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

TITLE: **A Pilot Study of ^{64}Cu-DOTA-Trastuzumab Positron Emission Tomography in Treatment of Advanced *HER2* Positive Breast Cancer with the Antibody Drug Conjugate Ado-Trastuzumab Emtansine**

CITY OF HOPE PROTOCOL NUMBER/VERSION: IRB # 14099 **PROTOCOL DATED: 07/18/18**

DATE(S)/ OF AMENDMENT(S)/REVISION(S):

COH Initial Approval	Protocol Dated 08/01/14	Version: 00
COH Amendment A	Protocol Dated 08/11/14	Version: 00
COH Amendment 01	Protocol dated 12/18/14	Version: 01
COH Amendment 02	Protocol dated 2/25/15	Version: 02
COH Amendment 03	Title page dated 08/03/2015	Version: 03
COH Amendment 04	Protocol dated 09/21/15	Version: 04
COH Amendment 05	Protocol dated 01/04/16	Version: 05
COH Amendment 06	Title page dated 04/08/2016	Version: 06
COH Amendment 07	Title Page dated 04/28/2016	Version: 07
COH Amendment 08	Protocol dated 06/02/2016	Version: 08
COH Amendment 09	Title Page/Personnel Update, dated 07/15/2016	Version: 09
COH Amendment 10	Title page dated 8/8/2016	Version: 10
COH Amendment 11	Title Page dated 09/10/16	Version: 11
COH Amendment 12	Title Page dated 10/24/16	Version: 12
COH Amendment 13	Title Page dated 01/31/2017	Version: 13
COH Amendment 14	Title Page dated 10/24/2017	Version: 14
COH Amendment 15	Protocol dated 11/02/17	Version: 15
COH Amendment 16	Protocol dated 07/18/18	Version: 16
COH Amendment 17	Title Page dated 01/22/19	Version: 17
COH Amendment 18 at Continuation	Title Page dated 12/17/19	Version: 18
COH Amendment 19 at Continuation	Protocol Dated 07/18/18 (TP)	Packet: 19

SITE: Breast
STAGE (If applicable): IV
MODALITY: Treatment/Imaging
TYPE: Pilot

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A Pilot Study of ^{64}Cu-DOTA-Trastuzumab Positron Emission Tomography in Treatment of Advanced *HER2* Positive Breast Cancer with the Antibody Drug Conjugate Ado-Trastuzumab Emtansine

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Federal-Wide Assurance Number: FWA 00000692

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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, an assessment 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.

Trastuzumab Emtansine (ado-trastuzumab emtansine – TDM1)

Antibody-drug conjugates (ADCs) are effective in the treatment of leukemia.[10] Ado-trastuzumab emtansine is a novel ADC specifically designed for the treatment of *HER2*-positive cancer. It is composed of the following: trastuzumab, a humanized antibody directed against the extracellular region of *HER2*; DM1, an anti-microtubule agent derived from maytansine; and succinimidyl 4-[N-maleimidomethyl] cyclohexane-1-carboxylate (SMCC), a thioether linker molecule used to conjugate DM1 to trastuzumab.[11, 12] Ado-trastuzumab emtansine binds to *HER2* with an affinity similar to that of unconjugated trastuzumab.[13] It is hypothesized that after binding to *HER2*, ado-trastuzumab emtansine undergoes receptor-mediated internalization, resulting in intracellular release of DM1 and subsequent cell death. DM1 is an inhibitor of tubulin polymerization; it binds to tubulin competitively with vinca alkaloids.[12] Ado-trastuzumab emtansine has been shown in preclinical studies to retain the activities of unconjugated trastuzumab including inhibition of *HER2* shedding, inhibition of PI3K/AKT signaling pathways, and antibody dependent cellular cytotoxicity.[13]

Ado-trastuzumab emtansine is the first ADC to be used in breast cancer. EMELIA was a randomized, Phase III study of ado-trastuzumab emtansine versus lapatinib + capecitabine for the treatment of

patients with *HER2*-positive, unresectable, locally advanced or metastatic breast cancer previously treated with trastuzumab and a taxane.[14] Patients received ado-trastuzumab emtansine (3.6 mg/kg IV on Day 1 of a 21-day cycle) or lapatinib (1250 mg orally once per day) plus capecitabine (1000 mg/m^2 orally twice daily on Days 1−14 of a 21-day cycle) until PD or unmanageable toxicity. Eligible patients had confirmed *HER2*-positive metastatic breast cancer (IHC 3+ and/or FISH positive) and had received prior therapy with trastuzumab and a taxane. Primary endpoints were progression-free survival (PFS) by independent review, overall survival (OS), and safety.

From February 2009 through October 2011, a total of 991 patients were enrolled; 496 were assigned to lapatinib + capecitabine, and 495 were assigned to ado-trastuzumab emtansine. Median duration of follow-up for the first and second interim analysis was approximately 13 months and 19 months, respectively. Baseline patient demographics, prior therapy, and disease characteristics were balanced. The study met the primary endpoint with an improvement in PFS by independent review with a hazard ratio (HR) of 0.65, [95% confidence interval (CI) 0.55 to 0.77, p<0.001]. The median PFS was 9.6 months in the ado-trastuzumab emtansine arm and 6.4 months in the lapatinib + capecitabine arm. A strong trend in OS was observed in favor of the ado-trastuzumab emtansine arm [HR = 0.62, (95% CI 0.48-0.81), p = 0.0005]. At the first interim analysis, median OS was not reached in the ado-trastuzumab emtansine arm and was 23.3 months for lapatinib + capecitabine; the interim efficacy stopping boundary for OS was not crossed. However, at the second interim analysis, OS data crossed the pre-specified boundary that showed patients receiving ado-trastuzumab emtansine (median OS = 30.9 months) survived significantly longer than the control group (median OS=25.1 months), with a HR=0.68, 95% CI 0.55-0.85, p<0.001). The objective response rate (ORR) was 43.6% for the ado-trastuzumab emtansine arm versus 30.8% for the lapatinib + capecitabine arm, with a median duration of objective response (DOR) of 12.6 months versus 6.5 months, respectively.

