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[⁸⁹Zr]Trastuzumab: Evaluation of Radiation Dosimetry, Safety and Optimal Imaging Parameters in Women with HER2-Positive Breast Cancer

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Abstract

Purpose—To evaluate safety, human radiation dosimetry and optimal imaging time of [⁸⁹Zr]trastuzumab in patients with HER2-positive breast cancer.

Procedures—Twelve women with HER2-positive breast cancer underwent [⁸⁹Zr]trastuzumab-PET/CT twice within 7 days postinjection. Biodistribution data from whole-torso PET/CT images, and organ time-activity curves were created using data from all patients. Human dosimetry was calculated using OLINDA with the adult female model.

Results—High-quality images and the greatest tumor-to-nontumor contrast were achieved with images performed 5 ± 1 day postinjection. Increased [⁸⁹Zr]trastuzumab uptake was seen in at least one known lesion in 10 patients. The liver was the dose-limiting organ (retention of ~12% of the injected dose and average dose of 1.54 mSv/MBq. The effective dose was 0.47 mSv/MBq. No adverse effects of [⁸⁹Zr]trastuzumab were encountered.

Conclusion—[⁸⁹Zr]trastuzumab was safe and optimally imaged at least 4 days post-injection. The liver was the dose-limiting organ.

Keywords

dosimetry; HER2; zirconium-89; trastuzumab; PET imaging

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Conflict of Interest

RB is a consultant for Genentech and has received an honorarium from Genentech and Novartis.

Ethical Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Introduction

Human epidermal growth factor receptor 2 (HER2) has an important role in cell survival, proliferation, angiogenesis, and development of metastasis [1]. HER2-positive tumors accounts for approximately 20–25% of all cases of breast cancer. Prior to the development of HER2-targeted therapy, HER2-positive breast cancer had the worst prognosis of all breast cancer subtypes [2–3]. Trastuzumab, a humanized monoclonal antibody (mAb) targeting HER2, has significantly improved patient survival, but the development of drug resistance is still a significant medical challenge [4–5]. In fact, the majority of metastatic HER2-positive breast cancer will develop trastuzumab resistance over the course of treatment [6].

Currently, two types of tests are available to measure HER2 in breast cancer biopsies. Immunohistochemistry (IHC) detects HER2 overexpression and fluorescence in situ hybridization (FISH) detects HER2 gene amplification [7–9]. There is evidence that HER2 status may change during the course of the disease and after chemotherapy [10]. In addition, discordance in HER2 expression across tumor lesions in the same patient has been reported [11–14]. Thus, the use of repeated biopsies during the course of the disease is encouraged [15]. However, repeated invasive biopsies are often not clinically feasible, and not all lesions are readily accessible for biopsy [16]. Therefore, a method that can address tumor heterogeneity and reliably determine both the quantity and the functional status of tumor HER2 in individual lesions in a non-invasive manner is of critical importance in identifying patients who would benefit from HER2-targeted therapy and to monitor the change in HER2 status during therapy.

Positron emission tomography (PET)-based measurement of the HER2 expression in breast cancer offers several advantages over current biopsy-based methods. These include assessing HER2 expression of the entire tumor volume rather than just a part of the tumor (addressing the intrinsic heterogeneity of HER2 expression), directly assessing the binding of the therapeutic mAb (trastuzumab) to HER2, and evaluating the effects of therapy on HER2 expression of the tumor. In addition, imaging can simultaneously assess HER2 expression of primary and metastatic sites, which may vary across lesions within any given patient. PET is a highly sensitive functional imaging technique that has the ability for quantification at sub-nanomolar concentrations. PET with radiolabeled mAbs can be used for non-invasive detection and quantification of specific targets throughout the body, and ultimately can help to better understand the behavior and effectiveness of treatment with mAbs in individual patients. Several PET radiopharmaceuticals have been developed for noninvasive evaluation of HER2 expression and localization of HER2-overexpressing tumors, including ^{64}Cu -trastuzumab, ^{68}Ga -trastuzumab $\text{F(ab}')_2$ fragments, ^{68}Ga -ABY-002 and ^{89}Zr -trastuzumab [17–24].

Of the positron-emitting radiometals, Zr-89 ($\text{EC} = 76.6\%$, $\beta^+ = 22.3\%$, $E_{\text{ave.}}(\beta^+) = 396.9$ keV, $R_{\text{ave.}}(\beta^+) = 1.18$ mm) has an optimal half-life (78.4 h) for imaging mAbs, allowing for imaging up to 7 days after injection. In this study, we aimed to evaluate the human radiation dosimetry of [^{89}Zr]trastuzumab based on PET imaging results and to determine the optimal imaging time with this agent in patients with HER2-positive breast cancer.

