Status Page

PROTOCOL 14-409

Closed to New Accrual

Closure Effective Date: 02/09/2018
No new subjects may be enrolled in the study as described above. Any questions regarding this closure should be directed to the study's Principal Investigator

Date Submitted: ***[08/17/2016]***

Alert Page

DF/HCC Protocol #: **14-409**

Protocol Clarifications (non-drug related e.g. eligibility criteria, study assessments)

Protocol Section 7.4.3: **Reminder to study teams:**

- Infusion associated reactions or hypersensitivity of any grade must be reported to Genentech as an AE of Special Interest (AESI) within fifteen (15) calendar days of the awareness date of the event.

Table 10.1 Required Tests and Procedures

- Serum β-HCG test is permitted in lieu of urine pregnancy test

DANA-FARBER CANCER INSTITUTE
DANA-FARBER / HARVARD CANCER CENTER

Office for Human Research Studies

Protocol Front Sheet

DFCI Protocol No.: 14-409

1. PROTOCOL INFORMATION

Title: The impact of HER2 heterogeneity on the treatment of early-stage HER2-positive breast cancer: a phase II study of T-DM1 in combination with Pertuzumab in the preoperative setting

Phase: Phase 2 **Sponsor Study Number:** ML29408

2. DF/HCC STUDY CONTACT INFORMATION

Primary Study Contact: Eileen Wrabel **Email:** ewrabel@partners.org **Phone:** 617-632-3684

INVESTIGATORS: (List only those under DFCI IRB, *i.e.*, from institutions listed in Section 6 below)

Overall PI:	Ian Krop, MD, PhD	**Phone:**	617.632.6973	**Institution(s):**	DFCI	**Mgmt group:**	Breast Oncology
Site Responsible PI:	Ian Krop, MD, PhD	**Phone:**	617.632.6973	**Institution(s):**	BWH @ FH	**Mgmt group:**	Breast Oncology
	Aditya Bardia		617-643-2208		MGH		

3. DRUG / DEVICE INFORMATION N/A:

☒ **Drug(s), Biologic(s)**: Pertuzumab and T-DM1
Provided by: Genentech Inc.
IND Exempt: ☒ -or-
IND#: N/A **Holder Type:** [pull down]
IND Holder Name:

☐ **Device(s) Name**:
Provided by:
IDE Exempt: ☐ -or-
IDE #: **Holder Type:** [pull down]
IDE Holder Name:

4. PROTOCOL COORDINATION, FUNDING, MODE

Regulatory Sponsor:
DF/HCC Investigator Ian Krop, MD, PhD

CTEP Study: No

Funding/Support (check all that apply):
☒ Industry: Genentech, Inc.
☐ Federal Organization:
Grant #:
☐ Internal Funding:
☐ Non-Federal:
☐ Other:

Protocol Involves (check all that apply as listed in the protocol document, even if not part of the research but is mandated by the protocol document):

☒ Chemotherapy
☐ Immunotherapy
☒ Surgery
☐ Bone Marrow/Stem Cell Transplant
☐ Cell Based Therapy
☐ Gene Transfer (use of recombinant DNA or synthetic nucleic acid molecules)
☐ Radiation Therapy
☐ Hormone Therapy
☐ Vaccine
☐ Engineered Cell Therapy (ECT)
☐ Data Repository
☐ Exercise/Physical Therapy
☒ Genetic Studies
☒ Human Material Banking
☒ Human Material Collection
☐ Medical Record Review
☐ Questionnaires/Surveys/Interviews
☒ Radiological Exams
☒ Required Biopsy Study
☐ Human Embryonic Stem Cell
☐ Quality of Life
☐ Other:

5. SUBJECT POPULATION (also applies to medical record review and specimen collection studies)

Total Study-Wide Enrollment Goal: 165 **Greater than 25% of the overall study accrual will be at DF/HCC:** ☒ Yes ☐ No

Total DF/HCC Estimated Enrollment Goal: 80 **Adult Age Range:** 18+ **Pediatric Age Range:** N/A

Will all subjects be recruited from pediatric clinics? ☐ Yes ☒ No

If enrolling both adults and pediatric subjects, anticipated percent of pediatric subjects: N/A

Retrospective Medical Record Reviews only (Please provide date range): from to

6. SITES UNDER DFCI IRB (check all that apply)

DF/HCC Main Sites:
☐ Beth Israel Deaconess Medical Center (BIDMC)
☐ Boston Children's Hospital (BCH)
☒ Brigham and Women's Hospital (BWH)
☒ Dana-Farber Cancer Institute (DFCI)
☒ Massachusetts General Hospital (MGH)

DF/PCC Affiliate Sites:
☐ Cape Cod Healthcare (CCH)
☐ Lowell General Hospital (LGH)
☐ New England Cancer Specialists (NECS)

☐ **Broad Institute**

Harvard Catalyst Member(s): (list institution/location)

DF/HCC Satellite Sites:
☐ Beth Israel Deaconess Medical Center – Needham (BIDMC-Needham)
☐ Dana-Farber/New Hampshire Oncology-Hematology (DFCI @ NHOH)
☐ Dana-Farber at Steward St. Elizabeth's Medical Center (DFCI @ SEMC)
☐ Dana-Farber at Milford Regional Cancer Center (DFCI @ MRCC)
☐ Mass General/North Shore Cancer Center (MGH @ NSCC)
☐ Mass General at Emerson Hospital – Bethke (MGH @ EH)
☐ Mass General at Newton-Wellesley Hospital (MGH @ NWH)
☐ DF/BWCC in Clinical Affiliation with South Shore Hospital (DFCI @ SS)
☒ Brigham and Women's Hospital at Faulkner Hospital (BWH @ FH)

7. DF/HCC INITIATED STUDIES ONLY - SITES UNDER OTHER IRB (N/A:)

DF/HCC Multi-Center Sites: (list institution/location)

Sarah Cannon Research Institute	Nashville, TN
Tennessee Oncology Franklin	Franklin, TN
Tennessee Oncology Hermitage	Hermitage, TN
Vanderbilt University Medical Center	Nashville, TN
MidAmerica Division, Inc. c/o Menorah Medical Center	Kansas City, MO

Multicenter Site(s) with Master CTA: (list institution/location)

Protocol Number: 14-409

Approval Date: 10/30/14 (IRB meeting date when protocol/consent approved or conditionally approved)

Activation Date: 12/29/14 (Date when protocol open to patient entry)

Approval signatures are on file in the Office for Human Research Studies, tel. 617-632-3029.

Date Posted	Revised Sections	IRB Approval Date	OHRS Version Date
12/29/14	Front Sheet revised due to Amendment #1	11/25/14	N/A
01/06/15	Delayed Activation Alert Page replaced: BIDMC now ready for activation (note: previously activated at DFCI/BWH on 12/29/14; MGH site still pending)	N/A	N/A
03/03/15	Eligibility Checklist replaced due to Amendment #2	02/26/15	N/A
03/05/15	Delayed Activation Alert Page removed: MGH now ready for activation (note: previously activated at DFCI/BWH on 12/29/14; BIDMC on 01/06/15)	N/A	N/A
04/20/15	Protocol/PES, Eligibility Checklist, Consent Form and Front Sheet replaced due to Amendment #3	03/19/15	04/20/15
Date Posted	**Revised Sections**	**IRB Approval Date**	**OnCore Version Date**
10/20/15	Study renewal/ Consent Form footer replaced due to Continuing Review #1	10/01/15	10/13/15
10/29/15	Front Sheet replaced due to Amendment #4	10/27/15	N/A
11/23/15	Front Sheet replaced due to Amendment #5	11/10/15	N/A
01/11/16	Consent Form, Protocol and Front Sheet replaced; Alert Page added - due to Amendment #6	01/07/16	01/08/16
03/29/16	Protocol and Eligibility Checklist replaced due to Amendment #7	03/24/16	N/A
04/12/16	Correction Am #7: Protocol replaced (wrong protocol was submitted with original Am #7)	(03/24/16)	N/A
06/03/16	Consent Form and Front Sheet replaced due to Amendment #8	06/02/16	06/02/16
09/08/16	Alert Page replaced due to Amendment #9	08/26/16	N/A
09/22/16	Study renewal/ Consent Form footer replaced due to Continuing Review #2	09/08/16	09/12/16
10/06/16	Consent Form and Front Sheet replaced due to Amendment #10	09/30/16	10/05/16
04/14/17	Front Sheet replaced due to Amendment #11	04/10/17	N/a
08/22/17	Study renewal/ Consent Form footer replaced due to Continuing Review # 3	08/17/17	08/22/17
10/12/17	Protocol, Front Sheet and Eligibility Checklist replaced due to Amendment #12	09/14/17	N/A
02/16/2018	Permanent Closure to New Accrual: due to Objective(s)/Accrual Met (effective date: 02/09/2018; Amendment #13	02/15/2018	n/a
07/17/2018	Study renewal/ Consent Form footer replaced due to Continuing Review # 4	07/12/2018	07/16/2018
05/07/2019	Study renewal/ Consent Form footer replaced due to Continuing Review #5	05/07/2019	05/07/2019

Protocol Date: **July 28, 2017**

Local Protocol #: *14-409*

Title: *The impact of HER2 heterogeneity on the treatment of early-stage HER2-positive breast cancer: a phase II study of T-DM1 in combination with Pertuzumab in the preoperative setting*

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Agent(s): T-DM1, *supplied by Genentech*; Pertuzumab*, supplied by Genentech*

Study Exempt from IND Requirements per 21 CFR 312.2(b).

Protocol Type/Version #/Version Date: *Sponsor Amendment 4 /Version 5/Version Date: July 28, 2017*

SCHEMA

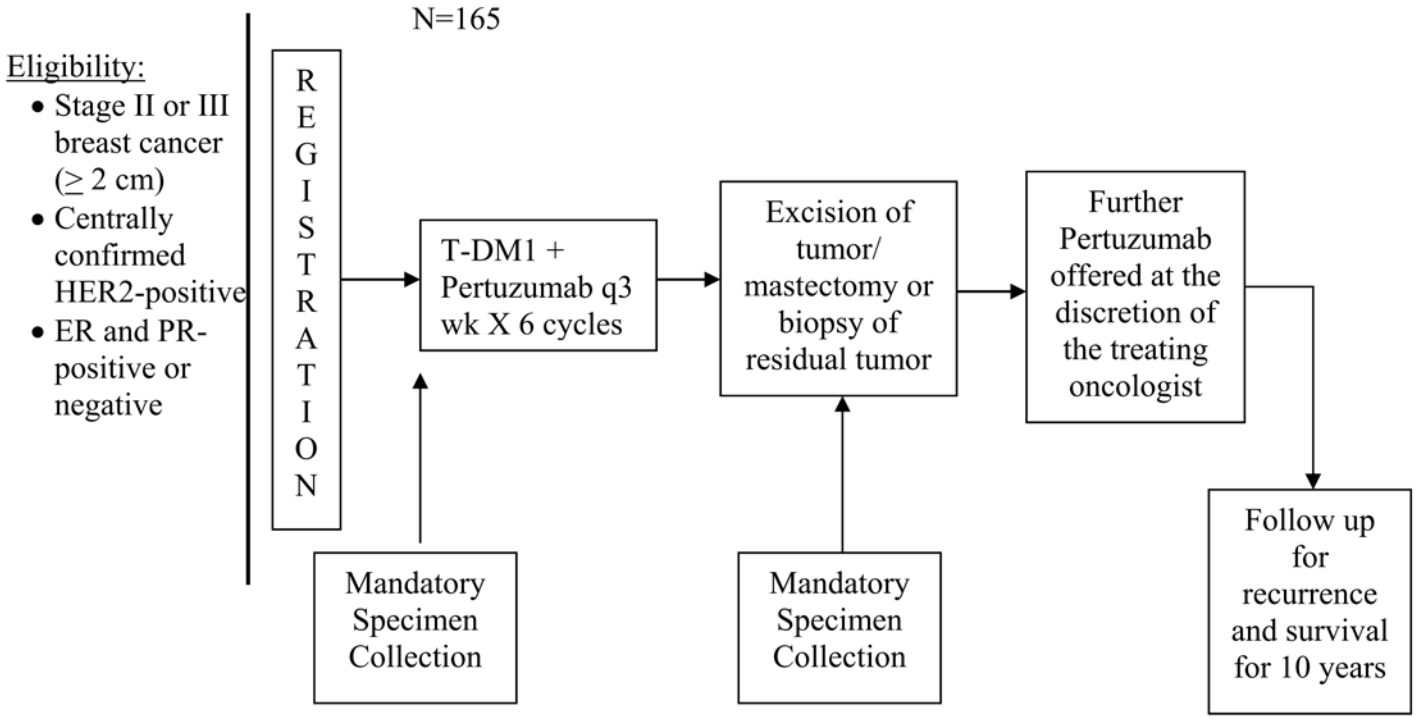

N=165
Eligibility:
• Stage II or III breast cancer (≥ 2 cm)
• Centrally confirmed HER2-positive
• ER and PR-positive or negative
REGISTRATION
T-DM1 + Pertuzumab q3 wk X 6 cycles
Excision of tumor/ mastectomy or biopsy of residual tumor
Further Pertuzumab offered at the discretion of the treating oncologist
Follow up for recurrence and survival for 10 years
Mandatory Specimen Collection
Mandatory Specimen Collection

TABLE OF CONTENTS

1. OBJECTIVES

1.1 Study Design

This is an open label phase II neoadjuvant clinical trial of trastuzumab emtansine (T-DM1) given in combination with pertuzumab for HER2-positive early-stage breast cancer. The planned sample size is 165 patients. Two ultrasound-guided core biopsies from different geographic areas of the same tumor will be performed to assess intratumor heterogeneity of HER2 prior to initiation of study therapy. Patients will receive T-DM1 3.6 mg/kg IV every 3 weeks for 6 doses plus pertuzumab loading dose 840 mg IV on Cycle 1 Day 1 followed by maintenance dose 420 mg IV every 3 weeks for a total of 6 doses, for a total of 18 weeks of treatment. Disease response assessment will be evaluated at baseline and prior to surgery with imaging, and clinically every three weeks during study therapy. Routine imaging is not required during the neoadjuvant treatment, but is recommended for patients not experiencing a clinical response. Definitive breast cancer surgery (excision or mastectomy) marks the end of protocol mandated therapy. Pathologic response determined by the Residual Cancer Burden calculator will be used for the primary and secondary endpoints of this study. Decisions regarding choice of post-surgical systemic therapy will be made by the treating oncologist. However, participants enrolled in this study should receive trastuzumab-based chemotherapy after surgery since long-term data using T-DM1/pertuzumab alone as neoadjuvant treatment of breast cancer is not available. Additional pertuzumab may be given after surgery at the discretion of the treating physician.

1.2 Primary Objective

To evaluate the relationship between pathologic complete response (pCR) and intratumor heterogeneity of HER2 amplification

1.3 Secondary Objectives

- To assess the rate of pCR after preoperative T-DM1 plus pertuzumab in HER2-positive breast cancer
- To determine the clinical response rate, defined as the number of partial and complete responses after preoperative T-DM1 plus pertuzumab in HER2-positive breast cancer
- To determine the relationship between hormone receptor (HR) status and intratumor heterogeneity of HER2 amplification
- To characterize safety and tolerability of T-DM1 plus pertuzumab
- To evaluate the relationship between pCR and HER2 heterogeneity assessed as a continuous variable
- To determine if T-DM1 plus pertuzumab preoperative therapy enriches for HER2-negativity or HER2 heterogeneity in residual tumors
- To determine whether T-DM1 plus pertuzumab activity defined by pCR is affected by the degree of intratumor heterogeneity of HER2 expression determined by Immuno-FISH
- To describe disease-free and overall survival in patient groups defined by HER2 heterogeneity who are treated with T-DM1 plus pertuzumab.

2. BACKGROUND

2.1 Study Disease(s)

HER2-positive breast cancers usually display several genomic aberrations, but remain critically dependent on HER2 signaling for survival and growth. Trastuzumab was the first agent developed to target the HER2 pathway. The addition of trastuzumab to adjuvant chemotherapy has resulted in a striking reduction in the risk of relapse and death by 50% and 30% respectively [1-4]. These results led to the approval of trastuzumab for use in the early disease setting.

In addition to large registration studies, clinical trials conducted in the preoperative setting have contributed to the understanding of the activity of anti-HER2 therapies with the ability to provide an early read-out of drug efficacy. In the "first generation" of neoadjuvant anti-HER2 trials, patients were randomized to receive chemotherapy with or without trastuzumab [5-7]. These seminal studies confirmed the superiority of neoadjuvant trastuzumab in combination with chemotherapy, showing pCR rates at least two times higher in the trastuzumab containing arms (summarized in Table 1). In the "second generation" of neoadjuvant anti-HER2 trials, patients were randomized to receive one of two different anti-HER2 agents or to receive trastuzumab with or without another anti-HER2 agent in an effort to further improve the clinical benefit achieved with trastuzumab alone (Table 1) [8-10].

Table 1: Representative first and second generation neoadjuvant trials of HER2-targeted therapies

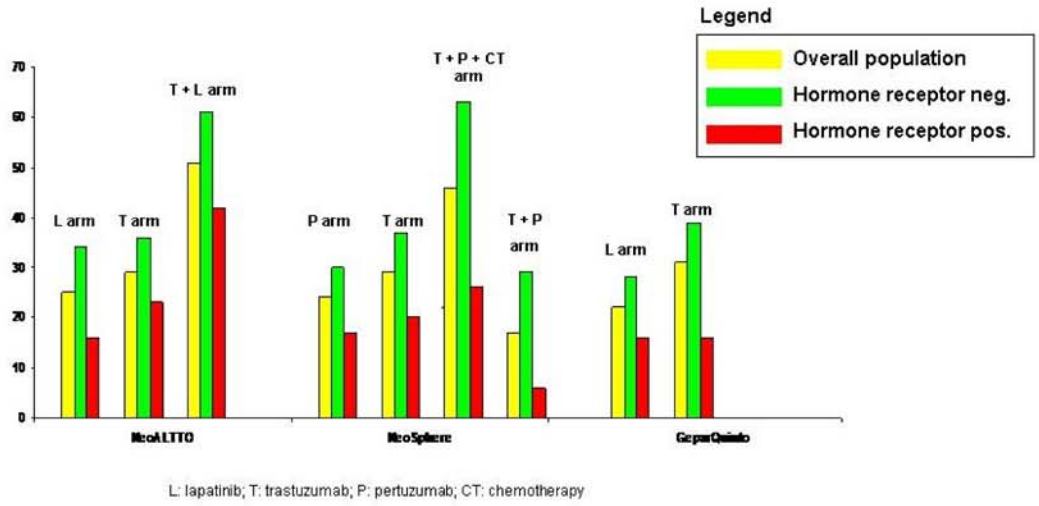
Studies and sample size	Study design	pCR * rates, p values
M.D. Anderson [5] N = 64	1) T→FEC	26.3
	2) T + H→FEC + H	60 (P = NR)
NOAH [11] N = 235	1) AT→T→CMF	19
	2) AT +H→T+ H→CMF +H	38 (P = 0.001)
NeoALTTO [12] N = 455	1) H → T + H → surgery → FEC → H until w 52	29.5
	2) L → T → surgery → FEC → L until w 52	24.7 (2 vs. 1; P = 0.34)
	3) H + L → T + H + L → surgery → FEC → H + L until w 52	51.3 (3 vs. 1; P = 0.0001)
NeoSphere [13] N = 417	1) H + D → surgery → FEC → H until w 52	29.0
	2) H + PZ + D → surgery → FEC → H until w 52	45.8 (2 vs. 1; P = 0.0141)
	3) H + PZ → surgery → H + D → FEC → H until w 52	16.8 (3 vs. 1; P = 0.019)
	4) PZ + D → surgery → FEC → H until w 52	24.0 (4 vs. 2; P = 0.03)
NSABP B41 N = 529 [14]	1) AC → T + H → surgery → H until w 52	52.5
	2) AC → T + L → surgery → H until w 52	53.2 (2 vs. 1; P = 0.99)
	3) AC → T + H+ L → surgery → H until w 52	62.0 (3 vs. 1; P = 0.09)
CALGB 40601 N = 301 [15]	1) T + H → surgery → ddAC → H until w 52	46.0
	2) T + L → surgery → ddAC → H until w 52	37.0 (2 vs. 1; P =0.12)
	3) T + H + L → surgery → ddAC → H until w 52	56.0 (3 vs. 1; P = 0.12)
GeparQuinto [16] N = 620	1) EC + H → D + H → surgery	31.3
	2) EC + L → D + L → surgery	21.7 P < 0.05

Abbreviations: A = doxorubicin; C = cyclophosphamide; D= docetaxel; E = epirubicin; F = fluorouracil; H = trastuzumab; L = lapatinib; NR = not reported; PZ = pertuzumab; pCR = pathologic complete response; T = paclitaxel; X = capecitabine. Comments: MD Anderson and NOAH studies have chemotherapy-only arms compared with chemotherapy plus trastuzumab (first generation studies). NeoALTTO, NeoSphere, GeparQuinto, NSABP-B41 and CALGB 40601 represent the second generation of neoadjuvant studies with anti-HER2 regimens in all study arms. pCR definitions: MD Anderson – No evidence of invasive cancer in breast or axilla; NOAH – Total pCR in breast and axillary nodes; NeoALTTO – non invasive cancer in the breast or only non invasive in situ cancer; NeoSphere – pathologic complete response in the breast GeparQuinto – no microscopic evidence of residual viable cells in any specimen.

While these second generation neoadjuvant studies hold considerable promise, it must be acknowledged that the pCR definitions used across these trials were not homogeneous (pCR

definitions are described in Table 1), limiting cross-trial analysis. However, one result which has been consistent across all of these studies is that patients with HER2-positive hormone receptor-positive cancers have lower rates of pCR than those with HER2-positive hormone receptor-negative cancers (detailed in Figure 1).

Figure 1: Pathologic complete response rates to neoadjuvant anti-HER2 therapies according to hormone receptor status



Abbreviations: Chemo = chemotherapy; neg = negative; pos = positive; T = trastuzumab, L = lapatinib, P = pertuzumab.

Over the past years there have been significant improvements in our understanding of the biology of HER2-positive disease and several novel anti-HER2 drugs have been approved for the treatment of HER2-positive breast cancer.

Pertuzumab, another HER2-targeted humanized monoclonal antibody, represents the first of a novel class of drugs that have the ability to block the heterodimerization of HER2 with other members of the HER family (e.g., HER1, HER3). The resulting complimentary and enhanced efficacy of HER2 blockade that is provided by the combination of trastuzumab plus pertuzumab has been demonstrated in both the preoperative setting (i.e. NeoSphere) and in the metastatic setting where this combination of antibodies along with docetaxel leads to improve overall survival compared with trastuzumab and docetaxel [17].

2.2 Agent(s)

The use of T-DM1 and Pertuzumab in the early-stage setting in the current study follows safety data provided by studies conducted in both advanced and early setting as summarized below.

2.2.1 Trastuzumab emtansine (T-DM1)

T-DM1 in the early disease setting

In the (neo)adjuvant setting, a phase 2 clinical trial (TDM4874g) [18] evaluated safety and rate of pre-specified cardiac events occurring within the first 12 weeks of T-DM1 treatment. Patients were treated with a total of 12 months of T-DM1 after completion of anthracycline-containing chemotherapy as adjuvant or neoadjuvant therapy for early-stage HER2-positive breast cancer. A cardiac event was defined as a death from a cardiac cause or severe CHF (New York Heart Association [NYHA] Class III or IV) with a decrease in LVEF of 10 percentage points or more from baseline to an LVEF of less than 50%. Among the 148 T-DM1-treated patients, no protocol pre-specified cardiac events occurred in the 143 cardiac-evaluable patients.

T-DM1 in advanced breast cancer

Two Phase II studies—TDM4258g and TDM4374g [19, 20]—evaluated the safety and efficacy of T-DM1 administered at a dose of 3.6 mg/kg (maximum tolerated dose [MTD] in Phase I) every 3 weeks until progressive disease (PD) or unacceptable toxicity in HER2-positive metastatic breast cancer patients who had progressed on previous HER2-directed therapy and conventional chemotherapy. Although patients enrolled into both studies had received multiple agents for the treatment of breast cancer, patients enrolled onto Study TDM4374g were specifically required to have received an anthracycline, trastuzumab, a taxane, lapatinib, and capecitabine in the neoadjuvant, adjuvant, or metastatic setting or as treatment for locally advanced disease; additionally, patients must have been treated with two or more HER2-directed regimens in the metastatic or locally advanced setting and must have progressed on their most recent treatment. Patients were required to have known HER2 overexpression as previously determined by local laboratory testing with immunohistochemistry (IHC) or HER2 gene amplification by fluorescence in situ hybridization (FISH).