Ado-trastuzumab emtansine was well tolerated, with no unexpected safety signals. The most common Grade ≥3 adverse events (AEs) in the ado-trastuzumab emtansine arm were thrombocytopenia (12.9% vs. 0.2%), increased AST (4.3% vs. 0.8%), and increased ALT (2.9% vs. 1.4%); the most common Grade ≥3 AEs in the lapatinib + capecitabine arm were diarrhea (20.7% vs. 1.6%) palmar plantar erythrodysesthesia (16.4% vs. 0), and vomiting (4.5% vs. 0.8%). The incidence of Grade 3/4 AEs in the ado-trastuzumab emtansine arm was 40.8% versus 57.0% in the lapatinib + capecitabine arm.

On the basis of this trial, ado-trastuzumab emtansine is now FDA approved as second line therapy in women with metastatic HER2 positive breast cancer who have undergone prior therapy with trastuzumab and a taxane.

Molecular Imaging in HER2 Positive Breast Cancer

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 before tumor regression can be documented by clinical examination or anatomical imaging.[15-17] 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. [18] 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 [19]. 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.

The underlying hypothesis of our project is that tumor uptake of ^{64}Cu-DOTA-trastuzumab as measured by PET-CT can be used to predict response to ado-trastuzumab emtansine.

2.0 Study Objectives

We propose to evaluate the potential of ^{64}Cu-DOTA-trastuzumab/PET-CT to identify women with advanced *HER2*-positive breast cancers who are likely to benefit from treatment with ado-trastuzumab emtansine. Because trastuzumab targets both ado-trastuzumab emtansine and ^{64}Cu-DOTA-trastuzumab to tumors, we hypothesize that ^{64}Cu-DOTA-trastuzumab uptake can be used as a surrogate for relative tumor uptake of ado-trastuzumab emtansine during treatment. Thus, we expect that ^{64}Cu-DOTA-trastuzumab/PET-CT can be used to identify *HER2*-positive disease that is unlikely to benefit from ado-trastuzumab emtansine therapy due to inadequate uptake of the ADC. Conversely, the current study of women whose disease is classified as *HER2*-positive, combined with prior observations of ^{64}Cu-DOTA-trastuzumab tumor uptake in HER2-negative patients, may engender evaluation of ^{64}Cu-DOTA-trastuzumab/PET-CT for identifying patients classified as HER2-negative who might nonetheless have adequate tumor uptake to benefit from ado-trastuzumab emtansine.

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 focus only on treatment with ado-trastuzumab emtansine. Patients entered in the trial will have experienced disease progression after prior taxane and trastuzumab chemotherapy. The fact that trastuzumab is rarely used as a single agent presents an obstacle in the evaluation of ^{64}Cu-DOTA-trastuzumab/PET-CT for prediction of response. In contrast, treatment with ado-trastuzumab emtansine as a single agent affords a unique opportunity for evaluating ^{64}Cu-DOTA-trastuzumab/PET-CT. While emtansine delivery to tumor is of primary interest, ^{64}Cu-DOTA-trastuzumab uptake is also relevant to potential effectiveness of the trastuzumab component of the ADC. Success in the proposed trial would have implications regarding the potential use of ^{64}Cu-DOTA-trastuzumab/PET-CT to aid patient selection for any treatment regimen that includes trastuzumab.

The goal of this pilot study is to correlate ^{64}Cu-DOTA-trastuzumab/PET-CT tumor uptake measurements with response both at the patient and individual tumor levels. In addition to inadequate tumor uptake, another potential cause of non-response is biological or "molecular" mechanisms of resistance (MMRs) to ado-trastuzumab emtansine. Consistent with our prior study protocol, all patients will undergo a pretreatment biopsy to ensure positive *HER2* status. Residual tumor will be stored for future studies that examine the role of putative MMRs in determining response to ado-trastuzumab emtansine.

The Specific Study Objectives are:

2.1 Correlated uptake of ^{64}Cu-DOTA-trastuzumab PET by individual tumors with subsequent tumor response to ado-trastuzumab emtansine as assessed by serial ^{18}F-FDG/PET-CT.

2.2 Compare tumor uptake of ^{64}Cu-DOTA-trastuzumab PET between patients who do and patients who do not respond to ado-trastuzumab emtansine.

2.3 Obtain tumor tissue for subsequent assessment of the presence of putative MMRs to ado-trastuzumab emtansine. When funding becomes available, those samples will be used to explore the correlation between the presence of MMRs as assessed by histopathology and tumor response to ado-trastuzumab emtansine both in univariate analysis and in combination with tumor uptake of ^{64}Cu-DOTA-trastuzumab as measured with PET/CT.

3.0 Research Design

This prospective clinical pilot trial will enroll 10 women with metastatic *HER2* positive breast cancer who will be treated with ado-trastuzumab emtansine. Patients will undergo ^{64}Cu-DOTA-trastuzumab/PET-CT just prior to initiation of therapy with ado-trastuzumab emtansine. The objective is to examine, both at the patient and individual tumor levels, the relationship between tumor uptake of ^{64}Cu-DOTA-trastuzumab and response to ado-trastuzumab emtansine.

4.0 Eligibility

Women with metastatic *HER2*-positive breast cancers whose disease will be treated with ado-trastuzumab emtansine will be considered eligible for study participation if they meet the following criteria:

4.1. Participants must be women who have histological confirmation of metastatic invasive breast cancer that has metastasized outside the region of the primary tumor and axilla. Biopsy must be obtained prior to initiation of chemotherapy. It should be performed within 28 days prior to enrollment (Patients with a biopsy of recurrent disease that is HER2-positive and have not received HER2-directed therapy since the biopsy can exceed the 28-day windowup to 6 months. Patients must have metastatic disease in lung, liver, soft-tissue or bone to qualify for the study (more than one site is permissible).

