Study Title: 64Cu-DOTA-trastuzumab Positron Emission Tomography in Patients with Gastric Cancer

NCT01939275

Protocol dated 04/26/2017

CITY OF HOPE NATIONAL MEDICAL CENTER
1500 E. DUARTE ROAD
DUARTE, CA 91010

DEPARTMENT OF SURGERY

TITLE: ^{64}Cu-DOTA-trastuzumab Positron Emission Tomography in Patients with Gastric Cancer

CITY OF HOPE PROTOCOL NUMBER: 13229 VERSION: 11

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COH Amendment	Protocol Amendment dated 7/31/14	Version 02
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COH Amendment	PI Change dated 07/17/15	Version 04
COH Amendment	Protocol Amendment dated 09/03/15	Version 05
COH Amendment	Title Page dated 4/22/16 (Personnel Update)	Version 06
COH Amendment	Protocol Amendment dated 04/28/16	Version 07
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COH Amendment	Title Page Dated 12/27/2016	Version 09
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SPONSOR/IND NUMBER: Amending current IND
DISEASE SITE: Gastric Cancer
STAGE (if applicable): I-IV
MODALITY: Intravenous
PHASE/TYPE: Pilot

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STUDY SPONSOR AND MONITOR:	City of Hope

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STUDY SPONSOR AND MONITOR: City of Hope

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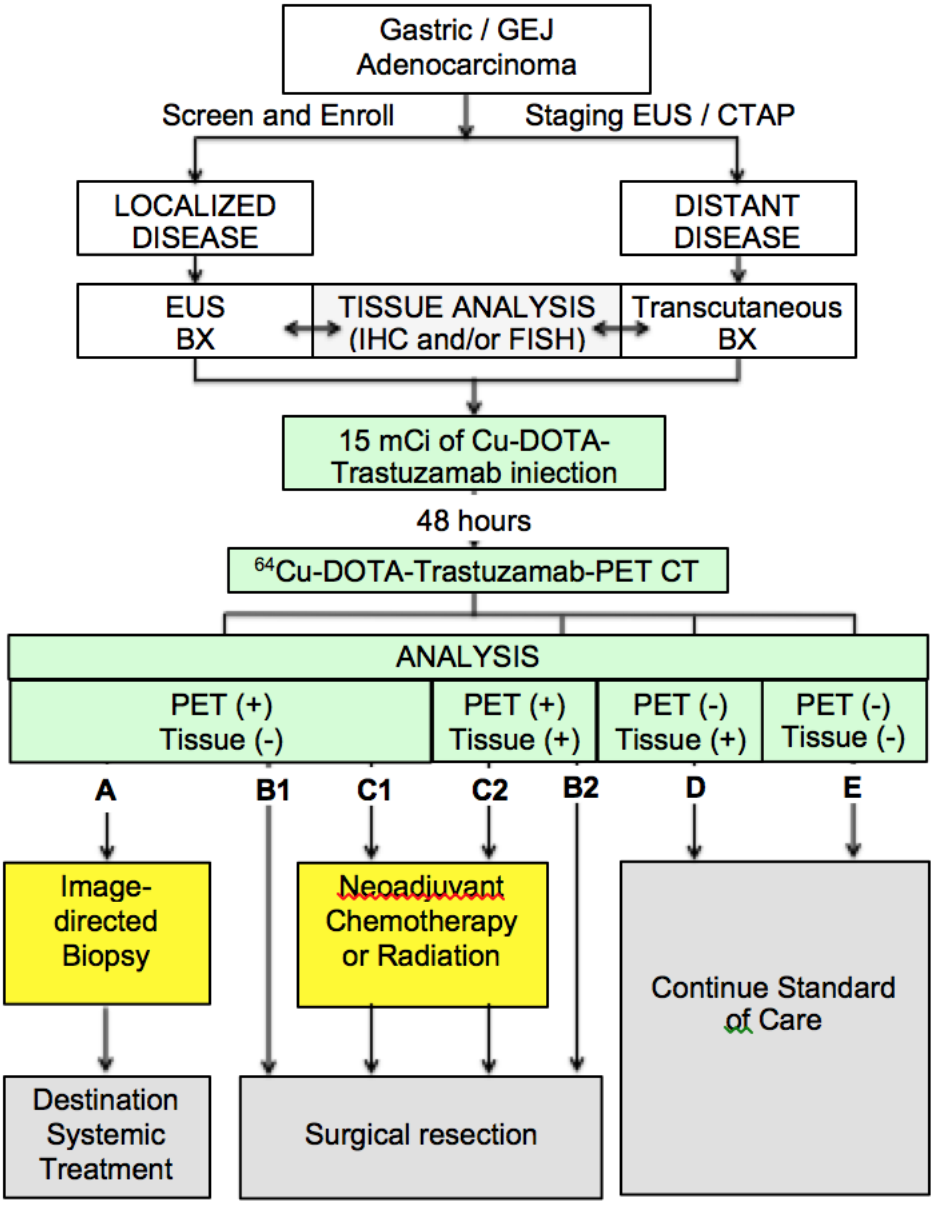
COORDINATING CENTER: N/A

Experimental Design Schema



Protocol Synopsis

Protocol Title:
^{64}Cu-DOTA-trastuzumab Positron Emission Tomography in Patients with Gastric Cancer
Brief Protocol Title for the Lay Public (if applicable):
Study Phase:
Pilot
Participating Sites:
City of Hope
Rationale for this Study:
Recently, in a Phase III randomized trial on patients with HER2 positive gastric cancer, trastuzumab was shown to significantly improve survival for patients with locally advanced or metastatic disease(1). Notably, the percent of HER2 positive disease in the literature ranges from ~7% to 34% depending on the clinical study and methodology used to assess HER2 status. Surgical blocks have established that, on a microscopic scale, gastric cancer is heterogeneous, making it more difficult to establish HER2 positivity when compared to breast cancer. This has resulted in recommendations ranging from the use of multiple blocks(2), to the use of bright-field methodologies(3) and larger tissue samples(4). Each of these approaches has limitations. Our research team has developed radiolabeled trastuzumab to study *HER-2/neu* positive breast cancers in animal models and in humans using PET, in part because trastuzumab has a high rate of internalization, which results in trapping of radiometals within the cell. We have used radiolabeled trastuzumab for PET imaging in women with advanced *HER2* positive invasive breast cancer and shown that tumor uptake of the radiotracer correlates with pathologic HER2 positivity/negativity. We hypothesize that ^{64}Cu-DOTA-trastuzumab PET can be used to improve assessment of HER2 status in gastric cancer by providing non-invasive, macroscopic measurements of average receptor expression (on a scale of approximately 4 cc). Our long term goal is to use functional imaging with ^{64}Cu-DOTA-trastuzumab PET to better predict which gastric patients will benefit from HER2-targeted therapy. This pilot study represents the first imaging of gastric cancer with ^{64}Cu-DOTA-trastuzumab PET. It will allow us (1) To compare tumor uptake of ^{64}Cu-DOTA-trastuzumab in gastric cancer patients with pathologic evaluation of tumor HER2/neu expression; and (2) To compare ^{64}Cu-DOTA-trastuzumab-PET scan with standard radiographic imaging for staging patients with gastric cancer.
Objectives:
1. To compare tumor uptake of ^{64}Cu-DOTA-trastuzumab in gastric cancer patients with pathologic evaluation of tumor HER2/neu expression **2.** To compare ^{64}Cu-DOTA-trastuzumab-PET-CT scan with standard radiographic imaging for staging patients with gastric cancer

