VI. PROTOCOL

Assessment of HER2 Receptors in Breast Carcinoma by Positron Emission Tomography (PET) using ^{89}Zr-Trastuzumab

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Protocol Revision History

10/29/2012	Initial PRMC Submission
11/15/2012	PRMC suggested changes • Section 6.4 clarified eligibility criterion #6 • Section 6.5.3 added clarification for use of small dose of unlabeled antibody • Section 6.5.5 added clarification to revising dose of unlabeled antibody • Section 6.5.9 corrected mistake in QT interval • Section 6.11 updated table for data submission
07/01/2013	Updated protocol to match FDA submission • Title Page updated sub-investigators and study team members • Section SCHEMA – removed reference to pilot study and updated to 2 cohorts and removed references to testing for trastuzumab resistance • Updated table of contents • 6.1.0 Background removed references to testing for trastuzumab resistance. Added references to Washington University's experiences with HER2 positive and HER2 negative cell lines. • 6.2.0 Objectives: study objectives updated • 6.4.0 Inclusion Criteria/ Exclusion Criteria updated to include 2 cohorts of subjects and statement on women and minority recruitment • 6.5.2 Patient Population: updated to include 2 cohorts of subjects. Total number of subjects increased from 15 to 52 • 6.5.4 PET Imaging: updated to include 2 cohorts of subjects • 6.5.5 PET Image Processing and Analysis: updated to include 2 cohorts of subjects • Removed section 6.5.6 "Analysis of markers of Trastuzumab Resistence" updated remaining sections for numbering. • 6.7.0 Statistics: updated to include 2 cohorts of subjects • 6.8.0 Risks and Benefits: updated to include radiation dosimetry for 2 cohorts of subjects • 6.11 Data Forms and Submission Schedule: updated to include 2 cohorts of subjects • 6.12 References: updated
07/24/2013	• Title Page updated to current version number and date. Added IND #, clarified registration to Clinical trials.gov is not applicable to this phase I study. Updated study team members • 6.1.0 Background :updated maximum injected dose from 4 mCi to 2.5 mCi revised total mass of trastuzumab based on revised maximum

	injected dose, updated table 1 maximum human absorbed radiation dosimetry based on revised maximum injected dose • 6.2.0 Objectives: updated maximum injected dose to 2.5 mCi • 6.5.4 PET Imaging: updated maximum injected dose to 2.5 mCi, revised CT for attenuation correction imaging parameters to a maximum effective mAs of 30 • 6.8.0 Risks and Benefits: updated radiation dosimetry based on revised maximum injected dose and CT for attenuation correction imaging parameters.
08-22-2013	• 6.4.0 Inclusion Criteria/ Exclusion Criteria: clarified HER2 positive and negative patients are included as stratified by cohort 1 vs cohort 2. • Fixed spelling and grammar errors.
12-27-2013	• Title Page updated to current version number and date. Added HRPO#, Corrected formatting, Added study coordinator pager numbers • 6.5.4 PET Imaging clarification added to imaging time points to allow a window of ± 6 hours around each imaging time point. • 6.5.7 Clinical Laboratory Testing revised window for obtaining baseline laboratory samples • 6.11 Data Forms and Submission Schedule updated to clarify timing for baseline lab and urine samples.
06-27-2014	• Title Page added NCT# updated version date to IRB renewal date • 6.1.0 Background Dosimetry table corrected error in column 3 rad/2.5mCi injection. Recorded dose was falsely elevated because it included radiation dosimetry from CT scanning and did not accurately represent the column title.
12-10-2014	• Title Page updated version number and date and footer date throughout entire document • 6.4.0 Inclusion Criteria/Exclusion Criteria added Cohort 2 HER2 negative subjects must have normal baseline ejection fraction to be eligible for study participation • 6.5.6 Vital Signs: fixed inconsistency in protocol vital signs will be obtained 3 times on injection day • 6.5.6, 6.5.7 & 6.5.8 Vital Signs, Lab Testing and ECG testing: clarified where clinically significant findings are recorded and when they are also recorded on the adverse event log • 6.8.0 Risks and Benefits: Added risks for baseline cardiac function testing (ECHO or RVG) for cohort 2 HER2 negative subjects • 6.10.1 Adverse Events (AEs): clarified AE assessments are made to assess relationship to ^{89}Zr-Trastuzumab injection and PET/CT imaging

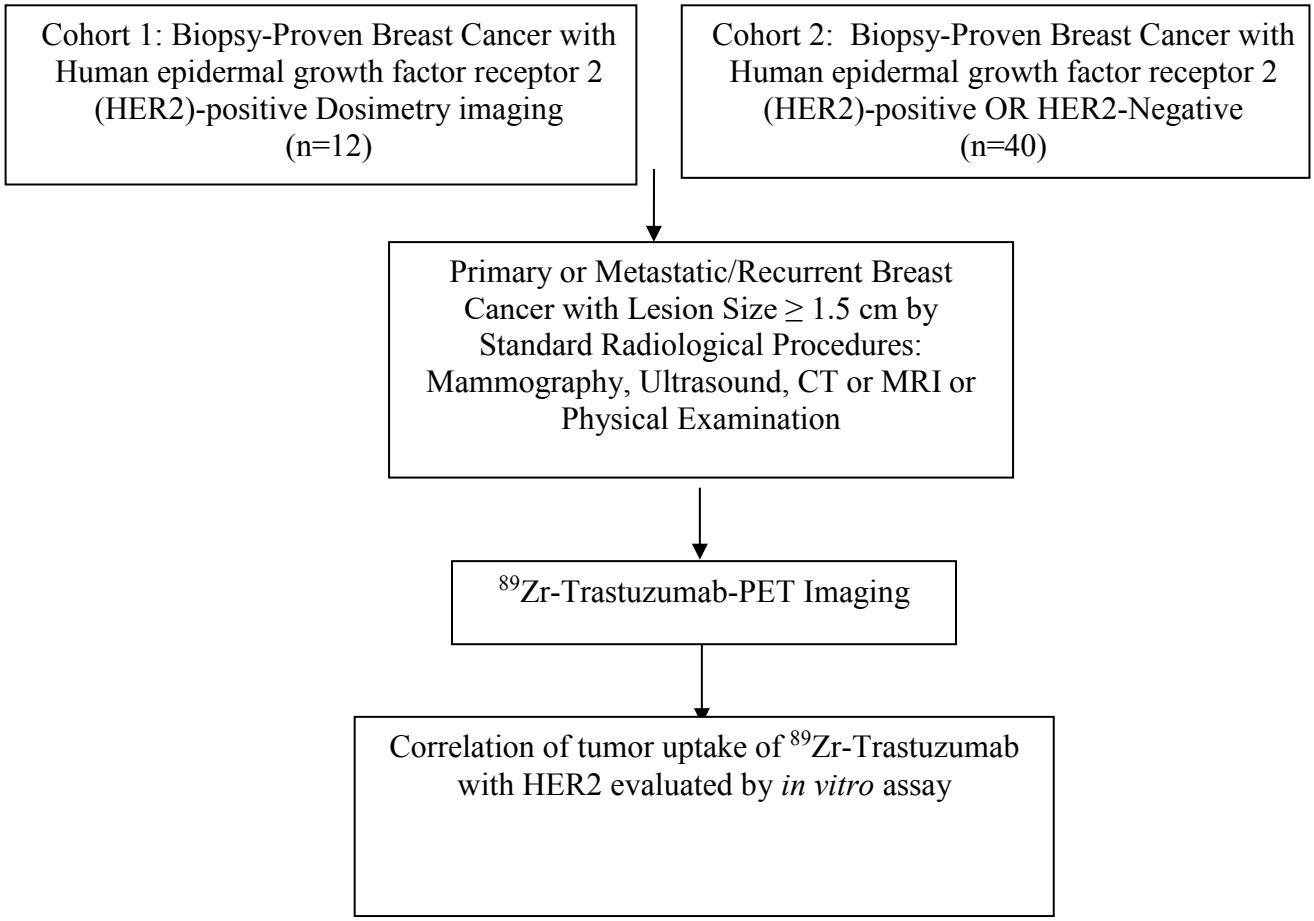
	• 6.10.5 Protocol Exceptions: Clarified that due to the nature of radioactivity and imaging, some protocol exceptions may occur. • 6.11 Data Forms and Submission Schedule: Updated footnotes in cohort 1 and cohort 2 schedule to clarify number and timing of ECG and Vital sign measurements.
05-18-2015	• Title Page updated version number and date and footer date throughout entire document to match renewal request
02-03-2016	• Title Page updated version number and date and footer date throughout entire document • SCHEMA - Continued added schema for cohort 3 • 6.1.0 Background updated to include TDM1 • 6.2.0 Objectives updated to include cohort 3 • 6.4.0 Inclusion Criteria/ Exclusion Criteria updated to include cohort 3 and to clarify stable disease is eligible • 6.5.0 METHODS updated all sections to include cohort 3 • 6.7.0 Statistics updated to add cohort 3 • 6.8.0 Risks and Benefits updated to add risks for cohort 3 including biopsy and additional radiation exposure if CT guided biopsy is needed • 6.9.0 Record Keeping add up to 2 year follow up for cohort 3 subjects • 6.11 Data Forms and Submission Schedule add cohort 3 submission schedule • 6.12 References add cohort 3 references
03-04-2016	• Title Page updated version number and date and footer date throughout entire document • 6.8.0 Risks and Benefits updated to revise the radiation dosimetry from CT guided biopsy if applicable to cohort 3 subjects
04/12/2016	CHANGES MADE AT ANNUAL RENEWAL • Title Page updated version number and date and footer date throughout entire document NOTE THESE CHANGES WERE REVIEWED BY PRMC ONLY

12/12/2016	Amendment for Cohort 3 Subjects • Protocol Page – update study coordinators • Footer updated date • SCHEMA – Updated Schema for cohort 3 • Table of Contents- updated page numbers • 6.1.0 Background- add FDG-PET/CT and breast cancer • 6.1.0 Background- update human dosimetry tables • 6.2.0 Objectives add FDG-PET/CT imaging for cohort 3 • 6.4.0 Inclusion Criteria/ Exclusion Criteria clarified cohort 3 procedures which must be completed prior to start of T-DM1 therapy • 6.5.2 Patient Population – added FDG-PET/CT to cohort 3 study patient description • 6.5.4 PET Imaging updated number of cohort 3 subjects to be studied added FDG-PET/CT imaging parameters and how results will be used • 6.5.5 PET Image Processing and Analysis Added FDG-PET/CT information • 6.7.0 Statistics updated to include FDG-PET/CT • 6.8.0 Risks and Benefits updated to include FDG-PET/CT • 6.11 Data Forms and Submission Schedule added FDG-PET to cohort 3 and clarified foot notes. • 6.12 References Updated to include published human dosimetry and other information
03/28/2017	Renewal Request update footer and version to date of request • Footer updated date • SCHEMA –updated to indicate cohort 1 and cohort 2 closed to enrollment 12/31/2016 • SCHEMA – Continued Cohort 3 updated to clarify cohort 3 specific study schema • Table of Contents- updated page numbers
06/16/2017	Protocol Amendment • Footer updated date • 6.5.4 PET Imaging revised to indicate that FDG-PET/CT imaging results after the 2nd cycle of therapy will be included in the subject's medical record.