4.2 At least 1 site of metastasis $\geq$ 20 mm in mean diameter must be identified.

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

4.4 Patients may not have received trastuzumab within 6 weeks of projected ^{64}Cu-DOTA-trastuzumab/PET-CT.

4.5 Participants must have normal cardiac ejection fraction.

4.6 Ability to provide informed consent.

4.7 Patients $\geq$18 years of age

4.8 Patients that may need dose reduction to commence Cycle 1 treatment.

4.9 ECOG Performance status of 0-2

4.10 Negative serum pregnancy test (female patient of childbearing potential only)

4.11 Patients must have adequate cardiac function. Left ventricular ejection fraction (LVEF) ≥ 50% as determined by MUGA scan or echocardiogram.

5.0 Ineligibility

5.1 Participants who have received trastuzumab within the prior 36 days.

5.2 Participants who are not considered candidates for ado-trastuzumab emtansine.

5.3 No metastatic sites $\geq$20 mm.

5.4 Concurrent malignancy other than skin cancer.

5.5 Inability to provide informed consent.

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 and Patient Registration Procedures

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 (include as applicable) their future care, their employment at City of Hope or any relationship they have with City of Hope. After signing the study consent form, HIPAA authorization form and the Experimental Subject's Bill of Rights, research subjects will undergo an assessment of their understanding of the study by the Research Subject Advocate, followed by eligibility testing. 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.

Eligible subjects will be registered on the study centrally by the Data Coordinating Center (DCC) at City of Hope. Staff (including physicians, protocol nurses and/or CRCs) should call the DCC at (626) 256-4673, ext. 64267 if there are any questions regarding subject registration.

Issues that would cause treatment delays should be discussed with the Principal Investigator. If a subject does not receive protocol therapy within two weeks following registration, the subject's registration on the study may be canceled. The Data Coordinating Center should be notified of cancellations as soon as possible.

Once the signed informed consent has been obtained, all pretreatment evaluations have been performed, and subject's eligibility has been confirmed by the Data Coordinating Center, a subject will be registered on study.

To register a subject, the treating physician should contact the protocol nurse or the responsible Clinical Research Coordinator (CRC) in the Clinical Trial Office (CTO) to complete the eligibility checklist.

The protocol nurse or CRC will contact the Data Coordinating Center at City of Hope (626-256-4673, ext. 64267 or via e-mail at dcc@coh.org), scan and EMAIL a copy of the completed and signed eligibility checklist, a copy of the signed Informed Consent (including a copy of signed subject's bill of Rights and HIPAA authorization form) and copies of any required pre-study test results that are not readily available in the COH electronic medical record to dcc@coh.org.

The protocol nurse or CRC may then call the Data Coordinating Center (626-256-4673 ext. 64267) to confirm receipt of all registration documents. To complete the registration process, the Data Coordinating Center will:

- Verify and confirm the subject's eligibility.
- Assign a subject accession number (RPN or for example, COH-001, COH-002, etc.).
- Register the subject on study centrally (the City of Hope CRC assigned to the trial will still be responsible for accessioning via MIDAS).
- Complete and email a Confirmation of Registration form within 24 hours to include the COH subject study number to the study team, which will include the Principal Investigator, treating physician, protocol nurse, CRC and COH IDS Pharmacy.
- Call the protocol nurse and/or CRC to verbally confirm registration.
- Enter the subject into Medidata RAVE.
- A subject failing to meet all protocol requirements will not be registered.

8.0 Study Procedures/Research Interventions

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

8.1.1 Ten women with metastatic breast cancer that is *HER2* positive by IHC and/or FISH will be recruited from the Medical Oncology Clinics at City of Hope.

8.1.2 As patients will ultimately receive ado-trastuzumab emtansine 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). Venous blood samples (each sample to be 7ml in red top tubes will be drawn just prior to the trastuzumab pre-infusion, 1 h after administration of ^{64}Cu-DOTA-trastuzumab, and shortly before each of the two PET scans of ^{64}Cu-DOTA-trastuzumab. The pre-infusion sample will be used both to determine levels of residual circulating trastuzumab from prior treatment and to obtain background measurements for radioactivity assays performed on the subsequent blood samples. The samples taken at 1 h and later are used to measure the blood clearance of ^{64}Cu-DOTA-trastuzumab.

8.1.5 A PET scan of regions of interest will be performed 24 and 48 hours after injection of ^{64}Cu-DOTA-trastuzumab. Systemic therapy with ado-trastuzumab emtansine will be initiated after completion of the ^{64}Cu-DOTA-trastuzumab PET-CT imaging. The conventional dose of ado-trastuzumab emtansine is 3.6 mg/kg every 3 weeks until disease progression or at the discretion of the treating oncologist.

8.1.6 Participants will be observed for toxicity for one year.

8.1.7 Subjects will undergo restaging by whole body^{18}F-FDG/PET-CT every 6 weeks for one year after initiation of treatment until disease progression. After one year a PET/CT or CT scan can be performed at the discretion of the treating physician.

8.1.8 An algorithm for the administration of ado-trastuzumab will be dosed according to the package insert.

8.1.9 Rules for dose modification of ado-trastuzumab are listed according to package insert

8.2 Handling of specimens: All specimens will be handled in accordance with RSC 09001 and OSBC 90029.

9.0 Study Calendar

	≤ 28[1] days prior to study entry	≤14 days prior to [18F]FDG PET-CT	Days -16 to -3	Day -2	Day -1	Day 0	Day 7	Day 14	Day 21	Day 28	Day 42	Day 63	Day 84	Day 105	Every 21 Days (+/- 7 days) Until Complete Response or Disease Progression
Biopsy of metastasis for diagnosis and *HER2* assessment	X														
History and Physical Exam		X				X	X	X	X		X	X	X	X	X
Cardiac ejection fraction (by MUGA or ECHO) q3 months		X					X[2]	X[2]					X		
CBC, CMP, Tumor markers		X				X	X[3]	X[3]	X		X	X	X	X	X
Pregnancy test if premenopausal		X													
[18F]FDG/PET-CT[2]			X								X		X		X[2]
Trastuzumab serum level				X	X	X									
Cold trastuzumab				X											
64Cu-DOTA-trastuzumab injection				X											
[2]PET-CT Imaging					X	X									
Ado-trastuzumab emtansine						X			X		X	X	X	X	X
Toxicity assessment and conmeds				X	X	X			X	X[5]					
Hematocrit[4]				X											

Treatment with ado-trastuzumab emtansine is administered every 21 days (+/- 7 days) until complete response or disease progression

[1]Pretreatment PET/CT and biopsy may be performed between 28 days and up to 6 months prior to study treatment for patients that have not received systemic therapy.