The clinical activity of T-DM1 was similar in the two studies. In Study TDM4258g, on the basis of the final analysis approximately 12 months after the last patient was enrolled, the overall response rate (ORR) in efficacy evaluable patients was 38.9% (95% confidence interval [CI], 29.7%, 48.5%) by investigator and 26.9% (95% CI, 19.2%, and 35.8%) by independent review. The clinical benefit rate (CBR) (defined as complete response [CR], partial response [PR], or stable disease for > 6 months) was 46.3% by investigator assessment (95% CI, 36.7%, 56.2%) and 40.7% by independent review (95% CI, 31.8%, 50.6%). The median progression-free survival (PFS) was 4.6 months by both the investigators and the independent review facility (IRF) assessment. In Study TDM4374g, on the basis of clinical data collected through 1 January 2010, approximately 9 months after the last patient had enrolled, the ORR among all treated patients was 34.5% (95% CI, 26.1%, 43.9%) by IRF assessment and 32.7% (95% CI, 24.1%, 42.1%) by investigator assessment. The CBR was 48.2% (95% CI, 38.8%, 57.9%) by IRF assessment and 46.4% (95% CI, 37.1%, 56.1%) by investigator assessment. The median duration of response was not reached (95% CI, 4.6 months, not reached) by IRF assessment and 9.7 months (95% CI, 6.6 months, not reached) by investigator assessment. In this study population, there was a median PFS of 6.9 months

(95% CI: 4.2, 9.5) as assessed by the IRF and 5.5 months (95% CI: 4.1, 7.5) by investigator review.
The safety profile of T-DM1 was also similar between the two studies.
In Study TDM4258g, the five most common adverse events (AEs) were fatigue (65.2%), nausea (50.9%), headache (40.2%), epistaxis (35.7%), and pyrexia (34.8%). Most of these events were Grade 1–2. The three most common Grade 3–4 AEs observed in this trial were hypokalemia (8.9%), thrombocytopenia (TCP) (8.0%), and fatigue (4.5%). In Study TDM4374g, a total of 49 patients (44.5%) experienced at least one Grade ≥ 3 AE. The three most common Grade ≥ 3 adverse events (by Medical Dictionary for Regulatory Activities [MedDRA] preferred terms) were TCP (7.3%), fatigue (4.5%), and cellulitis (3.6%). Serious adverse events (SAEs) were reported in 25 patients (22.7%). No single SAE was reported in more than 4 patients. No Grade ≥ 3 left ventricular systolic dysfunction (LVSD) events (symptomatic congestive heart failure [CHF] and/or left ventricular ejection fraction [LVEF] of < 40%) were reported in either study.
TDM4450g [20] is a randomized, multicenter, phase II study of the efficacy and safety of T-DM1 versus trastuzumab plus docetaxel (control arm) in patients with metastatic HER2-positive breast cancer who have not received prior chemotherapy for metastatic disease. This study completed enrollment in December 2009 (n = 137). The primary objectives are to assess the efficacy of T-DM1 compared with the combination of trastuzumab and docetaxel, as measured by PFS on the basis of investigator assessments, and to characterize the safety of T-DM1 compared with the combination of trastuzumab and docetaxel in this population. Secondary endpoints include ORR, survival, and duration of response. No new T-DM1 safety signals were observed. The incidence of Grade ≥ 3 AE on the control arm (75.0%) was twice that of T-DM1 (37.3%). Efficacy data demonstrated a 47.8% ORR in the T-DM1 arm compared to 41.4% in the trastuzumab plus docetaxel arm. Patients on T-DM1 had significantly longer progression-free survival compared with those on standard therapy (14.2 months vs 9.2 months), which amounted to a 41% relative risk reduction in progression (HR 0.59, p=0.035).
EMILIA is a randomized phase III study where T-DM1 monotherapy proved superior when compared to the approved regimen lapatinib plus capecitabine [21]. A total 991 patients were randomized to receive T-DM1 every 3 weeks at 3.6mg/Kg monotherapy or lapatinib (1,250 mg PO daily) plus capecitabine (1000 mg/m2 PO bid, day 1–14 every 3 weeks). All patients had received prior trastuzumab and taxane before entering in the study. Median progression-free survival was 9.6 months in the T-DM1 arm compared with 6.4 months in the capecitabine and lapatinib arm (HR = 0.65, 95% CI; 0.55 to 0.77; p <0.001), and median overall survival at the second interim analysis crossed the stopping boundary for efficacy (30.9 months vs. 25.1; HR = 0.68, 95% CI; 0.55 to 0.85; p <0.001). Overall, fewer Grade 3 ≥ adverse events were observed in the T-DM1 arm (40.8%) than in the lapatinib arm (57%). The most common Grade ≥ 3 adverse events were thrombocytopenia (12.9%), elevated AST (4.3%) and ALT (2.9%), anemia (2.7%) and fatigue (2.4%)
Refer to the Full Prescribing Information for T-DM1 for complete safety information: http://www.accessdata.fda.gov/scripts/cder/drugsatfda/ in addition to information provided in Section 8 of the protocol.

2.2.2 Pertuzumab

Pertuzumab in the early disease setting

Pertuzumab was combined with trastuzumab and/or chemotherapy in two neoadjuvant studies for the treatment of early-stage HER2-positive breast cancer. NEOSPHERE (Neoadjuvant Study of Pertuzumab and Herceptin in an Early Regimen Evaluation) evaluated the efficacy of 12 weeks of preoperative treatment with docetaxel plus pertuzumab or trastuzumab, or the combination of trastuzumab and pertuzumab in 417 women with early-stage HER2-positive breast cancer [22]. A fourth arm in this study assessed the activity of the trastuzumab/pertuzumab combination without accompanying chemotherapy. The primary endpoint of the study was pathologic complete response (pCR) in the breast. Patients treated with the dual anti-HER2 combination plus docetaxel experienced a higher pCR rate (45.8%) compared to those who received only docetaxel/trastuzumab (29%) or only docetaxel/pertuzumab (24%). Interestingly, 17% of patients treated with trastuzumab plus pertuzumab for 12 weeks (without chemotherapy) also achieved pCR, suggesting that a subset of HER2 patients is highly sensitive to these anti-HER2 agents. In a subgroup analysis, lower pCR rates were observed across the four study arms among patients whose cancers were hormone receptor positive, compared to those patients with hormone receptor negative disease. Overall, neutropenia and febrile neutropenia were the most common serious adverse, and occurred at similar frequencies in the chemotherapy-containing arms of the study. The mean maximum decrease in LVEF was 4% to 5% and was similar across the four treatment arms. A total of 6 patients experienced a decrease in LVEF of >10% from baseline, and to less than 50%; five of these 6 patients recovered LVEF to more than 50% by cycle 4, and one patient with a previous history of cardiac disease had to discontinue treatment because of CHF.
In the TRYPHAENA (Trastuzumab plus Pertuzumab in Neoadjuvant HER2-positiveBreast Cancer) study, a total of 225 HER2-positive breast cancer patients were randomized to receive trastuzumab and pertuzumab in combination with one of three chemotherapy regimens: concurrently with an anthracycline-taxane containing regimen (FEC [fluorouracil, epirubicin cyclophosphamide]-docetaxel); after FEC, but concurrently with docetaxel; or concurrently with the docetaxel/carboplatin combination [23]. The primary endpoint of the study was safety and tolerability. LVEF dropped by 6% in the concurrent anthracycline arm; by 4% in the sequential arm; and 3% in the carboplatin/docetaxel arm. Symptomatic LVEF dysfunction (grade 3 or higher) was recorded in 2.7% of the patients. pCR rates were 62%, 57% and 66% in the concurrent anthracycline, sequential and docetaxel/carboplatin treatment arms, respectively.

Pertuzumab in advanced breast cancer

In a single arm phase II study, a total of 11 patients with HER2-positive breast cancer were treated with pertuzumab/trastuzumab [24]. The study had a target accrual of 37 patients but was stopped early when 6 of 11 (54%) patients experienced a decline in left ventricular ejection fraction (LVEF). Cardiotoxicity was more commonly observed among patients who had previously developed LVEF decline while on trastuzumab

therapy, and in all 6 of the patients who had been previously received anthracycline-containing regimens. In addition, all patients had received trastuzumab treatment prior to entering the study, with a cumulative median duration of 82 weeks. The overall response rate (ORR) to the combination treatment was 18% and the median time to progression (TTP) was 6 weeks.

A subsequent single arm phase II study aimed at evaluating the same combination (trastuzumab and pertuzumab) enrolled a total of 66 HER2-positivepatients who had documented disease progression on a previous trastuzumab-based regimen [25]. Patients were excluded if they had experienced a symptomatic decrease in LVEF to less than 50% absolute value during prior trastuzumab therapy. Cardiac safety on the study was carefully monitored, and all echocardiograms were centrally re-evaluated. Three patients experienced a LVEF drop of ≥ 10 percentage points and to less than 50%, but no patient experienced symptoms related to cardiac toxicity. Two of the three patients that experienced LVEF drop remained on trastuzumab/pertuzumab treatment, and experienced subsequent recovery of cardiac function. The ORR was 24%, and the clinical benefit rate (ORR plus stable disease ≥ 6 months) was 50%. The observed benefit was durable, with an overall median PFS of 5.5 months (range 0.9 to 17 months). The promising results of the trastuzumab/pertuzumab combination led to the recruitment of an additional cohort of patients who were treated with pertuzumab monotherapy, in order to investigate whether the trastuzumab was actually required [26]. A total of 29 patients whose disease progressed during prior trastuzumab therapy were treated with single agent pertuzumab until disease progression or unacceptable toxicity. A minimum of four weeks from the last dose of trastuzumab was required to reduce the confounding effect of the prior trastuzumab treatment on pertuzumab efficacy. The ORR and CBR for pertuzumab monotherapy were 3.4% and 10.3%, respectively. Seventeen of 29 patients progressing on pertuzumab monotherapy continued treatment with the addition of trastuzumab. The ORR and CBR for the combination in this population were 17.6% and 41.2%, respectively. These objective responses observed with the addition of trastuzumab to patients progressing on single agent pertuzumab appear to confirm the synergistic interaction of the two antibodies that was demonstrated in preclinical models.

In addition to the cardiac safety issues, the most frequent adverse events observed in the phase II setting with pertuzumab/trastuzumab, or with pertuzumab monotherapy, were diarrhea, nausea, fatigue, and rash, with most being of mild to moderate intensity (grade 1 or grade 2).

CLEOPATRA (Clinical Evaluation Of Pertuzumab And Trastuzumab) is a randomized double-blind, placebo controlled phase III study that was designed to evaluate the effectiveness of the trastuzumab/pertuzumab combination [27]. A total of 808 patients with advanced HER2-positive breast cancer were randomized to receive trastuzumab and docetaxel, with either pertuzumab or placebo. Eligible patients could not have received prior chemotherapy or targeted therapy in the metastatic setting and only 10% of enrolled patients had received trastuzumab in the early-stage setting. The study primary endpoint was independently assessed PFS; secondary endpoints were overall survival OS, ORR, and safety. The addition of pertuzumab to trastuzumab and docetaxel significantly prolonged PFS, from a median of 12.4 to 18.5 months (hazard ratio [HR] 0.62; 95% CI

0.51-0.75; P < 0.001). LVEF dysfunction was more common in the control arm (trastuzumab/docetaxel) than in the pertuzumab-containing arm (8.3% versus 4.4%, respectively). The following adverse events were more common in the pertuzumab than placebo-containing arm: diarrhea (66.8% v 46.3%), mucosal inflammation (33.7% v 24.2%), febrile neutropenia (13.8% v 7.6%), and dry skin (10.6% v 4.3%). In a subsequent analysis with 30 months of median follow-up, the addition of pertuzumab to the trastuzumab/docetaxel combination conferred a 34% reduction in the risk of mortality (HR = 0.66; p =0.0008) [28]. In a subgroup analysis by age, treatment benefit was similar in patients ≥ 65 years compared to that in younger patients [29]. Quality of life assessment showed no detrimental effect with the addition of pertuzumab [30]. In the biomarker analysis of the CLEOPATRA study, PIK3CA gene mutations were identified in 32% of patients (176/557 patients), and patients with PIK3CA gene mutation had worse outcome when compared to those with wild type PIK3CA gene status. However, PIK3CA mutation(s) were not associated with resistance to pertuzumab and the magnitude of benefit of pertuzumab was independent of PIK3CA mutational status [31]. The positive results from CLEOPATRA led to approval by the U.S. Food and Drug Administration for use of pertuzumab, in combination with trastuzumab and docetaxel, for the treatment of patients with HER2+ advanced breast cancer who have not received prior anti-HER2 therapy or chemotherapy for metastatic disease.
Refer to the Full Prescribing Information for Pertuzumab for complete safety information: http://www.accessdata.fda.gov/scripts/cder/drugsatfda/ in addition to information provided in Section 8 of the protocol.

2.3 Rationale

T-DM1 represents a new approach to targeting HER2, coupling an anti-HER2 monoclonal antibody (trastuzumab) with a cytotoxic agent (DM1), thereby specifically directing the cytotoxicity to the HER2-positive cancer cell. In T-DM1, a highly stable thioether linker, N-maleimidomethyl cyclohexane-1-carboxylate (MCC) joins trastuzumab with DM1, a highly potent microtubule inhibitor. Activation of cytotoxicity of this conjugate requires that is internalized into the cell after binding to HER2. The non-internalized conjugate remains inactive, thus limiting the systemic toxicity. In T-DM1, trastuzumab not only delivers the drug to HER2- overexpressing cells, but it also retains its native properties, namely the inhibition of HER2 signaling and induction of ADCC [32]. With its innovative mechanism of action, T-DM1 acts primarily in HER2-positive tumor cells and limits off-target toxicity.

Pertuzumab and T-DM1 have demonstrated high levels of clinical activity in the advanced setting and are now approved drugs for the first- and second-line treatment of advanced HER2-positive breast cancer, respectively (summarized below). Recently, the US Food and Drug Administration (FDA) granted pertuzumab accelerated approval for the treatment of patients with HER2-positive early stage breast cancer prior to surgery. In the MARIANNE study, T-DM1 plus pertuzumab and T-DM1 plus placebo are being compared with trastuzumab plus taxane, for the first-line treatment of patients with advanced HER2-positive breast cancer.

The ability of T-DM1 to deliver the cytotoxic agent DM1 specifically to HER2-positive cancer

cells raises the hypothesis that T-DM1 may not be sufficient if HER2-negative subclones are present within a HER2-positive tumor (i.e. HER2 heterogeneity). Similar concern is shared for pertuzumab as it is a HER2-specific monoclonal antibody. In other words, a therapeutic regimen that consists only of HER2-targeted agents could be ineffective against HER2-negative subclones within a tumor, potentially resulting in failure to completely eradicate the tumor. To address this concern, we propose a neoadjuvant clinical trial dedicated to evaluating the impact of HER2 heterogeneity on the response to T-DM1 given in combination with pertuzumab. Spatially-separated tumor biopsies and a detailed evaluation of HER2 status will be of paramount value to understand the role of intratumoral heterogeneity on the efficacy of T-DM1 plus pertuzumab when given in combination. The primary hypothesis of this study is built upon HER2 heterogeneity defined according to the College of American Pathologists (CAP) and the United Kingdom (UK) guidelines [33, 34] with additional guidance provided by recently published studies [35, 36].
By definition, heterogeneous amplification of HER2 includes the existence of at least 2 distinct clones of breast cancer cells with different patterns of gene amplification (usually 1 clone amplified and 1 clone non-amplified) [37]. A considerable number of publications have reported on HER2 heterogeneity [33, 35, 36, 38-45], but the lack of uniform definition or standard measurements limits cross-studies comparison. The College of American Pathologists (CAP) guideline [33] suggest that all cases with between 5% and 50% of cells with HER2/CEP17 ratios greater than 2.2 be regarded as heterogeneous amplified, but does not provide detailed guidance on how to select tumor areas. In the UK guideline [34] HER2 assessment requires scanning the entire tumor section before selecting at least three separate tumor fields and counting the number of chromosome 17 (CEP17) and HER2 signals.

In the present study HER2 heterogeneity will be assessed in two spatially separated core biopsies from each tumor to understand whether different geographic regions have distinct patterns of HER2 overexpression. Experience with NeoALTTO and I-SPY1 suggest that taking four core biopsy samples from one area is feasible and acceptable to patients and ethics committees [46]. Intratumor heterogeneity of HER2 expression will be primarily assessed by FISH, followed by other techniques in the translational research component of this study (e.g. Immuno-FISH).

The entire slide of each core biopsy will be scanned before selecting 3 separate tumor areas per core bx and counting the number of chromosome 17 (CEP17) and HER2 signals for 50 cells in each area (The HER2 IHC staining will be used to guide pathologists to possible areas of heterogeneity). HER2 genetic heterogeneity will be defined as the existence of an area of tumor cells with a HER2/CEP17 ratio ≥2.0 or a gene copy number of >6 and representing more than 5% but less than 50% of infiltrating tumor cells. HER2 regional heterogeneity will be defined as at least one of the six tumor areas being HER2 negative by ISH (HER2/CEP17 ratio <2 and CN <6, if IHC is <3+). A HER2-positive tumor will be considered HER2 heterogeneous if genetic heterogeneity and/or HER2 regional heterogeneity is identified.

In a recently published manuscript 18% of the 96 evaluated HER2-positive tumors had regional and/or genetic heterogeneity [35].

Of note, the evaluation of heterogeneity will be performed in the lab of Dr Giuseppe Viale at the European Institute of Oncology which will function as the Central Pathology Core for this study.

In this study we will also evaluate whether hormone receptor status impacts the frequency of HER2 heterogeneity. Previous studies have reported an inverse correlation between hormone

receptor (ER and PgR) expression and HER2 copy number determined by FISH [47]

2.4 Correlative Studies Background

2.4.1 Genetic diversity and therapeutic outcome

The analyses will follow observations from Dr. Kornelia Polyak's laboratory evaluating the effects of neoadjuvant chemotherapy on the extent of genetic and phenotypic diversity within breast subtypes, and the associations between intratumor genetic diversity/heterogeneity and therapeutic outcomes [48]. Intratumor diversity was assessed using immunoFISH (iFISH - immunofluorescence combined with FISH) analyses on human primary breast tumor samples before and after neoadjuvant chemotherapy. iFISH allows for the combined analysis of phenotypic markers (e.g., CD24 and CD44 cell surface markers) and chromosomal copy number gains at the single cell level. Intratumor genetic heterogeneity was assessed by scoring the copy number of commonly gained chromosomal regions in breast cancer (e.g., 8q24). Preliminary results have demonstrated that HER2-positive tumors had the highest diversity for 8q24 when compared to other major breast cancer subtypes. Of importance, the subsets of HER2-positive tumors with low diversity were more likely to achieve a pCR following trastuzumab-based treatment than HER2-positive tumors with high diversity Intratumor genetic heterogeneity varied in different breast tumor subtypes but did not change significantly during treatment. By contrast pronounced changes were observed within phenotypically distinct cell populations. The analysis of tumor topology revealed that adjacent cells within residual tumors were more likely to be genetically divergent yet phenotypically more similar compared to pre-treatment samples. Furthermore, residual tumors were enriched for cells with lower proliferation rates, and higher pre-treatment genetic heterogeneity was associated with resistance to treatment.

2.4.2 Additional Biomarker Analysis

Tumor samples collected from the research biopsies and pathology specimen will be used to assess the potential prognostic or predictive value of candidate markers or biomarker panels, improve diagnostic tests, improve understanding of breast cancer biology, and potentially discover new biomarker profiles related to treatment benefit and/or safety or disease characteristics. The pathology material will be used to assess the potential relationship between the degree of HER2 gene amplification and the level of HER2/3 mRNA and response to study treatment. Other markers that may be selected for exploratory analyses include, for example, other HER family members, markers that are involved in downstream signaling of HER2 (e.g., PIK3CA mutations, PTEN), related receptor tyrosine kinases that could serve as salvage routes for an inhibited HER2 pathway, and ligands of HER family proteins that induce activation of the HER pathway.

The study also requires mandatory blood samples for biomarker research, which may include, among other analyses, the assessment of circulating tumor DNA. There is increasing evidence that circulating DNA can be obtained from the blood specimens of

cancer patients, which represents the DNA and mutational status of tumor cells [49-54]. Another example of markers that could be assessed in serum or plasma is circulating ligands of HER family members. The serial sampling gives the opportunity to evaluate changes in circulating biomarker levels over time that may allow further understanding of potential resistance mechanisms or of (early) indicators of recurrence. Additional candidate markers of response to treatments that emerge from other clinical or nonclinical studies may also be assessed in this study and can be assessed with different types of technologies.

In addition, a mandatory whole blood sample for clinical genotyping will be collected. Both safety and efficacy questions will be explored with the clinical genotyping analyses. The following are examples describing potential questions that could be explored by clinical genotyping. For example, reversible thrombocytopenia has been observed in completed and ongoing studies with T-DM1 in a subset of patients. It is hypothesized that this effect may be related to an interaction between T-DM1 and proteins expressed in megakaryocytes and/or platelets: Fcγ receptor (FcγRIIa). FcγRIIa is the only FcγR expressed in platelets and it has been shown to bind to and internalize IgG. The H131R polymorphism in FcγRIIa has been associated with differential affinity for binding of IgG Fc and, in some cases, with heparin-induced thrombocytopenia [55, 56] One possible hypothesis to explain the observation of T-DM1−induced thrombocytopenia in some but not all patients is a differential ability of megakaryocytes and/or platelets to bind to and internalize the drug conjugate. In relationship to efficacy, it has been hypothesized that the mechanism of action of antibody therapeutics, such as rituximab and trastuzumab, could include Fc-mediated attraction of immune effector cells known as antibody dependent cell-mediated cytotoxicity (ADCC). The affinity of IgG to the Fcγ receptors is influenced by the FcγRIIa and FcγRIIIa polymorphisms and may cause a difference in the efficacy of trastuzumab, T-DM1, or pertuzumab or in the efficacy by the combination of T-DM1 and pertuzumab, as a result of different FcγR-mediated ADCC (Shields et al. 2001 [66]).

P-glycoprotein (P-gp), which is encoded in the ABCB1 (also known as MDR1) gene, is known to act as an energy-dependent drug efflux pump for various chemotherapeutic drugs, such as anthracycline, vinca alkaloids, and taxanes [57].
Previous studies showed that ABCB1 3435CC genotype carriers had significantly higher P-gp expression in the duodenum and in breast cancer tissue [58], which may result in a decreased drug concentration in cells. Additional data support the theory that ABCB1 polymorphisms may predict PFS after first-line trastuzumab and taxane therapy in patients with HER2-positive MBC [59]. Polymorphisms in this gene and its correlation to efficacy in this trial may also be explored. Examples of other safety markers may also include polymorphisms in the human leukocytic antigen or genes involved in T-DM1 metabolism to evaluate their correlation to hepatotoxicity [60].

3. PARTICIPANT SELECTION

Baseline evaluations (laboratory tests and other non-laboratory tests) must be performed within 28 days of study entry except for the following items: Pregnancy test must be done within 7 days before planned treatment start, EKG must be performed within 12 weeks of study entry, and

Hepatitis B and C serology evaluations must be within 12 weeks of planned study treatment start.

3.1 Eligibility Criteria

3.1.1 Patients must have HER2-positive Stage II or III histologically confirmed invasive carcinoma of the breast. A minimum tumor size of 2 cm determined by physical exam or imaging (whichever is larger) is required.

3.1.2 HER-2 positive by ASCO CAP 2013 guidelines, confirmed by central testing (NeoGenomics Laboratories):

- IHC 3+ based on circumferential membrane staining that is complete, intense

-AND/OR-

- FISH positive based on one of the three following criteria:
  - Single-probe average HER2 copy number ≥ 6.0 signals/cell; **OR**
  - Dual-probe HER2/CEP17 ratio <2.0 with an average HER2 copy number ≥ 6.0 signals/cell; **OR**
  - Dual-probe HER2/CEP17 ratio ≥2.0

NOTE: HER-2 status must be confirmed to be positive by central review prior to patient starting protocol therapy. Patients previously having had HER2 testing at Neogenomics Laboratories, Inc. (formerly Clarient Laboratories) do not need to undergo retesting for central confirmation of HER2 status. DCIS components should not be counted in the determination of HER2 status. See Section 5.5 for information on NeoGenomics testing.

3.1.3 ER/PR determination is required. ER- and PR-assays should be performed by immunohistochemical methods according to the local institution standard protocol.

3.1.4 Bilateral breast cancers are allowed as long as both cancers are HER2-positive; however, only the primary cancer's status requires central review

3.1.5 Patients with multifocal or multicentric disease are eligible as long as at least one area meets eligibility criteria.

3.1.6 Breast imaging should include imaging of the ipsilateral axilla. For subjects with a clinically negative axilla, a sentinel lymph node biopsy will be performed either before or after preoperative therapy at the discretion of the subject's physicians. For subjects with a clinically positive axilla, a needle aspiration, core biopsy or SLN procedure will be performed to determine the presence of metastatic disease in the lymph nodes.

3.1.7 Men and women (with any menopausal status) ≥ 18 years of age

3.1.8 ECOG performance status 0 or 1

3.1.9 Required laboratory values:

- ANC ≥ 1500/mm^3
- Hemoglobin ≥ 9 g/dl
- Platelets ≥ 100,000/mm^3
- Serum creatinine < 1.5 X ULN (institutional)
- Total bilirubin ≤ 1.0 X ULN (institutional) For patients with Gilbert syndrome, the direct bilirubin should be within the institutional normal range.
- AST and ALT ≤ 1.5x ULN (institutional)
- Alkaline phosphatase ≤ 1.5x ULN (institutional)
- Only for patients who test positive for hepatitis B virus or hepatitis C virus: PTT/INR ≤ ULN (institutional)

3.1.10 Documentation of hepatitis B virus (HBV) and hepatitis C virus (HCV) serologies is required: this includes hepatitis B surface antigen (HBsAg) and/or total hepatitis B core antibody (HBcAb) in addition to HCV antibody testing. The most recent serologic testing must have occurred within 12 weeks prior to registration. If such testing has not been done, it must be performed during screening.

Note: Patients who have positive HBV (i.e., HBsAg or HBcAb) or HCV serologies without known active disease must meet the eligibility criteria for ALT, AST, total bilirubin (TBILI), INR, aPTT/ PTT, and alkaline phosphatase (ALP) on at least two consecutive occasions, separated by at least 1 week, within the 30-day screening period. The second of these evaluations must be performed within 3 days prior to the first treatment administration.

3.1.11 Left ventricular ejection fraction (LVEF) ≥ 55%

3.1.12 Premenopausal women must have a negative serum pregnancy test, including women who have had a tubal ligation and for women less than 12 months after the onset of menopause.

3.1.13 Women of childbearing potential and men with partners of childbearing potential must be willing to use one highly effective form of non-hormonal contraception or two effective forms of non-hormonal contraception by the patient and/or partner and continue its use for the duration of the study treatment and for 7 months after the last dose of study treatment.

3.1.14 Potent CYP3A4 inhibitors, such as ketoconazole and erythromycin, should be avoided during the study treatment period with T-DM1.

3.1.15 Excessive alcohol intake should be avoided (occasional use is permitted).

3.1.16 Patients with a history of ipsilateral DCIS are eligible.

3.1.17 Patients undergoing breast conservation therapy (i.e. lumpectomy) must not have any contraindications to radiation therapy.

3.1.18 Willing and able to sign informed consent.

3.1.19 Willing to provide tissue for research purposes.

3.2 Exclusion Criteria

3.2.1 Pregnant or nursing women due to the teratogenic potential of the study drugs.

3.2.2 Active, unresolved infection.

3.2.3 Receipt of intravenous antibiotics for infection within 7 days prior to enrollment.

3.2.4 Patients with active liver disease, for example, due to hepatitis B virus, hepatitis C virus, autoimmune hepatic disorder, or sclerosing cholangitis.

3.2.5 Uncontrolled hypertension (systolic >180 mm Hg and/or diastolic >100 mm Hg) or clinically significant (i.e. active) cardiovascular disease: cerebrovascular accident/stroke or myocardial infarction within 6 months prior to first study medication, unstable angina, congestive heart failure (CHF) of New York Heart Association (NYHA) Grade II or higher, or serious cardiac arrhythmia requiring medication.

3.2.6 Significant symptoms (Grade ≥ 2) peripheral neuropathy.

3.2.7 Other concurrent serious diseases that may interfere with planned treatment, including severe pulmonary conditions/illness, uncontrolled infections, uncontrolled diabetes.

3.2.8 Any prior treatment for the current breast cancer, including chemotherapy, hormonal therapy, radiation or experimental therapy.

