CITY OF HOPE NATIONAL MEDICAL CENTER
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DEPARTMENT OF MEDICAL ONCOLOGY AND THERAPEUTICS RESEARCH

TITLE: Use of ^{64}cu-DOTA-trastuzumab PET Imaging and Molecular Markers for Prediction of Response to Trastuzumab and Pertuzumab-based Neoadjuvant therapy

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1.0 Background

HER2-Positive Breast Cancer

The term “breast cancer” encompasses a number of different diseases that are clinically defined by hormone receptor status and *HER2* expression. *HER2* is a transmembrane protein in the epidermal growth factor family. *HER2* has tyrosine kinase activity that results in intracellular signaling and activation of genes for cell growth and survival. Women whose cancers overexpress the *HER2* protein have a distinct natural history and are candidates for treatment with trastuzumab. Trastuzumab is a humanized IgG-1 antibody that binds to the ectodomain of *HER2*. When combined with chemotherapy, trastuzumab significantly improves the survival of women with both early stage and advanced disease. [1-3]

The pathologic assessment of HER2 status is made on the primary tumor or metastatic foci. *HER2* overexpression and candidacy for trastuzumab have been defined as 3+ staining by immunohistochemistry (IHC) or gene amplification by fluorescence in situ hybridization (FISH). [4, 5] The American Society of Clinical Oncology and American College of Pathology have recently updated the guidelines for measurement of HER2. The new definition requires that the area of tumor contain at least 10% contiguous and homogeneous tumor cells which are identified as having protein over expression by IHC (3+) or gene amplification in a sample of 20 cancer cells.[6]

Trastuzumab in combination with chemotherapy improves the overall survival for women with early stage and metastatic HER2-positive breast cancers. However, the activity of trastuzumab as a single agent in metastatic disease is modest. Objective tumor responses are reported in 35% of women treated with trastuzumab as first-line therapy and 18% in those who have been previously treated.[7, 8] Thus, trastuzumab therapy does not benefit all patients with HER2-positive breast cancers. Given the cost and potential toxicity of trastuzumab, a clinical assessment of HER2 is needed that predicts benefit from trastuzumab.

A central pathology review of HER2 tumor status from NSABP B31, a controlled trial of adjuvant trastuzumab, identified 103 women whose tumors were HER2 negative. Women with *HER2*-negative disease who received adjuvant trastuzumab experienced fewer breast cancer recurrences than *HER2*-negative women who did not receive adjuvant trastuzumab.[5, 9] The CLEOPATRA trial tested the addition of pertuzumab to docetaxel and trastuzumab in women with metastatic *HER2*-positive breast cancers. A central pathology review identified HER2-negative patients who benefited from the addition of pertuzumab (personal communication, Genentech). These observations suggest that the use of trastuzumab-based therapy should be broadened to include at least some nominally HER2-negative women and confirm the discordance in results from different institutions. Counterbalancing the potential benefit, however, is the fact that treatment with trastuzumab is expensive and may cause cardiac toxicity.

Treatment of HER2 positive breast cancer.

Since the approval of trastuzumab in 1998, three additional HER2-directed therapies have been added to the armamentarium - lapatinib, ado-trastuzumab emtansine, and pertuzumab.[10, 11] The benefit of adding pertuzumab to neoadjuvant trastuzumab + chemotherapy was tested in the NEOSPHERE and TRYPAENA trials.[12, 13] At the time of surgery, higher pathologic complete response rates were reported with the addition of pertuzumab. As a result, the FDA expanded the indications for pertuzumab to include both the metastatic and neoadjuvant settings. Chemotherapy combined with trastuzumab and pertuzumab is considered standard of care for neoadjuvant therapy of HER2 positive breast cancer.

The most common use of neoadjuvant chemotherapy is to decrease the size and increase the use of breast conserving therapy.[14] Response to neoadjuvant chemotherapy also provides prognostic information. Those women who obtain complete disappearance in the primary tumor and the axillary nodes have a more favorable outcome than those who have residual disease in the primary and or nodes. [12, 15] With trastuzumab + chemotherapy regimens, pathologic complete remissions are

observed in approximately 50% of women with locally advanced HER2 positive disease. The addition of pertuzumab to trastuzumab + chemotherapy has increased the pathologic response rate to 60%.[13]

Molecular Imaging in HER2 Positive Breast Cancer

Positron Emission Tomography (PET) provides a non-invasive way of studying tumor location, metabolic function, and response to therapy. In breast cancer, PET imaging with ^{18}F-flourodeoxyglucose (FDG) has been used to stage women with advanced disease and to assess response to chemotherapy and endocrine therapy even before tumor regression can be documented by clinical examination or anatomical imaging.[16-18] The ability to identify patients who may benefit from systemic therapy is critically important to their quality of life; toxicities from ineffective therapies are prevented and medical costs are contained.

In a previous study, we developed a novel functional imaging approach using ^{64}Cu-DOTA-trastuzumab and PET to characterize the biodistribution and tumor uptake of trastuzumab 24-48 hours after systemic intravenous administration. We showed that, with pre-infusion of non-labeled trastuzumab (45 mg) to suppress liver uptake, this approach produces high-quality images and detects tumors with exquisite sensitivity in patients with HER2-positive metastatic breast cancer. [19] More recently, we extended the study to include patients with advanced HER2-negative disease. We found that tumor uptake as measured with ^{64}Cu-DOTA-trastuzumab/PET-CT is generally higher in patients classified as HER2-positive vs. HER2-negative, but with a good deal of inter- and intra-patient variability [20]. The observations were consistent between tumors that were biopsied and histopathologically assessed for HER2 expression and those that were not. This suggests that, while ^{64}Cu-DOTA-trastuzumab uptake is positively correlated with tumor *HER2* expression, other factors such as blood-tissue transport also have significant effect on tumor uptake of intravenously administered trastuzumab and therefore on response to trastuzumab-based therapy.

