MSK PROTOCOL COVER SHEET

Pilot Trial of PET Imaging with ^{89}Zr-DFO-trastuzumab in Esophagogastric Cancer

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Table of Contents

1.0 PROTOCOL SUMMARY AND/OR SCHEMA

Primary Objectives:

1) Evaluate the safety of ^{89}Zr-DFO-trastuzumab and the ability to detect tumors using PET imaging in patients with HER2 positive esophagogastric (EG) cancer

2) Determine the biodistribution and pharmacokinetics in normal organs and tumor of ^{89}Zr-DFO-trastuzumab in patients with esophagogastric cancer, and perform dosimetry estimates based on the data.

Secondary Objectives:

1) Evaluate changes in biodistribution of ^{89}Zr-DFO-trastuzumab in patients receiving HER2 directed therapy.
2) Patients who have archived tissue or will be having a clinically indicated biopsy will be asked to consent to have this tissue made available for molecular analysis of their tumor to correlate with imaging results.
3) Evaluate imaging results with response to treatment.

Patient Population:

The study group will be comprised of 55 patients with primary or metastatic HER2 positive EG cancer with evidence of measurable or evaluable disease.

Design:

PET imaging studies with ^{89}Zr-DFO-trastuzumab will be carried out in 55 patients. All patients will receive ^{89}Zr-DFO-trastuzumab radiotracer and then undergo PET scanning to determine if tumor localization is observed. Ten patients will undergo injection of ^{89}Zr-DFO-trastuzumab and undergo serial imaging, serum sampling and whole body counts for determination of biodistribution, dosimetry, pharmacokinetics and identification of optimal imaging time. The patients will be asked to consent to provide molecular analysis data obtained from archived tissue from their tumors, which will be correlated with ^{89}Zr-DFO-trastuzumab imaging results once funding is available. No molecular analysis or tissue procurement will be done under this protocol. In both cohorts (up to 55 patients), those receiving HER2 directed therapy (e.g. trastuzumab, afatinib or lapatinib), will receive up to two injections of ^{89}Zr-DFO-trastuzumab, prior to commencing treatment and within 6 weeks of start of HER2 directed therapy. The second injection will require a single timepoint scan.

Treatment Plan:

For each PET study: patients will receive 5 mCi $\pm$ 0.5 mCi of ^{89}Zr-DFO-trastuzumab given IV over 5-10 min. Because of well described, dose dependent pharmacokinetics in the literature, injection of cold trastuzumab will be mixed with ^{89}Zr-DFO-trastuzumab so that total mass is equal to 50 mg [1]. In the first ten patients we wish to obtain normal organ dosimetry, pharmacokinetics and determine optimal imaging time, therefore these patients will undergo imaging at 4 time points post injection, whole body counts and blood draws. Subsequent patients will receive the antibody and will only undergo imaging at a single time point (based on the first 10 patients) and will not have whole body counts or serial bloods for pharmacokinetics. The administration of ^{89}Zr-DFO-trastuzumab to patients undergoing a second study will be identical as for their baseline study. Patients undergoing a second injection will only have one scan that will be performed within 1 day before or 2 days after their optimum imaging time point, determined from their baseline imaging study.

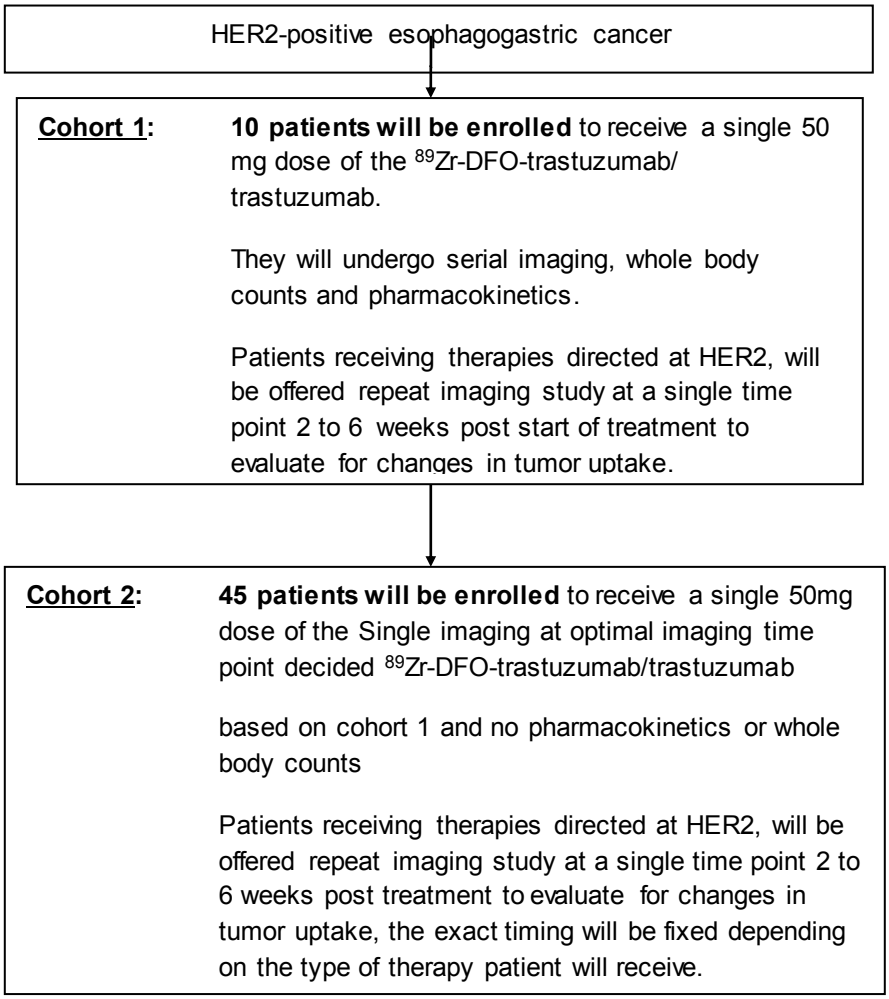
Duration of Study:

Accrual to this study should be complete in 24 months.

Regulatory Issues:

^{89}Zr-DFO-trastuzumab PET studies will be performed under the auspices of an IND (US FDA), no studies will be performed until the IND is obtained. No patient management decisions will be made based on these imaging studies.

Protocol schema:



Timing of repeat study will depend on treatment being administered and will be selected in conjunction with the treating PI based on what is thought to be most appropriate.

2.0 OBJECTIVES AND SCIENTIFIC AIMS

2.1 Primary Objectives

- Evaluate the safety of ^{89}Zr-DFO-trastuzumab and the ability to detect tumors using PET imaging in patients with HER2 positive esophagogastric (EG) cancer
- Determine the biodistribution and pharmacokinetics in normal organs and tumor of ^{89}Zr-DFO-trastuzumab in patients with esophagogastric cancer, and perform dosimetry estimates based on data

2.2 Secondary Objectives

- Evaluate changes in biodistribution of ^{89}Zr-DFO-trastuzumab in patients receiving HER2 directed therapy.
- Patients who have archived tissue or will be having a clinically indicated biopsy will be asked to consent to have this tissue made available for molecular analysis of their tumor to correlate with imaging results.
- Evaluate imaging results with response to treatment.

3.0 BACKGROUND AND RATIONALE

INTRODUCTION

The HER2 receptor is a 185-kDa transmembrane receptor tyrosine kinase that is expressed in a wide variety of human epithelial cancers including breast, bladder, ovarian, endometrial, cervical, lung, stomach, prostate as well as head and neck and pancreatic cancers [2]. In preclinical studies, HER2 over-expression has been found to contribute to oncogenic transformation, tumorigenesis, and metastatic potential [3, 4]. In human breast cancers, HER2 is overexpressed in 20-30% of cases, and this has been correlated with resistance to therapy, shorter disease-free and overall survival in patients with this disease [5, 6]. The ErbB2/HER2 oncogene encodes a transmembrane tyrosine kinase receptor that belongs to the epidermal growth factor receptor (EGFR) family and plays an essential role in promoting cell growth, migration, differentiation, proliferation, and survival. The family is comprised of ErbB1 (EGFR/HER1), ErbB2 (HER2/neu), ErbB3 (HER3), and ErbB4 (HER4). Each receptor has an extracellular domain, a lipophilic transmembrane domain, and an intracellular tyrosine kinase domain. Activation of the kinase occurs with ligand binding and hetero- or homodimerization of these receptors. Ligand-independent activation of HER2 may occur due to mutations in HER2 or receptor overexpression [7]. Activation plays a pivotal role in cell proliferation and survival which has been shown to be mediated largely through activation of the phosphatidylinositol 3'-kinase-AKT-mammalian target of rapamycin (PI3K-AKT-mTOR) pathway[8]. Hence HER2 has become a very important target of anticancer drug development.

Trastuzumab, a humanized anti-HER2 monoclonal antibody (Herceptin; Genentech, South San Francisco, CA), inhibits the growth of breast cancer cells with HER2 over-expression [9]. In a landmark randomized clinical trial, the addition of trastuzumab to chemotherapy improved response rates and prolonged survival for patients with HER2-overexpressing metastatic breast cancer [10]. Four separate, large, multicenter randomized adjuvant trials have shown a 50% reduction in the risk of recurrence for women with HER2+ early stage breast cancer treated with trastuzumab plus chemotherapy versus chemotherapy alone [11, 12]. In spite of these remarkable results, trastuzumab has activity in only a minority of breast cancer patients with HER2 over-expression, with response rates for trastuzumab monotherapy in the range of 12- 26% [13-15]. Moreover, resistance to trastuzumab eventually develops in the majority of cases.

3.1 The Problem Being Addressed:

3.1.1 Esophagogastric adenocarcinoma

World-wide, esophagogastric cancer is diagnosed in nearly one million individuals each year and is the second most common cause of cancer-related death [16, 17]. Most patients with esophagogastric cancer present with stage IV disease, which is incurable. Despite this, systemic chemotherapy can lead to a decrease in cancer-related symptoms and prolongs survival [11, 18-20]. However, even with treatment, most patients with advanced gastric cancer have a median survival of less than 1 year. More specific molecularly targeted therapies are anticipated to improve the current status of systemic treatment beyond conventional cytotoxic therapy.

3.1.2 Human Epidermal Growth Factor Receptor (HER2) in esophagogastric adenocarcinoma

HER2 is a validated treatment target in esophagogastric cancer, based on results of the trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-esophageal junction cancer (ToGA): a phase III, open-label, randomized controlled trial demonstrating improved response and survival when trastuzumab is added to chemotherapy. Approximately 30% of stomach card ia/gastroesophageal junction (GE junction) tumors harbor *HER2* gene amplification (assessed by fluorescent *in situ* hybridization [FISH] and/or HER2 oncoprotein overexpression (assessed by immunohistochemistry [IHC]) [21-23]. The rise in the incidence of gastroesophageal junction tumors in western countries [24, 25] underscores the significance of this new target in esophagogastric cancer, and opens a new drug development strategy for this disease.

There is limited data on the heterogeneity of expression of HER2 in gastric cancer between primary and metastatic sites [26]. In one report a discrepancy between primary and metastatic lesions was seen in 2 of 60 cases [27], with discrepancy seen between different tumor blocks. There is some data that suggest heterogeneity between primary and metastatic lesion in breast cancer [28]. There have been report of discrepancy between FISH and protein expression, this may allow imaging to more effectively select patients who are better candidates for trastuzumab therapy.