[2]Re-staging with ^{18}F-FDG/PET-CT is performed every 6 weeks for one year after 1st ado-trastuzumab emtansine dose. After one year a PET/CT or CT scan can be performed at the discretion of the treating physician.

[3]Participants will be examined by the treating physician on days 7 and 14, and a CBC will be obtained. If there are any clinical concerns about cardiac toxicity, a MUGA will be obtained.

[4]Blood sample for hematocrit will be collected just prior to the trastuzumab pre-infusion.

[5]AEs/SAEs, toxicities and conmeds will only be gathered for 30 days post cold trastuzumab/Cu-DOTA injection.

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 1.2% 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: The research participant/research participant's insurance carrier will be responsible for the cost of Trastuzumab.

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 use. 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 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 (36°-46° F).

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.

10.4.1.2 *Administration*: 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. Patients need to stay for 2 hours post ^{64}Cu-DOTA-trastuzumab administration with vital signs (Temp, Pulse, BP, Resp.) to be done at: Pre-start, 15 min post start, 30 min post start, 45 min post start , 60 min post start and 2 hours post start of ^{64}Cu-DOTA-trastuzumab. After measurement of the vital signs have been completed, discharge patient home if stable. If unstable, notify MD.

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.[18, 20] The study protocol also requires ^{18}F-fluorodeoxyglucose (FDG)/PET-CT scans prior to ^{64}Cu-DOTA-trastuzumab/PET-CT and performed every 6 weeks after 1st ado-trastuzumab emtansine dose.

The FDG scans are used to assess treatment response and thus are standard of care. Patients will be imaged twice following injection of ^{64}Cu-DOTA-trastuzumab. We have used estimated CT radiation dose to arrive at the following estimates of overall radiation equivalent and effective doses from the ^{64}Cu-DOTA-trastuzumab/PET-CT research procedure. 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.[21]

**Low-dose CT procedure; 2 scans.

10.3 Ado-trastuzumab emtansine

10.3.1 Drug description: Ado-trastuzumab emtansine is a novel ADC specifically designed for the treatment of *HER2*-positive cancer. It is composed of (i) trastuzumab, a humanized antibody directed against the extracellular region of *HER2*, (ii) DM1, an anti-microtubule agent derived from maytansine; and (iii) succinimidyl 4-[N-maleimidomethyl] cyclohexane-1-carboxylate (SMCC), a thioether linker molecule used to conjugate DM1 to trastuzumab.[11, 12]

Ado-trastuzumab emtansine binds to *HER2* with an affinity similar to that of unconjugated trastuzumab.[13] It is hypothesized that after binding to HER2, ado-trastuzumab emtansine undergoes receptor-mediated internalization, resulting in intracellular release of DM1 and subsequent cell death. DM1 is an inhibitor of tubulin polymerization; it binds to tubulin competitively with vinca alkaloids. Ado-trastuzumab emtansine also has been shown in preclinical studies to retain the activities of unconjugated trastuzumab, including inhibition of *HER2* shedding, inhibition of PI3K/AKT signaling pathways, and antibody dependent cellular cytotoxicity.[13]

10.3.2 Procurement of ado-trastuzumab emtansine: Because this is a standard-of-care treatment for recurrent *HER2*-positive breast cancer, we anticipate that the cost of the drug will be provided by insurance.

10.3.3 Storage/stability: The diluted ado- trastuzumab emtansine infusion solution should be used immediately. If not used immediately, the solution may be stored in a refrigerator at 2°C to 8°C (36°F to 46°F) for up to 24 hours prior to use. This storage time is additional to the time allowed for the reconstituted vials. *Do not freeze or shake.*

10.3.4 Reconstitutions and administration

10.3.4.1 *Reconstitution*: Using a sterile syringe, 5mL of Sterile Water should be injected into the 100mg ado-trastuzumab emtansine vial, or 8mL of Sterile Water should be injected into the 160mg ado-trastuzumab emtansine vial to yield a solution containing 20mg/ml. The reconstituted solution should be clear to opalescent and free of visible particulates. The reconstituted solution should be colorless to pale brown. The reconstituted, lyophilized vials should be used immediately or stored for up to 4 hours in a refrigerator at 2-8°C (36-46°F); discard unused ado-trastuzumab

emtansine after 4 hours. Do not freeze. The reconstituted product contains no preservative and is intended for single-use only.

10.3.5. Administration: Ado-trastuzumab emtansine is administered as an IV infusion using a 0.22 micron in-line non-protein adsorptive polyethersulfone (PES) filter. It should be administered as an IV infusion never as a bolus. The drug should not be mixed or administered as an infusion with other medical products. The recommended dose is 3.6 mg/kg as an IV infusion every 21 days until disease progression or unacceptable toxicity.

10.3.6 Drug accountability: Ado-trastuzumab emtansine 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.

10.3.7 Discard of unused agent: Unused ado-trastuzumab emtansine will be discarded in the chemotherapy discard containers within the outpatient pharmacy.

