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\textbf{\textsuperscript{111}In-labeled Trastuzumab Scintigraphy in Patients with HER2-Positive Metastatic Breast Cancer}

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\textit{Submitted for publication}
ABSTRACT

Background
The cardiac and anti-neoplastic effects of trastuzumab may be related to specific uptake of trastuzumab in myocardium and tumor tissue, respectively. Our aim was to evaluate whether \(^{111}\)In-labeled trastuzumab scintigraphy can predict cardiotoxicity and identify tumor lesions.

Patients and Methods
Patients with HER2-positive metastatic breast cancer underwent gamma-camera imaging from 15 minutes to 7 days after injection of 150 MBq \(^{111}\)In-DTPA-trastuzumab, prior to and after 12 doses of trastuzumab (2 mg/kg weekly, following 4 mg/kg loading dose) and 4 cycles of concomitant paclitaxel (175 mg/m\(^2\) 3-weekly). Cardiac and tumor assessments were performed before treatment, after 4 and 6 cycles, tumor evaluation also after 2 cycles. \(^{111}\)In-DTPA-trastuzumab tumor uptake was expressed as tumor/background (T/BG) ratio and % injected dose index (%ID).

Results
15 of the 17 patients were available for cardiac and tumor uptake analysis. Pre-treatment myocardial \(^{111}\)In-DTPA-trastuzumab uptake was observed in one patient with pre-existent cardiac arrhythmias, who did not develop symptomatic heart failure during treatment. Three other patients developed severe symptomatic heart failure, without initial myocardial \(^{111}\)In-DTPA-trastuzumab uptake, while one showed weak myocardial uptake after 4 cycles. Uptake in bloodpool and organs such as spleen and kidneys decreased, while tumor and liver uptake increased between days 1-7. Mean tumor uptake was 1.2 (±SD 0.9) %ID and T/BG ratio was 3.0 (± 1.0), 5 days after injection. In 87% of the patients, pre-treatment scans revealed new tumor lesions.

Conclusions
Radiolabeled trastuzumab scintigraphy was not valuable in predicting trastuzumab-related cardiotoxicity in metastatic breast cancer patients, but can identify HER2-positive tumor.
INTRODUCTION

Tumor overexpression or amplification of the human epidermal growth factor receptor 2 (HER2, also known as erbB-2) occurs in 25-30% of patients with breast cancer and adversely affects their prognosis. The addition of trastuzumab to chemotherapy in HER2-positive metastatic breast cancer patients, resulted in an increased time to disease progression, higher objective response rates and longer overall survival. However, an increased incidence of cardiac dysfunction was observed as the most important adverse effect. Recently, interim analyses of three large randomized trials, evaluating trastuzumab after standard chemotherapy in HER2-positive breast cancer patients, showed beneficial effects of the addition of trastuzumab to standard adjuvant treatment. The European HERA trial and combined analysis of the NSABP-B31 (US) and the NCCTG N9831 (Canada) trials showed a prolongation of disease free survival. The combined analysis of NSABP-B31 and NCCTG N9831 also showed improved overall survival. However, cardiac interim analysis of one of these studies revealed that symptomatic heart failure had occurred in 3.3% after more than 6 months since the start of paclitaxel plus trastuzumab, compared to none of the patients who received paclitaxel alone. An additional 15% of the patients were taken off study for an asymptomatic decrease in left ventricular ejection fraction (LVEF). As trastuzumab is now entering the clinic for the adjuvant treatment of HER2-positive breast cancer patients as well, the issue of trastuzumab-related cardiotoxicity becomes increasingly relevant.

The mechanism of trastuzumab-induced cardiac toxicity is still unclear. In rodents, HER2 plays a critical role in the development of the heart. Mice deficient in erbB-2 or neuregulin, an erbB-2 activating growth factor, die early in embryonic development with severe cardiac abnormalities. Furthermore, mice with an erbB-2 gene deletion restricted to the heart develop a severe dilated cardiomyopathy shortly after birth. Myocardial HER2 mRNA expression increased after implantation of a left ventricular assist device in patients with severe heart failure, while prior to implantation, HER2 mRNA expression was similar to healthy controls. In addition, weak positive immunohistochemical HER2 staining was observed in myocardial biopsies from six of 60 patients with severe heart failure. Therefore, trastuzumab may induce cardiotoxicity by specific binding to HER2 expressed in the myocardium, leading to cardiomyocyte death.