Materials and Methods

Patient Population

This study was conducted under an investigational new drug application (IND # 118029) submitted to the U.S. Food and Drug Administration, and was approved by the Institutional Review Board and the Radioactive Drug Research Committee at Washington University School of Medicine. The human radiation dosimetry estimated from preclinical data submitted with the IND application is briefly summarized in the Supplementary data. All subjects gave written informed consent before participation. We studied 12 adult (18 years or older) women with biopsy-proven HER2-positive breast cancer (Clinicaltrials.gov Identifier: NCT02065609). HER2 positivity was defined as per the 2013 American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines which require tumors to be 3+ by HER2 IHC or have a FISH HER2:CEP17 ratio > 2 on primary, recurrent, or metastatic breast cancer tissue [25]. Patients with HER2-negative (0 or 1+, 2+ and FISH negative) were not eligible to participate. All subjects were required to have at least one lesion ≥ 1.5 cm, as determined by imaging studies (mammography, ultrasonography, CT or MRI) or physical examination. Patients who were receiving trastuzumab therapy with or without other types of systemic therapy were eligible to participate. Patients with other invasive malignancies, with the exception of non-melanoma skin cancer, who had (or have) any evidence of the other cancer present within the last 5 years were not eligible.

^{89}Zr -Trastuzumab Synthesis and Quality Control

^{89}Zr -trastuzumab was prepared as previously described by Vosjan et al. [26]. Briefly, ^{89}Zr -oxalate was produced via the $\text{Y-}^{89}(\text{p,n})\text{Zr-}^{89}$ reaction on the CS-15 cyclotron (Cyclotron Corporation, Berkeley, CA) and purified with an in-house automated module [27–28]. The resulting [^{89}Zr]oxalate was produced with a specific-activity of 8.1–15.4 GBq/ μmol (220–418 mCi/ μmol). Trastuzumab (HerceptinTM, Genentech, South San Francisco, CA) was incubated with 10 mol equivalents of Df-Bz-NCS (Macrocylics, Dallas, TX) in 0.1 M NaHCO_3 buffer pH 9.0 for 1 h at 37 °C. The resulting product, Df-Bz-NCS-trastuzumab, was purified and buffer exchanged into 1M HEPES buffer pH 7.1 via Zeba Spin Desalting Columns (Pierce Biotechnology, Rockford, IL). 5 mg of Df-Bz-NCS-trastuzumab was radiolabeled with 111 – 370 MBq of neutralized ^{89}Zr solution for 1.5 h at 37°C. The resulting product, [^{89}Zr]trastuzumab, was challenged with 2.5 μmol DTPA for 5 min and subsequently purified via buffer exchange in saline using Zeba spin desalting columns. The final product, [^{89}Zr]trastuzumab was sterile filtered through a 0.2 μm filter and diluted with saline through the same sterile filter to a final volume of 10 mL. The radiochemical yield was determined to be 95% by radio-ITLC using 50 mM DTPA as the developing solution. The antibody aggregation analysis was determined using size-exclusion chromatography (Superose 12 10/300 GL, GE Healthcare, Piscataway, NJ), resulting in 80% ^{89}Zr -trastuzumab monomer. Radionuclide identity, visual appearance, pH, specific activity and bubble point filter membrane integrity were determined pre-release. The immunoreactivity assay was performed on HER2+ SKBR3 human breast cancer cells following methods developed by Lindmo et. al. [29], where we achieved an average immunoreactive fraction of

89 ± 0.1 % (acceptance criterion is 65%). The immunoreactivity assay and sterility of the final product were determined post-release.