10.3.8 *Warnings and Contraindications*

10.3.8.1 *Pulmonary Toxicity*: Cases of interstitial lung disease (ILD), including pneumonitis, some leading to acute respiratory distress syndrome or fatal outcome have been reported in clinical trials with ado-trastuzumab emtansine. Pneumonitis at an incidence of 0.8% (7 out of 884 treated patients) has been reported, with one case of grade 3 pneumonitis. Signs and symptoms include dyspnea, cough, fatigue, and pulmonary infiltrates. These events may or may not occur as sequelae of infusion reactions. In the randomized trial TDM 4370g/BO21977 *(*EMILIA*)* the overall frequency of pneumonitis was 1.2%. Treatment included administration of steroids, oxygen, and study drug discontinuation (Data on File. Genentech, Inc). Permanently discontinue treatment with ado-trastuzumab emtansine in patients diagnosed with ILD or pneumonitis Patients with *dyspnea at rest due to complications of advanced malignancy and co-morbidities may be at increased risk of pulmonary toxicity.*

10.3.8.2 *Hepatotoxicity*: Hepatotoxicity, predominantly in the form of asymptomatic transient increases in the concentrations of serum transaminases (Grade 1-4 transaminitis), has been observed while during treatment with ado-trastuzumab emtansine in clinical trials . A cumulative effect of ado-trastuzumab emtansine on transaminases has been observed (Data on File. Genentech, Inc.). Cases of severe hepatotoxicity, including death due to drug-induced liver injury (DILI) and hepatic encephalopathy, have been observed in patients treated with ado-trastuzumab emtansine. While there is evidence of drug-induced liver toxicity (predominantly in the form of asymptomatic increases in the concentrations of serum transaminases) in patients treated with ado-trastuzumab emtansine, its potential to cause liver injury with clinically meaningful changes in liver function is unclear, as the observed cases were confounded by concomitant medications with known hepatotoxic potential and/or underlying conditions. Nevertheless, a contributory role of ado-trastuzumab emtansine in these cases cannot be excluded.

10.3.8.3 *Cardiotoxicity*: Patients treated with ado-trastuzumab emtansine are at increased risk of developing left ventricular dysfunction. A

decrease of LVEF to <40% has been observed in patients treated with ado-trastuzumab emtansine. In the randomized trial, TDM 4370g/BO21977 (EMILIA). Left ventricular dysfunction occurred in 1.8% of patients in the ado-trastuzumab emtansine -treated group and 3.3% of patients in the lapatinib plus capecitabine-treated group.

10.3.8.4 *Infusion Reaction*: Treatment with ado-trastuzumab emtansine has not been studied in patients who had trastuzumab permanently discontinued due to infusion-related reactions (IRR) and/or hypersensitivity; treatment with ado-trastuzumab emtansine is not recommended for these patients. Infusion-related reactions, characterized by one or more of the following symptoms - flushing, chills, pyrexia, dyspnea, hypotension, wheezing, bronchospasm, and tachycardia have been reported in clinical trials of ado-trastuzumab emtansine. In the randomized trial, TDM 4370g/BO21977 *(*EMILIA), the overall frequency of IRRs in patients treated with ado-trastuzumab emtansine was 1.4%. In most patients, these reactions resolved over the course of several hours to a day after the infusion was terminated. Ado-trastuzumab emtansine treatment should be interrupted in patients with severe IRR. Ado-trastuzumab emtansine treatment should be permanently discontinued in the event of a life-threatening IRR. Patients should be observed closely for IRR reactions, especially during the first infusion. One case of a serious, allergic/anaphylactic-like reaction has been observed in clinical trials of single-agent ado-trastuzumab emtansine. Medications to treat such reactions, as well as emergency equipment, should be available for immediate use.

10.3.8.5 *Thrombocytopenia*: Thrombocytopenia was reported in clinical trials of ado-trastuzumab emtansine (103 of 884 treated patients with Grade ≥3; 283 of 884 treated patients with any Grade). The majority of these patients had Grade 1 or 2 events (<LLN to ≥50,000/mm^3) with the nadir occurring by day 8 and generally improving to Grade 0 or 1 (≥75,000 /mm^3) by the next scheduled dose. In clinical trials of ado-trastuzumab emtansine, the incidence and severity of thrombocytopenia were higher in Asian patients. Independent of race, the incidence of severe hemorrhagic events in patients treated with ado-trastuzumab emtansine was low.

10.3.8.6 *Neurotoxicity*: Peripheral neuropathy, mainly as Grade 1 and predominantly sensory, was reported in clinical trials of ado-trastuzumab emtansine (14 of 884 treated patients with Grade ≥3; 196 of 884 treated patients with any Grade). In the randomized trial (Study 1), the overall frequency of peripheral neuropathy was 21.2% in the ado-trastuzumab emtansine-treated group and 13.5% in the lapatinib plus capecitabine-treated group. The incidence of Grade ≥3 peripheral neuropathy was 2.2% in the ado-trastuzumab emtansine-treated group and 0.2% in the lapatinib plus capecitabine-treated group. Ado-trastuzumab emtansine should be temporarily discontinued in patients experiencing Grade 3 or 4 peripheral neuropathy until resolution to Grade ≤2.

Patients should be clinically monitored on an ongoing basis for signs or symptoms of neurotoxicity.

10.3.8.7 *Extravasation*: In ado-trastuzumab emtansine clinical studies, reactions secondary to extravasation have been observed. These reactions, frequently observed within 24 hours of infusion, were usually mild and comprised erythema, tenderness, skin irritation, pain, or swelling at the infusion site. Specific treatment for ado-trastuzumab emtansine extravasation is unknown. The infusion site should be closely monitored for possible subcutaneous infiltration during drug administration.

11.0 Positron Emission Tomography (PET)

11.1 Imaging will be performed on a GE Discovery 16 Ste PET-CT scanner (axial field of view 15.4 cm). 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 with spatial resolution of approximately 9 mm full-width-at-half maximum (FWHM) 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 and every 6 weeks during treatment with ado-trastuzumab emtansine. Patients will be injected via a peripheral vein with ≤ 15 mCi of ^{18}F-FDG. 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: 2 or 3 bed positions encompassing known tumors; 30 minutes per bed position if 2 bed positions, 20 minutes per bed position if 3 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

The ^{64}Cu-DOTA-trastuzumab and baseline ^{18}F-FDG PET-CT examinations will be interpreted by Dr. Park. Baseline and follow-up ^{18}F-FDG PET-CT examinations will be interpreted for the purpose of evaluating tumor response by Dr. Gidwany who is blinded to the ^{64}Cu-DOTA-trastuzumab examinations.