3.1.3 ToGA study: trastuzumab in HER2-positive esophagogastric adenocarcinoma

With the results of the phase III ToGA study, the benefit of trastuzumab in combination with cisplatin and fluoropyrimidine (CF) chemotherapy in HER2-positive metastatic esophagogastric adenocarcinoma has been established [29]. This study comprised 584 patients with HER2-positive gastric or GE junction tumors. Patients were randomly assigned to receive cisplatin and capecitabine (fluorouracil infusion was given to patients who were unable to take oral medication) alone or with trastuzumab. Patients assigned to receive trastuzumab with chemotherapy had a significant improvement in all measures of efficacy including overall survival, progression-free survival, and overall response rate (CR+PR). Notably, patients with strongly HER2-positive tumors (IHC 2+/FISH+ or IHC3+) derived the greatest overall survival benefit with the addition of trastuzumab to chemotherapy [29]. The selection of high HER2 expressing patients may be a setting in which quantitative imaging with ^{89}Zr-DFO-trastuzumab may provide prognostic information for patient selection to HER2 directed therapy.

Trastuzumab is the first biological strategy to show a survival benefit in advanced esophagogastric adenocarcinoma. Trastuzumab is now approved by the FDA and European Union (EMA) for treatment of patients with HER2-positive advanced gastric cancer in combination with systemic chemotherapy.

3.1.4 Trastuzumab: mechanism of action and the emergence of resistance

Trastuzumab is approved by the FDA and is the standard of care for patients with HER2-positive advanced gastric cancer in combination with systemic chemotherapy. Resistance to trastuzumab is now emerging in HER2-positive esophagogastric cancer. There is no standard of care treatment at this time that has been shown to reliably induce a second response in these patients.

Whereas anti-HER2 therapy with trastuzumab has shown efficacy in some HER2-positive esophagogastric cancer, limitations to this approach can include coincident activation of downstream signaling pathways. In HER2-positive breast cancer, both *de novo* and acquired resistance to trastuzumab are now recognized [30]. Elucidating the molecular mechanisms of trastuzumab resistance will aid in the development of new targeted therapies and a number of putative models of resistance have been described in HER2-positive breast cancer [14]. In breast cancer, one of the best-characterized mechanisms of trastuzumab resistance involves increased signaling from other EGFR members of EGFR family of receptors. Other mechanism of resistance is the accumulation of a truncated form of HER2 (p95-HER2) which lacks the extracellular domain needed for trastuzumab binding. This truncated receptor maintains kinase activity independent of ligand binding and is able to activate downstream signaling pathways [31]. By imaging with ^{89}Zr-DFO-trastuzumab we may be able to detect patients who lack the extracellular domain of HER2, since they are expected to have a negative scan.

Activation of PI3K-AKT-mTOR pathway by two genetic mechanisms, loss of the PTEN tumor suppressor and mutational activation of PI3K, has been demonstrated to confer resistance to trastuzumab in animal models and a small series of clinical tumor samples in breast cancer [32]. Increased insulin-like growth factor-1 receptor (IGF-1R) signaling is also associated with PI3K-AKT activation and trastuzumab resistance [33, 34]. In gastric cancer tumors, IGF-1R is most commonly overexpressed in HER2-positive tumors and correlates with poor prognosis and increased propensity to metastasize [12, 35, 36]. Through a better understanding of these models of resistance, new targeted therapies will be developed to improve response rates in HER2-positive esophagogastric cancer patients.

3.1.5 EGFR/HER2 Tyrosine Kinase Inhibitors (TKIs)

A series of TKI's of HER 2 are being developed. Lapatinib is a reversible tyrosine kinase inhibitor of EGFR and HER2 that blocks receptor activation by binding to the intracellular ATP binding site of these kinases. It has shown some effect in HER2 positive breast cancer patients [37, 38]. In a number of studies of single agent lapatinib in esophagogastric adenocarcinomas, modest activity has been documented [39].

Lapatinib is currently being tested in two phase III trials in gastric cancer. TYTAN is an open-label, randomized phase III study comparing paclitaxel with paclitaxel plus lapatinib in patients with HER2 FISH-amplified gastric cancer as a second-line therapy.

In vitro data suggest that second-generation irreversible inhibitors covalently bind HER2 and EGFR (unlike lapatinib, which compete with ATP in a reversible manner) in a highly selective fashion may be able to overcome trastuzumab resistance.

Afatinib (BIBW 2992) is a highly selective and potent low molecular weight, irreversible inhibitor of the erbB-family tyrosine kinase receptors EGFR, HER2 and HER4. MSK preclinical data shows potent antitumor activity of single agent afatinib in HER2-positive esophagogastric cancer xenografts (Janjigian 2011 unpublished data and is the basis for an ongoing MSKCC protocol 11-166.).

3.2 Positron Emission Tomography (PET)

PET has advantages over conventional imaging methods because it quantitatively assesses biologic processes in vivo and can assess molecular based pathways using specific radiotracers. Processes that can be analyzed with currently available reagents include glycolysis, amino acid metabolism, proliferation, blood flow, and receptor status (i.e., androgen receptor). Most clinical studies have focused on the accumulation of FDG.

A large amount of work has been performed using radiolabeled monoclonal antibodies with single photon emitters such as I-131, I-123, Tc-99m and In-111. In contrast to single photon emitters, positron emitters have advantages over conventional imaging methods because it quantitatively assesses biologic processes in vivo, furthermore PET scanner have higher sensitivity and resolution that conventional scanner for single photon emitters and are quantitative.

Various imaging studies have been performed with positron labeled antibodies. Our group has been a pioneer in the use of both I-124 and ^{89}Zr-labeled MAb. We have shown the excellent imaging characteristics of I-124 cG250 in patients with clear cell renal cancer [40]. In that study there was a sensitivity of 90% and specificity of 100%. This has led to a Phase 3 multicenter trial with intent to seek FDA approval for imaging of renal cell cancer. In a separate trial we have reported the targeting, imaging and pharmacokinetic characteristics of I-124 A33 monoclonal antibody in colorectal cancer patients [41]. In the latter 2 reports these antibodies are not highly internalizing and thus I-124, a non residualizing label is adequate for imaging.

We have previously evaluated different Positron emitting versions of trastuzumab antibodies, one labeled with Ga-68 and the other with Cu-64 in 2 clinical trials at MSKCC. In prior animal studies, we have used Ga-68 DOTA F(ab)'2 trastuzumab for monitoring HER2 inhibition with HSP90 inhibitors, as a basis for non-invasive imaging of pharmacodynamics of drug action [42, 43]. Our initials studies using Cu-64 DOTA trastuzumab demonstrated the safety and tolerability of this reagent in 11 patients. Furthermore, those studies showed that tumor localization could be observed in selected patients receiving large doses of trastuzumab although sensitivity was limited (personal observation, data not published). The pharmacokinetics of the intact Cu-64 DOTA-trastuzumab showed a biologic half-life of approximately 3 days and a mean of ~81% was present in the plasma 24 h after tracer administration. Our current proposal will allow in vivo tracing of the pharmacokinetic behavior of the intact ^{89}Zr-DFO-trastuzumab antibody. The PET images will allow us to see the

distribution of trastuzumab into different tissues and organs as well as tumor sites. The longer T1/2 of ^{89}Zr of 78 h is more suitable for the pharmacokinetics of the antibody at the required 50 mg dose. This information may serve as a basis for understanding the mechanisms of action and resistance, and toxicities associated with trastuzumab and HER2 directed therapies, and would form the basis for predicting the pharmacokinetics of trastuzumab-based conjugate therapies, which are emerging as potential therapeutic agents for HER2-positive disease, selection of patients and evaluation of treatment response.

Clinical use ^{89}Zr labeled antibodies, including their successful use in patients with breast cancer and head and neck has been reported [1, 44]. Furthermore, in an our own ongoing trial with ^{89}Zr-DFO-J591 excellent targeting has been observed.

3.2.1 ^{89}Zirconium

^{89}Zr is an attractive metallo-radionuclide for use in immuno-PET due to favorable decay characteristics. It has a 78.4h half life and a 22.7% positron yield.

Standardized methods for the routine production and isolation of high-purity and high-specific-activity ^{89}Zr using a small cyclotron are reported. Optimized cyclotron conditions reveal high average yields of 1.52 ± 0.11 mCi/muA*h at a proton beam energy of 15 MeV and current of 15 muA using a solid, commercially available ^{89}Y-foil target (0.1 mm, 100% natural abundance). The effective specific activity of ^{89}Zr was found to be in the range of 5.28–13.43 mCi/microg (470–1195 Ci/mmol) of zirconium.

Radiolabeling studies using the trihydroxamate ligand desferrioxamine B (DFO) gave 100% radiochemical yields in <15 min at room temperature, and *in vitro* stability measurements confirmed that (^{89}Zr) Zr-DFO is stable with respect to ligand dissociation in human serum for >7 days. Small-animal show that ^{89}Zr chloride and ^{89}Zr -oxalate reached 15- 20% of injected dose in bone after 6 days whereas ^{89}Zr-phosphate was only 5% of ID in bone but high amounts in the liver and the spleen [45].

These results have important implications for the analysis of immuno-PET imaging of ^{89}Zr-DFO labeled monoclonal antibodies. The detailed methods described can be easily translated to other radiochemistry facilities and will facilitate the use of ^{89}Zr in both basic science and clinical investigations [46-48].

3.2.2 Pharmacokinetics and biodistribution of chimeric or humanized monoclonal antibodies.

We have based our antibody mass dose selection on previous dose seeking clinical studies using radiolabeled trastuzumab [1]. Furthermore we have drawn on the experience with various other antibodies to select our initial mass of ^{89}Zr-DFO-trastuzumab. The pharmacokinetics of a variety of radiolabeled antibodies has been determined in humans. Some antibodies have cross reactivity with normal antigens in vivo and thus require a certain mass amount in order to optimize pharmacokinetics and biodistribution. As an example clinical trials with ^{89}Zr-DFO-trastuzumab have shown that mass of about 50 mg is necessary

in trastuzumab naïve patients. Other reports using Cu-64 labeled trastuzumab have shown that doses much smaller than 50 mg resulted in poor biodistribution [49]. Studies in patients with lymphoma targeting CD20 with In-111 Ibritumomab (2B8) showed that in 6 of 10 patients more lesions were identified with 1mg/kg co-infusion than with 2 mg total of antibody [50]. A clinical trial using In-111 hPAM4 showed good targeting and similar pharmacokinetics of doses ranging from < 10 mg compared to 100 mg [51] with T1/2 of 91.2h. In contrast, studies with the chimeric ^{89}Zr-cmAb U36 found adequate targeting when using 10 mg of the IgG. We have utilized PET imaging of I-124 anti-huA33 antibody to target A33 antigen, a transmembrane glycoprotein with homology to tight junction-associated proteins that is present in normal colon and small bowel epithelium as well as in over 95% of human colon adenocarcinoma and approximately 50% of gastric and pancreatic cancers, while absent in most other human tissues and tumors [52]. Our studies utilizing 10 mg of I-124 A33 have shown excellent targeting and have demonstrated Vd close to that of plasma 3,257 $\pm$ 631 mL, T1/2 of ~ 65 h and Co of ~31.9%ID/L [41]. In our experience the use of 10 mg of I-124 chimeric antibody cG250 resulted in excellent imaging of renal cell tumors [40]. The pharmacokinetic analysis in a clinical trial that used 2, 5, 10, 25 and 50 mg of I-131 cG250 showed that at the 2 mg dose levels there was faster clearance but at all other levels there was no dose response with a median T1/2 of 68.5 h. When doses of 25 and 50 mg of cG250 were administered there was less tumor uptake than at the 10 mg dose [53]. Humanized antibody hPAM4 specifically binds a mucin glycoprotein expressed in pancreatic adenocarcinomas.