3.3 Inclusion Demographics

Both men and women of all races and ethnic groups are eligible for this trial.

4. REGISTRATION PROCEDURES

4.1 General Guidelines for DF/HCC and DF/PCC Institutions

Institutions will register eligible participants in the Clinical Trials Management System (CTMS) OnCore. Registrations must occur prior to the initiation of protocol therapy. Any participant not

registered to the protocol before protocol therapy begins will be considered ineligible and registration will be denied.

An investigator will confirm eligibility criteria and a member of the study team will complete the protocol-specific eligibility checklist.

Following registration, participants may begin protocol therapy. Issues that would cause treatment delays should be discussed with the Overall Principal Investigator (PI). If a participant does not receive protocol therapy following registration, the participant's registration on the study must be canceled. Registration cancellations must be made in OnCore as soon as possible.

4.2 Registration Process for DF/HCC and DF/PCC Institutions

DF/HCC Standard Operating Procedure for Human Subject Research Titled *Subject Protocol Registration* (SOP #: REGIST-101) must be followed.

4.3 General Guidelines for Other Investigative Sites

Eligible participants will be entered on study centrally at the Dana-Farber Cancer Institute by the Project Manager. All sites should email or fax the documentation listed in section 4.4 to the Project Manager to verify treatment availability.

Following registration, participants must begin protocol treatment within 7 business days. Issues that would cause treatment delays should be discussed with the Overall PI. If a participant does not receive protocol therapy following registration, the participant's registration on the study may be canceled. The Study Coordinator should be notified of cancellations as soon as possible.

4.4 Registration Process for Other Investigative Sites

To register a participant, the research nurse or study coordinator should fax or and email the following documentation to CTOPM@dfci.harvard.edu or faxed to 617-632-5152:

- Clinic visit note documenting history and physical exam
- Pathology report and documentation of ER/PR status
- HER2 results from NeoGenomics Laboratories
- MRI/Mammogram/Ultrasound report
- CT (chest/abdomen/pelvis) scan report for patients with Stage III disease
- Required laboratory test results including: Hematology (CBC with differential), serum chemistries (creatinine and/or creatinine clearance, bilirubin, ALT, and AST)
- ECHO/MUGA report
- ECG report
- Signed participant consent form
- HIPAA authorization form (if separate from the informed consent document)
- Completed Eligibility checklist

To complete the registration process, the Project Manager will

- follow the DF/HCC Standard Operating Procedure for Human Subject Research Titled *Subject Protocol Registration* (SOP #: REGIST-101) and register the participant on the protocol
- call or email the research nurse or data manager at the participating site with the participant study number, and to confirm registration

NOTE: Registration can only be conducted during the business hours of 8:00 AM and 5:00 PM Eastern Time Monday through Friday. Same day treatment registrations will only be accepted with prior notice and discussion with the DF/HCC Project Manager.

5. STUDY PROCEDURES

At the time of registration, the eligibility checklist and supporting documentation to verify eligibility must be provided prior to enrollment. Data will be collected and maintained on study specific case report forms. See Section 10 Study Calendar for additional details.

Patients will be assessed for safety, efficacy and biomarker during the study. All patients will be closely monitored for safety and tolerability during study treatment. Patients should be assessed for toxicity prior to each dose; dosing will occur only if the clinical assessment and local laboratory test values are acceptable. In this study, one cycle is defined as 3 weeks (21 ± 3 days). Study treatment will be administered in 21-day/3-week cycles if no additional time is required for reversal of toxicity. If the timing of a protocol-mandated procedure coincides with a holiday and/or weekend that preclude performance of the procedure within the allotted 3-day window, the procedure should be performed on the nearest following date.

5.1 Medical History and Demographic Data

Medical history includes past or current clinically significant conditions, surgeries, breast cancer surgery and diagnosis, reproductive status, and all medications (e.g., prescription drugs, over the counter drugs, herbal or homeopathic remedies, nutritional supplements) used by the patient within 7 days prior to registration. Demographic data will include age, sex, and self-reported race/ethnicity and may include local HER2 and hormonal receptor test information.

5.2 Physical examinations

Physical examination should include examination of breast and local-regional lymphatics. Clinical T and N staging should be documented. At subsequent visits (or as clinically indicated), breast examination and evaluation of local-regional lymphatics should be performed. Additional physical examinations should be focused on organ systems related to adverse events. Weight is to be measured on Day 1 of the specified cycles and compared with baseline. Changes from baseline abnormalities should be recorded in patient notes.

5.3 Vital signs

Vital signs will include measurements of pulse rate, and systolic and diastolic blood pressures while the patient is in a seated position, as well as temperature.

5.4 Laboratory assessments

Samples for the following laboratory tests will be sent to the study site's local laboratory for analysis according to the Study Calendar:

Hematology

Hemoglobin, hematocrit, platelet count, WBC count, and differential including absolute neutrophil count

Serum chemistry

Sodium, potassium, chloride, bicarbonate, glucose, BUN or urea, creatinine, total protein, albumin, TBILI (and direct bilirubin when TBILI >ULN), ALT, AST, and ALP

Viral serology at screening and as clinically indicated

At baseline: Documentation of hepatitis B virus (HBV) and hepatitis C virus (HCV) serologies is required: this includes hepatitis B surface antigen (HBsAg) and/or total hepatitis B core antibody (HBcAb) in addition to HCV antibody testing. The most recent serologic testing must have occurred within 12 weeks of treatment start. If such testing has not been done, it must be performed during screening. Patients who have positive HBV (i.e. HBsAg or HBcAb) or HCV serologies without known active disease must meet the eligibility criteria for ALT, AST, total bilirubin (TBILI), INR, aPTT/ PTT, and alkaline phosphatase (ALP) on at least two consecutive occasions, separated by at least 1 week, within the 30-day screening period. The second of these evaluations must be performed within 3 days prior to the first administration

Pregnancy test

All women of childbearing potential (including those who have had a tubal ligation) will have a serum pregnancy test at screening (within 7 days prior to starting study medications, with result available prior to first dosing). Urine or serum β- HCG pregnancy tests will be performed at specified subsequent visits. If a urine pregnancy test is positive, it must be confirmed by a serum pregnancy test.

Coagulation (INR and aPTT)

At screening; otherwise, as clinically indicated

5.5 Central testing for HER2 status

Central Testing for HER2 will be performed by NeoGenomics Laboratories (formerly called Clarient Laboratories). IHC for HER2 will be performed on all samples. A result of HER2 3+ by IHC will be consistent with HER2-positivity. If a patient is HER2 2+ by IHC, a FISH test will be performed. If HER2/CEP17 ratio is <2.0 with an average HER2 copy number ≥ 6.0 signals/cell, or HER2/CEP17 ratio is ≥2.0, the tumor with be declared HER2-positive.

HER2 IHC will be assessed by HercepTest (Dako). HER2 FISH for HER-2 gene amplification will be assessed utilizing the PathVysion assay (Vysis Corp., Downers Grove, Illinois). The

identification probes for the HER-2 (SpectrumOrange) and alpha satellite DNA sequence at the centromeric region of chromosome 17 (SpectrumGreen) were hybridized according to the manufacturer's guidelines. At least twenty non-overlapping nuclei containing at least one orange and one green signal were enumerated. The ratio of orange signals (HER-2 gene) to green signals (chromosome 17) was calculated. A ratio greater than or equal to 2.0 is considered as amplified based on the FDA approval in this kit. The ASCO CAP 2013 guidelines suggest that a dual-probe HER2/CEP17 ratio of less than 2.0 be considered positive if an average HER2 copy number is greater than or equal to 6.0 signals/cell. HER2 is considered to be borderline (equivocal) if a single probe FISH average HER2 copy number is greater than or equal to 4.0 and less than 6.0 signals/cell, or if a dual-probe HER2/CEP17 ratio is less than 2.0 and has an average HER2 copy number of greater than or equal to 4.0 and less than 6.0 signals/cell. . Intended Use: The PathVysion HER-2 DNA Probe Kit (PathVysion Kit) is designed to detect amplification of the HER-2/neu gene via fluorescence in situ hybridization (FISH) in formalin-fixed, paraffin-embedded human breast cancer tissue specimens.

NeoGenomics will supply kits containing all materials needed to submit tissue samples for central HER-2 testing. Included in the shipper will be the necessary supplies and instructions for shipment of slides, specimen/patient requisition forms, FedEx air bill. NeoGenomics kit reorder information is found inside each kit. Slides for NeoGenomics testing must be obtained from either biopsy or surgical specimen with evidence of invasive carcinoma that has tested HER-2 positive by local testing. DCIS–containing slides will not be accepted.

Submit the following:

- 6-8 unstained slides, each 4-5 µm thick or 1 block from the tissue obtained from either biopsy or surgical specimen with evidence of invasive carcinoma that has tested HER-2 positive by local testing.
- De-identified pathology report
- Completed NeoGenomics requisition form

Label slides (or block) with the Protocol Number, NeoGenomics patient ID, Specimen/Tissue Block ID, and collection date (preferably all on a printed label). No patient names on slides. Documentation of the patient ID, site, and contact info at the site should be included with the shipment. Ship the slides at ambient temperature using the shippers provided in the NeoGenomics kit to:

NeoGenomics Laboratories
Attn: BioPharma Services
31 Columbia
Aliso Viejo, CA 92656

Notify NeoGenomics when samples have been submitted by emailing the airbill/tracking number, Study ID, Sample ID, and Site ID to alisoviejo.biopharmprocessing@geneogenomics.com. To request additional testing supplies send an email to alisoviejo.biopharmprocessing@geneogenomics.com. If this email address does not

work, please contact ctopm@dfci.harvard.edu. Results of central review will be provided directly by email to sites within 7-10 business days of receipt of tissue.

5.6 Research Blood Sample Collection

Research blood collection is mandatory for all patients for DNA isolation and cell-free circulating DNA analysis. Collection tubes for research blood samples will be provided in the research biopsy/blood collection kit available from Core Prognostex. The samples will be banked in the DF/HCC Core and Blood Tissue Bank in order to extract germline and cell-free DNA to be used as normal DNA reference for tumor tissue-based studies and for future research purposes. These specimens will become the property of the DF/HCC.

The following research blood samples are required:

- Two 10 mL lavender top (EDTA Fisher #366643) tubes of whole blood at baseline (or at any time after registration but before surgery).
- Three 10 mL Streck blood collection tubes will be collected at baseline and at the pre-surgery visit (6 total)

All samples should be de-identified and labeled with the Participant initials, Participant Study ID number and date of collection and time point (e.g., "Baseline" or "Off Treatment").

Include a copy of the 14-409 Specimen Requisition Form (Appendix B) with your shipment.

It is recommend that the baseline specimens be collected on the same day as the research biopsy so the research blood can be shipped in the biopsy shipping container provided by the study. All blood samples should be shipped overnight Monday – Thursday at ambient temperature to:

DF/HCC Core Blood and Tissue Bank
Dana Farber Cancer Institute
Smith Building - SM 956
450 Brookline Ave
Boston, MA 02215

DO NOT FREEZE OR REFRIGERATE STRECK TUBES. Streck tubes must be stored at room temperature to preserve the sample.

If blood specimens must be collected on Friday, the specimens should be stored over the weekend and shipped on Monday. When storing tubes over a weekend lavender top (EDTA) tubes should be refrigerated and Streck tubes should be stored at room temperature until shipment.

Please email the DFCI Study Coordinator and DF/HCC Core Blood and Tissue Bank with the sample information and tracking information the day before shipping specimens: eileen_wrabel@dfci.harvard.edu and DFCIBreastBank@partners.org.

5.7 Tumor Staging

5.7.1 Breast MRI, mammogram, or breast ultrasound

All subjects are required to have a MRI, mammogram, or ultrasound performed at screening (within 28 days of registration and at pre-surgery (within 4 weeks of last dose of treatment). Breast imaging should include imaging of the ipsilateral axilla. MRI is strongly recommended, although other imaging modalities (mammogram, ultrasound) are permitted if practical or financial considerations preclude MRI, as long as the target lesion can be adequately measured. This same imaging modality must be used at screening and prior to surgery to assess tumor response.

5.7.2 CT scans

Subjects with Stage III disease according to AJCC Staging Manual edition 7 will have CT scans of chest, abdomen and pelvis and bone scans performed during screening to rule out metastatic disease.

5.8 Cardiac Assessments

5.8.1 Electrocardiograms

Single 12-lead ECGs will be collected locally and assessed at screening and otherwise as clinically indicated. For safety monitoring purposes, any abnormalities on any of the ECGs will be documented on the CRF. The investigator or designee must review, sign, and date all ECG tracings. Paper copies will be kept as part of the patient's permanent study file at the site. For ECG tracings that will fade over time (e.g., ECGs on thermal paper), lasting legible copies should be filed together with the original.

5.8.2 Left Ventricular Ejection Fraction (LVEF)

LVEF cardiac monitoring will be assessed by ECHO or MUGA in all patients. LVEF assessment by ECHO is preferred, but LVEF can also be assessed by MUGA. An ECHO or MUGA is required at baseline, Cycle 2 during the last week of the cycle (anytime from day 15-21), and pre-surgery (within 4 weeks after the final treatment). The Cycle 2 ECHO/MUGA results must be available and reviewed before Cycle 3 Day 1 treatment is administered. Cardiac evaluation should still occur at these time points even if there has been a treatment delay. If a patient comes off study treatment early, cardiac imaging should be performed within 30 days of coming off study treatment. The same modality should be used throughout the study for each patient and preferably performed and assessed by the same assessor. Results of ECHO/MUGA will be collected in the CRF.

5.9 Surgical Assessment

All subjects will be seen and examined by the treating surgeon at Screening and at the Pre-Operative Visit. Each visit will include a clinical breast and lymph node examination and review of the imaging studies (mammogram, MRI, and any other radiographic method) of the breast and axilla. After examining the subject and reviewing the pertinent radiographic studies at the Screening visit, the surgeon will determine whether the subject is a candidate for potentially curative surgery. At both the Screening and Pre-Operative visits, the surgeon will also determine whether subject is eligible for breast conservation surgery. If the subject is not a breast conservation candidate, the reason(s) will be documented in the CRF (multicentric tumor, tumor location, tumor size, other).

5.10 Axillary Assessment

An axillary assessment will be performed at screening. Ipsilateral axillary lymph nodes will be assessed as clinically normal or clinically suspicious by physical examination and will be assessed as clinically normal or clinically suspicious independently by imaging. Axillary imaging and/or biopsy do not need to be repeated if performed prior to the screening period. Subjects with suspicious nodes documented by physical exam OR by imaging will have a biopsy of the nodes (fine needle aspirate or core needle biopsy). If clinical evaluation and biopsy results are discordant, the biopsy may be repeated at the discretion of the Investigator.

5.11 Research Core Biopsy

5.11.1 Pre-treatment research core biopsy

Research breast core biopsies of the target lesion will be obtained from all participants prior to initiating protocol therapy. The pre-treatment biopsies represent an integrated assay that will be used in evaluating the primary objective of this study, thus justifying their mandatory collection.

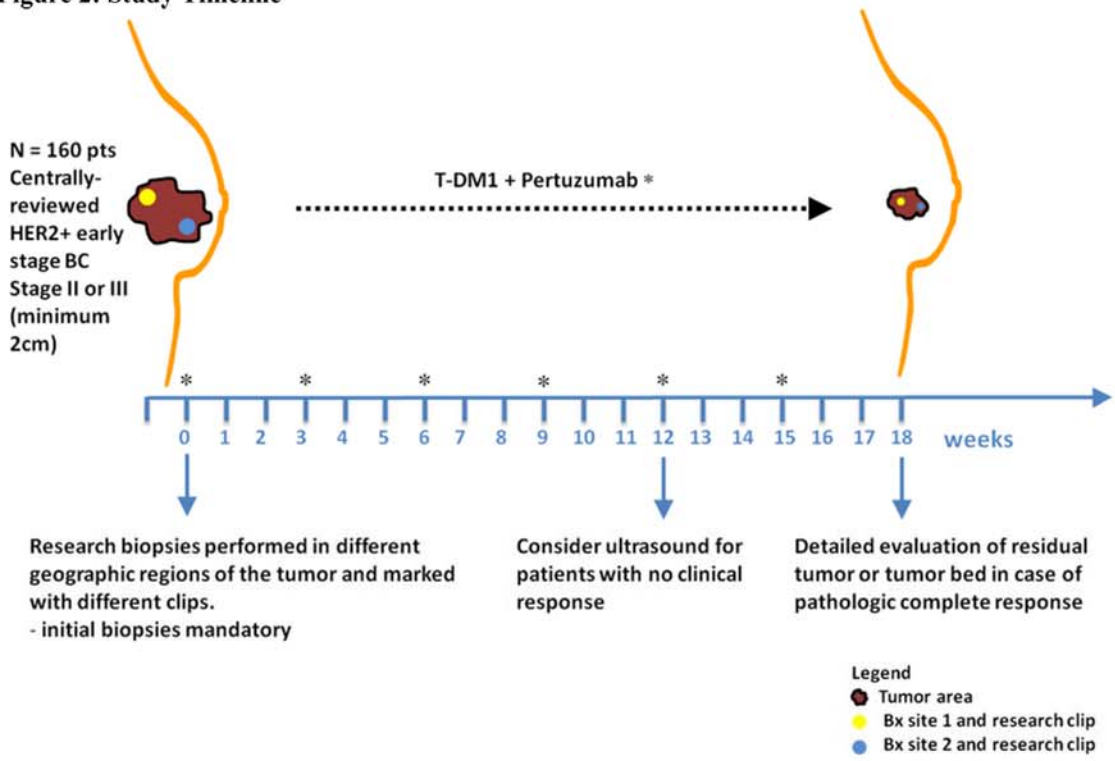
Tissue biopsies (at Screening) and tissue blocks from the definitive surgical specimen will be obtained from all subjects for correlative studies using the collection kits provided.

It is mandatory that core biopsies be image-guided and performed in two different geographical areas of the same tumor. Clips of different types should be placed in each of the two biopsy sites at the time of the research biopsy. Pre- and post-procedure 90-degree lateral and craniocaudal mammogram is recommended to ensure that the correct lesion has been biopsied and to determine the relationship of the clip to the lesion that was visualized prebiopsy. Clip migration following biopsy has been reported and the distance from the original biopsy cavity can be measured [61]. If sufficiently far away from the biopsy cavity, then an addendum should be made to the report documenting that the clip should NOT be used to guide post-treatment tumor sampling.

Tumor biopsy samples proposed for collection within the study timeline (Figure 2) will consist of at least two to three core biopsy samples from each of the two areas of the tumor (total of 4-6 core biopsy passes). Experience with NeoALTTO and I-SPY1 suggest that

taking four core biopsy samples from one area is feasible and acceptable to patients and ethics committees [46].

Figure 2: Study Timeline



Ideally three core biopsies from each area of the tumor will be obtained:

- Two cores should be placed in 10% neutral buffered formalin tube supplied by the study
- One core biopsy in an RNA Later tube supplied by the study

The order of specimen collection should be:

- Biopsy site 1: First and second core should be placed in formalin
- Biopsy site 1: Third core should be placed into RNA later.
- Biopsy site 2: First and second core should be placed in formalin
- Biopsy site 2: Third core should be placed into RNA later

Research biopsy kits will be provided by the study. Kits may be ordered from Core Prognostex.

5.11.2 Research biopsy shipping and storage

All research biopsy samples should be shipped Monday – Thursday at ambient

temperature to:

DF/HCC Core Blood and Tissue Bank
Dana Farber Cancer Institute
Smith Building - SM 956
450 Brookline Ave
Boston, MA 02215

If a biopsy must be performed on Friday, the specimens should be stored over the weekend and shipped on Monday. Specimens in RNA Later and formalin should be stored at room temperature until shipment.

All samples should be de-identified and labeled with the Participant initials, Participant Study ID number and date of procedure. Please email the DFCI Study Coordinator and DF/HCC Core Blood and Tissue Bank with the sample information and tracking information the day before shipping specimens: eileen_wrabel@dfci.harvard.edu and DFCIBreastBank@partners.org.

Include a copy of the 14-409 Specimen Requisition Form (Appendix B) with your shipment.

The DFCI study coordinator will track biopsy specimens using CaTissue. Upon receipt of an email notification of sample shipment, the coordinating center study coordinator will log in the information into CaTissue. CaTissue will store a complete record of the biopsy samples that are collected and analyzed as part of this study.

5.12 Immediate post surgical tumor sampling

5.12.1 Formalin-fixed Paraffin Embedded Tumor Blocks

Special care should be taken to identify the clips placed at the time of the research core biopsies, and to ensure that the area(s) containing the 2 clips are sampled. The corresponding block (or 2 blocks if the clips are sampled in 2 separate blocks) must be clearly identified with the suffix "C12" (if both clips are sampled in one block) or with "C1" and "C2", if the 2 clips are sampled in 2 different blocks.
The specimen may be blocked using regular cassettes or large cassettes. After the histological assessment of RCB, blocks should be selected for submission according to the following criteria:

- In case of pCR: submit the block(s) relative to the area(s) with the clips (i.e., C12 or C1 and C2)
- In case of residual tumor: submit the block(s) relative to the area(s) with the clips (i.e.: C12 or C1 and C2).

If these blocks do not include (or only include a minimal portion of) residual tumor, then submit an additional block representative of the main tumor residue.

If institutional policy prohibits the release of blocks, slides can be submitted in place of the block. Submit a minimum of 20 4-5 micron unstained slides relative to the area(s) with the clips (i.e., C12 or C1 and C2)

FFPE tissue block(s) and/or slides from surgery should be shipped to:

Eileen Wrabel
Dana-Farber Cancer Institute
Breast Oncology
450 Brookline Ave DA-157
Boston, MA 02215

All samples should be de-identified by assigning a unique sample ID number. Please email the DFCI Study Coordinator with the sample information and tracking information the day before shipping specimens: eileen_wrabel@dfci.harvard.edu.

Include a copy of the research specimen requisition with your shipment. (Appendix B)

The DFCI study coordinator will track specimens using CaTissue. Upon receipt of an email notification of sample shipment, the coordinating center study coordinator will log in the information into CaTissue. CaTissue will store a complete record of the biopsy samples that are collected and analyzed as part of this study.

5.12.2 Fresh Tissue

If it does not interfere with a site's clinical practice or the collection of FFPE block or slide collection noted above (Section 5.12.1), at the time of definitive surgery, an optional sample of fresh tissue may be collected. For this optional collection, 2-3 samples from the surgical specimen should be obtained using a core biopsy needle or biopsies from the specimen may be obtained with a 5-mm skin punch biopsy device. The tissue will be collected as a core by the surgeon in the operating room or sectioned by a pathologist (or pathology designee) in the institution's frozen section room. The operating surgeon should obtain biopsies preferably from the specimen ex vivo (using a core needle). If the pathologist obtains biopsies, the sample should be obtained within 1 hour, but may be collected up to 2 hours from the time of resection.

Biopsies should be placed in an RNA Later specimen tube, supplied by the study and shipped at ambient temperature.

All fresh tissue surgical samples should be shipped to:

DF/HCC Core Blood and Tissue Bank
Dana Farber Cancer Institute
Smith Building - SM 956
450 Brookline Ave
Boston, MA 02215

All samples should be de-identified by assigning a unique sample ID number. Please email the DFCI Study Coordinator DF/HCC Core Blood and Tissue Bank with the sample information and tracking information the day before shipping specimens: eileen_wrabel@dfci.harvard.edu and DFCIBreastBank@partners.org.

Include a copy of the research specimen requisition with your shipment. (Appendix B)

The DFCI study coordinator will track specimens using CaTissue. Upon receipt of an email notification of sample shipment, the coordinating center study coordinator will log in the information into CaTissue. CaTissue will store a complete record of the biopsy samples that are collected and analyzed as part of this study.

Note: Levels of HER2 heterogeneity in the pre- and post-treatment tissue samples will be assessed at the Research Pathology Laboratory at the European Institute of Oncology (EIO) in Milan under the coordination of Dr. Giuseppe Viale. Protocol defined assays will be performed during the course of this study. Upon completion of pre-defined study procedures all tissue samples will be shipped back to Dana-Farber and stored for at least 10 years.

5.13 Agent Administration

Treatment will typically be administered on an outpatient basis. Expected toxicities and potential risks as well as dose modifications for T-DM1 and pertuzumab are described. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the participant's malignancy.

Table 2: Treatment regimen

Agent	Pre-medications	Dose	Route	Schedule	Cycle Length

T-DM1*	No routine premedication required	3.6mg/kg	First infusion: IV, over 90min. Post-infusion observation period: 90 min. Subsequent infusions: IV, over 30-90 min as tolerated Post-infusion observation period: 30 min as tolerated	Day 1	3 weeks (21-days)*
Pertuzumab	No routine premedication required	First infusion: 840mg Subsequent infusions: 420mg	First infusion: IV, over 60min. Post-infusion observation period: 60 min. Subsequent infusions: IV, over 30min as tolerated Post-infusion observation period: 30 min as tolerated	Day 1	3 weeks (21-days)*

* Note: There is no requirement for the sequence of administration of the T-DM1 and pertuzumab

5.13.1 T-DM1

T-DM1 will be administered on Day 1 of a 3-week cycle at a dose of 3.6 mg/kg IV for a total of 6 cycles. The total dose will be calculated based on the patient's weight on Day 1 of (or up to 3 days before) each cycle with no upper limit.

T-DM1 doses may be reduced to as low as 2.4 mg/kg, according to the dose modification guidelines in section 6.1. Dose delays of up to 42 days from last dose are permitted. If the timing of a protocol-mandated procedure such as administration of T-DM1 coincides with a holiday or other scheduling conflict that precludes the procedure, the procedure should be performed within 3 days of the scheduled date and, when possible, on the earliest following date, with subsequent protocol-specified procedures rescheduled accordingly.

The first infusion of T-DM1 will be administered over 90 minutes (± 10 minutes). Infusions may be slowed or interrupted for patients experiencing infusion-associated symptoms. Vital signs must be assessed before and after dose administration in the first infusion. Following the initial dose, patients will be observed for at least 90 minutes for fever, chills, or other infusion-associated symptoms. If prior infusions were well tolerated (without any signs or symptoms of infusion reactions), subsequent doses of T-DM1 may be administered over 30-90 minutes (± 10 minutes), with a minimum 30-minute observation period after infusion. Local health authority guidelines must be followed with regard to further observation and monitoring, if applicable. Instructions for infusion reactions are listed in Table 4.

T-DM1 should be given even if Pertuzumab treatment is withheld or discontinued.

Premedication for nausea and infusion reactions are not commonly required but may be given at the investigator's discretion.

Dose delays and modifications for specific T-DM1–related toxicities are described in Section 6.1. T-DM1 will be continued up to 6 cycles, unless the following occur: intolerable toxicity, initiation of another anti-cancer therapy, patient discontinuation, or radiologic-proven evidence of disease progression.