Use of radiolabeled trastuzumab PET imaging to predict response to ado-trastuzumab emtansine

We and others have utilized radiolabeled trastuzumab to predict for response to the immunoconjugate ado-trastuzumab emtansine (TDM1).[21] In the four patients studied to date, we have observed significant heterogeneity of uptake on radiolabeled-trastuzumab PET imaging. Although the tumors were documented to be HER2 positive by pathologic assessment before therapy, the two patients who demonstrated low uptake on radiolabeled-trastuzumab PET imaging failed to benefit from TDM1.

We propose to utilize ^{64}Cu-DOTA-trastuzumab-PET to image women with newly diagnosed locally advanced HER2 positive breast cancer to predict for response to trastuzumab + pertuzumab + chemotherapy. We anticipate that the pretreatment ^{64}Cu-DOTA-trastuzumab/PET-CT will identify a large amount of heterogeneity in tumor uptake of trastuzumab. To the degree that low uptake of trastuzumab reflects difficulty in tumor penetration by large molecules, the observations may also predict low uptake of pertuzumab. All patients will undergo ^{18}F-FDG/PET-CT shortly before the ^{64}Cu-DOTA-trastuzumab procedure. Same-tumor comparison between ^{64}Cu-DOTA-trastuzumab (molecular weight 1.5×10^5) and ^{18}F-FDG (molecular weight 1.8×10^2) will elucidate the role of molecular size in determining tumor uptake of trastuzumab and pertuzumab.

We will also submit pretreatment biopsies of combining tumor for RPPA, RNAseq, SERPINA, and to develop PDX models. We anticipate that combining ^{64}Cu-DOTA-trastuzumab-PET with this additional biomarker assessment will further enable us to predict for benefit or lack of benefit from anti-HER2 therapies. This information will be used to develop strategies to improve treatment of HER2 positive breast cancer.

2.0 Study Objectives

While our ultimate goal is to utilize ^{64}Cu-DOTA-trastuzumab/PET-CT to aid "individualized" selection of *HER2*-directed therapies in general, the proposed pilot study will address imaging issues for neoadjuvant patients as its primary objective. Secondary objectives include combining with molecular markers to predict for complete response in locally advanced cancers. In addition to inadequate tumor uptake, another potential cause of non-response is biological or "molecular" mechanisms of resistance. Consistent with our prior study protocol, all patients will undergo a pretreatment biopsy to ensure positive *HER2* status. Tumor will also be submitted for assessment of molecular markers. The goal of this pilot study is to correlate ^{64}Cu-DOTA-trastuzumab/PET-CT tumor uptake measurements with molecular markers to predict for response both at the patient and individual tumor levels.

The Specific Study Objectives are:

2.1 Primary Aim

2.1.1

To evaluate the SUVmax in patients with previously untreated HER2+ breast cancer. Evaluating the tumor size sensitivity, and the ability to detect lymph node involvement will be assessed, along with comparing the SUVmax detected in previously untreated HER2+ breast cancer to the SUVmax detected in recurrent breast disease in our previous series on HER2+ patients with metastatic disease.

2.2 Secondary Aims

2.2.1 To evaluate if the uptake of ^{64}Cu-DOTA-trastuzumab , a proposed indicator of responsiveness to HER2-directed therapy, correlates with SERPINA1 expression, which has been shown to be associated with ER+/HER2+ patient survival, both in the HER2+/ER+ patients and in all the patients.

2.2.2 To compare uptake of ^{64}Cu-DOTA-trastuzumab in the patients with complete pathologic response (P_{CR}) vs non-P_{CR} patients.

3.0 Research Design

This prospective clinical pilot trial will enroll 20 women with locally advanced HER2 positive breast cancers who are considered candidates for neoadjuvant chemotherapy. Patients will undergo ^{18}F-FDG/PET-CT and ^{64}Cu-DOTA-trastuzumab/PET-CT prior to initiation of therapy with trastuzumab + pertuzumab + chemotherapy. The objective is to examine, both at the patient and individual tumor levels, the relationship among tumor uptake of ^{18}F-FDG, ^{64}Cu-DOTA-trastuzumab, molecular markers, and response to neoadjuvant therapy.

4.0 Inclusion Criteria

Women with locally advanced HER2-positive breast cancers will be considered eligible for study participation if they meet the following criteria:

4.1. Participants must be women, 18 years or older, who have histological confirmation of HER2 positive breast cancer

4.2 The primary tumor must be $\geq$ 2.0 cm in size and/or have biopsy proven axillary nodes that are $\geq$ 2.0 cm in size by mammography, ultrasound, or MRI

4.3 The current cancer must over express HER2 as determined by IHC and/or FISH.

4.4 Patients may not have received prior HER2 directed therapies

4.5 Participants must have normal cardiac ejection fraction (per label, as defined as institutional normal)

4.6 Planned neoadjuvant therapy with six cycles of combined pertuzumab, trastuzumab and chemotherapy

4.7 Ability to provide informed consent

4.8 Negative Serum Pregnancy test

5.0 Exclusion Criteria

5.1 Participants who are not considered candidates for pertuzumab + trastuzumab + chemotherapy.

5.2 Concurrent malignancy other than non-melanoma skin cancer.

5.3 Patients must not have known metastatic disease

5.4 Patients must not have received prior treatment for the current breast cancer

5.5

6.0 Recruitment Process

6.1 Participants will be recruited by the treating Medical Oncologists from patients receiving breast cancer treatment at City of Hope and its community practice sites. All imaging will occur on the main Duarte campus.