3.2.3 Rationale for ^{89}Zr-DFO-trastuzumab PET use in Esophagogastric cancer

Various radionuclides are available to label monoclonal antibody for imaging, pharmacokinetics and biodistribution. Antibodies labeled with positron emitters have significant advantage compared to single photon emitters. These include the higher sensitivity, spatial resolution of PET and its quantitative ability. At MSKCC we have used various positron emitters for labeling antibodies. It is well known that for antibodies that internalize there is significant improved tumor retention of radiometals compared to Iodinated antibodies [54]. ^{89}Zr has an ideal half-life (78h) that is well matched to the half life of intact IgG in the intravascular compartment, that in the case of trastuzumab varies from 2 to 12 days at a 10 and 500 mg dose level. Determining if ^{89}Zr-DFO-trastuzumab PET can predict presence of HER2 may also allow to quantitate HER2 antigen expression and perhaps allow one to rationally select patients that are more likely to respond to HER2 directed therapies. Furthermore, if high sensitivity for tumor detection is observed ^{89}Zr-DFO-trastuzumab PET may allow for staging of extent of disease.
The first in-human ^{89}Zr-DFO-trastuzumab PET in breast cancer showed excellent visualization and uptake in HER2-positive tumors.[1]

^{89}Zr-DFO-trastuzumab PET has a potential advantage over single site biopsies as it can non-invasively assess target engagement and inhibition reflecting the functional effects of HER2-targeted agents in the primary tumor and all sites of metastases simultaneously [55, 56]. In HER2-positive EG cancer, heterogeneity of HER2 expression within primary tumors and metastasis is a particular challenge and may contribute to trastuzumab resistance. Moreover, the biodistribution of trastuzumab varies in each patient and is heavily impacted by the extent of tumor load, which may contribute to variations in patient responses [57]. ^{89}Zr-DFO-trastuzumab PET can thus help elucidate the molecular basis of resistance to trastuzumab in

EG cancer and facilitate the development of an optimal dose and schedule of HER2 targeted agents tailored to individual patient's tumor burden and biology. Decrease in FDG-PET uptake of greater than 30% from baseline is a validated predictor of tumor response and survival with chemotherapy in EG cancer patients [58-60]. However, a proportion of EG cancers are not FDG-PET avid [61], thus ^{89}Zr-DFO-trastuzumab may provide a method to asses response. ^{89}Zr-DFO-trastuzumab PET showed excellent tumor visualization in patients with HER2-positive breast cancer [1, 62]. MSKCC data demonstrates that ^{89}Zr-DFO-trastuzumab PET can image the tumor response and PD changes in HER2-positive EG xenografts treated with afatinib (**Fig 1**), while the changes are not visualized with FDG-PET.

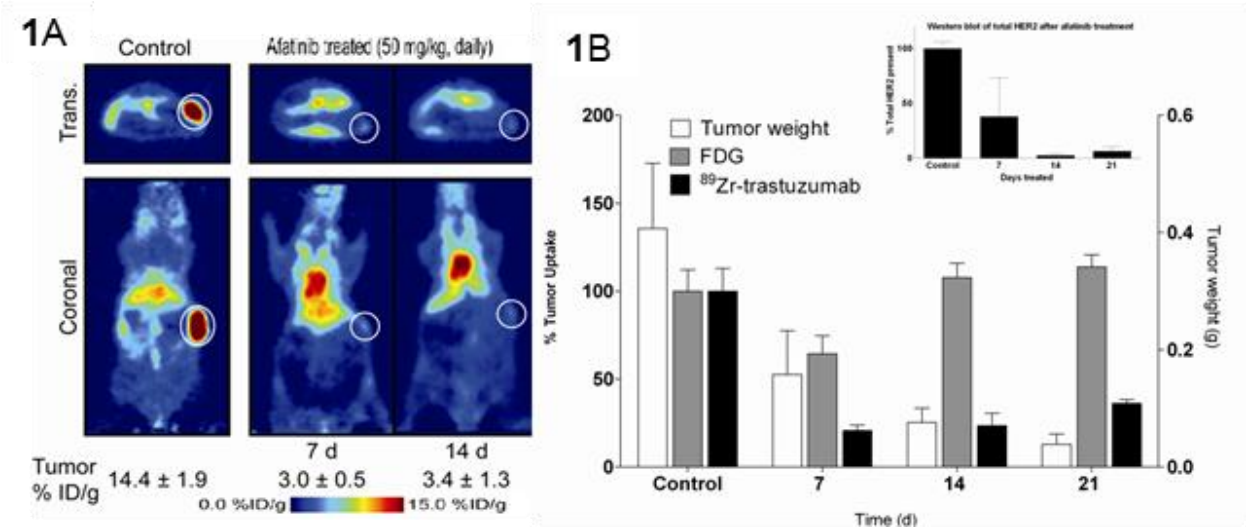


Figure 1. Imaging the effects of afatinib therapy with ^{89}Zr-DFO-trastuzumab PET. A. ^{89}Zr-DFO-trastuzumab PET images of control (left) and afatinib (right) treated mice bearing s.c. NCI-N87 tumors. **B.** Bar charts showing regions-of- interest (%ID tumor uptake) for ^{89}Zr-DFO-trastuzumab and FDG uptake recorded and tumor weight (at baseline, 7, 14 days post afatinib. Insert demonstrates PD studies on HER2 by immunoblot .

Currently IHC is used to determine HER2 status and select candidates for HER 2 directed therapy. If this pilot trial is successful new this technology may have an impact on various aspects of managing patients with esophagogastric cancers. For example ^{89}Zr-DFO-Trastuzumab may be incorporated into routine clinical practice for staging, diagnosis and monitoring treatment response in patients with HER2-positive tumors. ^{89}Zr-DFO-Trastuzumab may be used to aid in treatment planning by non- invasively selecting candidates for HER2-directed therapy. Whereas, IHC is a qualitative method limited to the biopsied site, if successful ^{89}Zr-DFO-Trastuzumab PET imaging will be implemented as a quantitative method to assess the primary tumor and all sites of metastatic disease providing the index of HER2 density. Currently patients are treated with HER2 directed therapies and assessed after a period of treatment for evidence of response by size change criteria. This method may facilitate early predictor of tumor response than decrease in tumor size on CT.

3.2.4 Tissue correlates with PET antibody imaging

We have documented with interventional radiology directed biopsies that sensitivity of tumor detection is greater than CT, PET-FDG or bone scanning using ^{89}Zr-DFO-J591 (unpublished data). Further studies are ongoing with image directed biopsy. The correlation of molecular characteristics, tissue histology and antibody imaging may give insight into mechanism of

uptake. We will use banked tissue for correlation of proteonomics or genomics with imaging results in a pilot fashion. We will not perform biopsies under the auspices of this protocol.

4.0 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.1 Design

This is a single institution, open-label, non-randomized, pilot evaluation of ^{89}Zr-DFO-trastuzumab PET imaging in patients with HER2-positive esophagogastric cancer. Patients will receive up to 5 mCi of ^{89}Zr-DFO-trastuzumab (initial 5 patients will receive 5 mCi, the activity may be adjusted downward if technically feasible). In order to optimize tumor targeting the activity of ^{89}Zr-DFO-trastuzumab will be brought up to a final 50mg dose by adding non-conjugated trastuzumab (example 5 mCi/3mg DFO-trastuzumab + 47 mg of trastuzumab). Data in the literature, in breast cancer patients, has shown that the optimal mass of trastuzumab is ~50 mg, in patients with no trastuzumab on board [1]. During the biodistribution, pharmacokinetics and optimization phase, 10 patients will undergo 4 serial PET scans for dosimetry and determination of optimal imaging time and scan parameters. The optimal time for imaging is probably 3 to 8 days, as has been observed with other intact IgG antibodies. In order to confirm these findings with our own preparation of ^{89}Zr-DFO-trastuzumab we will do pharmacokinetics and serial imaging in the first 10 patients enrolled. After the first 10 patients, all other patients will be injected with the tracer and imaged at the optimum time, selected based on the initial 10 patients and no pharmacokinetics will be performed.

Patients that will start a therapeutic regimen mediated through HER2 will be asked to have an additional ^{89}Zr-DFO-trastuzumab scan. If patient agree they will have a baseline study prior to start of therapy and if positive localization is observed they will have a optional second ^{89}Zr-DFO-trastuzumab repeated while on therapy following at least one cycle of therapy, or at a time point that the PI and CO-Pi's consider appropriate. At least 2 weeks will have elapsed between baseline and second injection to allow for decay and no more than 6 weeks. The second study will also be done using up to 5 mCi/ combined 50 mg of ^{89}Zr-DFO-trastuzumab and when feasible imaging will be performed on the same day post injection as on the baseline study or within ~1d prior or 2 days post optimal baseline imaging. In patients in cohort 1 (before selection of optimal imaging time) the study will be performed with ~1 to 2 days of best imaging on their baseline study. Images from both studies will be compared to determine similarities in biodistribution (concentration of activity in normal organs and tumor).

Baseline testing will be performed prior to injections with ^{89}Zr-DFO-trastuzumab. This includes history, hematology, chemistry, urinalysis, negative serum pregnancy test for female of child bearing potential, vital signs, ECG and baseline serum sample will be collected for possible analysis of complex formation if altered pharmacokinetics are observed and banked for testing of immune response to the DFO-trastuzumab. Patients will be assessed for toxicity.

Because ^{89}Zr-DFO-trastuzumab has only been evaluated in a small number of patients, and since no studies have been performed in the USA, toxicity assessment will be performed using Common Terminology Criteria for Adverse Events (CTCAE) v4.

4.2 Intervention

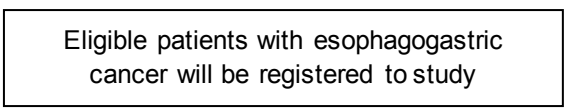
The intervention is the administration of a single dose of ^{89}Zr-DFO-trastuzumab tracer for imaging purposes. Patients will be approached by their oncologists for informed consent and enrollment to the study during their regularly scheduled follow-up appointments at MSK outpatient facilities or referred to Molecular Imaging Investigators for obtaining informed consent. The day of the study the patient will report to the Molecular Imaging and Therapy Service at the Main Campus. For the first 10 patients, two intravenous catheters will be inserted whenever feasible to allow for separate site for injection and blood draw for pharmacokinetics. For others who will receive scanning only, one IV will be inserted. The ^{89}Zr-DFO-trastuzumab tracer dose will be combined with non-radiolabeled trastuzumab for a total of 50 mg trastuzumab will be injected IV over 5-10 min using an in-line (0.22 micron) filter, followed by a flush of ~10 ml of normal saline. The infusion will be carried out under the supervision of the Principal Investigator (PI) or an authorized user assigned by the PI. The syringe will be measured for ^{89}Zr radioactivity prior to and at the end of infusion, and the net amounts of radioactivity will be recorded, along with the time of measurement, and the net amount of radioactivity administered calculated.