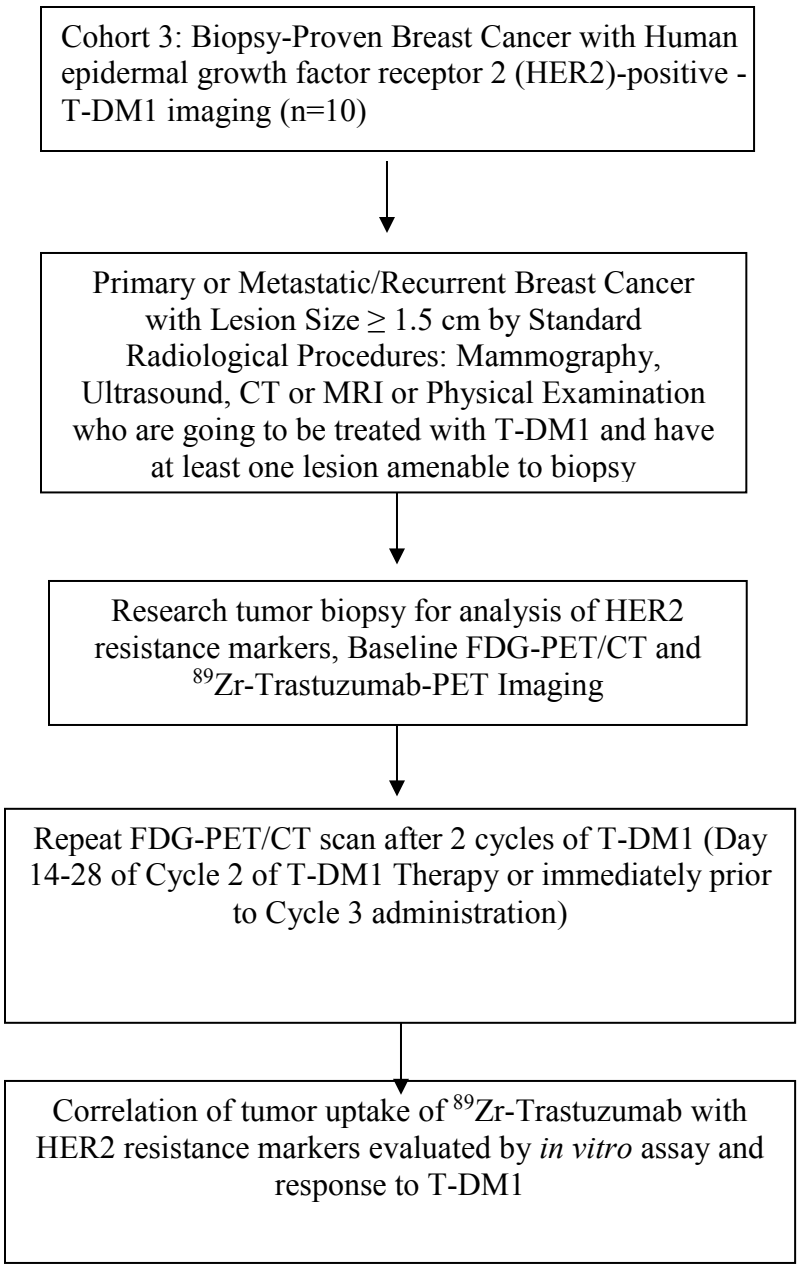
SCHEMA -

Assessment of HER2 Receptors in Breast Carcinoma by Positron Emission Tomography (PET) using ^{89}Zr-Trastuzumab

NOTE Cohort 1 and Cohort 2 closed to enrollment 12/31/2016



SCHEMA – Continued Cohort 3



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6.1.0 Background

Breast cancer remains the leading cause of cancer mortality among women in Western countries. Current estimates suggest one in eight American women will be diagnosed with breast cancer during their lifetime. Distinct characteristics of breast cancer can be exploited to help determine the overall prognosis and the likelihood of response to specific therapy. It is well established that several factors including steroid receptors, peptide growth factors, oncogenes, and tumor suppressor genes play a crucial role in the transformation of breast cancer.

Human epidermal growth factor receptor 2 (HER2) has an important role in cell survival, cell proliferation, cell maturation, metastasis and angiogenesis, as well as exerting antiapoptotic effects (1) HER2-positive breast cancer accounts for approximately 25% of all cases of breast cancer. In the setting of HER2 gene amplification or high levels of HER2 expression, the HER family of receptors and their associated signal transduction pathways play a dominant role in cell growth and survival. These tumors have a distinct natural history that, in the absence of HER2-directed therapy, is characterized by short disease-free survival and an aggressive course in the metastatic setting. Trastuzumab is monoclonal antibody directed against HER2. Its anti-tumor activity is likely attributable to several different effects. Trastuzumab downregulates HER2 expression on cell surface and activates p27 and p130. Trastuzumab also sensitizes tumor cells to TNF and inhibits tumor angiogenesis by decreasing the production of VEGF. In patients with HER2-amplified tumors, the overall response rate to single-agent trastuzumab as a first-line therapy for metastatic breast cancer is approximately 34%. In a randomized study, Slamon et al.have shown that the addition of trastuzumab improves survival in the adjuvant treatment of HER-positive breast cancer, although combined therapy with anthracycline-based regimens has been associated with cardiac toxicity (2). However, the non anthracycline-based regimens combined with trastuzumab have been associated with fewer acute toxic effects, and lower risks of cardiotoxicity and leukemia. Thus while trastuzumab therapy results in a significant improvement of disease free survival and overall survival, it is associated with cardiac toxicity resulting in symptomatic congestive heart failure and a significant drop in left ventricular ejection function (3). Identifying patients who are going to benefit from trastuzumab therapy will be of critical importance.

Markers of Trastuzumab Resistance: Multiple trastuzumab resistance mechanisms have been identified, including alternation of the of the HER2 protein that prevent trastuzumab binding and activation of downstream or parallel signaling pathways that support HER2+ breast cancer cell growth even in the presence of trastuzumab. A shorted isoform of HER2, called p95-HER2, lacks the trastuzumab binding site but is able to still promote breast cancer tumorigenesis(4). The frequency of p95-HER2 expression in patient samples has been reported to be up to 30% (5, 6). Loss of the trastuzumab binding site will result in loss of ^{89}Zr-trastuzumab binding by the tumor and this would be detectable by PET imaging.

Most studies to date have used Western blot, which has limited clinical utility, to identify the p95HER fragments. There is evidence that HER2 status may change during the course of the disease and after chemotherapy (7). In addition, discordance in HER2 expression across tumor lesions in the same patient has been reported (8-11). Thus, the use of repeated biopsies during the course of the disease is encouraged (12, 13). However, repeated biopsies are not clinically feasible due to their invasiveness and not all lesions are readily accessible to be biopsied (14). Therefore, a method that can reliably determine both the quantity and the functional status of tumor HER2 in individual lesions would be of critical importance in identifying patients who would benefit from HER2-targeted therapy.

Additional mechanisms for trastuzumab resistance include activation of pSrc or activation of PI3-kinase signaling either by PIK3CA gene mutation or deletion of PTEN and upregulation of signaling from other receptor tyrosine kinases, IGF1R, EGFR, HER3, MET and pMAPK (15, 16, 33) . These mechanisms of trastuzumab resistance would not be expected to alter trastuzumab binding.

Assessment of HER2: In order to select the patients that are likely to benefit from HER-2-targeted therapy, it is important to determine the status of HER-2 in breast cancer. Currently, two types of tests, i.e. immunohistochemistry (IHC) that detects receptor overexpression and fluorescence in situ hybridization (FISH) that identifies HER-2 gene amplification, are used for the purpose (17-19). Protein expression levels are scored as 0 or 1+ (zero or low), 2+ (intermediate) or high 3+. Recent reports indicate a considerable degree of concordance between the two methods of detection on same tumor specimens. While 100% concordance was observed in IHC 3+ readings when compared with FISH, cases with 2+ IHC score were not very reproducible (20) It is common practice to send scores of 2+ on IHC for FISH analysis, and patients whose tissue is positive on FISH (*i.e.* amplification >2.0) are offered trastuzumab. Thus, such patients must have a confirmatory FISH test before administration of trastuzumab therapy. Though detection of HER-2 by FISH is more accurate, it requires special equipment that make it more expensive. When compared to IHC, FISH test is also found to be a better predictor of response to trastuzumab therapy and overall prognosis.

Recently, ado-Trastuzumab Emtansine (T-DM1), a HER2-targeted ADC consisting of an antimicrotubule agent (DM1) linked to trastuzumab, has shown to be active in patients with trastuzumab and lapatinib refractory metastatic breast cancer. (34, 35, 36) As the mechanism for this therapy is via binding to the extracellular domain of HER2, the presence of this receptor is vital for this therapy to be efficacious.

There is evidence that HER2 status may change during the course of the disease and after chemotherapy (7). In addition, discordance in HER2 expression across tumor lesions in the same patient has been reported (8-11). Thus, the use of repeated biopsies during the course of the disease is encouraged (12, 13). However, repeated biopsies are not clinically feasible due to their invasiveness and not all lesions are readily accessible to be biopsied (14). Therefore, a method that can reliably determine both the quantity and the functional status of tumor HER2 in individual lesions would be of critical importance in identifying patients who would benefit from HER2-targeted therapy.

In vivo measurement of the HER2 expression in breast cancer could offer several advantages over current *in vitro* methods. These include assessing the HER2 expression of the entire tumor volume rather than just a part of the tumor (addressing the intrinsic heterogeneity of receptor expression), assessing the biologic availability of HER2 *in vivo*, and evaluating the effects of therapy on HER2 expression of the tumor. In addition, *in vivo* imaging can simultaneously assess HER2 expression of the primary and metastatic lesions, many of which may be inaccessible to biopsy. Efforts have been made to develop imaging agents for HER2 labeled with single photon radionuclides and positron emitting radionuclides for noninvasive *in vivo* evaluation of HER2 expression and localization of HER2-overexpressing tumor lesions including inaccessible metastatic foci. ^{111}In-trastuzumab, a single photon emission radiopharmaceutical, has been developed and tested in patients with breast cancer (21). Perik et al. have shown HER2-specific uptake in patients with HER2-positive metastatic breast cancer. ^{111}In-trastuzumab imaging discovered unsuspected HER2-positive lesions in 13 of 15 patients and was therefore considered to be of potential value as a clinical diagnostic tool in patients with metastatic breast cancer (21). In addition, there are several other radiopharmaceuticals that have been used for clinical HER2 imaging, such as ^{99m}Tc-labeled anti-HER2 rat antibody ICR12. ^{99m}Tc-ICR12 was administered to 8 patients with breast cancer, which suggested that this radiopharmaceutical could be used for imaging of HER2-positive disease (22). Currently, there are on-going clinical studies with both

gamma-emitting and positron emitting radiopharmaceuticals, such as ^{111}In- and ^{64}Cu-trastuzumab and with ^{68}Ga-trastuzumab F(ab′)$_2$ fragments (23, 24).

HER2 Imaging with PET: Positron emission tomography (PET) has been shown to have the ability to image radioligand binding quantitatively at tracer concentrations. This is a distinct advantage over conventional nuclear medicine using planar or SPECT imaging. This has encouraged considerable efforts to develop positron emitting radionuclides such as ^{64}Cu-trastuzumab and ^{68}Ga-trastuzumab F(ab′)$_2$ fragments, ^{68}Ga-ABY-002 and ^{89}Zr-trastuzumab (25). Limited clinical studies with ^{68}Ga-ABY-002 have shown that high liver and renal uptake interferes with lesion detection in patients with metastatic lesions in the abdomen (26). ^{89}Zr-trastuzumab using intact antibodies has the advantage over the smaller HER2-directed F(ab′)$_2$ fragments, as generally, large intact monoclonal antibodies penetrate slowly but constantly into solid tumor tissue, ultimately resulting in higher accumulation in the tumor than is the case with small proteins when antibody fragments penetrate faster into tumor tissue but show less tumor uptake because of more rapid clearance from the blood (10). In addition, for immunoPET imaging, positron-emitting radiometals such as ^{89}Zr have an advantage over radiohalogens such as ^{124}I because they are residualizing and are therefore retained within the target cell after internalization and intracellular degradation of the tracer. This results in higher uptake in the tumor when an internalized antibody such as trastuzumab is used (10). Of the positron-emitting radiometals, ^{89}Zr has the longest and therefore most favorable half-life (78.4 h), allowing antibody imaging up to 7 days after the injection, the time needed for *in vivo* circulation, optimal biodistribution and tumor targeting. Preclinical evaluation of ^{89}Zr-trastuzumab showed that it displays superior image quality as compared to ^{111}In-trastuzumab, given the high spatial resolution and sensitivity of PET (27). At Washington University, we have evaluated MDA435/LCCHER2/GFP/Luc (HER2-positive) and MDA435/Lcc6Vector (HER2-negative) cell lines, which overexpress and minimally express HER2, respectively, with 89Zr-trastuzumab. Using flow cytometry, MDA435/LCC6HER2GFP/Luc cells demonstrated elevated levels of HER2 expression in comparison to MDA435/LCC6Vector cells, which demonstrated minimal expression. Both cell lines also express luciferase for bioluminescence imaging to confirm tumor growth. In *in vitro* studies, the uptake of 89Zr-trastuzumab was 8.5 fold greater in HER2-positive cells compared to HER2-negative cells ($p < 0.01$).

Dijkers et al. studied 14 patients with HER2-positive metastatic breast cancer with ^{89}Zr-trastuzumab-PET (28). These patients received 37 MBq of ^{89}Zr-trastuzumab at one of three doses (10 or 50 mg for those who were trastuzumab-naive and 10 mg for those who were already on trastuzumab treatment). The patients underwent at least two PET scans between days 2 and 5 after ^{89}Zr-trastuzumab injection. The results of the study showed that the best time for assessment of ^{89}Zr-trastuzumab uptake by tumors was 4–5 days after the injection. The accumulation of ^{89}Zr-trastuzumab in lesions allowed PET imaging of most of the known lesions and some that had been undetected earlier. The relative uptake values (RUVs) (mean ± standard error of mean [SEM]) were 12.8 ± 5.8, 4.1 ± 1.6, and 3.5 ± 4.2 in liver, bone, and brain lesions, respectively, and 5.9 ± 2.4, 2.8 ± 0.7, 4.0 ± 0.7, and 0.20 ± 0.1 in normal liver, spleen, kidneys, and brain tissue, respectively. This first-in-human ^{89}Zr-trastuzumab HER2-PET imaging study showed excellent tumor uptake and visualization of HER2-positive metastatic liver, lung, bone, and even brain tumor lesions, when an adequate trastuzumab protein dose was administered. In trastuzumab-naive patients with HER2-positive metastatic breast cancer, 50 mg of trastuzumab was the dose that resulted in optimal biodistribution characteristics under the test conditions and proved adequate for ^{89}Zr-trastuzumab-PET imaging. The 50-mg trastuzumab dose in naive patients is a good starting point for further optimization in future studies. In patients undergoing treatment with trastuzumab at the time of tracer injection, 10 mg trastuzumab was adequate for PET imaging. Higher doses of trastuzumab were not expected to improve ^{89}Zr-trastuzumab-PET imaging; trastuzumab clearance was already minimal, and therefore a further increase in the dose of

trastuzumab could induce target saturation. Quantification of the PET images confirmed the dose-dependency of trastuzumab clearance and revealed a significantly higher uptake in metastatic tumor lesions as compared to corresponding normal tissue. In this study, lesions with HER2 overexpression can be distinguished from those without HER2 overexpression based on ^{89}Zr-trastuzumab uptake. However, further studies are needed to determine the exact amount of HER2 expression required for adequate imaging. Thus, due to these advantages, we have chosen ^{89}Zr-trastuzumab with PET to study HER2 expression in patients with breast cancer.