To date, LVEF measurement by multigated radionuclide angiography (MUGA) or cardiac ultrasound is generally accepted as the method of choice to detect cardiotoxicity induced by antineoplastic treatment. However, MUGA only detects cardiac functional loss, after the myocardial injury has occurred. Previously, we have described the development of radiolabeled trastuzumab for clinical use and shown in a xenograft model that tumor HER2 expression can be visualized with $^{111}$In-labeled trastuzumab scintigraphy. Moreover, in a
preliminary report, Behr and colleagues suggested that radiolabeled trastuzumab uptake in the myocardium and tumor, could predict cardiotoxicity and response to trastuzumab treatment, respectively.12 Molecular imaging of HER2 with radiolabeled trastuzumab may improve our understanding of the mechanism of action of trastuzumab. Up to now, data with regard to the degree of HER2 expression in different metastatic lesions within patients is limited. Nevertheless, HER2 status of metastatic sites may differ from primary tumors.13 Discordant HER2 expression levels has been described between primary tumors and asynchronous metastases or recurrence.14 Radiolabeled trastuzumab scintigraphy, may improve our insights into the intra- and interpatient variation in HER2 expression in metastatic lesions in vivo.

The primary aim of the current study was to evaluate whether radiolabeled trastuzumab can be used for the identification of patients at risk of developing cardiac dysfunction during treatment with trastuzumab and paclitaxel. The secondary aim was to evaluate whether this technique can be used to demonstrate localizations and uptake intensity of HER2 positive tumor localizations.

PATIENTS AND METHODS

Patients
Eligible patients were aged 18 years or older, diagnosed with HER2-positive metastatic or locally advanced breast cancer, suitable for treatment with paclitaxel and trastuzumab, and had an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2. Before initiation of therapy, all patients underwent routine staging evaluations, which included a complete history, physical examination and blood chemistry profile, in addition to a chest radiography, computed tomography or ultrasound of the liver and a bone scan. Tumors were considered HER2 positive, if immunohistochemistry (IHC) showed 2/3+-membrane overexpression. Exclusion criteria were treatment with any investigational drug within 30 days prior to the start of the study, radiotherapy within 4 weeks of enrollment, serious uncontrolled central nervous system metastases, LVEF < 40% (MUGA), or symptomatic heart failure New York Heart Association (NYHA) functional class III or IV. In addition, patients suffering from uncontrolled serious concurrent illness, dyspnea at rest due to malignant disease, dyspnea that required oxygen therapy, or any of the following abnormal laboratory tests: neutrophil count < 1.5 x 10⁹/L, platelets < 100 x 10⁹/L, serum total bilirubin > 1.5 x upper limit of normal (ULN), ALT or AST > 2.5 x ULN (> 5.0 x ULN in case of liver metastases), alkaline phosphatase > 2.5 x ULN (> 4.0 x ULN in case of liver or bone metastases), or serum creatinin > 1.5 x ULN, were not eligible.

The study was approved by the local medical ethics committee and written informed consent was obtained from all participants.
Treatment
Patients were assigned to six three-weekly cycles of trastuzumab and paclitaxel. After the loading dose of 4 mg/kg bodyweight, which was administered as an intravenous infusion during 90 minutes, trastuzumab was administered as a weekly intravenous infusion of 2 mg/kg bodyweight, in 30 minutes. Paclitaxel (175 mg/m²) was administered in 4 hours as an intravenous infusion, once every three weeks. The first dose of paclitaxel was given the day after the trastuzumab loading dose. Subsequent paclitaxel infusions were administered on the same day as the trastuzumab infusions. Toxicity was coded according to the NCI common toxicity criteria (CTC) scale V3.0.

Cardiac function
Assessment of left ventricular function (history and physical examination, LVEF measurement by MUGA scan and standard cardiac ultrasonography) was performed at baseline, after 4 treatment cycles and after completion of the treatment regimen. A 12-lead electrocardiogram was obtained before enrollment. Peripheral blood samples for measurement of serum cardiac troponin I (TnI) and plasma N-terminal pro-B-type natriuretic peptide (NT-proBNP) were collected before treatment, 1 and 7 days after the first trastuzumab infusion, and at the end of each treatment cycle. Serum TnI levels were measured with a micro-particle enzyme immunoassay (Abbott Axsym system, Abbott Diagnostics Division, Abbott Park, IL), with a detection limit of 0.1 g/L. Values of more than 0.5 g/L indicate myocardial injury. EDTA plasma NT-proBNP concentrations were analyzed with an electrochemoluminescence immunoassay (Roche Diagnostics, Vienna, Austria), with a cut-off value of 125 ng/L.15

In the patients who developed symptomatic left ventricular dysfunction and/or a LVEF < 40%, trastuzumab was discontinued and they were taken off study.