Study Design:
Pilot trial: see study schema
Endpoints:
^{64}Cu-DOTA-trastuzumab-PET-CT image assessment; HER2/neu assessment by pathology
Sample Size:
22
Estimated Duration of the Study
35months
Summary of Subject Eligibility Criteria:
Inclusion Criteria 1.1.1 Histologic diagnosis of gastric or gastroesophageal junction adenocarcinoma (Two of the 22 enrolled patients must be HER2 3+ by IHC or FISH positive) 1.1.2 Either the primary tumor or at least one of the metastatic lesions must be >2cm 1.1.3 Normal cardiac ejection fraction 1.1.4 Ability to provide informed consent **Exclusion Criteria** 1.1.1 Impaired cardiac function as evidenced by ejection fraction below institutional normal limits 1.1.2 Uncontrolled hypertension (mmHg >160 systolic or >90 diastolic) 1.1.3 Participants who have reached trastuzumab within the prior 36 days 1.1.4 Patients who are pregnant
Investigational Product Dosage and Administration:
^{64}Cu -DOTA-trastuzumab
Clinical Observations and Tests to be Performed:
After the diagnosis and HER 2 status have been determined, additional tumor will be stored in pathology for future assessment of PI3K, PTEN, IGF1-R, EGFR, and phospho-S6 by immunohistochemical staining. The DNA Sequencing Core will assess mutations in PI3K and PTEN.
Statistical Considerations:
Sample Size Determination:

As most of the objectives can obtain sufficient information for a pilot study with fewer patients, we powered this study to insure sufficient number of HER2 positive patients by IHC to serve as a positive control.

Specifically, with 22 patients, there is an 80% chance we'd enroll at least one HER2 positive patient by IHC if the true incidence of HER2 positivity is the lowest end of the reported rate of 7%. If the incidence is 16% (5), we would expect to observe 2 or more HER2+ patients with 89% probability.

Additional analysis:

1. In breast cancer, ^{64}Cu-DOTA-trastuzumab-PET-CT) (Cu-PET-CT) was able to identify the tumors with low HER2 expression. Gastric cancer has different adjacent tissue to provide a background noise comparison. As a result, with 22 patients, we will be able to determine the percent of patients whose tumors image with Cu-PET-CT with a 95% CI half-width of 21%.
2. A subset of patients will have tumors of sufficient size to have Cu-PET-CT SUV variation over the tumor. For patients with metastatic disease who have Cu-PET-CT positivity but negative initial testing for HER2 expression, additional image-directed biopsy of the Cu-PET-CT positive area will be performed for analysis prior to their destination systemic treatments (Track A on schema). In those cases when subjects undergo surgical resection (Track B on schema), the, positive and negative areas of the tumor will be sent to pathology for confirmation. These will be exploratory.
3. A subset of patients will receive neoadjuvant chemotherapy or chemotherapy and radiation as part of their standard of care treatment as determined by the surgeon. Once their neoadjuvant treatment is complete, they will undergo resection and pathologic correlation with the Cu-PET-CT. (Track C on Schema).
4. Correlation of Cu-PET-CT SUV (measured as peak SUV) and pathology will be explored. As correlation requires a continuous measure from pathology, and some pathology will use FISH and/or IHC, this will be exploratory. Comparing Cu-PET-CT in positive cases versus negative by pathology will depend on the percent of patients deemed positive. This is an exploratory aim.
5. Patients who are deemed negative by pathology, but appear positive by Cu-PET-CT is exploratory, as further evaluation will be needed to determine positivity by Cu-PET-CT.

Sponsor/Licensee:
City of Hope
Case Report Forms
Medidata Rave Electronic Data Capture
Study Design

We will enroll patients with gastric adenocarcinoma diagnosed either with endoscopic biopsy of the primary tumor or transcutaneous biopsy of a metastatic focus. After consenting and enrollment, the patient will have ^{64}Cu-DOTA-trastuzumab-PET-CT scan prior to any treatment. If the ^{64}Cu-DOTA-trastuzumab-PET-CT scan is negative and it correlates with a negative HER2 evaluation of the biopsied tissue then utility of scan is verified (E on Schema). If the ^{64}Cu-DOTA-trastuzumab-PET-CT scan is positive and correlates with positive HER2 staining of biopsied tissues then, again the utility of scan is verified (C2 and B2 on Schema). If the scan is positive but the staining of HER2 is negative, the hypothesis is that this discrepancy is due to the heterogeneity of the tumor (A, B1, C1 on Schema). Therefore these patients will undergo scan directed biopsy or scan directed evaluation of the surgical specimen after resection.

Table of Contents

Abbreviations

Abbreviation	Meaning
AE	Adverse Event
CFR	Code of Federal Regulations
COH	City of Hope
CR	Complete Response
CRA	Clinical Research Associate
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
DCE	Dynamic Contrast Enhanced
DLT	Dose Limiting Toxicity
DSMC	Data Safety Monitoring Committee
DWI	Diffusion-weighted Image
FDA	Food and Drug Administration
GCP	Good Clinical Practice
IB	Investigator Brochure
ICF	Informed Consent Form
IDS	Investigational Drug Services
IND	Investigational New Drug
IRB	Institutional Review Board
MTD	Maximum Tolerated Dose
NCI	National Cancer Institute
PD	Progressive Disease
PI	Principal Investigator
PMT	Protocol Monitoring Team
PR	Partial Response
SAE	Serious Adverse Event
SD	Stable Disease

1. Goals and Objectives (Scientific Aims)

1. To compare tumor uptake of ^{64}Cu-DOTA-trastuzumab in gastric cancer patients with pathologic evaluation of tumor HER2/neu expression
2. To compare ^{64}Cu-DOTA-trastuzumab-PET-CT scan with standard radiographic imaging for staging patients with gastric cancer

2. Background

2.1 Introduction/Rationale for Development

Gastric cancer remains the 2nd most frequent cause of cancer-related death worldwide(6). Although the incidence of gastric cancer is relatively low in the United States, it is a major cause of cancer-related deaths in California because of the state's high numbers of Asian and East European immigrants(7). Despite multimodality treatment approaches, the prognosis of patients with advanced gastric cancers has remained quite poor with median rates of overall survival approximately 7 months. Targeted therapies have been identified and tested in many cancers; and recently, the evaluation of HER2/neu in gastric cancer has resulted in treatment advances. As a member of the epidermal growth factor receptor family, *HER-2/neu* (c-erb-B2, ERBB2) is a transmembrane tyrosine kinase receptor that plays a key role in growth factor signal transduction regulating cell growth, survival, and differentiation.(8) It has been shown to be overexpressed in 15-40% of tumors evaluated.(9)