PET Imaging Procedures

All PET imaging was performed with a Siemens Biograph 40HD PET/CT scanner. Whole-body PET imaging was performed at two separate time points within 7 days (1 and 3 days, 2 and 5 days, 4 and 6 days) following ^{89}Zr -trastuzumab injection (day 0). Trastuzumab-naïve subjects were dosed with 50 mg “cold” trastuzumab, whereas those undergoing trastuzumab therapy received 10 mg, 30 min to 2 h before administration of 62 ± 2 MBq (1.76 ± 0.50 mCi) of [^{89}Zr]trastuzumab; these pre-doses have been shown by Dijkers et al. to be adequate for minimizing uptake of radiolabeled trastuzumab by normal organs [18]. The administered activity was expected to result in an effective dose (ED) of 50 mSv based on mouse radiation dosimetry (supplemental data). All subjects were monitored for possible infusion reaction (i.e., dyspnea, chest tightness, fever, rigors or hypotension) during trastuzumab and [^{89}Zr]trastuzumab administration. In addition, all patients underwent measurement of vital signs (blood pressure, heart rate, respiratory rate, and temperature), clinical laboratory testing (standard hematologic and comprehensive metabolic panels that included hemoglobin, white blood cells, neutrophils, lymphocytes, platelets, creatinine, blood urea nitrogen, calcium, sodium, potassium, carbon dioxide, alanine transaminase, aspartate aminotransferase, alkaline phosphatase, total bilirubin, and albumin), urinalysis, and electrocardiography before [^{89}Zr]trastuzumab administration and at the second imaging session.

A spiral CT scan for attenuation correction (120 kVp, 50 effective mAs at 5-mm slice thickness) was obtained from the top of the skull through the upper thighs with the subject in the supine position. Immediately after the attenuation CT scan, emission images beginning at the top of the skull and proceeding down through the upper thighs were obtained (1–10 min per bed position depending on the time post-injection). Imaging was performed over 6–7 bed positions with a total imaging duration of no more than 1 hour. Images were reconstructed with 3D-OSEM with 3 iterations, 24 subsets and a post-reconstruction Gaussian filter of 7 mm.

Image Analysis

All images were evaluated subjectively by one observer, who was blinded to the time of imaging for assessment of image quality (graded on a 4-point scale, with 1 being poor quality—not acceptable for diagnostic interpretation, and 4 being good image quality—similar to a routine clinical study). The images were then evaluated to assess the uptake of [^{89}Zr]trastuzumab in known sites of disease (based on all available imaging studies)

Image-Derived Patient Dosimetry

Organ activity concentration data were obtained by drawing regions of interest (ROIs) on the PET images for the following organs/tissues: liver, kidneys, blood (left ventricular chamber), spleen, muscle, subcutaneous fat and red-marrow. The red marrow activity concentrations were determined from the average activity concentration measured in the lumbar vertebrae. The total activity in each organ was then calculated as the product of the activity

concentration and the standard adult female organ weight. Organ time-activity curves were created by collating the decay-corrected data from all patients fitted by mono-exponential functions. Analytical integration of the fit provided the organ residence times. Total patient blood volume was determined by the method of Lemmens et al [30]. The remainder of the body residence time was determined from the maximum theoretical residence time minus the sum of the residence times observed in the organs (except for fat and blood; accordingly, this conservative approach, assumed no excretion. Human dosimetry estimates were obtained from the residence times using OLINDA/EXM version 1.1 software with the adult female model.

Results

Patient Characteristics

Twelve women (mean $\pm$ standard deviation: 57.0 ± 22.2 years; range 29 to 71 years) were enrolled in the study. Six had primary breast cancer and 6 had metastatic breast cancer. Eleven subjects underwent [^{89}Zr]trastuzumab-PET/CT during neoadjuvant or adjuvant therapy; the remaining subject was studied before initiation of neoadjuvant therapy. Ten of the 12 subjects were receiving trastuzumab while participating in this trial and, thus, received the 10 mg pre-dose of cold trastuzumab while the other 2 were not receiving trastuzumab therapy and received a 50 mg pre-dose.

Safety Evaluation

The mean administered activity was 65 ± 18 MBq (1.76 ± 0.50 mCi) (range 43.3 to 88.8 MBq) with a total mass of 5-mg of [^{89}Zr]trastuzumab. There were no adverse or clinically detectable pharmacological effects in any of the 12 subjects. No significant changes in vital signs or the results of laboratory studies or electrocardiograms were observed.

PET Results

Increased [^{89}Zr]trastuzumab uptake was seen in at least one known lesion in 10 of the 12 subjects (Table 1), including the primary tumor in 4 of 6 subjects before or undergoing neoadjuvant therapy and at least one lesion in all 6 subjects with metastatic disease. When the paired images were compared, the later time points had better image quality, with the best quality observed on days 4 and 6 after [^{89}Zr]trastuzumab administration (Fig. 1 and 2). In the 10 subjects who had abnormally increased ^{89}Zr -trastuzumab uptake in one or more lesions, the intensity of tumor uptake relative to surrounding tissues increased at the later imaging time in all but one case.

