation dose of 100 MBq ^{111}In.

We realize that this study has several shortcomings, mainly because it was originally not intended and powered to

look at tumor size and HER2 expression over time. However, although experience with molecular HER2 imaging is expanding, we wondered whether trastuzumab treatment affects imaging. The fact that this study showed that all tumor lesions remained visible during treatment means that HER2 imaging is feasible even during trastuzumab treatment.

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