Response evaluation
Tumor response was defined according to the Response Evaluation Criteria in Solid Tumors Group criteria.16 Patients with progressive disease were taken off study.

111In-DTPA-trastuzumab scintigraphy and pharmacokinetics
Trastuzumab was radiolabeled with 111In, using diethylenetriamine penta-acetic acid anhydride (DTPA) as a chelator, as described previously (radiochemical purity > 95%).17 Radiolabeled trastuzumab scintigraphy was performed at the start of the treatment and on day 15 of the fourth treatment cycle, after 12 trastuzumab infusions. 100-150 MBq 111In-DTPA-trastuzumab (5 mg) was injected intravenously, within 24 hours after the infusion of the trastuzumab loading (4 mg/kg) or maintenance dose (2 mg/kg). Planar whole body imaging, using a two-headed gammacamera equipped with medium-energy all purpose collimators at a scan speed of 12 centimeter/minute, were performed 15 minutes to 7 days after tracer injection. Single photon emission computed tomographic (SPECT) images of
the heart region were also obtained, using 2 x 32 projections of 60 seconds duration. For pharmacokinetic analysis heparin blood samples were obtained at 15, 30 minutes, 1, 2, 3, 6, 24, 96, 168 and 336 hours after injection of radiolabeled trastuzumab. Total whole blood and plasma radioactivity were determined in the samples using a gammacounter (well type LKB-1282-Compu-gamma system, LKB Wallac, Turku, Finland). Urine samples, obtained from collected 24 hours urines, collected separately for 7 days were also analyzed for radioactivity. To correct for physical decay, injection standards were counted simultaneously.

Pharmacokinetic parameters were derived using the KINFIT module of the MW/PHARM computer program package (version 3.50, MediWare, Groningen, The Netherlands). Clearance rates of $^{111}$In-DTPA-trastuzumab from the circulation were calculated using non-linear regression analysis. Based on the curves the number of hours required for 50% of the activity to be removed from the blood ($T_{1/2}$) in the distribution phase ($\alpha$) and elimination phase ($\beta$) of the curve was determined. Other calculated pharmacokinetic parameters were volume of distribution (Vd), total clearance (CL) and mean residence time (MRT).

**Image and data analysis**

SPECT reconstructions were performed with the ordered subset expectation maximization algorithm. Images were interpreted by a nuclear medicine specialist (PLJ), blinded for clinical information. Dosimetric data of $^{111}$In-DTPA-trastuzumab were obtained from the total body scans using visually determined regions of interest (ROIs). Myocardial uptake was assessed visually from spotviews of the cardiac region and SPECT short-axis reconstructions. In addition to the primary visual analysis, semi-quantitative methods were used to quantify $^{111}$In-DTPA-trastuzumab uptake in regions with increased uptake, determined on whole body images. Radioactive counts from visually determined ROIs of areas with increased radiolabeled trastuzumab uptake were compared with radioactive counts from a standardized and representative background area (shoulder/axillary region) in the same whole body image, to estimate the ratio of tumor versus background uptake (T/BG ratio). Furthermore, the tumor uptake was expressed as a percentage of the injected tracer dose (%ID), using the ratio between radioactive counts in visually determined ROIs of areas with increased uptake and the total radioactive counts of the whole body image, corrected for the radioactive decay of the tracer. Apart from analysis at the individual lesion level, tumor uptake was also assessed per body region. For this analysis, the following metastatic regions were recognized: primary tumor, skeleton, liver, lung, subcutis, non-skin soft tissue, pericardium and retina. Radiolabeled trastuzumab thoracic SPECT images were fused with conventional computed tomography images (if available) to validate regions of increased trastuzumab uptake as metastatic lesions. For this analysis a Siemens LEONARDO E-soft workstation (Erleranger, Germany) was used.
Serum HER2
Serum testing for HER2 was collected before treatment, 24 hours and 7 days after the start of treatment, and at the end of every cycle. Serum HER2 levels were determined with a sandwich enzyme immunoassay according to the manufacturer’s instructions (Oncogene Science, Inc, Cambridge, MA).