Trastuzumab is a humanized IgG-1 antibody that binds to the ectodomain of *HER2*. When combined with chemotherapy, trastuzumab significantly improves the survival of patients with biopsy-proven *HER2* overexpression. In the recent trastuzumab in gastric cancer (TOGA) Phase III randomized trial, the addition of trastuzumab to standard chemotherapy was shown to significantly improve survival. *HER2* overexpression and candidacy for trastuzumab is defined as 3+ staining by immunohistochemistry (IHC) or gene amplification by fluorescence in situ hybridization (FISH).(1) The greatest benefit appeared to occur in patients with 3+ overexpression on IHC, leading to median overall survival rates close to 18 months. However, significant problems exist with *HER2*. Unlike breast cancers which are largely homogeneous, the task of identifying *HER2* expression in gastric cancers is more difficult because of tumor heterogeneity. Although *HER2* overexpression in the TOGA trial was reported to be 22% (consistent with breast cancer), our City of Hope institutional data shows a rate closer to 10-15%. Therefore, the diagnostic workup for *HER2* overexpression must be improved, so that we can determine whether patients are eligible to receive trastuzumab which improves survival in patients with advanced gastric cancer.

Interestingly, trastuzumab has been shown to be of benefit even in patients whose cancers are *HER2* negative by IHC and have low amplification of *HER2* when associated with chromosome 17 polysomy. Additionally, data from NSABP B31 trial suggest that select women with *HER2* negative breast cancer may benefit from trastuzumab therapy.(10) Finally, it is noted that when multiple measures of *HER2* are performed at different institutions, the discordance rate (positive versus negative) may be as high as 20%. Taken together, the current measures of *HER2* in clinical practice for patients with gastric cancer (and breast cancer) remain inaccurate which may preclude the administration of an effective targeted agent.

2.2 Overview of Proposed Study

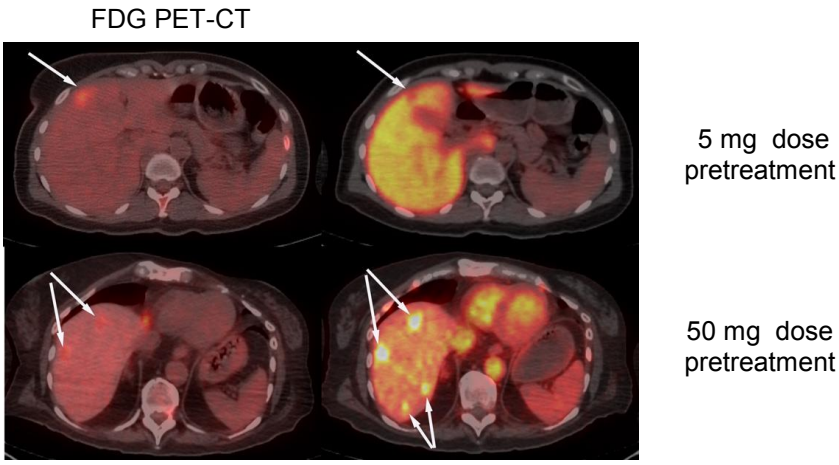
Positron emission tomography (PET) provides a non-invasive way of studying tumor metabolic functions and response to therapy. In gastric cancer, PET-CT imaging is used in staging patients to evaluate metastatic disease and to assess response to chemotherapy before tumor regression can be documented on

clinical or radiological exam.(11-13) Our research team has developed radiolabeled trastuzumab to study *HER-2/neu* positive breast cancers in animal models and in humans, in part because trastuzumab has a high rate of internalization, which results in trapping of radiometals within the cell.(14, 15) We have used radiolabled trastuzumab to image women with advanced *HER2* positive invasive breast cancer. ^{64}Cu-DOTA-trastuzumab, first developed at City of Hope, accurately identified *HER2* positive breast cancer in nude mice bearing an MCF7 *HER2* overexpressing xenograft.(16, 17) We hypothesize that functional imaging with ^{64}Cu-DOTA-trastuzumab PET-CT will improve the accuracy of *HER2* assessment in patients with gastric cancer.

An accurate assessment of *HER2* status is essential to select patients who would benefit the most from the targeted therapy with trastuzumab. However, tumor heterogeneity of *HER2* gene amplification has been reported between 5-53%.(4) Although other published studies report overexpression of *HER-2/neu* in 15-40% of gastric cancers, our institution has had a much lower *HER-2/neu* overexpression rate at approximately 10% in biopsy-proven gastric cancer. We hypothesize that imaging with ^{64}Cu-DOTA-trastuzumab-PET-CT can provide a non-invasive approach to more accurately evaluate *HER2* status in gastric cancer. Our proposed study is also highly innovative in three ways: (1) We will compare *HER2* expression in gastric cancers *in vivo* with ^{64}Cu-DOTA-trastuzumab-PET-CT; (2) the ^{64}Cu-DOTA-trastuzumab-PET-CT and the IHC and/or FISH expressions of HER2 will be correlated; (3) the possibility of ^{64}Cu-DOTA-trastuzumab-PET-CT directed identification of HER2 positive areas will be explored in both the biopsy of metastatic and resection of primary lesions; and (4) the effect of neoadjuvant treatment of gastric cancer on HER2 expression will be tested.

2.3 Human Studies

Trastuzumab, a recombinant humanized antibody that binds to the extracellular domain of HER2 protein, was conjugated to the active ester of DOTA (Macrocyclics). ^{64}Cu, provided by the Mallinckrodt Institute of Radiology, Washington University School of Medicine, was incubated with DOTA-conjugated antibody and purified on a size exclusion preparative column. Radiolabeled trastuzumab has been used safely and effectively to study *HER2* positive breast cancers in animal models and in humans. Researchers at City of Hope have utilized ^{111}In-DOTA-trastuzmab to image women with advanced *HER2* positive invasive breast cancer. In the City of Hope experience, ^{111}In-DOTA-trastuzmab identified areas of known metastasis in 4 of 7 patients; however, this agent has variable accuracy in identifying *HER2* positive disease on planar and SPECT scans. The positron emitting isotope ^{64}Cu has a number of potential advantages over ^{111}In including: improved image resolution with PET-CT and lower doses of radiation to the patient. ^{64}Cu-DOTA-trastuzumab, first developed at City of Hope, accurately identified *HER2* positive breast cancer in nude mice bearing an MCF7 *HER2* overexpressing xenograft. Because normal tissues that express *HER2* will bind the radioabeled (hot) antibody, administration of a “cold dose” of trastuzumab improved the tumor specificity of the radiographic image. Dr Joanne Mortimer (PI) recently completed a clinical trial with ^{64}Cu-DOTA-trastuzumab in patients with metastatic *HER2* positive breast cancer. The optimal dose of cold antibody required for ^{64}Cu-DOTA-trastuzumab PET-CT to produce a high image quality was determined to be 50 mg.