Dosimetry

The tracer was predominantly retained in the liver and blood. Liver activity remained nearly constant over time at $\sim 12\%$ of the injected dose (ID). Slow blood clearance was observed with a biological half-life of 113 hr and an initial level of 58% ID. Cumulative organ time-activity curves are presented in Fig. 3. For most organs, a rapid distribution of the tracer to the organ is observed immediately after injection (chiefly reflecting activity in blood) followed by constant activity concentration or slow clearance. The organ residence times are presented in Table 2 and the human radiation dose estimates are presented in Table 3.

Because of its high retention, the liver is the critical organ with a dose of 1.63 mSv/MBq (6.02 rad/mCi). The effective dose was 0.61 mSv/MBq (2.25 rem/mCi). The biodistribution of [^{89}Zr]trastuzumab did not significantly change during the 7 days of imaging (Fig. 2).

Discussion

Systemic therapy of breast cancer includes cytotoxic chemotherapy, hormonal therapy, and targeted therapy to HER2. Accurate determination of HER2 status is essential for breast cancer treatment. However, HER2 status may change during the course of a patient's disease and may vary from one tumor site to another. Lower et al. reviewed 382 paired primary tumors and subsequent metastases and reported discordance in HER2 status in 33.2% of paired samples overall; the primary tumor was positive and the metastatic site negative in 23.6% and the converse was found in 9.6% [14]. For these reasons, repeated biopsies have been encouraged; however, this is not always practical. Another challenge with biopsy-based determination of HER2 status is intratumoral heterogeneity [31–33]. Discordant HER2 expression within a single cancer or between different sites of cancer can introduce sampling error and confound treatment decision-making.

PET-based measurement of HER2 expression using the radiolabeled mAb, [^{89}Zr]trastuzumab, has several advantages over biopsy-based methods. PET directly assesses the availability of HER2 to be bound to trastuzumab; thus, it is potentially a more reliable predictor of response to trastuzumab therapy. In addition, PET has the ability to assess intratumoral heterogeneity of HER2 expression in a lesion (within the limits of resolution of PET) and intertumoral heterogeneity among multiple lesions in a patient. Moreover, because it is noninvasive, PET can be repeated during therapy to assess response. Several mechanisms of drug resistance to trastuzumab have been identified, and these can be divided into mechanisms that will alter trastuzumab binding versus intracellular protein or pathways changes that will not affect trastuzumab binding [34]. Drug resistance mechanisms that alter trastuzumab binding, such as production of a truncated HER2 protein that lacks the trastuzumab binding epitope, emergence of a low HER2 expressing subclone, or inhibition of trastuzumab binding by overexpression of the MUC4 sialomucin complex, are potentially detectable with ^{89}Zr -trastuzumab PET imaging [35–36]. We acknowledge that drug resistance mechanisms that do not affect trastuzumab binding, such as PI3-kinase mutations, PTEN deletions, or activation of other tyrosine kinases, will not be detectable with ^{89}Zr -trastuzumab PET imaging [37–38].

In this study, we evaluated the human radiation dosimetry, as well as the safety and imaging characteristics, of [^{89}Zr]trastuzumab in women with HER2-positive breast cancer. Human dosimetry estimates derived from murine biodistribution data were used to select the target injected dose for this human study. These data indicated a critical organ dose of 0.79 mSv/MBq to the heart wall reflecting the long retention time of the tracer in the blood and an ED of 0.44 mSv/MBq. Thus total ED for two PET/CT imaging time points for dosimetry evaluation (from up to 92.5 MBq of [^{89}Zr]trastuzumab and two low-dose CT scans) was estimated to be 48.6 mSv.

In humans, we observed overall higher tracer residence times in all organs. Notably the liver, kidney and heart muscle (estimated from blood content) residence times are higher in humans indicating slower kinetics in humans relative to rodents. Supplemental Fig. 1 presents the comparative dosimetry of the animal data against the present human dosimetry. [^{89}Zr]trastuzumab was found to be safe in this phase 0 study. We observed no adverse effects in any of our 12 subjects.