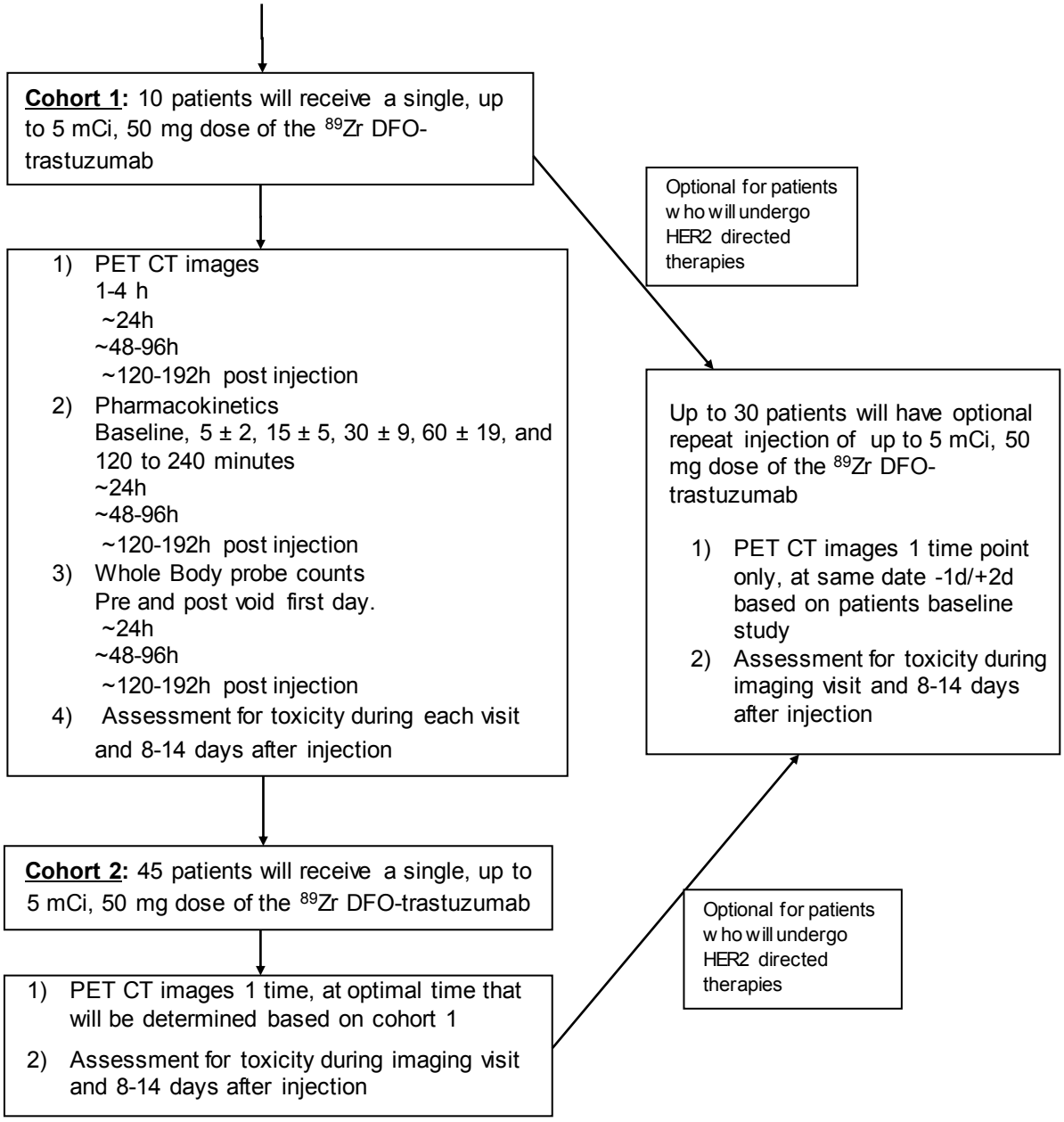
Patients undergoing serial imaging will have whole body count measurements using a NaI probe, placed at ~3.3 yards from the patient, to determine whole body retention: after antibody infusion, prior to voiding and post void, as well as at each time of return for imaging.

Imaging for the initial group undergoing serial scans will start within 1-4 hr of end of the ^{89}Zr-DFO-trastuzumab infusion, ~24h, ~48-96h and ~120-192h post injection. The administration of ^{89}Zr-DFO-trastuzumab to patients undergoing a second study will be identical as for their baseline study. Patients undergoing a second injection will only have one scan that will be performed within 1 day before or 2 days after their optimum imaging time point, determined from their baseline imaging study.

Pharmacokinetic blood sampling will be performed utilizing a separate IV site (if feasible). Blood samples for ^{89}Zr-DFO-trastuzumab levels will be drawn prior to the ^{89}Zr-DFO-trastuzumab injection and at approximately 5 ± 2, 15 ± 5, 30 ± 9, 60 ± 19, and 120 to 240 minutes and a sample at the time of each subsequent day of imaging (~24h, ~48-96h and ~120-192h) post injection these are ideal times and after the first day a window of ± 8h will be allowed. In all patients on the day of first infusion and prior to ^{89}Zr-DFO-trastuzumab injection baseline blood will banked for determination of complexes formation with ^{89}Zr-DFO-trastuzumab.

Intervention schema





5.0 THERAPEUTIC/DIAGNOSTIC AGENTS

The Investigational New Drug in this study is ^{89}Zr-DFO-trastuzumab. Trastuzumab is a humanized antibody that targets HER2 approved for the treatment of HER2+ metastatic gastric cancer. We will use ^{89}Zr, a positron-emitting radionuclide, labeled to trastuzumab via DFO. For ^{89}Zr labeling DFO will be attached to trastuzumab. To bring the total mass to 50 mg of antibody, trastuzumab kits will be reconstituted as per manufacture instructions by

MSKCC pharmacy and sufficient amount will be added to bring the combined total of radiolabeled plus cold to 50 mg at the time of radiolabeling compounding.

Drug Accountability

The investigator is obliged to keep sufficient documentation of the delivery and use of the investigational product. The documentation will include administration date/ time, calibration date/time, quantity, patient name, lot number. Destruction of non used doses will be by decay in storage. The investigator should maintain records that document adequately that the patients were provided the dose specified in the protocol and reconcile the medication received for the study. The label of each syringe/vial will contain the patient name and lot number, patient and radiopharmaceutical information will be entered in the radiopharmacy drug accountability forms. The investigator may assign some or all of the investigator's duties for drug accountability to an appropriate individual such as pharmacist, research assistant or Molecular Imaging and Therapy Technologist who is under the supervision of the investigator.

^{89}Zr-DFO-trastuzumab

Radiolabeling of ^{89}Zr-DFO-trastuzumab: The **^{89}Zr-DFO-trastuzumab** will be labeled in accordance with the MSKCC investigator sponsored IND. The study will be performed under an FDA approved IND for ^{89}Zr-DFO-trastuzumab, no studies will be performed until the IND is approved. The final product will contain up to 5 mCi and will have been brought up to desired total 50 mg of antibody quantity by adding cold carrier trastuzumab as outlined in the design section. The product will have met the release criteria specified in the IND. Cold trastuzumab will be obtained from the MSKCC pharmacy, this antibody will be prepared in the standard fashion currenly made for clinical administration.

Images will be corrected for attenuation and scatter and adjusted for system sensitivity and providing parametric images in terms of standardized uptake values (SUV) (= µCi found/gm tissue / (µCi injected/gm body mass).

Radiochemical Purity

The amount of free ^{89}Zr in radiolabeled DFO-trastuzumab preparations is evaluated using instant thin layer chromatography. Only samples which have a radiochemical purity of >95% will be used for clinical studies. Release criteria in accordance with IND, must be met prior to use.

Immunoreactivity

The immunoreactivity of the radiolabeled ^{89}Zr-DFO-trastuzumab preparation is assessed using Lindmo's method [63]. The method has been validated in at least 3 immunoreactivity assays, to meet the criteria specified in the IND. All radiolabeled preparations will be tested for immunoreactivity, though the ^{89}Zr-DFO-trastuzumab will be administered prior to these results being available.

Package Labeling and Formulation

Each vial will contain the following information on the label:

^{89}Zr-DFO-trastuzumab, Solution IV IRB xx-xxx

Lot #:_____ Activity (mCi):____ Volume (mL):_____ Mass (mg): _____
Production Date/Time:_____ Expiration Date/Time:_____
Store product at room temperarute in a shieled cotainer

Caution: New Drug Limited by Federal Law to Investigational Use. Radioactive Material, do not use if cloudy or contains particulate material.
Cyclotron-Radiochemistry Core Memorial Sloan Kettering Cancer Center

5. 1 Dose Adjustments

Expected dosimetry for this tracer is based on murine imaging studies: Dosimetry Table 2. The maximal administered dose of 5 mCi of ^{89}Zr DFO-trastuzumab is similar in absorbed dose from other ^{89}-Zr and I-124 labeled antibodies that are being used in clinical trials. The combined DFO-trastuzumab + trastuzumab mass will be fixed at 50 mg.

If the analysis of the first 10 patients undergoing serial imaging, in this study reveals an unexpectedly higher radiation dose to any particular organ, a dose reduction will be considered. If adequate imaging statistics can be obtained with lower acivity, with acceptable imaging times, an activity reduction may be performed to use less than 5 mCi of activity.

6.0 CRITERIA FOR SUBJECT ELIGIBILITY

6.1 Subject Inclusion Criteria

To be included in this study, patients should meet all of the following criteria:

- Registered patient at MSKCC
- Age ≥18 years
- Pathologically or cytologically confirmed metastatic or primary esophagogastric cancer HER2 positive status by FISH or IHC as currently being implemented for patients with esophagogastric cancer. HER2 overexpression and/or amplification as determined by immunohistochemistry (3+) or FISH (≥2.0)
- Measurable or evaluable disease, lesions that have not been previously radiated, with clinically indicated imaging evaluation performed within 4 weeks prior to study entry (CT, MRI, FDG PET or bone scan). Patients requiring concurrent radiation treatment are not eligible unless additional lesions that are not being irradiated and are assessable for targeting are present.
- Karnofsky Performance Score ≥ 60 (appendix 1)
- Ability to understand and willingness to sign informed consent
- Negative pregnancy test, to be performed on female patients of childbearing potential within 1week before administration of radioactive material.
- Life expectancy of at least three (3) months.
- Willingness to use birth control while on study.
- The patients will be asked to consent to provide access to data obtained from molecular analisys that has been done on archived tumor tissue that will be correlated with ^{89}Zr-DFO-trastuzumab imaging results.
- Concurrent therapy will be allowed.