Tumor uptake of ^{18}F-FDG will be measured in terms of SUL_{peak} for evaluation of response, as prescribed by PERCIST [21]. In order to avoid potential bias, those measurements will be performed by the “second” radiologist, who will not have knowledge of the ^{64}Cu-DOTA-trastuzumab scans. In a separate analysis performed by Dr. Bading, we will also evaluate baseline SUV_{wt} for ^{18}F-FDG within a 3D isocontour half way between the image intensity of

adjacent background and the maximum voxel.[22] For each baseline FDG-positive tumor, SUV_{wt} for ^{64}Cu-DOTA-trastuzumab will be determined by applying the FDG whole-tumor volume of interest (VOI) to the CT-coregistered ^{64}Cu image set. We denote to this metric of ^{64}Cu-DOTA-trastuzumab as SUV_{wt}(FDG-matched). Tumor sizes (product of maximum mutually perpendicular transaxial diameters as well as maximum axial diameter) will be estimated from coregistered CT.

Tumor uptake will be evaluated in terms of standardized uptake values (SUV or SUL = tumor activity concentration decay-corrected to time of injection x body weight or lean-body mass /injected activity). The standard protocol for metabolic response assessment [PET Response Criteria in Solid Tumors (PERCIST)] employs SUL as the metric for tumor uptake of ^{18}F-FDG. However, because ado-trastuzumab-emtansine dose is prescribed in terms of mg/body weight, we will use SUV for ^{64}Cu-DOTA-trastuzumab.

For ^{64}Cu, SUVs will be evaluated in tumors, adjacent non-tumor tissue and 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. Tumor uptake of ^{64}Cu-DOTA-trastuzumab will be parameterized in terms of single-voxel maximum values SUV_{max}, "peak" values SUV_{peak} (defined as the maximum average value within a 1.2 cm diameter sphere scanned over the PET image of the tumor), and whole-tumor volumes of interest (SUV_{wt}).[23]

Tumor uptake of ^{18}F-FDG will be measured in terms of SUV_{peak} for evaluation of response, as prescribed by PERCIST [23]. We will also evaluate SUV_{wt} for ^{18}F-FDG within a 3D isocontour half way between the image intensity of adjacent background and the maximum voxel.[22] For each baseline FDG-positive tumor, SUV_{wt} for ^{64}Cu-DOTA-trastuzumab will be determined by applying the FDG whole-tumor volume of interest (VOI) to the CT-coregistered ^{64}Cu image set. We denote to this metric of ^{64}Cu-DOTA-trastuzumab as SUV_{wt}(FDG-matched).

Relative ado-trastuzumab emtansine dose to individual tumors will be equated with ^{64}Cu-DOTA-trastuzumab SUV_{max}, SUV_{peak} or SUV_{wt} for those tumors. Overall relative ado-trastuzumab emtansine dose to tumor (DT) for a given patient will be equated with the ^{64}Cu-DOTA-trastuzumab VOI volume-weighted average SUV_{wt} over all evaluated tumors.

13.0 Response Assessment

Patient and individual tumor response to ado-trastuzumab emtansine will be determined from serial ^{18}F-FDG/PET-CT, using the specifications prescribed in PERCIST regarding patient preparation, scan acquisition, tumor selection for response assessment, and measurement of tumor uptake.[23] The relatively coarse image resolution of PET can lead to errors in uptake measurements. PERCIST seeks to limit such "partial volume effect" errors by requiring a minimum tumor size of 2 cm for quantitative assessment of response. An *ad hoc* compensation for statistical noise and variations in the^{18}F-FDG integral blood curve is made by comparing tumor uptake with liver uptake and the amount of random noise in the liver image.

Response assessment is based on the single evaluable tumor ("target lesion") with highest FDG uptake in the baseline and follow-up scans. Note that different tumors may qualify as the target lesion in the baseline and follow-up scans. Complete metabolic response (CMR) is defined as target lesion SUL_{peak} < liver SUL and no tumors (including the target lesion) with visually higher uptake than surrounding background in the follow-up scan. Partial metabolic response (PMR) is defined as reduction of target lesion SUL_{peak} by at least 30% with no increase >30% in the size or SUL_{peak} of any other lesion, and no new FDG-avid lesions. Progressive metabolic disease (PMD) is >30% increase in target lesion SUL_{peak}, or visible increase relative to baseline in the volumetric extent of FDG tumor uptake with no decline in SUL, or any new

FDG-avid lesions. Stable metabolic disease (SMD) is the absence of any of the other 3 possible responses.

PERCIST is designed to support response assessment at the patient level. Patients with metastatic breast cancer typically have multiple quantifiable lesions. (We evaluated as many as 10/patient in our preliminary study.) In order to leverage the statistical power inherent in that multiplicity, we will extend PERCIST to individual tumors by applying the rules as they would be were the patient to have only one evaluable tumor. Thus, an individual tumor will be classified as (i) CMR if its image intensity on follow-up is < liver and not > its surroundings; (ii) PMR if its SUL_{peak} declines more than 30% and its size does not increase more than 30% relative to baseline, (iii) PMD if its SUL_{peak} and/or size increases more than 30% relative to baseline, and (iv) SMD if not CMR, PMR or PMD.

Patients will be considered responders if they achieve CMR or PMR while on protocol therapy (prior to progressive disease or subsequent therapy). Patients with confirmed responses (meeting the criteria for response on two consecutive measurements) will be classified as being confirmed responders. ^{18}F-FDG PET-CT performed prior to ^{64}Cu-DOTA-trastuzumab PET-CT will provide the baseline scan for response assessment. Follow-up ^{18}F-FDG PET-CT will be obtained at 6-week intervals for one year, i. e., after each two cycles of ado-trastuzumab emtansine. After one year a PET/CT or CT scan can be performed at the discretion of the treating physican.