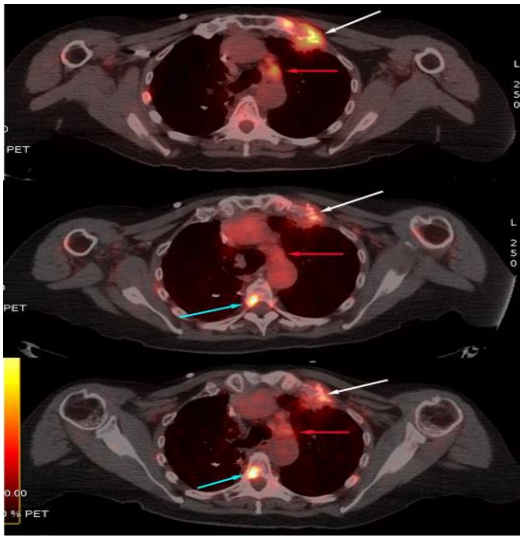
As ^{64}Cu-DOTA-trastuzumab is a radiolabeled conjugated ester analogue of trastuzumab, it is expected that it will have similar risks as the standard trastuzumab. Additional risks will include the radiation absorbed per dose (reference **Table A** for the estimated absorbed dose vs. the radiation from the standard PET-CT tracer molecule F18 Glucose [FDG] which has a low dose of radiation equivalent to a bone scan).

Table A: Estimated Radiation per Absorbed Dose

^{64}Cu Herceptin Absorbed Dose (AD) per 20 mCi injection		
Organ/Tissue	**AD (cGy)**	**STD (cGy)**
Heart Wall	17	2
Spleen	14	2
Liver	12	1
Kidneys	9.2	2
Red Marrow	3.8	0.2
Osteogenic cells	4.6	0.2
Other	$\leq$ 2.2	
Total Body	1.8	0.2

Currently, Dr. Mortimer is correlating tumor uptake of ^{64}Cu-DOTA-trastuzumab PET-CT with the degree of immunopositivity of *HER2* in women with metastatic disease. Patients with metastatic HER2+ breast cancer that had not received HER2 directed therapy in the last 4 months were considered eligible. The ^{64}Cu-DOTA-trastuzumab was infused intraveneously in 25 ml of saline over 10 minutes and patients were

watched closely for any adverse reactions. Eight patients were treated on study and tolerated it well without any unexpected adverse events. ^{64}Cu-DOTA-trastuzumab is a radiolabelled conjugate so any toxicities are expected from the trastuzumab. The estimated radiation dose was within the range of those established for radionuclear imaging procedures. The purpose of this study was to confirm the safety of this imaging technique which was shown. In addition as demonstrated in the following figure, FDG-PET-CT was able to visualize the rib lesion; however, the spinal metastasis was not obvious. With the ^{64}Cu-DOTA-trastuzumab PET-CT, the spinal lesion was clearly visible for up to 48 hours later.



^{18}FDG-PET

^{64}Cu-DOTA –Trastuzumab PET-CT
(at 23 h)

^{64}Cu-DOTA –Trastuzumab PET-CT
(at 48 h)

3. Patient Eligibility

3.1 Inclusion Criteria

3.1.1 Disease Status

Patient must have a histologic diagnosis of gastric or gastroesophageal junction adenocarcinoma. Two patients must be HER2 3+ by IHC or FISH positive

Either the primary tumor or at least one of the metastatic lesions must be ≥2cm

3.1.2 Age Criteria

Patients must be ≥ 18 years old

ECOG performance status of 0-2

Life expectancy of ≥ 3 months

3.1.3 Child Bearing Potential

Women of childbearing potential must have a negative serum pregnancy test within 14 days of ^{64}Cu-DOTA administrations and must have agreed to use an effective contraceptive method

The effects of ^{64}Cu-DOTA on the developing fetus are unknown. For this reason, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control or abstinence} prior to study entry and for four months following duration of study participation. Should a woman become pregnant or suspect that she is pregnant while participating on the trial, she should inform her treating physician immediately.

3.1.4 Protocol-Specific Criteria

CT/MRI scan must be obtained within 4 weeks prior to study entry

Patients must have normal cardiac ejection fraction

3.1.5 Informed Consent/Assent

All subjects must have the ability to understand and the willingness to sign a written informed consent.

3.1.6 For patients that have received prior therapy

All toxicities should recover to grade 0 or 1 prior to day 1.

3.2 Exclusion Criteria

3.2.1 Study-Specific Exclusion

Impaired cardiac function including any one of the following:

Complete left bundle branch block or use of a permanent cardiac pacemaker, congenital long QT syndrome, presence of ventricular tachyarrhythmias, clinically significant resting bradycardia (<50 beats per minute), QTcF > 450 msec on screening ECG, or right bundle branch block + left anterior hemiblock (bifascicular block)

Presence of atrial fibrillation

Previous history angina pectoris or acute MI within 6 months

Congestive heart failure (New York Heart Association functional classification III-IV)

Uncontrolled hypertension (mmHg >160 systolic or >90 diastolic)

Patients should not have active infections or concurrent neoplastic disease except for skin cancer.

Patients may not be receiving any other investigational agents

At the time of enrollment, patients may not have received any biological, chemotherapy, or radiation therapy.

Patients may not have received trastuzumab within the prior 6 months for any other reason.

Patients who are pregnant

3.2.2 Non-Compliance

Subjects, who in the opinion of the investigator, may not be able to comply with the safety monitoring requirements of the study.

3.3 Inclusion of Women and Minorities

The study is open to anyone regardless of gender or ethnicity. Efforts will be made to extend the accrual to a representative population, but in a trial which will accrue approximately 20 subjects, a balance must be struck between subject safety considerations and limitations on the number of individuals exposed to potentially toxic or ineffective treatments on the one hand and the need to explore gender, racial, and ethnic aspects of clinical research on the other. If differences in outcome that correlate to gender, racial, or ethnic identity are noted, accrual may be expanded or additional studies may be performed to investigate those differences more fully.

4. Screening and Registration Procedures

4.1 Screening Procedures

Diagnostic or laboratory studies performed exclusively to determine eligibility for this trial will be done only after obtaining written informed consent. Studies or procedures that were for clinical indications (not exclusively to determine study eligibility) may be used for baseline values, even if the studies were done before informed consent was obtained. Reference is made to Section 10.0 – Study Calendar.

4.2 Informed Consent

The investigational nature and objectives of the trial, the procedures and treatments involved and their attendant risks and discomforts, and potential alternative therapies will be carefully explained to the subject and a signed informed consent will be obtained. Documentation of informed consent for screening will be maintained in the subject's research chart and medical record.

4.3 Registration Requirements/Process

To register a patient, the treating physician should contact the protocol nurse or the responsible Clinical Research Associate (CRA) in Clinical Trial Office (CTO) to complete the eligibility/registration form. The protocol nurse or CRA will contact the Data Coordinating Center at the City of Hope (626-256-4673, ext. 64267 or e-mail dcc@coh.org), EMAIL a copy of the completed eligibility checklist, required pre-study tests (per protocol – and may include laboratory, CT and pathology reports), signed Informed Consent, signed Patients' Bill of Rights and HIPAA authorization form to dcc@coh.org.