FDG-PET/CT Imaging: The use of PET imaging with ^{18}F-fluorodeoxyglucose (FDG) for restaging breast cancer patients who have progressed on currently therapy and in predicting response to therapy in breast cancer is well documented and beneficial (37,38). FDG is transported into the cell via glucose transporters, but unlike glucose, FDG is not metabolized but is irreversibly phosphorylated by hexokinase and trapped within the cell. Glucose transport is upregulated in most cancers and the use of FDG PET imaging for restaging breast cancer patients who have progressed on current therapy is routinely used at our institution. Biologic correlates of FDG uptake in breast cancer include mitotic activity index, histologic grade, tumor cell density, as well as other markers of aggressiveness. FDG-PET imaging has also proven useful in monitoring response to chemotherapy in the metastatic setting.

Animal Toxicity Study

Animal Toxicity studies were conducted at Memorial Sloan Kettering Cancer Center The objective of the study was to evaluate the acute toxicity of a single high dose intravenous infusion of cold Zr-DFO-Trastuzumab in mice 250 times higher than the proposed clinical PET imaging dose for ^{89}Zr-DFO-Trastuzumab.

Zr-DFO-Trastuzumab was tested at a dose level of 10 mg/kg in B6D2F1mice (32 male and 32 female). No unacceptable adverse events were observed for the test article during acute dosing or the following 14-day observation period. The study concluded that 10 mg/kg ^{89}Zr-DFOtraztuzumab administered i.v. as a single dose is the No Observed Adverse Effect Level

Dosimetry (NOAEL in B6D2F1 mice)**:**

The human radiation dosimetry estimates for the imaging agent ^{89}Zr-trastuzumab was calculated from mice dissection biodistribution data and from human S-values for ^{89}Zr. Radiation dose estimates were calculated using the MIRD methodology. The human female and male adult model was used with the program OLINDA/EXM to calculate the normal organ radiation dose estimates. A dosage of maximum of 2.5 mCi is proposed for human studies. Assuming a worst case specific activity of 0.2 mCi/mg, the ^{89}Zr-trastuzumab mass injected for a 2.5 mCi dosage would be 12.5 mg. The dosage given to mice and hamsters in the toxicity study was more than 30 times the mass (10 mg/kg) as the proposed dosage to humans. Thus, favorable binding characteristics, animal biodistribution, dosimetry, and our ability to produce high-specific-activity product combine to make ^{89}Zr-trastuzumab a candidate for human breast cancer imaging.

Table 1. Extrapolated human radiation dose estimates for ^{89}Zr-Herceptin per unit of administered activity. (Cohort 1 and Cohort 2 Study Subjects)

Organ	**Dose (rad/mCi)**	**Dose (rad/ 2.5 mCi)**
Adrenals	1.99	4.98
Brain	0.53	1.33
Breasts	1.21	3.03
Gallbladder	1.66	4.15
LLI Wall	1.70	4.25
Small Intestines	1.61	4.03
Stomach	1.54	3.85
ULI Wall	1.74	4.35
Heart muscle	2.93	7.33
Kidneys	1.98	4.95
Liver	2.07	5.18
Lungs	2.23	5.58
Muscle	1.26	3.15
Ovaries	1.85	4.63
Pancreas	1.71	4.28
Red Marrow	1.45	3.63
Bones	1.86	4.65
Skin	0.94	2.35
Spleen	1.59	3.98
Thymus	1.73	4.33
Thyroid	1.43	3.58
Urinary Bladder	1.37	3.43
Uterus	2.36	5.90
Total Body	1.39	3.48
Effective Dose (rem)	1.63	4.08

We are planning to inject 2.5 mCi in order to provide images of sufficient quality for analysis and interpretation. For dosimetry patient, imaging is performed over 6-7 bed positions and at 5-8 minutes per bed position, resulting in an imaging duration of approximately 1 hour. We judge that 1 hour is the longest imaging duration a patient can tolerate in the scanner.

Based on dosimetry results from cohort 1 and the associated publication (39) the maximum injected dose to cohort 3 patients will be 1. 5 mCi

Table 2. Human Radiation Dosimetry for ^{89}Zr-Herceptin per unit of administered activity. (Cohort 3 Study Subjects)

Organ	**Dose (rad/mCi)**	**Dose (rad/ 1.5 mCi)**
Adrenals	2.96	4.44
Brain	1.44	2.16
Breasts	1.55	2.33
Gallbladder	3.18	4.77
LLI Wall	2.15	3.22
Small Intestines	2.11	3.16
Stomach	2.33	3.50
ULI Wall	2.41	3.61
Heart muscle	4.11	6.16
Kidneys	4.55	6.83
Liver	6.03	9.05
Lungs	2.18	3.27
Muscle	1.81	2.72
Ovaries	2.18	3.27
Pancreas	2.89	4.33
Red Marrow	2.55	3.83
Bones	2.92	4.38
Skin	1.26	1.89
Spleen	3.18	4.77
Thymus	2.11	3.16
Thyroid	1.59	2.39
Urinary Bladder	1.55	2.33
Uterus	2.15	3.22
Total Body	2.04	3.05
Effective Dose (rem)	2.26	3.39

6.2.0 Objectives

We are planning to perform a trial with goals to demonstrate the feasibility of imaging breast cancer patients with ^{89}Zr-trastuzumab-PET, to evaluate preliminarily the relationship between tumor ^{89}Zr-trastuzumab uptake and *in vitro* positivity of HER2, to assess the safety of ^{89}Zr-trastuzumab and human dosimetry of this tracer. In a pilot study, we are planning to study the correlation between ^{89}Zr-trastuzumab uptake and known markers of resistant to Her2-targeted therapies (such as PTEN, pAkt, pMAPK, and pSrc) and to assess whether ^{89}Zr-trastuzumab-PET can assist in identifying patients with HER2-positive breast cancer who are resistant to HER2-targeted therapy with T-DM1. This is to obtain pilot data for planning a future trial to more definitively evaluate the role of ^{89}Zr-trastuzumab in predicting response, assessed according to standard clinical response criteria, to anti HER2 therapies.

Study objectives:

6.2.1 To determine the diagnostic quality and lesion detection rate of ^{89}Zr-trastuzumab-PET images.

6.2.1.1 To assess the diagnostic quality and lesion detectability of ^{89}Zr-trastuzumab-PET images at a maximum of 2.5 mCi dose. Also to determine the imaging time after injection of ^{89}Zr-trastuzumab that yields best image quality and lesion detection rate.

6.2.2 To calculate human dosimetry of ^{89}Zr-trastuzumab and assess the safety ^{89}Zr-trastuzumab administration.

6.2.3 To evaluate the relationship between tumor ^{89}Zr-trastuzumab uptake and *in vitro* status of HER2.

6.2.4 To evaluate preliminary if the degree ^{89}Zr-trastuzumab uptake (and intra-patient lesion heterogeneity in uptake) can predict patient response to the T-DM1 antibody drug conjugate.

6.2.5 To identify metabolically active disease to map HER2+ disease and to predict response to T-DM1 therapy.

6.265 To evaluate if loss of tumor binding of trastuzumab is the mechanism for acquired resistance to T-DM1. The tumor uptake of ^{89}Zr-trastuzumab will be correlated with markers of HER2 resistance on patient's biopsy tissue obtained at the time of resistance to trastuzumab-targeted therapy prior to T-DM1 therapy initiation.

6.3.0 Statement of Qualifications

The FDA 1572 Form is provided in the IND submission along with curriculum vitae for the study's Principal Investigator, Farrokh Dehdashti, M.D. Washington University School of Medicine, St. Louis, Missouri.

6.4.0 Inclusion Criteria/ Exclusion Criteria

Adult women with biopsy- proven breast cancer who have undergone HER2-testing and who will be treated according to standard of care are eligible to participate in this study.

Inclusion Criteria:

- Female patients 18 years of age or older
- Cohort 1: Her2-positive (defined as 3+) or FISH HER2:CEP17 ratio > 2 biopsy-proven breast cancer
- Cohort 2: Her2-positive (defined as 3+) or FISH HER2:CEP17 ratio > 2 OR HER2-negative (0 or 1+, 2+ and FISH negative) biopsy-proven breast cancer
- Cohort 2 HER2 negative subjects must have normal baseline ejection fraction (as determined by standard echocardiogram (ECHO) or radionuclide ventriculogram (RVG) obtained within 3 months of ^{89}Zr-Trastuzumab infusion. If not performed as standard of care, a study specific ECHO or RVG may be scheduled after informed consent has been obtained as part of baseline screening.
- Cohort 3: Her2-positive (defined as 3+) or FISH HER2:CEP17 ratio > 2 biopsy-proven breast cancer who are going to be treated with T-DM1 and have at least one lesion amenable to biopsy. Biopsy, FDG-PET/CT prior to and after 2 cycles of T-DM1 and ^{89}Zr-Trastuzumab-PET/CT imaging must be completed prior to starting TDM-1 therapy.

- Primary or recurrent/metastatic lesion size ≥ 1.5 cm as determined by imaging studies (ultrasonography, mammography, CT or MRI) or physical examination. Subjects with stable disease whose lesion size meets criteria are also eligible to participate.
- Able to give informed consent
- Not currently pregnant or nursing: Subject must be surgically sterile (has had a documented bilateral oophorectomy and/or documented hysterectomy), post-menopausal (cessation of menses for more than 1 year), non-lactating, or of childbearing potential for whom a urine pregnancy test (with the test performed within the 24 hour period immediately prior to administration of ^{89}Zr-trastuzumab) is negative
- Cohort 1 and cohort 2 patients currently receiving trastuzumab therapy with or without other types of systemic therapy can participate during therapy provided lesion size criteria is met, or if their disease progresses (development of new lesion(s) or worsening of known lesion(s) based on imaging modalities or physical examination.
- Cohort 3 patients must complete all study related procedures (biopsy, injection of ^{89}Zr-Trastuzumab and imaging 5-7 days later) prior to starting treatment with TDM-1

Exclusion Criteria:

- 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
- Unable to tolerate up to 60 min of PET imaging per imaging session

6.4.1 Inclusion of Women and Minorities

Only women will be enrolled due to the anatomical specificity of breast cancer. Members of all races and ethnic groups are eligible for this trial.

6.5.0 METHODS

6.5.1 Study Design

A single center, open-label, baseline-controlled diagnostic imaging study designed to assess the diagnostic quality of ^{89}Zr-trastuzumab imaging with PET.

6.5.2 Patient Population

Cohort 1: 12 adult women with Her2-positive primary, recurrent/metastatic breast cancer with lesion size ≥ 1.5 cm by standard radiological procedures: mammography, ultrasound, CT or MRI or physical examination will be studied once with ^{89}Zr-trastuzumab-PET imaging.. Patients who are on trastuzumab based therapy can also participate. Patients with recurrent/metastatic disease are required to have at least one measurable lesion (as defined by RECIST 1.1).. Written informed consent will be obtained prior to the PET imaging session. ^{89}Zr-trastuzumab-PET images at 2 times points in each patient will be compared to identify the time point that yields the best lesion detection rate. ^{89}Zr-trastuzumab-PET images also will be correlated with all available imaging studies to assess lesion detection rate. . Cohort 1 subjects entered on study will be asked to undergo total body ^{89}Zr-trastuzumab-PET imaging at two separate time points for purposes of calculating human dosimetry.

Cohort 2: To evaluate whether tumor uptake ^{89}Zr-trastuzumab correlates with tumor expression of HER2, only adult women with at least one measurable (based on RECIST 1.1) primary or metastatic lesions, which has known HER2 status will be evaluated. HER2-negative (0 or 1+, 2+ and FISH negative) and HER2-positive lesions (3+ by IHC and FISH positive) will be evaluated. A total of 40 patients will yield a minimum of 40 evaluable lesions, 20 HER2-negative and 20 HER2-positive. Patients will undergo only one PET imaging study after ^{89}Zr-trastuzumab injection similar to the imaging protocol used Specific Aim 1, but at the best time established from Cohort 1.