5.13.2 Pertuzumab

Pertuzumab will be administered on Day 1 of a 3-week cycle for a total of 6 cycles. The current protocol does not specify a maximum dose delay for pertuzumab. The initial loading dose of Pertuzumab is 840 mg over 60 minutes (+/- 10 minutes) in cycle 1. The pertuzumab dose in subsequent cycles is 420 mg over 30 minutes (+/- 10 minutes). Patients should be observed for fever and chills or other infusion-associated symptoms for at least 60 minutes after the initial dose and for 30 minutes after subsequent doses. For delayed or missed doses of Pertuzumab, if the time between 2 sequential infusions is less than 6 weeks, the 420 mg IV dose of Pertuzumab should be administered. If the time between 2 sequential infusions is 6 weeks or more, the initial dose of 840 mg Pertuzumab should be re-administered as a 60-minute IV infusion followed every 3 weeks thereafter by a dose of 420 mg IV administered over 30 minutes.

Pertuzumab should be withheld or discontinued if T-DM1 treatment is withheld or discontinued. Missed doses of Pertuzumab are not made up.

Premedication for nausea and infusion reactions are not commonly required but may be given at the investigator's discretion. Additional pertuzumab may be given after surgery as part of a standard of care pertuzumab-containing regimen for up to four cycles at the discretion of the treating physician. Patients should begin this post-surgery pertuzumab within 6 months of surgery. Additionally, adverse events will not be collected nor reported during this treatment. Pertuzumab will be provided by Genentech.

5.13.3 Additional Pre-Surgical Therapy

In participants with evidence of disease progression, additional pre-surgical therapy is allowed. The selected treatment regimen will be at the discretion of the treating investigator.

In participants without evidence of disease progression, additional pre-surgical anti-cancer therapy will not be allowed unless the individual case was reviewed and approved by the Overall PI. In this case, a protocol deviation would not be required.

A research biopsy at time of treatment change, prior to initiation of new anti-cancer

therapy, is strongly recommended, but not required.

5.14 General Concomitant Medication and Supportive Care Guidelines

Concomitant therapy includes any medication (e.g., prescription drugs, over-the-counter drugs, herbal/homeopathic remedies, nutritional supplements) used by a patient from 7 days prior to registration to the end of treatment visit. All concomitant medications should be reported to the investigator and recorded on the Concomitant Medications section of the CRF. All concomitant medications are to be reported until the end of treatment visit.

The following treatments are permitted throughout the duration of the study treatment phase and during follow-up:

- Standard therapies for pre-existing medical conditions unless listed as prohibited therapy in section below. Any medication intended solely for supportive care (e.g., analgesics, anti-diarrheal, anti-depressants) may be used at the investigator's discretion. Patients on anti-coagulant treatment should have their platelet count monitored closely during treatment with T-DM1.
- Hematopoietic growth factors (e.g., G-CSF, granulocyte macrophage colony stimulating factor) may be used at investigator's discretion for the primary prophylaxis and/or management of treatment-emergent neutropenia and/or for secondary prophylaxis as per NCCN/European Society for Medical Oncology guidelines [62, 63] or local standard practice.
- Bisphosphonate or denosumab therapy to be used in accordance with the approved labeled indication and/or nationally recognized treatment guidelines.

Explicitly prohibited therapies prior to the end of treatment visit include:

- Anti-cancer therapies other than those administered in this study, including cytotoxic chemotherapy, radiotherapy (except for adjuvant radiotherapy for breast cancer after completion of chemotherapy), immunotherapy, biological, hormonal or targeted (e.g., lapatinib, neratinib) anti-cancer therapy
- Any systemically active oral, injected, or implanted hormonal method of contraception except for progesterone coated intrauterine devices (IUDs) that had been previously implanted
- Estrogen-replacement therapy
- Chronic immunosuppressive therapies, including systemic corticosteroids
- Any investigational agent, except those used for this study
- Concomitant use of strong CYP3A4 inhibitors (e.g., ketoconazole and itraconazole) with T-DM1 should be avoided. An alternate medication with no or minimal potential to inhibit CYP3A4/5 should be considered. If a strong CYP3A4/5 inhibitor is co-administered with T-DM1, patients should be closely monitored for adverse reactions.

5.15 Definitive Breast Surgery

Definitive breast surgery (excision and/or mastectomy) must be performed no later than 42 days

from administration of the last dose of T-DM1 plus pertuzumab. If contralateral mastectomy is performed concurrently, the pathology report from the contralateral breast must be reported if invasive disease is identified.

5.16 Post-operative Radiotherapy

Decisions regarding choice of post-surgical radiotherapy will be made by the treating team.

5.17 Post-operative Adjuvant Therapy

The treating team will make decisions regarding choice of post-surgical additional adjuvant therapy. The first Post-Surgery Follow-Up visit will be considered the subject's final study visit.

Considering that FDA granted accelerated approval to pertuzumab for use in combination with trastuzumab and chemotherapy for patients with HER2-positive breast cancer, treating physicians will be allowed to prescribe up to four cycles of standard of care pertuzumab-containing regimen as additional post-surgical additional therapy. Patients should begin this post-surgery pertuzumab within 6 months of surgery. Additionally, adverse events will not be collected nor reported during this treatment. Additional pertuzumab will be provided by Genentech.

5.18 Criteria for Taking a Participant off Protocol Therapy

Treatment will continue for 6 cycles or until one of the following criteria applies:

- Disease progression
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Participant demonstrates an inability or unwillingness to comply with the medication regimen and/or documentation requirements
- Participant decides to withdraw from the protocol therapy
- General or specific changes in the participant's condition render the participant unacceptable for further treatment in the judgment of the treating investigator

Participants will be removed from the protocol therapy when any of these criteria apply. The reason for removal from protocol therapy, and the date the participant was removed, must be documented in the case report form (CRF). Alternative care options will be discussed with the participant.

A QACT Treatment Ended/Off Study Form will be filled out when a participant is removed from protocol therapy. This form can be found on the QACT website or obtained from the QACT registration staff. For non-DF/HCC sites this form will be included in the Site Activation Packet.

In the event of unusual or life-threatening complications, treating investigators must immediately notify the Overall PI, Ian Krop, MD, PhD at 617-632-6157 or ikrop@partners.org.

5.19 Duration of Follow Up

The first post-surgery follow-up visit will be considered the subject's final study visit. If breast surgery is not performed the subject's final visit will be the 28 days after the last dose of protocol-specified therapy (i.e., T-DM1 and pertuzumab).

Simplified post-surgery follow-up information will be subsequently collected. Decisions regarding choice of post-surgical treatment and disease assessments will be at the discretion of the treating team and not mandated by the current protocol. Additional post-surgical pertuzumab may be given as part of a standard of care pertuzumab-containing regimen for up to four cycles at the discretion of the treating physician, but this is not mandated by the current protocol. The investigator is not required to actively monitor patients for adverse events after the final visit. However, the Sponsor should be notified if the investigator becomes aware of any death or other serious adverse event that is considered related to the study medication

Post-surgery follow-up information will be collected at assessments every 6 months until 5 years after surgery, then yearly until 10 years after surgery, or until a disease free survival event.

Disease-free survival (DFS) will be defined from the time of registration until the occurrence of the first of the following events:

- Local/regional recurrence: a recurrent or new invasive ipsilateral breast cancer, invasive breast cancer in the axilla, regional lymph nodes, chest wall, or skin of the ipsilateral breast.
- Contralateral invasive breast cancer,
- Distant recurrence: metastatic disease that has either been biopsy confirmed or clinically diagnosed as recurrent invasive breast cancer. A single new lesion on a bone scan without evidence of lytic disease on x-ray and without symptoms does not in and of itself constitute distant recurrence, but multiple new bone lesions, or increased isotope uptake associated with new bone symptoms are more likely due to metastases. Bone metastases must be documented with x-rays and clinical description.
- Death from any cause

In situ cancer is not included as DFS event. If a patient has in situ breast cancer (on the ipsilateral or contralateral side) diagnosed during follow-up before any of the DFS events above, then the patient should continue to be followed for DFS on study (even if she is given hormonal therapy after the in situ diagnosis). These patients will be followed for survival.
If a patient is diagnosed with a non-melanoma skin cancer or a vaginal carcinoma in situ, she will continue on this study and continue to be followed for DFS.

It is recommended that any disease free survival event should be biopsied to confirm recurrent disease. Information on breast cancer status, new anti-cancer therapy, and new onset malignancy diagnoses will be collected via simplified CRFs. Following an IDFS event, survival information (i.e., date and cause of death or last known alive date if not deceased and new onset malignancy information) will be collected.

5.20 Criteria for Taking a Participant off Study

Participants will be removed from study before study completion when any of the following criteria apply:

- Withdrawal of consent for data submission
- Death

The reason for taking a participant off study, and the date the participant was removed, must be documented in the case report form (CRF).

A QACT Treatment Ended/Off Study Form will be filled out when a participant comes off study. This form can be found on the QACT website or obtained from the QACT registration staff.

In the event of unusual or life-threatening complications, treating investigators must immediately notify the Overall PI, Ian Krop, MD, PhD at 617-632-6157 or ikrop@partners.org.

6. EXPECTED TOXICITIES AND DOSING DELAYS/DOSE MODIFICATIONS

A list of the important adverse events and potential risks associated with T-DM1 and Pertuzumab appear below and will determine whether dose delays and modifications will be made and whether the event requires expedited reporting in addition to routine reporting.

6.1 T-DM1

Identified and potential risks of treatment with T-DM1 are based on all available nonclinical and clinical data relating to T-DM1 as well as clinical toxicities related to its components (trastuzumab and maytansine), in addition to other DM1-containing ADCs.

Pulmonary toxicity, hepatotoxicity, cardiac toxicity (left ventricular dysfunction), infusion-related reaction/hypersensitivity, thrombocytopenia (including thrombocytopenia associated with severe hemorrhage), and peripheral neuropathy are important identified risks with T-DM1 and are detailed below. Fetal harm and impaired fertility are important potential risks with T-DM1.

Guidance on dose modifications and discontinuation upon toxicities are provided in Section 6.3-6.4.

Please refer to the Investigator's Brochure for full description of the T-DM1 safety profile, warnings, precautions, and guidance for investigators.

6.1.1 Pulmonary Toxicity

Cases of ILD, including pneumonitis, some leading to acute respiratory distress syndrome or fatal outcome, have been reported in clinical trials with T-DM1. Signs and symptoms include dyspnea, cough, fatigue, and pulmonary infiltrates. These events may or may not occur as sequelae of infusion reactions. Patients with dyspnea at rest as a result of complications of advanced malignancy and/or comorbidities may be at increased risk of pulmonary events. Treatment has included administration of steroids and oxygen, as well as study drug discontinuation.

6.1.2 Hepatotoxicity

Hepatotoxicity, predominantly in the form of asymptomatic increases in the concentrations of serum transaminases (Grade 1–4 transaminitis), has been observed in patients while on treatment with T-DM1 in clinical trials. Transaminase elevations were generally transient with peak elevation at Day 8 after therapy administration and subsequent recovery to Grade 1 or less prior to the next cycle. The incidence of increased AST was substantially higher than that for ALT. A cumulative effect of T-DM1 on transaminases has been observed: the proportion of patients with Grade 1 or 2 elevated transaminases increases with successive cycles; however, no increase in the proportion of Grade 3 abnormalities over time was observed. The majority of patients with elevated transaminases improved to Grade 1 or normal within 30 days of the last dose of T-DM1.

Rare cases of severe hepatotoxicity, including death due to DILI and associated hepatic encephalopathy, have been observed in patients treated with T-DM1. Some of the observed cases may have been confounded by concomitant medications with known hepatotoxic potential and/or underlying conditions. Acute severe liver injury (Hy's law) has the following components:

Aminotransferase enzymes are greater than 3 × ULN with concurrent elevation of serum TBILI to > 2 × ULN, without initial findings of cholestasis (elevated serum alkaline phosphatase).

Cases of NRH of the liver have been identified from liver biopsies in patients treated with T-DM1 and presenting with signs and symptoms of portal hypertension. NRH is a rare liver condition characterized by widespread benign transformation of hepatic parenchyma into small regenerative nodules; NRH may lead to non-cirrhotic portal hypertension and also may be fatal [64]. NRH should be considered in patients who develop clinical symptoms of portal hypertension and/or a cirrhosis-like pattern seen on CT scan of the liver but with normal transaminases and no other manifestations of cirrhosis or liver failure following long-term treatment with T-DM1. Diagnosis of NRH can only be confirmed by histopathology.

6.1.3 Cardiac Toxicity

Patients treated with T-DM1 are at increased risk of developing left ventricular dysfunction. LVEF < 40% has been observed in patients treated with T-DM1.

6.1.4 Infusion-Related Reactions/Hypersensitivity

Infusion-related reactions (anaphylactoid/cytokine release reactions) and hypersensitivity (anaphylactic/allergic reactions) may occur with the administration of monoclonal antibodies and have been reported with T-DM1. Treatment with T-DM1 has not been studied in patients who had trastuzumab permanently discontinued due to an IRR/hypersensitivity; treatment with T-DM1 is not recommended for these patients.

Infusion-related reactions (IRRs), 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 T-DM1. In general, these symptoms were not severe. In most patients, these reactions resolved over the course of several hours

to a day after the infusion was terminated. Serious hypersensitivity (anaphylactic-like reactions) have been observed in clinical trials of T-DM1.

Administration of T-DM1 will be performed in a setting with access to emergency facilities and staff who are trained to monitor and respond to medical emergencies. Patients will be observed closely for infusion related/hypersensitivity reactions during and after each T-DM1 infusion as detailed in Section 5.13. Pre-medication is allowed according to standard practice guidelines. In the event of a true hypersensitivity reaction (in which severity of reaction increases with subsequent infusions), T-DM1 treatment must be permanently discontinued.

6.1.5 Thrombocytopenia

Thrombocytopenia, or decreased platelet count, was reported in patients in clinical trials of T-DM1. The majority of these patients had Grade 1 or 2 events (≥ 50,000/mm3), with the nadir occurring by Day 8 and generally improving to Grade 0 or 1 (≥ 75,000/mm3) by the next scheduled dose. In clinical trials, the incidence and severity of thrombocytopenia were higher in Asian patients. Among Asian patients, the incidence of thrombocytopenia was higher (52.5%) compared with the overall population (30.4%) in Study TDM4370g. However, the incidence rate of Grade ≥ 2 hemorrhage did not increase in Asian patients compared to the overall population.

Rare cases of bleeding events with a fatal outcome have been observed. Independent of race, cases of severe hemorrhagic events, including central nervous system hemorrhage, have been reported in clinical trials with T-DM1. In some of the observed cases the patients were also receiving anti-coagulation therapy. Patients on anti-coagulant treatment have to be monitored closely during treatment with T-DM1. Platelet counts will need to be monitored prior to each T-DM1 dose.

6.1.6 Peripheral neuropathy

Peripheral neuropathy, mainly Grade 1 and predominantly sensory, has been reported in clinical trials of T-DM1. Patients should be examined for signs of peripheral neuropathy prior to each dose of T-DM1.

6.2 Pertuzumab

Overall, safety data indicates that pertuzumab is well-tolerated as monotherapy and that it can be given in combination with trastuzumab and a range of other therapeutic agents with manageable toxicities. No unexpected toxicities of pertuzumab were encountered other than those known for agents that target the HER family of receptors. Infusion related reactions (chills, fatigue, headache, nausea, pyrexia) hypersensitivity reactions and anaphylaxis, neutropenia/febrile neutropenia, diarrhea, mucositis, rash, and left ventricular dysfunction are adverse events (AEs) of particular clinical relevance for this study. Diarrhea has been observed in approximately 60% of patients (treatment-related diarrhea in 50% of patients) being treated with pertuzumab in Phase II single-agent studies, and in up to 90% of patients in combination therapy studies. Diarrhea was Grade 1 or 2 in the majority of cases. Rash has been observed in approximately 17% of patients receiving

pertuzumab in Phase II single-agent studies and up to 73% of patients in combination studies. The rash was generally of Grade 1 or 2 in severity. The toxicities described above are to be closely monitored during the course of the study as detailed in Section 6.3 and/or in the Investigator's Brochure for pertuzumab.

Serious or severe infusion-associated symptoms have been rarely observed in patients receiving pertuzumab. A low level of cardiac AEs, predominantly asymptomatic declines in LVEF, has been reported. In the pivotal Phase III Study WO20698/TOC4129g, the rates of symptomatic and asymptomatic LVSD were lower in patients receiving pertuzumab than in those receiving placebo.

Because of pertuzumab's role in inhibiting heterodimerization with EGFR, there is a potential risk of ILD with pertuzumab treatment. However, few reports of ILD have been received from patients receiving pertuzumab, and in all cases these indicated alternative possible causes for the events (e.g., concomitant medication, preceding/concurrent neutropenia with potential infection, relevant medical history). In Study WO20698/TOC4129g, 2.2% of patients receiving pertuzumab developed pneumonitis/ILD, compared with 1.5% of patients receiving placebo. The incidence of Grade ≥ 3 AEs was similar in both treatment arms (0.7% in the pertuzumab-treated arm vs. 0.5% in the placebo controlled arm).

6.2.1 Single-Agent Pertuzumab

The most commonly reported AEs in patients (N = 386) receiving single-agent pertuzumab were diarrhea, fatigue, nausea, vomiting, and decreased appetite. The majority of AEs reported were Grade 1 or 2 in severity, and the proportion of patients across the pertuzumab program who have discontinued study medication as a result of AEs is low.

6.2.2 Pertuzumab in Combination with Trastuzumab and Docetaxel

Pertuzumab was well tolerated in combination with trastuzumab (Study WO20697 and Study BO17929), with an increase in the incidence but not severity of the common AEs seen with single-agent pertuzumab (notably diarrhea, rash, and fatigue). Pertuzumab also added little toxicity (predominantly diarrhea and febrile neutropenia) to the AE profile of trastuzumab and docetaxel when all three drugs were used concurrently (Study WO20698/TOC4129g and Study WO20697), and had minor impact on the doses received, interruptions, discontinuations, or treatment-related mortality. Diarrhea, rash, mucosal inflammation, febrile neutropenia, pruritus, and dry skin were more common (> 5% difference) in patients receiving the pertuzumab + trastuzumab + docetaxel regimen than in patients in the placebo-controlled arm in Study WO20698/TOC4129g.

Importantly, despite targeting the same HER2 pathway, pertuzumab adds no significant cardiac toxicity when given with trastuzumab (with or without chemotherapy).

An increased incidence of febrile neutropenia was observed for Asian patients in both treatment arms compared with patients of other races and from other geographic regions. Among Asian patients, the incidence of febrile neutropenia was higher in the pertuzumab-treated group (26%) compared with the placebo-treated group (12%) in Study WO20698/TOC4129g.

Refer to the Investigator's Brochure for full description of the pertuzumab safety profile, warnings, precautions, and guidance for investigators.

6.3 Dose Modification Guidelines

Patients will be assessed for toxicity prior to each dose. Patients will be instructed to notify their physician immediately for any and all toxicities. NCI CTCAE v 4.0 must be used to grade the severity of AEs. Assessment of causality (chronology, confounding factors, concomitant medications, medical history, diagnostic tests, and previous experience with the study treatment) should be conducted by the investigator prior to dose modification and/or delay whenever possible.

As a general approach, it is suggested that all AEs be managed with supportive care when possible at the earliest signs of toxicity. If supportive care is ineffective, a dose delay or dose reduction may be considered to avoid worsening toxicity. Dosing will occur only if the clinical assessment and laboratory test values are acceptable.

In the event that any of the individual study drug(s) in a regimen is delayed as a result of toxicity, the administration of other agent(s) may be continued based on the following guidelines:

- If T-DM1 is delayed, pertuzumab should also be withheld.
- If pertuzumab is withheld T-DM1 may continue. Do not make up missed pertuzumab dose.

T-DM1 doses may be delayed and/or reduced and pertuzumab may be delayed as a result of toxicities. No dose reductions are allowed for pertuzumab.

General guidelines on dose delays for study treatment-related toxicity, other than those specified in below are as follows:

- If significant T-DM1 and/or Pertuzumab–related toxicities have not recovered to Grade ≤ 1 or baseline grade, the next scheduled dose may be delayed for up to 21 days from the last scheduled dose. "Significant" and "related" will be based on the judgment of the investigator. For example, alopecia, even if considered related, would most likely not be considered significant. Fatigue may or may not be considered either related or significant.
- After a treatment delay, study therapy should be resumed as soon as possible and within 21 days from the last schedule dose. In general, when the significant and related toxicity resolves to Grade 1 or baseline, the patient may resume study treatment. if the delay has not exceeded the timeframe defined above for corresponding dosing schedule. Patients should be re-evaluated at least weekly during the delay, whenever possible.

In general, when the significant and related toxicity (or any other toxicity for which the investigator chooses to delay dosing) resolves to Grade ≤ 1 or baseline, the patient may resume T-DM1 and/or Pertuzumab if the delay has not exceeded 21 days from the last scheduled dose. Patients should be re-evaluated weekly during the delay whenever possible. If dosing treatment resumes, the patient may receive T-DM1 either at the previous dose level or at one dose level lower (see Table3) based on the specific instructions in the sections below. No dose escalation is allowed after a dose

reduction. No dose reductions are allowed for pertuzumab. Future cycle intervals should remain every 21 days.

If a patient requires a dose reduction for hematologic or hepatic toxicity as described in the following sections, T-DM1 dosing will be reduced by one dose level per Table 3.

Table 3: Dose Reduction for T-DM1

Dose Level	Dose
0	3.6 mg/kg
−1	3.0 mg/kg
−2	2.4 mg/kg
Indication for further dose reduction	Off study treatment

Note: No dose escalation is allowed after a dose reduction.

6.4 Specific AEs for T-DM1 and/or Pertuzumab

During study treatment, some toxicity may be attributable to T-DM1 and/or Pertuzumab. It is important to evaluate the possible cause of toxicity and weigh risk versus benefit for each agent to determine the schema of dose modifications (e.g., which agent to prioritize for maintaining dose level and the sequence of dose modifications).

General guidelines are provided in the following subsections based on the known safety profile of study drugs.

Table 4: Management of Adverse Events

Event	Grade	Action to Be Taken
Infusion-Related Reactions	Infusion-related symptoms Grades 1–2.	Decrease infusion rate by 50% or interrupt infusion for patients who experience any other infusion-related symptoms (e.g., chills, fever). When symptoms have completely resolved, infusion may be restarted at ≤ 50% of prior rate and increased in 50% increments every 30 minutes as tolerated. Infusions may be restarted at the full rate at the next cycle, with appropriate monitoring. In the event of a true hypersensitivity reaction (in which the severity of reaction increases with subsequent infusions) to T-DM1 or pertuzumab treatment, the respective agent must be permanently discontinued. Supportive care with oxygen, β-agonists, antihistamines, antipyretics, or corticosteroids may be used as appropriate at the investigator's discretion. Premedication with corticosteroids, antihistamines, and antipyretics may be used before subsequent infusions at the investigator's discretion. Patients should be monitored until complete resolution of symptoms.
	Grade ≥ 3 allergic/hypersensitivity reaction	Stop infusion; discontinue study treatment permanently. Supportive care with oxygen, β-agonists, antihistamines, antipyretics, or corticosteroids may be used, as appropriate, at the investigator's discretion. Patients should be monitored until complete resolution of symptoms.
Pneumonitis/ Interstitial Lung Disease	Grade 1 or 2	Discontinue all study treatment regardless of attribution..
	Grade 3 or 4	Discontinue all study treatment regardless of attribution.
Paresthesias/ Dysesthesias (Persistent for > 7 Days or Caused the Next Cycle to be Delayed)	Grade 1 or 2	No dose modification
	Grade 3 or 4	Withhold therapy dose until neuropathy < Grade 3 Reduce T-DM1 one dose level Discontinue therapy if event does not resolve to Grade < 3 within 42 days
Ejection fraction decreased	Grade 3 or 4	Discontinue all study treatment.
Heart Failure	Grade 3 or 4	Discontinue all study treatment.
Heart Failure Accompanied by LVEF <45%	Grade 2-4	Discontinue all study treatment.

Asymptomatic decrease in LVEF	See Figure 3 for dose modifications	
ALT	Grade 2 or 3 on Day 1 of a cycle or on the planned day of dosing	Hold T-DM1 and Pertuzumab until ALT recovers to ≤ Grade 1. Assess ALT weekly or as medically indicated. Reduce T-DM1 one dose level.
	Grade 2 or 3 noted between doses	No dose modification if resolved to ≤ Grade 1 by next planned dose.
	Grade 4	Discontinue all protocol treatment. Repeat lab evaluation (within 24 hours) may be done to exclude lab error prior to discontinuing study treatment.
AST	Grade 2 on Day 1 of a cycle or on the planned day of dosing	Hold T-DM1 and Pertuzumab until AST recovers to ≤ Grade 1. Assess AST weekly or as medically indicated. Resume T-DM1 without dose reduction when recovered.
	Grade 2 or 3 noted between doses	No dose modification if resolved to ≤ Grade 1 by next planned dose.
	Grade 3 or 4 on Day 1 of a cycle or on the planned day of dosing	Hold T-DM1 and Pertuzumab until AST recovers to ≤ Grade 1. Reduce T-DM1 one dose level.
Bilirubin (Total)	TBILI > 1.0 X ULN to ≤ 2.0 × ULN on Day 1 of a cycle or on the planned day of dosing	Hold T-DM1 and Pertuzumab until TBILI recovers to ≤ 1.0 × ULN (or direct bilirubin recovers to ≤ 1.0 × ULN for patients with Gilbert's syndrome). Assess TBILI weekly or as medically indicated until recovery. Reduce T-DM1 by one dose level.
	TBILI > 2 × ULN at any time (or direct bilirubin > 2 × ULN for Gilbert's syndrome)	Discontinue all protocol treatment.