Statistics
The statistical power of the study was based on the incidence of trastuzumab-related cardiotoxicity. Retrospective analysis of the pivotal phase III trial showed an incidence of trastuzumab-related cardiac toxicity of 27%. As a consequence, approximately 20 evaluable patients were considered to be needed to detect cardiotoxicity in five. A sensitivity of the trastuzumab scan (fraction of patients with cardiotoxicity who have a positive scan) of at least 50%, was considered to be required for the technique to be of clinical value. If none out of five patients with a significantly reduced LVEF (decrease of > 10% or value < 40%) would have a positive scan, the sensitivity would be below 0.5 (P < .05). With an estimate that 80% of the patients who will undergo at least 4 cycles of trastuzumab treatment and thus have two cardiac evaluations, the estimated total number of patients needed in the study was 25. An interim analysis was planned after recruitment of 13 evaluable patients. Quantitative variables were compared between two groups using a Mann-Whitney-U test for skewed distributed variables. Paired analyses were performed with a Wilcoxon paired samples test. Correlations between variables were calculated using Pearson’s correlation coefficient test. A P-value of < 0.05 was considered statistically significant.

RESULTS
Seventeen patients entered the study. Patients characteristics are presented in table 1. All patients had received anthracycline-containing chemotherapy prior to inclusion in the current study (Table 1). The median time interval between the last dose of anthracycline-based chemotherapy and the start of treatment in the current study was 11 (range 5-59) months.
Two patients (patients no. 9 and 10, see table 1) were not evaluable for the cardiac and tumor uptake analysis, due to premature withdrawal caused by their clinical condition, resulting in incomplete pre-treatment scan procedures. Both subjects received the first \(^{111}\text{In-DTPA-trastuzumab tracer dose. One of these patients only underwent gammacamera imaging at 15 minutes and 24 hours after tracer injection. For the second patient, the imaging protocol was limited to gammacamera imaging at 15 minutes and 168 hours after tracer injection. As a result, 15 patients were evaluable for the cardiac and tumor uptake analysis, with regard to the pre-treatment scan.}
## Table 1. Patient characteristics and cardiac evaluations

<table>
<thead>
<tr>
<th>Pre-treatment</th>
<th>111In-DTPA-trastuzumab uptake</th>
<th>Pre-treatment serum HER2 (ng/mL)</th>
<th>Highest NT-proBNP during treatment (ng/L)</th>
<th>Elevated pre-treatment NT-proBNP</th>
<th>Elevated LVEF change (%)</th>
<th>NYHA class II-IV heart failure during treatment</th>
<th>Prior chest wall irradiation</th>
<th>Total epirubicin dose (mg/m²)</th>
<th>Total doxorubicin dose (mg/m²)</th>
<th>Age (years)</th>
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<td>111In-DTPA-trastuzumab uptake</td>
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<td>Pre-treatment serum HER2 (ng/mL)</td>
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<td>Highest NT-proBNP during treatment (ng/L)</td>
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<td>Elevated LVEF change (%)</td>
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<td>NYHA class II-IV heart failure during treatment</td>
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</table>
Due to progressive disease, treatment was discontinued in one patient after 2 cycles and after 4 cycles in another. Only the patient with disease progression after 2 cycles did not undergo the second radiolabeled trastuzumab scan. The second scan was performed in 14 patients.

**Cardiac functional assessment**

Three out of 15 evaluable patients developed severe symptomatic left ventricular dysfunction (CTC grade 3; NYHA functional class II-IV). This occurred during the second cycle in two patients and during the fourth cycle in the third patient. Table 1 summarizes the cardiac evaluation according to the separate patients. The development of heart failure was the reason for discontinuation of trastuzumab treatment in all three patients. One of them died due to severe left ventricular failure and massive pulmonary embolism. Paclitaxel alone was continued for 6 cycles in the second patient, and no further treatment was given in the third patient. Pre-treatment plasma NT-proBNP levels were higher in the patients who developed symptomatic left ventricular dysfunction during treatment (mean 534 (± SD 236) ng/L), than in the women without heart failure (mean 105 (± SD 79) ng/L, P = 0.009). During treatment, NT-proBNP values remained higher in these three patients.

**111In-DTPA-trastuzumab pharmacokinetics**

The kinetics of radiolabeled trastuzumab in plasma could satisfactorily be described by a two-compartment model. Curve fitting of the pharmacokinetic data with a three-compartment model did not improve the fit. Table 2 summarizes the pharmacokinetic parameters for 111In-DTPA-trastuzumab in plasma, assuming a two-compartment model. The mean urinary excretion (± SD) during the first 24 h was 9.2 ± 3.1 %ID. Mean excretion during day 2-7 was 2.4 ± 0.8 %ID. Total excretion during the first week was 23.4 %ID. These data match with the scintigraphic data that show a total body retention of 75% after 1 week.