Cohort 3: To evaluate preliminarily if ^{89}Zr-trastuzumab can predict patient response to the T-DM1 antibody drug conjugate. To evaluate if loss of tumor binding of trastuzumab is the mechanism for acquired resistance to T-DM1. Also, to identify metabolically active tumor by FDG-PET/CT in order to map HER2+ metabolically active disease sites by ^{89}Zr-trastuzumab. In this Cohort, only adult women with at least one measurable (based on RECIST 1.1) HER2-positive primary or metastatic/recurrent lesion which is amenable to biopsy will be evaluated. A total of 5 patients will be evaluated. Patients will undergo FDG-PET/CT followed by ^{89}Zr-trastuzumab injection and imaging at the best time established from Cohort 1 (5 to 7 days post ^{89}Zr-trastuzumab injection). These patients will also undergo a research tumor biopsy for in vitro analysis of known markers of resistant to HER2-targeted therapies (such as PTEN, pAkt, pMAPK, and pSrc) prior to starting T-DM1-therapy. Optimal pre-therapy scheduling will be FDG-PET/CT, biopsy, followed by injection, and imaging 5 to 7 days later. However, due to anticipated scheduling conflicts other scheduling options will also be acceptable. In addition, cohort 3 subjects will undergo FDG-PET/CT an day 14-28 of cycle 2 of T-DM1 therapy or immediately prior to administration of cycle 3 of therapy.

6.5.3 Infusion of Trastuzumab and ^{89}Zr-Trastuzumab

As suggested by Dijkers et al. (28). To minimize uptake of the radiotracer in normal tissues, trastuzumab-naïve patients (OR patients who have received their last dose of transtuzumab therapy ≥ 4 weeks prior to planned ^{89}Zr-trastuzumab injection will receive a 50 mg dose of trastuzumab and patients already on trastuzumab treatment will receive a 10 mg dose, typically 30 min to 2 hours, prior to ^{89}Zr-trastuzumab injection. For subjects who are currently receiving trastuzumab therapy a standard of care therapy dose can be substituted for the 10 mg dose if scheduling permits. The administration of a fixed small dose of unlabeled antibody to improve tumor-to-normal-tissue (T/N) uptake of the radiolabeled antibody is standard in this type of imaging procedure. Infusion of trastuzumab will be prepared and administered in the treatment area of the Siteman Cancer Center according to normal treatment protocol by personnel appropriately trained and certified to administer chemotherapeutic agents. Following the infusion of trastuzumab subjects will be monitored for approximately 30 minutes for infusion reactions and side effects

Because the dose of trastuzumab being administered is a very small fraction of the dose normally administered as standard treatment to this patient population and it is being administered one time on study, no dose modifications for trastuzumab are allowed. We do not expect any side effects as a result of the administration of the small dose of trastuzumab for this study. However, as it is suggested for therapeutic dose, subjects who experience serious infusion reaction (i.e., dyspnea, chest tightness, fever, rigors or hypotension) during trastuzumab administration will have the infusion stopped. Continuation of dosing and continuation on study will be based on the severity and

resolution of the event and will be at the discretion of the investigator and the referring/treating physician. Any treatment for an infusion reaction should be administered per institutional practices.

Immediately following release from the treatment area subjects will be transported to the clinical nuclear medicine facility or the CCIR for administration of ^{89}Zr-trastuzumab. A maximum of 2.5 mCi (range 1-2.5 mCi) of ^{89}Zr-trastuzumab will be injected intravenously over a period of at least 60 seconds. Injection will be followed by a normal saline flush of 20-30 ml. Patients will be monitored for 30 min after injection to detect any infusion-related anaphylactic reactions or adverse events. Whenever possible and as applicable, the injection site should be on the arm opposite of the known breast lesion

6.5.4 PET Imaging

A total of 42 adult women with Her2-positive breast cancer and 20 adult women with HER2-negative breast cancer will be studied with ^{89}Zr-trastuzumab-PET imaging in cohorts 1 and 2. An additional 5 subjects will be scanned in cohort 3. In all cases subjects will have a minimum of 24 hours to review the consent form before agreeing to participate in this research project. All PET imaging will be performed with a CTI/Siemens Biograph 40 PET/CT scanner, located on the 10th floor West Pavilion of Barnes-Jewish Hospital in the Center for Clinical Research Imaging (CCIR). The Biograph 40 is a 4-ring PET scanner made up of a multi-LSO-detector ring system with 3D acquisition and reconstruction and 109 image planes with an extended 21.6 cm axial field of view, enabling the detection of 78% more photons (compared with a conventional-field-of-view scanner). The scanner features high spatial resolution (less than 5 mm in transaxial and axial dimensions) with Pico 3D ultra fast electronics for decreased dead-time and high signal-to-noise ratio.

All patients will undergo routine clinical staging as dictated by the treating medical oncologist or surgeon. The results of ^{89}Zr-trastuzumab-PET will not be provided to the patient or the treating oncologist/surgeon unless, in the judgment of the principal investigator, the images demonstrate an unsuspected abnormality that may warrant further evaluation. The results of baseline FDG-PET/CT for cohort 3 subjects will be reported clinically and made a part of a subject's medical record as this information is considered useful and relevant to patient management. The results of the FDG-PET/CT scan performed after 2 cycles of T-DM1 therapy will also be reported due to the fact FDG-PET/CT imaging which clearly shows progression after two cycles of therapy should be known so that the treating physician can make an informed decision about continuing on therapy. .

6.5.4.1 PET Imaging Procedure

All subjects entered on study will be asked to undergo total body (brain to upper thighs or extended to include metastatic disease in the lower legs) ^{89}Zr-trastuzumab-PET imaging.

Subjects who enter on study in Cohort 1 will undergo whole body imaging twice within 7 days following administration of ^{89}Zr-trastuzumab to measure HER2 density and calculate human dosimetry. 4 Subjects will be imaged at each of the following time points:

1 and 3 days post injection.

2 and 5 days post injection.

4 and 6 days post injection

A window of ± 6 hours is allowed for all imaging time points

Subjects who enter on study in cohort 2 or cohort 3 will undergo total body imaging a single time at the optimal time point as determined from cohort 1 image assessment.

For all cohorts receiving ^{89}Zr-trastuzumab-PET imaging, the subject will be placed supine on the imaging table with arms resting above the head or secured by the sides. A spiral CT scan for attenuation correction will be obtained from the top of the skull through the upper thighs. The CT will consist of a 10-20 second topogram for determining correct anatomical positioning followed by a spiral CT at a maximum of 30 mAs (or care dose calculated dose if less than 30 mAs) and 120 kVp (Biograph 40). Average CT scan time is 15-30 seconds. Scans are acquired using a 5-mm-slice thickness. Immediately after the attenuation CT scan, emission images beginning at the top of the skull and proceeding down through the upper thighs will be obtained (1-10 min per bed position).

In addition to ^{89}Zr-trastuzumab-PET imaging, Cohort 3 subjects will also undergo FDG-PET imaging at two time points. For FDG-PET imaging patients will fast for at least 4 hours and have their glucose checked prior to FDG administration. Fasting blood glucose must be ≤ 200 mg/dL (or approved by attending NM physician if greater) for FDG administration to occur. 10-15 mCi of FDG will be administered intravenously followed by a 50-70 min uptake period. Oral hydration or IV hydration (up to 500 ml) may be given during the uptake period. Oral and IV contrast will not be routinely administered for FDG-PET imaging.

Cohort 3 FDG scanning parameters will vary depending on timing of scan within protocol. The subject will be placed supine on the imaging table with arms resting above the head or secured comfortably by the side. A spiral CT scan for attenuation correction will be obtained from the base of the brain to the upper thighs (or adjusted higher or lower to include known sites of disease). As described above a topogram will be obtained followed by a spiral CT scan. The baseline CT scan (done prior to starting TDM-1 therapy) will be a maximum of 111 mAs (or care dose if calculated to be less) the follow up scan performed after 2 cycles of therapy will be a maximum of 50 mAs or case dose calculated. Immediately after the attenuation scan emission imaging will be obtained at 2-5 minutes per bed position.

For safety analysis, all subjects will undergo vital sign measurement, clinical laboratory testing, and ECG testing as specified in sections 6.5.6, 6.5.7 and 6.5.8 of the protocol. No additional safety analysis is needed for FDG-PET/CT.

6.5.5 PET Image Processing and Analysis

The emission images will be corrected for measured attenuation using CT data according to the provided scanner manufacturer software package. PET images will be evaluated semiquantitatively by the use of standardized uptake value (SUV) and tumor-to-normal tissue (T/N) ratio or tumor-to-muscle (T/M) ratio. The SUV is widely used for assessment of regional tracer accumulation in oncological studies, is technically simple to perform, and makes imaging easier for the patient because longer dynamic imaging is not required. SUV is a decay-corrected measurement of activity per volume of tissue (nCi/mL) divided by the average activity per unit mass in the entire body.

Cohort 1: Images will be assessed by the principal investigator, who will initially be blinded to all clinical information available (tumor size, location, and HER2 status). Overall image quality will be graded (using 4-point scale with 1 being the worse and poor quality, not acceptable for diagnostic interpretation and 4 being good image quality, similar to routine clinical studies), the number of lesions (up to 5 most intense lesions and in patients with multiple lesions, 2 in each organ), and the degree of confidence for malignancy of each lesion (tumor foci will be graded on a five-point scale regarding the presence or absence of abnormal uptake: 1 = definitely normal; 2 = probably normal; 3 = equivocal; 4 = probably abnormal; and 5 = definitely abnormal). Semiquantitative analysis of the images will also be obtained. For all tumor foci (primary and metastases, if identified and grade 4 or 5 on the malignancy confidence scale) and main organs (i.e., blood pool, liver, kidney, bladder, etc.), an SUV calculation (or T/N, T/M) will be performed.

Human Dosimetry (results published (39): Calculation from Cohort 1 images: Using our standard image analysis software, regions of interest (ROIs) will be traced on the visible organs as visible on the PET/CT whole body images. Average organ activity concentration will be measured from large 3D ROI encompassing most of the organ volume. Decay corrected to injection time activity concentrations will be expressed as fraction of injected activity and plotted as a function of time combing all data points from all twelve patients. Activity organ residence times will be calculated by numerical or analytical integration of the time-activity curves. Uptake/clearance functional fits of mono- or bi-exponential functions will be performed and analytical integration, accounted for physical decay, will be performed.

It will be assumed that the lung and heart chamber residence times to be determined in proportion of their respective blood content which will be taken as 300 and 550 mL with respect to the whole body blood volume *(29)*. The blood content of the major organ are taken to be: 550mL in heart, 300 mL in the lungs, 250 mL in the liver, 190mL in the bone, 77mL in the spleen, and 72 ml in the kidneys for a standard human size of 70 kg with 5.1 liter of blood *(29)*. The red marrow residence times will be calculated from the formula of Wessels et al. *(30)* for intact antibody.

$A_{RM} = \left(\frac{RMECFF}{1 - HCT} \right) A_{blood} \frac{M_{RM}}{M_{blood-patient}}$ where RMCEFF = 0.19 for the red marrow extra cellular fluid fraction (RMECFF), HCT, the hematocrit value taken as 0.39 and M_{RM} and $M_{blood\text{-}patient}$ are the mass of red marrow and mass of blood in the patient *(31)* Since activity in the blood is assigned to each major organ, 72% of the blood residence time will be assigned to the remainder of the body. All unaccounted for activity will be assumed to be excreted. This value is expected to be small due to the long retention time of mono-clonal antibodies.

The calculated residence times will be used with the program OLINDA/EXM for 89Zr and using the adult human female model to calculate the individual organ radiation dose, the whole-body dose and the effective dose. Doses will be reported in rad/rem per mCi injected or rad/rem

Cohort 2: Images will be evaluated by one observer qualitatively (graded on a five-point scale regarding the presence or absence of abnormal uptake: 1 = definitely negative; 2 = probably negative; 3 = equivocal; 4 = probably positive; and 5 = definitely positive) and semiquantitatively (SUV and T/N) with the knowledge of the location of

the lesion with known HER2 status. The uptake of ^{89}Zr-trastuzumab will be correlated to the HER2 status of each lesion. It is expected that a significant difference ($p < 0.05$) in ^{89}Zr-trastuzumab tumor uptake between HER-positive and HER2-negative lesions will be seen.

Cohort 3: ^{89}Zr-trastuzumab images will be evaluated by one observer qualitatively (graded on a five-point scale regarding the presence or absence of abnormal uptake: 1 = definitely negative; 2 = probably negative; 3 = equivocal; 4 = probably positive; and 5 = definitely positive) and semiquantitatively (SUV and T/N) with the knowledge of the location of the lesion with known HER2 status. The uptake of ^{89}Zr-trastuzumab will be correlated to response to T-DM1 therapy and the markers of resistant to HER2-targeted therapies. FDG-PET/CT images will be evaluated according to standard clinical practice with generation of a clinical report, and inclusion of images and report to the participant's medical record (only for the baseline FDG-PET/CT). The second FDG-PET/CT is considered research and the results of the study will not be reported, however, the treating oncologist will be informed of the results if there is a finding that may impact patient management or is life threatening. Additional study specific analysis will consist of SUV measurement of six most intense lesions (not more than 2 lesions per organ) in order to assess change in FDG uptake after therapy and assess metabolic response.