6.2 Subject Exclusion Criteria

- Inability to lie still for the duration of the scanning procedure.
- Patients with known sensitivity or contraindication to any of the component of ^{89}Zr-DFO-trastuzumab (^{89}Zr or Desferroxiamine (DFO) or trastuzumab)
- Patients who have received trastuzumab must have at least a washout period for trastuzumab of 14 days, this will not apply to ^{89}Zr-DFO-trastuzumab repeat, post treatment assessment where patients may be receiving trastuzumab.
- HIV positive or active hepatitis.
- History or presence of clinically relevant cardiovascular abnormalities such as uncontrolled hypertension, congestive heart failure NYHA classification of 3, unstable angina or poorly controlled arrhythmia. Myocardial infarction within 6 months prior to study entry
- Hematologic
  - Platelets <50K/mcL
  - ANC <1.0 K/mcL
- Hepatic laboratory values
  - Bilirubin >2 x ULN (institutional upper limits of normal), with exception of patients with Gilberts disease.
  - AST/ALT >2.5 x ULN (institutional upper limits of normal); >5 x ULN if liver metastasis
- Renal laboratory values
  - Estimated GFR (eGFR) < 30mL/min/1.73m2

7.0 RECRUITMENT PLAN

Recruitment Plan (with Limited Waiver of Authorization)

Patients who meet the above inclusion and exclusion criteria will be invited to participate in the study by their primary oncologist. We will invite men and women, and all minorities on an equal basis.

Consenting professionals will meet with the patients and obtain informed consent in the oncologist's or Molecular Imaging and Therapy clinic. All subjects who agree to participate will be registered in the Office of Clinical Research. No advertisements or payments will be offered to the patients.

Candidates must conform to all inclusion and exclusion criteria to be accepted into the study.

If the investigator is a member of the treatment team, s/he will screen their patients' medical records for suitable research study participants and discuss the study and their potential for enrolling in the research study. Potential subjects contacted by their treating physician will be referred to the investigator/ research staff of the study.

The principal investigator may also screen the medical records of patients with whom they do not have a treatment relationship for the limited purpose of identifying patients who would be eligible to enroll in the study and to record appropriate contact information in order to approach these patients regarding the possibility of enrolling in the study.

During the initial conversation between the investigator/ research staff and the patient, the patient may be asked to provide certain health information that is necessary to the recruitment and enrollment process. The investigator/ research staff may also review portions of their medical records at MSKCC in order to further assess eligibility. They will use the information provided by the patient and/or medical record to confirm that the patient is eligible and to contact the patient regarding study enrollment. If the patient turns out to be ineligible for the research study, the research staff will destroy all information collected on the patient during the initial conversation and medical records review, except for any information that must be maintained for screening log purposes.

In most cases, the initial contact with the prospective subject will be conducted by either the treatment team, investigator or the research staff working in consultation with the treatment team. The recruitment process outlined presents no more than minimal risk to the privacy of the patients who are screened and minimal protected health information (PHI) will be maintained as part of a screening log. For these reasons, we seek a (partial) limited waiver of authorization for the purposes of (1) reviewing medical records to identify potential research subjects and obtain information relevant to the enrollment process; (2) conversing with patients regarding possible enrollment; (3) handling of PHI contained within those records and provided by the potential subjects; and (4) maintaining information in a screening log of patients approached (if applicable).

Estimated breakdown of target population:

% Native American/Alaskan	0%
% Asian/Pacific Islander	16%
% Black Non Hispanic	12%
% Hispanic	8%
% White Non-Hispanic	64%
% Other	0%

8.0 PRETREATMENT EVALUATION

- There will be a chart review to ensure the patient meets eligibility criteria. Primary pre-treatment evaluation will be based on the evaluation performed as part of the patient's regular medical care at MSKCC. Additional pre-treatment evaluation will include:
- History and physical examination
- Laboratory studies (within 2 weeks before study entry):
- Chemistry: Comprehensive metabolic panel including glucose, urea nitrogen, creatinine, eGFR, calcium, protein, albumin, bilirubin, AST, ALT, LDH, alkaline phosphatase

- Hematology: CBC, differential count, platelet count.
- Pregnancy test to be performed on female patients of childbearing potential within 1 week before administration of radioactive material.
- Clinically available imaging studies will be reviewed, including CT, FDG and or MRI and other available studies, such as bone scans. These studies must be from within 4 weeks of ^{89}Zr-DFO-trastuzumab injection.

9.0 TREATMENT/INTERVENTION PLAN

9.1 Pharmacokinetics

Patients are not required to fast before ^{89}Zr-DFO-trastuzumab injection or imaging.

Catheter insertion and Pharmacokinetics

If feasible, patients in cohort 1 will have 2 intravenous catheters (Hep-Lock) placed by staff in the Molecular Imaging and Therapy Service for radiopharmaceutical administration and blood sampling, or alternatively a central line may be used for injection or sampling. The intravenous placed catheter for injection and/or blood draw during study will be removed at the conclusion of the imaging session. If two lines are not feasible, one line will be used for both injection and PK sampling with intervening 10 cc saline flush and change of catheter hub. Patients in Cohort 2 (no pharmacokinetics), will not require a second catheter.

To determine pharmacokinetic and for possibly metabolite analysis of the drug, patients undergoing pharmacokinetics will have serial blood samples drawn:

- Blood sample, 4-6 mL of blood will be drawn in a speckled top serum separator tube.
- Blood will be centrifuged in Dr. Carrasquillo's lab, and the serum pipetted and counted. Samples may be tested for breakdown products by TCA or HPLC.
- Blood draws will be done prior to injection of ^{89}Zr-DFO-trastuzumab (baseline), approximately baseline, 5 ± 2, 15 ± 5, 30 ± 9, 60 ± 19, and 120 to 240 minutes after injection of the tracer. One sample at the time of each subsequent day of imaging (24 , ~48-96h and ~120-192h post injection, in order to build in flexibility after the initial injection day we will allow ± 8h of stated times).
- Pharmacokinetic parameters will be obtained including Concentration at time 0, Volume of distribution of central compartment (Vc), clearance,T1/2 and AUC.

9.2 Imaging

PET scanning: In order to provide reproducible clinical data, we will acquire all ^{89}Zr-DFO-trastuzumab PET scans on the GE PET-CT scanner DSTE at MSKCC or the same type or a newer generation scanner. Once a study is begun in one scanner, it will be continued on the same scanner unless malfunction/emergency issues prevent this. Intravenous injection of ^{89}Zr-DFO-trastuzumab (maximum of 5 mCi ± 10%) will be given over 5-10 minutes. Initially we will start with ~ 5 mCi of ^{89}Zr-DFO-trastuzumab; the activity may be adjusted downward

based on visual review of image quality. A PET-CT scan extending from mid skull to proximal thighs will be performed to determine the biodistribution. For patient undergoing serial scanning one scan will be performed utilizing low mA (80 mA) and on subsequent days, we will use 10 mA in order to minimize radiation exposure.

Patients undergoing pharmacokinetics will also have imaging at 4 time points post-injection to allow for selection of optimal imaging time and dosimetry determination.

- Within one to four hours following injection of tracer on Day 0
- ~24 ± 8hours post-injection (Day 1)
- ~48–96 hours post-injection (once during Days 2–4) ±8h
- ~120–192hours post injection (once during Days 5–8)±8h

Imaging will be in 3D, typically from mid skull to midthigh (approximately 6-7 bed positions) to allow for estimates of whole organ uptake and excretion patterns. These whole body studies will vary in duration according to the height of the patient but will typically be 21-60 minutes in duration.

Active lesions will be identified (Molecular Imaging investigator) based on review of the patients conventional images and treatment history (i.e. external radiation). A PET positive lesion will be considered present if there is greater localization than that expected based on blood pool distribution of the tracer. Given that at present we do not know the heterogeneity of the antigen between lesions or between primary and metastatic disease a patient will be considered positive if any of the active lesions are identified with the ^{89}Zr-DFO-trastuzumab. We will, nonetheless tabulate the number of lesions and their status based on ^{89}Zr-DFO-trastuzumab. When pathologic material that has been evaluated for the presence of HER2 is available for any specific lesion, we will correlate the presence of HER2 with imaging results.

Images will be analyzed with ROI and SUVmean and max measurements will be obtained. The serial quantitative data obtained over the various organs of interest (heart, liver, spleen, kidney, lung and any other organ that exhibits uptake will be collected. These data can then be utilized to determine organ time activity curves and the dosimetry can be calculated. Uptake and clearance values will be summarized using descriptive statistics (mean, median, standard deviation). Once the biodistribution and pharmacokinetics have been characterized, subsequent patients will not require serial imaging and pharmacokinetics, but rather will have a single scan at an optimal time point determined from the initial cohort. We will determine the time at which the greatest number of lesions are seen. This is likely to be between 3-8 days, but choice will be based on the first Cohort 1 patients' data, using the most frequent day at which the most lesions are seen and/or at times at which best contrast is observed, determined visually. Thus, for the remainder of the patients, PET scan will be performed once.

Images will be stored as Digital Imaging and Communications in Medicine (DICOM) on the PACS and HERMES. No decision on the patients' health care management will be made based on imaging with ^{89}Zr-DFO-trastuzumab scan.

9.3 Whole body probe measurements (Cohort 1)

Patients undergoing pharmacokinetics and serial imaging will undergo whole body activity measurement using a NaI probe placed at ~3.3 yards from the patient using our standard procedure. These measurements will be performed the day of injection of ^{89}Zr DFO-trastuzumab before voiding (if patient has to void before the initial measurement, urine will be collected and counted together with the patient) and after first void, and at each time the patient returns for imaging (typically probe count are acquired in less than 6 minutes).

9.4 Assessment for Toxicity

Safety Outcome Measures (CTCAE v4)

Infusion Reaction

Infusion reactions with ^{89}Zr-DFO-trastuzumab at these low milligram administration are expected to be infrequent. In the event of fever, rigors, shortness of breath, or other evidence of infusion reaction, patients may receive diphenhydramine 25-50 mg IV and Tylenol 650 mg PO as felt to be clinically appropriate by the treating physician.

Patients will be monitored during and after ^{89}Zr-DFO-trastuzumab infusion for 60 minutes after the infusion. Patients who experience infusion-related symptoms should be managed as directed in Table 1.

The safety and tolerability of ^{89}Zr-DFO-trastuzumab will be assessed using the following primary safety outcome measure:

- Incidence, nature, and severity of adverse events up to day 14 by phone following antibody administration.
- Change in vital signs during administration

Table 1
Management of Infusion-Related Symptoms

Infusion-Related Symptoms	Guidance
Grade 1	• Slow or hold infusion • Give supportive treatment • Upon symptom resolution, may resume infusion rate escalation at the investigator's discretion
Grade 2	• Slow or hold infusion • Give supportive treatment • Upon symptom resolution, may resume infusion-rate escalation at the investigator's discretion
Grade 3 or Grade 4	• Discontinue infusion • Give supportive treatment Patient may be imaged if sufficient tracer has been administered and the patient is stable, at the discretion of the administering investigator.

Given that this is a humanized antibody and that it is administered repetitively to patients, no determination of human anti human antibody will be performed as part of this study.

10.0 EVALUATION DURING TREATMENT/INTERVENTION

Laboratory Safety Assessments

Blood testing:

Hematology and blood chemistry assessments (CBC, serum BUN, creatinine, AST, ALT, total bilirubin, alkaline phosphatase, albumin and blood glucose levels) will be done at the time points described in Flowchart 1. Additional blood tests may also be performed at the discretion of the investigator for the purposes of planning treatment administration, dose modification, or investigation and follow-up of adverse events.