7.0 Informed Consent Process

7.1 The Principal Investigator or IRB-approved named designate will explain to prospective enrollees the nature, duration, purpose of the study, potential risks, alternatives and potential benefits, and all other information contained in the informed consent document as well as their rights as a research subject (Experimental Subjects Bill of Rights) and the HIPAA research authorization form. Research participants will be informed that they may withdraw from the study at any time and for any reason without jeopardizing their future care, their employment at City of Hope or any relationship they have with City of Hope.. Should sufficient doubt be raised regarding the adequacy of comprehension, further clarifications will be made and the consent comprehension assessment may be repeated until a satisfactory result is obtained. Prospective research subjects who cannot adequately comprehend the fundamental aspects of the research study with a reasonable amount of discussion, education and proctoring will be ineligible for enrollment. Following this procedure, the protocol management team will review the results of eligibility testing and determine if the research subject is a candidate for study enrollment.

8.0 Study Procedures/Research Interventions

8.1 Use of PET imaging to predict response to neoadjuvant therapy

8.1.1 Twenty women with locally advanced breast cancer that is *HER2* positive by IHC and/or FISH will be recruited from the Medical Oncology and Surgical Clinics at City of Hope and its community network.

8.1.2 As patients will ultimately receive pertuzumab and trastuzumab-based therapy, a baseline cardiac ejection fraction, by MUGA or ECHO cardiogram, will be performed.

8.1.3 All participants will undergo a history and physical exam and radiographic staging workup with whole body ^{18}F-FDG/PET-CT.

8.1.4 All subjects will receive an IV dose of trastuzumab (45 mg) immediately before infusion of ^{64}Cu-DOTA-trastuzumab (≤15 mCi; protein dose 5 mg).

8.1.5 A PET scan of regions of interest will be performed 24 - after injection of ^{64}Cu-DOTA-trastuzumab. Systemic therapy may be initiated after completion of the ^{64}Cu-DOTA-trastuzumab PET-CT imaging. The regimen must include both pertuzumab and trastuzumab at approved doses. The chemotherapy utilized is up to the treating physician's discretion.

8.1.6 Participants will be observed for toxicity for one year.

8.1.7 Subjects will undergo surgery after 6 cycles of treatment.

8.2 Collecting and Processing of specimens: All specimens will be collected and processed in accordance with the study teams SOPs.

9.0 Study Calendar

	Baseline (-28 days)	Day -2	Day -1	Day 1 (+3 days)	Day 30 (post cold trastuzumab/ Cu-DOTA injection)	Day 1 of C2-C6 (+/- 3 days)	Prior to C5D1 (-14 days)	After C6 (+21-48 days)	Follow up for one year post Day -2 [m]
History and Physical [a]	x			x		x			
Staging ^{18}F-FDG/PET-CT	x								
Archival Tissue [b]	x								
Tumor Submission Biopsy [c]				x					
MUGA or Echocardiogram[d]	x						x		
Serum Pregnancy test [e]	x					x			
CBC	x			x		x			
CMP	x			x		x			
ctDNA[f]	x					x		x	
Coagulation Panel [g] ?				x					
IV (cold) Trastuzumab administration		x							
64Cu-DOTA-Trastuzumab administration		x							
64Cu-DOTA-Trastuzumab PET [h]			x						

MRI [i]	X						X		
Pertuzumab and trastuzumab containing regimen [j]				X (+3 days)		x			
Surgery								x	
Response Assessment								x	
Residual tumor submission (if any) [k]								x	
Toxicity Assessment [l]		x	x	x	X				

a. H&P may be completed by MD or NP listed on the protocol.
b. Archival tissue is required during baseline. Archival sample must be from untreated tissue and patients must not have received any chemotherapy after the tissue was collected.
c. Two tumor submission biopsies will be collected after the CuDOTA scan D1 (one sample from a “hot” area – meaning an area (in the breast or regional lymph node) that illuminates with CuDOTA on the scan; and one sample from a “cold” area – meaning an area (in the breast or regional lymph node) that does not illuminate with CuDOTA on the scan).
d. MUGA or cardioECHO will be done at baseline, prior to C5, and then q 3 months.
e. Serum pregnancy test will be collected during screening for all women of childbearing potential (see sect.14.3). At the discretion of the treating physician, pregnancy test will be repeated prior to each tx for women of childbearing potential.
f. 10 cc of ctDNA (liquid bx) will be collected immediately post-op in OR and prior to every cycle. ctDNA will be collected and processed per SOP of the study team.
g. PT w/ INR and PTT will be collected within 1 day (24 hours) days prior to bx.
h. ^{64}Cu-DOTA-Trastuzumab PET will be obtained 18-32 hrs post ^{64}Cu-DOTA-Trastuzumab administration (confirm w/ J. Bading, PhD).
i. MRI (of breast) will be performed at baseline (up to - 42 days) and prior to C5D1 (-14 days)
j. Pertuzumab and trastuzumab containing regimen will begin within 4 days after the completion of Day 1 ^{64}Cu-DOTA-Trastuzumab PET. The regimen will consist of Pertuzumab, trastuzumab, and a chemotherapy. Regimen will be given in accordance with sect. 10.3 of the protocol.
k. If residual cancer is found at the time of definitive surgery, any remaining tumor tissue will be handled in accordance to sect. 14.2 of the protocol.
l. Toxicities, AE’s/SAE’s, and conmeds will be assessed for 30 days post cold trastuzumab/Cu-DOTA injection.
m. After the completion of C6, follow-up visits/assessments will be performed according to COH SOC surgical and medical oncology schedules for one year from post Day-2.