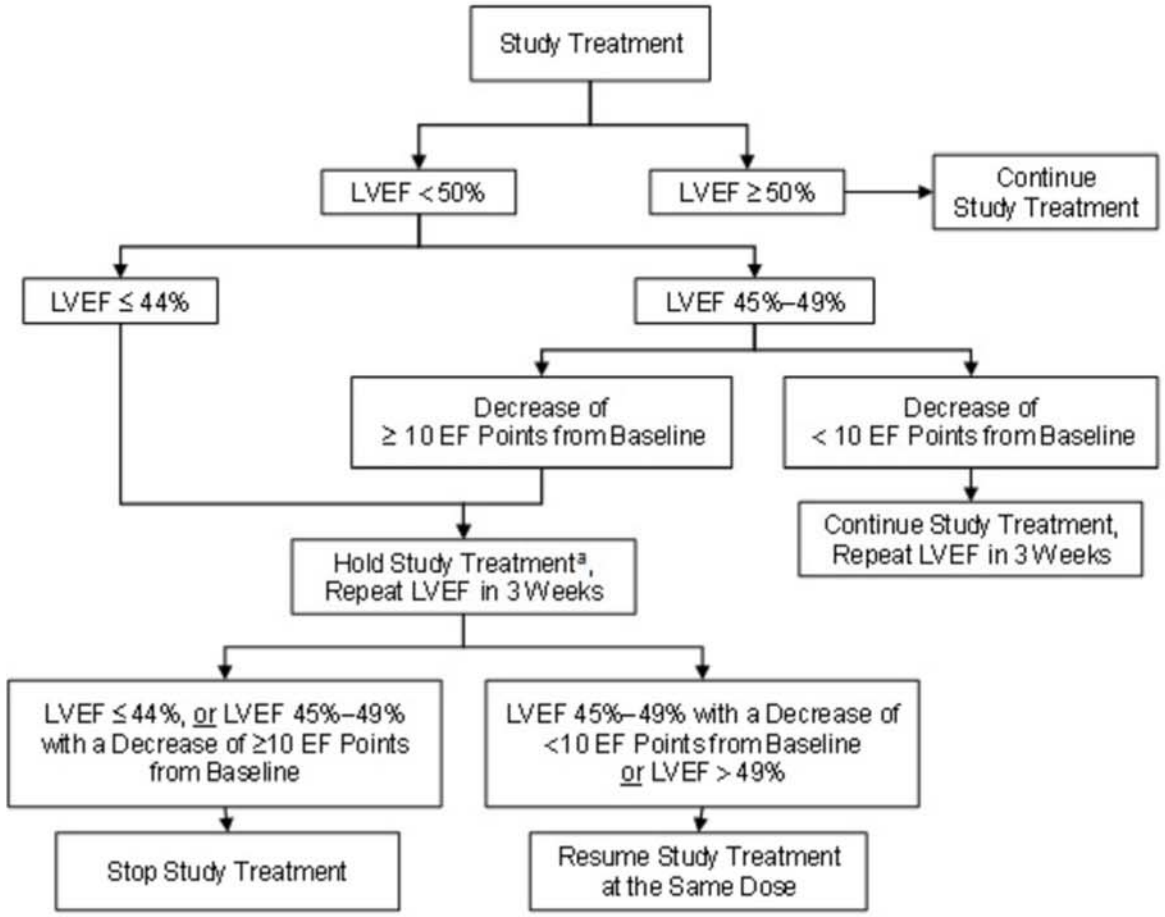
	ALT or AST > 3.0 × ULN concurrent with TBILI > 2.0 × ULN	Discontinue all protocol treatment
Nodular regenerative hyperplasia (NRH)		For any clinical signs of liver dysfunction, discontinue T-DM1 and have the patient evaluated by a hepatologist. If there are signs of portal hypertension (e.g., ascites and/or varices) and a cirrhosis-like pattern is seen on CT scan of the liver, the possibility of NRH should be considered. For liver biopsy guidelines, please see Appendix C . All protocol treatment should be discontinued in the event of a diagnosis of NRH.
Neutropenia	Grade 3	Hold T-DM1 and Pertuzumab until recovered to Grade ≤ 2. Assess ANC weekly or as medically indicated until recovery. Resume treatment without dose reduction.
	Grade 4	Hold T-DM1 and Pertuzumab until recovered to Grade ≤ 2. Assess ANC weekly or as medically indicated until recovery. Reduce T-DM1 one dose level.
Thrombocytopenia	Grade 2 or 3 on day of scheduled treatment	Hold T-DM1 and Pertuzumab until recovered to Grade ≤ 1. Assess platelet counts weekly or as medically indicated until recovery. Resume treatment without dose reduction. If a patient requires two delays due to thrombocytopenia, consider reducing dose by one level.
	Grade 4 at any time	Hold T-DM1 and Pertuzumab until Grade ≤ 1. Assess platelet counts weekly or as medically indicated until recovery. Reduce T-DM1 by one dose level. If event occurs with 2.4 mg/kg dose, discontinue study all protocol treatment.

Cardiovascular Safety Assessments and Dose Modifications Due to Cardiovascular Events
All patients will undergo scheduled LVEF assessments by ECHO or MUGA scans. The results of the LVEF assessments will be used to determine if T-DM1 and pertuzumab administration can be continued (Schedule of Assessments).

Asymptomatic Decrease in LVEF

Refer to Figure 3 for the algorithm for continuation and discontinuation of study treatment on the basis of asymptomatic LVEF assessment.

Figure 3: HER2-Directed Therapy Management Based on LVEF Assessments



LVEF = left ventricular ejection fraction; EF = ejection fraction % points

Note: Baseline refers to the screening LVEF.

[a] Three intermittent holds of study treatment will lead to discontinuation.

Pertuzumab

Pertuzumab dose may be delayed due to toxicities. Pertuzumab dose reductions are not permitted.

Diarrhea and rash are considered EGFR-related risks based on the mechanism of action of pertuzumab. To prevent dehydration, early treatment of diarrhea with anti-diarrheal medication should be considered, and patients should be treated with fluids and electrolyte replacement, as clinically indicated. Treatment recommendations for EGFR-associated rash include topical or oral antibiotics, topical pimecrolimus, and topical or (for severe reactions) systemic steroids. These agents may be used in patients experiencing pertuzumab-related rash, as clinically indicated; although, they have not been studied in this context.

Please refer to the Investigator's Brochure for full description of pertuzumab-related AE management and dose delay guidelines.

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of reported and/or potential AEs (Section 7.1) and the characteristics of an observed AE (Section 7.2) will determine whether the event requires expedited reporting **in addition** to routine reporting.

7.1 Expected Toxicities

7.1.1 Adverse Event List(s) for Trastuzumab emtansine (T-DM1)

In clinical trials, ado-T-DM1 has been evaluated as single-agent in 884 patients with HER2- positive metastatic breast cancer. The most common (frequency ≥ 25%) adverse drug reactions (ADRs) seen in 884 patients treated with ado-T-DM1 were fatigue, nausea, musculoskeletal pain, thrombocytopenia, headache, increased transaminases, and constipation.

Adverse events were identified in patients with HER2-positive metastatic breast cancer treated in a randomized trial, TDM 4370g/BO21977 *(EMILIA)*. Patients were randomized to receive T-DM1 or lapatinib plus capecitabine The median duration of study treatment was 7.6 months for patients in the ado-T-DM1 -treated group and 5.5 months and 5.3 months for patients treated with lapatinib and capecitabine, respectively. Two hundred and eleven (43.1%) patients experienced ≥ Grade 3 adverse events in the ado-T-DM1 -treated group compared with 289 (59.2%) patients in the lapatinib plus capecitabine-treated group. Dose adjustments for ado-T-DM1 were permitted. Thirty-two patients (6.5%) discontinued ado-T-DM1 due to an adverse event, compared with 41 patients (8.4%) who discontinued lapatinib, and 51 patients (10.5%) who discontinued capecitabine due to an adverse event. The most common adverse events leading to ado-T-DM1 withdrawal were thrombocytopenia and increased transaminases. Eighty patients (16.3%) treated with ado-T-DM1 had adverse events leading to dose reductions. The most frequent adverse events leading to dose reduction of ado-T-DM1 (in ≥ (1% of patients) included thrombocytopenia, increased transaminases, and peripheral neuropathy. Adverse events that led to dose delays occurred in 116 (23.7%) of ado-T-DM1 treated patients. The most frequent adverse events leading to a dose delay of ado-T-DM1 (in ≥ 1% of patients) were neutropenia, thrombocytopenia, leukopenia, fatigue, increased transaminases and pyrexia.

The most common ADRs seen with ado-T-DM1 in the randomized trial, *EMILIA* (frequency > 25%) were nausea, fatigue, musculoskeletal pain, thrombocytopenia, increased transaminases, headache, and constipation. The most common NCI–CTCAE (version 4.0) ≥ Grade 3 ADRs (frequency >2%) were thrombocytopenia, increased transaminases, anemia, hypokalemia, peripheral neuropathy and fatigue.

7.1.2 Adverse Event List(s) for Pertuzumab

As of 7 November 2011, 1757 patients with cancer have been treated with pertuzumab in all company-sponsored pertuzumab trials, and an additional 114 patients have received pertuzumab in combination studies with T-DM1. Overall, data indicate that pertuzumab is well-tolerated as monotherapy and that it can be given in combination with trastuzumab and a range of other therapeutic agents with manageable additional toxicity. No new or unexpected toxicities were encountered other than those that are known for agents that target the HER family of receptors. Serious or severe infusion-associated symptoms have been rarely observed in patients receiving pertuzumab. A low level of cardiac toxicities, predominantly asymptomatic declines in left ventricular ejection fraction (LVEF), has been reported. In the pivotal Phase III trial WO20698/TOC4129g the rates of symptomatic and asymptomatic left ventricular systolic dysfunction (LVSD) were not higher in patients receiving pertuzumab, trastuzumab and docetaxel than in those receiving placebo, trastuzumab and docetaxel.
No fetal studies in humans have been performed but pertuzumab caused oligohydramnios, delayed renal development and embryo-fetal deaths in pregnant cynomolgus monkeys. Moreover, in the post-marketing setting, cases of oligohydramnios, some associated with fatal pulmonary hypoplasia of the fetus, have been reported in pregnant women receiving trastuzumab. Therefore, pertuzumab should not be used in pregnant women. Protocols for ongoing pertuzumab studies indicate that one highly effective or two effective contraceptive measures must be used; continuous pregnancy monitoring must be performed during the trials and for six months after the last dose of pertuzumab is administered. Because of the long half-life of pertuzumab women should be warned not to become pregnant for at least seven months after completion of treatment.

7.2 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

- **For expedited reporting purposes only:**
  - AEs for the agent(s) that are listed above should be reported only if the adverse event varies in nature, intensity or frequency from the expected toxicity information which is provided.
  - Other AEs for the protocol that do not require expedited reporting are outlined in the next section (Expedited Adverse Event Reporting) under the sub-heading of Protocol-Specific Expedited Adverse Event Reporting Exclusions.

- **Attribution** of the AE:
  - Definite – The AE *is clearly related* to the study treatment.
  - Probable – The AE *is likely related* to the study treatment.
  - Possible – The AE *may be related* to the study treatment.
  - Unlikely – The AE *is doubtfully related* to the study treatment.
  - Unrelated – The AE *is clearly NOT related* to the study treatment.

7.3 Expedited Adverse Event Reporting

7.3.1 Investigators **must** report to the Overall PI any serious adverse event (SAE) that occurs after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment on the local institutional SAE form.

7.3.2 For multi-institution studies where a DF/HCC investigator is serving as the Overall Principal Investigator, each participating institution must abide by the reporting requirements set by the DF/HCC. This applies to any medical event equivalent to an unexpected grade 2 or 3 with a possible, probable or definite attribution, unexpected grade 4 toxicities, and grade 5 (death) regardless of study phase or attribution.

7.3.3 DF/HCC Expedited Reporting Guidelines

Investigative sites within DF/HCC will report AEs directly to the DFCI Office for Human Research Studies (OHRS) per the DFCI IRB reporting policy.

Other investigative sites will report AEs to their respective IRB according to the local IRB's policies and procedures in reporting adverse events. A copy of the submitted institutional AE form should be forwarded to the Overall PI within the timeframes detailed in the table below.

Table 5: DF/HCC Reportable Adverse Events

Attribution	DF/HCC Reportable AEs				
	Gr. 2 & 3 AE Expected	**Gr. 2 & 3 AE Unexpected**	**Gr. 4 AE Expected**	**Gr. 4 AE Unexpected**	**Gr. 5 AE Expected or Unexpected**
Unrelated Unlikely	Not required	Not required	5 calendar days#	5 calendar days	24 hours*
Possible Probable Definite	Not required	5 calendar days	5 calendar days#	5 calendar days	24 hours*
# If listed in protocol as expected and not requiring expedited reporting, event does not need to be reported.					
* For participants enrolled and actively participating in the study ***or*** for AEs occurring within 30 days of the last intervention, the AE should be reported within 1 business day of learning of the event.					

The Overall PI will submit SAE reports from outside institutions to the DFCI OHRS according to DFCI IRB policies and procedures in reporting adverse events.

7.4 Expedited Reporting to Genentech

7.4.1 Assessment of safety

Specification of Safety Variables

Safety assessments will consist of monitoring and reporting adverse events (AEs) and serious adverse events (SAEs) that are considered related to T-DM1 and/or pertuzumab, all events of death, any pregnancy that occurs during treatment of within seven months following the last dose of T-DM1 and pertuzumab, and any study specific issue of concern.

Adverse Events

An AE is any unfavorable and unintended sign, symptom, or disease temporally associated with the use of an investigational medicinal product (IMP) or other protocol-imposed intervention, regardless of attribution.

This includes the following:

- AEs not previously observed in the subject that emerge during the protocol-specified AE reporting period, including signs or symptoms associated with the participant's disease that were not present prior to the AE reporting period.
- Complications that occur as a result of protocol-mandated interventions (e.g., invasive procedures such as cardiac catheterizations).
- Preexisting medical conditions (other than the condition being studied) judged by the investigator to have worsened in severity or frequency or changed in character during the protocol-specified AE reporting period.

Serious Adverse Events

For the purposes of reporting to Genentech an AE should be classified as an SAE if the following criteria are met:

- It results in death (i.e., the AE actually causes or leads to death).
- It is life threatening (i.e., the AE, in the view of the investigator, places the subject at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death.).
- It requires or prolongs inpatient hospitalization.
- It results in persistent or significant disability/incapacity (i.e., the AE results in substantial disruption of the subject's ability to conduct normal life functions).

- It results in a congenital anomaly/birth defect in a neonate/infant born to a mother exposed to the IMP.
- It is considered a significant medical event by the investigator based on medical judgment (e.g., may jeopardize the subject or may require medical/surgical intervention to prevent one of the outcomes listed above).

7.4.2 Methods and timing for assessing and recording safety variables

The investigator is responsible for ensuring that all AEs and SAEs that are observed or reported during the study, are collected and reported to the FDA, appropriate IRB(s), and Genentech in accordance with CFR 312.32 (IND Safety Reports).

Adverse Event Reporting Period

The study period, during which AEs and SAEs must be reported begins after initiation of study treatment and ends 30 days following the last administration of study treatment or study discontinuation/termination, whichever is earlier. After this period, investigators should only report SAEs that are attributed to study treatment. See Section 7.4.3 for additional reporting requirements for pregnancy and post-study adverse events.

The following adverse events will be reported on the appropriate study specific case report forms:

- Toxicities defined by Genentech as toxicities of special interest per section 7.4.3
- Any other toxicity grade 2 or higher (except for abnormal labs that do not meet reporting criteria defined in section 11.1 or 12.1.1)
    - Abnormal laboratory values or diagnostic test results constitute adverse events only if they induce clinical signs or symptoms or require treatment or further diagnostic tests or require a dose modification.

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP website at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

Assessment of Adverse Events

All AEs and SAEs whether volunteered by the subject, discovered by study personnel during questioning, or detected through physical examination, laboratory test, or other means will be assessed and reported when appropriate. Each reported AE or SAE will be described by its duration (i.e., start and end dates), regulatory seriousness criteria if applicable, suspected relationship to the T-DM1 and pertuzumab (see following guidance), and actions taken.

Expected adverse events are those adverse events that are listed or characterized in the Package Insert or current Investigator Brochure.

Unexpected adverse events are those not listed in the Package Insert (P.I.) or current Investigator Brochure (I.B.) or not identified. This includes adverse events for which the specificity or severity is not consistent with the description in the P.I. or I.B. For example, under this definition, hepatic necrosis would be unexpected if the P.I. or I.B. only referred to elevated hepatic enzymes or hepatitis.

7.4.3 Procedures for Reporting Adverse Events

Deaths

All deaths that occur during the protocol-specified AE reporting period (see Section 7.5.2), regardless of attribution, will be reported to the appropriate parties. When recording a death, the event or condition that caused or contributed to the fatal outcome should be reported as the single medical concept. If the cause of death is unknown and cannot be ascertained at the time of reporting, report "Unexplained Death".

Hospitalizations for Medical or Surgical Procedures

Any AE that results in hospitalization or prolonged hospitalization should be documented and reported as an SAE. If a subject is hospitalized to undergo a medical or surgical procedure as a result of an AE, the event responsible for the procedure, not the procedure itself, should be reported as the SAE. For example, if a subject is hospitalized to undergo coronary bypass surgery, record the heart condition that necessitated the bypass as the SAE.

Hospitalizations for the following reasons do not require reporting:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for preexisting conditions
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study or
- Hospitalization or prolonged hospitalization for scheduled therapy of the target disease of the study.

Pregnancy

If a female subject becomes pregnant while receiving investigational therapy or within 7 months after the last dose of study drug, a report should be completed and expeditiously submitted to the Genentech. Follow-up to obtain the outcome of the pregnancy should also occur. Abortion, whether accidental, therapeutic, or spontaneous, should always be classified as serious, and expeditiously reported as an SAE. Similarly, any congenital anomaly/birth defect in a child born to a female subject exposed to the T-DM1 should be reported as an SAE. Additional information on any pertuzumab-exposed pregnancy and

infant will be requested by Genentech/Roche Drug Safety at specific time points (i.e., after having received the initial report during the first trimester, at the end of the second trimester, 2 weeks after the expected date of delivery, and at 3, 6, and 12 months of the infant's life).

Post-Study Adverse Events
The investigator should expeditiously report any SAE occurring after a subject has completed or discontinued study participation if attributed to prior T-DM1 and/or pertuzumab exposure. If the investigator should become aware of the development of cancer or a congenital anomaly in a subsequently conceived offspring of a female subject who participated in the study, this should be reported as an SAE.

Reconciliation
The Sponsor agrees to conduct reconciliation for the product. Genentech and the Sponsor will agree to the reconciliation periodicity and format, but agree at minimum to exchange monthly line listings of cases received by the other party. If discrepancies are identified, the Sponsor and Genentech will cooperate in resolving the discrepancies. The responsible individuals for each party shall handle the matter on a case-by-case basis until satisfactory resolution.

AEs of Special Interest (AESIs)
AEs of Special Interest are defined as a potential safety problem, identified as a result of safety monitoring of the Product.

The T-DM1 Events of Special Interest are:

- Cardiac events including asymptomatic declines in LVEF requiring treatment or leading to discontinuation of study medication
- Thrombocytopenia (Grade ≥ 3)
- Hepatic events (Grade ≥ 3 AST, ASLT or total bilirubin elevations or Drug-Inducted Liver injury (non-serious and serious))
- Infusion Associated Reactions, Hypersensitivity
- Embryofetal Toxicity or Birth Defects

7.4.4 Procedures for reporting adverse events to Genentech

Investigators must report all AEsof Special Interest (AESIs) and SAEs to Genentech within the timelines described below. The completed Medwatch/case report should be faxed immediately upon completion to Genentech Drug Safety at:

(650) 225-4682
OR

(650) 225-5288

- AEs of Special Interest (AESI), regardless of causality, will be transmitted to Genentech within fifteen (15) calendar days of the Awareness Date.
- Serious AE reports that are unrelated to the ado-T-DM1 or pertuzumab will be transmitted to Genentech within thirty (30) calendar days of the Awareness Date.
- Relevant follow-up information should be submitted to Genentech Drug Safety as soon as it becomes available and/or upon request.

Additional Reporting Requirements to Genentech include the following:
Any reports of pregnancy following the start of administration with the ado-T-DM1 will be transmitted to Genentech within thirty (30) calendar days of the Awareness Date.
All Non-serious Adverse Events originating from the Study will be forwarded in a quarterly report to Genentech.

MedWatch 3500A (Mandatory Reporting) form is available at:
http://www.fda.gov/AboutFDA/ReportsManualsForms/Forms/default.htm

7.5 Study Close-Out

Any study report submitted to the FDA by the Sponsor-Investigator should be copied to Genentech. This includes all IND annual reports and the Clinical Study Report (final study report). Additionally, any literature articles that are a result of the study should be sent to Genentech. Copies of such reports should be mailed to the assigned Clinical Operations contact for the study.

7.6 Expedited Reporting to Hospital Risk Management

Participating investigators will report to their local Risk Management office any participant safety reports or sentinel events that require reporting according to institutional policy.

7.7 Routine Adverse Event Reporting

Adverse Events that meet the adverse event reporting criteria are entered on the toxicity case report forms during routine study data submissions. The reporting period begins after initiation of study treatment and ends 30 days following the last administration of study treatment or study discontinuation/termination, whichever is earlier. See Section 7.4.3 for additional reporting requirements for pregnancy and post-study adverse events. **AEs reported through expedited processes (e.g., reported to the IRB, FDA, etc.) must <u>also</u> be reported in routine study data submissions.**

The following adverse events will be reported on the appropriate study specific case report forms:

- Toxicities defined by Genentech as toxicities of special interest per section 7.4.3
- Any other toxicity grade 2 or higher (except for abnormal labs that do not meet reporting

criteria defined in section 11.1 or 12.1.1)

- Abnormal laboratory values or diagnostic test results constitute adverse events only if they induce clinical signs or symptoms or require treatment or further diagnostic tests or require a dose modification.

8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational or other agents administered in this study can be found in Section 7.1.

8.1 Trastuzumab emtansine (T-DM1)

Refer to the Investigator's Brochure for detailed pertuzumab information and FDA approved package for more information.

8.1.1 Formulation, Preparation, and Storage

Trastuzumab-MCC-DM1 (T-DM1) is provided as a single-use lyophilized formulation in a colorless 20-mL Type I glass vial closed by means of a FluroTec-coated stopper and an overseal with flip-off cap. Upon receipt of T-DM1 vials should be refrigerated at 2°C–8°C. All vials of T-DM1 should be handled by appropriately trained site staff wearing gloves and visually inspected upon receipt to ensure they are intact without exterior contamination. Drug from any vials that appear abnormal upon inspection should not be administered to patients.

The lyophilized product should be reconstituted using Sterile Water for Injection (SWFI). Using a new syringe, 8 mL SWFI should be added to the vial and the vial swirled gently until the product is completely dissolved. The vial should not be shaken. The resulting product contains 20 mg/mL T-DM1, 10 mM sodium succinate, pH 5.0, 60 mg/mL sucrose, and 0.02% (w/v) polysorbate 20. Each 20 mL vial contains enough T-DM1 to allow delivery of 160 mg T-DM1. The reconstituted product contains no preservative and is intended for single use only. The vial should be inspected to ensure the reconstituted product is a clear colorless solution, and is free of particulates before proceeding. Drug from any vial that appears abnormal upon inspection should not be administered to patients. Using a new syringe, the indicated volume of T-DM1 solution should be removed from the vial(s) and added to the IV bag containing at least 250 mL of 0.45% sodium chloride (preferred) or 0.9% sodium chloride injection and gently inverted to mix the solution. A 0.22 micron non-protein adsorptive polyethersulfone (PES) in-line filter is recommended when using 0.45% sodium chloride and required when using 0.9% sodium chloride injection. The solution of T-DM1 should not be shaken.

The solution of T-DM1 for infusion should be used immediately. If not used immediately, storage times should not be longer than 24 hours at 2°C−8°C (36°F−46°F) for solutions of T-DM1 diluted in polyvinyl chloride (PVC) or latex-free PVC-free polyolefin, polypropylene, or polyethylene bags containing 0.45% or 0.9% Sodium Chloride Injection, USP.

For additional details, please refer to the current version of the T-DM1 Investigator

Brochure.

8.1.2 Availability

T-DM1 is an investigational agent and will be supplied free-of-charge from Genentech.

8.1.3 Administration

The first infusion of T-DM1 will be administered over 90 minutes (± 10 minutes). Infusions may be slowed or interrupted for patients experiencing infusion-associated symptoms. Vital signs must be assessed before and after dose administration. Following the initial dose, patients will be observed for at least 90 minutes for fever, chills, or other infusion-associated symptoms. If prior infusions were well tolerated (without any signs or symptoms of infusion reactions), subsequent doses of T-DM1 may be administered over 30 minutes (± 10 minutes), with a minimum 30-minute observation period after infusion.

8.1.4 Ordering

The investigator or those named as sub-investigators agree to supply study drug only to those subjects who are enrolled in the study. The investigator or designee will keep a current and accurate inventory of all clinical drug supplies provided by Genentech. The study site will maintain a dispensing log and compliance with the treatment regimen will be calculated at the site from this information at each visit. Each DFHCC center will order its own inventory of drug. Drug will be shipped directly to that participating site by Genentech.

8.1.5 Treatment Compliance

Pertuzumab will be administered by trained medical personnel at the investigational site. Treatment compliance will be monitored through drug accountability documentation, as well as the recording of study treatment administration in the subject‘s medical record. In addition, all details pertaining to the administration of pertuzumab, including but not limited to date, actual dose, and start and end times of each dose, will be recorded on the Study Drug Administration page of the subject‘s eCRF.

Clinical research associates (CRAs) will review treatment compliance during investigational site visits conducted during and at the completion of the study.

8.1.6 Drug Supplies and Accountability

In compliance with local regulatory requirements, drug supplies will not be sent to the investigator until the following documentation has been received by the sponsor:

- A signed and dated Confidentiality Agreement
- A copy of the final protocol signature page signed and dated by both the sponsor and investigator
- Written proof of approval of the protocol and its ICFs by the Institutional Review Board

(IRB) or Independent Ethics Committee (IEC) for the institution where the study is to be conducted

• A copy of the IRB/IEC-approved patient information and ICF to be used in this study

• The IRB/IEC membership list and Health and Human Services Assurance number (US)

• A copy of the certification and a table of the normal laboratory range for the reference laboratory conducting the clinical laboratory test required by this protocol

• An investigator-signed and dated FDA form 1572, and signed and dated current curriculum vitae and medical license for the PI

• Disclosure of financial interests by the PI and all sub-investigators listed on FDA Form 1572

• A signed and dated Clinical Trial Agreement

The investigator and his or her study staff will be responsible for the accountability of all clinical supplies (dispensing, inventory, and record keeping) following the sponsor‘s instructions and adherence to GCP guidelines as well as local and regional requirements.

Under no circumstances will the investigator allow the study treatment to be used other than as directed by this protocol. Clinical supplies will not be dispensed to any individual who is not enrolled in the study. An accurate and timely record of the receipt of all clinical supplies, dispensing of study treatment to the subject, collection of unused supplies returned by the subject, and subsequent return of unused study treatment to the sponsor must be maintained. This includes, but may not be limited to: (a) documentation of receipt of clinical supplies, (b) study treatment dispensing/return reconciliation log, (c) study treatment accountability log, (d) all shipping service receipts, and (e) documentation of drug returned to the sponsor. All forms will be provided by Genentech. Any comparable forms that the investigational site wishes to use must be approved by Genentech.