6.5.6 Vital Signs

All vital signs will be recorded on the case report form. Vital signs may be obtained with the subject in the supine or upright position. Care will be taken to obtain subsequent recordings with the subject in the same position (supine or upright). Although allergic or other immediate adverse reactions are not anticipated, subjects will be monitored for at least 30 min post injection in an area where emergency equipment is available. Vital signs will be obtained pre-injection (within 30 minutes prior to injection of ^{89}Zr-trastuzumab), within 10-15 min post injection, and at study discharge on injection day. At each imaging session vital signs will be obtained prior to placing the subject on the imaging table and at discharge after the scan has been completed. . Vital signs will include the following: heart rate, systolic blood pressure, diastolic blood pressure, respiratory rate, and body temperature. The following changes from baseline will be considered noteworthy:

Heart rate:	> 20 beats per minute
Systolic blood pressure	> 20 mm Hg
Diastolic blood pressure	> 10 mm Hg

Noteworthy changes will be documented on the PI Data Safety Review Form. The Principal Investigator will indicate on the form whether or not the changes in vital signs are clinically significant. If clinically significant, the principal investigator will assess the causality of the change to the injection of ^{89}Zr-trastuzumab. Clinically significant changes in vital signs will be followed up hourly until they return to baseline or normal levels, or until follow-up is no longer warranted. If a clinically significant change of a vital sign is noted and assessed as being attributable to ^{89}Zr-Trastuzumab injection by the PI on the Data Safety and Review CRF, it will be reported on the adverse event log.

6.5.7 Clinical Laboratory Testing

Laboratory tests will consist of the following: standard CBC, comprehensive metabolic panel and urinalysis obtained at the following time points:

Baseline: Baseline labs may be obtained anytime within 21 days prior to ^{89}Zr-trastuzumab injection. .

Subsequent imaging days up to 7 days (2 time points for cohort 1 subjects and 1 time point for cohort 2 and cohort 3 subjects): Approximately 14 ml of blood and a urine sample will be collected at each imaging time point.

Additional standard of care laboratory testing obtained up to 30 days post injection will be collected and reviewed. Analysis of this will take into account whether or not the subject has undergone surgical removal of tumor or neoadjuvant treatment including hormonal therapy or chemotherapy.

The following changes from baseline clinical laboratory values are considered to be noteworthy and require assessment as to clinical significance when they fall outside of normal limits. Clinically significant changes in laboratory values which are attributed to ^{89}Zr-Trastuzumab injection should be followed up daily until they return to baseline or normal levels, or until follow-up is no longer warranted. Laboratory values that are abnormal at baseline but move into normal range will not be considered clinically significant. If a clinically significant change of a laboratory value is noted, it will be reported on the PI Data Safety and Review Form so that the PI can assess the change as being attributable to ^{89}Zr-Trastuzumab injection. All clinically significant laboratory changes as identified below which are identified by the PI as being attributable to ^{89}Zr-Trastuzumabwill be recorded on the adverse event log.

Clinically Significant Laboratory Values

Analyte	Change from baseline
Hemoglobin	> 2g/dL
WBCs	> 1 K/mm^3
Neutrophils	> 10 %
Lymphocytes	> 10%
Platelets	> 50 K/mm^3
Creatinine	> 0.75 mg/dL
BUN	> 20 mg/dL
Calcium	> 1mg/dL
Sodium	> 5 mmol//L
Potassium	> 0.5 mmol/L
CO_2	> 4 mmol/L
ALT (SGPT)	> 150 IU/L
AST (SGOT)	> 100 IU/L
Alkaline Phosphatase	> 150 IU/L
Total Bilirubin	> 0.5 mg/dL
Albumin	> 1g/dL

Changes in pre and post injection urinalysis will be noted but due to variability will not be used to determine clinical significance for changes due to the injection of ^{89}Zr-trastuzumab.

6.5.8 Electrocardiograms (ECGs)

A standard 12-lead ECG will be obtained on all subjects at baseline (within 30 minutes prior to injection of ^{89}Zr-trastuzumab), 5-10 min post injection, and prior to discharge on injection day. ECG will also be obtained at both imaging sessions.

The following table lists criteria for normal limits and clinically notable limits for ECGs in adults.

Criteria for Normal Limits and Clinically Notable Limits for ECGs in Adults

	Normal Limits (msec)		**Notable Limits (msec)**	
ECG Variables	Low	High	Low	High
PR interval	120	200	<120	>200
QRS interval	50	100	< 50	>100
RR interval	600	1000	<600	>1000
QT interval	No lower limit	≥ 460	No lower limit	≥ 460

ECG's which have limits noted to be outside of the table above will be recorded on the PI Data Safety and Review Form where the PI will review and assess as being attributable to ^{89}Zr-Trastuzumab injection. Any ECG recordings identified by the PI as being attributable to ^{89}Zr-Trastuzumabwill be recorded on the adverse event log

6.5.9 Cohort 3 Research Biopsy Procedure

All subjects enrolled in cohort 3 will undergo a biopsy of the suspected primary or recurrent/metastatic lesion prior to initiation of T-DM1 therapy. If a biopsy has already been scheduled as part of the subject's standard of care she will be asked to provide additional tissue at the time of biopsy. If biopsy is not needed per standard of care the biopsy will be required for research purposes only. The type of biopsy (CT guided, ultrasound guided, etc) will be determined by the treating physician based on what is most likely to yield a diagnostic specimen. It is preferred that the biopsy be the first research procedure followed by baseline FDG-PET/CT, ^{89}Zr-trastuzumab injection and imaging 5 to 7 days after injection. However, other scheduling options will be determined based on availability of the various modalities on an individual basis.

Biopsy specimens will be sent to the AMP Core lab at the Washington University School of medicine where they will be formalin-fixed paraffin embedded (FFPE). Upon completion samples will be transferred to the secure lab of one of the primary breast oncologists (Dr. Bose or Dr. Ma) where they will be securely stored at room temperature until all cohort 3 participants have been enrolled and completed study procedures. Batch processing of all samples for phospho-MAPK, phospho-Akt,, phospho-Src, PTEN and HER2 will occur for research purposes only. If applicable and if informed consent for storage of future research was obtained any remaining tissue samples will be transferred to the lab manager for enrollment into an applicable tissue bank or for use in retrospective or current research projects. Remaining tissue samples for those who did not consent to future use will be destroyed per current lab practices.

6.6.0 Drug Preparation

^{89}Zr-trastuzumab will be prepared as previously described by Vosjan et al (29). ^{89}Zr-oxalate will be produced via the ^{89}Y(*p,n*)^{89}Zr transmutation reaction on the CS-15 cyclotron (Cyclotron Corporation, Berkeley, CA) as described previously(30, 31). The resulting ^{89}Zr-oxalate is produced with a specific-activity of 8.1-15.4 GBq/μmol (220-418 mCi/μmol). Trastuzumab (Herceptin^{TM}, Genentech, South San Francisco, CA) is incubated with Df-Bz-NCS (Macrocyclics, Dallas, TX) in 0.1 M $NaHCO_3$ buffer pH 9.0. The resulting product, Df-Bz-NCS-trastuzumab, is purified via Zeba Spin Desalting Columns (Pierce Biotechnology, Rockford, IL). ^{89}Zr is complexed with Df-Bz-NCS-trastuzumab in 0.5 M HEPES buffer pH 7.0 at 37^{o}C for 1h with constant agitation at 850 RPM. ^{89}Zr-Df-Bz-NCS-trastuzumab is purified with Zeba Spin Desalting Columns and radiochemical purity determined by radio-ITLC with 50 mM DTPA and analytical size-exclusion chromatography (Superose 12 10/300 GL, GE Healthcare, Piscataway, NJ).

6.7.0 Statistics

Study Endpoints:

The primary endpoint of the study is the ^{89}Zr-trastuzumab uptake in tumors and normal structures including the blood pool, liver, intestine, kidneys and bladder evaluated by the standardized uptake value (SUV) and tumor-to-normal tissue ratio (T/N). The secondary endpoint of the study is the tumor expression of HER2 that is HER2-negative (0 or 1+, 2+ by IHC and FISH negative) and HER2-positive (3+ by IHC and FISH positive) correlates with ^{89}Zr-trastuzumab tumor uptake.

Study Design:

This is a study aiming to demonstrate the feasibility of using ^{89}Zr-trastuzumab-PET in breast cancer patients by identifying HER2-positive tumors. The sampling method is non-random. For Specific Aim 1, twelve HER-positive patients will be imaged to determine the imagining time for optimal image quality based on visual and semi-quantitative analysis of the images. Images will be independently evaluated by two readers. Inter-rater reliability will be examined. For Specific Aim 2, the differentiating power of ^{89}Zr-trastuzumab-PET on HER2 expression will be examined on 20 HER2-negtive and 20 HER2-positive patients using the optimal imaging time established by Specific Aim 1. The 5 patients enrolled to cohort 3 will be used as pilot data to design a larger trial specific to this patient population only.

Accrual:

The rate of accrual for the study is expected to be about 2-3 patients per month. It is expected that the accrual period of the study will be completed in 24 months with total 52 patients enrolled to cohorts 1 and 2. Accrual to cohort 3 is expected to be much slower as this is a very specific patient population. Enrollment is expected to be one patient every two to three months. .

Power Analysis

There is no power analysis for Specific Aim 1 considering its descriptive nature. For Specific Aim 2, using a 2-sample t-test with 80% power at a 2-sided 0.05 significance level, the designed sample size (n=20 patients/group) will allow us to detect a minimum of 91% SD between-group difference, where SD represents the standard deviation of ^{89}Zr-trastuzumab uptake in a pooled data. Previous study on ^{89}Zr-trastuzumab-PET and HER2-positive tumors in breast cancer (29) showed that the coefficients of variance (CV=SD/Mean) of relative uptake values (RUVs) on normal tissues ranged from .65 –

1.66, assuming similar variability will be seen in this study, this sample size will allow us, with 80% power at 2-sided 0.05 significance level, to detect a between group difference of 60% - 150% normal tissue means.

Data Analysis
Demographic and clinical characteristics of the sample will be summarized using descriptive statistics. For Specific Aim 1, Cohen's kappa coefficient will be calculated to measure inter-rater agreement on images quality and lesion detection. For Specific Aim 2, the association between ^{89}Zr-trastuzumab uptake values (e.g., SUV, T/N) and HER2 expression on tumor will be examined via Wilcoxon rank sum tests, Spearman-Brown correlation coefficients, and scatter plots. Bootstrapping techniques will be used as a method of inference which does not rely on a specific underlying distribution considering sample size is small. The changes in vital signs and clinical laboratory tests will be listed to evaluate the safety of ^{89}Zr-trastuzumab-PET.

Cohort 3: The association between tumor uptake of ^{89}Zr-trastuzumab and response to T-DM1 and markers of HER2 resistance will be examined via Pearson and/or Spearman's rank correlation coefficients. Imaging measurements such as SUV for ^{89}Zr-trastuzumab and FDG uptake will be collected on all patients at baseline and after 2 cycles of T-DM1. Descriptive statistics will be used to summarize each type of measurements at each time point, including mean, median, range, overall across all patients and by response. Given 40~44% response rate, we may have 3 non-responding patients and 2 responding patients and measurements will be compared by response using two sample t-test at each time point.

6.8.0 Risks and Benefits

The potential risks from this imaging protocol are expected to be very low. Expected risks include discomfort from the placement of intravenous catheter(s) for injection of trastuzumab, and ^{89}Zr-trastuzumab (and FDG for cohort 3), radiation exposure from the injection of ^{89}Zr-trastuzumab (and FDG for cohort 3) and from low dose CT scan used for attenuation correction and discomfort from lying still on the imaging table. Whenever possible, injection site will be contra lateral to the known tumor site if a primary breast or axillary lesion is present. Blood samples will be obtained for safety analysis. There is a slight risk of bruising and a remote risk of infection from the placement of the intravenous catheter(s). Although unlikely, there is a rare chance of having patients suffer an allergic or other immediate adverse reaction to trastuzumab or ^{89}Zr-trastuzumab. To minimize the risks to subjects, intravenous catheters will only be placed by trained personnel. All studies will take place under the supervision of the Principal Investigator or a collaborating physician. Additionally, the PET scanner is located within the hospital where advanced life support personnel and equipment are available for immediate use.

At standard therapeutic doses Trastuzumab can be cardiotoxic. HER2 negative subjects who enter on study as part of cohort 2 will be required to have a normal ejection fraction as determined by cardiac imaging such as ECHO or RVG. Although, a significantly higher risk of congestive heart failure has been reported in regimens in which trastuzumab was given for more than six months than in regimens without trastuzumab (risk ratio (RR) 5.39; 90% confidence interval (CI) 3.56 to 8.17, $p < 0.00001$). A shorter treatment period did not appear to be associated with an increase in the risk of congestive heart failure (RR 0.50; 90% CI 0.07 to 3.74, $p = 0.57$) (32). In addition, the risk of congestive heart failure has been reported to be significantly higher when trastuzumab was given sequentially with chemotherapy (RR 11.05; 90% CI 3.46 to 35.29, $p < 0.0007$) and concurrently (RR 3.90; 90% CI 2.42 to 6.28, $p < 0.00001$) (32). Establishing baseline cardiac function for this subject population is done as a precaution in case abnormal cardiac

function is suspected after study participation has concluded and subject is receiving standard chemotherapy agents which could also be cardiotoxic. Risks from ECHO include discomfort from lying on the imaging table and from the imaging probe which may be pressed firmly into the chest during the scan. Risks from RVG include radiation exposure, discomfort from lying on the imaging table and from the IV injection of stannous pyrophosphate and technetium-99m necessary to perform the imaging procedure.