The patient registration process will be handled by the Department of Clinical Research Information Support (CRIS) Data Coordinating Center (DCC) at City of Hope. Documentation of current IRB approval must be on file with the DCC prior to registration of patients on this study for participating institution.

The steps below are to be taken when registering a **patient:**

- The research staff must assure they have the most current and updated version of the protocol and informed consent prior to enrolling a patient. If a question arises, please contact the Data Coordinating Center at 626-256-4673 extension 64267 or via email at dcc@coh.org.
- The study staff must assure that all prestudy laboratory tests, scans and x-rays have been completed prior to registration according to the study calendar
- The study staff must assure that the patient has signed an approved informed consent prior to registration/randomization, including the Experimental Subject Bill of Rights and appropriate HIPAA authorization.

- The study staff must confirm that the patient meets all inclusion and exclusion eligibility criteria for the protocol. The eligibility checklist (provided by the COH DCC) must be completed in its entirety.
- Patients must be registered prior to initiation of treatment but no more than 5 working days prior to planned start of treatment. A patient failing to meet all protocol requirements may not be registered.
- Once a patient is eligible, all the pre-study requirements have been fulfilled, and the informed consent obtained, the research nurse or the data manager (study coordinator) will inform the COH Data Coordinating Center at (626) 256-4673, extension 64267; email dcc@coh.org and FAX (fax number 626 256-8794) a copy of the patient's signed informed consent, , completed eligibility checklist and corresponding source documentation confirming eligibility (including pathology reports, lab reports, x-ray reports, etc.).

The City of Hope Data Coordinating Center will:

- Review all materials/source documentation to ensure the patient is eligible.
- Ensure the consent form is valid and is signed correctly by all parties. If additional information is needed or should there be any questions, the Data Coordinating Center will immediately contact the study staff and registration will not occur until all issues are resolved.
- If there are questions regarding exceptions to the eligibility criteria, please contact he study Principal Investigator, as well as the COH DCC. Documentation of IRB approval of exception will need to be submitted as well as the COH DCC.
- Confirmation of Registration will be emailed/faxed to the study staff noting the patient's study number within 24 hours post receipt of a complete eligibility packet.
- The COH DCC will call the research nurse or data manager (study coordinator) and verbally confirm the registration (if needed).
- If the patient does not receive protocol therapy following registration, the patient's registration on the study may be cancelled. The COH DCC should be notified of cancellations as soon as possible.

5. Treatment Program

5.1 Treatment Overview

Patients with histology confirmed gastric or gastroesophageal junction adenocarcinoma are eligible for the study. Informed consent will be obtained and patients screened. If eligibility criteria are met, patient will be enrolled in the trial and undergo ^{64}Cu -DOTA-trastuzumab PET CT scan.

Treatment Plan

Patients will receive 45 mg trastuzumab over 15 minutes prior to the administration of ^{64}Cu -DOTA-trastuzumab, which includes 5 mg of trastuzumab. See section 8.1 for information regarding preparation of ^{64}Cu -DOTA-trastuzumab. Imaging will be performed on a GE Discovery 16 Ste PET-CT scanner (axial field of view 15.4 cm). PET-CT images will be performed in 3D mode (septa retracted) and corrected for tissue attenuation based on co-registered CT acquired during the same examination. PET-CT images will be reconstructed with spatial resolution of approximately 9 mm full-width-at-half maximum (FWHM) using an iterative algorithm (OSEM). Patients will be injected via a peripheral vein with 15

mCi of ^{64}Cu-DOTA-trastuzumab. PET-CT scanning of ^{64}Cu will be peformed between 24 and 48 hours post injection (Day 2 or Day 3). At 24 hours, the concentration of ^{64}Cu-DOTA-trastuzumab will allow for whole body PET-CT imaging within the same amount of time required to image the abdomen and pelvis at 48 hours due to the half life of the radiotracer. PET-CT images will be compared to standard MRI or CT scans.

5.2 Planned Duration of Therapy

This is a nontherapeutic study and patients will be on study for the duration of screening and then PET-CT imaging with ^{64}Cu -DOTA-trastuzumab. If a patient experiences any adverse events related to the administration of ^{64}Cu -DOTA-trastuzumab, their medical condition will continue to be followed until resolution of the adverse effects. Participants requiring additional study related treatment such as the image directed transcutanteous biopsy of the area of PET and IHC/FISH discordance will continued to be monitored as part of the study until first postprocedure follow-up or until any complications from the study related procedures are resolved. Those who undergo surgical resection will be followed until the first postoperative clinic appointment or until any complications from the study related procedures are resolved.

5.3 Criteria for Removal from Treatment

This is a nontherapeutic trial. Patients will be removed from the trial if they experience an infusion reaction or are unable to comply with the research visits.

5.4 Subject Follow-Up

There is no formal follow-up after 15 days. Progress notes on the patient's condition with standard therapy may be collected for data up to 6 months after the completion of the trial.

5.5 Supportive Care, Other Concomitant Therapy, Prohibited Medications

Patients will be instructed not to take any additional medications (including over-the-counter products) during the course of the study without prior consultation with the investigator. At each visit, the investigator will ask the patient about any new medications he/she is or has taken after the start of the study drug.

All concomitant medications/Significant non-drug therapies taken ≤ 30 days prior to start and after start of study drug, including physical therapy and blood transfusions, should be recorded.

The following restrictions apply during the entire duration of the study (Days 1-15):

No other investigational therapy should be given to patients. The patient may proceed with therapeutic treatment after day 15.

No anticancer agents should be given to patients. If such agents are required for a patient then the patient must first be withdrawn from the study.

5.6 Additional Studies

5.6.1 Laboratory Studies

Prestudy laboratory tests to determine eligibility should be done within 30 days of day 1 on protocol.

6. Dose Delays/Modifications for Adverse Events

N/A

7. Data and Safety Monitoring

7.1 Definition of Risk Level

This is a Risk Level 4 study, as defined in the "City of Hope Data and Safety Monitoring Plan", http://www.coh.org/dsmc/Pages/forms-and-procedures.aspx involving COH as IND holder and the first-in-human use in gastric patients of ^{64}Cu-DOTA.

7.2 Monitoring and Personnel Responsible for Monitoring

The Protocol Management Team (PMT) consisting of the PI, Collaborating Investigator, CRA/protocol nurse, and statistician is responsible for monitoring the data and safety of this study, including implementation of the stopping rules for safety and efficacy.

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).

Table 1: City of Hope PMT Reporting Timelines for the DSMC

Risk Level	Phase	Standard Reporting Requirement
RL 1, RL2, and Expanded Access Studies	No reports required	
3	I	Every 3 months from activation date, as indicated in MIDAS
3	Pilot, Feasibility, II-IV	Every 6 months from activation date, as indicated in MIDAS
4	Pilot, Feasibility, I-IV	Every 3 months from activation date, as indicated in MIDAS

Data and safety will be reported to the COH DSMC using the PMT report and submitted quarterly from the anniversary date of activation. Protocol specific data collection will include the following items: any UPs or AEs that occur on this study along with any treatment related deaths.