The supplies and inventory records must be made available, upon request, for inspection by the designated representative of the sponsor or a representative of the FDA. All unused study drug, are to be returned to Genentech at the conclusion of the study, unless provision is made by Genentech for destruction of supplies and containers at the investigational site. In that case, unused supplies of T-DM1 should be destroyed according to institutional policies.

Destruction will be documented in the Drug Accountability Record Form. Upon completion of drug accountability and reconciliation procedures by investigational site personnel and documentation procedures by Genentech personnel, study drug that is to be returned to Genentech, if necessary, must be boxed, sealed and shipped back to Genentech following all local regulatory requirements. In some regions, study drugs may be removed from the site and hand delivered to the sponsor‘s specified location by sponsor representatives. The CRAs will review drug accountability during investigational site visits and at the completion of the study. The investigator, or a responsible party designated by the investigator, will maintain a careful record of the inventory and disposition of the agent (investigational or free of charge) using the NCI Drug Accountability Record or another comparable drug accountability form. (See the CTEP website at

http://ctep.cancer.gov/protocolDevelopment for the ―Policy and Guidelines for Accountability and Storage of Investigational Agents‖ or to obtain a copy of the drug accountability form.)

8.1.7 Destruction and Return

At the end of the study, unused supplies of T-DM1 should be destroyed according to institutional policies. Destruction will be documented in the Drug Accountability Record Form.

8.2 Pertuzumab

Refer to the Investigator's Brochure for detailed pertuzumab information and FDA approved package for more information.

8.2.1 Description

Pertuzumab drug product is provided as a single use formulation containing 30 mg/mL pertuzumab in 20 mM L-histidine acetate (pH 6.0), 120 mM sucrose and 0.02% polysorbate 20. Each 20 mL vial contains 420 mg of Pertuzumab (14.0 mL/vial). **DEXTROSE (5%) SOLUTION SHOULD NOT BE USED.** The preparation of pertuzumab solution for infusion, using aseptic technique, should be as follows:

- Parenteral drug products should be inspected visually for particulates and discoloration prior to administration.
- Withdraw the appropriate volume of pertuzumab liquid concentrate from the vial(s).
- Dilute into the 250 mL 0.9% sodium chloride PVC or non PVC polyolefin infusion bags. Do not withdraw saline out of the infusion bag.
- Mix diluted solution by gentle inversion. Do not shake.
- Administer immediately once prepared.

8.2.2 Storage Conditions

Upon receipt, pertuzumab vials are to be refrigerated at 2°C–8°C (36°F–46°F) until use. Pertuzumab vials should not be used beyond the expiration date provided by the manufacturer. Because the formulation does not contain a preservative, the vial seal may only be punctured once. Any remaining solution should be discarded. Vial contents should be protected from light, and should not be frozen or shaken. The solution of pertuzumab for infusion, diluted in PVC or non-PVC polyolefin bags containing 0.9% Sodium Chloride Injection, USP, may be stored at 2°C–8°C for up to 24 hours prior to use. Diluted pertuzumab has been shown to be stable for up to 24 hours (up to 30°C). However, since diluted pertuzumab contains no preservative, the diluted solution should be stored refrigerated (2°C–8°C).

8.2.3 Handling

Qualified personnel, familiar with procedures that minimize undue exposure to themselves and the environment, should undertake the preparation, handling, and safe disposal of the chemotherapeutic agent in a self-contained and protective environment.

8.2.4 Availability

Pertuzumab is an investigational agent and will be supplied free-of-charge from Genentech. Pertuzumab will be supplied for neo-adjuvant protocol treatment and adjuvant treatment after surgery for up to four cycles.

8.2.5 Administration

Pertuzumab will be administered on Day 1 of a 3-week cycle for a total of 6 cycles. The initial loading dose of Pertuzumab is 840 mg over 60 minutes (+/- 10 minutes) in cycle 1. The pertuzumab dose in subsequent cycles is 420 mg over 30 minutes (+/- 10 minutes). Patients should be observed for fever and chills or other infusion-associated symptoms for at least 60 minutes after the initial dose and for 30 minutes after subsequent doses. For delayed or missed doses of Pertuzumab, if the time between 2 sequential infusions is less than 6 weeks, the 420 mg IV dose of Pertuzumab should be administered. If the time between 2 sequential infusions is 6 weeks or more, the initial dose of 840 mg Pertuzumab should be re-administered as a 60-minute IV infusion followed every 3 weeks thereafter by a dose of 420 mg IV administered over 30 minutes. Additional pertuzumab may be administered after surgery for up to 4 cycles.

8.2.6 Ordering

The investigator or those named as sub-investigators agree to supply study drug only to those subjects who are enrolled in the study. The investigator or designee will keep a current and accurate inventory of all clinical drug supplies provided by Genentech. The study site will maintain a dispensing log and compliance with the treatment regimen will be calculated at the site from this information at each visit. Each DFHCC center will order its own inventory of drug. Drug will be shipped directly to that participating site by Genentech.

8.2.7 Treatment Compliance

Pertuzumab will be administered by trained medical personnel at the investigational site. Treatment compliance will be monitored through drug accountability documentation, as well as the recording of study treatment administration in the subject‘s medical record. In addition, all details pertaining to the administration of pertuzumab, including but not limited to date, actual dose, and start and end times of each dose, will be recorded on the Study Drug Administration page of the subject‘s eCRF.

Clinical research associates (CRAs) will review treatment compliance during investigational site visits conducted during and at the completion of the study.

8.2.8 Drug Supplies and Accountability

In compliance with local regulatory requirements, drug supplies will not be sent to the investigator until the following documentation has been received by the sponsor:

- A signed and dated Confidentiality Agreement
- A copy of the final protocol signature page signed and dated by both the sponsor and investigator
- Written proof of approval of the protocol and its ICFs by the Institutional Review Board (IRB) or Independent Ethics Committee (IEC) for the institution where the study is to be conducted
- A copy of the IRB/IEC-approved patient information and ICF to be used in this study
- The IRB/IEC membership list and Health and Human Services Assurance number (US)
- A copy of the certification and a table of the normal laboratory ranges for the reference laboratory conducting the clinical laboratory test required by this protocol
- An investigator-signed and dated FDA form 1572, and signed and dated current curriculum vitae and medical license for the PI
- Disclosure of financial interests by the PI and all subinvestigators listed on FDA Form 1572
- A signed and dated Clinical Trial Agreement

The investigator and his or her study staff will be responsible for the accountability of all clinical supplies (dispensing, inventory, and record keeping) following the sponsor‘s instructions and adherence to GCP guidelines as well as local and regional requirements.

Under no circumstances will the investigator allow the study treatment to be used other than as directed by this protocol. Clinical supplies will not be dispensed to any individual who is not enrolled in the study. An accurate and timely record of the receipt of all clinical supplies, dispensing of study treatment to the subject, collection of unused supplies returned by the subject, and subsequent return of unused study treatment to the sponsor must be maintained. This includes, but may not be limited to: (a) documentation of receipt of clinical supplies, (b) study treatment dispensing/return reconciliation log, (c) study treatment accountability log, (d) all shipping service receipts, and (e) documentation of drug returned to the sponsor. All forms will be provided by Genentech. Any comparable forms that the investigational site wishes to use must be approved by Genentech.

The supplies and inventory records must be made available, upon request, for inspection by the designated representative of the sponsor or a representative of the FDA. All unused study drug, are to be returned to Genentech at the conclusion of the study, unless provision is made by Genentech for destruction of supplies and containers at the investigational site. In that case, unused supplies of pertuzumab should be destroyed according to institutional policies.

Destruction will be documented in the Drug Accountability Record Form. Upon completion of drug accountability and reconciliation procedures by investigational site personnel and documentation procedures by Genentech personnel, study drug that is to be

returned to Genentech, if necessary, must be boxed, sealed and shipped back to Genentech following all local regulatory requirements. In some regions, study drugs may be removed from the site and hand delivered to the sponsor‘s specified location by sponsor representatives. The CRAs will review drug accountability during investigational site visits and at the completion of the study. The investigator, or a responsible party designated by the investigator, will maintain a careful record of the inventory and disposition of the agent (investigational or free of charge) using the NCI Drug Accountability Record or another comparable drug accountability form. (See the CTEP website at http://ctep.cancer.gov/protocolDevelopment for the ―Policy and Guidelines for Accountability and Storage of Investigational Agents‖ or to obtain a copy of the drug accountability form.)

8.2.9 Destruction and Return

At the end of the study, unused supplies of Pertuzumab should be destroyed according to institutional policies. Destruction will be documented in the Drug Accountability Record Form.

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

Biomarker Analysis

Tumor samples obtained from pre-treatment core biopsies and surgical specimen will be used to assess the potential prognostic or predictive value of candidate markers or biomarker panels, improve diagnostic tests, improve understanding of breast cancer biology, or discover new biomarker profiles related to treatment benefit and/or safety or disease characteristics.

9.1 Biomarker linked to study primary objective

9.1.1 Assessment of HER2 heterogeneity

Research breast core biopsies of the target lesion will be obtained from all participants prior to initiating protocol therapy as detailed in section 5.11.

Central pathology evaluation for ER, PgR, and HER2 will be performed by Dr. Giuseppe Viale in the Central Pathology Laboratory, European Institute of Oncology, Milan, Italy. ER, PR and HER2 assessment will be performed following current guidelines [65, 66].

HER2 heterogeneity will be defined according to the College of American Pathologists (CAP) and the United Kingdom (UK) guidelines [33, 34].
The entire slide of each core biopsy site will be scanned before selecting 3 separate tumor areas per core bx and counting the number of chromosome 17 (CEP17) and HER2 signals for 50 cells in each area (The HER2 IHC staining will be used to guide pathologists to possible areas of heterogeneity).

HER2 genetic heterogeneity will be defined as the existence of an area of tumor cells with a HER2/CEP17 ratio ≥2.0 or a gene copy number of >6 and representing more than 5% but less than 50% of infiltrating tumor cells. HER2 regional heterogeneity will be defined as at least one of the six tumor areas being HER2 negative by ISH (HER2/CEP17 ratio <2 and CN <6, if IHC is <3+). A HER2-positive tumor will be considered HER2 heterogeneous if genetic heterogeneity and/or HER2 regional heterogeneity is identified

9.2 Biomarkers linked to correlative studies

The following hypotheses will be tested: (1) tumors with higher genetic and/or phenotypic diversity are less likely to respond to T-DM1 treatment, (2) higher post-treatment diversity for a specific genomic locus indicates a causal role in resistance mechanisms, (3) T-DM1 therapy will enrich for tumor cells that have low HER2 levels and/or have low proliferation index

Experimental design:
To assess intratumor genetic and phenotypic heterogeneity at the single cell level and to correlate these with response to treatment we will perform FISH and iFISH analyses and calculate Shannon and Simpson diversity indices based on the evaluation of populations of individual cancer cells within tumors. We will perform FISH and iFISH analyses on 4μ slides of FFPE tissue from tumors essentially as described in our prior publications[67] and in the preliminary data [48]. For FISH we will select HER2 itself, and other probes that may causally contribute to resistance to HER2-targeted therapies such as EGFR, 8q24 (c-MYC), and IGFR, as well as "neutral" regions that are not known to harbor resistance-causing genes but display copy number variation in HER2+ tumors (e.g., 1q32). For immunofluorescence, we will select markers that reflect the activity of a particular pathway relevant to the treatment (e.g., HER2 itself and PI3K/AKT) or reflect the differentiation state of the cells (e.g., ER+ or ER-, more stem-like or more luminal epithelial). In addition, we will also assess the proliferation rate in the phenotypically and genetically distinct tumor cell subpopulations by performing combined staining for Ki67 (or other proliferation marker) and the above listed genetic and phenotypic markers. Determining the proliferation index and its changes after treatment in distinct cell populations could help us identify cancer cells that do or do not respond to treatment.
While these techniques do not allow unbiased and genome-wide assessment of genetic and epigenetic diversity, they are sufficient to detect changes that mark divergent populations of tumor cells and they are technically feasible to apply to core biopsies comprised of small cell numbers. Our preliminary data support the applicability of this approach to address associations between intratumor diversity score and response to neoadjuvant chemotherapy. However, we will also conduct genome-wide studies on single cells or combine genome-wide approaches on bulk tumors with analyses at single cell level. We will assess three different types of diversity: (1) genetic-gene-specific BAC and corresponding CEP copy numbers, (2) phenotypic - IF markers used, and (3) spatial-topology of distinct clones within tumors. Assays will be performed as described in the preliminary data and the enclosed manuscripts (Almendro NEO manuscript).

Other markers that may be selected for exploratory analyses are, for example, other HER family members—markers that are involved in downstream signaling of HER2, belong to a group of related receptor tyrosine kinases that could serve as salvage routes for an inhibited HER2 pathway

(e.g., PIK3CA mutations, PTEN), or are ligands of HER family proteins that induce activation of the HER pathway.

Analysis of the tissue obtained from patients will include the following markers
• RNA extraction (core biopsies collected on RNA later and/or paraffin)
• DNA extraction (paraffin embedded biopsies)
• Additional markers or alternative technologies (based on scientific developments and/or novel technologies) to explore potential prognostic or predictive candidate markers/panels or markers related to treatment benefit and/or safety, to improve diagnostic tests, or to understand breast biology

The study also requires mandatory plasma/serum samples for biomarker research, which may include, among other analyses, the assessment of circulating tumor DNA. Additional candidate markers of response to treatments that emerge from other clinical or nonclinical studies may also be assessed in this study and can be assessed with different types of technologies.

10. STUDY CALENDAR

Baseline evaluations are to be conducted within 4 weeks (28 days) prior to start of protocol therapy. Scans and x-rays must be done $\leq$4 weeks prior to the start of therapy.

Assessments must be performed within 3 days prior to administration of any study agent. Agents should be administered within $\pm$ 3 days of the protocol-specified date, unless otherwise noted.

10.1 Required Tests and Procedures

Tests and procedures	**≤ 28 days prior to study entry (except as noted)**	**Day 1 of each cycle (cycles 1-6)**	**Cycle 2 Day 15-21**	**Pre-surgery Assessment (within 4 weeks of last treatment)**	**Surgery**	**Post-surgery Assessment (within 6 weeks after surgery)**	**Post-surgery follow-up**
Central testing of tumor for HER2 [1]	X						
History + exam, ECOG PS (ECOG at baseline only)	X	X		X			
Vital signs, weight, height (height at baseline only)	X	X					
Hematology (CBC with diff)	X	X		X			
Chemistry [2]	X	X					
INR/PTT[2]	X						
Hepatitis B surface Antibody, Hepatitis B surface Ag, and Hepatitis C Antibody[11]	X						
EKG [3]	X						
MUGA or echocardiogram [4]	X		X[4]	X			
Pregnancy test [5]	X	Cycle 4 only				X	X
Mammogram, breast US, or breast MRI [6]	X			X			
Image-guided Research biopsy [7]	X						
Research specimen collection[8]	X			X	X		
AE evaluation		X		X		X	
CT scan, (chest/abd/pelvis) abd bone scan or PET-CT scan [9]	X						
Study treatment		X					

CRF Submission	X	X		X		X	X[10]

1 See Section 5.5. Central HER2 testing does not need to be performed within 28 days of enrollment.
2 Sodium, potassium, chloride, bicarbonate, BUN, creatinine, total protein, albumin, total bilirubin, SGOT (AST), SGPT (ALT), Alkaline Phosphatase. INR and aPTT at screening only, then as clinically indicated.
3 EKG should be performed within 12 weeks of enrollment.
4 LVEF assessment by ECHO is preferred. The same method should be used throughout the study for each patient and preferably performed and assessed by the same assessor. At baseline, LVEF must be done within 28 days prior to enrollment. ECHO/MUGA should be obtained during the last week of Cycle 2 (Days 15-21), and at the pre surgery visit. Cycle 2 ECHO/MUGA results must be available and reviewed before Cycle 3 Day 1 treatment is administered. Cardiac evaluation should still occur at these time points even if there has been a treatment delay. If a patient comes off study treatment early, cardiac imaging should be performed within 30 days of coming off study treatment.
5 For women of childbearing potential and for those who do not meet the definition of postmenopausal or who have not undergone surgical sterilization, a serum β- HCG must be performed within 7 days prior to treatment start (with the result available prior to first study dosing). During the treatment period, a urine or serum β- HCG pregnancy test in women of childbearing potential must be performed within 72 hours prior to Cycle 4 Day 1 (with the result available prior to dosing), at the follow-up visit 2 to 6 weeks after surgery, at 4 and 7 months (+/-2 weeks) and as clinically indicated. All positive urine pregnancy tests must be confirmed by a serum β-HCG test.
6 Affected breast imaging that measures the tumor must be done within 28 days prior to enrollment; MRI is strongly recommended, although other imaging modalities (mammogram, ultrasound) are permitted if practical or financial considerations preclude MRI, as long as the target lesion can be adequately measured. This same imaging modality should be performed again prior to surgery (within 4 weeks after the last dose of study treatment) to assess tumor response.
7 See Section 5.11
8 See section 10.2 for more information
9 Subjects with Stage III disease according to AJCC Staging Manual edition 7 will have CT scans of chest, abdomen and pelvis and bone scans or PET-CT scans performed during screening to rule out metastatic disease**.**
10 Post-surgery follow-up information will be collected at assessments every 6 months until 5 years after surgery, then yearly until 10 years after surgery, or until a disease free survival event. No tests or procedures are required during follow-up (other than pregnancy test) but clinical data will be collected on CRFs. These data should be based on exams, tests, or procedures done at the registering institution or at a local facility.
11 Should be performed within 12 weeks of beginning study treatment.

10.2 Required Research Specimen Submissions

Specimen Type	Time Point				Kit provided	Shipping Condition	Ship to
	Screening	**Pre-treatment**	**Pre-surgery** (within 4 weeks after last treatment	**Surgery**			
5-8 Unstained slides for central HER-2 testing[a]	X				Yes	Ambient temperature	NeoGenomics
20 mL whole blood in lavender top (EDTA) tube[b]		X			Yes	Ambient temperature	DFCI Laboratory
Three 10 mL Streck tubes of whole blood[b]		X	X		Yes	Ambient temperature	DFCI Laboratory
Core biopsies in formalin[c]		X			Yes	Ambient temperature	DFCI Laboratory
Core biopsies in RNA Later[c]		X			Yes	Ambient temperature	DFCI Laboratory
Surgical block[d]				X	No	Ambient temperature	DFCI Eileen Wrabel
Fresh tumor tissue in RNA Later[d]				X	Yes	Ambient temperature	DFCI Laboratory

a See section 5.5 for more information.
b See section 5.6 for more information.
c See section 5.11 for more information.
d See section 5.12 for more information.

11. MEASUREMENT OF EFFECT

11.1 Antitumor Effect – Solid Tumors

A baseline and presurgical radiographic study of the breast is required; MRI is recommended. The same radiographic modality should be used consistently. The baseline scan must be obtained within 21 days of beginning therapy. The presurgical scan should occur 2-4 weeks after the last chemotherapy administration. If the participant clinically progresses, repeat imaging is required. If there is discordance (clinical progression, but radiographic stable disease or response), contact the study chair.

11.2 Radiographic assessment

Each participant will have pre- and post-therapy radiographic tumor measurements, preferably by MRI, however if logistic or practical issues preclude MRI use, mammogram or ultrasound may be substituted. The longest diameter (LD) of the target lesion at the time of study initiation will be reported as the baseline LD. The baseline LD of the target lesion will be used as reference to further characterize the objective tumor response of the measurable dimension of the disease.

Response criteria are based on the RECIST 1.1 criteria:

Radiographic Complete Response (CR): Complete disappearance of the target lesion

Radiographic Partial Response (PR): Greater than or equal to 30% decrease in the longest diameter (LD) of the target lesion taking as reference the baseline LD.

Radiographic Progressive Disease (PD): Greater than or equal to 20% increase in the LD of target lesion taking as reference the baseline LD or the appearance of one or more new lesions
.
Radiographic Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD taking as reference the baseline LD

11.3 Clinical assessments

Both target and, in the event of multifocal or multicentric invasive cancer, nontarget lesions should be followed clinically and their clinical size recorded at baseline. Measurements thereafter are required; these lesions should be categorized at subsequent visits regarding whether there is evidence of progression. If "yes", the study chair should be notified in order to determine whether the participant should come off protocol treatment.

11.4 Pathologic Response

Pathologic response will be reported using the Residual Cancer Burden calculator [68] M.D Anderson http://www.mdanderson.org/breastcancer_RCB.

The following parameters are required from pathologic examination in order to calculate Residual Cancer Burden (RCB) after neoadjuvant treatment:

- The largest two dimensions (mms) of the residual tumor bed in the breast (largest tumor bed if multicentric disease)
- Histologic assessment of the percentage of the tumor bed area that contains carcinoma (all carcinoma, i.e. invasive and in situ), select one of the following: 0%, 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%
- To assess cellularity it is helpful to scan across the sections of tumor bed and then estimate the average cellularity from the different microscopic fields.
- When estimating percentage cancer cellularity in any microscopic field, compare the involved area with obvious standards, e.g. more or less than half, one quarter, one fifth, one tenth, one twentieth, etc.
- Expect there to be variable cellularity within the cross section of any tumor bed, but estimate the overall cellularity from the average of the estimates in different microscopic fields of the tumor bed. E.g., if cellularity in different fields of the tumor bed were estimated as 20%, 10%, 20%, 0%, 20%, 30%, then an average estimate of overall cellularity would be 20%.
- Histologic estimate of the percentage of the carcinoma in the tumor bed that is in situ, select one of the following: 0%, 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%
- The number of positive (metastatic) lymph nodes
- The largest diameter (mm) of the largest nodal metastasis

The primary objective of this study is to determine the impact of intratumor heterogeneity of HER2 on the pathologic complete response rate (pCR) to T-DM1 plus pertuzumab**. For the purpose of this study pCR will be defined as RCB=0.**

12. DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.0 (Adverse Events: List and Reporting Requirements).

12.1 Data Reporting

The QACT will collect, manage, and perform quality checks on the data for this study.

12.2 Responsibility for Data Submission

Investigative sites within DF/HCC or DF/PCC are responsible for submitting data and/or data forms to the QACT according to the schedule set by the QACT.

12.3 Data Safety Monitoring

The DF/HCC Data and Safety Monitoring Committee (DSMC) will review and monitor toxicity and accrual data from this study. The committee is composed of clinical specialists with experience in oncology and who have no direct relationship with the study. Information that raises any questions about participant safety will be addressed with the Overall PI and study team.

The DSMC will review each protocol up to four times a year or more often if required to review toxicity and accrual data. Information to be provided to the committee may include: up-to-date participant accrual; current dose level information; DLT information; all grade 2 or higher unexpected adverse events that have been reported; summary of all deaths occurring with 30 days of intervention for Phase I or II protocols; for gene therapy protocols, summary of all deaths while being treated and during active follow-up; any response information; audit results, and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

This is an open label phase II neoadjuvant clinical trial of T-DM1 given in combination with pertuzumab for HER2-positive early-stage breast cancer. The planned sample size is 160 patients.

The primary objective is to evaluate the relationship between pathologic complete response (pCR) and intratumor heterogeneity of HER2 amplification. Intratumor heterogeneity will be assessed from two core biopsies at baseline from different geographic areas, and a dichotomous determination of heterogeneity will be made according to the methods given in Section 9. Pathologic response is determined by Residual Cancer Burden (RCB) as RCB= 0.

Secondary clinical endpoints include clinical response, safety and tolerability defined under CTCAE version 4.0, disease-free survival from the date of definitive surgery and overall survival from the date of registration. Secondary laboratory endpoints include continuous measures of intratumor heterogeneity at baseline and in residual tumor using FISH and Immuno-FISH assessments.

13.2 Sample Size, Accrual Rate and Study Duration

The sample size of the study is determined by the percentage of the study population classified as HER2 heterogeneous, the overall pCR rate and its ability to demonstrate differences in pCR rates between heterogeneous and non-heterogeneous subsets. Based on available historical data in HER2 positive disease (described in Section 2), we anticipate the overall rate of pCR with T-DM1 + pertuzumab will be 40%, and approximately 20% of the population will be detected as heterogeneous for HER2. With that prevalence, N = 136 evaluable subjects, there will be 80% power to detect a difference in proportions of pCR of 44.9% in the HER2 homogenous tumors to 20.3% in the HER2 heterogeneous subset (odds ratio = 3.2) which would represent a clinically meaningful difference to warrant further development as a predictive biomarker. Assuming that 85% of enrolled patients will be evaluable for intratumor heterogeneity and pCR the total target sample size is N=165 patients.

As a sensitivity analysis of the study design, the following table gives the effect-sizes (pCR rate of HER2 heterogeneous versus pCR rate of HER2 homogeneous) for which there will be 80% and 90% power to detect under varying observed marker prevalences.

Prevalence of HER2 heterogeneity	80% power	90% power
0.1	12.4% versus 43.1 %	8.8% versus 43.4%
0.2	20.3% versus 44.9%	17.3% versus 45.7%
0.3	24.6% versus 46.6%	22.0% versus 47.7%
0.4	27.5% versus 48.4%	25.3% versus 49.8%
0.5	29.6% versus 50.4%	27.9% versus 52.1%

For all other endpoints of safety and efficacy with T-DM1 plus pertuzumab, a target sample size of N=165 patients will allow for a level of precision such that the maximum half-width to a 95% confidence interval is 8.0%.

13.3 Analysis of Primary Endpoints

For the primary objective to evaluate the association between pCR and HER2 heterogeneity (dichotomous), we will use a stratified Mantel-Haenszel chi-squared test across HR+ and HR- patients using a two-sided alpha = 0.1. Odds ratios in each stratum will be reported with a 90% confidence interval using logistic regression models and Wald-type estimates. Use of the stratified model is critical to prevent confounding due to the known relationship between HR status and pCR [69].

13.4 Analysis of Secondary Endpoints

For a secondary objective to explore the association between continuous measures of HER2 heterogeneity and pCR, descriptive statistics will be used to summarize the distribution of marker values in the study population, and a non-parametric Wilcoxon rank sum test will contrast marker levels between patients who achieve or fail to achieve a pCR.

The proportion of patients characterized as HER2 heterogeneous in HR+ and HR- disease will be summarized with 95% confidence intervals and contrasts will be explored using Fisher's exact test.

For patients with residual tumor, changes in the proportion of cells that are HER2 negative and changes in measures of HER2 heterogeneity will be summarized using descriptive statistics.