**Table 2. Pharmacokinetic parameters 111In-DTPA-trastuzumab (mean ± SD)**

<table>
<thead>
<tr>
<th></th>
<th>T1/2(α) (hours)</th>
<th>T1/2(β) (hours)</th>
<th>Vd (central) (L)</th>
<th>Vdss (L)</th>
<th>CL (L/hour)</th>
<th>MRT (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Scan 1 (n=11)</strong></td>
<td>13.7 ± 2.5</td>
<td>117.4 ± 15.2</td>
<td>2.8 ± 0.3</td>
<td>5.2 ± 0.6</td>
<td>0.045 ± 0.009</td>
<td>140.0 ± 18.1</td>
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<tr>
<td><strong>Scan 2 (n=8)</strong></td>
<td>16.0 ± 3.2</td>
<td>142.2 ± 15.2</td>
<td>3.2 ± 0.4</td>
<td>5.7 ± 0.8</td>
<td>0.034 ± 0.006</td>
<td>183.3 ± 18.0</td>
</tr>
</tbody>
</table>

T1/2(α) = 111In-DTPA-trastuzumab half-life in the distribution phase; T1/2(β) = 111In-DTPA-trastuzumab half-life in the elimination phase; Vd (central) = volume of distribution; Vdss = volume of distribution in steady state; CL = total clearance; MRT = mean residence time.

**111In-DTPA-trastuzumab imaging**

17 patients underwent the first radiolabeled trastuzumab scan, 14 patients also underwent the second scan. Initially (15 minutes after radiolabeled trastuzumab
infusion), high bloodpool activity was observed, in addition to immediate high uptake in the liver. Less uptake was observed in other organs, such as the spleen, kidneys and bladder. Liver intensity (Figure 1) increased over the following days, whereas bloodpool activity in the spleen, kidneys and bladder disappeared. Pretreatment non-tumoral radiolabeled trastuzumab biodistribution patterns were similar to the patterns observed on the second scans. Pre-treatment serum HER2 levels, available in 15 of the 17 patients, correlated positively with \(^{111}\text{In-DTPA-trastuzumab} \) liver uptake 15 minutes, 24 hours and 168 hours after tracer injection on pre-treatment scans (R = 0.609, P = 0.016; R = 0.647, P = 0.017; R = 0.541 P = 0.046, respectively). Furthermore, a weak negative correlation was observed between \(^{111}\text{In-DTPA-trastuzumab} \) plasma elimination half life and liver uptake at 120 hours and 168 hours after tracer injection, R = -0.630, P = 0.038 and R = -0.604, P = 0.049, respectively.

In three patients, the radiolabeled trastuzumab pre-treatment scan was performed approximately 24 hours prior to the unlabeled trastuzumab predose of 4 mg/kg, to evaluate the effect of the unlabeled trastuzumab predose on the radiolabeled trastuzumab biodistribution. In these three patients, who were enrolled as patient three to five, liver uptake was higher from 15 minutes to 168 hours after tracer injection, compared to the 14 patients who received the trastuzumab loading dose prior to the \(^{111}\text{In-DTPA-trastuzumab} \) tracer (Figure 1). No differences were observed with regard to radiolabeled trastuzumab uptake in other organs.

![Liver uptake vs time after tracer injection](image)

**Figure 1.** \(^{111}\text{In-DTPA-liver} \) uptake expressed as percentage of the injected dose. Solid lines: pre-treatment scan. Closed circles: trastuzumab loading dose prior to tracer injection. Open circles: trastuzumab loading dose 24 hours after tracer injection. Dotted line with closed squares: second scan. Values are expressed as mean (+/- SD). * P < 0.05 compared to patients with “cold” pre-dose.

In one of the 15 patients who were evaluable for the cardiac analysis, myocardial uptake was observed on the pre-treatment scan at 48 hours after tracer injection. (Figure 2). Due to progressive liver metastases, she was taken off study after two cycles, without symptoms of cardiac dysfunction (pre-treatment LVEF 54%).
this patient, the time interval between the last dose of anthracycline-based chemotherapy and the start of trastuzumab and paclitaxel treatment was 17 months. None of the three patients who developed severe left ventricular dysfunction showed myocardial $^{111}$In-DTPA-trastuzumab uptake on their pre-treatment scans. In the patient who developed severe left ventricular dysfunction during the fourth cycle, myocardial uptake was observed on the second radiolabeled trastuzumab scan, 24 hours after tracer injection. The patient died during the second scan period as a consequence of severe left ventricular dysfunction and no further images could be obtained beyond 24 hours after tracer injection.