Radiation Exposure: This research project involves radiation exposure to participants from the i.v. injection of ^{89}Zr-trastuzumab and 2 whole-body PET/CT scans. The total amount of radiation exposure received, when averaged over the entire human body, is equivalent to a uniform whole-body exposure of approximately 4.86 rem for cohort 1 subjects and 4.47 rem for cohort 2 HER2 positive subjects (4.08 rem from ^{89}Zr-trastuzumab and 0.39 rem from one whole-body CT and 0.78 rem from two whole-body CT). Cohort 2 HER2 negative subjects who receive RVG imaging at screening to determine study eligibility will receive an additional 0.65 rem of exposure for a total of 5.12 rem. Cohort 3 subjects (with updated radiation dosimetry based on calculations and published data from cohort 1 subjects (39) will receive up to 7.83 rem of total exposure (3.39 rem from ^{89}Zr-trastuzumab, 1.71 rem from 2 FDG injections, 2.44 rem from 3 whole-body CT scans, and 0.16 to 0.29 rem additional exposure depending on the type of biopsy procedure performed). .

Research tumor biopsy: Research biopsy will be performed by surgeon or radiologist under imaging-guidance according to standard of care procedure. The risks related to the biopsy are described below

Likely/Common
- Pain or discomfort
- Bruising around the biopsy site
- Redness around the biopsy site
- Discomfort from the pressure of the probe during the ultrasound procedure
- Pain, discomfort, bleeding, bruising, and allergic reaction from the numbing medication
- Drowsiness, slurred speech, staggering gait (clumsy or uncoordinated walking), poor judgment, and poor reflexes from the sedative

Less Likely/Less Common
- Swelling
- Excessive bleeding
- Drainage

Rare
- Infection at the site where the biopsy needle penetrates though the skin
- There is also the possibility that having this procedure may shift some cells from the tumor into the surrounding tissues that come in contact with the biopsy needle. This means that if there are cancer cells present there is a slight risk they could spread to that area.

Although subjects who participate in this early phase 1 imaging trial are not expected to directly benefit from ^{89}Zr-trastuzumab-PET imaging, the overall and long term benefits to future women with breast carcinoma far outweigh the minimal risks anticipated. It is anticipated that this study will contribute to the understanding of the performance of the 89Zr-trastuzumab-PET/CT in evaluating HER2 status and the interaction of HER2 with trastuzumab based therapy in breast

cancer. Ultimately, ^{89}Zr-trastuzumab-PET/CT may provide clinically important information about HER2 expression that can be used to select patients with trastuzumab or T-DM1 therapy and to assess the effectiveness of therapeutic strategies targeted to treat HER2-positive cancer.

6.9.0 Record Keeping

It is anticipated that 62 subjects will be entered into this pilot study within a 60-month period. No long-term follow-up is required for subjects entered into cohort 1 and cohort 2, as the primary end point for these subjects do not require it. Subjects who enter into cohort 3 will be followed for a maximum of 2 years or disease progression whichever comes first. A patient file will be kept in the Research Coordinators' office (Room 1017C) located on the 10th floor of the Mallinckrodt Institute of Radiology in Barnes-Jewish Hospital South Campus. Each file will contain a copy of the original signed consent form, as well as documentation necessary to prove patient eligibility to participate in this research study. Specific documentation of imaging procedures will also be maintained in this patient file. Original signed consent forms will be maintained in a separate file also located in the coordinators' office. All digital data associated with ^{89}Zr-trastuzumab-PET imaging is maintained on the PET acquisition computer. Raw data files are backed up on password protected encrypted external hard drive(s). All imaging data is also backed up on DVD after it has been transferred to a desktop PC. Processed data will be stored on the Principal Investigator's password protected desktop. CRF's and patient files will be stored in the study coordinators' office. Entrance to the PI's and coordinators' offices requires admission through two separate locked doors.

6.10.0 Regulatory and Reporting Requirements

6.10.1 Adverse Events (AEs)

Definition: any unfavorable medical occurrence in a human subject who receives ^{89}Zr-Trastuzumab and PET/CT imaging, including any abnormal sign, symptom, or disease. The event does not necessarily have to be causally related to injection of ^{89}Zr-Trastuzumab or PET/CT imaging to qualify as an adverse event, just temporally related.

Grading: the descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for all toxicity reporting. A copy of the CTCAE version 4.0 can be downloaded from the CTEP website.

Attribution (relatedness), Expectedness, and Seriousness: the definitions for the terms listed that should be used are those provided by the Department of Health and Human Services' Office for Human Research Protections (OHRP). A copy of this guidance can be found on OHRP's website:
http://www.hhs.gov/ohrp/policy/advevntguid.html

6.10.2 Unanticipated Problems

Definition:

- unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
- related or possibly related to participation in the research (in this guidance document, possibly related means there is a reasonable possibility that the incident,

experience, or outcome may have been caused by the procedures involved in the research); and

- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

6.10.3 Noncompliance

Definition: failure to follow any applicable regulation or institutional policies that govern human subjects research or failure to follow the determinations of the IRB. Noncompliance may occur due to lack of knowledge or due to deliberate choice to ignore regulations, institutional policies, or determinations of the IRB.

6.10.4 Serious Noncompliance

Definition: noncompliance that materially increases risks, that results in substantial harm to subjects or others, or that materially compromises the rights or welfare of participants.

6.10.5 Protocol Exceptions

Definition: A planned deviation from the approved protocol that are under the research team's control. Exceptions apply only to a single participant or a singular situation.

Research imaging protocols which involve the injection of radioactive tracers can produce unique situations not common to standard treatment protocols. In the event a situation occurs which requires deviation from this protocol – for example less than expected tracer production, problems with the scanner, patient unable to tolerate the imaging protocol as described, the principal investigator will have final authority over whether or not a study is completed. Any protocol deviations will be documented on the PET imaging data form. Deviation such as less than expected tracer production can be accounted for during data analysis and will not necessarily result in cancellation of the scan. Similarly, adjusting imaging procedures to accommodate patient comfort can also be accounted for at the time images are reviewed.

Except as described above, pre-approval of all protocol exceptions must be obtained prior to the event.

6.10.6 Reporting to the Human Research Protection Office (HRPO) and the Quality Assurance and Safety Monitoring Committee (QASMC) at Washington University

The PI is required to promptly notify the IRB of the following events:

- Any unanticipated problems involving risks to participants or others which occur at WU, any BJH or SLCH institution, or that impacts participants or the conduct of the study.
- Noncompliance with federal regulations or the requirements or determinations of the IRB.
- Receipt of new information that may impact the willingness of participants to participate or continue participation in the research study.

These events must be reported to the IRB within **10 working days** of the occurrence of the event or notification to the PI of the event. The death of a research participant that qualifies as a reportable event should be reported within **1 working day** of the occurrence of the event or notification to the PI of the event.

6.10.7 Reporting to the FDA

The conduct of the study will comply with all FDA safety reporting requirements. **PLEASE NOTE THAT REPORTING REQUIREMENTS FOR THE FDA DIFFER FROM REPORTING REQUIREMENTS FOR HRPO/QASMC.** It is the responsibility of the investigator to report any unanticipated problem to the FDA as follows:

- Report any unexpected fatal or life-threatening adverse experiences associated with use of the drug (i.e., there is a reasonable possibility that the experience may have been caused by the drug) by telephone or fax no later than **7 calendar days** after initial receipt of the information. A life-threatening adverse experience is defined as any adverse drug experience that places the subject (in the view of the investigator) at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that, had it occurred in a more severe form, might have caused death.
- Report any serious, unexpected adverse experiences, as well as results from animal studies that suggest significant clinical risk within **15 calendar days** after initial receipt of this information. A serious adverse drug experience is defined as any adverse drug experience occurring at any dose that results in any of the following outcomes:
    - Death
    - A life-threatening adverse drug experience
    - Inpatient hospitalization or prolongation of existing hospitalization
    - A persistent or significant disability/incapacity (i.e., a substantial disruption of a person's ability to conduct normal life functions)
    - A congenital anomaly/birth defect
    - Any other experience which, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above

  An unexpected adverse drug experience is defined as any adverse drug experience, the specificity or severity of which is not consistent with the current investigator brochure (or risk information, if an IB is not required or available).

For the purposes of regulatory reporting, a causality assessment of definitely related, probably related, or possibly related will be managed as "related," and unrelated or unlikely related will be managed as unrelated."

All MedWatch forms will be sent by the investigator or investigator's team to the FDA at the following address or by fax:

Food and Drug Administration

Center for Drug Evaluation and Research
Division of Oncology Drug Products
5901-B Ammendale Rd.
Beltsville, MD 20705-1266
FAX: 1-800-FDA-0178

6.10.8 Timeframe for Reporting Required Events

Reportable adverse events will be tracked for 30 days following the last day of study treatment.

Deaths	
Any reportable death while on study or within 30 days of study	Immediately, within 24 hours, to PI, Daiichi Sankyo, and the IRB
Any reportable death while off study	Immediately, within 24 hours, to PI, Daiichi Sankyo, and the IRB
Adverse Events/Unanticipated Problems	
Any reportable adverse events as described in Sections 7.1 and 7.2 (other than death) and 7.9	Immediately, within 24 hours to PI , within 10 working days to the IRB, and within 7 or 15 calendar days to the FDA and Daiichi Sankyo
All adverse events regardless of grade and attribution should be submitted cumulatively	Include in DSM report
Noncompliance and Serious Noncompliance	
All noncompliance and serious noncompliance as described in Sections 7.3 and 7.4	Immediately, within 24 hours, to PI and within 10 working days to the IRB

6.12 References6.11 Data Forms and Submission Schedule

Case report forms for PET imaging, image analysis, and laboratory test results are located in the appendix 1

Cohort 1

Procedure	Baseline[(a)] Day 0	First Imaging Day 2[(b)]	Second Imaging Day[(b)]	30 Day follow-up[(c)]
Informed Consent	**X**			
Medical History	**X**			**X**
Radiology Reports	**X**			**X**
Pathology Report(s)	**X**			**X**
Blood for Laboratory Analysis	**X**	**X**	**X**	
Urine for Laboratory Analysis	**X**	**X**	**X**	
Vital Signs[(d)]	**X**	**X**	**X**	
ECG[(e)]	**X**	**X**	**X**	
Total Body Imaging		**X**	**X**	

(a) Baseline is defined as period from initial subject contact until injection of ^{89}Zr-trastuzumab Baseline for vital signs and ECG is within 30 minutes prior to injection of ^{89}Zr-trastuzumab. Baseline for blood and urine laboratory samples is within 21 days prior to trastuzumab injection.

(b) Imaging day is defined as period of time from injection of ^{89}Zr-trastuzumab through end of last imaging session. Each subject will be imaged on two separate days.

(c) Subjects' medical records are followed for 30 days after study entry to ensure no adverse events have occurred. Any standard of care clinical exams obtained during this time period will be reviewed to correlate with scan results. There are no protocol requirements during this time frame

(d) obtained at each time point (baseline, within 30 min post injection, and at discharge on injection day and at beginning and end of each imaging session)

(e) obtained at baseline, 5-10 minutes post injection and prior to discharge on injection day and at each imaging session

Cohort 2

Procedure	Baseline[a] Day 0	Imaging Day [b]	30 Day follow-up[c]
Informed Consent	**X**		
Medical History	**X**		**X**
Radiology Reports	**X**		**X**
Pathology Report(s)	**X**		**X**
Blood for Laboratory Analysis	**X**	**X**	
Urine for Laboratory Analysis	**X**	**X**	
Vital Signs[d]	**X**	**X**	
ECG[e]	**X**	**X**	
Total Body Imaging		**X**	

(a) Baseline is defined as period from initial subject contact until injection of ^{89}Zr-trastuzumab Baseline for vital signs and ECG is within 30 minutes prior to injection of ^{89}Zr-trastuzumab. Baseline for blood and urine laboratory samples is within 21 days prior to trastuzumab injection HER2 negative cohort 2 subjects are allowed to be completed cardiac assessment to confirm eligibility on the same day as baseline study drug injection.

(b) Imaging day as defined from cohort 1. Imaging day period will be defined as period of time beginning after study day 0 procedures are completed (injection of 89Zr-trastuzumab through completion of the imaging session).