All participants will be evaluated for toxicity from the time of their first treatment, and rates of adverse events and treatment modification or discontinuation will be reported with 95% binomial exact confidence intervals.

The distribution of disease-free survival and overall survival will be summarized using Kaplan-Meier estimators with 95% confidence bands.

The association of correlative biomarkers to levels of intratumor heterogeneity and clinical outcomes will be exploratory and hypothesis-generating, and will not adjust for multiple comparisons in any statistical inferences.

All secondary objectives will use two-sided alpha = 0.05 for inferential tests.

14. PUBLICATION PLAN

The results should be made public within approximately 6 months of the end of data collection.

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APPENDIX A PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (*e.g.*, light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

APPENDIX B 14-409 SPECIMEN REQUISITION FORM

Complete this form and include with the specimen shipment. Label ALL materials with participant initials, DFCI participant study ID, and the date the specimen was obtained. Include a pathology report with any tissue specimens being submitted.

- Ship fresh tumor tissue or blood samples to: DF/HCC Core Blood and Tissue Bank, DFCI, 450 Brookline Ave, Smith Bldg- SM 956 Boston, MA 02215
- Ship surgical block(s) and/or slides to: Eileen Wrabel, DFCI Breast Oncology, 450 Brookline Ave. Dana Bldg DA-157 Boston, MA 02215

Specimen Information

Participant Initials (FML): _____ DFCI Participant Study ID Number: _____ DFCI Assigned MRN: _____

Date specimen(s) shipped: _____ Time Point: ☐ Pre-treatment ☐ Pre-surgery ☐ Surgery

Site of tumor: ☐ Right breast ☐ Left breast Pathology reports included (Mark all that apply) ☐ Pre-chemo ☐ Post-chemo

Specimen Type *(indicate inclusion in shipment by checking box)*	**Pathology Number(s) or Serial Coding**	**Quantity submitted**	**Date specimen obtained**	**Time from resection to fixative immersion**
☐ Two 10 ml Whole Blood Lavender (EDTA) Top Tubes				
☐ Three 10 ml Whole Blood Streck Tubes				
Core biopsy site 1				
Clip description (C1):				
☐ Core biopsies in formalin				Minutes
☐ Core biopsies in RNA Later				Minutes
☐ Surgical block ☐ Slides				
☐ Fresh tissue in RNA Later (from Surgery)				Minutes
Core biopsy site 2				
Clip description (C2):				
☐ Core biopsies in formalin				Minutes

☐ Core biopsies in RNA Later				Minutes
☐ Surgical block ☐ Slides				
☐ Fresh tissue in RNA Later (from Surgery)				Minutes
☐ Other, specify:				

Note: Special care should be taken to identify the clips placed at the time of the research core biopsies, and to ensure that the area(s) containing the 2 clips be sampled. The corresponding block (or 2 blocks if the clips are sampled in 2 separate blocks) must be clearly identified with the suffix C12 (if both clips are sampled in one block) or with C1 and C2, if the 2 clips are sampled in 2 different blocks. The specimen may be blocked using regular cassettes or large cassettes.
After the histological assessment of RCB, blocks should be selected for submission to the central lab according to the following criteria:
1. In case of pCR: submit the block(s) relative to the area(s) with the clips (i.e.: C12 or C1 and C2)
2. In case of residual tumor: submit the block(s) relative to the area(s) with the clips (i.e.: C12 or C1 and C2). If these blocks do not include (or only include a minimal portion of) residual tumor, then submit an additional block representative of the main tumor residue.
Please make sure the description of clips (i.e., C1 and C2) used in core biopsies 1 and 2 are clear to allow adequate matching with clips identified in the surgical specimen.

Responsible contact: _____

Email: _____

Phone Number: _____

APPENDIX C GUIDELINES FOR LIVER BIOPSY

As nodular regenerative hyperplasia (NRH) can be a very subtle diagnosis to make on liver biopsy, every attempt should be made to maximize the amount of tissue obtained. A minimum size of an 18 gauge needle and percutaneous biopsies of at least 1.5 cm in length are recommended, if clinically appropriate. In order to diagnose NRH, reticulin and trichrome stains are necessary. Smaller biopsies obtained via a transjugular approach, as well as smaller biopsy gun needle biopsies, are discouraged. Small wedge biopsies should also be discouraged

APPENDIX D DF/HCC MULTI-CENTER DSMP

DFCI IRB Protocol #: 14-409

Dana-Farber/Harvard Cancer Center Multi-Center Data and Safety Monitoring Plan

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1. INTRODUCTION

The Dana-Farber/Harvard Cancer Center Multi-Center Data and Safety Monitoring Plan (DF/HCC DSMP) outlines the procedures for conducting a DF/HCC Multi-Center research protocol. The DF/HCC DSMP should serve as a reference for any sites external to DF/HCC that will be participating in the research protocol.

1.1 Purpose

To establish standards that will ensure that a Dana-Farber/Harvard Cancer Center Multi-Center protocol will comply with Federal Regulations, Health Insurance Portability and Accountability Act (HIPAA) requirements and applicable DF/HCC Standard Operating Procedures.

1.2 Multi-Center Data and Safety Monitoring Plan Definitions

DF/HCC Multi-Center Protocol: A research protocol in which one or more outside institutions are collaborating with Dana-Farber/Harvard Cancer Center where a DF/HCC investigator is the sponsor. DF/HCC includes Dana-Farber/Partners Cancer Care (DF/PCC) Network Clinical Trial Affiliates.

Lead Institution: One of the Dana-Farber/Harvard Cancer Center consortium members (Dana-Farber Cancer Institute (DFCI), Massachusetts General Hospital (MGH), Beth Israel Deaconess Medical Center (BIDMC), Boston Children's Hospital (BCH), Brigham and Women's Hospital (BWH)) responsible for the coordination, development, submission, and approval of a protocol as well as its subsequent amendments per the DFCI IRB and applicable regulatory guidelines (Food and Drug Administration (FDA), etc.). The Lead Institution is typically the home of the DF/HCC Sponsor. The Lead Institution also typically serves as the Coordinating Center for the DF/HCC Multi-Center Protocol.

DF/HCC Sponsor: The person sponsoring the submitted Multi-Center protocol. Within DF/HCC, this person is the Overall Principal Investigator who takes responsibility for initiation, management and conduct of the protocol at all research locations. In applicable protocols, the DF/HCC Sponsor will serve as the single liaison with any regulatory agencies (i.e. FDA). The DF/HCC Sponsor has ultimate authority over the protocol and is responsible for the conduct of the study at DF/HCC and all Participating Institutions. In most cases the DF/HCC Sponsor is the same person as the DF/HCC Overall Principal Investigator; however, both roles can be filled by two different people.

Participating Institution: An institution that is outside the DF/HCC and DF/PCC consortium that is collaborating with DF/HCC on a protocol where the sponsor is a DF/HCC Investigator. The Participating Institution acknowledges the DF/HCC Sponsor as having the ultimate authority and responsibility for the overall conduct of the study.

Coordinating Center: The entity (i.e. Lead Institution, Medical Monitor, Contract Research Organization (CRO), etc) that provides administrative support to the DF/HCC Sponsor in order that he/she may fulfill the responsibilities outlined in the protocol document and DSMP, and as specified in applicable regulatory guidelines. In general, the Lead Institution is the Coordinating Center for the DF/HCC Multi-Center Protocol. Should the DF/HCC Sponsor decide to use a CRO, the CRO will be deemed the Coordinating Center.

DF/HCC Quality Assurance Office for Clinical Trials: A group within DF/HCC responsible for registering human subjects for trials, ensuring high-quality standards are used for data collection and the ongoing management of clinical trials, auditing, and data and safety monitoring. QACT also coordinates quality assurance efforts related to multi-center clinical research.

2. GENERAL ROLES AND RESPONSIBILITIES

For DF/HCC Multi-Center Protocols, the DF/HCC Sponsor, the Coordinating Center, and the Participating Institutions are expected to adhere to the following general responsibilities:

2.1 DF/HCC Sponsor

The DF/HCC Sponsor, Ian Krop, MD, PhD, will accept responsibility for all aspects of conducting a DF/HCC Multi-Center protocol which includes but is not limited to:

- Oversee the coordination, development, submission, and approval of the protocol as well as subsequent amendments.
- Ensure that the investigators, study team members, and Participating Institutions are qualified and appropriately resourced to conduct the protocol.
- Include the Multi-Center Data and Safety Monitoring Plan as an appendix to the protocol.
- Ensure all Participating Institutions are using the correct version of the protocol.
- Ensure that each participating investigator and study team member receives adequate protocol training and/or a Site Initiation Visit prior to enrolling participants and throughout trial's conduct as needed.
- Ensure the protocol will be provided to each participating site in a language understandable to all applicable site personnel when English is not the primary language.
- Monitor progress and overall conduct of the study at all Participating Institutions.
- Ensure all DFCI Institutional Review Board (IRB), DF/HCC and other applicable (i.e. FDA) reporting requirements are met.
- Review data and maintain timely submission of data for study analysis.
- Act as the single liaison with FDA.
- Ensure compliance with all requirements as set forth in the Code of Federal Regulations, applicable DF/HCC requirements, HIPAA requirements, and the approved protocol.

- Commit to the provision that the protocol will not be rewritten or modified by anyone other than the DF/HCC Sponsor.
- Identify and qualify Participating Institutions and obtain accrual commitments prior to extending the protocol to that site.
- Monitor accrual and address Participating Institutions that are not meeting their accrual requirements.

2.2 Coordinating Center

The general responsibilities of the Coordinating Center may include but are not limited to:

- Assist in protocol development.
- Maintain FDA correspondence.
- Review registration materials for eligibility and register participants from Participating Institutions with DF/HCC QACT.
- Distribute protocol and informed consent document updates to Participating Institutions as needed.
- Oversee the data collection process from Participating Institutions.
- Maintain documentation of Serious Adverse Event (SAE) reports and deviations/violation submitted by Participating Institutions and provide to the DF/HCC Sponsor for timely review.
- Distribute serious adverse events reported to the DF/HCC Sponsor that fall under the DFCI IRB Adverse Event Reporting Policy to all Participating Institutions.
- Provide Participating Institutions with information regarding DF/HCC requirements that they will be expected to comply with.
- Carry out plan to monitor Participating Institutions either by on-site or remote monitoring.
- Maintain Regulatory documents of all Participating Institutions which includes but is not limited to the following: local IRB approvals/notifications from all Participating Institutions, confirmation of Federalwide Assurances (FWAs) for all sites, all SAE submissions, Screening Logs for all sites, IRB approved consents for all sites
- Conduct regular communications with all Participating Institutions (conference calls, emails, etc) and maintain documentation all relevant communications.

2.3 Participating Institution

Each Participating Institution is expected to comply with all applicable federal regulations and DF/HCC requirements, the protocol and HIPAA requirements.

The general responsibilities for each Participating Institution may include but are not limited to:

- Document the delegation of research specific activities to study personnel.
- Commit to the accrual of participants to the protocol.
- Submit protocol and/or amendments to their local IRB.
- Maintain regulatory files as per sponsor requirements.

- Provide the Coordinating Center with regulatory documents or source documents as requested.
- Participate in protocol training prior to enrolling participants and throughout the trial as required (i.e. teleconferences).
- Update Coordinating Center with research staff changes on a timely basis.
- Register participants through the Coordinating Center prior to beginning research related activities.
- Submit Serious Adverse Event (SAE) reports to local IRB per local requirements and to the Coordinating Center, in accordance with DF/HCC requirements.
- Submit protocol deviations and violations to local IRB per local requirements and to the DF/HCC Sponsor in accordance with DF/HCC requirements.
- Order, store and dispense investigational agents and/or other protocol mandated drugs per federal guidelines and protocol requirements.
- Have office space, office equipment, and internet access that meet HIPAA standards.
- Participate in any quality assurance activities and meet with monitors or auditors at the conclusion of a visit to review findings.
- Promptly provide follow-up and/or corrective action plans for any monitoring queries or audit findings.

3. DF/HCC REQUIREMENTS FOR MULTI-CENTER PROTOCOLS

The following section will clarify DF/HCC Requirements and further detail the expectations for participating in a DF/HCC Multi-Center protocol.

3.1 Protocol Distribution

The Coordinating Center will distribute the final DFCI IRB approved protocol and any subsequent amended protocols to all Participating Institutions.

3.2 Protocol Revisions and Closures

The Participating Institutions will receive notification of protocol revisions and closures from the Coordinating Center. It is the individual Participating Institution's responsibility to notify its IRB of these revisions.

- **Non life-threatening revisions:** Participating Institutions will receive written notification of protocol revisions regarding non life-threatening events from the Coordinating Center. Non-life-threatening protocol revisions must be IRB approved and implemented within a timely manner

- **Revisions for life-threatening causes:** Participating Institutions will receive immediate notification from the Coordinating Center concerning protocol revisions required to protect lives with follow-up by fax, mail, e-mail, etc. Life-threatening

protocol revisions will be implemented immediately followed by IRB request for approval.

- **Protocol closures and temporary holds:** Participating Institutions will receive notification of protocol closures and temporary holds from the Coordinating Center. Closures and holds will be effective immediately. In addition, the Coordinating Center, will update the Participating Institutions on an ongoing basis about protocol accrual data so that they will be aware of imminent protocol closures.

3.3 Informed Consent Requirements

The DF/HCC approved informed consent document will serve as a template for the informed consent for Participating Institutions. The Participating Institution consent form must follow the consent template as closely as possible and should adhere to specifications outlined in the DF/HCC Guidance Document on Model Consent Language for PI-Initiated Multi-Center Protocols. This document will be provided separately to each Participating Institution.

Participating Institutions are to send their version of the informed consent document and HIPAA authorization, if a separate document, to the Coordinating Center for review and approval prior to submission to their local IRB. The approved consent form must also be submitted to the Coordinating Center after approval by the local IRB for all consent versions.

The Principal Investigator (PI) at each Participating Institution will identify the physician members of the study team who will be obtaining consent and signing the consent form for therapeutic protocols. Participating institutions must follow the DF/HCC requirement that only attending physicians obtain informed consent and re-consent to interventional trials (i.e. drug and/or device trials).

3.4 IRB Documentation

The following must be on file with the Coordinating Center:

- Initial approval letter of the Participating Institution's IRB.
- Copy of the Informed Consent Form(s) approved by the Participating Institution's IRB.
- Participating Institution's IRB approval for all amendments.
- Annual approval letters by the Participating Institution's IRB.

3.5 IRB Re-Approval

Verification of IRB re-approval from the Participating Institutions is required in order to continue research activities. There is no grace period for continuing approvals.

The Coordinating Center will not register participants if a re-approval letter is not received from the Participating Institution on or before the anniversary of the previous approval date.

3.6 Participant Confidentiality and Authorization Statement

In 1996, congress passed the first federal law covering the privacy of health information known as the Health Insurance Portability and Accountability Act (HIPPA). Any information, related to the physical or mental health of an individual is called Protected Health Information (PHI). HIPAA outlines how and under what circumstances PHI can be used or disclosed.

In order for covered entities to use or disclose protected health information during the course of a study, the study participant must sign an authorization statement. This authorization statement may or may not be separate from the informed consent document. The Coordinating Center, with approval from the DFCI IRB, will provide a consent template, with information regarding authorization for the disclosure of protected health information.

The DF/HCC Sponsor will use all efforts to limit its use of protected health information in its trials. However, because of the nature of these trials, certain protected health information must be collected. DF/HCC has chosen to use authorizations, signed by the participant in the trial, rather than limited data sets with data use agreements.

DF/HCC Multi-Center Protocol Confidentiality

All documents, investigative reports, or information relating to the participant are strictly confidential. Whenever reasonably feasible, any participant specific reports (i.e. Pathology Reports, MRI Reports, Operative Reports, etc.) submitted to the Coordinating Center should be de-identified. It is recommended that the assigned DF/HCC QACT case number (as described below) be used for all participant specific documents. Participant initials may be included or retained for cross verification of identification.

3.7 DF/HCC Multi-Center Protocol Registration Policy

3.7.1 Participant Registration

Refer to section 4.4 of the protocol for participant registration process.

3.7.2 Initiation of Therapy

Participants must be registered with the DF/HCC QACT before receiving treatment. Treatment may not be initiated until the Participating Institution receives confirmation of the participant's registration from the Coordinating Center. The DF/HCC Sponsor and DFCI IRB must be notified of any violations to this policy.

3.7.3 Eligibility Exceptions

The DF/HCC QACT will make no exceptions to the eligibility requirements for a protocol without DFCI IRB approval. The DF/HCC QACT requires each institution to fully comply with this requirement.

3.7.4 DF/HCC Protocol Case Number

At the time of registration, QACT requires the following identifiers for all subjects: initials, date of birth, gender, race and ethnicity. Once eligibility has been established and the participant successfully registered, the participant is assigned a unique protocol case number. Participating Institutions should submit all de-identified subsequent communication and documents to the Coordinating Center, using this case number to identify the subject.

3.8 Protocol Deviations, Exceptions and Violations

Federal Regulations require an IRB to review proposed changes in a research activity to ensure that researchers do not initiate changes in approved research without IRB review and approval, except when necessary to eliminate apparent immediate hazards to the participant. DF/HCC requires all departures from the defined procedures set forth in the IRB approved protocol to be reported to the DF/HCC Sponsor, who in turn is responsible for reporting to the DFCI IRB.

For reporting purposes, DF/HCC uses the terms "violation", "deviation" and "exception" to describe departures from a protocol. All Participating Institutions must adhere to these requirements for reporting to the DF/HCC Sponsor and will follow their institutional policy for reporting to their local IRB.

3.8.1 Definitions

Protocol Deviation: Any departure from the defined procedures set forth in the IRB-approved protocol which is *prospectively approved* prior to its implementation.

Protocol Exception: Any protocol deviation that relates to the eligibility criteria, e.g. enrollment of a participant who does not meet all inclusion/exclusion criteria.

Protocol Violation: Any protocol deviation that was not *prospectively approved* by the IRB prior to its initiation or implementation.

3.8.2 Reporting Procedures

DF/HCC Sponsor: is responsible for ensuring that clear documentation is available in the medical record and/or regulatory documents to describe all protocol exceptions, deviations and violations. The DF/HCC Sponsor will also be responsible for ensuring that all protocol violations/deviations are promptly reported per DFCI IRB guidelines.

Participating Institutions: Protocol deviations require prospective approval from the DFCI IRB. The Participating Institution must submit the deviation request to the Coordinating Center who will then submit the deviation request to the DFCI IRB. Upon DFCI IRB approval the deviation is submitted to the Participating Institution IRB, per

institutional policy. A copy of the Participating Institution's IRB report and determination will be forwarded to the Coordinating Center within 10 business days after the original submission.

All protocol violations must be sent to the Coordinating Center in a timely manner.

Coordinating Center: Upon receipt of the violation/deviation report from the Participating Institution, the Coordinating Center will submit the report to the DF/HCC Sponsor for review. Subsequently, the Participating Institution's IRB violation/deviation report will be submitted to the DFCI IRB for review per DFCI IRB reporting guidelines. DF/HCC will forward all violation reports via an internal DF/HCC process, as applicable.

3.9 Safety Assessments and Toxicity Monitoring

The study teams at all participating institutions are responsible for protecting the safety, rights and well-being of study participants. Recording and reporting of adverse events that occur during the course of a study help ensure the continuing safety of study participants.

All participants receiving investigational agents and/or other protocol mandated treatment will be evaluated for safety. The safety parameters include all laboratory tests and hematological abnormalities, physical examination findings, and spontaneous reports of adverse events reported by participants. All toxicities encountered during the study will be evaluated according to the criteria specified in the protocol. Life-threatening toxicities must be reported immediately to the DF/HCC Sponsor via the Coordinating Center.

Additional safety assessments and toxicity monitoring will be outlined in the protocol.

3.9.1 Guidelines for Reporting Serious Adverse Events

Guidelines for reporting Adverse Events (AEs) and Serious Adverse Events (SAEs) are detailed in protocol section 7.

Participating Institutions must report the SAEs to the DF/HCC Sponsor and the Coordinating Center following the DFCI IRB Adverse Event Reporting Policy.

The Coordinating Center will maintain documentation of all Participating Institution Adverse Event reports and be responsible for communicating to all participating investigators, any observations reportable under the DFCI IRB Reporting Requirements. Participating Institutions will review and submit to their IRB according to their institutional policies and procedures

3.9.2 Guidelines for Processing IND Safety Reports

The DF/HCC Sponsor will review all IND Safety Reports and ensure that all IND Safety Reports are distributed to the Participating Institutions. Participating Institutions will review and submit to their IRB according to their institutional policies and procedures.

3.10 Data Management

The DF/HCC QACT develops case report forms (CRF/eCRFs), for use with the protocol. These forms are designed to collect data for each study. The DF/HCC QACT provides a web based training for eCRF users.

3.10.1 Data Forms Review

Data submissions are monitored for timeliness and completeness of submission. Participating Institutions are notified of their data submission delinquencies in accordance with the following:

3.10.2 Incomplete or Questionable Data

If study forms are received with missing or questionable data, the submitting institution will receive a written or electronic query from the DF/HCC QACT Data Analyst, Coordinating Center or designee. Responses to all queries should be completed and submitted within 14 calendar days. Responses may be returned on the written query or on an amended paper case report form, or in the case of electronic queries, within the electronic data capture (eDC) system. In the case of a written query for data submitted on a paper case report form, the query must be attached to the specific data being re-submitted in response.

3.10.3 Missing Forms

If study forms are not submitted on schedule, the Participating Institution will receive a Missing Form Report from the Coordinating Center noting the missing forms. These reports are compiled by the DF/HCC QACT and distributed on a monthly basis.

4. REQUISITIONING INVESTIGATIONAL DRUG

The ordering of investigational agent is specified in the protocol section 8.

Participating Institutions should order their own agent regardless of the supplier.

Ensure that the pharmacy will be able to receive and store the agents according to state and federal requirements. The local IRB should be kept informed of who will supply the agent (i.e., Genentech) so that any regulatory responsibilities can be met in a timely fashion.

5. MONITORING: QUALITY CONTROL

The quality control process for a clinical trial requires verification of protocol compliance and data accuracy. The Coordinating Center, with the aid of the QACT provides quality control oversight for the protocol.

5.1 Ongoing Monitoring of Protocol Compliance

The Participating Institutions may be required to submit participant source documents to the Coordinating Center for monitoring. Participating Institution may also be subject to on-site monitoring conducted by the Coordinating Center.

The DF/HCC Lead Institution will implement on-site as well as virtual monitoring activities to ensure that Participating Institutions are complying with regulatory and protocol requirements, data quality, and subject safety. At a minimum, the DF/HCC Lead Institute, or designee, will monitor each participating site twice a year while patients are receiving treatment. Should a Participating Institution be monitored once and then not accrue any additional patients or participant visits, then a second monitoring visit may not be necessary.
Monitoring practices may include but are not limited to; source verification, review and analysis of the following: eligibility requirements of all participants, informed consent procedures, adverse events and all associated documentation, study drug administration / treatment, regulatory records and site trial master files, protocol deviations, pharmacy records, response assessments, and data management. Additionally, regular and ongoing communication with Participating Institutions, will be accomplished by holding all site bi-weekly teleconferences. The Lead Institution will keep in close touch with the Participating Institutions via email and phone. Source documents from Participating Institutions, will be collected at specific data points that support the primary and or secondary endpoints.

On-Site Monitoring: On-site monitoring will occur on an as-needed basis. Participating Institutions will be required to provide access to participants' complete medical record and source documents for source documentation verification during the on-site visit. In addition, upon request from a monitor or auditor, Participating Institutions should provide access to regulatory documents, pharmacy records, local policies related to the conduct of research, and any other trial-related documentation maintained by the participating site. If there are concerns for protocol compliance, issues that impact subject safety or the integrity of the study are found, or trends identified based on areas of need, additional monitoring visits may be scheduled. On site monitoring visits can be supplemented with virtual monitoring assessments, provided that the minimum monitoring frequencies are adhered to.

Virtual Monitoring: The Coordinating Center will request source documentation from Participating Institutions as needed to complete monitoring activities. Participating Institutions will be asked to forward de-identified copies of participants' medical record and source documents to the Coordinating Center to aid in source documentation verification.

The Overall PI, or designee, will review all monitoring reports for on-site and virtual monitoring of Participating Institutions to ensure protocol compliance and ability to fulfill responsibilities of participation in the study. The Coordinating Center may increase the monitoring activities at

Participating Institutions that are unable to comply with the protocol, DF/HCC requirements or federal and local regulations. Participating Institutions may also be subject to an audit as determined by the Coordinating Center.

In addition to monitoring performed by the Coordinating Center, DF/HCC QACT may monitor data for timeliness of submission, completeness, and adherence to protocol requirements. The Lead Institution or designee and, if applicable, QACT Data Analysts assigned to the Protocol will perform the ongoing protocol data compliance monitoring with the support of the Participating Institution's Coordinators, the Principal Investigators, and the Protocol Chair.

5.2 Monitoring Reports

The DF/HCC Sponsor will review all monitoring reports for on-site and remote monitoring of Participating Institutions to ensure protocol compliance. The DF/HCC Sponsor may increase the monitoring activities at Participating Institutions that are unable to comply with the protocol, DF/HCC Sponsor requirements or federal and local regulations. Participating Institutions may also be subject to an audit as determined by the DF/HCC Sponsor.

5.3 Accrual Monitoring

Prior to extending a protocol to an external site, the DF/HCC Sponsor will establish accrual requirements for each participating institution. Accrual will be monitored for each participating institution by the DF/HCC Sponsor or designee. Sites that are not meeting their accrual expectations may be subject to termination. Sites are expected to accrue at least 3 patients per year.

6. AUDITING: QUALITY ASSURANCE

Auditing is a method of Quality Assurance. Its main focus is to measure whether standards and procedures were followed. Auditing is the systematic and independent examination of all trial related activities and documents. Audits determine if evaluated activities were appropriately conducted and whether data was generated, recorded and analyzed, and accurately reported per the protocol, Standard Operating Procedures (SOPs), and the Code of Federal Regulations (CFR).

6.1 Audit Plan: DF/HCC Sponsored Trials

One on-site audit will be scheduled by the QACT, assuming at least three participants have been treated on protocol at the site. Approximately 3-4 participants will be audited at the site over a 2 day period. If violations which impact participant safety or the integrity of the study are found, more participant records may be audited.

6.2 Audit Notification

It is the Participating Institution's responsibility to notify the Coordinating Center of all scheduled audit dates and re-audit dates (if applicable), which involve this protocol. All institutions will forward a copy of final audit and/or re-audit reports and corrective action plans (if applicable) to the Coordinating Center, within 12 weeks after the audit date.