PT w/ INR and PTT will be collected within 1 days (24 hours) prior to bx.

10.0 Protocol Drugs and Radiolabeled Imaging Agent

10.1 Trastuzumab (for "cold" trastuzumab infusions)

10.1.1 Drug description: Trastuzumab is a recombinant humanized monoclonal antibody that binds to the extracellular domain of the *HER2*. Trastuzumab is a sterile, white to pale yellow, preservative-free lyophilized powder for IV administration. The nominal content of each trastuzumab vial is 440mg trastuzumab, 440mg α,α-trehalose dehydrate, 9,9mg L-histidine HCl, 6.4mg L-histidine, and 1.8mg polysorbate 20, USP. Reconstitution with 20mL of the supplied Bacteriostatic Water for Injection (BWFI), USP, containing 1.1% benzyl alcohol as a preservative, yields a multi-dose solution containing 21mg/mL trastuzumab, at a pH of approximately 6.

10.1.2 Procurement of trastuzumab: Trastuzumab will be supplied free of charge as part of the study.

10.1.3 Storage/stability: Vials of trastuzumab are stable at 2-8°C (36-46° F) prior to reconstitution. Do not use beyond the expiration date stamped on the vial. A vial of trastuzumab reconstituted with BWFI, as supplied, is stable for 28 days after reconstitution when stored refrigerated at 2°-8°C (36°-46° F), and the solution is preserved for multiple uses. Discard any remaining multi-dose reconstituted solution after 28 days. If unpreserved Sterile Water for Injection (SWFI) (not supplied) is used, the reconstituted trastuzumab solution should be used immediately and any unused portion must be discarded. Do not freeze trastuzumab that has been reconstituted. The solution of trastuzumab for infusion diluted in polyvinylchloride or polyethylene bags containing 0.9% Sodium Chloride Injection, USP, may be stored at 2-8°C (36-46° F) for up to 24 hours.

10.1.4 Reconstitutions and administration

10.1.4.1. Reconstitution

10.4.1.1.1 The diluent provided has been formulated to maintain the stability and sterility of trastuzumab for up to 28 days. Other diluents have not been shown to contain effective preservatives for trastuzumab. Each vial of trastuzumab should be reconstituted with 20mL of BWFI, USP, 1.1% benzyl alcohol preserved as supplied, to yield a multi-dose solution containing 21mg/mL trastuzumab.

10.1.4.1.2 Immediately upon reconstitution with BWFI, the vial of trastuzumab must be labeled in the area marked "Do not use after:" with the future date that is 28 days from the date of reconstitution.

Note: When administering trastuzumab to a patient with a known hypersensitivity to benzyl alcohol, trastuzumab must be reconstituted with SWFI, and only one dose per trastuzumab vial should be used. Trastuzumab which has been reconstituted with SWFI must be used immediately and any unused portion must be discarded. Use of other reconstitution diluents should be avoided.

Shaking the reconstituted trastuzumab or causing excessive foaming during the addition of diluent may result in problems with dissolution and the amount of

trastuzumab that can be withdrawn from the vial. Use appropriate aseptic technique when performing the following reconstitution steps:

- Using a sterile syringe, slowly inject the 20mL of diluent into the vial containing the lyophilized cake of trastuzumab. The stream of diluent should be directed into the lyophilized cake.
- Swirl the vial gently to aid reconstitution. Trastuzumab may be sensitive to shear-induced stress, e.g., agitation or rapid expulsion from a syringe. Do not shake.
- Slight foaming of the product upon reconstitution is not unusual. Allow the vial to stand undisturbed for approximately 5 minutes. The solution should be essentially free of visible particulates, clear to slightly opalescent and colorless to pale yellow.

10.1.4.1.3 *"Cold dose" of trastuzumab*: The "cold" dose is prepared using **50ml NS**. The recommended 45 mg cold infusion dose of trastuzumab is administered intravenously over 15 minutes. The cold trastuzumab will be injected prior to the injection of ^{64}Cu-DOTA-trastuzumab.

10.1.5 Drug accountability: Trastuzumab will be purchased by the City of Hope Pharmacy, which will store and control the drug. It will be prepared for administration in the outpatient pharmacy.

10.1.6 Discard of unused agent: Unused trastuzumab will be discarded in the chemotherapy discard containers within the outpatient pharmacy.

10.1.7 Warnings and Contraindications

10.1.7.1 *Cardiotoxicity*: Administration of trastuzumab can result in the development of ventricular dysfunction and congestive heart failure. Signs and symptoms of cardiac dysfunction, such as dyspnea, increased cough, paroxysmal nocturnal dyspnea, peripheral edema, S3 gallop, or reduced ejection fraction have been observed in patients treated with trastuzumab. Congestive heart failure associated with trastuzumab therapy may be severe and has been associated with disabling cardiac failure, death, and mural thrombosis leading to stroke.

10.1.7.1 Hypersensitivity reactions including anaphylaxis; severe hypersensitivity reactions have been infrequently reported in patients treated with trastuzumab. Signs and symptoms include anaphylaxis, urticaria, bronchospasm, angioedema, and/or hypotension. In some cases, the reactions have been fatal. Trastuzumab infusion should be interrupted in all patients with severe hypersensitivity reactions. In the event of a hypersensitivity reaction, appropriate medical therapy should be administered, which may include epinephrine, corticosteroids, diphenhydramine, bronchodilators, and oxygen. Patients should be evaluated and carefully monitored until complete resolution of signs and symptoms.