7.3 Definitions

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.

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.

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.

Serious Adverse Event (SAE) [21 CFR 312.32] - defined as *any expected or unexpected adverse event* that results in any of the following outcomes:

- Death
- Is life-threatening experiences (places the subject at immediate risk of death from the event as it occurred)
- Unplanned hospitalization equal 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).

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

1. Unexpected (in term 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.

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).

The PI will be responsible for determining the event name, assessing the severity (grade), expectedness, and attribution of all adverse events using the CTCAE version 4.

7.4 Reporting of Unanticipated Problems and Adverse Events

Unanticipated Problems - Most unanticipated problems must be reported to the COH DSMC and IRB **within 5 calendar days** according to definitions and guidelines at http://www.coh.org/policy/Policies%20and%20Procedures/REVIEWING_AND_REPORTING_UNANTICIPATED_PROBLEMS.pdf. Any unanticipated problem that occurs during the study conduct will be reported to the DSMC and IRB by submitting electronically in iRIS (http://iris.coh.org).

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 definitions and guidelines at http://www.coh.org/policy/Policies%20and%20Procedures/REVIEWING_AND_REPORTING_UNANTICIPATED_PROBLEMS.pdf and Table 2 below. Those SAEs that require expedited reporting will be submitted electronically in iRIS (http://iris.coh.org).

Adverse Events - 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 continuation reports and PMT reports (see Table 2 below).

Table 2: City of Hope Adverse Event and Unanticipated Problem Reporting Timelines for the DSMC and IRB

DSMC Risk Level 3 and Risk Level 4 Protocol Reporting Timelines

Required Reporting Timeframe to the DSMC		
Attribution	**Unexpected**	**Expected**
Death while on active treatment or within 30 days of last day of treatment		
Possibly, Probably, Definitely	5 calendar days	
Unlikely, Unrelated		
Death after 30 days of last active treatment/therapy		
Possibly, Probably, Definitely	5 calendar days	No reporting required
Unlikely, Unrelated	No reporting required	No reporting required
Within 30 days of last active treatment/therapy		
	Grades 3 and 4 AND meeting the definition of "serious"	
Possibly, Probably, Definitely	5 calendar days	10 calendar days
Unlikely, Unrelated	5 calendar days	10 calendar days
	Grades 1 and 2 AND resulting in "hospitalization"	
Possibly, Probably, Definitely	10 calendar days	10 calendar days

Required Reporting Timeframe to the DSMC		
Attribution	**Unexpected**	**Expected**
Unlikely, Unrelated	10 calendar days	10 calendar days
After 30 days of last active treatment/therapy		
	Grades 3 and 4 AND meeting the definition of "serious"	
Possibly, Probably, Definitely	10 calendar days	10 calendar days
Unlikely, Unrelated	No reporting required	No reporting required
	Grades 1 and 2 AND resulting in "hospitalization"	
Possibly, Probably, Definitely	10 calendar days	10 calendar days
Unlikely, Unrelated	No reporting required	No reporting required

DSMC Risk Level 1 and Risk Level 2 Protocol Reporting Timelines

Required Reporting Timeframe to DSMC		
Attribution	**Unexpected**	**Expected**
	Death while on active treatment or within 30 days of last day of treatment	
Possibly, Probably, Definitely	5 calendar days	5 calendar days
Unlikely, Unrelated	No reporting required	No reporting required
	Death after 30 days of last active treatment/therapy	
Possibly, Probably, Definitely	5 calendar days	No reporting required
Unlikely, Unrelated	No reporting required	No reporting required
	Grades 3 and 4 AND meeting the definition of "serious"	
Possibly, Probably, Definitely	5 calendar days	10 calendar days
Unlikely, Unrelated	No reporting required	No reporting required
	Grades 1 and 2 AND resulting in "hospitalization"	
Possibly, Probably, Definitely	5 calendar days	10 calendar days
Unlikely, Unrelated	No reporting required	No reporting required

COH IRB Adverse Event Reporting Timelines

Required Reporting Timeframe to COH IRB		
Attribution	**Unexpected**	**Expected**
	Death while on active treatment/therapy or within 30 days of the last day of active treatment/therapy	
Possibly, Probably, Definitely	5 calendar days[1]	Annual
Unlikely, Unrelated	Annual	Annual
	Grades 3 and 4	
Possibly, Probably, Definitely	5 calendar days[1]	Annual
Unlikely, Unrelated	Annual	Annual
	Grade 1 and 2	
Possibly, Probably, Definitely	5 calendar days[1]	Annual[2]
Unlikely, Unrelated	Annual[2]	Annual[2]

[1] These events must be reported in the time frame if they meet the definition of an unanticipated problem.

[2] For studies that are not first in human, Phase I and first in pediatric trials, only grades 3-5 must be reported at annual review.

ADDITIONAL REPORTING REQUIREMENTS

SAEs meeting the requirements for expedited reporting to the 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 which can found at:
http://www.fda.gov/Safety/MedWatch/HowToReport/DownloadForms/default.htm

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 following:

- 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)]

8. Agent Information and Risks

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

8.1.1 Description

Trastuzumab will be 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 COH biologics production facility (CBG) under cGMP compliant conditions. Each lot of trastuzumab-DOTA will undergo testing for sterility, potency, purity and lack of pyrogenicity. Vialing of the conjugated materials will be done in COH biologics production facility Fill and Finish area. An IND application will be filed with the FDA. 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 under a separate NCI grant. Labeling will be accomplished by incubating conjugated antibody with the ^{64}Cu for 45 minutes at 43^{o} 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. Protein dose to the patient will be approximately 50 mg. The total trastuzumab content per ^{64}Cu -DOTA-trastuzumab injected dose is less than 50 mg. All procedures will be done as specified in an IND that will be filed with the FDA to cover the studies proposed in the protocol. Based on our previous work with ^{111}In-DOTA-trastuzumab and anticipating that the normal tissue biodistribution of ^{64}Cu -DOTA-trastuzumab will be the same as that of ^{111}In-DOTA-trastuzumab, we calculate that the radiation doses to normal tissues will be:

^{64}Cu Herceptin Absorbed Dose (AD)* per 15 mCi injection estimated from ^{111}In-DOTA-trastuzumab in 8 patients (18)	
Organ/Tissue	**AD (cGy)**
Heart Wall	12 ± 1
Spleen	10 ± 3
Liver	9 ± 1
Kidneys	7 ± 1
Red Marrow	2.8 ± 0.3
Osteogenic cells	3.5 ± 0.5
Other	≤ 3.5
Total Body	1.4 ± 0.1

* Values are mean ± standard deviation.