(c) Subjects' medical records are followed for 30 days after study entry to ensure no adverse events have occurred. Any standard of care clinical exams obtained during this time period will be reviewed to correlate with scan results. There are no protocol requirements during this time frame

(d) obtained at each time point (baseline, within 30 min post injection and at study discharge on injection day and at beginning and end of the imaging session

(e) obtained at baseline, 5-10 minutes post injection and at discharge on injection day and once on the day of imaging

Cohort 3

Procedure	Baseline[a]	^{89}Zr-Trastuzumab Scan [b]	Post 2 Cycles T-DM1	Follow-up at 30 Day up to approximately 2 years[c]
Informed Consent	X			
Medical History	X			X
Radiology Reports	X			X
Pathology Report(s)	X			X
Blood for Laboratory Analysis	X	X		
Urine for Laboratory Analysis	X	X		
Vital Signs[d]	X	X		
ECG[e]	X	X		
^{89}Zr-Trastuzumb PET Scan		X		
FDG-PET Scan	X		X	
Tumor Biopsy	X[f]			

(a) Baseline is defined as period from initial subject contact until injection of ^{89}Zr-trastuzumab Baseline for vital signs and ECG is within 30 minutes prior to injection of ^{89}Zr-trastuzumab. Baseline for blood and urine laboratory samples is within 21 days prior to trastuzumab injection

(b) Imaging day as defined from cohort 1. Imaging day period will be defined as period of time beginning after study day 0 procedures are completed (injection of ^{89}Zr-trastuzumab through the imaging session which will occur 5 to 7 days post ^{89}Zr-Trastuzumab injection).

(c) Subjects' medical records are followed for 30 days after study entry to ensure no adverse events have occurred. Follow up will continue for up to two years or disease progression whichever comes first to document response and for correlation with HER2 markers of resistance. Any standard of care clinical exams obtained during this time period will be reviewed to correlate with scan results. There are no protocol requirements during this time frame

(d) Obtained at each time point (baseline, within 30 min post injection and at study discharge on injection day and at beginning and end of the imaging session)

(e) Obtained at baseline, 5-10 minutes post injection and at discharge on injection day and once on the day of imaging

(f) Optimal scheduling will be baseline FDG-PET/CT, biopsy, ^{89}Zr-trastuzumab injection, and imaging 5 to 7 days later. However, due to anticipated scheduling conflicts other scheduling options will be acceptable

(g) FDG-PET scan may be performed on days 14-28 of Cycle 2 or immediately before Cycle 3 of T-DM1 therapy is administered.

6.12 References

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Washington University in St. Louis

FOR IRB USE ONLY

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APPROVAL DATE: 03/14/18
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EXPIRATION DATE: 03/13/19

INFORMED CONSENT DOCUMENT

Project Title: Assessment of HER2 Receptors in Breast Carcinoma by Positron Emission Tomography (PET) using 89Zr-Trastuzumab (Cohorts 1 & 2)

Principal Investigator: Farrokh Dehdashti

Research Team Contact: Dr. Farrokh Dehdashti 314-362-1474

This consent form describes the research study and helps you decide if you want to participate. It provides important information about what you will be asked to do during the study, about the risks and benefits of the study, and about your rights as a research participant. By signing this form you are agreeing to participate in this study.

- If you have any questions about anything in this form, you should ask the research team for more information.
- You may also wish to talk to your family or friends about your participation in this study.
- Do not agree to participate in this study unless the research team has answered your questions and you decide that you want to be part of this study.

WHAT IS THE PURPOSE OF THIS STUDY?

This is a research study. We invite you to participate in this research study because you have been diagnosed with new or recurrent breast cancer which has been tested for a protein called human epidermal growth factor (HER2). HER2 is a protein which helps cancer cells to grow. HER2 positive breast cancer cells have excess HER2. Currently, there are treatments approved by the United States Food and Drug Administration (FDA) to target HER2. One approved treatment is a drug called trastuzumab (also known as Herceptin). Trastuzumab targets HER2 and kills the cancer cells. Trastuzumab can be used alone or with other drugs in the treatment of breast cancer.

One problem with the current way breast cancers are diagnosed and treatment decisions are made is that we must rely on biopsies or small samples of tissue that are taken from the tumor to tell us what types of cancer cells are present. A tumor may not be uniform in the type of cells present so the biopsy sample may not accurately represent the actual function of the cells within the tumor. Additionally, just because a tumor was classified as, for example, HER2 positive at initial diagnosis, recurrent or metastatic tumors may not have the same characteristics.

The primary goal of this research imaging study is to see if a radioactive labeled form of trastuzumab called ^{89}Zr-trastuzumab can provide more information about the location and function of HER 2 positive cells within the body. A radioactive labeled form of trastuzumab is the FDA approved medicine trastuzumab which has a very small amount of a radioactive element called zirconium attached to it. This labeled form of trastuzumab allows us to image it within the body to confirm it is going to known cancer cells within the body. We will also be looking at how accurate ^{89}Zr-trastuzumab imaging is in tumors classified has HER-2 negative. The safety of ^{89}Zr-trastuzumab and the amount of the radioactive tracer that is found in normal cells when it is injected into the blood stream and how the amount of ^{89}Zr-trastuzumab in the tumor cells and normal tissues changes over time is also being studied.

^{89}Zr-trastuzumab is considered investigational, which means that it has not been approved by the U.S. Food and Drug Administration.

WHAT WILL HAPPEN DURING THIS STUDY?

To participate in this study you will be asked to read and sign this consent form before any study procedures are performed. Your study doctor will determine if you are eligible to participate by reviewing your medical history, medical records, current medications, and imaging scans.

Standard medical procedures that are part of your regular cancer care and probably would be done even if you do not join the study will be reviewed along with those procedures that are done for research purposes only.

If you are one of the first 12 patients to enter on study you will be assigned to cohort 1. If you enter into the study after the 12th patient, you will be in cohort 2. The only differences between cohort 1 and cohort 2 is that cohort 1 subjects will be asked to return on 2 separate times for imaging while those in cohort 2 will only be asked to return for imaging once. The optimal imaging time used in cohort 2 will be determined from the cohort 1 images. HER2 negative patients in cohort 2 will also be asked to undergo a test of their heart to confirm study eligibility.

Cohort 2 HER2 negative patients Screening

If you are a cohort 2 patient who is HER2 negative, you will be asked to undergo a test to evaluate how well your heart is functioning before you will be entered on study. Trastuzumab, when given over a period of time at treatment doses (dose much higher than you will receive in this study) can cause heart failure (inability of the heart to properly pump blood). You may receive either a MUGA (multi-gated acquisition scan) or an ECHO (echocardiogram). Both of these tests take pictures of the blood as it moves through the heart. A MUGA scan uses a small amount of a radioactive tracer to label the blood cells so the scanner is able to take the pictures. For the MUGA scan you will receive two injections about 20 minutes apart. The second injection is a small amount of a radioactive material that labels your blood cells so the camera can see your heart. An ECHO uses sound waves to take pictures of your beating heart. If this test has been done within the past 3 months it will not need to be repeated.

All Patients: Infusion/Injection Visit Day 0

Your first visit to the hospital will consist of two parts: trastuzumab infusion followed by ^{89}Zr-trastuzumab injection. For both parts of the visit an IV will be needed. The IV will be inserted into an upper extremity vein such as one in your arm or hand. If you are a woman capable of becoming pregnant, you will have a blood or urine pregnancy test performed to confirm you are not currently pregnant before you will be allowed to receive the infusions. Blood, if not previously obtained in the past 21 days, will be drawn from the IV line for standard laboratory testing (routine testing of your blood counts and organ function will be obtained. Standard laboratory testing is used to monitor the safety of ^{89}Zr-trastuzumab so these tests will be repeated several times during the study. Each time, approximately 3 teaspoons of blood will be drawn from a vein in your arm or the IV line. If a pregnancy test is needed an additional 1 teaspoon of blood may be obtained for this test one time only before you receive trastuzumab.

- You will be asked to provide a urine sample (if not previously obtained in the past 21 days) for standard laboratory testing.

- Your weight will be measured and your vital signs (blood pressure, heart rate, breathing rate and temperature) will be collected.
- You will receive an infusion of trastuzumab into the IV line. The amount of trastuzumab you will receive is less than what is given as a standard treatment dose. This dose of trastuzumab is not intended to treat you but only to saturate or fill up the normal cells in your body so that the cancer cells will be ready to receive the ^{89}Zr-trastuzumab.
- Following the infusion of trastuzumab you will be monitored for approximately 30 minutes to ensure you do not have any reactions. Your vital signs may be checked several times during this monitoring period.
- You will be transported to the positron emission tomography (PET) facility for injection of ^{89}Zr-trastuzumab.
- At the PET facility and before the injection of ^{89}Zr-trastuzumab, an electrocardiogram (ECG or tracing of your heart) will be obtained along with your vital signs. For this test, up to 12 self-adhesive electrodes (small blunt pieces of metal) will be attached to your skin on your arms, legs and chest. The areas where the electrodes will be placed will be cleaned and sometimes the area may need to be shaved.
- The injection of ^{89}Zr-trastuzumab will be given to you into your IV line over a period of 1-2 minutes. After the injection you will be monitored for approximately 30 minutes. You vital signs will be measured and additional ECG tracings of your heart will be taken during this time.

The total amount of time you will need to be at the hospital on the infusion day is 3 – 3 ½ hours. No PET imaging will occur on the infusion day. You will be transported from the chemotherapy area of the hospital after the trastuzumab infusion for the injection of ^{89}Zr-trastuzumab. This is done so the amount of ^{89}Zr-trastuzumab you will receive can be measured in a special counter immediately before the injection.

PET Imaging – Days 1-7

If you are in cohort 1, you will be scheduled to return to the PET facility on 2 separate days for imaging. You will be asked to return at one of the following time points: 1 and 3 days post injection OR 2 and 5 days post injection, OR 4 and 6 days post injection. The best imaging time point will be determined from these first set of images.

If you are in cohort 2 you will be scheduled to return for imaging one time 1-7 days after injection. The exact imaging time point will be determined from the cohort 1 images.

Visual Chart of Study Visits based on cohort

Infusion / Injection Day 0 for All Participants

- Trastuzumab infusion
- Move to PET facility for ^{89}Zr- Trastuzumab injection

Cohort 1 Subjects

Return to PET facility on 2 different assigned imaging days

- day 1 & 3 OR
- day 2 & 5 OR
- day 4 & 6

Cohort 2 Subjects

Return to PET facility for imaging one time on day assigned

On each imaging(s) day you will be questioned to see if you have had any changes in your medication or health since your last visit. Your vital signs and an ECG recording of your heart will be obtained along with a sample of blood and urine for standard laboratory testing (approximately 3 teaspoons of blood will be drawn from a vein in your arm).

The scanner will be a combination PET/CT scanner and is used to take pictures of your body. PET scanners allow us to image the function of different cells and organs in the body. The CT scan (computed tomography) is a type of x-ray scan that images the anatomy (size or structure) of the body in two dimensions. The combined PET/CT scanner is a special type of scanner that allows us to image both structure (CT) and function (PET) following the injection of ^{89}Zr-trastuzumab. You will be asked to lie on the PET imaging table for the pictures. Your arms will be positioned to rest at your side or comfortably above your head. Your body will be scanned from your head through your upper thighs. Each scan will take approximately 30-60 minutes to complete.

The total amount of time you will need to be at the PET facility on each of the 2 imaging days is approximately 90-120 minutes.

Will you save my samples or research data to use in future research studies?

As part of this study, we are obtaining PET/CT images of you. By agreeing to be part of this study you give up any property rights you may have in the images and the data. We would like to use your PET/CT images for other research projects in the future. These future studies may provide additional information that will be helpful in understanding your cancer type or how the PET/CT scanner obtains and processes the images, but it is unlikely that what we learn from these studies will have a direct benefit to you. It is possible that your images might be used to develop tests, determine future treatments. There are no plans to provide financial compensation to you should this occur. This means we will store your images and may use it for studies going on right now as well as studies that are conducted in the future. Your images may be used to develop investigational tests, treatments, drugs, or devices that are not yet approved by the U.S. Food and Drug Administration.

HOW MANY PEOPLE WILL PARTICIPATE?

Approximately 52 people (12 in cohort 1 and 40 in cohort 2) will take part in this study conducted by investigators at Washington University.

HOW LONG WILL I BE IN THIS STUDY?

If you agree to take part in this study, your involvement will last for approximately 6-8 hours spread out over two or three different days.

- The infusion visit will take approximately 3 – 3 ½ hours
- Each of the PET imaging visits will take approximately 1 ½ - 2 hours. If you are in cohort 1 you will undergo PET imaging on 2 days separate from your injection day. If you are in cohort 2 you will return for imaging once on a day separate from your injection.
- Your medical records will be followed for approximately 30 days after you complete imaging to ensure no problems developed as a result of your participation in this research project.

WHAT ARE THE RISKS OF THIS STUDY?

You may experience one or more of the risks indicated below from being in this study. In addition to these, there may be other unknown risks, or risks that we did not anticipate, associated with being in this study. Some risks described in this consent document, if severe, may cause death.

MUGA Scan:

Mild / Likely: You may feel discomfort from the IV injections and from lying on your backside for the pictures. .

Life Threatening/Rare: allergic reaction to the tracer is rare. The most common symptoms of an allergic reaction are rash or hives, tightening of the throat, wheezing, difficulty breathing or shortness of breath.

ECHO Scan:

Mild/Likely: You may feel discomfort from lying on the imaging table or from the scanner probe which may be pressed into your skin and moved across your chest while the pictures are being taken.