Fourteen patients underwent both radiolabeled trastuzumab scans and LVEF measurement after 4 cycles or earlier if symptomatic heart failure developed. No myocardial uptake was observed on any of the pre-treatment scans. Based on 14 negative scans, the confidence interval for a positive radiolabeled trastuzumab scan, with regard to myocardial uptake, is 0 - 23%. Furthermore, three of the 14 patients developed cardiac dysfunction. Myocardial uptake was not observed in any of these three patients. As a consequence, the study was closed after this interim analysis.

Figure 2. Myocardial uptake in a patient with pre-existent cardiac ventricular arrhythmias. Transversal SPECT slice. The arrow indicates the characteristic horseshoe shape of the myocardium of the left ventricle. Dotted arrow indicates (normal) liver uptake.

Next to myocardial uptake, radiolabeled trastuzumab tumor uptake was evaluated. Tumor uptake was best visualized 5 days after tracer injection, since semiquantitative uptake parameters and the number of lesions visualized reached a maximum at that time. Beyond 5 days following tracer injection image quality decreased due to decay of $^{111}$In. Figure 3 shows the validation of regions with increased $^{111}$In-DTPA-trastuzumab uptake by fusion of the radiolabeled trastuzumab SPECT with conventional computed tomography images.
Figure 3. (A) Fused computed tomography with $^{111}$In-DTPA-trastuzumab single photon emission tomography (SPECT) image (96 hours after tracer injection). (B) Conventional CT images (top) of a patient with a large liver metastasis as indicated by the arrow. Fusion with $^{111}$In-DTPA-trastuzumab SPECT (bottom) shows correspondence of liver metastases and SPECT hotspot.
One or more known tumor lesions, assessed with routine staging examinations, were readily discernable in 14 out of the 15 evaluable patients. In 13 out of the 15 enrolled patients (87%), lesions not previously identified by routine staging examinations, were detected on the pre-treatment scan. Previously undetected skeletal lesions were found in nine patients, soft tissue lesions in four, and in one patient increased uptake was observed in the contralateral breast. In four of the 13 patients in whom new lesions were identified, one or more of the previously unknown lesions were confirmed with routine staging examinations at the time of response evaluation. This concerned skeletal lesions in two and a lesion in the contralateral breast in one patient. The detection rate of all known single tumor lesions on the pre-treatment scan was 45%.

In 13 out of the 15 patients, visually determined ROIs of sites with increased $^{111}$In-DTPA-trastuzumab uptake could be determined, at 120 hours after tracer injection. At this time, the mean %ID of all identified lesions (including lesions not previously identified by routine staging) was 1.2 (SD 0.9) and T/BG ratio was 3.0 (SD 1.0). %ID and T/BG ratio of known metastatic lesions only, identified with routine staging, were 1.3 (SD 1.1) and 3.2 (SD 1.4), respectively. Great variation in the degree of tumor uptake was observed between separate patients (range of mean tumor uptake 0.3 - 3.4 %ID and range of mean T/BG ratio 1.4 - 4.9). For 11 patients, in whom multiple lesions were visualized, large variation in uptake was observed between separate lesions in one patient. In most patients, more tumor lesions were visualized on the pre-treatment scan, compared to the second scan. Figure 4 shows that, for most patients, the cumulative tumor lesion uptake as %ID per patient is higher on the pre-treatment, than on the second scan. $^{111}$In-DTPA-trastuzumab uptake on the pre-treatment and second scans was not clearly associated with tumor response. Figure 5 shows an example of decreased tumor uptake on the second scan, compared to pre-treatment uptake.

**Figure 4.** Total body tumor load estimated on cumulative uptake (%ID) of all ROIs at 120 hours after tracer injection of individual patients on pre-treatment scans compared to the second scans, according to tumor response. Black circles: patients with a partial response after 4 cycles; open circles: response not evaluable; squares (with dotted line): progressive disease.
Figure 5. SPECT maximum intensity projection images with sagittal, coronal and transversal views before treatment (A) and corresponding images after 4 cycles (B). Arrows denote $^{111}$In-DTPA-trastuzumab uptake in the lower central and left chest wall. Liver and spleen uptake is normal.