8.2 Herceptin

8.2.1 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 intravenous (IV) administration. The nominal content of each trastuzumab vial is 440 mg trastuzumab, 440 mg α,α-trehalose dehydrate, 9,9 mg L-histidine HCl, 6.4 mg L-histidine, and 1.8 mg polysorbate 20, USP. Reconstitution with 20 mL of the supplied Bacteriostatic Water for Injection (BWFI), USP, containing 1.2% benzyl alcohol as a preservative, yields a multi-dose solution containing 21 mg/mL trastuzumab, at a pH of approximately 6.

8.2.2 Pharmacology – Handling, Storage, Dispensing and Disposal

Storage/stability: Vials of trastuzumab are stable at 2°-8° C (36°-46° F) prior to reconstitution. They will not be used 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 use. We will 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. We will 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 at room temperature 2°-25° C. However, because diluted trastuzumab contains no effective preservative, the reconstituted and diluted solution should be stored refrigerated 2°-8° C.

8.2.3 Reconstitutions and administration

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 20 mL of BWFI, USP, 1.1% benzyl alcohol preserved as supplied, to yield a multi-dose solution containing 21 mg/mL trastuzumab. 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 20 mL 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.

Administration

- The cold trastuzumab will injected prior to the injection of ^{64}Cu -DOTA-trastuzumab.

9. Correlative/Special Studies

Specimen Collection / Documentation

Antibody assessment will be performed in the laboratory of Dr. Colcher. Left over serum from the antibody assessment will be stored in the Translational Research Core of the Cancer Center for future research. After the diagnosis and HER 2 status have been determined, additional tumor will be stored in pathology for future assessment of PI3K, PTEN, IGF1-R, EGFR, and phospho-S6 by immunohistochemical staining. The DNA Sequencing Core will assess mutations in PI3K and PTEN. Both IHC and FISH assessment of HER2 status will be performed by both IHC and FISH. Left over tumor will be stored in pathology for future analysis.

10. Study Calendar

FOR ALL PATIENTS	Pre-Study	Day 1	Day 2 or 3		Within one month
Informed consent	R				
Demographics	R				
Medical history	R				
Concurrent meds	R				
Pretreatment Biopsy of Tumor	R				
Staging EUS	S				
Staging CT AP Chest	S				
"Cold" Trastuzumab		R			
^{64}Cu -DOTA-trastuzumab [a]		R			
^{64}Cu -DOTA-trastuzumab PET/CT			R		
Antibodies to DOTA					R
Cardiac ECHO[c]	R				
Start Neoadjuvant Treatment*					*S
Surgical Resection*					*S
Correlation analysis					R
Image directed biopsy of tumor[d]					R
DATA COLLECTION					
Physical exam	S	S			S
Vital signs	S	S	S		S
Height	S				S
Weight	S				S
Performance Status	S	S			S
CBC w/diff. Platelets[b]	S	S	S		S
Serum chemistry[b]	S	S	S		S
B-HCG[e]	S				
Diagnostic Pathology	S				

HER2 Testing on tumor	S				R
Adverse event evaluation	R	R			

a: Dose as assigned
b: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, phosphorus, potassium, total protein, SGOT[AST], SGPT[ALT], sodium.
c: Cardiac ECHO will be done prestudy
d. More frequent evaluations and laboratory tests will be done at the discretion of the treating physician.
e: Serum pregnancy test (women of childbearing potential).
*: For certain patients, surgery upfront will be standard; others will require neoadjuvant treatment prior to surgery. The initial treatment whether surgery or neoadjuvant chemotherapy will receive their planned treatment as determined by the surgeon or medical oncologist within the month after enrollment in the study
R: Represents tests that are done purely for research purposes
S: Indicates tests that are done for standard of care. If these tests are done for standard of care purposes, the data from these tests will be collected, however they are not required for research purposes. If they are not done, it will not exclude the patient from participation on the trial, and will not constitute a protocol deviation.

11. Endpoint Evaluation Criteria/Measurement of Effect

11.1 Response Criteria

The PET-CT examinations will be analyzed and interpreted by the City of Hope Image Response Assessment Team (IRAT). Tumors will be selected for analysis on the basis of the pretreatment CT examination and pathological analysis of the surgical specimen. The $^{Cu}64$ SUVs will be evaluated in tumors, adjacent non-tumor tissue and selected non-tumor organs and tissues (heart, extracardiac mediastinum, liver, skeletal muscle). Tumor sizes (product of maximum mutually perpendicular transaxial diameters as well as maximum axial diameter) will be estimated from coregistered CT. Tumor uptake of ^{64}Cu-DOTA-trastuzumab will be parameterized in terms of single-voxel maximum values SUVmax and whole-tumor volumes of interest (SUVwhtum) as defined from the coregistered CT images. Ratios of tumor to non-tumor activity concentration will also be calculated for adjacent tissue, extracardiac mediastinum, liver, and skeletal muscle. Receiver-operator curve (ROC) analysis will be performed to estimate optimal cutoff values of SUVmax, SUVwhtum, tumor:background and tumor:organ ratios for classifying tumors as "HER2 positive" or "HER2 negative." The optimal cold antibody dose required for ^{64}Cu -DOTA-trastuzumab to produce high quality images was determined to be 45 mg.

12. Data Reporting/Protocol Deviations

12.1 Data Reporting

12.1.1 Confidentiality and Storage of Records

The original data collection forms will be submitted into electronic data capture (EDC) system Medidata Rave. Data will be stored in a encrypted, password protected, secure computers that meet all the HIPAA requirements. When results of this study are reported in medical journals or at meetings, identification of those taking part will not be disclosed. Medical records of subjects will be securely maintained in the strictest confidence, according to current legal requirements. They will be made available for review, as required by the FDA, HHS, or other authorized users such as the NCI, under the guidelines established by the Federal Privacy Act and rules for the protection of human subjects.

12.1.2 Subject Consent Form

At the time of registration, the original signed and dated Informed Consent form, HIPAA research authorization form, and the California Experimental Subject's Bill of Rights (for the medical record) and three copies (for the subject, the research record, and the Coordinating Center) must be available. All Institutional, NCI, Federal, and State of California requirements will be fulfilled.

12.1.3 Data Collection Forms and Submission Schedule

All data will be collected on the case report forms in Medidata Rave. The CRFs will be submitted within 1 week from baseline, completion of each treatment and each subsequent follow-up visit. Data will be sent to the location identified in Section 12.1.1 and stored in a secure location.

12.1.3.1Eligibility Checklist

The Eligibility Checklist must be completed by a protocol nurse or clinical research associate and signed by an authorized investigator prior to registering the subject. See Section 4.3 for the registration procedure.

12.1.3.2Prior Therapy Forms and On-Study Forms

Within one week of registration, the clinical research associate will submit baseline Case Report Forms to Medidata EDC.

12.2 Protocol Deviations

12.2.1 Deviation Policy

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].

This protocol will be conducted in accordance with COH's "Clinical Research Protocol Deviation Policy" located at http://www.coh.org/dsmc/Documents/Institutional%20Deviation%20Policy.pdf.

Deviations from the written protocol that could increase patient risk or alter protocol integrity require prior IRB approval of a single subject exception (SSE) request. In addition, if contractually obligated, the sponsor must also approve the deviation. IRB pre-approved SSE protocol modifications are considered an amendment to the protocol and not a deviation. The submission of a deviation report is not required.