Trastuzumab Infusion:

The amount of trastuzumab you will be receiving is less than what is normally given for treatment. The followings are side effects collected from patients who receive normal treatment doses of trastuzumab. Side effects have been reported during the actual infusion and up to 24 hours following the infusion.

Mild / Likely: pain at the injection site, generalized feeling of weakness, muscle aches, fever, chills, nausea, headaches and coughing

Serious / Less likely: back pain, stomach pain, bone or joint pain, vomiting, loss of appetite, dizziness

Life Threatening / Rare: irregular heartbeat, difficulty breathing and shortness of breath, high blood pressure, weakening of the heart muscle, allergic reaction-unexplained rash, itching, hives, swelling, low red blood cell counts, which can cause tiredness and shortness of breath, and low white blood cell counts, which can increase the risk of infection.

^{89}Zr-Trastuzumab Injection: because the amount of trastuzumab used in making the radioactive form of trastuzumab is even less than what you will receive from the trastuzumab infusion no additional side effects other than those listed above are expected.

PET/CT Imaging:

Mild / Likely: Discomfort from lying still on the imaging table

Life Threatening / Rare: Malfunction of worn or implanted electronic medical devices from the CT scanner

Radiation Exposure: This study will expose you to radiation from the injection of an investigational radioactive drug, ^{89}Zr-Trastuzumab and from CT scanning used as part of each PET/CT scan. Cohort 2 HER2 positive patients who receive MUGA testing will have additional exposure from that test. The radiation exposure you will receive is equal to a uniform whole body exposure which is about 97 % (cohort 1) or 89% (cohort 2 HER2 positive or cohort 2 HER2 negative patients who receive ECHO or no cardiac testing) or 99% (cohort 2 HER2 negative patients who receive MUGA scan) of the allowable annual dose for radiation workers. The risk from this level of radiation exposure is too small to be measured and is small when compared with other everyday risks. If you want to know more about radiation exposure, please see the "Radiation Fact sheet" on the Guidelines page of the Human Research Protection Office website, at http://hrpo.wustl.edu or ask the study staff for a copy.

Because certain research studies are subject to specific radiation exposure limits, it is important that you inform us if you have been in any other research studies in the last 12 months that involved exposure to radiation for research purposes (from x-rays, CT scans, PET scans or other nuclear medicine procedures). It is also important that you tell future investigators about your participation in this research study if you are asked to participate in another research study.

IV Placement and Blood Drawing:

Mild / Likely: discomfort from placement of the IV in your arm or hand or from the needle used to draw blood

Serious /Less Likely: There is a slight risk of bruising, some people feel dizzy or faint when an IV is placed or blood is drawn from them

Life Threatening / Rare: There is a rare risk of infection at the site of IV placement or blood drawing.

ECG: You may feel discomfort from the placement and removal of the electrode patches from the skin. Remaining still while the ECG is taken (less than 1 minute) may cause anxiety.

Vital Signs: You may feel discomfort from the squeezing of the blood pressure cuff around your arm.

Reproductive Risks:

If you are a woman of childbearing potential, you will be required to have a pregnancy test before you may receive the infusion of Trastuzumab or the ^{89}Zr-Trastuzumab.

Because PET scans can be harmful to an unborn baby, you should not become pregnant while on this study. There is not enough medical information to know what the risks might be to an unborn child in a woman who takes part in this study. It is important that you understand that you need to use birth control while on this study. Ask your study doctor about what kind of birth control methods to use and how long to use them. If you are a woman who can become pregnant, you must agree to a blood or urine pregnancy test on the infusion day. Please discuss with your research physician how long you need to wait before becoming pregnant.

Participation in future research studies: There is a rare risk that you would not be eligible to participate in other research or treatment protocols or that you would need to complete a waiting period a this study before you would be allowed to participate in other research projects or studies.

One risk of participating in this study is that confidential information about you may be accidentally disclosed. We will use our best efforts to keep the information about you secure, and we think the risk of accidental disclosure is very small. Please see the section in this consent form titled "*How will you keep my information confidential?"* for more information.

WHAT ARE THE BENEFITS OF THIS STUDY?

You will not benefit from being in this study.

However, we hope that, in the future, other people might benefit from this study because being able to accurately identify the active HER2 receptor sites in the body could lead to better and more individualized treatment for patients with HER2 positive breast cancer.

WILL IT COST ME ANYTHING TO BE IN THIS STUDY?

You may have additional costs for being in this research study.

- You will be asked to visit the hospital for two or three separate visits. If you are making the trip to the hospital only for the research visits you will have travel related expenses.

You and/or your medical/hospital insurance provider will remain responsible for your regular medical care expenses.

WILL I BE PAID FOR PARTICIPATING?

You will be paid for being in this research study. You will need to provide your social security number (SSN) in order for us to pay you. You may choose to participate without being paid if you do not wish to provide your social security number (SSN) for this purpose. You may also need to provide your address because payment will be made in the form of a check mailed to you. It can take up to 4 weeks for processing and delivery of the check to the address you provide. If your social security number is obtained for payment purposes only, it will not be retained for research purposes.

We recognize and appreciate the time and effort to participate in a research study. If you are in cohort 1 you will receive $350.00. If you are in cohort 2 and HER2 positive or HER2 negative patient who does not need to undergo cardiac assessment, you will receive $250.00. If you are a HER2 negative patient who needs to have cardiac testing done to confirm eligibility you will receive $300. This is for

additional costs you may encounter, such as meals, extra time at hospital away from work or family, and/or other unforeseen expenses related to your participation and completion of the imaging visits of study. The amount you will be compensated is broken down into $150 for the infusion day, $100 for each day of imaging and $50 if you need to have cardiac testing done to confirm eligibility. Overnight hotel stay(s) may be available if needed as well.

WHO IS FUNDING THIS STUDY?

The National Institutes of Health (NIH) is funding this research study. This means that Washington University is receiving payments from the NIH to support the activities that are required to conduct the study. No one on the research team will receive a direct payment or increase in salary from the NIH for conducting this study.

WHAT IF I AM INJURED AS A RESULT OF THIS STUDY?

Washington University investigators and staff will try to reduce, control, and treat any complications from this research. If you feel you are injured because of the study, please contact the investigator **Dr. Farrokh Dehdashti at 314-362-2809** and/or the Human Research Protection Office at 1-(800)-438-0445.

Decisions about payment for medical treatment for injuries relating to your participation in research will be made by Washington University**.** If you need to seek medical care for a research-related injury, please notify the investigator as soon as possible.

HOW WILL YOU KEEP MY INFORMATION CONFIDENTIAL?

We will keep your participation in this research study confidential to the extent permitted by law. However, it is possible that other people such as those indicated below may become aware of your participation in this study and may inspect and copy records pertaining to this research. Some of these records could contain information that personally identifies you.

- Government representatives, (including the Office for Human Research Protections) to complete federal or state responsibilities
- The U.S. Food and Drug Administration
- Your primary care physician if a medical condition that needs urgent attention is discovered
- Hospital or University representatives, to complete Hospital or University responsibilities
- Information about your participation in this study may be documented in your health care records and be available to your health care providers who are not part of the research team.
- The last four digits of your social security number may be used in hospital or University systems to track billing information for research procedures
- Washington University's Institutional Review Board (a committee that oversees the conduct of research involving human participants.) The Institutional Review Board has reviewed and approved this study.

New health information will be created as a result of your participation. The images from the PET scans are obtained and stored in a digital format in a computer and kept on protected computer hard drives and/or disks. Study Protected Health Information (PHI) will be kept in your research record and the information obtained from your 89Zr-Trastuzumab PET scans will not be shared with your doctor unless a life threatening condition is found. Your research records and images may be kept permanently on file

at Washington University and may be used for future research. All personal identifiers are removed and replaced with a unique identifying number. The research that may be done with the information will not specifically help you. But, it might help people who have cancer and other diseases in the future.

The Siteman Cancer Center at Washington University School of Medicine and Barnes-Jewish Hospital is supported by funding from the National Cancer Institute (NCI). To meet NCI requirements, your PHI relating to your participation in this study (including your social security number) will be stored in a secure database at the Siteman Cancer Center. This database and also your treatment records may be reviewed by Siteman Cancer Center personnel. All information will be securely and confidentially maintained.

To help protect your confidentiality, we will ensure that all patients identified and recruited for this study are within the Health Insurance Portability and Accountability Act (HIPAA) protected population of the research team at Washington University. You will have an opportunity to ask questions about this study privately. The research location will be in the clinic and treatment area of the Center for Advanced Medicine, the clinical PET facility and in the Center for Clinical Imaging Research where precautions are made to protect the privacy of all patients, not only research or non-research patients. If we write a report or article about this study or share the study data set with others, we will do so in such a way that you cannot be directly identified.

This consent form or similar documentation that you are participating in a research study may be included in your clinical medical record. Anyone with access to your medical record, including your health insurance company may be able to see that you are participating in a research study.

A description of this clinical trial may be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

Are there additional protections for my health information?

Protected Health Information (PHI) is health information that identifies you. PHI is protected by federal law under HIPAA (the Health Insurance Portability and Accountability Act). To take part in this research, you must give the research team permission to use and disclose (share) your PHI for the study as explained in this consent form. The research team will follow state and federal laws and may share your health information with the agencies and people listed under the previous section titled, "How will you keep my information confidential?"

Once your health information is shared with someone outside of the research team, it may no longer be protected by HIPAA.

The research team will only use and share your information as talked about in this form. When possible, the research team will make sure information cannot be linked to you (de-identified). Once information is de-identified, it may be used and shared for other purposes not discussed in this consent form. If you have questions or concerns about your privacy and the use of your PHI, please contact the University's Privacy Officer at 866-747-4975.

Although you will not be allowed to see the study information, you may be given access to your health care records by contacting your health care provider.

If you decide not to sign this form, it will not affect

- your treatment or the care given by your health provider.
- your insurance payment or enrollment in any health plans.
- any benefits to which you are entitled.

However, it will not be possible for you to take part in the study.

If you sign this form:

- You authorize the use of your PHI for this research
- Your signature and this form will not expire as long as you wish to participate.
- You may later change your mind and not let the research team use or share your information (you may revoke your authorization).
  - To revoke your authorization, complete the withdrawal letter, found in the Participant section of the Human Research Protection Office website at http://hrpo.wustl.edu/participants//withdrawing-from-a-study/ or you may request that the Investigator send you a copy of the letter.
    - **If you revoke your authorization:**
      - The research team may only use and share information already collected for the study.
      - Your information may still be used and shared if necessary for safety reasons.
      - You will not be allowed to continue to participate in the study.

IS BEING IN THIS STUDY VOLUNTARY?

Taking part in this research study is completely voluntary. You may choose not to take part at all. If you decide to be in this study, you may stop participating at any time. If you decide not to be in this study, or if you stop participating at any time, you won't be penalized or lose any benefits for which you otherwise qualify.

What if I decide to withdraw from the study?

You may withdraw by telling the study team you are no longer interested in participating in the study or you may send in a withdrawal letter. A sample withdrawal letter can be found at http://hrpo.wustl.edu/participants/ under Withdrawing from a Research Study.

Will I receive new information about the study while participating?

If we obtain any new information during this study that might affect your willingness to continue participating in the study, we'll promptly provide you with that information.

Can someone else end my participation in this study?

Under certain circumstances, the researchers might decide to end your participation in this research study earlier than planned. This might happen because staying in the study would be harmful, you need treatment that is not allowed while on the study, you fail to follow instructions, you become pregnant, you develop a major side effect, or the study is canceled.

FOR IRB USE ONLY

IRB ID #: 201307037
APPROVAL DATE: 03/14/18
RELEASED DATE: 03/14/18
EXPIRATION DATE: 03/13/19

WHAT IF I HAVE QUESTIONS?

We encourage you to ask questions. If you have any questions about the research study itself, please contact: **Dr. Farrokh Dehdashti at 314-362-1474** If you experience a research-related injury, please contact: Dr. Dehdashti as well.

If you have questions, concerns, or complaints about your rights as a research participant, please contact the Human Research Protection Office, 660 South Euclid Avenue, Campus Box 8089, St. Louis, MO 63110, 1-(800)-438-0445, or email hrpo@wustl.edu. General information about being a research participant can be found on the Human Research Protection Office web site, http://hrpo.wustl.edu. To offer input about your experiences as a research subject or to speak to someone other than the research staff, call the Human Research Protection Office at the number above.

This consent form is not a contract. It is a written explanation of what will happen during the study if you decide to participate. You are not waiving any legal rights by agreeing to participate in this study.

Your signature indicates that this research study has been explained to you, that your questions have been answered, and that you agree to take part in this study. You will receive a signed and dated copy of this form.

Do not sign this form if today's date is after EXPIRATION DATE: 03/13/19.

_____ _____

(Signature of Participant) (Date)

_____

(Participant's name – printed)

Statement of Person Who Obtained Consent

The information in this document has been discussed with the participant or, where appropriate, with the participant's legally authorized representative. The participant has indicated that he or she understands the risks, benefits, and procedures involved with participation in this research study.

_____ _____

(Signature of Person who Obtained Consent) (Date)

_____

(Name of Person who Obtained Consent - printed)