**DISCUSSION**

In the current study, we evaluated the use of radiolabeled trastuzumab scanning for predicting trastuzumab-related cardiotoxicity and visualization of tumor lesions. To date, data with regard to molecular HER2 imaging in humans is limited. In patients with HER2-positive metastatic breast cancer, $^{111}$In-DTPA-trastuzumab scintigraphy could not predict trastuzumab-related cardiotoxicity, since none of the patients who developed severe left ventricular dysfunction showed myocardial uptake on their pre-treatment scan. Pre-treatment plasma NT-proBNP levels were higher in the patients who did, than in the patients who did not develop cardiac dysfunction during treatment. However, this technique detected (HER2-positive) tumor lesions and showed variation in radiolabeled trastuzumab tumor lesion uptake between patients, and between lesions within one patient. In most patients new lesions were identified, that were not previously identified with routine staging examinations.

We hypothesized that trastuzumab-related cardiotoxicity is based on a direct effect of trastuzumab on HER2 expressed in the myocardium. In a preliminary report, Behr et al. observed myocardial uptake of $^{111}$In-labeled trastuzumab in seven out of
20 patients. Six of them developed NYHA functional class II to IV heart failure, and the seventh patient had episodes of cardiac arrhythmia during trastuzumab administration. In contrast to these results, we observed myocardial uptake at the start of trastuzumab treatment in only one patient, who had received extensive anthracycline pre-treatment and who had cardiac ventricular arrhythmias prior to trastuzumab treatment. The second scans revealed myocardial uptake in one patient who died shortly thereafter as a consequence of severe left ventricular failure. However, no myocardial uptake was observed in the three patients who developed severe symptomatic left ventricular dysfunction during trastuzumab and paclitaxel treatment. A possible explanation for this finding may be the variation in HER2 expression in the myocardium, in analogy with the variation in HER2 expression ranging from 1+ to 3+ overexpression. Myocardial HER2 however, if present, is not likely to be in the range of tumor HER2 overexpression as was suggested by a report that showed only weak immunohistochemical HER2 expression in the myocardium in six of sixty patients with heart failure. An additional factor, may be the contrast resolution of the planar and SPECT imaging techniques. Alternatively, the administration of the unlabeled trastuzumab pre-dose may also have influenced myocardial 111In-DTPA-trastuzumab uptake. However this is contradicted by the fact that both patients who showed myocardial uptake had trastuzumab “on board” at the time of tracer injection.

NT-proBNP and TnI measurement were included in the cardiac functional analysis, in addition to the radiolabeled trastuzumab scintigraphy, cardiac ultrasound and MUGA scan. A remarkable finding of the current study was that pre-treatment NT-proBNP levels were above normal values and were almost 5-fold higher in the patients who developed heart failure during trastuzumab treatment in comparison to the patients who did not experience symptomatic left ventricular dysfunction. This could suggest that prior anthracycline treatment induced subclinical myocardial injury and reduced compensatory reserves in these patients, which has been described previously. Subsequently, myocardial distress induced by trastuzumab treatment resulted in heart failure. These findings are in line with in vitro data in doxorubicin-treated rat cardiomyocytes, in which myofibrillar disarray increases with the addition of trastuzumab.

The issue of trastuzumab-related cardiotoxicity becomes increasingly relevant, since the reported effect of trastuzumab in the adjuvant treatment of breast cancer. Perez et al. recently reported that, despite strict cardiac eligibility criteria, cardiac dysfunction occurred in 3.3% of the patients in the paclitaxel plus trastuzumab-treated group after more than six months follow-up, whereas no cardiovascular events were observed in the paclitaxel alone subgroup. Interim analysis of a large European randomized trial evaluating observation versus one or two years of 3-weekly trastuzumab, following standard adjuvant chemotherapy, showed that a decrease in LVEF of more than 10% to an absolute value below 50% occurred in 7.1% during the first year of trastuzumab treatment, compared to 2.2% of patients not receiving trastuzumab. The findings of the current study suggest that plasma
markers such as NT-proBNP may be of particular value for the early detection or prediction of cardiotoxicity. A large variation was observed in $^{111}$In-DTPA-trastuzumab tumor uptake between patients and between tumor lesions within one patient. Furthermore, we observed an overall detection rate of 45% at the single lesion level, and previously unidentified lesions were visualized in 13 out of the 15 evaluable patients on the first scans. There are several explanations for these findings. First of all, we estimated the degree of tumor uptake from visually determined ROIs, using the two dimensional whole body images. On these images, uptake intensity increases with increasing tumor size. In addition, the localization of lesions influences the intensity of radiolabeled trastuzumab uptake; sites closer to the body surface are more readily discernable than lesions that are localized deeper. An alternative possible explanation is that the degree of HER2 expression differs between separate metastatic lesions within one patient. Estrogen receptor expression for instance, can change between primary and asynchronous recurrences and estrogen receptor status of metastatic sites was suggested to be more predictive of endocrine responsiveness. The HER2 status of lymph node metastases can also differ from their primary tumors.