Physical examination:

A physical examination including, but not limited to, general appearance, skin, neck, eyes, ears, throat, lungs, heart, abdomen, lymph nodes, extremities, and nervous system will be performed. The physical examination will include examination of known and suspected sites of disease. Physical examination results, height and weight will be recorded at baseline.

Performance Status

Performance status will be assessed using the Karnofsky Performance Status scale (Appendix 1).

Vital Signs

Blood pressure and heart rate will be obtained at the time points outlined in the flowchart.

Flowchart Cohort 1

	Screening[a]		Day 0	Day 1	Days 2-4	Days 5-8	~14 days post dose
	Within 4 weeks	Within 14 days					
Informed consent	X						
Demographics	X						
Medical history	X						
Concomitant medications	X						
Physical exam	X						
Performance status	X						
Vital signs[b]	X		X				
Laboratory studies[c]		X				X	
Radiographic studies[d, e]	X						
IHC and or FISH for HER2[f]	Prior to enrollment						
^{89}Zr DFO-trastuzumab tracer administration			X				
Adverse events on day of imaging			X	X	X	X	
Phone assessment[g]							X
Serial whole-body scans[h]			X	X[i]	X[i]	X[i]	
Serial whole-body probe[j]			X	X[i]	X[i]	X[i]	
Serial blood samples[j]			X	X[i]	X[i]	X[i]	

[a] Screening evaluation should be performed following informed consent and within 4 weeks before study entry, except when noted otherwise. Evaluations performed as standard of care before informed consent but within the 4-week window need not be repeated.

[b] Vital signs will be monitored prior to infusion and approximately every 30 minutes (± 5 minutes) for at least 2 h or later if the patient is still in the department for imaging. If no changes are observed in the first 10 patients subsequent patiens will be monitored for 1 h post injection.

[c] All laboratory studies are to be performed up to 2 weeks before study entry .

[d] Radiographic evaluations include CT of chest, abdomen and pelvis (MRI is acceptable), and modified MRI of the pelvis, if clinically indicated. All radiographic studies are to be performed within 4 weeks before.

[e] Radiographic post treatment work up will be performed as per patient's therapy protocol or as per standard of care.

[f] HER2 status performed at MSKCC or elsewhere will be acceptable.

[g] A phone call will be used to assess for changes in concomitant medications and any adverse events after all scans have been completed.

[h] Following injection with the ^{89}Zr DFO-trastuzumab tracer, the patients enrolled will have serial whole-body PET-CT scans (WBS) performed to determine the biodistribution of the tracer. PET scans will be acquired: 1–4 hours following injection of the tracer, ~24 hours post-injection, ~48–96 hours post injection, and ~120–192 hours post injection the 24 h and other later scans will be performed within a ±8h of stated times.

[i] Only serial measurement in first 10 patients. All subsequent patients will not have serial blood draws, probe measurements or imaging, but rather one single imaging at a time point that is optimum based on the initial cohort of patients

[j] For the first 10 patients enrolled, serial blood samples will be obtained for biodistribution, metabolite analysis of the ^{89}Zr DFO-trastuzumab compound will be performed on selected samples. Samples will be obtained just prior to injection of the ^{89}Zr DFO-trastuzumab tracer this sample will be banked at -80degree C for future testing for immune response (HAHA) if altered biodistribution is observed., and at 5 ± 2 minutes, 15 ± 5, 30 ± 9, 60 ± 19 minutes, and 120 – 240 minutes after the injection of the

tracer 1, and at the time of each subsequent day of imaging. Additionally, whole body counts (WBC) using a NaI probe will be performed during the imaging in the first 10 patients and the exact time recorded:

- Within 0.1 to 3 hours following injection of tracer on Day 1 (prior to voiding) and post voiding
- ~24 hours post-injection (Day 1)±8h
- ~48–96 hours post-injection (once during Days 2–4)±8h
- ~120–192 hours post injection (once during Days 5–8) ±8h

Documentation of actual times of blood draw and probe measurement is required.

Flowchart Cohort 2

	Screening[a]		Day 0	Day TBD	~14 days post dose
	Within 4 weeks	Within 7 days			
Informed consent	X				
Demographics	X				
Medical history	X				
Concomitant medications	X				
Physical exam	X				
Performance status	X				
Vital signs[b]	X		X		
Laboratory studies[c]		X	X	X[c]	
Radiographic studies[d]	X			X	
IHC and or FISH for HER2[e]	Prior to enrollment				
^{89}Zr DFO-trastuzumab tracer administration			X		
Adverse events			X	X	
Phone assessment[f]					X
Single whole-body scans				X[g]	

[a] Screening evaluation should be performed following informed consent and within 4 weeks before study entry, except when noted otherwise. Evaluations performed as standard of care before informed consent but within the 4-week window need not be repeated.

[b] Vital signs will be monitored prior to infusionand approximately every 30 minutes (± 5 minutes) for at least 2 h or later if the patient is still in the department for imaging.

[c] Within 1 week of second injection and day of imaging. On Day 0, on injection 1 serum sample 3cc, will be collected and frozen at -80degree for testing of immune (HAHA) response if altered biodistribution is observed.

[d] Radiographic studies as per clinical routine for assessment of response.

[e] HER2 status performed at MSKCC or elsewhere will be acceptable.

[f] A phone call will be used to assess for changes in concomitant medications and any adverse events after all scans have been completed.

[g] Single imaging time point based on cohort 1 (likely to be from day 3 to 8)

11.0 TOXICITIES/SIDE EFFECTS

Risks

^{89}Zr-DFO-trastuzumab

Overall our experience with other radiolabeled antibodies and radiolabeled trastuzumab are that allergic reactions are uncommon. Therefore no pretreatment will be provided before antibody injection. Potential risks associated with ^{89}Zr-DFO-trastuzumab are similar to those of other humanized antibody and include allergic reactions, characterized by fever, chills, and sometimes rashes or hives. Less likely shortness of breath or gastrointestinal upset can occur. Rare allergic reactions can present as shock or death or swelling of the throat. Heart failure, although observed with therapeutic doses of trastuzumab are not expected at these lower ~ 50mg x 2 doses. Allergic reactions are usually addressed by stopping the administration of the drug, administering diphenhydramine , acetaminophen and/or steroids, and restarting the drug at a slower rate if the reaction is not severe. We will also follow MSKCC guidelines for “adult hypersensitivity orders for chemotherapy/biologic therapy”, which intstitutes therapy according to the severity of the reaction.

There is a very small risk of infection. This could cause itching or redness at the area on the patient’s arm where the IV was placed.

^{89}Zr DFO-trastuzumab PET/CT Scans

As part of this scan there is radiation delivered from the ^{89}Zr and from the low dose CT scan that are performed as part of the CT for attenuation correction and co-registration. Although any exposure to ionizing radiation has the potential to cause some harm to tissue, the radiation exposures in this study are comparable to the low-level exposures associated with common diagnostic procedures such as CT scanning. There remains a low theoretical risk of developing a cancer at some point later in life because of the radiation exposure received in this study. This risk is particularly low for adults given the fact that the radiation from the scans will be over several weeks. Participants should avoid pregancy while on this study. Acceptable birth control methods include abstinence, double barrier method, surgically sterilized patient or partner. This risk is much smaller that the clinical risks posed by the patient’s current cancer.

Radiation Dosimetry

Normal-organ radiation absorbed doses and the effective dose for ^{89}Zr-DFO-trastuzumab in human were estimated based on measured time-activity data in mice. The time-activity data (percent of the injected dose per gram (%ID/gm) versus time (t) post-administration) for each organ were fit to an exponential functions and the resulting function were analytically integrated (incorporating the radioactive decay of ^{89}Zr) to yield the residence time (cumulated activity) of ^{89}Zr in each organ. The mouse organ ^{89}Zr residence times were converted to ^{89}Zr residence times in human organs (for the 70-kg Standard Man anatomic model) by adjusting for the difference in fractional organ masses between mice and humans. The resulting human residence times were then entered into the OLINDA/EXM radionuclide dosimetry program, which implements the MIRD (Medical Internal Radionuclide Dosimetry) algorithm, to yield the normal-organ radiation absorbed doses (in cGy) and effective dose (in rem) for ^{89}Zr-DFO-trastuzumab in man. Assuming a 5-mCi administered activity of ^{89}Zr-DFO-trastuzumab and a low-dose (80 mA) and three very-low-dose (10-mA) CT scans are to be performed, with approximate CT effective doses of 0.90 rem and 0.11 rem the radiation dosimetry is tabulated below to include the second injection of ^{89}Zr-DFO-trastuzumab, that some patients may receive (Table 2).

Table 2

			# of Injections/CT scans
Activity of ^{89}Zr-DFO-trastuzumab	5	mCi	2
low dose CT scan (80mA)	0.9	cGy	2
ultra-low dose CT scan (10mA)	0.11	cGy	3

	Absorbed Dose		
	^{89}Zr-DFO-trastuzumab [1]		Total dose from CT and up to 2 ^{89}Zr-DFO-trastuzumab + CT
Target Organ	cGy/mCi	cGy per inj	cGy
Adrenals	1.47	7.4	**16.8**
Bone Surfaces	3.36	16.8	**35.7**
Brain	0.64	3.2	**8.5**
Heart Wall	1.32	6.6	**15.3**
Kidneys	2.45	12.3	**26.6**
Large Intestine - Lower Wall	1.08	5.4	**12.9**
Large Intestine - Upper Wall	1.06	5.3	**12.7**
Liver	2.96	14.8	**31.7**
Lungs	2.07	10.4	**22.8**
Ovaries	1.00	5.0	**12.1**
Pancreas	1.33	6.7	**15.4**
Red Marrow	2.76	13.8	**29.7**
Small Intestine	1.08	5.4	**12.9**

Spleen	2.30	11.5	**25.1**
Stomach Wall	1.33	6.7	**15.4**
Testes	0.62	3.1	**8.3**
Thyroid	0.75	3.8	**9.7**
Urinary Bladder Wall [2]	1.83	9.2	**20.4**
Uterus	0.98	4.9	**11.9**
Total Body	1.06	5.3	**12.7**
Effective Dose (rem)	1.48	7.4	**16.9**

[1] Based on biodistribution of ^{89}Zr-89-DFO-trastuzumab in nude mice (Holland et al, PLoS ONE 5(1): e8859. doi:10.1371/journal.pone.0008859) OLINDA/EXM-based absorbed dose estimates by Pat Zanzonico

[2] (assumed 3-hr voiding interval)

In accordance with local and Federal regulations (USNRC), patients administered ^{89}Zr can be released from hospital control when it can be demonstrated that a member of the public is unlikely to receive greater than 1 mSv. Conservative regulatory guidance suggests that in the case of ^{89}Zr, a patient can be released from hospital control if either of the following conditions apply: they receive < 5 mCi or the dose rate at 1 meter from the patient is < 4 mrem/h. In a subset of 10 patients from a previous protocol who received a mean activity of 5 mCi ^{89}Zr-J591, the measured dose rate at 1 meter averaged 1.9 mrem/h and the maximum dose rate was 2.3 mrem/h. As these values are demonstrated to be well below 4 mrem/h, patients administered 5 mCi ^{89}Zr for this current diagnostic protocol can be released from hospital control without the need for specific radiation safety measurements.