10.1.7.3 *Infusion reactions*: In the post-marketing setting, rare occurrences of severe infusion reactions leading to fatal outcome

have been associated with the use of trastuzumab. In clinical trials, infusion reactions consisted of a symptom complex characterized by fever and chills, and on occasion included nausea, vomiting, pain, headache, dizziness, dyspnea, hypotension, rash, and asthenia. These reactions were usually mild to moderate in severity. However, in post-marketing reports, more severe adverse reactions to trastuzumab infusion were observed and included bronchospasm, hypoxia, and severe hypotension. These severe reactions were usually associated with the initial infusion of trastuzumab and generally occurred during or immediately following the infusion. However, the onset and clinical course were variable. Delayed post-infusion events with rapid clinical deterioration have also been reported. Rarely, severe infusion reactions culminated in death within hours or up to one week following an infusion.

10.7.1.4 *Pulmonary events*: Severe pulmonary events leading to death have been reported rarely with the use of trastuzumab in the post-marketing setting. Signs, symptoms, and clinical findings include dyspnea, pulmonary infiltrates, pleural effusions, non-cardiogenic pulmonary edema, pulmonary insufficiency and hypoxia, and acute respiratory distress syndrome. These events may or may not occur as a sequelae of infusion reactions. Patients with symptomatic intrinsic lung disease resulting in dyspnea at rest may be at greater risk of severe reactions.

10.1.7.5 Other severe events reported rarely in the post-marketing setting include pneumonitis and pulmonary fibrosis.

10.2 Preparation of ^{64}Cu-DOTA-trastuzumab

10.2.1 All procedures are performed as specified in FDA-approved IND #109971. Trastuzumab is purchased from the City of Hope pharmacy and conjugated with active ester of DOTA (1,4,7,10-tetraazadodecane-1,4,7,10-tetracetic acid) in the City of Hope biologics production facility (CBG) under cGMP compliant conditions. Each lot of trastuzumab-DOTA underwent testing for sterility, potency, purity and lack of pyrogenicity. Vialing of the conjugated materials will be done in City of Hope biologics production facility Fill and Finish area. Radiolabeling with ^{64}Cu will be carried out in the City of Hope Radiopharmacy under the direction of David Colcher, PhD. The ^{64}Cu will be purchased from the Mallinckrodt Institute of Radiology at the Washington University School of Medicine, which is preparing the radiolabel for clinical use. Labeling will be accomplished by incubating conjugated antibody with the ^{64}Cu for 45 minutes at 43°C, followed by a chase with DTPA and subsequent purification on a size exclusion preparative grade Superdex-200 column. Appropriate fractions will be pooled and filtered to make up the patient dose, which will be formulated with human serum albumin. Patients will be injected via a peripheral vein with ≤ 15mCi of ^{64}Cu-DOTA-trastuzumab. The total trastuzumab content per ^{64}Cu-DOTA-trastuzumab injected dose is less than 5 mg.

We have estimated radiation dose from ^{64}Cu-DOTA-trastuzumab based on our previous work with ^{64}Cu-DOTA-trastuzumab and ^{111}In-MxDTPA-trastuzumab.[19, 22] The study protocol also requires ^{18}F-fluorodeoxyglucose

(FDG)/PET-CT scans prior to ^{64}Cu-DOTA-trastuzumab/PET-CT. The following table compares that procedure with ^{18}F-FDG/PET-CT.

Organ/Tissue	Estimated Equivalent or Effective Dose (mSv)*			
	^{64}Cu-Trastuzumab (15 mCi)	**CT****	**^{64}Cu-Trastuzumab PET-CT**	**^{18}F-FDG/PET-CT** (15 mCi)
Heart wall	88	6	94	9
Spleen	55	6	61	9
Liver	65	6	71	9
Bladder wall	10	6	16	93
Kidneys	52	6	58	9
Red marrow	21	6	27	9
Other	12	6	18	9
Effective dose	15	6	21	14

* Values for ^{18}F-FDG and CT were obtained from Brix, 2005.[23]

**Low-dose CT procedure; 2 scans.

10.3 Trastuzumab + Pertuzumab + chemotherapy

Treatment will be administered in the outpatient setting. A number of treatment regimens have been approved for use in the neoadjuvant setting, all of which contain trastuzumab and pertuzumab. The chemotherapy regimen is at the discretion of the treating oncologist. The treatment regimen must include both trastuzumab and pertuzumab. Therapeutic doses will be prepared per package insert and toxicities will be managed per standard of care and/or physician discretion.

10.3.1 Trastuzumab may be administered weekly or every three weeks. The schedule is not dictated. (See additional warning 10.1.7)

10.3.1.1 For the three week schedule, the loading dose is 8 mg/kg/90 minutes followed at three week intervals by a maintenance dose of 6 mg/kg over 30 minutes.

10.3.1.2 With the weekly schedule, a loading dose of 4 mg/kg/90 minutes is administered followed by a weekly dose of 2 mg/kg/30 minutes.

10.3.2 Pertuzumab

A loading dose of pertuzumab 840 mg IV is administered over 60 minutes with the first cycle. Every 21 days thereafter the maintenance dose of pertuzumab is 420 mg IV. Patients are observed for fever, chills, and infusion related side effects for 1 hour following the loading dose and 30 minutes after each subsequent dose. If the patient experiences an infusion reaction, the infusion rate is decreased or discontinues until the patient's symptoms have resolved. The infusion is resumed at a rate 50% of the rate at the time of the infusion.

10.3.2 Chemotherapy

The chemotherapy regimen is not dictated by the protocol as long as the patients receive both trastuzumab and pertuzumab in the regimen.