Brief interruptions and delays may occasionally be required due to travel delays, airport closure, inclement weather, family responsibilities, security alerts, government holidays, etc. This can also extend to complications of disease or unrelated medical illnesses not related to disease progression. The PI has the discretion to deviate from the protocol when necessary so long as such deviation does not threaten patient safety or protocol scientific integrity. Examples include, but are not limited to: a) dose adjustments based on excessive patient weight; b) alteration in treatment schedule due to non-availability of the research participant for treatment; c) laboratory test results which are slightly outside the protocol requirements but at levels that do not affect participant safety. These instances are considered to be deviations from the protocol. A deviation report will be submitted to the DSMC/IRB within five days.

12.2.2 Reporting of Deviations

Protocol deviations using the Protocol Deviation Form must be submitted by the protocol nurse or the clinical research associate to the Coordinator (cccp@coh.org) for distribution. The clinical research associate at City of Hope will also submit copies to the Protocol Management Team and the City of Hope Data and Safety Management Board. All deviations will be reported to the COH DSMC within five days. The DSMC will forward to report to the IRB following review.

12.2.3 Resolving Disputes

The COH Investigational Drug Service (IDS) cannot release a research agent that would cause a protocol deviation without approval by the PI. Whenever the protocol is ambiguous on a key point, the IDS should rely on the PI to clarify the issue.

In situations where there is misperception or dispute regarding a protocol deviation among the persons involved in implementing the protocol, it is the responsibility of the PI to resolve the dispute and the PI may consult with the DSMC chair (or designee) to arrive at resolution.

13. Statistical Considerations

13.1 Study Design

As most of the objectives can obtain sufficient information for a pilot study with fewer patients, we powered this study to insure sufficient number of HER2 positive patients by IHC to serve as a positive control.

Specifically, with 22 patients, there is an 80% chance we'd enroll at least one HER2 positive patient by IHC if the true incidence of HER2 positivity is the lowest end of the reported rate of 7%. If the incidence is 16% (Marx, et al, 2009), we would expect to observe 2 or more HER2+ patients with 89% probability.

For the other objectives:

1. In breast cancer, Cu-PET-CT (^{64}Cu-DOTA-trastuzumab-PET-CT) was able to identify the tumors with low HER2 expression. Gastric cancer has different adjacent tissue to provide a background noise comparison. As a result, with 22 patients, we will be able to determine the percent of patients whose tumors image with Cu-PET-CT with a 95% CI half-width of 21%.
2. A subset of patients will have tumors of sufficient size to have Cu-PET-CT SUV variation over the tumor. For patients with metastatic disease who have Cu-PET-CT positivity but negative initial testing for HER2 expression, additional image-directed biopsy of the Cu-PET-CT positive area will be performed for analysis prior to their destination systemic treatments (Track A on schema). In those cases when subjects undergo surgical resection (Track B and C on schema), the positive and negative areas of the tumor will be sent to pathology for confirmation. These will be exploratory.
3. Correlation of Cu-PET-CT SUV (measured as peak SUV) and pathology will be explored. As correlation requires a continuous measure from pathology, and some pathology will use FISH and/or IHC, this will be exploratory. Comparing Cu-PET-CT in positive cases versus negative by pathology will depend on the percent of patients deemed positive. This is an exploratory aim.
4. Patients who are deemed negative by pathology, but appear positive by Cu-PET-CT is exploratory, as further evaluation will be needed to determine positivity by Cu-PET-CT.

13.2 Sample Size Accrual Rate

22 patients

Accrual rate 1-2 patients per month

14. Human Subject Issues

14.1 Institutional Review Board

In accordance with City of Hope policies, an Institutional Review Board (IRB) that complies with the federal regulations at 45 CFR 46 and 21 CFR 50, 56 and State of California Health and Safety code, Title 17, must review and approve this protocol and the informed consent form prior to initiation of the study. All institutional, NCI, Federal, and State of California regulations must be fulfilled.

14.2 Recruitment of Subjects

The gastric cancer team, most often the gastric surgeon who is the PI of this study or the medical oncologist who is a co-investigator of this study will screen patients for this study. From analysis of the gastric cancer patient volume at City of Hope in the previous three years, 65-67 gastric cancer patients underwent surgery for gastric cancer and another similar volume non-surgical patients seen by medical

oncologists. The inclusion criteria should allow for majority of these patients to be eligible to this study and both the surgical and medical oncologists treating gastric cancer patients at COH will be recruiting to this study.

14.3 Advertisements

Advertisements to include print, media (radio, television, billboards), telephone scripts, lay summary to be posted on City of Hope's public Clinical Trials On-Line^{SM} website, etc., will be reviewed and approved by the IRB prior to their use to recruit potential study subjects.

14.4 Study location and Performance Sites

This study will be performed at COH.

14.5 Confidentiality

This research will be conducted in compliance with federal and state of California requirements relating to protected health information (PHI). The principal investigator, co-investigators, and laboratory technicians will have access to this information, but all information will be treated confidentially. No identifiers will be used in any subsequent publication of these results.

14.6 Financial Obligations and Compensation

The investigational drug, ^{64}Cu-DOTA-trastuzumab , will be provided free of charge by City of Hope. The standard of care procedures provided will be the responsibility of the research participant and/or the insurance carrier. The research participant will be responsible for all copayments, deductibles, and other costs of treatment and diagnostic procedures as set forth by the insurance carrier. The research participant and/or the insurance carrier will be billed for the costs of treatment and diagnostic procedures in the same way as if the research participant were not in a research study. However, neither the research participant nor the insurance carrier will be responsible for the research procedures related to this study.

In the event of physical injury to a research participant, resulting from research procedures, appropriate medical treatment will be available at the City of Hope to the injured research participant, however, financial compensation will not be available.

The research participant will not be paid for taking part in this study.

14.7 Informed Consent Processes

The Principal Investigator or IRB approved named designate will explain the nature, duration, purpose of the study, potential risks, alternatives and potential benefits, and all other information contained in the informed consent document. In addition, they will review the experimental subject's bill of rights and the HIPAA research authorization form. Research subjects will be informed that they may withdraw from the study at any time and for any reason without prejudice, including as applicable, their current or future care or employment at City of Hope or any relationship they have with City of Hope. Research subjects will be afforded sufficient time to consider whether or not to participate in the research.

Should sufficient doubt be raised regarding the adequacy of comprehension, further clarifications will be made and the questionnaire 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. For those subjects who do comprehend the fundamental aspects of the study, consent will be obtained and documented, followed by eligibility testing. The research team will review the results of eligibility testing and determine if the subject is a candidate for study enrollment.

15. References

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2. Asioli S, Maletta F, Verdun di Cantogno L, Satolli MA, Schena M, Pecchioni C, et al. Approaching heterogeneity of human epidermal growth factor receptor 2 in surgical specimens of gastric cancer. Hum Pathol. 2012;43:2070-9.

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