12.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

We will determine the toxicity, optimum imaging time and the feasibility of using ^{89}Zr-DFO-trastuzumab as an imaging agent in esophagogastric tumors that express HER2. In a pilot manner we will quantitatively evaluate changes in tumor concentration compared to baseline scan, once patients undergo HER2 directed therapy and a second ^{89}Zr-DFO-trastuzumab imaging study. Decay corrected images will be assessed, SUVmax and mean values for regional dose distribution will be calculated, and converted using excel into values that can be entered into the OLINDA software package that will ultimately calculate the absorbed doses.

Positive lesions on ^{89}Zr-DFO-trastuzumab will be defined visually as a focus of accumulation of radioactivity that is deemed to not be physiologic and is higher than adjacent or contralateral background. Antibody scan will be read blinded from clinical history and conventional imaging modalities. Two different experienced, blinded readers will visually analyze the ^{89}Zr-DFO-trastuzumab scans, and SUVmax/mean will be determined in lesions identified and in normal organs and blood pool. To study the accumulation and biodistribution of ^{89}Zr-DFO-trastuzumab in patients with esophagogastric cancer, the accumulation of ^{89}Zr-DFO-trastuzumab will be assessed for each site and compared with the clinically established lesion in the baseline radionuclide bone scan, FDG PET/CT, CT, and/or MRI imaging. The definition of a positive lesion will be a lesion identified by an experienced reader on conventional imaging CT/MRI, and/or FDG scan consistent with metastatic

disease, as is currently done clinically. Lesions on ^{89}Zr-DFO-trastuzumab or conventional imaging will be read in a scale of 1 to 5, 1= negative, 2 = probably negative, 3= not sure, 4= probably positive and 5= definitively positive. Once this is done, a consensus master list of lesions will be created that compares all imaging conventional imaging modalities(CT/MRI, FDG PET, bone scan), only those lesions that are conspicuously positive (i.e., "4 or 5") by imaging will be considered as a positive site of disease. Discrepant findings between findings on conventional imaging and ^{89}Zr-DFO-trastuzumab modalities will be considered false positive or false negative. Although it would be ideal to biopsy any site that is considered abnormal this is not logistically feasible or ethically acceptable. Successful imaging will be considered a positive imaging in $\geq$ 70% of patients . One or 2 non-blinded readers (access to clinical, laboratory and imaging data) will identify "positive lesions" .

As a first level, we will compare site by site. Since the individual modalities are based on different mechanisms of uptake, they may not have exact correspondence in space, since bone scan is a 2D modality and ^{89}Zr looks at tumor antigen expression and is a 3D modality. For this purpose, we have made a list of standardized anatomic sites, to make sure that a positive lesion would have a positive ^{89}Zr-DFO-trastuzumab uptake. As a second level, we will compare lesion by lesion.

12.1 CT and MRI

Available CT and/or MRI imaging done clinically will be used as a correlative diagnostic imaging tool to compare to results of ^{89}Zr-DFO-trastuzumab PET scanning. Furthermore soft tissue lesions greater than 2 cm on CT or MRI will also be compared to ^{89}Zr-DFO-trastuzumab in order to overcome the issues of partial volume.

13.0 CRITERIA FOR REMOVAL FROM STUDY

Participation in the study is strictly voluntary. Patients have the right to withdraw from the study at any time. If a patient chooses to withdraw, he or she must inform the investigator immediately. In addition, the investigator has the right to terminate participation of any patient at any time if it is deemed in the patient's best interest. The reason and circumstances for premature discontinuation will be documented in the patient's medical records. Possible examples for reasons of premature study withdrawal include withdrawal of consent, SAE or intolerable AE, or any other medical illness at investigator's discretion.

14.0 BIOSTATISTICS

14.1 Primary Objective

This is a pilot trial. The primary objective of this protocol is to determine the feasibility, safety and tolerability of administration of ^{89}Zr-DFO-trastuzumab antibody.

Definition of Feasibility: Antibody imaging is considered feasible if 70% of the patients are antibody-imaging positive as defined below.

Defintion of Antibody-Positive: A patient with HER2 positive tumor will be considered antibody-imaging-positive if 50% or more of the active lesions (FDG positive, bone scan positive or CT positive >2cm) are detectable in any of the scanning time points (4 scans in the first 10 patients and 1 scan in all others). Active lesion means any lesion that was identified by conventional imaging methods and clinical data at baseline felt to represent

tumor. We have selected a threshold of 50% for this first imaging study for 3 reasons: 1) we do not know what the heterogeneity of antigen expression is within a number of lesions in the same patient; 2) we will consider positive any lesion based on conventional clinical imaging criteria, in which case some lesions considered positive may be false positive and more importantly 3) our main long term emphasis is to evaluate whether this imaging will identify HER2 positive patients and whether identification of these patients for treatment with HER2 directed therapies will be of benefit. Thus, we do not want to discard this agent if we can use it as a **theranostic** (defined as an agent that is integrated into the drug development process to guide patient selection and drug development), for the development of therapeutic regimens aimed at HER2. Nonetheless, we intend to evaluate treatment response of all available lesions in this way we will maximize our ability to determine the relationship between uptake of antibody and response.

The optimal time for imaging will be estimated based on results from the first 10 patients studied. In each of the first 10 patients the proportion of lesions idenfied on each imaging day will be determined. The imaging day in which the greatest proportion of lesions are identified for each patient will be identified. The optimal day for imaging will be the one most frequently associated with the visualization of the largest proportion of lesions in the first 10 patients. Since it is possible that the same proportion of lesions may be identified over several days and that contrast may vary between the different imaging days a consensus visual assessment of a group of experienced Molecular Imaging and Therapy investigator who will grade the studies separately (JAC, SML and NPT, 2 of the readers will be blinded to clinical information), will be used to make the final decision.

Evaluation of Feasibility in the First Cohort: Antibody imaging will be considered feasible if 7 or more of the 10 patients in the first cohort are antibody-imaging-positive.We will also require that none of these patients experience severe toxicity attributable to the initial antibody. The following table provides the one-sided 90% confidence limit for the true feasibility rate based on the number of feasible patients:

Number of Feasible Patients	7	8	9	10
One-sided 90% CL	45%	55%	66%	79%

If less than 70% of patients are considered positive for imaging in either the first cohort or combination of first and second cohort the study will be closed.

Tolerability: If the study proceeds to cohort B, one-sided exact confidence limits will be used to estimate the toxicity and feasibility rates at the end of the study using all the 55 patients. The following tables give some of the likely outcomes to be observed:

Number of Patients with Toxicity	0	1	2
One-sided 90% CL	9%	15%	20%

Evaluation of Feasibility in the Second Cohort:

Number of Feasible Patients	20	21	22	23	24	25
One-sided 90% CL	66%	71%	75%	80%	85%	91%

Dosimetry and PK: Another objective is to determine the organ/tissue uptake and dosimetry following IV injection of ^{89}Zr-DFO-trastuzumab in patients with HER2 positive tumors. The group for this analysis will comprise 10 HER2+ patients in the cohort 1 ^{89}Zr-DFO-trastuzumab study the 10 patients that will be recruited in the treatment assessment arm. Standardized uptake value (SUV based on weight and lean body mass) in various organs will be measured and summarized over patients to describe the organ and tissue uptake of the tracer. The SUVmean, max and peak will be summarized with descriptive statistics. This data will be converted to residence time and will be used in OLINDA dosimetry program to obtrain dosimetry measurements. The dosimetry estimates will be summarized with descriptive statistics.

Pharmacokinetic analysis will be performed using a non-compartmental analysis (SAAM or GraphPad V5); standard parameters such as AUC, clearance, volume of distribution of central compartment, and Co will be reported. Descriptive statistics will be tabulated. These patients will undergo a single whole-body scan at the optimal time point. The ability of ^{89}Zr-DFO-trastuzumab to detect tumors will be estimated from this cohort using the proportion of antibody-imaging-positive patients (within +/-23% if the true detection rate is 70%).

Toxicity: This study does not intend to find the maximal tolerated dose of ^{89}Zr-DFO-trastuzumab. This is a diagnostic agent that is expected to have low incidence of adverse events. Patients will be monitored closely looking for evidence of adverse events. Because most patients are expected to start new therapies within 2 weeks of receiving ^{89}Zr-DFO-trastuzumab only short term toxicity will be evaluated. The safety and tolerability of ^{89}Zr-DFO-trastuzumab will be assessed using the following primary safety outcome measures: Incidence and nature and severity of adverse events; and change in vital signs and clinical laboratory results. Incidence and severity of adverse events will be summarized with descriptive statistics.

If severe adverse effect (grade 3 or 4 CTCAE) attributable to ^{89}Zr-DFO-trastuzumab is observed in any cohort we will review with MSKCC-DSMC. A secondary objective is to correlate ^{89}Zr-DFO-trastuzumab imaging to response to treatment in those patients recruited for repeat imaging following therapy. Outcome measures to be correlated include those that are outlined in any parent protocol under which the patient is receiving treatment (typically RECIST,survival or progression free survival).

Secondary Analysis: Preliminary quantitative data will be obtained using SUV measurements on baseline study compared to that of patients having repeat ^{89}Zr-DFO-trastuzumab study(n=10). Paired T-test will be performed to estimate the differences. With 10 patients having repeat imaging.

Imaging result based on mean SUV measurements will be tabulated and compared to clinical response (stable, regression or progression). Furthemore we will be identify lesion outcome (stable, regression or progression) based on the individual lesion SUV. The data will be expressed in terms of stable, regression and progression versus mean SUV measurement and summarized in terms of descriptive statistics.

15.0 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES

15.1 Research Participant Registration

Confirm eligibility as defined in the section entitled Criteria for Patient/Subject Eligibility.

Obtain informed consent, by following procedures defined in section entitled Informed Consent Procedures.

During the registration process registering individuals will be required to complete a protocol specific Eligibility Checklist.

All participants must be registered through the Protocol Participant Registration (PPR) Office at Memorial Sloan-Kettering Cancer Center. PPR is available Monday through Friday from 8:30am – 5:30pm at 646-735-8000. Registrations must be submitted via the PPR Electronic Registration System (http://ppr/). The completed signature page of the written consent/RA or verbal script/RA, a completed Eligibility Checklist and other relevant documents must be uploaded via the PPR Electronic Registration System.

15.2 Randomization

This research study does not require randomization procedures.

16.0 DATA MANAGEMENT ISSUES

A Research Study Assistant (RSA) will be assigned to the study. The responsibilities of the RSA include project compliance, data collection, abstraction and entry, data reporting, regulatory monitoring, problem resolution and prioritization, and coordinate the activities of the protocol study team.

The data collected for this study will be entered into a secure database. Source documentation will be available to support the computerized patient record.

Study personnel will record clinical data in each patient's source documents (i.e., the patient's medical record).

The investigator will maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. After study closure, the investigator will maintain all source documents, study-related documents, and the data stored in the database used for data collection. Records will be retained and securely stored for a minimum of 2 years after the completion of all study activities.

Data will be entered throughout the duration of the trial as patients are enrolled. Accrual is expected to last 18 month.