14.0 Laboratory Evaluations

14.1 Biomarker assessment: Although analysis of biomarkers is not included in the current pilot study, left over tumor from the required biopsy will be stored in Pathology for future analysis. We anticipate that high uptake of ^{64}Cu-DOTA-trastuzumab will not predict response in some tumors and patients due to the presence of molecular mechanisms of resistance (MMRs) to ado-trastuzumab emtansine. A core biopsy or an excisional biopsy will be performed to confirm recurrent disease within 28 days of ado-trastuzumab emtansine infusion. After the diagnosis and *HER2* status have been determined, residual tumor tissue will be stored in Pathology for future assessment of putative biomarkers of DM1 and trastuzumab resistance by IHC staining and/or genomic analysis.

14.2 Residual circulating trastuzumab from prior treatment: Patients will have recently been treated with trastuzumab and chemotherapy. Residual trastuzumab from those treatments could interfere with tumor uptake of ^{64}Cu-DOTA-trastuzumab, although prior experience suggests that will not be the case. We address this issue in the study protocol by (i) restricting time since last trastuzumab administration to $\geq$ 6 wk prior to the ^{64}Cu-DOTA-trastuzumab procedure, and (ii) collecting blood samples which could be analyzed for residual trastuzumab. 1 Red-top tube (7 ml) will be collected, kept at room temperature and sent to Dr. Colcher's lab for processing and storage each time. We will store venous blood taken from patients just prior to infusion of the trastuzumab pre-dose and compare tumor uptake of ^{64}Cu-DOTA-trastuzumab with our prior results in HER2-positive patients.[18] If uptake is, on average, lower than expected, we will attempt to account for that by measuring trastuzumab concentrations in the blood samples and correlating those data with tumor uptake in the respective patients.

15.0 Statistical Analysis

This is a pilot study of 10 patients to explore the relationship between tumor uptake of ^{64}Cu-DOTA-trastuzumab as measured by PET-CT and metabolic tumor response to ado-trastuzumab emtansine as assessed by ^{18}F-FDG/PET-CT. Results from this study will help guide future evaluations of ^{64}Cu-DOTA-trastuzumab/PET-CT for use in

individualizing treatment of metastatic breast cancer with ado-trastuzumab emtansine or other trastuzumab-antibody drug conjugates.

Specifically, we are seeking to:

1. Correlate uptake of ^{64}Cu-DOTA-trastuzumab by individual tumors with subsequent tumor response to ado-trastuzumab emtansine as assessed by serial ^{18}F-FDG/PET-CT. We expect an average of 5 evaluable tumors per patient. Thus, we expect to have an initial evaluation of 50 tumors. This will provide sufficient power to detect a correlation between pre-treatment tumor uptake of ^{64}Cu-DOTA-trastuzumab and change in tumor uptake of ^{18}F-FDG.

   Percentage change in SUL_{peak} [$\%\Delta SUL_{peak}$(FDG) = 100 x (follow-up – baseline)/baseline] will be used as the metric for change in tumor uptake of ^{18}F-FDG. We will evaluate $\%\Delta SUL_{peak}$(FDG) vs. several alternative metrics of ^{64}Cu-DOTA-trastuzumab uptake [SUV_{max}, SUV_{peak}, SUV_{wt}(FDG-matched) and the ratio SUV_{wt}(FDG-matched)/ SUV_{wt}(FDG)]. If we observe appreciable changes in tumor size between baseline and follow-up, we will also correlate the various metrics of ^{64}Cu uptake with percentage change in ^{18}F-FDG "total lesion glycolysis" [23] [TLG = SUV_{wt}(FDG) x volume within which SUV_{wt}(FDG) is evaluated].

   With approximately 50 lesions, we will have 90% power to detect a difference between a null hypothesis regression slope of 0 (comparing selected ^{64}Cu-DOTA-trastuzumab metric and change in FDG SUL_{peak} or TLG and an alternative regression slope of 0.47, assuming that the standard deviation of the predictor variable is 4.3 (the observed standard deviation from preliminary data on SUV_{max} for ^{64}Cu-DOTA-trastuzumab/PET-CT) and the standard deviation of the residuals equal that of the predictor variable, using a t-test with a 0.05 two-sided significance level.

   In addition, we will also compare ^{64}Cu-DOTA-trastuzumab uptake metrics between "responding" (i. e. CMR + PMR) and "non-responding" (SMR + PMD) tumors. We expect about 40% (i. e. about 20) of the 50 tumors to respond [14]. With 20 responding tumors and 30 non-responding tumors, using a t-test with 0.05 one-sided significance level, there is 85% power to detect a difference in means between responding and non-responding tumors, assuming a common standard deviation of 4.3.

   A hierarchical model will be also be explored to evaluate the role of tumor-within-patient effects.

   Assuming that correlation is observed, the metric(s) of ^{64}Cu-DOTA-trastuzumab tumor uptake that best correlates with change in FDG uptake and/or best separates responding from non-responding tumors will be used in studies going forward.

2. Compare tumor uptake of ^{64}Cu-DOTA-trastuzumab PET between patients who do and patients who do not respond to ado-trastuzumab emtansine.

   We will compare ^{64}Cu-DOTA-trastuzumab uptake between "responding" (i. e. CMR + PMR) and "non-responding" (SMR + PMD) patients. Metrics of overall upake will include (i) VOI volume-weighted average SUV_{wt}(FDG-matched) over all evaluated tumors (a putative measure of relative overall ado-trastuzumab emtansine dose per unit tumor volume); and (ii) VOI volume-weighted average SUV_{wt}(FDG-matched)/SUV_{wt}(FDG) over all evaluated tumors (a putative measure of relative overall ado-trastuzumab emtansine dose per viable tumor cell). We will also explore the response criteria based on the single tumor with max uptake (per PERCIST).

We expect 4 responders among the 10 patients [14]. Although such a small sample is unlikely to yield a significant difference between the 2 groups, we may see a trend (responders substantially > non-responders or vice-versa) that either encourages or discourages further investigation. This comparison is aimed at all responders, but we will also separately evaluate confirmed responders, and responders at the first evaluation.