Discordance in HER2 expression between the primary tumors and asynchronous metastases or recurrences has also been described. This may account partly for the low overall tumor detection rate. Next to the variation in tumor lesion uptake, we observed that in most patients lesions were visualized, irrespective of the tumor response to trastuzumab treatment, although several lesions had disappeared on the second scan. In addition, in most patients one or more tumor lesions were still visualized on the second scan. This implies that HER2 remains present on the cell membrane and is not lost due to shedding of the extracellular domain or receptor internalization.

High liver uptake is a phenomenon normally observed with monoclonal antibody imaging, especially with $^{111}$In-DTPA labeled antibodies, and may limit visualization of liver metastases. Immediately after injection, we observed high $^{111}$In-DTPA-trastuzumab liver uptake, which increased during the first week to up to 29 %ID. The plasma half-life of the $^{111}$In-DTPA-trastuzumab tracer used in this study was 4.9 days. This is in line with evidence from phase I and II trials, reporting a half-life of trastuzumab varying from 1.1 at a dose of 10 mg weekly to 23 days with 500 mg weekly. Remarkably, in the three patients in whom the trastuzumab loading dose prior to administration of the tracer dose of $^{111}$In-DTPA-trastuzumab was omitted, particularly high liver uptake was observed (Figure 1). A possible partial explanation for this finding is that radiolabeled trastuzumab targets the extracellular domain of HER2, which is present in the circulation after being shed from the cell membrane. The circulating serum HER2-radiolabeled trastuzumab immune complexes may subsequently be cleared by the liver. The weak correlation between serum HER2 and the degree of $^{111}$In-DTPA-trastuzumab uptake in the liver may point into the direction of this hypothesis. $^{111}$In-DTPA-trastuzumab scintigraphy may become of value for clinical practice. Since only patients with HER2-positive breast cancer are expected to benefit from
trastuzumab treatment, selection of patients is essential. Currently, the most widely applied techniques are immunohistochemistry and FISH/CISH. The most important disadvantage of both techniques is the need for tumor tissue. Furthermore, due to heterogeneity of tumor tissue, HER2 positivity (or negativity) can be missed by tissue analysis. Based on the results of the current study, radiolabeled trastuzumab scintigraphy may prove of value as a non-invasive technique for assessment of HER2 tumor status. Another promising application of our In-labeled trastuzumab scan is its potential use in staging of HER2-positive breast cancer. A limitation of In-DTPA-trastuzumab SPECT imaging in this regard however, might be its spatial resolution. Implication of positron emission tomography (PET), with a higher spatial resolution than planar and SPECT imaging, may improve tumor imaging. The use of trastuzumab or analogues radiolabeled with PET isotopes may develop as an exciting new tool for molecular tumor imaging in PET scanning. For the use of trastuzumab for PET scanning however, a relatively long-lived isotope, such as 124I or 89Zr, is required. This entails a high radiation dose, all the more based on the high photon energy of PET imaging compared to traditional nuclear imaging. Also interesting in this regard is the recent development of smaller engineered anti-HER2 antibody fragments, the so called diabodies. The most important advantage of imaging with these smaller antibody fragments is faster tumor targeting and clearance kinetics, compared to intact monoclonal antibody imaging, thereby improving target-non target ratio. In a xenograft model, Robinson and colleagues recently reported tumor imaging from 4 hours after injection of the radiolabeled anti-HER2 diabody. In another report, imaging with radiolabeled trastuzumab fragments in a human breast cancer xenograft model showed tumor visualization 24 hours after injection. Tumor targeting of these smaller fragments is inferior to intact monoclonal antibodies. Although recently a study with a new type of HER2 affinity ligand showed equally high HER2 binding affinity, compared to trastuzumab. Anti-HER2 diabody imaging may assist in the localization of sites with HER2 overexpression however, a disadvantage is that no insights will be provided into the mechanisms of trastuzumab tumor uptake. Currently, PET tumor imaging especially focuses on fluorodeoxyglucose (FDG) as the tracer. FDG uptake is representative of glucose metabolism, which is higher in malignant cells than in normal tissue. However, the concept of new molecular targeting PET tracers, that addresses more specific cellular processes than glucose metabolism, such as radiolabeled trastuzumab, are very interesting for the near future of oncology PET imaging.
REFERENCES