11.0 Positron Emission Tomography (PET)

11.1 PET images will be acquired in 3D mode (septa retracted) and corrected for tissue attenuation based on co-registered CT acquired during the same examination. PET images will be reconstructed using an iterative algorithm (OSEM).

11.2 **^{18}F-Fluorodeoxyglucose (^{18}F-FDG)**: Standard ^{18}F-FDG PET-CT examinations will be performed prior to the ^{64}Cu-DOTA-trastuzumab PET-CT research procedure. Large-area (eyes to mid-thigh) PET-CT scans will be obtained beginning at 1 hour post-injection; time per bed position during the PET scan will be 2-3 minutes, depending on patient body habitus.

11.3 **^{64}Cu-DOTA-trastuzumab**: Patients will be injected via a peripheral vein with ≤ 15 mCi of ^{64}Cu-DOTA-trastuzumab. In order to allow the antibody to accumulate in tumor, PET-CT scanning of ^{64}Cu will be delayed until 18-24 hours post injection (Day 1). A second scan will be obtained 42-48 hours post injection (Day 2). Because of the limited amount of activity to be injected and the fact that only 18% of ^{64}Cu decays produce a positron, the count rates will be low. To compensate, time per bed position will be relatively long and the axial field of view relatively short. (Day 1: 1 or 2 bed positions encompassing known tumors; 30 minutes per bed position if 2 bed positions. Day 2: 1 or 2 bed positions, 60 or 30 minutes per bed position). Based on our prior clinical study, we know the scanning protocol defined above will yield adequate tumor visualization and signal-to-noise ratio in measurements of tumor uptake. For tumors at least 2 cm in diameter and average SUV (= tumor activity concentration/injected activity per unit body weight) of at least 3 in body regions for which tumor: background contrast is at least 4, the precision (coefficient of variation) of tumor SUV and tumor: background contrast measurements are expected to be about 10% and 15%, respectively.

12.0 Image Analysis

Tumor uptake of ^{18}F-FDG will be measured by Dr. Bading in terms of standardized uptake value (SUV = tumor activity concentration x patient body weight/injected activity decay-corrected to time of scan). Measurements will include maximum single-voxel SUV (SUV_{max}) within the tumor PET image. We will also evaluate tumor average SUV (SUV_{mean}) for ^{18}F-FDG within a 3D isocontour encompassing voxels with SUV $\geq$ 50% of SUV_{max}. For each FDG-positive tumor, a corresponding SUV for ^{64}Cu-DOTA-trastuzumab will be determined by applying the FDG volume of interest (VOI) to the CT-coregistered ^{64}Cu image set. We denote this metric of ^{64}Cu-DOTA-trastuzumab uptake as SUV_{mean}(FDG-matched). Tumor sizes (product of maximum mutually perpendicular transaxial diameters as well as maximum axial diameter) will be estimated from coregistered CT.

For ^{64}Cu, SUVs will also be evaluated in selected non-tumor organs and tissues [heart, blood (cardiac ventricles), liver, skeletal muscle]. PET quantification for ^{64}Cu will be validated by comparing image-derived measurements of blood activity concentrations from the Day 1 and Day 2 scans with direct assays performed on whole blood samples taken just prior to each of those scans.

13.0 Response Assessment

The response assessment is performed at the time of definitive surgery. Patients disease will be staged in the conventional manner according to the AJCC. The primary endpoint is complete pathologic response defined as no evidence of residual invasive cancer in either the breast or regional lymph nodes.

14.0 Laboratory Evaluations

14.1 **Biomarker assessment**: A biopsy will be performed to confirm the diagnosis of breast cancer and assessment of hormone receptors and HER2 status. If a biopsy has been performed at another institution, tumor block will be obtained for the pretreatment analyses. The pretreatment tumor analyses include:

14.1.1 RPPA (Performed at MD Anderson)

14.1.2 RNAseq (Cancer Center Core)

14.1.3 SERPINA (Dr. Shiuan Chen's Lab)

14.2 **Residual Disease** If there is residual cancer at the time of definitive surgery, tumor will be submitted for the following. In the event of a complete pathologic response, there will be no reassessment of tumor markers.

14.2.1 RPPA

14.2.2 RNAseq

14.2.3 SERPINA

14.3 **Pregnancy Tests:** WOCBP must have a negative serum or urine pregnancy test result within 0 to 72 hours prior to each dose of study drug.

14.3.1 WOCBP comprises women who have experienced menarche and who have not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation, or bilateral oophorectomy) or who are not post-menopausal (for purposes of this study, post-menopausal will be defined as women who have not had a menstrual period for a minimum of 12 consecutive months).

14.3.2 WOCBP must agree to use an acceptable method of contraception to avoid pregnancy throughout the study and for up to 4 weeks after the last dose of study drug in such a manner that the risk of pregnancy is minimized.

14.4

14.4 **CBC:** CBC w/ differential to be collected per study calendar (sect. 9)

14.5 **CMP:** to be collected per schedule of assessments (sect. 9)

15.0 Statistical Analysis

This is a pilot study of 20 patients to explore the tumor uptake of ^{64}Cu-DOTA-trastuzumab as measured by PET-CT in patients who are candidates for Her2-directed neoadjuvant therapy. In addition, we will obtain preliminary data on the role of the uptake and pathologic complete response from trastuzumab, pertuzumab-based chemotherapy. With 20 subjects, we can estimate the percent of tumors detected with a standard error of 11% or less, and describe the observed impact of tumor size on this sensitivity in this small pilot study. The secondary endpoints are exploratory endpoints in the context of this small pilot study.