Further analysis on cut-points, ROC analysis, considering patients as responders or not, will also be analyzed in an exploratory fashion.

3 Obtain tumor tissue for subsequent assessment of the presence of putative molecular mechanisms of resistance (MMRs) to ado-trastuzumab emtansine.

Tumor biopsy material will be evaluated when funding becomes available, to help reduce the variability in response by adjusting for putative MMRs to ado-trastuzumab emtansine. The samples will be used to explore the correlation between the presence of MMRs as assessed by histopathology and tumor response to ado-trastuzumab emtansine both in univariate analysis and in combination with tumor uptake of ^{64}Cu-DOTA-trastuzumab as measured with PET/CT.

The information gained from this pilot study will determine whether ^{64}Cu-DOTA-trastuzumab/PET-CT warrants further evaluation for individualizing treatment with ado-trastuzumab emtansine. If so, the pilot study will provide measures of variability and help select the proper metrics) for larger studies.

16.0 Toxicity Assessment / Follow Up

16.1 Patients will be monitored for toxicity during and immediately after infusion of the trastuzumab imaging pre-dose and ^{64}Cu-DOTA-trastuzumab according to the study calendar.

16.2 Participants will begin ado-trastuzumab emtansine therapy within 3 days after completion of ^{64}Cu-DOTA-trastuzumab/PET-CT. Side effects not known to be related to ado-trastuzumab emtansine therapy will be recorded.

16.3 Participants will be examined by the treating physician on days 7 and 14, and a complete blood count will be obtained. If there are any clinical concerns about cardiac toxicity, a MUGA will be obtained.

16.4 Adverse events, serious adverse events, toxicities, conmeds, will be collected 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 (Mary Carroll

RN, Phyllis Broene RN) 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 Confidentiality and Storage of Records: Data will be stored in encrypted, password protected, secure computers that meet all HIPAA requirements.

When results of this study are reported in medical journals or at meetings, identification of those taking part will be withheld. Medical records of subjects will be maintained in 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.

17.4.2 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.

^{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 COH data collection forms via an electronic data capture system, Medidata RAVE. Any original data collections forms will reside in the Clinical Trials Office.

17.4.3.1 The Eligibility Checklist must be completed by a protocol nurse or clinical research coordinator 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 coordinator will submit all baseline forms located in Medidata RAVE.

17.4.3.3 Within two weeks of imaging or end of cycle, the clinical research coordinator will submit the following forms:

- Treatment Form
- Response/Follow-Up
- Adverse Events Collection Form
- Please refer to Medidata RAVE for a complete listing of all case report forms associated with this protocol.

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 Data and Safety Monitoring Plan", http://www.coh.org/dsmc/Pages/forms-and-procedures.aspx involving COH as IND holder.

18.2 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

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

Risk Level	Phase	Standard Reporting Requirement
RL 1, RL2, and Compassionate Use 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: tumor uptake on Cu-DOTA trastuzumab PET and infusion reactions.

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18.3 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] is 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 dyscrasisas 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.

18.4 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/hrpp/Pages/hrpp-policies.aspx. 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 - 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/hrpp/Pages/hrpp-policies.aspx 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

Required Reporting Timelines to DSMC for AE/SAEs
Investigator Initiated Studies

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	
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
	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	5 calendar days	10 calendar days
Unlikely, Unrelated	10 calendar days	10 calendar days

Externally Sponsored Studies

Required Reporting Timeframe to DSMC		
Attribution	**UNEXPECTED[1]**	**EXPECTED**
	Death while on active treatment or within 30 days of last day of treatment	
Possibly, Probably, Definitely	No DSMC reporting required - IRB reporting may be necessary	
Unlikely, Unrelated		
	Death after 30 days of last active treatment/therapy	
Possibly, Probably, Definitely	No DSMC reporting required - IRB reporting may be necessary	
Unlikely, Unrelated		
	Grades 3 and 4 AND meeting the definition of "serious"	
Possibly, Probably, Definitely	No DSMC reporting required - IRB reporting may be necessary	
Unlikely, Unrelated		
	Grades 1 and 2	
Possibly, Probably, Definitely	No DSMC reporting required - IRB reporting may be necessary	

An event determined by the IRB of record to be an Unanticipated Problem (UP) will be communicated to the Investigator and COH DSMC through the COH IRB Operations Director. The DSMC will review the case and make a determination as to whether the study will be suspended, terminated, amended, or allowed to continue without amendment.

Required Reporting Timeframe to IRB of Record		
Attribution	**UNEXPECTED**	**EXPECTED**
	Death	
Possibly, Probably, Definitely	5 calendar days	Annual
Unlikely, Unrelated	Annual	Annual
	Grades 3 and 4 AND meeting the definition of a UP	
Possibly, Probably, Definitely	5 calendar days	Annual
Unlikely, Unrelated	Annual	Annual
	Grade 1 and 2 AND meeting the definition of a UP	
Possibly, Probably, Definitely	5 calendar days	Annual
Unlikely, Unrelated	Annual	Annual

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)]

19.0 Protocol Deviations

Protocol deviations using the Protocol Deviation Form must be submitted by the protocol nurse or the clinical research coordinator to the Coordinator (cccp@coh.org) for distribution. The clinical research coordinator at City of Hope will also submit copies to the Protocol Management Team and the City of Hope Data and Safety Management Board.

19.1 In accordance with the City of Hope Policy on Clinical Research Protocol Deviation, there will be a “no deviations” rule for this protocol. However, for subject safety or unforeseen scheduling problems, planned deviations from this protocol will be permitted with approval from the IRB in the form of a Single Subject Amendment. In addition, the sponsor (the CDMRC) must also approve any planned deviations.

19.2 All unplanned deviations will be reported to the City of Hope DSMC.

19.3 If there is a dispute among the persons involved in the provision of research treatment, in regard to whether a treatment deviates from the protocol, the facts of the case will be reported to the DSMC which will be serve as the arbiter of whether a deviation exists.

19.4 **Study attrition**: Participants who withdraw from study before completion of both imaging procedures will be replaced by another study participant.

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