6.3 Audit Reports

The DF/HCC Sponsor will review all final audit reports and corrective action plans if applicable. The Coordinating Center, must forward these reports to the DF/HCC QACT per DF/HCC policy for review by the DF/HCC Audit Committee. Based upon the audit assessments the DF/HCC Audit Committee could accept or conditionally accept the audit rating and final report. Conditional approval could require the DF/HCC Sponsor to implement recommendations or require further follow-up. For unacceptable audits, the DF/HCC Audit Committee would forward the final audit report and corrective action plan to the DFCI IRB as applicable.

6.4 Participating Institution Performance

The DF/HCC Sponsor and the DFCI IRB, is charged with considering the totality of an institution's performance in considering institutional participation in the protocol.

Corrective Actions

Participating Institutions that fail to meet the performance goals of accrual, submission of timely and accurate data, adherence to protocol requirements, and compliance with state and federal regulations, may be recommended for a six-month probation period. Such institutions must respond with a corrective action plan and must demonstrate during the probation period that deficiencies have been corrected, as evidenced by the improved performance measures. Participating Institutions that fail to demonstrate significant improvement will be considered by the DF/HCC Sponsor for revocation of participation. A DF/HCC Sponsor and/or the DFCI IRB may terminate a site's participation if it is determined that a site is not fulfilling its responsibilities as described above.

DANA-FARBER CANCER INSTITUTE
Nursing Protocol Education Sheet

Protocol Number:	**14-409**
Protocol Name:	**The impact of HER2 heterogeneity on the treatment of early-stage HER2-positive breast cancer: a phase II study of T-DM1 in combination with Pertuzumab in the preoperative setting**
DFCI Site PI:	Ian Krop, MD
DFCI Research Nurse:	Peg Haldoupis, RN; Liz Kasparian, RN; Mary O'Driscoll, RN; Kathy Roche, RN; Myra St. Amand, RN

Page the DFCI research nurse or DFCI site PI if there are any questions/concerns about the protocol.
Please also refer to ONC 15: Oncology Nursing Protocol Education Policy
***** Remember to check the ALERT PAGE*****

SPECIAL NURSING CONSIDERATIONS UNIQUE TO THIS PROTOCOL

Study Design	**T-DM1** is an anti-HER2 monoclonal antibody (trastuzumab) coupled with a cytoxic agent (DM1). **Pertuzumab** is a HER2-targeted monoclonal antibody. **Study Design** – Section 1.1; **Study Rationale** – Section 2.3; **A cycle is defined as 3 weeks** – Section 5
Dose Calc.	• **T-DM1** is dosed in mg/kg – Section 5.13 • **Pertuzumab** is a fixed dose in mg – Section 5.13 • COE will use our institutional standard of practice for dose calculations.
Study Drug Administration	**T-DM1** • IV, administered on Day 1 of each cycle – Section 5.13 • A 0.22 micron in-line filter is recommended for infusion– Section 8.1.1 • No routine premedication is required – Section 5.13 • Pre- and post-infusion vital signs are required for the first infusion – Section 5.13.1 • Please see Section 5.13 for detailed administration instructions and (required post-infusion observation times). **Pertuzumab** • IV, administered on Day 1 of each cycle – Section 5.13 • DEXTROSE (5%) solution should NOT BE USED – Section 8.2.1 • No routine premedication is required – Section 5.13 • Please see Section 5.13 for detailed administration instructions and(required post-infusion observation times.) • A new section for post-operative adjuvant therapy has been added – Section 5.17
Dose Mods & Toxicity	*Dose Modifications/Dosing Delay for Toxicity* are outlined in Section 6 • This protocol uses NCI CTCAE criteria, version 4.0 – Section 6.3 • Dose modification guidelines are in Section 6.3
Con Meds	*Concomitant Therapy* Guidelines are in Section 5.14 • Please review the cited sections for permitted, prohibited, and "use with caution" medications/therapies/foods
Required Data	*Study Calendar and Assessment Required data* are outlined in Sections 5 and 10 • The study calendar is in Section 10 • **V/S:** Obtain in seated position – Sections 5.3 • **Research Blood samples:** Sections 5.6 and 10.2 • **ECGs:** Single, at screening only and then as clinically indicated – Section 5.8.1
Charting Tips	**All study drugs require documentation of exact administration time.** Please be sure to DOCUMENT study medication **actual** UP/DOWN times in medical record (e.g. LMR, eMAR, nursing notes). **Edit eMAR as needed to match the exact time given**. • If there is a discrepancy in the infusion time, delay in administration, or infusion takes longer than is permitted by the guidelines of the protocol, please **document the reason for the discrepancy in the medical record.** Please be sure to also DOCUMENT the required observation periods, any additional vital signs, routes of administration, injection sites, and exact time of research blood sample collections.

DFCI IRB PROTOCOL #: 14-409

APPENDIX D: DANA-FARBER/HARVARD CANCER CENTER MULTI-CENTER DATA AND SAFETY MONITORING PLAN

TABLE OF CONTENTS

1. INTRODUCTION

The Dana-Farber/Harvard Cancer Center Multi-Center Data and Safety Monitoring Plan (DF/HCC DSMP) outlines the procedures for conducting a DF/HCC Multi-Center research protocol. The DF/HCC DSMP should serve as a reference for any sites external to DF/HCC that will be participating in the research protocol.

1.1 Purpose

To establish standards that will ensure that a Dana-Farber/Harvard Cancer Center Multi-Center protocol will comply with Federal Regulations, Health Insurance Portability and Accountability Act (HIPAA) requirements and applicable DF/HCC Standard Operating Procedures.

1.2 Multi-Center Data and Safety Monitoring Plan Definitions

DF/HCC Multi-Center Protocol: A research protocol in which one or more outside institutions are collaborating with Dana-Farber/Harvard Cancer Center where a DF/HCC investigator is the sponsor. DF/HCC includes Dana-Farber/Partners Cancer Care (DF/PCC) Network Clinical Trial Affiliates.

Lead Institution: One of the Dana-Farber/Harvard Cancer Center consortium members (Dana-Farber Cancer Institute (DFCI), Massachusetts General Hospital (MGH), Beth Israel Deaconess Medical Center (BIDMC), Boston Children's Hospital (BCH), Brigham and Women's Hospital (BWH)) responsible for the coordination, development, submission, and approval of a protocol as well as its subsequent amendments per the DFCI IRB and applicable regulatory guidelines (Food and Drug Administration (FDA), etc.). The Lead Institution is typically the home of the DF/HCC Sponsor. The Lead Institution also typically serves as the Coordinating Center for the DF/HCC Multi-Center Protocol.

DF/HCC Sponsor: The person sponsoring the submitted Multi-Center protocol. Within DF/HCC, this person is the Overall Principal Investigator who takes responsibility for initiation, management and conduct of the protocol at all research locations. In applicable protocols, the DF/HCC Sponsor will serve as the single liaison with any regulatory agencies (i.e. FDA). The DF/HCC Sponsor has ultimate authority over the protocol and is responsible for the conduct of the study at DF/HCC and all Participating Institutions. In most cases the DF/HCC Sponsor is the same person as the DF/HCC Overall Principal Investigator; however, both roles can be filled by two different people.

Participating Institution: An institution that is outside the DF/HCC and DF/PCC consortium that is collaborating with DF/HCC on a protocol where the sponsor is a DF/HCC Investigator. The Participating Institution acknowledges the DF/HCC Sponsor as having the ultimate authority and responsibility for the overall conduct of the study.

Coordinating Center: The entity (i.e. Lead Institution, Medical Monitor, Contract Research Organization (CRO), etc) that provides administrative support to the DF/HCC Sponsor in order that he/she may fulfill the responsibilities outlined in the protocol document and DSMP, and as specified in applicable regulatory guidelines. In general, the Lead Institution is the Coordinating

Center for the DF/HCC Multi-Center Protocol. Should the DF/HCC Sponsor decide to use a CRO, the CRO will be deemed the Coordinating Center.

DF/HCC Quality Assurance Office for Clinical Trials: A group within DF/HCC responsible for registering human subjects for trials, ensuring high-quality standards are used for data collection and the ongoing management of clinical trials, auditing, and data and safety monitoring. QACT also coordinates quality assurance efforts related to multi-center clinical research.

2. GENERAL ROLES AND RESPONSIBILITIES

For DF/HCC Multi-Center Protocols, the DF/HCC Sponsor, the Coordinating Center, and the Participating Institutions are expected to adhere to the following general responsibilities:

2.1 DF/HCC Sponsor

The DF/HCC Sponsor, Ian Krop, MD, PhD, will accept responsibility for all aspects of conducting a DF/HCC Multi-Center protocol which includes but is not limited to:

- Oversee the coordination, development, submission, and approval of the protocol as well as subsequent amendments.
- Ensure that the investigators, study team members, and Participating Institutions are qualified and appropriately resourced to conduct the protocol.
- Include the Multi-Center Data and Safety Monitoring Plan as an appendix to the protocol.
- Ensure all Participating Institutions are using the correct version of the protocol.
- Ensure that each participating investigator and study team member receives adequate protocol training and/or a Site Initiation Visit prior to enrolling participants and throughout trial's conduct as needed.
- Ensure the protocol will be provided to each participating site in a language understandable to all applicable site personnel when English is not the primary language.
- Monitor progress and overall conduct of the study at all Participating Institutions.
- Ensure all DFCI Institutional Review Board (IRB), DF/HCC and other applicable (i.e. FDA) reporting requirements are met.
- Review data and maintain timely submission of data for study analysis.
- Act as the single liaison with FDA.
- Ensure compliance with all requirements as set forth in the Code of Federal Regulations, applicable DF/HCC requirements, HIPAA requirements, and the approved protocol.
- Commit to the provision that the protocol will not be rewritten or modified by anyone other than the DF/HCC Sponsor.
- Identify and qualify Participating Institutions and obtain accrual commitments prior to extending the protocol to that site.
- Monitor accrual and address Participating Institutions that are not meeting their accrual requirements.

2.2 Coordinating Center

The general responsibilities of the Coordinating Center may include but are not limited to:

- Assist in protocol development.
- Maintain FDA correspondence.
- Review registration materials for eligibility and register participants from Participating Institutions with DF/HCC QACT.
- Distribute protocol and informed consent document updates to Participating Institutions as needed.
- Oversee the data collection process from Participating Institutions.
- Maintain documentation of Serious Adverse Event (SAE) reports and deviations/violation submitted by Participating Institutions and provide to the DF/HCC Sponsor for timely review.
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- Conduct regular communications with all Participating Institutions (conference calls, emails, etc) and maintain documentation all relevant communications.

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Each Participating Institution is expected to comply with all applicable federal regulations and DF/HCC requirements, the protocol and HIPAA requirements.

The general responsibilities for each Participating Institution may include but are not limited to:

- Document the delegation of research specific activities to study personnel.
- Commit to the accrual of participants to the protocol.
- Submit protocol and/or amendments to their local IRB.
- Maintain regulatory files as per sponsor requirements.
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The following section will clarify DF/HCC Requirements and further detail the expectations for participating in a DF/HCC Multi-Center protocol.

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The Coordinating Center will distribute the final DFCI IRB approved protocol and any subsequent amended protocols to all Participating Institutions.

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The Participating Institutions will receive notification of protocol revisions and closures from the Coordinating Center. It is the individual Participating Institution's responsibility to notify its IRB of these revisions.

- **Non life-threatening revisions:** Participating Institutions will receive written notification of protocol revisions regarding non life-threatening events from the Coordinating Center. Non-life-threatening protocol revisions must be IRB approved and implemented within a timely manner

- **Revisions for life-threatening causes:** Participating Institutions will receive immediate notification from the Coordinating Center concerning protocol revisions required to protect lives with follow-up by fax, mail, e-mail, etc. Life-threatening protocol revisions will be implemented immediately followed by IRB request for approval.

- **Protocol closures and temporary holds:** Participating Institutions will receive notification of protocol closures and temporary holds from the Coordinating Center. Closures and holds will be effective immediately. In addition, the Coordinating Center, will update the Participating Institutions on an ongoing basis about protocol accrual data so that they will be aware of imminent protocol closures.

3.3 Informed Consent Requirements

The DF/HCC approved informed consent document will serve as a template for the informed consent for Participating Institutions. The Participating Institution consent form must follow the

consent template as closely as possible and should adhere to specifications outlined in the DF/HCC Guidance Document on Model Consent Language for PI-Initiated Multi-Center Protocols. This document will be provided separately to each Participating Institution.

Participating Institutions are to send their version of the informed consent document and HIPAA authorization, if a separate document, to the Coordinating Center for review and approval prior to submission to their local IRB. The approved consent form must also be submitted to the Coordinating Center after approval by the local IRB for all consent versions.

The Principal Investigator (PI) at each Participating Institution will identify the physician members of the study team who will be obtaining consent and signing the consent form for therapeutic protocols. Participating institutions must follow the DF/HCC requirement that only attending physicians obtain informed consent and re-consent to interventional trials (i.e. drug and/or device trials).

3.4 IRB Documentation

The following must be on file with the Coordinating Center:

- Initial approval letter of the Participating Institution's IRB.
- Copy of the Informed Consent Form(s) approved by the Participating Institution's IRB.
- Participating Institution's IRB approval for all amendments.
- Annual approval letters by the Participating Institution's IRB.

3.5 IRB Re-Approval

Verification of IRB re-approval from the Participating Institutions is required in order to continue research activities. There is no grace period for continuing approvals.

The Coordinating Center will not register participants if a re-approval letter is not received from the Participating Institution on or before the anniversary of the previous approval date.

3.6 Participant Confidentiality and Authorization Statement

In 1996, congress passed the first federal law covering the privacy of health information known as the Health Insurance Portability and Accountability Act (HIPPA). Any information, related to the physical or mental health of an individual is called Protected Health Information (PHI). HIPAA outlines how and under what circumstances PHI can be used or disclosed.

In order for covered entities to use or disclose protected health information during the course of a study, the study participant must sign an authorization statement. This authorization statement may or may not be separate from the informed consent document. The Coordinating Center, with approval from the DFCI IRB, will provide a consent template, with information regarding authorization for the disclosure of protected health information.

The DF/HCC Sponsor will use all efforts to limit its use of protected health information in its trials. However, because of the nature of these trials, certain protected health information must be collected. DF/HCC has chosen to use authorizations, signed by the participant in the trial, rather than limited data sets with data use agreements.

DF/HCC Multi-Center Protocol Confidentiality

All documents, investigative reports, or information relating to the participant are strictly confidential. Whenever reasonably feasible, any participant specific reports (i.e. Pathology Reports, MRI Reports, Operative Reports, etc.) submitted to the Coordinating Center should be de-identified. It is recommended that the assigned DF/HCC QACT case number (as described below) be used for all participant specific documents. Participant initials may be included or retained for cross verification of identification.

3.7 DF/HCC Multi-Center Protocol Registration Policy

3.7.1 Participant Registration and Randomization

Refer to section 4.4 of the protocol for participant registration process.

3.7.2 Initiation of Therapy

Participants must be registered with the DF/HCC QACT before receiving treatment. Treatment may not be initiated until the Participating Institution receives confirmation of the participant's registration from the Coordinating Center. The DF/HCC Sponsor and DFCI IRB must be notified of any violations to this policy.

3.7.3 Eligibility Exceptions

The DF/HCC QACT will make no exceptions to the eligibility requirements for a protocol without DFCI IRB approval. The DF/HCC QACT requires each institution to fully comply with this requirement.

3.7.4 DF/HCC Protocol Case Number

At the time of registration, QACT requires the following identifiers for all subjects: initials, date of birth, gender, race and ethnicity. Once eligibility has been established and the participant successfully registered, the participant is assigned a unique protocol case number. Participating Institutions should submit all de-identified subsequent communication and documents to the Coordinating Center, using this case number to identify the subject.

3.8 Protocol Deviations, Exceptions and Violations

Federal Regulations require an IRB to review proposed changes in a research activity to ensure that researchers do not initiate changes in approved research without IRB review and approval,

except when necessary to eliminate apparent immediate hazards to the participant. DF/HCC requires all departures from the defined procedures set forth in the IRB approved protocol to be reported to the DF/HCC Sponsor, who in turn is responsible for reporting to the DFCI IRB.

For reporting purposes, DF/HCC uses the terms "violation", "deviation" and "exception" to describe departures from a protocol. All Participating Institutions must adhere to these requirements for reporting to the DF/HCC Sponsor and will follow their institutional policy for reporting to their local IRB.

3.8.1 Definitions

Protocol Deviation: Any departure from the defined procedures set forth in the IRB-approved protocol which is *prospectively approved* prior to its implementation.

Protocol Exception: Any protocol deviation that relates to the eligibility criteria, e.g. enrollment of a participant who does not meet all inclusion/exclusion criteria.

Protocol Violation: Any protocol deviation that was not *prospectively approved* by the IRB prior to its initiation or implementation.

3.8.2 Reporting Procedures

DF/HCC Sponsor: is responsible for ensuring that clear documentation is available in the medical record and/or regulatory documents to describe all protocol exceptions, deviations and violations. The DF/HCC Sponsor will also be responsible for ensuring that all protocol violations/deviations are promptly reported per DFCI IRB guidelines.

Participating Institutions: Protocol deviations require prospective approval from the DFCI IRB. The Participating Institution must submit the deviation request to the Coordinating Center who will then submit the deviation request to the DFCI IRB. Upon DFCI IRB approval the deviation is submitted to the Participating Institution IRB, per institutional policy. A copy of the Participating Institution's IRB report and determination will be forwarded to the Coordinating Center within 10 business days after the original submission.

All protocol violations must be sent to the Coordinating Center in a timely manner.

Coordinating Center: Upon receipt of the violation/deviation report from the Participating Institution, the Coordinating Center will submit the report to the DF/HCC Sponsor for review. Subsequently, the Participating Institution's IRB violation/deviation report will be submitted to the DFCI IRB for review per DFCI IRB reporting guidelines. DF/HCC will forward all violation reports via an internal DF/HCC process, as applicable.

3.9 Safety Assessments and Toxicity Monitoring

The study teams at all participating institutions are responsible for protecting the safety, rights and well-being of study participants. Recording and reporting of adverse events that occur during the course of a study help ensure the continuing safety of study participants.

All participants receiving investigational agents and/or other protocol mandated treatment will be evaluated for safety. The safety parameters include all laboratory tests and hematological abnormalities, physical examination findings, and spontaneous reports of adverse events reported by participants. All toxicities encountered during the study will be evaluated according to the criteria specified in the protocol. Life-threatening toxicities must be reported immediately to the DF/HCC Sponsor via the Coordinating Center.

Additional safety assessments and toxicity monitoring will be outlined in the protocol.

3.9.1 Guidelines for Reporting Serious Adverse Events

Guidelines for reporting Adverse Events (AEs) and Serious Adverse Events (SAEs) are detailed in protocol section 7.

Participating Institutions must report the SAEs to the DF/HCC Sponsor and the Coordinating Center following the DFCI IRB Adverse Event Reporting Policy.

The Coordinating Center will maintain documentation of all Participating Institution Adverse Event reports and be responsible for communicating to all participating investigators, any observations reportable under the DFCI IRB Reporting Requirements. Participating Institutions will review and submit to their IRB according to their institutional policies and procedures

3.9.2 Guidelines for Processing IND Safety Reports

The DF/HCC Sponsor will review all IND Safety Reports and ensure that all IND Safety Reports are distributed to the Participating Institutions. Participating Institutions will review and submit to their IRB according to their institutional policies and procedures.

3.10 Data Management

The DF/HCC QACT develops case report forms (CRF/eCRFs), for use with the protocol. These forms are designed to collect data for each study. The DF/HCC QACT provides a web based training for eCRF users.

3.10.1 Data Forms Review

Data submissions are monitored for timeliness and completeness of submission. Participating Institutions are notified of their data submission delinquencies in accordance with the following:

3.10.2 Incomplete or Questionable Data

If study forms are received with missing or questionable data, the submitting institution will receive a written or electronic query from the DF/HCC QACT Data Analyst, Coordinating Center or designee. Responses to all queries should be completed and submitted within 14 calendar days. Responses may be returned on the written query or on an amended paper case report form, or in the case of electronic queries, within the electronic data capture (eDC) system. In the case of a written query for data submitted on a paper case report form, the query must be attached to the specific data being re-submitted in response.

3.10.3 Missing Forms

If study forms are not submitted on schedule, the Participating Institution will receive a Missing Form Report from the Coordinating Center noting the missing forms. These reports are compiled by the DF/HCC QACT and distributed on a monthly basis.

4. REQUISITIONING INVESTIGATIONAL DRUG

The ordering of investigational agent is specified in the protocol section 8.

Participating Institutions should order their own agent regardless of the supplier.

Ensure that the pharmacy will be able to receive and store the agents according to state and federal requirements. The local IRB should be kept informed of who will supply the agent (i.e., Genentech) so that any regulatory responsibilities can be met in a timely fashion.

5. MONITORING: QUALITY CONTROL

The quality control process for a clinical trial requires verification of protocol compliance and data accuracy. The Coordinating Center, with the aid of the QACT provides quality control oversight for the protocol.

5.1 Ongoing Monitoring of Protocol Compliance

The Participating Institutions may be required to submit participant source documents to the Coordinating Center for monitoring. Participating Institution may also be subject to on-site monitoring conducted by the Coordinating Center.

The DF/HCC Lead Institution will implement on-site as well as virtual monitoring activities to ensure that Participating Institutions are complying with regulatory and protocol requirements, data quality, and subject safety. At a minimum, the DF/HCC Lead Institute, or designee, will monitor each participating site twice a year while patients are receiving treatment. Should a Participating Institution be monitored once and then not accrue any additional patients or participant visits, then a second monitoring visit may not be necessary.
Monitoring practices may include but are not limited to; source verification, review and analysis of the following: eligibility requirements of all participants, informed consent procedures, adverse events and all associated documentation, study drug administration / treatment, regulatory records and site trial master files, protocol deviations, pharmacy records, response assessments, and data management. Additionally, regular and ongoing communication with Participating Institutions, will be accomplished by holding all site bi-weekly teleconferences. The Lead Institution will keep in close touch with the Participating Institutions via email and phone. Source documents from Participating Institutions, will be collected at specific data points that support the primary and or secondary endpoints.

On-Site Monitoring: On-site monitoring will occur on an as-needed basis. Participating Institutions will be required to provide access to participants' complete medical record and source documents for source documentation verification during the on-site visit. In addition, upon request from a monitor or auditor, Participating Institutions should provide access to regulatory documents, pharmacy records, local policies related to the conduct of research, and any other trial-related documentation maintained by the participating site. If there are concerns for protocol compliance, issues that impact subject safety or the integrity of the study are found, or trends identified based on areas of need, additional monitoring visits may be scheduled. On site monitoring visits can be supplemented with virtual monitoring assessments, provided that the minimum monitoring frequencies are adhered to.

Virtual Monitoring: The Coordinating Center will request source documentation from Participating Institutions as needed to complete monitoring activities. Participating Institutions will be asked to forward de-identified copies of participants' medical record and source documents to the Coordinating Center to aid in source documentation verification.

The Overall PI, or designee, will review all monitoring reports for on-site and virtual monitoring of Participating Institutions to ensure protocol compliance and ability to fulfill responsibilities of participation in the study. The Coordinating Center may increase the monitoring activities at Participating Institutions that are unable to comply with the protocol, DF/HCC requirements or federal and local regulations. Participating Institutions may also be subject to an audit as determined by the Coordinating Center.

In addition to monitoring performed by the Coordinating Center, DF/HCC QACT may monitor data for timeliness of submission, completeness, and adherence to protocol requirements. The Lead Institution or designee and, if applicable, QACT Data Analysts assigned to the Protocol will perform the ongoing protocol data compliance monitoring with the support of the Participating Institution's Coordinators, the Principal Investigators, and the Protocol Chair.

5.2 Monitoring Reports

The DF/HCC Sponsor will review all monitoring reports for on-site and remote monitoring of Participating Institutions to ensure protocol compliance. The DF/HCC Sponsor may increase the monitoring activities at Participating Institutions that are unable to comply with the protocol, DF/HCC Sponsor requirements or federal and local regulations. Participating Institutions may also be subject to an audit as determined by the DF/HCC Sponsor.

5.3 Accrual Monitoring

Prior to extending a protocol to an external site, the DF/HCC Sponsor will establish accrual requirements for each participating institution. Accrual will be monitored for each participating institution by the DF/HCC Sponsor or designee. Sites that are not meeting their accrual expectations may be subject to termination. Sites are expected to accrue at least 3 patients per year.

6. AUDITING: QUALITY ASSURANCE

Auditing is a method of Quality Assurance. Its main focus is to measure whether standards and procedures were followed. Auditing is the systematic and independent examination of all trial related activities and documents. Audits determine if evaluated activities were appropriately conducted and whether data was generated, recorded and analyzed, and accurately reported per the protocol, Standard Operating Procedures (SOPs), and the Code of Federal Regulations (CFR).

6.1 Audit Plan: DF/HCC Sponsored Trials

One on-site audit will be scheduled by the QACT, assuming at least three participants have been treated on protocol at the site. Approximately 3-4 participants will be audited at the site over a 2 day period. If violations which impact participant safety or the integrity of the study are found, more participant records may be audited.

6.2 Audit Notification

It is the Participating Institution's responsibility to notify the Coordinating Center of all scheduled audit dates and re-audit dates (if applicable), which involve this protocol. All institutions will forward a copy of final audit and/or re-audit reports and corrective action plans (if applicable) to the Coordinating Center, within 12 weeks after the audit date.

6.3 Audit Reports

The DF/HCC Sponsor will review all final audit reports and corrective action plans if applicable. The Coordinating Center, must forward these reports to the DF/HCC QACT per DF/HCC policy for review by the DF/HCC Audit Committee. Based upon the audit assessments the DF/HCC Audit Committee could accept or conditionally accept the audit rating and final report.

Conditional approval could require the DF/HCC Sponsor to implement recommendations or require further follow-up. For unacceptable audits, the DF/HCC Audit Committee would forward the final audit report and corrective action plan to the DFCI IRB as applicable.

6.4 Participating Institution Performance

The DF/HCC Sponsor and the DFCI IRB, is charged with considering the totality of an institution's performance in considering institutional participation in the protocol.

Corrective Actions

Participating Institutions that fail to meet the performance goals of accrual, submission of timely and accurate data, adherence to protocol requirements, and compliance with state and federal regulations, may be recommended for a six-month probation period. Such institutions must respond with a corrective action plan and must demonstrate during the probation period that deficiencies have been corrected, as evidenced by the improved performance measures. Participating Institutions that fail to demonstrate significant improvement will be considered by the DF/HCC Sponsor for revocation of participation. A DF/HCC Sponsor and/or the DFCI IRB may terminate a site's participation if it is determined that a site is not fulfilling its responsibilities as described above.