Specifically, we are seeking to:

15.1 To evaluate the percentage of patients with a positive ^{64}Cu-DOTA-trastuzumab scan. If any tumors are missed, we will describe their size, lymph node involvement, and other biological correlatives. In addition, we will compare the SUVmax detected in previously untreated HER2+ breast cancer to the SUVmax detected in recurrent breast disease in our previous series on HER2+ patients with metastatic disease.

15.2 To evaluate if the SUV, a proposed indicator of responsiveness to HER2-directed therapy, correlates with SERPINA1 expression, which has been shown to be associated with ER+/HER2+ patient survival, both in the HER2+/ER+ patients and in all the patients.

15.3 To compare SUV in the P_{CR} patients vs non-P_{CR} patients.

The information gained from this pilot study will determine whether ^{64}Cu-DOTA-trastuzumab/PET-CT warrants further evaluation in the treatment of patients with locally advanced HER2 positive breast cancers. We anticipate that we will be able to prospectively identify patients who will not obtain a Pcr with pertuzumab and trastuzumab-based chemotherapy regimens. Subsequent studies will focus on ways to develop individualized treatments that address the "resistant population" within the cancer.

16.0 Toxicity Assessment

16.1 Patients will be monitored for acute reactions during and immediately after infusion of the trastuzumab imaging pre-dose and ^{64}Cu-DOTA-trastuzumab.

16.2 Participants will begin systemic therapy within 4 days after completion of ^{64}Cu-DOTA-trastuzumab/PET-CT. Side effects <u>not</u> known to be related to the chemotherapy regimen will be recorded.

n. **16.3** Toxicities, AE's, and conmeds will be assessed for 30 days post cold trastuzumab/Cu-DOTA injection.

17.0 <u>Data Management</u>

17.1 Methods used for data collection: All clinical data is captured from the patient's medical record.

17.2 Volunteer Identification: Participants identity will be linked to unique patient numbers (UPN).

17.3 Confidentiality: This research will be conducted in compliance with federal and state of California requirements relating to protected health information (PHI). The study will record individual imaging results and any side effects, and this will be linked to the subject's identity using a UPN. The Protocol Management Team (PMT) consisting of the PI (Joanne Mortimer), statistician (Paul Frankel PhD), and study nurses are eligible to review research records, but all information will be treated confidentially. No identifiers will be used in any publication of the study results.

17.4 Data Reporting

17.4.1 <u>Confidentiality of Records:</u> The original data collection forms will be stored at City of Hope in secure cabinets by Clinical Trials Office. . All records will be retained for a minimum of 2 years.

When results of this study are reported in medical journals or at meetings, identification of those taking part will be withheld. Medical records of patients will be maintained in strictest confidence, according to current legal requirements. However, they will be made available for review, as required by the FDA or other authorized users such as the NCI, under the guidelines established by the Federal Privacy Act.

17.4.2 <u>Patient Consent Form</u>: At the time of registration, the original signed and dated patient's Informed Consent with the Experimental Subject's Bill of Rights (for

the medical record) and 2 copies (for the patient, the research record,) must be available. All Institutional, NCI, Federal, and State of California regulations concerning the Informed Consent form will be fulfilled. ^{64}Cu-DOTA trastuzumab is administered under IND #109971, which is approved by and subject to the regulations of the FDA.

17.4.3 Data Collection Forms and Submission Schedule: All data will be collected using City of Hope Biostatistics data collection . (See Appendix D for a summary of the forms submission schedule.) . The original data collection forms will reside at City of Hope in a secure location.

17.4.3.1 The Eligibility Checklist must be completed by a protocol nurse or clinical research associate and signed by a participating investigator prior to registering the patient. See Appendix 1.

17.4.3.2 Within two weeks of registration, the clinical research associate will submit Prior Therapy forms (Prior Therapy Summary - COH1987 and supplemental forms, as necessary) and On-Study forms (On-Study Hematology/Other - COH2658 and supplemental forms, as necessary).

17.4.3.3 Within one week of imaging, the clinical research associate will submit the following forms:

- Protocol Treatment – Drug Agent (COH2698).
- Monitoring & Follow-Up Summary (COH2001).
- Adverse Events Collection Form (COH2000).

17.4.4 Results Reporting: City of Hope, as sponsor of IND #109971, will submit reports annually to the FDA within 60 days of the anniversary date that the IND went into effect (December 27, 2010) in accordance with 21 CFR 312.33.

17.5 Sharing results with participants: Information from the ^{64}Cu-DOTA trastuzumab PET-CT images will be shared with the patient and the treating physician if that information may impact the patient's medical care.

18.0 Data Safety Monitoring Plan

18.1 Definition of Risk Level

This is a Risk Level 4 study as defined in the City of Hope Institutional Data and Safety Monitoring Plan [policy dated 07/09/2014]. This determination was made because the study involves COH held IND imaging agent.

18.2 Monitoring and Personnel Responsible for Monitoring

The Protocol Management Team (PMT) is responsible for monitoring the data and safety of this study. The PMT consists of the Principal Investigator (PI), Biostatistician, Research Protocol Nurse, and Clinical Research Coordinator.

The PMT is required to submit periodic status reports (i.e., the PMT Report) according to the frequency prescribed in the City of Hope Institutional Data and Safety Monitoring Plan [policy dated 07/09/2014]. Important decisions made during PMT meetings (i.e., dose escalation, de-escalation, etc.) only need to be noted in the PMT Report submitted to the Data and Safety Monitoring Committee (DSMC).

18.3 Adverse Events and Serious Adverse Events

The PI will be responsible for determining the event name, assessing the severity (i.e., grade), expectedness, and attribution of all adverse events.