16.1 Quality Assurance

Regularly scheduled registration reports will be generated to monitor patient accruals and the completeness of registration data. Routine data quality reports will be generated to assess missing data and inconsistencies. Accrual rates and the extent and accuracy of evaluations and follow-up will be monitored periodically throughout the study period, and potential problems will be brought to the attention of the principal investigator for discussion and action.

Random-sample data quality and protocol compliance audits will be conducted by the study team at least once a year, more frequently if indicated.

16.2 Data and Safety Monitoring

The data and safety monitoring (DSM) plans at MSKCC were approved by the National Cancer Institute in September 2001. The plans address the policies set forth by the NCI in the document entitled *Policy of the National Cancer Institute for Data and Safety Monitoring of Clinical Trials*, which can be found at http://cancertrials.nci.nih.gov/clinicaltrials. The DSM plans at MSKCC were established and are monitored by the Office of Clinical Research. The MSKCC DSM plans can be found on the MSKCC Intranet at: http://mskweb5.mskcc.org/intranet/html/70775.cfm.

There are several different mechanisms by which clinical trials are monitored for data, safety, and quality. There are institutional processes in place for quality assurance (e.g., protocol monitoring, compliance and data verification audits, therapeutic response, and staff education on clinical research quality assurance) and departmental procedures for quality control, and there are two institutional committees that are responsible for monitoring the activities of our clinical trials programs. The committees: *Data and Safety Monitoring Committee (DSMC)* for Phase I and II clinical trials, and the *Data and Safety Monitoring Board (DSMB)* for Phase III clinical trials, report to the MSKCC Research Council and Institutional Review Board.

During the protocol development and review process, each protocol will be assessed for its level of risk and degree of monitoring required. Every type of protocol (e.g., NIH sponsored, in-house sponsored, industrial sponsored, NCI cooperative group, etc.) will be addressed, and the monitoring procedures will be established at the time of protocol activation.

For this protocol, we will use the following patient safety monitoring plan and monitoring for adverse events, in terms of the test performed and their timing relative to injection (see Flowchart 1 and Flowchart 2). Patient will be observed closely during infusion and serial vital signs will be monitored every half hour for at least 2 h or later if the patient remains in the department. Patients will be queried for adverse events up to ~14 days after infusion.

Safety reviews will be performed by the MSKCC-DSMC with yearly review by the IRB.

16.3 DOD Requirements

Per DoD Directive 3216.02, all greater than minimal risk studies require a Medical Monitor.

Responsibilities of the Medical Monitor

The medical monitor is required to review all unanticipated problems involving risk to subjects or others, serious adverse events, and all subject deaths associated with the protocol and provide an unbiased written report of the event.

Medical Monitor: Lisa Bodei, MD

Lisa Bodei, MD will serve as an independent clinical monitor of the research trial and will oversee the conduct and expectations of fulfilling its research goals and obligations. She will be meeting with the Principal Investigator to review any relevant toxicities incurred by patients and to determine whether the study accrual is being conducted in a timely and efficient manner. Lisa Bodei, MD is a qualified physician, other than the Principal Investigator, not associated with this particular study who possesses sufficient educational and professional experience to serve as the subject/patient advocate. As the medical monitor, Dr. Lisa Bodei may:

- Stop a research study in progress
- Remove an individual from the study
- Take any steps to protect the safety and well-being of participants until the IRB can assess

The DOD funding supporting this study expired in 09/2019.

17.0 PROTECTION OF HUMAN SUBJECTS

This study will be conducted in compliance with the protocol, GCP guidelines established by the International Conference on Harmonization, and the ethical standards set forth in the Declaration of Helsinki 2004 (available at: www.laakariliitto.fi/e/ethics/helsinki.html).

A limited waiver of authorization will be utilized to effectively screen and track patients in screening, however the recruitment process outlined presents no more than minimal risk to the privacy of the patients who are screened and minimal protected health information (PHI) will be maintained as part of a screening log.

This protocol does not have therapeutic intent and does not offer patients therapeutic benefit. This will be clearly conveyed to patients when communicating the potential toxicities/side effects of participating in this trial. Participation in the trial is voluntary and there will be no financial benefit (or burden) for the patients. Participants will not be charged for ^{89}Zr-DFO-trastuzumab PET scans, whole body probe counts, radiotracer drugs, and extra blood draws (to measure radiotracer activity).

When all other possibilities are exhausted, if an eligible patient lives outside of New York City, then the patient will be eligible for reimbursement for the costs of parking/ public transportation (up to $50 per day with receipt) and a two-night stay at an MSKCC approved hotel (up to $1000 total), when those costs are incurred during the days that the patient visits the Main Campus Radiology Department for study injection and scans.

17.1 Privacy

MSKCC's Privacy Office may allow the use and disclosure of protected health information pursuant to a completed and signed Research Authorization form. The use and disclosure of protected health information will be limited to the individuals described in the Research Authorization form. A Research Authorization form must be completed by the Principal Investigator and approved by the IRB and Privacy Board (IRB/PB).

17.2 Serious Adverse Event (SAE) Reporting

An adverse event is considered serious if it results in ANY of the following outcomes:

- Death
- A life-threatening adverse event
- An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition

Note: Hospital admission for a planned procedure/disease treatment is not considered an SAE.

SAE reporting is required as soon as the participant signs consent. SAE reporting is required for 30-days after the participant's last investigational treatment or intervention. Any events that occur after the 30-day period and that are at least possibly related to protocol treatment must be reported.

If an SAE requires submission to the IRB office per IRB SOP RR-408 'Reporting of Serious Adverse Events', the SAE report must be sent to the IRB within 5 calendar days of the event. The IRB requires a Clinical Research Database (CRDB) SAE report be submitted electronically to the SAE Office as follows:

For IND/IDE trials: Reports that include a Grade 5 SAE should be sent to saegrade5@mskcc.org. All other reports should be sent to saemskind@mskcc.org.

For all other trials: Reports that include a Grade 5 SAE should be sent to saegrade5@mskcc.org. All other reports should be sent to sae@mskcc.org.

The report should contain the following information:

Fields populated from CRDB:

- Subject's initials
- Medical record number
- Disease/histology (if applicable)
- Protocol number and title

Data needing to be entered:

- The date the adverse event occurred
- The adverse event
- The grade of the event
- Relationship of the adverse event to the treatment (drug, device, or intervention)
- If the AE was expected
- The severity of the AE
- The intervention
- Detailed text that includes the following
    - A explanation of how the AE was handled
    - A description of the subject's condition
    - Indication if the subject remains on the study
- If an amendment will need to be made to the protocol and/or consent form
- If the SAE is an Unanticipated Problem

The PI's signature and the date it was signed are required on the completed report.

For IND/IDE protocols:
The CRDB SAE report should be completed as per above instructions. If appropriate, the report will be forwarded to the FDA by the SAE staff through the IND Office.

The radiation doses (on the order of 10 rem) lie well within the "low-dose" range—well below the threshold doses (typically on the order of 100 rem or greater) for any known deterministic effects. A detailed description of the expected dosimetry from the ^{89}Zr-DFO-trastuzumab is included (Table 2). The dose of ^{89}Zr-DFO-trastuzumab per administration (~5mCi) gives the lowest amount that is expected to be consistent with the research goal, namely, biodistribution, metabolism, and pharmacokinetics of the compound.

17.2.1

This protocol will have an IND. SAE will also be reported to the FDA through the IND Office and the report will include the FDA assigned BB-IDE, BB-IND or IND number and name.

Patients will be monitored for adverse events following agent administration. Beginning immediately after tracer-injection, patients will be observed for 1 hour in the Radiology Department, to monitor for acute adverse reactions.

Toxicities will be assessed according to the National Cancer Institute CTC Scale (Version 4.0).

An adverse event is defined by the GCP (Good Clinical Practice guidelines) as an undesirable experience occurring to a subject during a clinical trial, whether or not considered related to the investigational product(s). It is the responsibility of the Principal Investigator and his/her research team to identify, review and record all necessary adverse events.

17.3 SAE Grading

17.3.2 Grading Severity

All adverse events will be graded for intensity on a scale of 0 to 5. Severity grades will be recorded and based on the CTCAE v4.0.

17.3.3 Attributing Causality

The investigator will evaluate the potential relationship between all clinical AEs, abnormal laboratory values, and the ^{89}Zr-DFO-trastuzumab , and categorize the relationship according to the descriptions in Table 3. Abnormal laboratory values of clinical significance that were present at baseline and did not change in either severity or frequency during the experimental therapy or intervention and/or that can obviously be attributed to the underlying disease will be evaluated by the investigator and recorded in the "unrelated" category.

Table 3. Relationship of Adverse Event to ^{89}Zr-DFO-trastuzumab

Relationship	Description
Unrelated	AE is clearly not related to the ^{89}Zr-DFO-trastuzumab
Unlikely	AE is unlikely related to the ^{89}Zr-DFO-trastuzumab
Possible	AE may be related to the ^{89}Zr-DFO-trastuzumab
Probable	AE is likely related to the ^{89}Zr-DFO-trastuzumab
Definite	AE is clearly related to the ^{89}Zr-DFO-trastuzumab

Adverse events will be defined graded using CTCAE V4.0.

18.0 INFORMED CONSENT PROCEDURES

Before protocol-specified procedures are carried out, consenting professionals will explain full details of the protocol and study procedures as well as the risks involved to participants prior to their inclusion in the study. Participants will also be informed that they are free to withdraw from the study at any time. All participants must sign an IRB/PB-approved consent form indicating their consent to participate. This consent form meets the requirements of the Code of Federal Regulations and the Institutional Review Board/Privacy Board of this Center. The consent form will include the following:

1. The nature and objectives, potential risks and benefits of the intended study.
2. The length of study and the likely follow-up required.
3. Alternatives to the proposed study. (This will include available standard and investigational therapies. In addition, patients will be offered an option of supportive care for therapeutic studies.)
4. The name of the investigator(s) responsible for the protocol.
5. The right of the participant to accept or refuse study interventions/interactions and to withdraw from participation at any time.

Before any protocol-specific procedures can be carried out, the consenting professional will fully explain the aspects of patient privacy concerning research specific information. In addition to signing the IRB Informed Consent, all patients must agree to the Research Authorization component of the informed consent form.

Each participant and consenting professional will sign the consent form. The participant must receive a copy of the signed informed consent form.

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20.0 APPENDICES

APPENDIX 1: Karnofsky Performance Status

Karnofsky Performance Scale

Condition	%	Comments
Able to carry on normal activity and to work; no special care needed.	100	Normal; no complaints; no evidence of disease.
	90	Able to carry on normal activity; minor signs or symptoms of disease.
	80	Normal activity with effort; some signs or symptoms of disease.
Unable to work; able to live at home and care for most personal needs; varying amount of assistance needed.	70	Cares for self; unable to carry on normal activity or to do active work.
	60	Requires occasional assistance, but is able to care for most of his personal needs.
	50	Requires considerable assistance and frequent medical care.
Unable to care for self; requires equivalent of institutional or hospital care; disease may be progressing rapidly.	40	Disabled; requires special care and assistance.
	30	Severely disabled; hospital admission is indicated although death not imminent.
	20	Very sick; hospital admission necessary; active supportive treatment necessary.
	10	Moribund; fatal processes progressing rapidly.
	0	Dead