We found excellent delineation of tumor lesions by [^{89}Zr]trastuzumab PET/CT in woman with HER2-positive breast cancer, similar to the findings of the first-in-human study of [^{89}Zr]trastuzumab, conducted by Djikers et al. [18] and to the findings of the ZEPHIR clinical trial recently published by Gebhart et. al [24]. The optimal imaging times were found to be comparable despite differences in the method of conjugation of the metal chelate for Zr-89 to trastuzumab. Another difference is the administered activity of [^{89}Zr]trastuzumab. Djikers et al. administered 37 MBq, whereas we administered almost twice that to allow for imaging up to 6 days post injection (day 0 = injection day) without compromising image quality. Our studies show that a dosage of approximately 62 MBq of [^{89}Zr]trastuzumab in the presence of unlabeled trastuzumab (50 mg of trastuzumab in trastuzumab-naïve and 10 mg in patients already receiving trastuzumab) yielded good visualization of tumor foci at 6 days post injection with acceptable dosimetry in normal organs (Fig. 1). Finally, absence of [^{89}Zr]trastuzumab uptake in known lesions was observed in 7 of 14 subjects in the Djikers study, and in 2 of 12 subjects of our study (Table 1). More investigations are needed to determine whether the absence of tumor uptake reflects a reduction in HER2 overexpression as a consequence of trastuzumab therapy. The high dose rate to the liver is a limitation of this radiotracer. At 1.63 mSv/MBq, the 62 MBq injection yield an estimated dose to the liver of more than 100 mSv, twice the 50 mSv limit from 21CFR161.1 recommended by FDA. However, a maximal injected dose of 30 MBq [^{89}Zr]trastuzumab would require longer acquisition times, which can result in patient motion, to maintain similar tumor visualization.

Conclusion

PET imaging of HER2 expression using [^{89}Zr]trastuzumab is safe and has acceptable dosimetry. It provides a noninvasive means of assessing HER2 status of individual lesions in patients with breast cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1. Anterior and posterior reprojection PET images of a patient (#1) with HER2-positive metastatic breast cancer on Day 3 and Day 6 following [^{89}Zr]trastuzumab administration demonstrate greater tracer uptake in the right femoral metastatic lesion without other significant change in tracer biodistribution on Day 6 compared to Day 3. The area of intense activity in the right chest is related to administration of [^{89}Zr]trastuzumab via a port catheter.

Fig. 2.

Axial PET (top) and fused PET/CT (bottom) images of a patient (#9) with HER2-positive metastatic breast cancer at the chest level on Day 2 and Day 4 following [^{89}Zr]trastuzumab administration demonstrate greater tracer uptake in the right humeral metastatic lesion on Day 4 compared to Day 2.

Fig. 3. Time activity curves created by collating the percentage injected dose in all patients (A–G). When appropriate, data were fitted by a mono-exponential function.

Table 1Patient clinical and imaging results (based on the second [^{89}Zr]trastuzumab scan)

Subject number	Number/type of prior HER2-targeted therapeutic regimens	Number of known sites of disease based on conventional imaging	Number of positive known disease sites on [^{89}Zr]trastuzumab PET (qualitative assessment)
1	7/metastatic disease	5	2
2	1/metastatic disease	4	3
3	1/neoadjuvant	1	0
4	2/metastatic disease	5	4
5	3/metastatic disease	1	1 [*]
6	1/neoadjuvant	2	2
7	1/neoadjuvant	1	0
8	3/metastatic disease	2	1
9	1/neoadjuvant	1	1
10	none	5	5
11	1/neoadjuvant	1	1
12	6/metastatic disease	>5	1

^{*} 3 additional lesions were seen on the [^{89}Zr]trastuzumab-PET/CT scan, one of which became apparent on follow-up CT 3 months later.

Table 2

Organ residence time measured from PET/CT images

Organ	Residence Time (hr)
Liver	13.6
Blood Pool	38.7
Fat	8.0
Spleen	0.9
Kidneys	2.1
Muscle	25.2
Heart Content	3.3
Red Marrow	6.4
Remainder	61.6

Table 3Organ radiation doses for [^{89}Zr]trastuzumab

Organ	Organ Dose (mGy/MBq)
Adrenals	0.80
Brain	0.39
Breasts	0.42
Gallbladder Wall	0.86
Lower Large Intestine Wall	0.58
Small Intestine Wall	0.57
Stomach Wall	0.63
Upper Large Intestine Wall	0.65
Heart Muscle	1.11
Kidneys	1.23
Liver	1.63
Lungs	0.59
Muscle	0.49
Ovaries	0.59
Pancreas	0.78
Red	0.69
Osteogenic Cells	0.79
Skin	0.34
Spleen	0.86
Thymus	0.57
Thyroid	0.43
Urinary Bladder Wall	0.42
Uterus	0.58
Whole Body	0.55
Effective Dose Equivalent (mSv/MBq)	0.74
Effective Dose (mSv/MBq)	0.61