18.4 **Adverse Event (AE) -** An adverse event is any untoward medical experience or change of an existing condition that occurs during or after treatment, whether or not it is considered to be related to the protocol intervention.

18.5 **Reporting Non-serious Adverse Events –** Adverse events will be collected after the patient is given the study treatment or any study related procedures. Adverse events will be monitored by the PMT. Adverse events that do not meet the criteria of serious OR are not unanticipated problems will be reported only in the PMT Report.

18.6 **Serious Adverse Event (SAE)** [Modified from the definition of unexpected adverse drug experience in 21 CFR 312.32] - defined as *any expected* or *unexpected adverse event*s that result in any of the following outcomes:

- Death
- Is life-threatening experience (places the subject at immediate risk of death from the event as it occurred)
- Unplanned hospitalization (equal to or greater than 24 hours) or prolongation of existing hospitalization
- A persistent or significant disability/incapacity
- A congenital anomaly/birth defect
- Secondary malignancy
- Any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require

medical or surgical intervention to prevent one of the outcomes listed above (examples of such events include allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasias of convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse).

18.7 **Reporting Serious Adverse Events -** begins after study treatment or any study related procedures. All SAEs occurring during this study, whether observed by the physician, nurse, or reported by the patient, will be reported according to the approved City of Hope's Institutional policy [policy effective date: 05/14/14]. Serious Adverse Events that require expedited reporting will be submitted electronically using iRIS.

18.8 **Adverse Event Name and Severity**

The PI will determine the adverse event name and severity (grade) by using the CTCAE version 4.

18.9 **Expected Adverse Event -** Any event that does not meet the criteria for an unexpected event, OR is an expected natural progression of any underlying disease, disorder, condition, or predisposed risk factor of the research participant experiencing the adverse event.

18.10 **Unexpected Adverse Event [21 CFR 312.32 (a)] –** An adverse event is unexpected if it is not listed in the investigator's brochure and/or package insert; is not listed at the specificity or severity that has been observed; is not consistent with the risk information described in the protocol and/or consent; is not an expected natural progression of any underlying disease, disorder, condition, or predisposed risk factor of the research participant experiencing the adverse event.

18.11 **Adverse Event Attribution**

The following definitions will be used to determine the causality (attribution) of the event to the study agent or study procedure.

Definite - The AE is clearly related to the investigational agent or study procedure and unrelated to any other cause.

Probable - The AE is likely related to the investigational agent or study procedure and unlikely related to other cause(s).

Possible -The AE may be related to the investigational agent or study procedure and may be related to another cause(s).

Unlikely -The AE is doubtfully related to the investigational agent or study procedure and likely related to another cause(s).

Unrelated -The AE is clearly not related to the investigational agent or study procedure and is attributable to another cause(s).

18.12 **COH Held IND –**

Serious Adverse Events meeting the requirements for expedited reporting to the Food and Drug Administration (FDA), as defined in

21 CFR 312.32, will be reported as an IND safety report using the MedWatch Form FDA 3500A for Mandatory Reporting.

The criteria that require reporting using the Medwatch 3500A are:

- Any unexpected fatal or life threatening adverse experience associated with use of the drug must be reported to the FDA no later than 7 calendar days after initial receipt of the information [21 CFR 312.32(c)(2)]
- Any adverse experience associated with use of the drug that is both serious and unexpected must be submitted no later than 15 calendar days after initial receipt of the information [21 CFR 312.32(c)(1)]
- Any follow-up information to a study report shall be reported as soon as the relevant information becomes available. [21 CFR 312.32(d)(3)]

The PI or designee will be responsible for contacting the Office of IND Development and Regulatory Affairs (OIDRA) at COH to ensure prompt reporting of safety reports to the FDA. OIDRA will assist the PI with the preparation of the report and submit the report to the FDA in accordance with the approved City of Hope's Institutional policy [policy effective date: 05/14/14].

18.13 **Deviations and Unanticipated Problems**

Deviation - A deviation is a divergence from a specific element of a protocol that occurred without prior IRB approval. Investigators may deviate from the protocol to eliminate immediate hazard(s) for the protection, safety, and well-being of the study subjects without prior IRB approval. For any such deviation, the PI will notify the COH DSMC and IRB within 5 calendar days of its occurrence via iRIS in accordance with the Clinical Research Protocol Deviation policy [policy effective date: 11/07/11].

18.14 **Single Subject Exception (SSE)**

An SSE is a planned deviation, meaning that it involves circumstances in which the specific procedures called for in a protocol are not in the best interests of a specific patient. It is a deviation that is anticipated and receives prior approval by the PI and the IRB. The SSE must be submitted as a "Single Subject Exception Amendment Request" via iRIS in accordance with IRB guidelines and the Clinical Research Protocol Deviation policy [policy effective date: 11/07/11]. An IRB approved SSE does not need to be submitted as a deviation to the DSMC.

18.15 **Unanticipated Problem (UP) –** Any incident, experience, or outcome that **meets all three** of the following criteria:

1. Unexpected (in terms of nature, severity, or frequency) given the following: a) the research procedures described in the protocol-related documents such as the IRB approved research protocol, informed consent document or Investigator Brochure (IB); and b) the characteristics of the subject population being studied; **AND**
2. Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the

incident, experience, or outcomes may have been caused by the drugs, devices or procedures involved in the research); **AND**

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

Any UP that occurs during study conduct will be reported to the DSMC and IRB in accordance with the City of Hope's Institutional policy [policy effective date: 05/14/14] using iRIS.

18.16 **COH Held IND**

The Office of IND Development and Regulatory Affairs (OIDRA) will assist the PI in reporting the event to the Food and Drug Administration (FDA).

19.0 References

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